SYSTEMATICS AND GENETICS OF THE LEUCAENA DIVERSIFOLIA (SCHLECHT.) BENTH. COMPLEX

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ABSTRACT

The Leucaena diversifolia complex has thirteen described species which are found mainly in southern Mexico and northern Central America. Living plants were cultivated at Waimanalo Research Station, Hawaii. The present study, involving morphological, cytological and isozymic examinations, and an extensive program of hybridization, including genetic study of F_1 and F_2 hybrid progenies, makes it possible for the first time to clarify taxonomic relationships within.

Cytological investigation showed that two gametic chromosome numbers occur within the complex. Diploid counts with n = 26 were obtained for 15 accessions. Fourteen accessions were found to be tetraploids with n = 52, among which four accessions were morphologically different from other accessions. All accessions of the complex observed frequently carried 1 to 8 extra chromosomes which may be B-chromosomes. Gametic chromosome numbers of 8 other species of Leucanea were also presented. One species, L. leucocephala, was confirmed as tetraploid (n = 52). Numbers of n= 26 were observed for L. collinsii, L. lanceolata, L. macophylla, L. shannoni and L. trichodes, and n = 28 for L. pulverulenta and L. retusa. The F_1 chromosome behavior of sesven hybrid combinations involving six taxa revealed that 26 chromosomes of all the diploid species examined were highly homologous to each other, and to half the genome of each of the tetreaploid species.

Study of herbarium specimens indicated that the Leucaena diversifolia complex could be divided into three groups. Diagnostic pubescence on leaflets and stem tips attributed the complex were shown to variate even within a single accession. An investigation of 30 accessions of living plants, utilizing 8 qualitative and 5 quantitative characteristics also revealed the presence of three groups in the complex: diploid L. diversifolia (DIV2N), tetraploid PSL. diversifoliaPS (DIV4N) and L. pallida (PAL). Morphological features serving to distinguish these groups were corola/calyx length ratio, lengths of stamens and styles, and inflorescence (head) size. DIV2N was found to be highly variable in most characters observed. Tetraploids occurred only in Veracruz, Mexico whereas diploids had much wider areas of distribution occurring from southern Mexico to northern Nicaragua. They were allopatric. Pollen siza and size of stomatal guard cells were correlated with ploidy level of L. diversifolia.

The self-pollination program revealed that diploid Leucaena diversifolia and L. pallida were self-compatible while tetraploid L. diversifolia were self-compatible. Analysis of F_1 progeny in diploid L. diversifolia indicated that the self-incompatibility was of the gametic type. The crossability of different groups within the complex was determined. There was no indication of any genetic crossing barrier between different groups, and even between morphologically different species. The F_1 hybrids of both

intra-specific and inter-specific crosses were remarkably vigorous. The F_2 hybrids resulting from F_1 sib-crossing of diploid L. diversifolia and two other diploid species, L. lanceolata and L. shannoni, however, showed genetic breakdown. Controlled pollination using F_1, F_2 and backcross hybrids of two different accessions of L. diversifolia showed single gene Mendelian inheritance of vesture on leaflets, rachilla and stem tips, with the pubescence alele dominant over the glabrous allele. Genetics of leaflet pairs per pinna and petiolar gland type were also discussed. Two cytotypes of L. diversifolia were separated by strong sterility barriers.

A study of peroxidase polymophism in different tissues and ages of Leucaena leucocephala was presented. Some peroxidase isozymes and their intensiy in corresponding tissues were specific to age or developmental stage. The analysis of isozyme polymorphism in Leucaena tissues revealed at least 6 bands, which appeared to represent 4 loci. The comparison of the peroxidase frequencies in seven taxa, including three groups of the L. diversifolia complex, indicated that accessions of diploid L. diversifolia were highly variable in peroxidase isozyme pattern, in agreement with their morphological variation. However peroxidase isozyme patterns in three tetraploid taxa, L. pallida, L. diversifolia and L. leucocephala were found to be invariable.

The available morphological, cytological and distributional evidence suggests that Leucaena pallida is

the amphiploid derivative of diploid L. diversifolia and L. esculenta. Based on morphological and distributional data, an accession from Guatemala, K740, and L. greggii are also considered to be amphiploids.

The studies indicate that the Leucaena diversifolia complex includes two species, L. pallida and L. diversifolia. The two cytotypes of the latter species are treated subspecies, L. diversifolia (Schletch.) Benth. ssp. diversifolia (tetraploids), and L. diversifolia (Schletch.) ssp. trichandra Pan et Brewbaker, stat. nov. (diploids).

The evolution of the observed pattern of differentiation and divergence in the genus Leucaena is also discussed.

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CHAPTER 1

INTRODUCTION

Leucaena Benth. is a genus of subtropical and tropical woody legumes originating in Mexico and Central America. The L. diversifolia complex involves 13 taxa that are distributed from Nicaragua to north Central Mexico in the highlands. These were used here for systematic studies that were based on herbarium materials and a living collection in Hawaii of mature trees grown from 30 accessions of this complex.

Leucaena leucocephala (Lam.) de Wit is the only commercial species, used widely throughout the world for forage and fuelwood (Brewbaker and Hutton, 1979). Its potential for firewood, timber, organic fertilizers and food have also been recognized (National Academy of Science, 1984). It is a drought tolerant species, adapted to marginal tropical lands and used by low income farmers. Disadvantages of L. leucocephala include high mimosine in foliage, which is toxic to nonruminants (National Academy of Science, 1984), and limited adaptation to low-elevation tropics and non-acid soils.

Increased interest in Leucaena diversifolia (Schlecht.) Benth. as a timber and forage tree was reflected in a recent symposium summarizing current knowlege of the growth and ecology of leucaenas (Brewbaker, 1982). Certain accessions of L. diversifolia were noted as complementing the genetic

variation of L. leucocephala and extending the range of the latter to cooler montane climates and the acid soils, and lower mimosine contents. Brewbaker and Hylin (1965) found that the lowest mimosine value were obtained from several strains of different species of Leucaena. A single accessions of L. diversifolia (referred to as "L. buitenzorg") was one of them. Hutton (1981), who conducted acid soil tolerance tests of Leucaena species in Colombia, observed two lines of L. diversifolia that had relatively high acid tolerance. Their growth in acid soils (pH 4.5) was much more vigorous than that of L. leucocephala (Hutton, 1981). Brewbaker and Hutton (1979) suggested that L. diversifolia would be better adapted to the cool tropics since some accessions of this species are fast-growing in their native locations at high elevations. The notably fast-growing nature of L. diversifolia at 1200 m confirmed its superiority of growth rate in cool areas (Brewbaker et al., 1981). This study was designed to examine the systematics of one portion of the genus -- the L. diversifolia complex.

The Leucaena diversifolia complex is defined here as plants of the genus Leucaena possessing pink flowers and small leaflets (less than 10 mm in length). The complex comprises thirteen described species which are morphologically quite similar to each other when compared with other species of the genus. A general description of this complex is as follows: shrubs or trees ranging to 15 m

in height; twigs usually terete; bark freckled with lenticels, brown or brownish purple: rachis and petioles minutely puberulent to densely pubescent, sometimes glabrous; rachis bearing glands at basal and terminal pairs of pinnae; glands orbicular, subrobicular or oblong, cupulate or depressed; heads (inflorescences) 8-20 mm in diameter at anthesis; peduncles 1-3 cm long; florets 80-120 per inflorescence; anthers pilose; and pods glabrous or puberulent. The complex is widespread, involving small populations in Honduras, El Salvador, Guatemala and southern Mexico, at elevations of 600-2300 m.

Many of the previously listed chracters are found to overlap in most described species which also display a great deal of interspecific and intraspecific variation. Some Mexican accessions of the complex (e.g. K178 and K376) planted at the Waimanalo Research Station were observed to have morphological characters intermediate between components of the Leucaena diversifolia complex and another very different species, L. esculenta (Moc. & Sesse) Benth., suggesting that these accessions might be hybrid derivatives. Therefore, taxonomic problems in the L. diversifolia complex are not resolvable by traditional methods. Utilization of modern taxonomic techniques is thus necessary.

The present study of the Leucaena diversifolia complex was accordingly approached through experimental hybridization, isozyme analysis and cytological studies. The

main objective of this study is to critically analyze the wide variation in the complex in order to produce a meaningful classification scheme. The evolution of the observed pattern of differentiation and divergence in the genus Leucaena is also discussed.

CHAPTER 2

LITERATURE REVIEW

2.1. Systematics of the L. diversifolia Complex

A review of literature indicated that 55 species were described in the genus Leucaena Benth (Index Kewensis, 1886-1950; Index Londonensis, 1930-1941). Most of these, however, are ill-defined and are considered to be of doubtful validity (Brewbaker et al., 1972; Brewbaker and Ito, 1980; Brewbaker, 1985). The L. diversifolia complex is a diverse segment of this genus, with a wide geographical range extending from Central America to southern Mexico. There are striking differences in plant growth habit, leaf size, nature of pubescence, head size, peduncle length, color and size of seeds, and pod shape. The magnitude of these differences is so great that the following 13 species have been relegated to the complex:

L. diversifolia (Schlecht.) Benth.,

L. brachycarpa Urban,

L. dugesiana Britton & Rose,

L. guatemalensis Britton & Rose,

L. laxifolia Urban,

L. oaxacana Britton & Rose,

L. pallida Britton & Rose,

L. paniculata Britton & Rose,

L. pueblana Britton & Rose,

L. revoluta Britton & Rose,

L. stanleyi Britton & Rose,

L. stenocarpa Urban and

L. trichandra (Zucc.) Urban.

Leucaena diversifolia was first described in 1838 as Acacia diversifolia by Schlechtendal. Bentham (1842) made a new combination of this taxon and treated it correctly as a species of Leucaena. L. brachycarpa was applied by Urban in 1900 based on the plant from cultivated tree in Hope, Jamaica. Britton and Rose (1928) later found this taxon distributed in Veracruz, Mexico and suggested that it was an introduction to Jamaica. L. dugesiana, L. oaxacana, L. pallida and L. paniculata were species applied by Britton and Rose in the same paper in 1928. L. pallida was first mentioned in the paper. Type specimens of L. guatemalensis were characterized by densely villose leaflets. Britton and Rose (1928) used this character todiagnose the species from others. L. laxifolia was described by Urban in 1900. No date and locality was recorded on the type specimen. Britton and Rose (1928) suggested it was from Mexico. Standley (1946) even indicated that it was of Veracruz origin. L. pueblana Britton & Rose had puberulent rachis and puberulent young branches. Britton and Rose (1928) established the species only by its rounded tip leaflets which were different from other species. L. revoluta Britton & Rose was

characterized by its revolute margin leaflets. Type specimens were collected on mountain slopes near Fenix, Chiapas, Mexico. L. standleyi Britton & Rose was collected in a pine forest at about 655-900 m elevation at Santa Ana, El Slavador. The species was with glabrous leaflets. L. stenocarpa was applied by Urban in 1900. The type speciemen was collected in the foothills of Sierra San Felipe at about 2000 m altitude, Oaxaca, Mexico. Young branches, rachis and rachilla of the type specimens were covered with dense pubescence. L. trichandra (Zucc.) Urban was first described as Acacia trichandra by Zuccarini (1838) on the plant grown in the Munich Garden in 1838 from Mexican seeds, origin unknown. Urban (1900) recognized it as a species of Leucaena.

Morphological distinctions among these species are not always clear-cut and problems in identification arise. It is evident that use of morphological features in the demarcation of species by taxonomists in the past has resulted in uncertain determinations in this complex. This raises the question of whether or not more than one species is included in the complex. Because all members of this complex possess pink flower heads, Brewbaker (1983a) viewed them as a single, large, polymorphic species which comprises several infraspecific taxa. He also visualized Leucaena diversifolia as a complex central to the evolution of many species of the genus (Brewbaker, 1985). A comprehensive study of all

species in the genus including the L. diversifolia complex has never been undertaken.

2.2. Higher Plant Speciation and Isolation Mechanisms

2.2.1. Isolation and the Origin of Species

Speciation involves the development of new and different gene combinations in separated populations. When populations are incapable of gene exchange under natural conditions, and are phenotypically distinct, species then can be formed (Lewis, 1969). Therefore, the differentiation of species is maintained by genetic barriers to gene exchange (Lewis, 1969). Hybridization and gene exchange among species are prevented or reduced by isolating mechanism of many different kinds.

Isolation mechanisms can be grouped into three main classes: geographical, ecological and reproductive (Stebbins 1950; Grant, 1981). Geographical isolation exists between any two allopatric populations or species which are separated geographically. The gaps that separate them are greater than the normal radius of dispersal of their pollen or seeds. Ecological isolation results from two populations or species occupy different habitats where they coexist in the same area. If there are blocks to gene exchange between populations or species which have genotypic differences in

relationships, the mechanism is called reproductive isolation.

Geographical isolation of populations is normally a necessary or even essential condition for the development of isolation mechanisms and thereby species formation (Heywood, 1976). Differentiation of populations by this mechanism is a common phenomenon in species of wide spread distribution.

Local differentiation in plant populations does not require geographic discontinuity. Antlfinger (1981) demonstrated that different taxa could be maintained by strong selection in the face of gene flow over short distances. Ecotypic differentiation in response to edaphic factors has been described in many plant species. For example, high soil concentrations of zinc was shown by Miller (1982) to be a contributing factor in the development of an adapted population.

New species may arise abruptly through the acquisition of reproductive isolation caused by gene mutations leading to chromosomal rearrangements. Hybrids formed between the new individuals and the parents would be sterile due to the inability of the chromosomes to pair at meiosis. For example, evidence from morphology, cytogenetics and isozyme analyses suggested that the composite species Stephanomeria malheurensis was immediately isolated subsequent to its origin with only little genetic change involved (Gottlieb, 1973a).

The other method of species formation is usually gradual and consists of the step-by-step accumulation of small differences caused by mutation, recombination and selection, often accompanied by a slow build-up of isolation mechanisms. If ecologically and geographically isolated populations have not reached a level of differentiation or degree of reproductive isolation for them to be treated as species, they may be referred to as races (Heywood, 1976) or varieties (Grant, 1981).

2.2.2. Taxonomic Species vs. Biological Species

Groups of plants recognized as species are in practice defined largely by morphological criteria. Species based on morphological similarities and differences are called taxonomic species (Grant, 1981). Taxonomic species has to be a usable unit in practical classification and identification. A disadvantage of this species concept is that, when using the criterion of morphological characteristics, there is no way to decide objectively whether a given taxon should be considered a species, a subspecies, or a variety. Different taxonomists may produce different systems of classification for the same group of organisms.

Biological species are groups of actually or potentially interbreeding populations which are reproductively isolated

from other such groups (Heywood, 1976; Grant, 1981). Inbreeding between biological species may be prevented by many kinds of isolation mechanisms.

During the past three decades the concept of biological species has raised a good deal of controversy about what species are, assuming that they are indeed real and not just taxonomic categories (Heywood, 1976). Ideally, it is preferable to equate taxonomic species with biological species wherever possible, but this can not always be done. It is suggested that species be defined on the basis of genetic or chromosomal incompatibility rather than adaptive discontinuites of which the incompatibility may be independent.

2.3. Hybridization in Higher Plants without Changing Chromosome Number

Hybridization has been known since antiquity to occur in both plants and animals. Only by the middle of this century, however, had the evolutionary significance of hybridization, based on genetic and cytological criteria, been authoritatively treated in plants (Anderson, 1949; Stebbins, 1950). Introgressive hybridization, as defined by Anderson (1949), is the repeated backcrossing of a natural hybrid to one or both parental populations. It results in the transfer of genes from one population to another across a breeding

barrier. The ability of populations to hybridize and exchange genes is a criterion that has been used in the formulation of the biological species concepts (Grant, 1981).

Hybridization between populations which have undergone a previous history of divergence to different taxa, and which are separated by certain isolation mechanism, represents a reversal in the process of evolutionary divergence. Grant (1981) therefore termed hybridization as "a reversal divergence". The divergence between the separated lines of populations may eventually reach a point of reproductive isolation when interbreeding is no longer possible at all.

2.3.1. The Role of Hybridization in Plant Evolution

During the first half of this century it was thought that mutation has the major role in effecting diversity in nature (Knobloch, 1972). Considering hybridization as the major force in producing variation, Knobloch (1972) listed most of the known intergeneric hybrids in flowering plants. Many reports have demonstrated the importance of hybridization in speciation (e.g. Grant, 1953; 1966).

Hybridization between populations with different adaptive norms, followed by introgressive hybridization, may greatly enhance the level of variation. Rattenbury (1962) argued that introgressive hybridization might have permitted the tropical forest elements in New Zealand to survive periodic cooling during the late Pleiocene and early

Pleistocene. This might account for the high incidence of hybridization and usually high levels of variation in the New Zealand flora (Rattenbury, 1962). The great diversity in the Hawaiian species of Wikstroemia was also thought to be due to hybridization between species (Gupta and Gillett, 1969). In his studies of Hawaiian flora, Gillett (1966) concluded that weak barriers to hybridization and extensive hybridization in plants were evident in the Hawaiian Islands. Hybridization had blurred the morphological discontinuities between species. Grant (1953) distinguished various types of hybrid complexes of genus Gilia. He suggested that the taxonomic complexity of the groups was due to occasional hybridization between populations. The wide variation noted in populations of Flaveria linearis and F. floridana was also attributed to introgression (Long and Rhamstine, 1968).

Hybridization may also set the stage for the evolution of novel lineages via stabilization of intermediate transgressive hybrid derivatives (Anderson, 1949; Stebbins, 1950). With self-fertilization, hybrids may proceed through several generations of inbreeding, with the resultant stabilization of certain combinations (Levin, 1979). The stabilization of hybrid dervatives could account for the evolution of new species. Through hybridization, sympatric speciation in angiosperms becomes a possible and plausible mechanism. Straw (1955) presented a strong case for the hypothesis of sympatric speciation. He described the

stabilization of hybrid derivatives by pollinators in Penstemon, and concluded that all the hybrids among species of this genus had their own normal range of selective pollinators. The formation of new species without change in ploidy level via experimental manipulation was also studied by Grant (1966). The fertile hybrid dèrivative from the usually sterile hybrid Gilia malior x modocensis has its own distinctive character combinations and is intersterile with both parental species. It represents an experimental case of the hybrid origin of a newly isolated species.

Lewis and Epling (1959) also presented evidence that Delphinium gypsophilum had its origin from a sequence of hybridizations and the fixations of a limited number of hybrid genotypes. He suggested that this species originated by a more direct process of recombination than that ordinarily referred to as introgression. Derived species from natural hybridization between different genera have also been reported. Purshia glandulosa was shown to be a stabilized segregant from the hybridization products of Cowania stansburyana and Purshia tridentata (Stutz and Thomas, 1964).

2.3.2. Adaptation of Hybrids

In a stable environment in which no ecological niches are opened to colonization, all of the gene combinations generated by hybridization are likely to be less adaptive than those of the parental races or species, and are discarded by natural selection (Stebbins, 1971). Since the hybrids are not so well adapted to their habitats as their parental races or species, the species remain distinct in nature in the face of hybridization (Anderson, 1949). When products of hybridization are exposed to a changing environment, in which many new ecological niches are being opened, some of the new combinations are more likely to be better adapted to these new conditions than are any genotypes present in the old established populations (Stebbins, 1971). A varied array of ecological niches is required for the survival of F_2 and later generations of hybridization because these progenies contain a great diversity of recombinational types with repect to physiological traits. This is referred to Anderson's "hybridization of habitat" (Anderson, 1949).

Hybrids are often associated with disturbed habitats where the natural vegetation communities have been disturbed (Anderson, 1949). Human activities often create new habitats. In disturbed habitats formed by deforestation, clearing, cultivation, construction, etc., some hybrids were found to be better adapted than their parents. For example, stabilized populations of a species of hybrid origin

(Flaveria latifolia, Asteraceae) become adapted to the fill material dredged from the ocean and used in road building and land development in southern Florida (Long and Rhamstine, 1968). In prehuman times, geological, climatic and floristic shifts, which may have opened new habitats, may also have greatly increased opportunities for hybridization (Levin, 1979). Anderson and Stebbins (1954) postulated that the speed of evolution and bursts of innovative diversification at various times in the past may be the consequence of hybridization.

2.3.3. Hybridization in Leucaena Species

As mentioned earlier, natural hybridization is not uncommon in higher plant populations. If a variant character is intermediate between the suspected parental species in most or all of the characters in which they differ, the case for hybridity is strong (Maze, 1968).

Species hybridization in Leucaena was found to occur between L. leucocephala and L. pulverulenta in Indonesia where man had introduced these species for shading trees or crops (Brewbaker et al., 1972). Significant natural hybridization was reported in a number of other Leucaena species (Hutton, 1981). Barriers to interspecific hybridization in the genus Leucaena seem to be lacking. Under experimental conditions, Brewbaker (1983b) found that

at least some gene exchange took place between most species in the genus. It is apparent that hybridization has contributed to some of the taxonomic difficulties in Leucaena.

2.4. Polyploidy

Plants with three or more complete sets of chromosomes are collectively termed polyploids. Polyploidy is a common and widespread genetic system in higher plants. Love (1964) estimated that polyploidy occurs in approximately 30 percent of the dicotyledons and 50 percent of the monocotyledons. Stebbins (1950) estimated that approximately one-third (30-35 percent) of the species of angiosperms are polyploids. The estimates of polyploids in dicotyledons by Grant (1981) are even higher. He classified species with 14 or more pairs chromosome as polyploids and those with fewer than 14 pairs as diploids, and estimated that about 43 percent of the dicotyledons are polyploids. Wright (1976) postulated that polyploidy seems to be as frequent in woody plants as in herbaceous plants.

Polyploids can be subdivided according to whether the chromosome sets are derived from the same parental species (autopolyploid) or different parental species (allopolyploid), and defined as alloploids (Stebbins, 1947). Distinguishing between these categories and agreeing on their boundaries, however, is very difficult. Sometimes polyploids

derived from hybridization between well-differentiated populations within a species may be as successful phylogenetically as are polyploids derived from hybridization between related species (de Wet, 1971).

2.4.1. Evolutionary Significance of Polyploids

The best known mechanism to produce polyploids is by disruption of the mitotic spindle and failure of cytokinesis (Jackson, 1976). Unreduced gametes are produced in a low frequency in all sexually reproducing organisms, and this phenomenon offers a mechanism for polyploid increase in chromosomes (Jackson, 1976).

Most polyploids are formed as a result of a doubling of the chromosomes of hybrids produced between separate species, or at least between different accessions or races of the same species. Stebbins (1950) suggested that the subfamily Pomoideae (n = 17) of Rosaceae arose through ancient hybridization and subsequent chromosome doubling between a spiraea-like plant (n = 9) and a cherry-like plant (n = 8). The genus Tilia has n = 41 chromosomes, whereas all its relatives have n = 7 chromosomes. Wright (1976) thus postulated that a hexaploid (2n = 42) plant was produced from an n = 7 ancestor. This hexaploid then lost a single chromosome to produce an n = 41 plant. Stebbins (1947) was convinced that euploids are primarily of the alloploid type,

due to a doubling of the chromosome number following hybridization.

The primary phylogenetic effect of polyploidy is to stabilize select hybrid genotypes. Mooring (1975) suggested that the major role of polyploidy in Eriophyllum lanatum was to stabilize the products of intra- and inter-varietal hybridization. Another study showed that the hybrid derivatives of Chaenactis douglasii complex was stabilized by polyploidy (Mooring, 1980).

Incipient species may be formed by polyploidy, followed by a gradual process of further differentiation under suitable circumstances. Cytogenetic evidence suggested that isolation between two sections of Lipochaeta was due to differences in ploidy level (Rabakonandrianina, 1980). Studies of Malvaceae (cited by Menzel and Wilson, 1969) strongly suggested that allopolyploidy was a major mechanism of speciation in this family. By means of cytological analysis, Abdel-Hammeed and Snow (1972) concluded that the diploid species Clarkia amoena spp. huntiana and C. lassenensis donated genomes to the tetraploid species C. gracilis.

Polyploidy provides a mechanism by which daughter and parental populations become immediately isolated from each other. In practice most taxonomists do not give species status or other formal taxonomic recognition to the morphologically identical populations differing in ploidy

level. Polyploids are normally unable to form fertile hybrids with diploids due to inability of many of chromosomes to pair (Lewis, 1967). A diploid-tetraploid pair is separated by the formation of sterile triploids. In this case, polyploidy may be regarded as a mechanism of abrupt species formation. If such polyploids are able to find suitable ecological niches, and establish themselves as differentiated populations, they can be definitively treated as independent species, even though there may be little morphological difference between the diploids and polyploids (Lewis, 1967; Heywood, 1976; Jackson, 1976). Lewis (1967) presented data from Delphinium to illustrate the effect of barriers to gene exchange between diploid and tetraploid populations which are indistinquishable from each other in external morphology. Love (1964) also suggested that polyploidy represents a primary genetic isolation mechanism, and different polyploid levels deserve specific rank. However, hybridization of diploid and tetraploid entities sometimes can give rise to a series of combinations and recombinations to produce a great variety of polyploid forms. For example, in Dactylis, triploid hybrids produce appreciable quantities of good seeds when backcrossed to either diploid or tetraploid male parents (Jones, 1959). In this case, the incidence of polyploidy does not justify treating the different cytotypes as separate species.

2.4.2. Adaptation of Polyploids

Polyploids, especially allopolyploids, generally have different ecological requirements than their diploid parents. Hybrids need new habitats in which to become established. Success of polyploids will depend on the availability of their adaptation to habitats, as well as their ability to eliminate diploid immigrants.

Many individual polyploid genotypes have phenotypes which are able to tolerate a wide range of environmental conditions (Stebbins, 1971). Polyploid duplication of chromosomes enhances the production of new gene combinations that can be acted upon by natural selection and may allow the polyploids to become adapted to new habitats (Reese, 1959; de Wet, 1971). The potential increase in favorable gene combinations in polyploids may permit them to colonize disturbed habitats far more readily than diploids (Bayer and Stebbins, 1981). Increased size of organs, particularly seeds, which accompanies polyploidy may also help in the process of stabilization and establishment in new habitats, since it increases seedling vigor (Stebbins, 1971).

Major climatic and geologic changes may promote rapid evolution of new taxa. Stebbins (1950) thought that polyploidy confers a degree of evolutionary variability that permits a species to adapt readily to new habitats or vigorous growth conditions. Thus, he considered that plants found in arctic and glaciated regions are apt to have high

chromosome numbers. Diploids tend to be found on more ancient substrates while polyploids tend to be found on relatively modern ones. Polyploid species of the Chaenactis douglasii complex, thought to be derivatives of other diploid species, are tolerant of increased aridity and occupy habitats of recent availability following disturbance by volcanic and glacial activities or by glacially related processes (Mooring, 1980).

Polyploids tend to occur on massively disturbed substerates. Stebbins (1971) suggested that polyploidy-disturbance correlations are more significant than polyploidy-climatic conditions. Woody angiosperms such as Rosa crataegus and Rubus were reported to be successful in colonizing open habitats created by human destruction of forests (Stebbins, 1971).

Many polyploids of Antennaria (Asteraceae) occur only in glaciated regions, and increase in frequency with increase in latitude in the eastern United States (Bayer and Stebbins, 1981). They found all of the diploids are predominant below the glacial margin. Love and Love (1943) and Johnson and Packer (1965) also noted that the frequency of polyploidy increased with increasing latitude. This may have been due to a selective superiority of polyploids over their diploid parents to invade pioneer habitats, or they may have been albe to survive extreme conditions during the Pleistocene glaciation (reviewed by de Wet, 1971).

2.4.3. Polyploids of Woody Plants

There are modal differences in chromosome numbers between woody and herbaceous angiosperms. In the flora of the temperate and tropical zones, trees and shrubs have higher basic numbers and lower frequencies of polyploidy with genera than perennial herbs (Stebbins, 1971). The basic chromosome numbers of these woody plants are similar with x = 11, 12, 13, and 14 (Stebbins, 1971). Most groups (families or genera) of woody dicotyledons listed by Wright (1976) also had basic numbers in the polyploid range (chromosome number n = 13). Stebbins (1971) suggested that basic numbers of modern woody plants were derived from ancient polyploidy, and that the original basic numbers of angiosperms were x = 6 and x = 7. These plants evidently had undergone "secondary cycles of polyploidy" and represented old polyploid complexes (Stebbins, 1950, 1971; Grant, 1981). Stebbins (1971) hypothesized that the polyploidy which gave rise to the basic numbers of woody plants took place at various times during the Cretaceous and the earliest part of the Tertiary period, while the diversification of species on the basis of secondary basic numbers is largely a product of the Tertiary and Quaternary periods.

2.4.4. Effects of Polyploidy on Pollen and Grard Cells

Effects of polyploidy on morphology have long been known. It is believed that an increase in cell size is roughly equivalent to the increase in nuclear volume that results from chromosome doubling and there often appears to be a direct causal relation between increased cell size and enlargement of fully differentiated organs (Randolph, 1941). Thus, polyploids often have statistically significant differences in features such as pollen diameter, epidermal cell size, stomatal guard cell size, seed size, root and stem meristems, etc. Johnson and Sass (1944) reported that tetraploid plants usually have larger vegetative and floral organs, larger leaf and root cells and larger somatic nuclei than diploids. Sizes of pollen grains and stomata have been widely used as morphological indicators of ploidy level, and are used to determine the ploidy level of the uncounted species by comparing the sizes of stomatal guard cells or pollen from known diploids, tetraploids, etc.

Pollen grains of tetraploid and diploid plants are usually distinctly different with respect to size, volume and shape and can be easily distinguished under the microscope. Pollen diameter is positively correlated with chromosome number and has been successfully employed in the identification of ploidy levels in many species. An average increase in pollen diameter of 27% and increase in pollen volume of 103% were noted in Melilotus alba tetraploids in

comparison with diploids (Johnson and Sass, 1944). In the same investigation, the tetraploid pollen grains were in all cases somewhat triangular in shape in contrast to the oval shape of the diploid pollen grains. In Townsendia (Asteraceae), pollen grains of the polyploids were distinctly larger than those of the diploids (Beaman, 1954). Pollen size was also correlated with ploidy level in the Bothriochloa saccharioides complex (Poaceae) (Allred and Gould, 1983). Pollen measurements indicated that pollen size in Lipochaeta was correlated with doubling of the chromosome number (Rabakonandrianina and Carr, 1981).

Pollen size proved useless in indicating ploidy level in Epilobium latifolium (Onagraceae), however, the number of pores in the pollen grains was found useful for that purpose (Small, 1968). Tetraploids of the species were found to have a high percentage of 4-pored pollen, while the diploids usually had only 3-pored pollen (Small, 1968). In Ambrosia dumosa (Asteraceae), pollen size can be used to separate the diploids from the tetraploids, but the separation of tetraploids from hexaploids is difficult (Raven et al., 1968).

Although it is possible to separate diploid and tetraploid populations in any one locality, the features by which they may be recognized tend to vary from one locality to another (Small, 1968). Moreover, mineral nutrition appears to affect the extent of variability of pollen size

(Bell, 1959). Anyone using pollen size as an indicator of polyploidy or as a taxonomic characteristic should consider these factors.

The stomatal guard cell size is also known to be a useful indicator of polyploidy. Leaf material is generally available in herbarium specimens while pollen grains may be absent. Thus, the measurement of guard cells has greater potential as an indicator of differences in ploidy.

The size of stomatal guard cells in tetraploid Melilotus alba showed an increase of 36.7% in comparison with the diploid (Johnson and Sass, 1944). Differences in the size of guard cells of diploids and triploids were found to be highly significant in pineapples (Hernandez, 1954). A positive relationship exists between ploidy level and the length of stomatal guard cells in Bromus inermis an and Dunn, 1973; 1975). Tetraploids had the smallest guard cells and highest stomatal density, while octoploids had the largest guard cells and lowest stomatal density. Hexaploids were intermediate in these features. None of the ploidy levels overlap in these measurements. High correlations between stomatal guard cell size and ploidy level are also found in Galax (Neson, 1983) and Carya (Stone, 1961).

2.5. Cytological Analyses in Higher Plant Systematics

Cytotaxonomy refers to the use of chromosome number and morphology as a data source for classification (Jones and Luchsinger, 1979). Cytogenetics is the study of observations of chromosome pairing or behavior (Jones and Luchsinger, 1979). Both are regarded as modern techniques for cytological study.

In recent years, cytological data have been used successfully as important criteria in systematic work. Information from cytological studies has proved to aid in the resolution of genetic, taxonomic and phylogenetic problems within small groups. This technique may also aid in the interpretation of difficult species complexes such as eucaena diversifolia.

2.5.1. Chromosome Numbers and Plant Taxonomy

Chromosome number alone is useful in systematic studies. Since it is relatively easy to obtain and interpret, by far the largest number of cytological observations are limited to this aspect of chromosomes. Chromosome number often shows a high degree of stability within a species and often correlates with the natural grouping of species. Compared with any single morphological feature, the chromosome number is also more stable. Therefore, chromosome number may be a good marker for the delimitation of taxonomic groups.

Changes in chromosome number are often associated with speciation. Two sets of basic chromosome numbers occur within the species Coreopsis nuecensis. These chromosome numbers are correlated with some morphological differences, with distributional differences and with strong sterility barriers in the F_1 hybrids. Smith (1972) proposed that the x = 9,10 segment of this species be recognized as a new species. Similarily, in accord with its cytological and morphological distinctiveness and its geographical disjunction, the Sierran element of Calycadenia pauciflora was described as the new species, C. hooveri by Carr (1975). This new species has a chromosome number of n = 7, while others have n = 6.

Chromosome numbers may help in clarifying taxonomic confusion. Certain taxonomic problems in Cactaceae have been elucidated by the investigation of chromosome numbers and meiotic behavior (Pinkava and Mcleod, 1971; Pinkava et al., 1973). On this basis, these authors reduced several species to a single species with several varieties. Similarily, the morphologically diverse weedy populations of Euphorbua were classified as a single species on the basis of chromosome numbers (Pritchare, 1959).

2.5.2. Cytologicval Evidence in Phylogenetic Analysis

Within taxonomically complex genera of flowering plants, chromosome numbers are often helpful in indicating natural relationship (Stuessy, 1971). In Hawaiian Wikstroemia, a limited cytological study together with morphological evidence showed that W. pulcherrima (x = 18) could be an autopolyploid race of W. phillyreifolia (x = 9). (Gupta and Gillet, 1969).

Aneuploid and polyploid changes in chromosome number, often result in linear relationships. The chromosomal relationships between a number of species of Clarkia have been demonstrated by Lewis (1953). The original basic chromosome number of this genus was suggested to be x = 7. Subsequent changes in chromosome number had involved the addition of the equivalent of whole chromosomes to produce genomes of 8 and 9. Additional species are characterized by an aneuploid reduction in chromsome number from the original base of x = 7. The same pattern of chromosomal relationships was also presented by Stace (1978) in the genus Calotis (Compositae) which had n = 4,5,7 and 8.

The study of the process of meiosis and of the production of gametes in plants is of considerable importance in understanding the processes of genetic transmission from generation to generation. Meiotic analyses can tell much about the mechanisms producing sterility in hybrids and thus

provide evolutionary information. Such studies are usually made by observing chromosomes at metaphase and diakinesis stages of meiosis. Chromosomal behavior at meiosis may be used to assess relationships between species. For example, judging by chromosome pairing of the hybrids, Lewis and Raven (1958) were able to propose that Clarkia amoena was an ancient species which gave rise to C. rubicunda and which was the progenitor of C. franciscana. Numerous naturally occurring polyploids are characterized by chromosomes that usually associate into bivalents during sporogenesis, but occasionally fail to pair or may associate into multivalents. Multivalents, usually rings or chains, were nearly always associated with polyploids of the Chaenactis douglasii complex (Mooring, 1980). The origin of polyploid species can thus be predicted by cytological investigation.

As a consequence of the intense selection pressure that may exist in severe environments, rapid rates of speciation may occur, resulting in the origin of species differentiated genetically from their progenitors in only minor ways. Chromosomal structural differentiation between related species is considered to be a rapid mode of speciation which occurs in many plant groups. Carr (1980) crossed two aneuploid chromosome races of Calycadenia pauciflora and observed the pairing configuration of chromosomes in the F_1, F_2, and F_3 generations. From the chromosome behavior in each generation, he concluded that the derived race could have

originated directly from the ancestral race in nature through a single saltational event involving multiple chromosome breaks. Another cytological study by the same anthor indicated that saltational events have also lead to the formation of other species in the genus (Carr, 1977). Several other papers describe the chromosomal relationships between progenitor and derivative species of Asteraceae (Stephanomeria- Gottilieb, 1973b, Coreopsis-Lasthenia- Ornduff, 1976). From these reports, it can be concluded that simple chromosome rearrangements can be much more important in contributing to hybrid sterility than genetic differences in some cases.

2.5.3. Chromosome Evolution in Leguminosae

The Leguminosae is predominantly a family of tropical and subtropical affinities. The family lives in diverse habitats, and varies in growth type from herbs to vines, shrubs and trees. It is usually divided into three subfamilies: Caesalpinioideae, Faboideae and Mimosoideae. The range of chromosome numbers is different for each subfamily. For example, Bandel (1974) reported that the most frequent haploid numbers in Caesalpinioideae are n = 14 and 12, in Faboideae n = 8, and in Mimosoideae are

are n = 13 and 14. Variation in chromosome number occurs at the subfamily level as well as at tribe and genus levels. This indicates that the evolution of tribes proceeded independently within the subfamily level. Variation in chromosome number within the tribes is evidence that several evolutionary branches were formed at the genus level (Bandel, 1974). Senn (1938) and Turner and Fearing (1960) postulated a number of chromosomal evolutionary trends in this family.

2.5.4. Chromosome Numbers in Mimosoideae

In order of evolutionary speciation, the Mimosoideae is usually considered the most primitive of the three subfamilies of the family. The following haploid chromosome numbers in Mimosoideae were presented by Bandel (1974): 8, 11, 12, 13, 14, 15, 16, 18, 20, 22, 25, 26, 28, 38, 52 and 104. The most frequent haploid numbers and their percentages are: n = 13 (56.8%), n = 14 (14.6%), n = 26 (12.3%) and n = 52 (5.0%) (Bandel, 1974). The most frequent basic numbers are n = 13 and 14. These high basic numbers seem to be secondary polyploid derivatives (Stebbins, 1966, 1971). Stebbins (1966) suggested that both aneuploid alternations of basic number and polyploidy took place during the early evolution of the angiosperms, including this subfamily. Senn (1938) postulated that aneuploid loss had probably been more frequent than aneuploid gain.

2.5.5. Chromosomes in the Genus *Leucaena*

Leucaena is a genus of Mimosoideae. Only limited cytological studies have been reported for this genus. The chromosome numbers of 8 species have been reported: These include *L. leucocephala* (2n = 104 - Tijo, 1948), *L. pulverulenta* (2n = 56 - Turner and Fearing, 1960; Gonzalez et al., 1967), *L. collinsii* (2n = 102 - Hutton, 1981) and *L. lanceolata*, *L. diversifolia*, *L. esculenta*, *L. shannoni*, *L. trichodes* (2n = 52 - Gonzalez et al., 1967; Hutton, 1981). Except for the *L. collinsii* count which is doubtful, the chromosome numbers of the most studied species in the genus can be interpreted as a multiple of x = 13, while *L. pulverulenta* is based on x = 14. The ancient basic numbers of this genus seem also to be x = 6 and 7.

Usually, chromosome uniformity is frequent among the tropical woody legumes (Atchison, 1951). This stability is characteristic not only of species within a genus but also commonly of genera within a tribe (Atchison, 1951). The genus *Leucaena* has at least 3 different chromosome numbers (n = 26, 28, 52). It should thus be considered unusually variable in this respect. Indeed, together with morphological characters, chromosome numbers may provide a basis for classification within the genus.

2.6. Electrophoresis as an Important Tool of Systematic Study in Higher Plants

Gel electrophoresis, first described by Smithies (1955), provides a simple method for the detection of minute protein differences from a relatively small quantity of crude extract. With this technique, proteins are separated on the bases of their net charge and molecular sizes. Methods have been developed for staining specific proteins. Hunter and Markert (1957) proposed the term "zymogram" to describe the visible patterns produced after staining for specific proteins. These zymograms are sometimes described as the "fingerprints" of a particular material (e.g. Qualset and Wrigley, 1979).

Enzymes extracted from plants can be separated into different molecular forms called isozymes, which are capable of catalyzing the same reaction (Markert and Moller, 1959). Isozymes may differ in primary structure because they are encoded in different genes, either allelic or nonallelic (Nelson and Burr, 1973). Many reports have described methods of how to analyze various kinds of isozymes effectively in higher plant studies. For example, Brewbaker et al. (1968, 1975) have provided convenient methods and recipes in their "Isoenzyme Polymorphism in Flowering Plants" series. Use of electrophoretic techniques to measure isozyme heterogeneity for taxonomic, evolutionary and other genetic studies has

been investigated intensively by many researchers (reviews by Scandalios, 1969; Pierce and Brewbaker, 1973; Gottlieb, 1977 and 1981a).

2.6.1. Taxonomic Applications of Isozyme Data

Application of isozyme assays in plant taxonomy is a relatively recent development. Several investigators have examined the possibility of separating proteins or enzymes of higher plants by electrophoresis in starch or acrylamide gel, and using the resultant patterns (zymograms) as taxonomic evidence. Sibley (1968) pointed out that one of the two activities of genes is to direct protein synthesis. It follows that the genotyope of an organism is reflected in its protein complement and that genetic control of morphology must be exerted through protein metabolism. It seems reasonable then to assume that a close relationship exists between the protein complement of an organism and its morphology (Sibley, 1968). Thus, there is a theoretical basis for attempting to apply gel electrophoresis to taxonomic studies.

Electrophoretic analyses have shown that some species differ from one another in band frequencies or patterns. Studies in Chenopodium (Crawford and Wilson, 1979) indicated that isozyme data were concordant with information from morphology, flavonoid chemistry and seed protein profiles. This demonstrated that the electrophoretic technique might be

a valuable approach in plant systematics in which the plants are phenotypically plastic and where patterns of morphological variability might be difficult to interpret. Bell and Lester (1980) confirmed the existence of the doubted species Sabatia formosa Buckl, by using morphological measurement and isozyme patterns obtained by starch gel electrophoresis. Gottlieb (1973a, 1974) used the isozyme technique to differentiate species of Clarkia and confirmed the origin of one species of Clarkia. The genus Hordeum was surveyed for distinct forms of 5 enzymes. The similarities between zymograms of the species examined agreed with the affinities based on cytogenetic analysis (Mitra et al. 1970). Sheen (1972) used leaf peroxidase band patterns to distinguish many of the 60 Nicotiana species in his study. Comparisons of six Nicotiana species showed that each species exhibited distinct and characteristic isozyme and protein patterns (Hart and Bhatia, 1967). The same study also revealed that variation within a species of Nicotiana is much less than between species (Hart and Bhatia, 1967).

The isozyme technique is an important supplemental method for identification of lower taxa such as subspecies, variety, race or cultivar. Oliver and Rejon (1980) used esterase isozymes of Muscari atlanticum for racial and biogeographical analysis in Spain, and indicated the usefulness of the electrophoretic technique in race identification. Isozyme data are available for quickly and

positively identifying wheat varities, clones of citrus (Button et al., 1976), and turfgrass (Green et al., 1981). Recently, Cardy and Kannenberg (1982) proved that isozyme patterns were useful for identification of maize populations, including inbred lines and hybrids. Cultivar identification by zymograms has been particularly feasible, as in wheats (Qualset and Wrigley, 1979), roses (Kuhns and Fretz, 1979), and ryegrasses (Kranski and Bula, 1970). The technique was used in identifying breeding material in alfalfa (Quiros, 1980).

These investigations show that enzyme electrophoresis is a dependable method for the identification of many plant species as well as varieties or cultivars within the species. Lecture and Garber (1974) suggested that morphological characters and experimental crosses or other systematic methods should be used in conjunction with isozyme techniques before conclusions are shown.

2.6.2. Electrophoretic Evidence of Progenitor and Derivative Species

Electrophoretic evidence allows predictions concerning progenitor and derivative species in some taxa. In recent years this technique was used by researchers to elucidate relationships of many species pairs. For example, Quiros (1980) applied the technique of starch gel electrophoresis

for the identification of alfalfa (Medicago sativa) maternal plants by using four enzyme systems. Isozyme patterns of peroxidase, malate dehydrogenase (MDH), and esterase provided evidence concerning the origin of Nicotiana tabacum (Sheen, 1972). Gottlieb (1973b,c) was able to demonstrate that Stephanomeria exigua ssp. coronaria was very likely the sympatric progenitor of another species S. malheurensis. Reddy and Garber (1971) observed that some tetraploid Nicotiana species possessed most peroxidase and esterase isozymes of their putative parental diploid species and indicated that the pertinent diploid species might have been involved in the origin of each tetraploid species. Isozyme data strongly support the contention that the grapefruit originated from a cross between pummelo, Citrus grandis, and sweet orange, C. sinensis (Scora et al., 1982).

When evidence from morphology, cytology, geographical distributions and ecology combine to suggest that one species has recently originated from another in a rapid series of events, both species are expected to possess in common a high proportion of their alleles as detected by electrophoretic analysis of enzyme variation (Gottlieb, 1974). Gottlieb (1974) provided additional evidence that Clarkia lingulata originated recently from C. biloba. The related progenitor and recent derivative species Coreopsis nuecensoides and C. nuecensis exhibited very high similarity for genes coding for enzymes (Crawford and Smith, 1982). Isozyme data also

supported the hypothesis that C. basalia and C. wrightii are more closely related to each other than to other species (Crawford and Smith, 1982). These studies indicated that speciation occurred via chromosomal repatterning with minimal divergence at genes coding for isozymes.

Theoretically, the alleles of the derivative should still be present in the progenitor and the derivative should have very few or no unique ones (Gottlieb, 1981a). However, new alleles may result from mutation and eventually may become estabished in the derivative (Gottlieb, 1981a). In any case, the smaller the number of generations elapsed, the more genetically similar it should be to its parent (Gottlieb, 1973c).

2.6.3. Electrophoretic Evidence in Polyploid Plants

Polyploid individuals contain more than one copy of each gene, and have the potential to express more isozymes than diploids (Roose and Gottlieb, 1976). The presence of additional isozymes in a polyploid is a direct consequence of its mode of origin via chromosome doubling (Gottlieb, 1981a).

To determine the probable origin of polyploid species of Phaseolus, Lecture and Garber (1974) found that the .pa amphiploid exhibited all esterase and leucine aminopeptidase (LAP) zymograms present in the putative parental species. Roose and Gottlieb (1976) used 11 enzyme systems to discuss the relationship between diploid and tetraploid species in

Tragopogon. Every one of the enzymes detected in each tetraploid species was fully accounted for by simple additivity of polypeptide subunits specified by alleles inherited from its diploid parents. Studies of five enzyme systems in Triticinae revealed that the number of variant enzyme bands increased with the level of ploidy, and the amphiploid isozyme pattern was additive of the parental diploid species (Mitra and Bhatia, 1971). Sing and Brewer (1969) studied different ploidy levels of wheats by examining 9 enzyme systems. The results with phosphoglucomutase (PGM) suggested that the hexaploid (AABBDD), the (BB) or (DD) pattern, but not all enzyme systems, revealed such a additive pattern. Gottlieb (1981b) studied 17 enzyme systems in five species of Machaeranthera, in which n = 4, 5 and 9 and two species of Aster in which n = 5 and 9. The data demonstrated that all of these species had the same number of gene loci specifying the tested enzymes. The absence of isozyme multiplicity in the species in which n = 9 suggested that they did not arise by polyploidy. Tetraploids also express novel heteromorphic enzymes not produced in either one of their parents. Electrophoretic analysis of tetraploid Chenopodium for leucine aminopeptidase (LAP) and phosophoglucoisomerase (PGI) showed tetraploid gene duplication which did not exist in diploid parents.

2.6.4. Isozyme Data and Hybrid Analyses

Isozyme patterns are also useful for identifying or analyzing plant hybrids. In the past few years this technique has been used by workers to detect interspecific hybrids in natural populations of plants.

In some instances, the technique provides a method for analysis of introgression in populations peripheral to areas of hybridization. Zymograms provided a means of distinguishing interspecific hybrids in Phaseolus (Lecture and Garber, 1974). They described electrophoresis as a new procedure to study introgressive hybridization in nature. Copes and Beckwith (1977) found three enzyme systems (LAP, GDH, TO) were very useful in the identification of populations of Picea glauca and P. sitchensis. and of intermediate populations in which introgressive hybridization had apparently occurred.

2.6.5. Genetic Control of Isozyme Polymorphisms

Enzymes and proteins are gene products which can serve as easily determined gene markers. Isozymes are ideal genetic markers because they are colinear with the gene, commonly codominant in effect and relatively unaffected by the environment (Torres and Bergh, 1980). In certain types of segregations, isozymes are far more efficient than morphological markers.

Genetic control of isozyme variation has been studied and reported for a wide variety of enzymes in many higher plants. The first example of genetic control of a plant enzyme separated by electrophoresis seems to be reported by Schwartz (1960) for an alkaline phosphatase in maize endosperm. The synthesis of the enzyme was found to be under the control of the Sh gene (Schwartz. 1960).

The genetic control of leucine aminopeptidases (LAP) was demonstrated in loblolly pine by Adams and Joly (1980). The enzyme was shown to be controlled by single locus with codominant alleles. Heterozygotes were characterized by two isozymes, with no hybrid isozyme presence, and homozygotes by a single isozyme. This presence of both parental bands in a hybrid is called the "fast and slow band" pattern by Peirce and Brewbaker (1973). An enzyme with this pattern of genetic control is considered to be monomeric. Phospho-glucomutase (PGM) is also reported to be monomeric in avocado (Torres et al., 1978).

Homozygous progenies contain only one electrophoretically distinguishable isozyme while heterozygotes contain hybrid isozymes in addition to the two parental types. Glutamate oxaloacetate transaminase (GOT) isozymes in Stephanomeria exigua are controlled by three unlinked gene loci (Gottlieb, 1973b). All the alleles are codominant and heterozygotes for any pair of them produce a more darkly staining enzyme with intermediate mobility, suggesting that

the enzymes have a dimeric subunit structure. Acid phosphatase was also reported to be dimeric in sunflower seeds (Helianthus annuus) by Torres and Diedenhofen (1976).

Two esterase loci in maize (E6 and E8) were found to control only 1 allele, with presence of the allele being dominant and absence being recessive (MacDonald land Brewbaker, 1974). The absence of a single band is often controlled monogenically, with heterozygotes showing presence of the band. The absence of an isozyme, however, sometimes may simply reflect a concentration too low for recognition on the gels (Peirce and Brewbaker, 1973).

Scandalios (1965) found that maize catalase isozymes were also under strong genetic control. His results strongly supported the hypothsis that the maize catalase is a tetramer. When two homozygous individuals, each with a single but dissimilar catalase were crossed, the progeny showed the presence of both parental enzymes and three new hybrid enzymes. Scandalios suggested that each monomer of the tetramer was controlled by a single allele, and that random association of monomers gave rise to five isozyme types (Scandalois, 1965).

It is not uncommon for a single allele to be associated with more than one band on gels. A series of bands with equal spaces could be due to old tissue age or other environmental factors (Peirce and Brewbaker, 1973). The peresence of secondary bands caused by complexes with

cofactors (e.g. irons), conformers or even incompletely transcribed or translated polypeptides was reported by Schwartz (1975).

2.7. Morphological Studies in Systematics

2.7.1. Evaluation of Morphological Characters as Criteria in Systematic Studies

Comparative morphology is the basic approach of systematics. Systematists still rely to a very large extent on morphological characters, both for producing classifications and for diagnostic purposes. Especially when working on herbarium specimens, with which most taxonomic work has to be performed, morphological characters can be assessed with a high degree of success.

Using morphological criteria, however, different populations might be included in the same species even though they are separated from each other by a sterility barrier; conversely interfertile populations might be separated as different species on morphological grounds. For example, the Luzula multiflora complex is distributed in .pa Europe as well as in eastern North America. The taxa cannot be separated morphologically and they have the same chromosome numbers (2n = 24 or 36). When crossed with each other, however, they gave rise to almost sterile F_1 hybrids. On the other hand, the morphologically distinguished hexaploid L.

frigida of Scandinavia gives wholly fertile hybrids with L. multiflora of the same region (Nordenskiold, 1959).

Sometimes, morphological data only demonstrate phenotypic plasticity across a wide range of habitats (Heywood, 1976). It is dangerous to treat different, highly plastic genotypes as different species. Morphological characters frequently provide the only available evidence, and whatever other characters may be employed in the taxonomic constructions, the results must be morphologically expressible (Davis and Heywood, 1963). Morphological characters have the great advantage that they can be seen and their variability can be more readily appreciated than other kinds of characters.

Present system for classifying most plants are based mainly on morphological criteria. To some extent such systems may simply be ones of convenience, but ideally the arrangement of species should reflect genetic similarities and differences.

2.7.2. Morphological Characters as Markers in Genetic Studies

The application of genetic studies to evolutionary and systematic questions has produced the biological definition of the species, and insights into the production and maintenance of variability (Grant, 1981). Morphological characteristics have long been taken as genetic markers. For example, Griffiths et al. (1977) described a leaf spot polymorphism in Collinsia grandiflora populations. Genetic studies demonstrated that heavy spotting was controlled by a single gene, and the heavy-spotting system was genetically distinct from that of faint spotting.

Ideally, markers should be available from vegetative tissues, so that progeny can be scored while very young (Wright, 1976). It has also been suggested that a marker should be controlled by dominant alleles with each genotype producing a distinct phenotype, rather than codominant ones, so that formal genetic studies can be carried out with extant F_1s (Davis and Heywood, 1963).

In the genus Leucaena, genetic studies have been conducted only with L. leucocephala. The studies of Gray (1960, 1966) showed that growth habit differences had a genetic basis, tallness being dominant over shortness. Stem-tip pubescence was found to be inherited as a single recessive and it was suggested that this character is very

pigmentation was also reported to be fairly simply inherited, but variation in expression made it somewhat difficult to define (Gray, 1960).

CHAPTER 3

MATERIALS AND METHODS

3.1. Morphological Analyses

3.1.1. Herbarium Investigations

Herbarium specimens were observed from the following institutions: U.S. National Herbarium (US) in Washington, the New York Botanical Garden (NY) in New York, John G. Searle Herbarium of Field Museum of Natural History (F) in Chicago and Gray Herbarium of Harvard University (GH) in Boston. Some specimens including type specimen of Leucaena trichandra were also observed from the Herbarium Regium Monacense (M) in Munich.

3.1.2. Measurements of Morphological Characteristics

A worldwide collection of Leucaena species has been assembled by Brewbaker in Hawaii since 1962 (Brewbaker, unpublished). Each accession has been assigned a number prefixed with a "K". Thirty accessions of members of the L. diversifolia complex were used intensively in the studies. Table 1 gives the accession number, the USDA P.I. (Plant Introduction) number and geographical origin of each accession. These plants were grown for at least 4 years at Waimanalo Research Station, 20°N latitude, 60 m elevation,

Table 1. Localities and USDA plant introduction (P.I.) numbers of accessions (K number) of the Leucaena diversifolia complex at Waimanalo Research Station, Hawaii in analysis.

Access. number	P.I. number	Area of origin
K145	324352	5 km west of Fortin des Flores, Veracruz, Mexico.
K146	324353	5 km west of Fortin des Flores, Veracruz, Mexico.
K154	324354	2 km west of Cordoba, Veracruz, Mexico.
K155	324355	3 km north of Fortin des Flores (at 1000 m), Veracruz, Mexico.
K156	324356	3 km north of Fortin des Flores (at 1000 m), Veracruz, Mexico.
K157	324357	16 km north of Fortin des Flores, Veracruz, Mexico.
K159	324358	3 km west of Huatusco, Veracruz, Mexico.
K160	324359	10 km north of Huatusco, Veracruz, Mexico.
K164	324360	10 km east of Dos Rios, Veracruz, Mexico.
K165	324361	10 km east of Dos Rios, Veracruz, Mexico
K166	324362	10 km east of Jalapa, Veracruz, Mexico.
K174	324364	10 km north of Oaxaca City, Oaxaca, Mexico.
K177	324366	20 km north of Oaxaca City, Oaxaca, Mexico.
K178	324367	20 km north of Oaxaca City, Oaxaca, Mexico.
K186	324369	near Jalapa, Veracruz, Mexico.
K376c*	--	10 km north of Oaxaca City, Oaxaca, Mexico.
K406	443489	12 km east of Guatemala City, Guatemala.
K407c	443490	14 km east of Guatemala City, Guatemala.
K408	443491	91 km east of Guatemala City (at 900 m), Guatemala.
K409	443492	15 km east of Mexico border near La Democracia, Guatemala.
K410	443493	20 km east of Guatemala City, enroute to Puerto Barrios, Guatemala.
K411c	443494	21 km east of Guatemala City, Guatemala.
K412	443495	21 km east of Guatemala City, Guatemala.
K413	443497	3 km west of Copan, Honduras.
K422	443476	Volcan de Izalco (at 1500 m), El Salvador.
K423c	443477	Cerro Verde area, above Lake Coatepeque, El Salvador.
K454	443521	3 km northwest of Tuxtla, Chiapas, Mexico.
K465	443523	5 km south of Trinitaria, Chiapas, Mexico.
K478c	443498	Mt. Uyuca, near Zamorano, Honduras.
K480c	443500	Mt. Uyuca, near Zamorano, Honduras.
K483	443501	15 km west of Danli (at 1200 m), Honduras.

* "c" represents seeds from a composite of several trees.

mean annual temperature of 24°C. Each accession was scored for both quantitative and qualitative characteristics. Quantitative characteristics included length of rachis, pairs of pinna per leaf, length of pinnae, pairs of leaflet per pinna, leaflet length; diameter of inflorescence (head), length of peduncle, floret number per inflorescence; length of sepal; and length of pistil; length and width of pods; number of seeds per pod, etc. Measurement of leaf parts was made on the third or fourth leaf from the stem apex, the first fully-opened leaf. Middle pinnae from that leaf were measured for pinna length. Leaflet pairs and length were measured on the middle pinna. Qualitative characteristics include habit; bark color; pubescence of leaflet, petal, ovary, and pod; shape and color of pods and seeds; gland types; nature of peduncle (slender or not) etc. Means, standard deviations and ranges of the quantitative characteristics were calculated. Measurements of calyx, corolla, stamen and pistil were made with a transparent celluloid ruler to the nearest tenth of a millimeter under a dissecting microscope.

Growth rate of tetraploid and diploid of Leucaena diversifolia were also measured for comparison. Diameters at breast height (dbh) and tree height of five trees of ten accessions of each cytotype were selected and measured. Diameters at breast height of multiple-stemmed trees were combined following the method of Van Den Beldt (1983). All

trees studied were planted at the Waimanalo Research Station in August, 1978, and mearsurement data collected in August, 1982.

3.1.3. Pollen Size Measurement

In order to determine the size of pollen grains, microscope slides were made from fresh pollen collected in the garden and stained with acetocarmine and then embedded in glycerine jelly. Twenty well-formed and stained grains from each of 5 randomly selected trees were measured for each of 14 accessions. Diameters of pollen were measured under a magnification of 400x with a calibrated ocular micrometer. Pollen diameter was measured from the middle of a pore to the middle of the opposite wall of the grain, or in case of 4-pored pollen, between two nonadjacent pores.

3.1.4. Stomatal Guard Cell Measurement

The area of ten pairs of guard cells from one leaf of each of five plants was measured for each accession planted in the garden. A strip of the lower epidermis at the middle portion of a fresh leaflets was removed with forceps and was used for the guard cell measurements. All measurements were taken only from those stomata located midway between the leaflet edge and midrib. The tissue was mounted in acetocarmine, which produced uniform expansion of the

stomatal guard cells regardless of the state of turgor of the specimen when collected. A measure of guard cell size was obtained by calculating the elliptical area of guard cell pairs from length (a) and width (b) using the formula (a+b) x 3.14/4.

From herbarium specimens whose chromosome numbers could not be predicted by morphology of inflorescences, ten guard cells per plant were measured. Since epidermis was difficult to remove from dry leaflets of specimens, nail polish was applied on the under surface of leaflets to obtain replicate. Measurements of guard cells were based on the middle part of the replicate. The mean guard cell size and an estimate of the ploidy level was recorded for the plants on each of the sheets studied.

3.2. Experimental Hybridization

It seems obvious that a systematic program of genetic analysis is not only desirable but is also required if progress is to be made in solving taxonomic and breeding problems in the Leucaena diversifolia complex. One traditional means of assessing the number of genetic differences between related taxa is a hybridization program followed by an examination of F_1 and F_2 progenies.

Thirty accessions of this complex planted at the Waimanalo Research Station were divided into seven groups based on morphological and distributional data. An

experimental hybridization program was then attempted.

Before intercrossing, heads of each parent were bagged and selfed to determine self-compatibility. The crosses to self-incompatible accessions then were done without emasculation. Flower heads of self-compatible accessions were emasculated before crosses were made. Emasculation of unopened buds was found to cause some damage and therefore was not used. Emasculation was accomplished early in the morning before the sun rose, (usually before 6 A.M.), when the flowers were widely open but before the anthers had dehisced.

The mature stigmas were pollinated by scraping them individually with pollen on a blade from the desired male parent. The blade was repeatedly sterilized in a vial of 95% alchohol. Pollen was applied to about 15 individual stigmas of each inflorescence.

Materials available for intercrossing are listed in Table 1. All accessions listed were used as pollen parents. Due to tree height, however, only some accessions were selected as female plants. All possible crosses were made among 12 selected accessions including reciprocals. Intercrosses were also made between accession of the same entity (group). In this study, each intercross consisted of 5 to 10 pollinated inflorescences.

Pollen stainability was also calculated. Fresh pollen from cultivated trees was mounted in cotton blue. The grains

that filled out and took up a deep blue stain were counted as good, while the smaller, irregular, transparent, unstained grains were considered to be aborted. The pollen grains were assessed from one edge of the cover slip to the other. At least 200 pollen grains were counted on each slide. Every individual plant was replicated by counting pollen from 5 different inflorescences collected at different times.

3.3. Cytological Study

Chromosome studies were made from microsporocytes. Young flower buds were collected before noon from plants cultivated at the Waimanalo Research Station, and fixed in modified Carnoy's solution (chloroform-ethanol-glacial acetic acid, 6:3:1 v/v/v following Smith (1947). The buds were kept in the fixing solution at room temperature for 6 days and then stored at about -15C until analyzed. Anthers were removed from the flowers by dissection and squashed in acetocarmine. Chromosome number and behavior at diakinesis and metaphase I or sometimes later stages of meiosis were observed. Pictures were taken of cells in acceptable preparations using a Zeiss Photoscope III equipped with phase contrast optics.

3.4. Isozyme Study

In order to assay comparable tissue from plants growing in uniform conditions at the same developmental stage, seeds were placed on moist paper towels, which were rolled and stored in beakers in the laboratory at room temperature (25°C). Tissues of 7-15 day old seedlings were used for analysis. The electrophoretic procedures have been reported previously by Brewbaker et al. (1968).

A wide variety of tissues both in mature plants and seedlings was studied for peroxidase isozyme polymorphisms. Fourteen tissues of mature Leucaena leucocephala (K8), planted in Waimanalo Research Station were analyzed. These include leaflet, rachillus, gland, young stem, green bark, young pod, cotyledon of dry seed, embryo, perianth, peduncle, and pollen. Cotyledons and embryos were observed from dry seeds, while the other tissues were collected from the respective female parental trees and refrigerated until extracted.

Samples of tissues were first macerated in 0.2 M phosphate buffer (1:1 of 0.2 M dibasic sodium phosphate and 0.2 M monobasic sodium phosphate) at pH 7.0, and then ground with a small pestle. The enzyme solution was absorbed into paper wicks through lens paper barriers to remove fibrous tissue residues.

Twenty five seedlings of each of sixteen accessions of Leucaena diversifolia, and several accessions of L.

lanceolata, L. shannoni, L. leucocephala, and L. esculenta were used for isozyme comparison and genetic study. Five enzyme systems were assessed: peroxidase, esterase, acid phosphotase, leucine aminopeptidase (LAP) and malate dehydrogenase (MDH). Stainining methods of the first four enzymes followed the methods of Brewbaker et al. (1968). MDH was stained according to the method of Scandalios (1969). Acrylamide gel (6%) was used for assaying peroxidase while starch gel (12%) was used for other enzymes. Isozyme zymograms were documented by drawings and photographs.

CHAPTER 4

CYTOLOGY

Few chromosome studies of the genus Leucaena have been reported. The following cytotaxonomic study was initiated in an attempt to obtain a better understanding of cytogenetic relationships within the genus, and of the L. diversifolia complex in particular.

4.1 Chromosome Numbers of the Leucaena diversifolia Complex

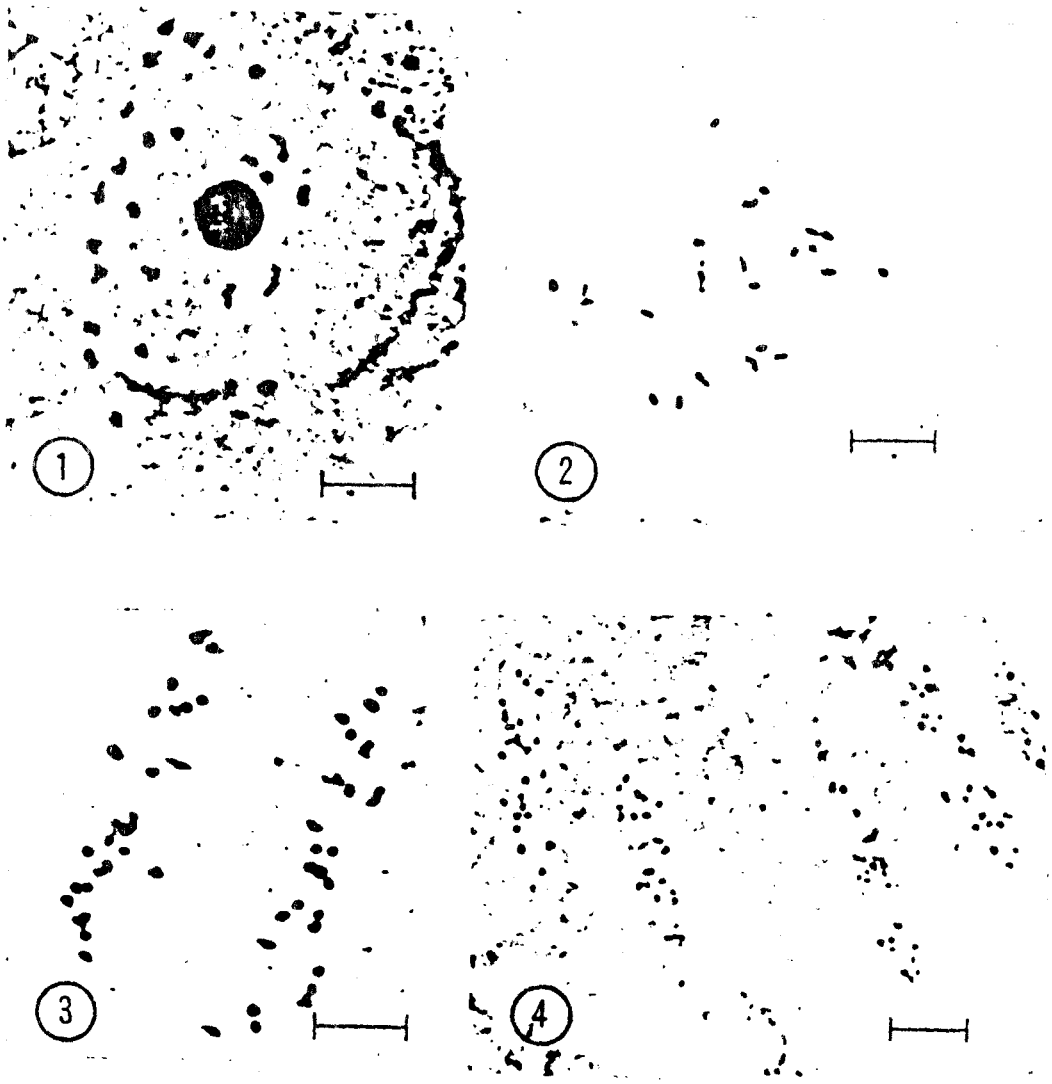
The primary purpose of the cytological study was to determine the chromosome numbers of the unknown accessions rather than investigating interesting cytological phenomena in individual plants. Consequently, chromosome number determinations were generally based on analysis of only 10-20 pollen mother cells per plant in all taxa. Nevertheless, when a surprising number of cytological aberrations were observed, more intensive study of the Leucaena diversifolia complex was conducted which involved more pollen mother cells and more individuals. The results will be discussed in the following sections.

Fifteen accessions from Central America were found to be diploids (Table 2). Diploids of Leucaena diversifolia usually formed 26 bivalents at diakinesis and metaphase I, as illustrated in Figs. 1-4.

Previous studies had identified only Leucaena

Table 2. Chromosome numbers in the Leucaena diversifolia complex.

Accession No.	Locality Province (Dept.), Country	Gametic No.
K406	Guatemala, Guatemala	26
K407	Guatemala, Guatemala	26
K408	Guatemala, Guatemala	26
K409	La Democracia, Guatèmala	26
K410	Guatemala, Guatemala	26
K411	Guatemala, Guatemala	26
K412	Guatemala, Guatemala	26
K413	Copan, Honduras	26
K422	Izalco, El Salvador	26
K423	Cerro Verde, El Salvador	26
K454	Chiapas, Mexico	26
K465	Chiapas, Mexico	26
K478	Zamorano, Honduras	26
K480	Zamorano, Honduras	26
K483	Danli, Honduras	26
K146	Veracruz, Mexico	52
K155	Veracruz, Mexico	52
K156	Veracruz, Mexico	52
K157	Veracruz, Mexico	52
K159	Veracruz, Mexico	52
K160	Veracruz, Mexico	52
K164	Veracruz, Mexico	52
K165	Veracruz, Mexico	52
K166	Veracruz, Mexico	52
K186	Veracruz, Mexico	52
K174	Oaxaca, Mexico	52
K177	Oaxaca, Mexico	52
K178	Oaxaca, Mexico	52
K376	Oaxaca, Mexico	52

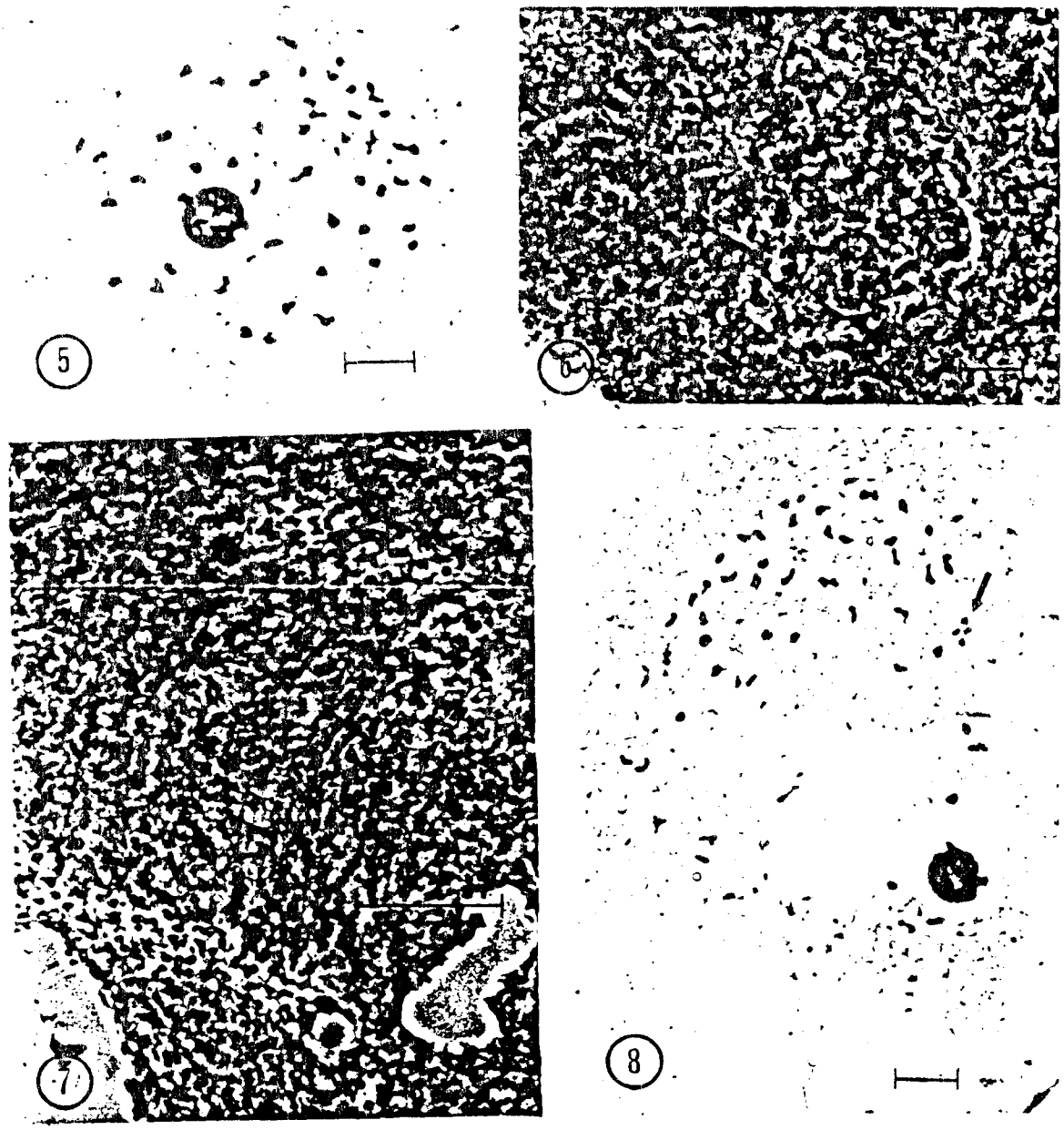


Figs. 1-4. Meiosis in pollen mother cells of diploid Leucaena diversifolia, all figures indicate n = 26. Fig. 1. Diakinensis of K478. Fig. 2. Metaphase I of K411. Fig. 3. Anaphase I of K408. fig. 4. Telophase II of K411. (Bars = 10 u).

leucocephala as a polyploid (2n = 104) member of the genus (Tjio, 1948; Gonzalez et al., 1967). An early finding of the present study was that some accessions of L. diversifolia also had 2n = 104, while others were diploid (2n = 52) as observed in Table 2. Ten of the 30 accessions (K146, K155, K156, K157, K159, K160, K164, K165, K166 and K186) were found to be tetraploids, all of them from Veracruz, Mexico (Table 2). Chromosome counts at diakinesis and metaphase I showed that the normal chromosome number was n = 52 (Figs. 5-8). Quadrivalents during meiosis were occasionally observed, in less than 40% of cells (Fig. 8).

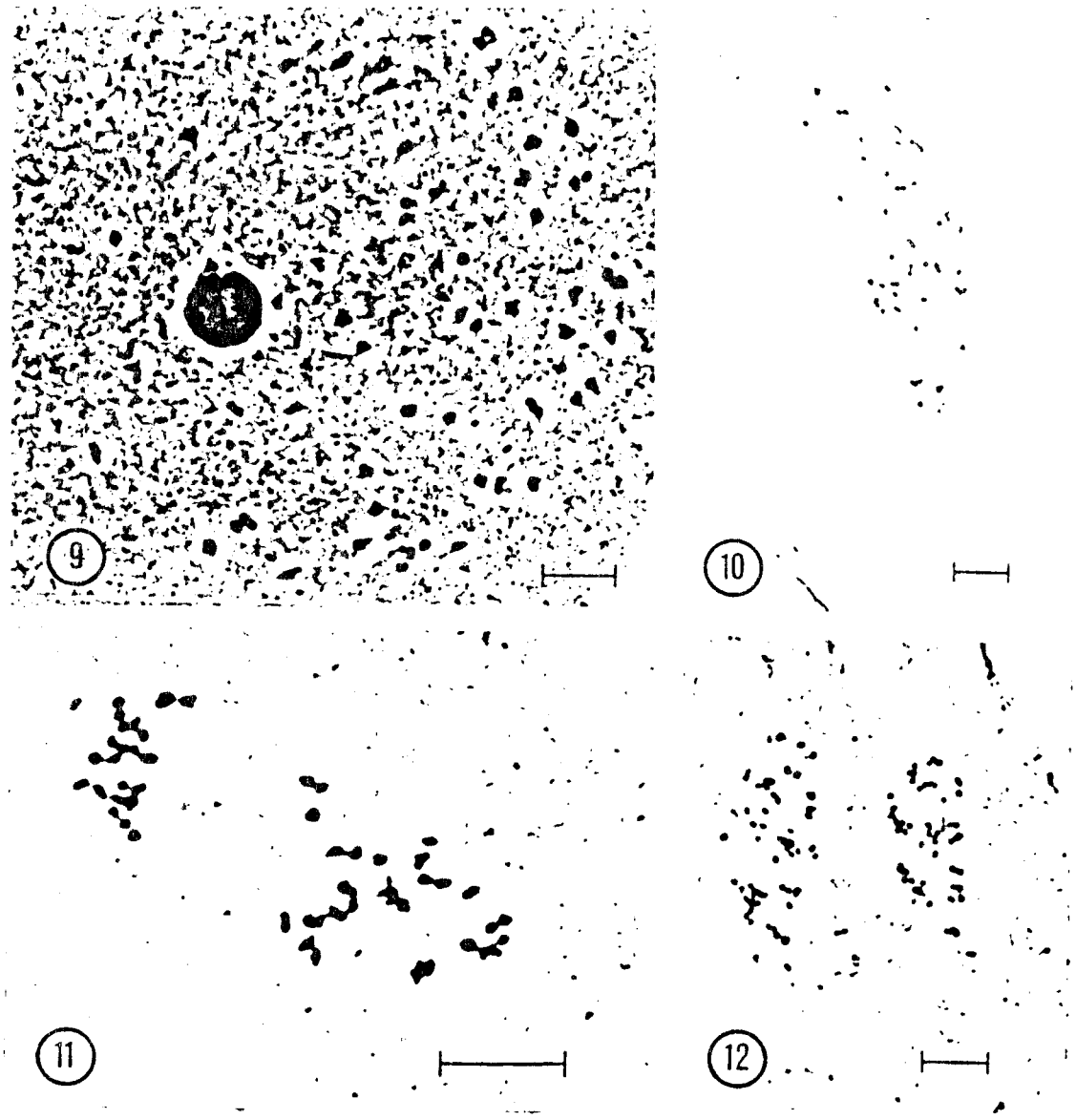
An examination of microsporogenesis in four morphologically distinct accessions of the complex from Oaxaca, Mexico (cf. Leucaena pallida K174, K177, K178 and K376) showed they were also tetraploid with n = 52. The chromosomes of these accessions were frequently clumped, making it difficult to obtain definitive counts. However, some well-spread cells of these accessions allowed firm determination of n = 52 (Figs. 9-12).

Based on morphological and cytological characters, it is hypothized that the Leucaena diversifolia complex composes of three groups: L. diversifolia (diploid), L. diversifolia (tetraploid) and L. pallida. For convenience, the codes DIV2N, DIV4N and PAL are used in the following chapters to represent Leucaena diversifolia (diploids), L. diversifolia (tetraploids) and L. pallida, respectively.



Figs. 5-8. Meiosis in pollen mother cells of tetraploid Leucaena diversifolia, indicate n = 52.

Fig. 5. Diakinesis of K165. Fig. 6. Metaphase I of K156. Fig. 7. Anaphase I of K156. Fig. 8. Diakinesis of K164, note the chain of four (arrow). (Bars = 10 u).



Figs. 9-12. Meiosis in pollen mother cells of Leucaena pallida, all figures indicate n = 52.
Fig. 9. Diakinesis of K174. Fig. 10. Metaphase I of K177. Fig. 11. Metaphase I of K376. Fig. 12. Anaphase I of K178.
(Bars = 10 u).

4.2. Chromosome Numbers of Other *Leucaena* Species

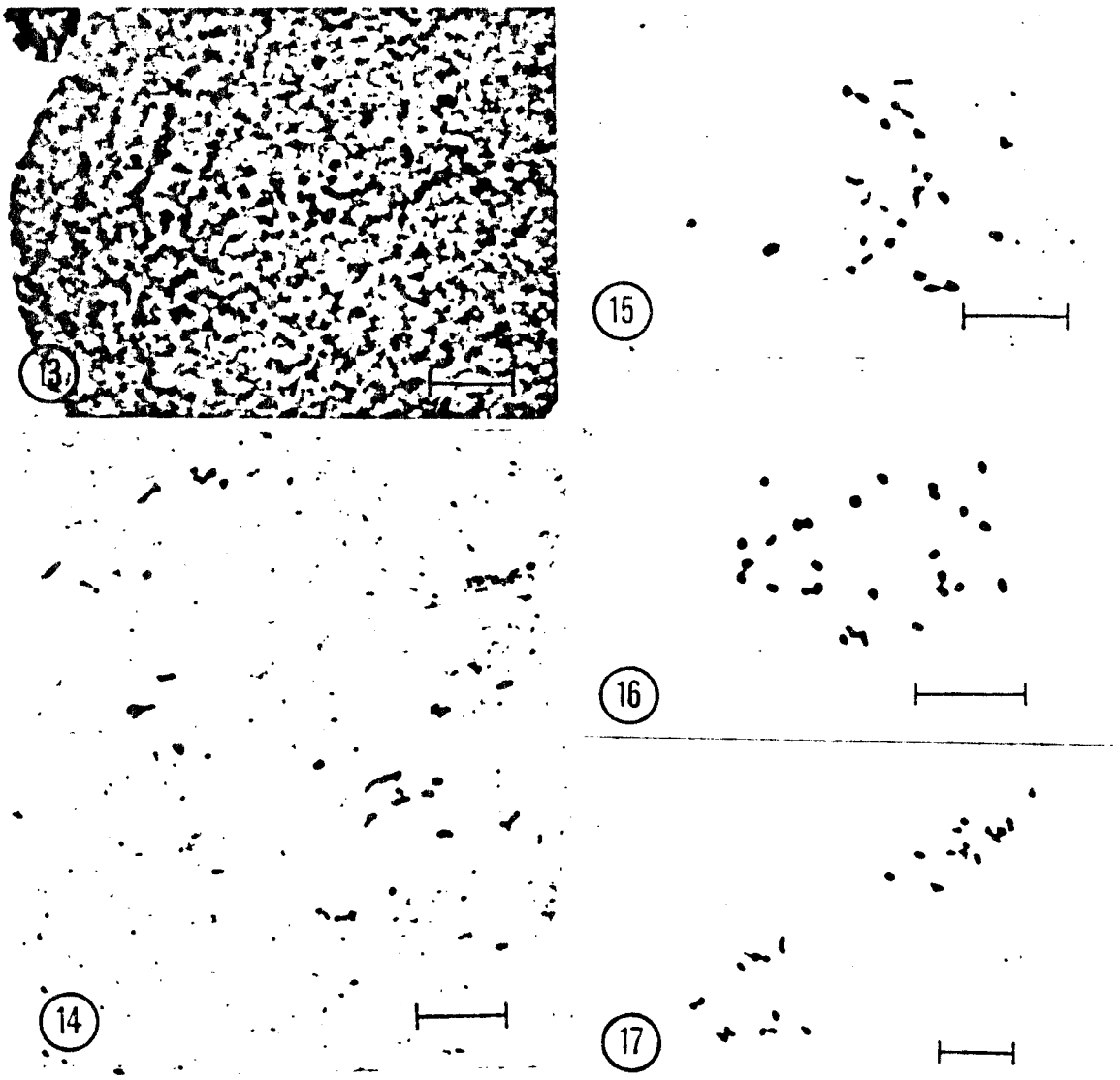
Chromosome counts and locality data for other species ofthe genus *Leucaena* are reported in Table 3. *L. collinsii* is a species with small leaflets, convex petiolar glands and large white flower heads. It is restricted to the province of Chiapas, Mexico and only a few specimens were seen in herbaria. Chromosome counts were made of two accessions, K180 and K461. Metaphase I and telophase I regularly indicated gametic number of 26 chromosomes for these accessions of this species (Fig. 13). The somatic number of 2n = 102 reported by Hutton (1981) could not be verified.

Leucaena esculenta is a species with small leaflet, corky bark and oblong concave petiolar glands. It is distributed in southern Mexico, largely at high elevations. Tow accessions of the species were determined chromosomally. The gametic number for all of them was n = 26 (Fig. 14). No irregularities or other numbers were found. Gonzalez et al.(1967) counted a variety of *L. leucocephala* attributed by the collector H. Gray to "*L. esculenta*" with 2n = 104 and n = 52. It was later determined to be misclassified, and is now known as the variety K8 (Brewbaker, unpublished).

Leucaena lanceolata has large leaflets (2-4 cm long) and is distributed from eastern to western Mexico. Chromosome numbers were observed in two accessions, K11 and K401. Both

Table 3. Chromosome numbers of miscellaneous *Leucaena* species.

Species	Access. No.	Locality	Gametic No.
L. collinsii	K180	Chiapas, Mexico	26
	K450	Chiapas, Mexico	26
L. esculenta	K138	Puebla, Mexico	26
	K342	Not known, Mexico	26
L. lanceolata	K401	Guerrero, Mexico	26
L. leucocephala	K8	Zacatecas, Mexico	52
L. macrophylla	K379	Oaxaca, Mexico	26
L. pulverulenta	K75	San Luis Potosi, Mexico	28
L. retusa	K280	Texas, USA	28
	K502	Texas, USA	28
L. shannoni	K405	unkonwn, planted in Hawaii	26
	K487	Salvador, El Salvador	26
L. trichodes	K90	Caracas, Venezuela	26
	K738	Cesar, Colombia	26



Figs. 13-17. Meiosis in pollen mother cells of Leucaena species. Fig. 13. Metaphase I of L. collinsii (K180), n = 26 II. Fig. 14. Metaphase I of L. esculenta (K138), n = 26 II. Fig. 15. Metaphase I of L. lanceolata (K411), n = 26 II. Fig. 16. Metaphase I of L. retusa (K280), n = 28 II. Fig. 17. Metaphase I of L. trichodes (K738), n = 26 II. (Bars = 10 u).

accessions showed 26 pairs in metaphase I (Fig. 15) with no irregularities, in agreement with a former report for the species (Gonzalez, 1966).

Chromosome counts of Leucaena macrophylla were made only from one accession, K379, and 26 bivalents at meiotic metaphase were seen (Table 3).

Leucaena retusa Benth. is a unique species in the genus. It is distributed at about 2000 m elevation in southern Texas and northeastern Mexico, the northern limit of the genus. It has reticulate leaflets and yellow flower heads. The chromosome counts of two accessions (K280 and K502) revealed n = 28 chromosomes in this species (Fig. 16).

Leucaena shannoni is a lowland shrubby species of southeast Mexico that has fragrant white flowers. Two accessions (K405 and K487) of this species were studied. Twenty-six bivalents were observed at metaphase I, and no irregularities were found in microsporogenesis (Table 3).

Leucaena trichodes, like L. lanceolata and L. macrophylla, is a species with large leaflets. Its natural distribution is in South America, from Panama to Equador, the southern limit of the genus. Three accessions (K90, K737 and K738) were observed cytologically and were found to have a gametic number of n = 26 (Fig. 17). None of the pollen mother cells observed in these accessions showed irregularities at metaphase I or anaphase I.

Cytological observation of six species thus showed

counts of n = 26 in meiotic figures. Among them were Leucaena esculenta, L. lanceolata, L. shannoni and L. trichodes, all reported with 2n = 52 in previous works (Genzalez et al., 1967; Hutton, 1981). Two species, L. collinsii and L. macrophylla, had not previously been counted.

Leucaena retusa and L. pulverulenta were found to have n = 28. The counts of the latter are in agreement with previously published reports of 2n = 56 (Turner and Fearing, 1960; Gonzalez et al., 1967). The chromosome count of the former represents a first determination for that species.

Leucaena leucocephala was previously reported as 2n = 104 (Tjio, 1948). The observation herein of n = 52 confirmes the previous report.

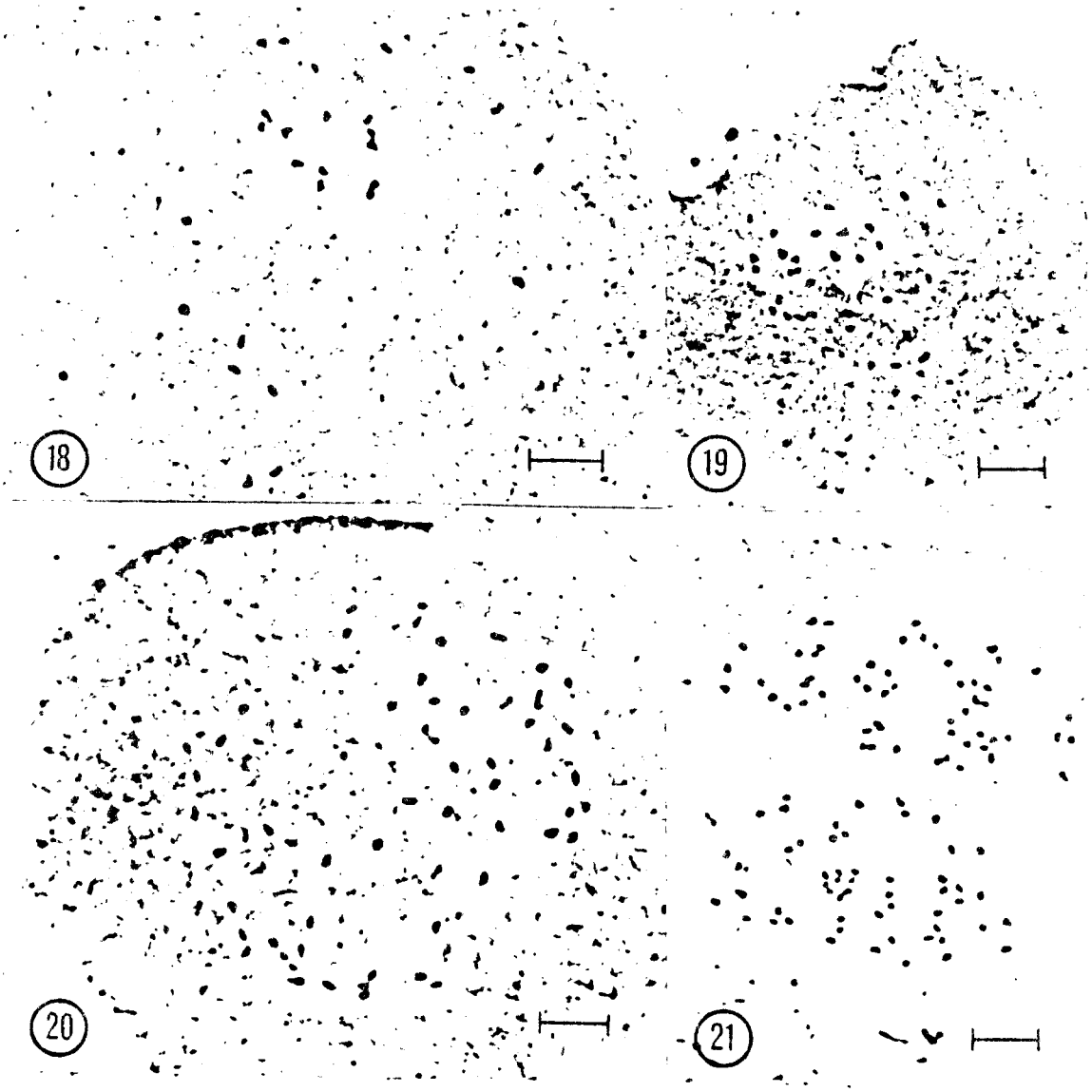
4.3. Extra Chromosomes in the Leucaena diversifolia Complex

In addition to the normal number n = 26, chromosome numbers of n = 29 or 30 were also observed in accessions of the Leucaena diversifolia complex. These numbers do not fit in a polypolid series based on 13 or 14. An intensive study of chromosome in the complex was then conducted.

Two hundred and eighty-four pollen mother cells, representing 23 plants from 15 diploid accessions (DIV2N), were examined critically. About sixty percent of the cells showed extra chromosomes (Table 4, Figs. 18-19). Normal chromosome pairing was 26 II; therefore, 27 II or 26 II + 2 I

Table 4. Meiotic cells with extra chromosomes in the *Leucaena diversifolia* complex.

Group	Access. No.	No. plant observed	No. cells resolved	Cells with extra chrom.	Cells with normal chrom.	Frequency with extra chrom.
	K156	1	15	5	10	
	K160	1	10	6	4	
	K164	1	4	0	4	
	K165	1	8	1	7	
DIV4N	K166	1	4	1	3	
	K186	1	2	0	2	
	total	6	43	13	30	30.2%
	K174	2	11	5	6	
	K177	1	6	3	3	
PAL	K178	1	3	2	1	
	K376	5	31	15	16	
	total	9	51	25	26	49.0%
	K406	1	1	0	1	
	K407	1	2	1	1	
	K408	2	36	22	14	
	K409	1	7	3	4	
	K410	1	5	1	4	
	K411	1	14	8	6	
	K412	1	7	6	1	
	K413	1	28	5	23	
DIV2N	K422	1	42	27	15	
	K423	1	8	6	2	
	K454	6	28	25	3	
	K465	2	61	41	20	
	K478	1	30	16	14	
	K480	2	1	1	0	
	K483	1	14	4	10	
	total	23	284	166	118	58.4%



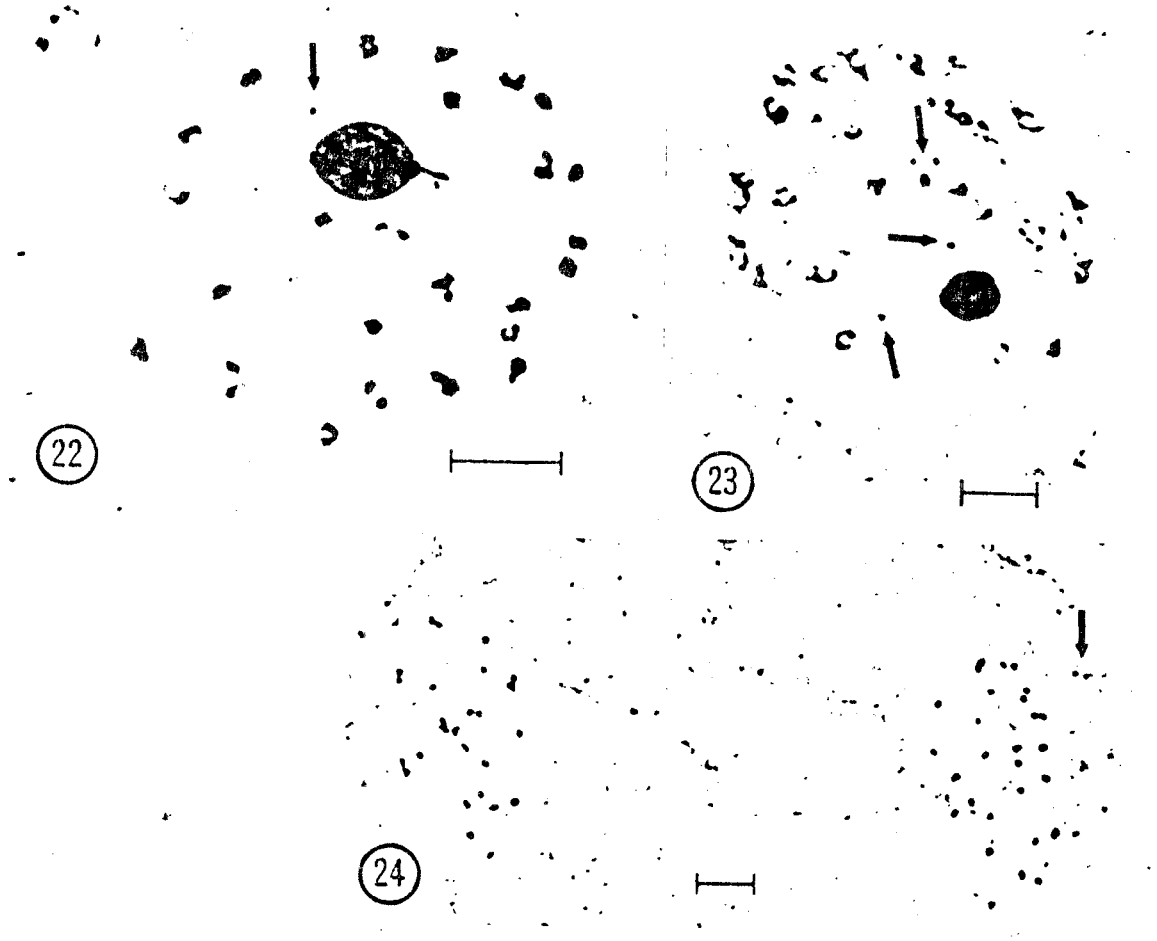
Figs. 18-21. Meiotic cells with extra chromosomes. Fig. 18. Metaphase I of diploid Leucaena diversifolia (K411), n = 30 II. Fig. 19. Metaphase I of diploid L. diversifolia (K465), n = 28 II. Fig. 20. Diakinesis of tetraploid L. diversifolia (K156), n = 59 II. Fig. 21. Anaphase I of L. pallida (K174), 56 + 58 chromosomes. (Bars = 10 u).

represent plants with two extra chromosomes, while 28 II, 27 II + 2 I or 26 II + 4 I means four extra chromosomes, and so on. This high freguency of extra chromosomes was found in almost all accessions examined, rather than in certain populations and geographic regions. High percentages of cells with extra chromosomes were also found in tetraploids (DIV4N) and in the four Oaxaca accessions (PAL) (Figs. 20-21), with 30% and 50%, respectively. The occurrence of extra chromosomes was the most frequent aberration. These chromosomes appeared normal in size and appearance in some cells (Figs. 18-19). They usually did not pair with numbers of the basic complement and were distinguishable at diakinesis (Figs. 22-23). This behavior suggests that the extra chromosomes might represent supernumerary chromosomes.

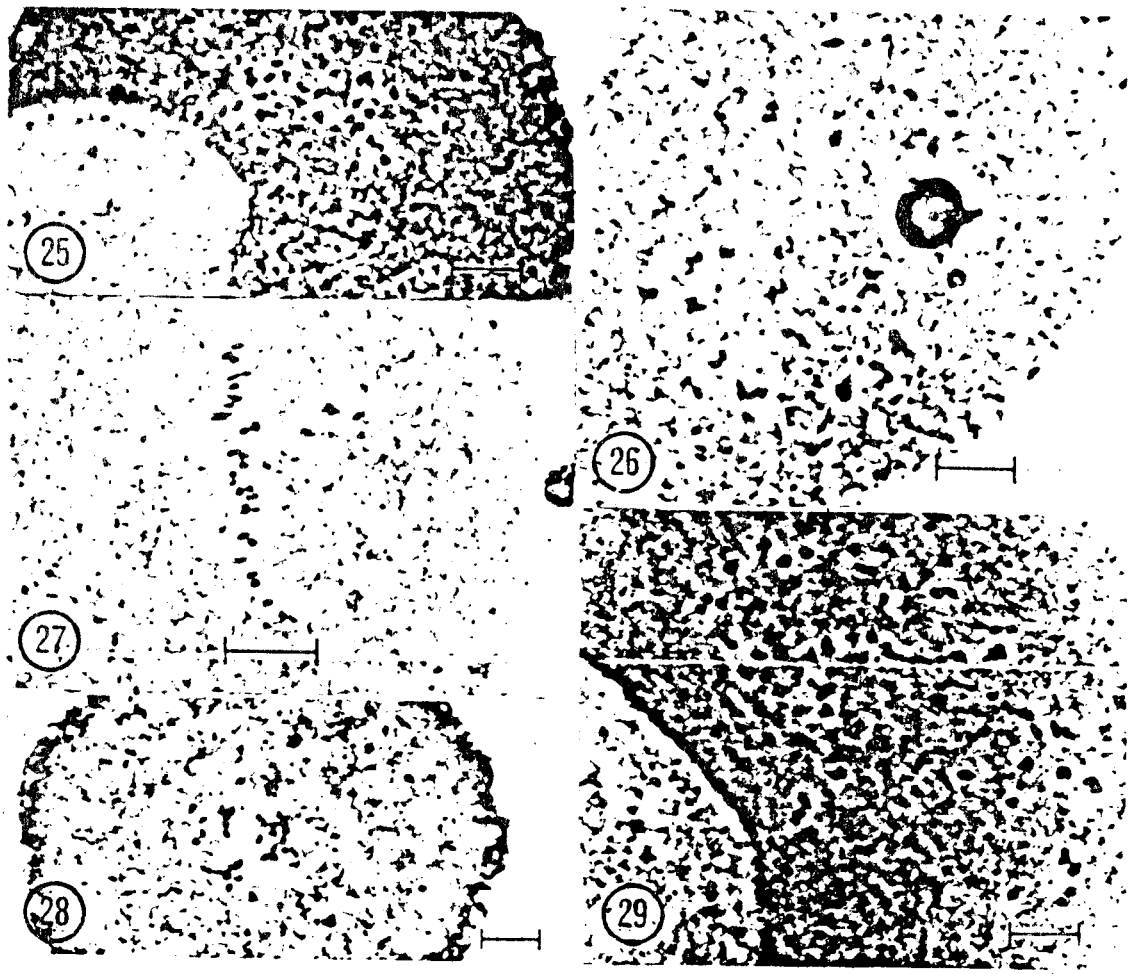
4.4. Chromosome Pairing in F_1 Hybrids of Some *Leucaena* Species

Fifty F_1 hybrids were grown from crosses between two DIV2N accessions, K409 and K480. These hybrids generally had high mean pollen stainability. Cytological analyses of 10 of these hybrids revealed a high degree of homology in chromosome structure. No cytological abnormalities were observed in the pollen mother cells examined from these plants (Fig. 25).

Meiosis was examined in 46 pollen mother cells of the F_1



Figs. 22-24. Meiosis in pollen mother cells of the Leucaena diversifolia complex. Fig. 22. Diakinesis of K478, arrow shows an unpaired, probable B chromosome. Fig. 23. Diakinesis of K422, with several unpaired small chromosomes (arrows). Fig. 24. Telophase I of K454, with 26 + 28 chromosomes and one pair of probable B chromosomes (arrow). (Bars = 10 u).



Figs. 25-29. Meiosis in pollen mother cells of F_1 hybrids involving the Leucaena diversifolia complex. Fig. 25. Metaphase I of L. diversifolia (K409, 2N) x (K480, 2N), n = 26 II. Fig. 26. Diakinesis of L. diversifolia (K409) x L. shannoni (K405), n = 26 II. Fig. 27. Metaphase I of the same hybrid as Fig. 26, 26 II. Fig. 28. Metaphase I of L. diversifolia (K409) x L. lanceolata (K401), n = 26 II. Fig. 29. Metaphase I of L. diversifolia (K156, 4N) x L. leucocephala (K8), n = 52 II. (Bars = 10 u).

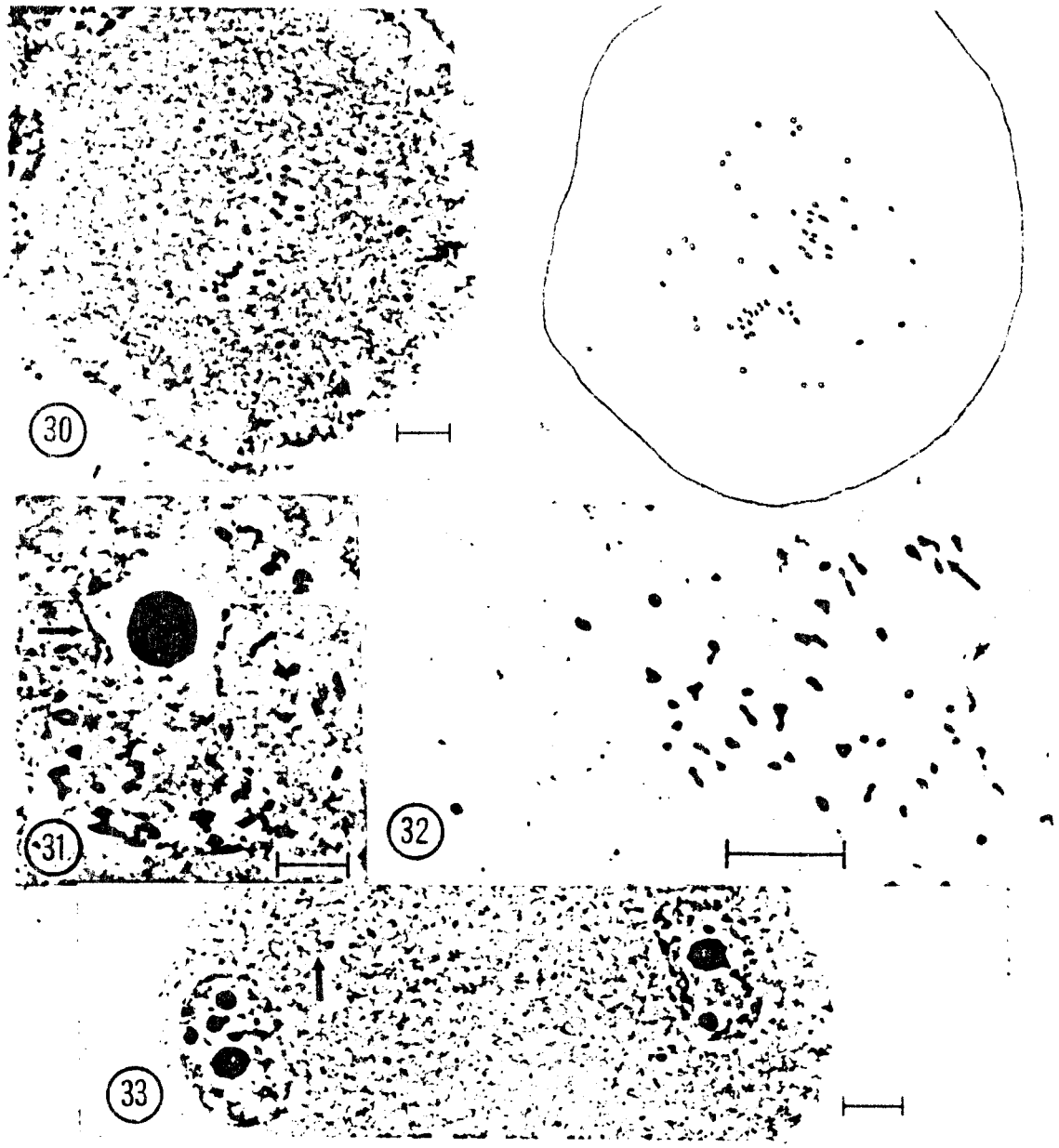
hybrids between DIV2N (K409, n = 26) and L. shannoni (K405, n = 26), and was found to be perfectly regular (Figs. 26, 27).

Ten progenies of the cross of DIV2N (K409) x L. lanceolata (K401, n = 26) were grown to flowering; meiosis in five of these was studied. The most common chromosomal configuration seen at diakinesis and metaphase I in these hybrids was 26 bivalents (Fig. 28). No irregularities were observed in this F_1 hybrid.

DIV4N (K156, n = 52) and L. leucocephala (K8, n = 52) were observed to cross freely and gave rise to fertile hybrids. The chromosome pairing of the hybrids was regular at both diakinesis and metaphase I stages with 52 II (Fig. 29). Only bivalents (52 II) were observed.

Of the twelve triploid hybrids of DIV2N (K409) x DIV4N (K156), five were analyzed meiotically. Data on meiotic abnormalities in the hybrids were collected at diakinesis, metaphase I and II, anaphase I and II and the tetrad stages. In general, univalents usually were difficult to distinguish from bivalents in Leucaena species. Nevertheless, in favorable material, these could be indentified by their different sizes.

Although most microsporocytes of the hybrids exhibited some degree of stickiness, the apparent maximum chromosome association of 26 bivalents and 26 univalents was seen in 43 resolvable cells at diakinesis and metaphase I (Fig. 30). Trivalents were also observed in some cells of these stages



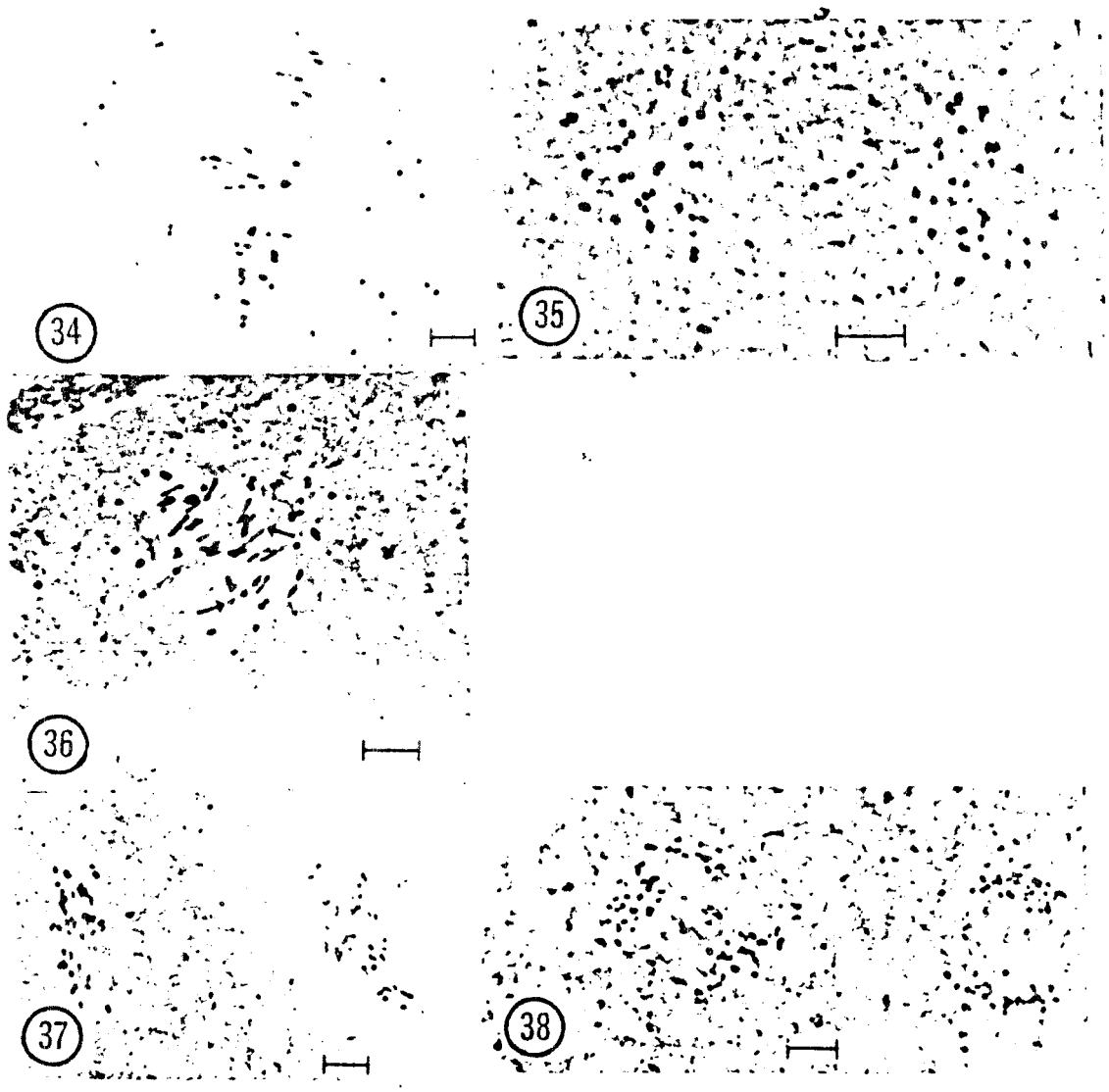
Figs. 30-33. Meiotic behavior of diploid Leucaena diversifolia (K409) x tetraploid L. diversifolia (K156). Fig. 30. Metaphase I, 26 II + 26 I. Fig. 31. Diakinensis, note poosible trivalent (arrow). Fig. 32. Metaphase I, note possible trivalent (arrow). Fig. 33. Telophase II, note the laggards (arrow). (Bars = 10 u).

(Figs. 31, 32). The subsequent stages were greatly disturbed due to the presence of laggard chromosomes, and their uneven distribution resulted in polyad formation. At anaphase I and telophase I, segregation of 39 chromosomes to each pole was noted in most cells while several cells showed a 45-33 distribution. A number of lagging chromosomes were also seen in these stages (Fig. 33).

The subsequent stages were difficult to describe, due to the tiny chromosomes which were usually stuck together. It might be inferred that most cells after telophase II with a chromosome number lower than 26 would fail to form viable gametes due to the loss of chromosomes. This also help to account for the low pollen stainability in pollen grains.

The hybrid Leucaena lanceolata (K264) x L. leucocephala (K8) represented a cross between different species at different ploidy level. Leucaena lanceolata is a diploid species with n = 26 while L. leucocephala is a tetraploid with n = 52. The maximum chromosome association of the hybrid was also 26 bivalents and 26 univalents as seen in Metaphase I, and shown in Fig. 34. No trivalents were seen at either diakinesis or metaphase I stages. The subsequent stages showed laggards which contributed to uneven distribution of chromosomes (Fig. 35).

The chromosome number of the species Leucaena pulverulenta (K19) was n = 28. When crossed with a tetraploid species, L. leucocephala (K8) with n = 52, the F_1



Figs. 34-38. Meiosis in pollen mother cells of F_1 hybrids in some Leucaena species. Fig. 34. Metaphase I of L. lanceolata (K264) x L. leucocephala (K8), 26 II + 26 I. Fig. 35. Anaphase I of the same hybrid as Fig. 34, note laggards. Fig. 36. Metaphase I of L. pulverulenta (K19) x L. leucocephala (K8), 24 II + 2 III + 26 I, arrows indicate trivalents. Fig. 37-38. Telophase I and II of the same cross as Fig. 36.

had a very interesting chromosome figure. The maximum chromosome association of the hybrid was found to be 24 bivalents, 2 trivalents and 26 univalents instead of 26 bivalents and 28 univalents from twelve resovable cells (Fig. 36). Two extra pairs of chromosomes in L. pulverulenta appeared to be homologous to part of L. leucocephala. This might interpret that the extra chromosome pairs in aneuploid species of genus Leucaena were from loss of centromeres in certain chromosomes from their ancestral species with n = 26. However, more study is needed before arriving at any solid conclusion. The subsequent stages of the meiosis of the hybrid were found to have laggard chromosomes (Figs. 37, 38).

4.5. Discussion

Most species of the genus Leucaena observed were n = 2x = 26; three species were tetraploids with n = 4x = 52. However, two species, L. pulverulenta and L. retusa, were n = 2x = 28. It is generally agreed that the basic chromosome numbers for the genus Leucaena are x = 13 and x = 14 (Atchison, 1951; Goldblatt, 1978).

It is fairly obvious that x = 13 was the ancestral number in the genus. The species with n = 26 or n = 52 were directly evolved from the x = 13 ancestors. The species with n = 28 might be directly evolved from the ancestral plants with x = 13, or from recent derivatives from n = 26 plants. It is also possible that the Leucaena species with n = 28

were evolved from n = 14 ancestors. The present study, however, can not prove either hypothesis.

The phenomenon of the extra chromosomes (more than 26 in diploids and 52 in tetraploids and Leucaena pallida) is quite complicated. It is likely that supernumerary chromosomes exist in these taxa, as suggested in the previous section, due to the occurrence of unpaired small fragments in diakinesis (Figs. 22-23).

The cytotypes 2n = 52 and 2n = 104 of Leucaena diversifolia or other combinations such as DIV2N (2n = 52) and L. leucocephala (2n = 104), are able to give rise to triploid hybrids with 2n = 78 chromosomes. These appear to be fertile and successfully backcross with either parent, resulting in hybrids with 2n = 65 and 91 chromosomes, respectively. The F_2 or backcrosses would lead to all numbers between 2n = 52 and 104 or n = 26 and 52, as observed by Gonzalez et al. (1967), resulting in highly variable hybrid swarms with all possible chromosome numbers between n = 26 and n = 52. This may account for the chromosome counts with n = 29 or 30 in diploid accessions and n = 59 or 60 in tetraploid ones.

At the present stage, it is possible to deduce two possible alternatives for tetraploids (DIV4N). It is either an autotetraploid, originating by chromosome doubling in the diploids (DIV2N), or it is an amphiploid derived from a cross

between DIV2N and another diploid species followed by chromosome doubling.

Evidence that DIV4N is of autoploid origin include: (1) DIV4N is very similar morphologically to DIV2N. Few characters served to distinguish these two, and are quantitative rather than qualitative. The two taxa are much closer to each other than to other species of the genus. (2) Quadravalents were found in meiotic cells of the tetraploid accessions. (3) Both bivalents and trivalents were found in the same pollen mother cells of F_1 hybrids between these two cytotypes.

Cytological analysis of seven hybrid combinations involving six taxa revealed that 26 chromosomes of all the diploid species are highly homologous to each other, and to half the genome of each of the tetraploid species, based on their F_1 chromosome behavior.

CHAPTER 5

MORPHOLOGICAL ANALYSES

Three groups of the Leucaena diversifolia complex emerge from cytological investigation and some distinctive morphological characters as suggested in Chapter 4. They are DIV4N (tetraploid L. diversifolia), DIV2N (diploid L. diversifolia) and PAL (L. pallida). Detailed investigation of morphological characteristics of the complex were made from specimens in four major herbaria as well as live plants cultivated at the Waimanalo Research Station. The purpose of this chapter was to see whether these were in agreement with the preliminary groupings.

5.1. Herbarium Observations

Morphological characteristics used to delimit the described species of the L. diversifolia complex by the previous authors are shown in Table 5. These include shape and length of leaflets, vesture of the leaflets and involucre position. These species were mostly described by two authors--Britton & Rose and Urban. Many workers recognize only one species (e.g. Brewbaker, 1982).

The non-type specimens of the Leucaena diversifolia complex in the herbaria were found frequently assigned incorrect names. Most of these specimens were treated under the name of Leucaena guatumalensis whether the leaflets were

Table 5. Major morphological differences among described species of the *Leucaena diversifolia* complex based on measurements taken from Type specimens.

Described species	*Leaflet length	**Vesture leaflet	**Vesture twigs	Pod length	Other unique characters
L. brachycarpa Urban	4-5 mm	G	P	ca.12 cm	involucre appressed
L. diversifolia (Schlecht.) Benth.	4-6 mm	G	P	--	involucre distinct
L. laxifolia Urban	4-6 mm	G	P	--	involucre appressed
L. dugesiana Britton & Rose	5-8 mm	P	G (subterete)	7-11 cm	peduncles glandula
L. oaxacana Britton & Rose	5-7 mm	G	G (subterete)	8-10 cm	
L. pallida Britton & Rose	3-10 mm	P	G (subterete)	12-18 cm	
L. paniculata Britton & Rose	4-7 mm	G	G (subterete)	ca.20 cm	
L. guatemalensis Britton & Rose	3-6 mm	Pi	Pi	--	
L. pueblana Britton & Rose	2-3 mm	G	Pr	8-10 cm	leaflet apex rounded
L. revoluta Britton & Rose	4-5 mm	G	P	--	
L. standleyi Britton & Rose	3-5 mm	G	G+Pr	ca.9 cm	leaflets glossy
L. stenocarpa Urban	2-4 mm	P	P	11-13 cm	pods puberulent
L. trichandra (Zucc.) Urban	3-5 mm	G	P	--	

* measured from leaflets of middle pinnae in third or fourth leaf from branch top.

** Vesture: G = glabrous; P = pubescent; Pi = pilose; Pr = puberulent.

pubescent or not. In most cases, I succeeded in matching the herbarium types with living materials by using the characters listed in Table 5.

5.1.1. *Leucaena diversifolia* (Schlecht.) Benth. (DIV4N) and Related Species

The Type specimen of *Leucaena diversifolia* (Schlech.) Benth. was from Jalapa, Veracruz, Mexico, in a region where several tetraploid accessions of U. Hawaii have been made (Table 1). The type specimen had leaves 6-9 cm long, pinna 9-16 pairs and 3-4.5 cm long, leaflets 41-53 pairs and 4-6 mm long, peduncle 1-2 cm long. Pods of the type specimen appeared to be immature and 6-10 cm long, 1.3 cm wide.

Leaves of the type specimen of *Leucaena bracycarpa* were about 10 cm long, pinnae 11-12 pairs and about 4 cm long, leaflets about 30 pairs and 4-5 mm long, with long peduncle (1.8 cm long); pods sparsely pubescent, 12 cm long and about 1.5-1.7 cm wide, with about 20 seeds.

Fragments of the type specimen of *Leucaena laxifolia* Urban were seen in the herbarium of New York Botanical Garden (N.Y.), with 50 pairs of leaflets, 4-6 mm long. It was described to have minutely pilose young branches, 12-20 pairs pinnae, 25-40 pair leaflets. The heads were 10-12 mm in diameter. The ovaries were observed to be glabrous by the original author (Urban, 1900).

5.1.2. *Leucaena pallida* Britton & Rose (PAL) and Related Species

The type specimen of *Leucaena pallida* Britton & Rose was from Huejuquila, Jalisco, Mexico, with glabrous to minutely puberulent young twigs, rachis and rachilla. The species has leaves 14-16 cm long, pinnae 14-16 pairs and 6-7 cm long, leaflets pubescent on both sides, 40-55 pairs and 3-10 mm long, flower heads 2.0 cm in diameter, peduncles 1.5-2.5 cm long, pods 12-16 cm long, 1.3-1.5 cm wide with about 20 seeds.

The type specimen of *Leucaena dugesiana* Britton & Rose was collected from Guanajuato, Mexico, with young branches, rachis and rachilla minutely puberulent. The leaves were 14-16 cm long, pinnae 12-16 pairs and about 7 cm long, leaflets glabrous 40-50 pairs and 5-8 mm long; flower heads 1.5 cm in diameter; peduncles 1.7-20 cm long. The pods of this species were very small, only 7-11 cm long, 1.0-1.2 cm wide, with about 11 seeds.

The type specimen of *Leucaena oaxacana* Britton & Rose was collected near the city of Oaxaca, Oaxaca, Mexico. The specimen had glabrous young branches, rachis and rachilla, leaves about 20 cm long, pinnae up to 24 pairs and 5-8 cm only, leaflets glabrous underneath, 40-50 pairs per pinna and 5-7 mm long, flower heads 1.8-2.0 cm in diameter, peduncles 2-2.5 cm long, pods 8-9.5 cm long, 1.1-1.2 cm wide and about 11 seeds.

The type specimen of *Leucaena paniculata* Britton & Rose

was collected near Cuernavaca, Morelos, Mexico. The specimen has angulate and glabrous twigs, and glabrous rachis and rachilla, leaves about 15 cm long, pinnae 14-18 pairs and 5-6 cm long, leaflets glabrous, 45-61 pairs and 4-7 mm long, flower heads about 1-5 cm in diameter, peduncles 1.2-1.5 cm long. Only one pod was on the specimen; it was 20 cm long, 1.4 cm wide and with 22 seeds.

These plants had a number of distinctive features in common which could distinguish them from other groups in the L. diversifolia complex: they had glabrous or sparsely puberulent and subterete young branches, large flower heads (1.5-2.2 cm in diameter), very long peduncles (2-3.5 cm long) and very large seeds compared with other members of the complex.

All of the type specimens of these four species displayed an array of character combinations intermediate to Leucaena esculenta and L. diversifolia plants, leading to the suggestion that they might be derived from the hybridization of the latter two species. This is discussed in chapter 8. Herbarium specimens of these species were usually identified as L. divesifolia or L. esculenta in the four herbaria visited.

5.1.3. Leucaena trichandra (Zucc.) Urban and Related Species

This investigation of Leucaena trichandra (Zucc.) Urban was based on the pictures of the holotype from the Herbarium Regium Monacense in Munich, and on segments of holotype and drawing pictures in herbarium of New York Botanic Garden. The species had pubescent young branches, rachis and rachilla, pinnae 4-9 cm long, 3-10 pairs, leaflets 15-30 pairs, 3-5 mm long, glabrous. Heads were small, 0.6-0.7 cm in diameter. No pods were on the specimen.

The unique features of the type specimens of Leucaena guatemalensis Britton & Rose were the dense pubescence of young branches, rachis and rachilla, densely villsoe leaflets and the puberulent pods. Populations of these plants occur mainly in Guatemala. The type was collected on plains near Guatemala City. However, individual specimens that had the same characteristics were also collected from Chiapas and Oaxaca, Mexico (Fig. 39). The type specimen had leaves about 12 cm long, pinnae 11-14 pairs, and 5-6 cm long, leaflets about 35 pairs (20 to 38) and 3-6 mm long, flower heads 0.8-0.9 cm in diameter, peduncles 1 to 2 cm long.

The species of Leucaena pueblana Britton & Rose was described to have puberulent leaflets beneath, however, the holotype in the US National Herbarium seemed to be glabrous. Type specimen of the species was collected in the valley of Cuicatlan, about 1000-1200 m, Oaxaca, Mexico. Leaves of the type specimen were 10-12 cm long, pinnae 15-25 pairs, 3-3.5

cm long, leaflets 30-50 pairs, 2-3 cm long, flower heads were infected by galls, nevertheless, the estimated size was 0.7-0.8 cm in diameter. Pods were 8-10 cm long, 0.7 cm wide, about 16 seeds.

Young branches, rachises and rachilla of the type specimens of Leucaena revoluta Britton & Rose were densely pilose. Leaves were 5-7 cm long, pinnae 7-14 pairs, 5-6 cm long, leaflets glabrous on under surface, 20-30 pairs and 4-5 mm long, flower heads were 0.7-0.8 cm in diameter. No pods was seen on the type specimens. The revolute leaflet margins of the type specimens appear to be caused by poorly pressed specimen. I have several specimens which also show revolute and curved leaflets that were due to their not being pressed hard. However, the specimens from the same individual appeared to have flat leaflets when press well.

The type specimen of Leucaena standleyi Britton & Rose had glabrous or sparsely puberulent young branches, sparsely pubescent rachis and rachilla. Leaves were 8-11 cm long, pinnae 14-19 pairs, 3-4.5 cm long, leaflets glabrous, shiny, 33-45 pairs, 3-5 mm long. Pods were shiny and about 9 cm long, 1.2 cm wide, seeds 16. No flower heads were on the specimen. Plants with glossy or glabrous leaflets which should be designated as L. standleyi were primarily in the populations of Honduras and in the apparently disjunct populations of Western Guatemala, but they were also scattered through the range of the complex (Fig. 39). It

definitely matches K409 of Dr. Brewbaker's collections from La Democracia, Guatemala.

Young branches, rachis and rachilla of the type specimens of Leucaena stenocarpa Urban were covered with dense pubescence. Leaves 5-7 cm long, pinnae 13-15 pairs and 7-10 cm long, leaflets densely pubescent on both sides, 25-35 pairs and size variable, 2-4.5 mm long. Flower heads were 6-7 cm in diameter, peduncles 0.5-1.6 cm long. Pods were sparsely puberulent, 11-13 cm long, 1-1.2 cm wide, about 15 seeds. The ovaries were described to be pubescent on the type specimen.

Leucaena guatemalensis Britton & Rose, L. pueblana Britton & Rose, L. revoluta Britton & Rose, L. standleyi Britton & Rose, L. stenocarpa Urban and L. trichandra (Zucc.) Urban tended to have smaller sizes of leaves and flower parts than the species of two other groups. They all had compact flower heads in common.

The distribution map (Fig. 39) was based on specimens from four herbaria. The approximate distribution of the diploid species of the Leucaena diversifolia complex is shown. The figure reveals that these taxa occur in more or less continuous colonies of varying size.

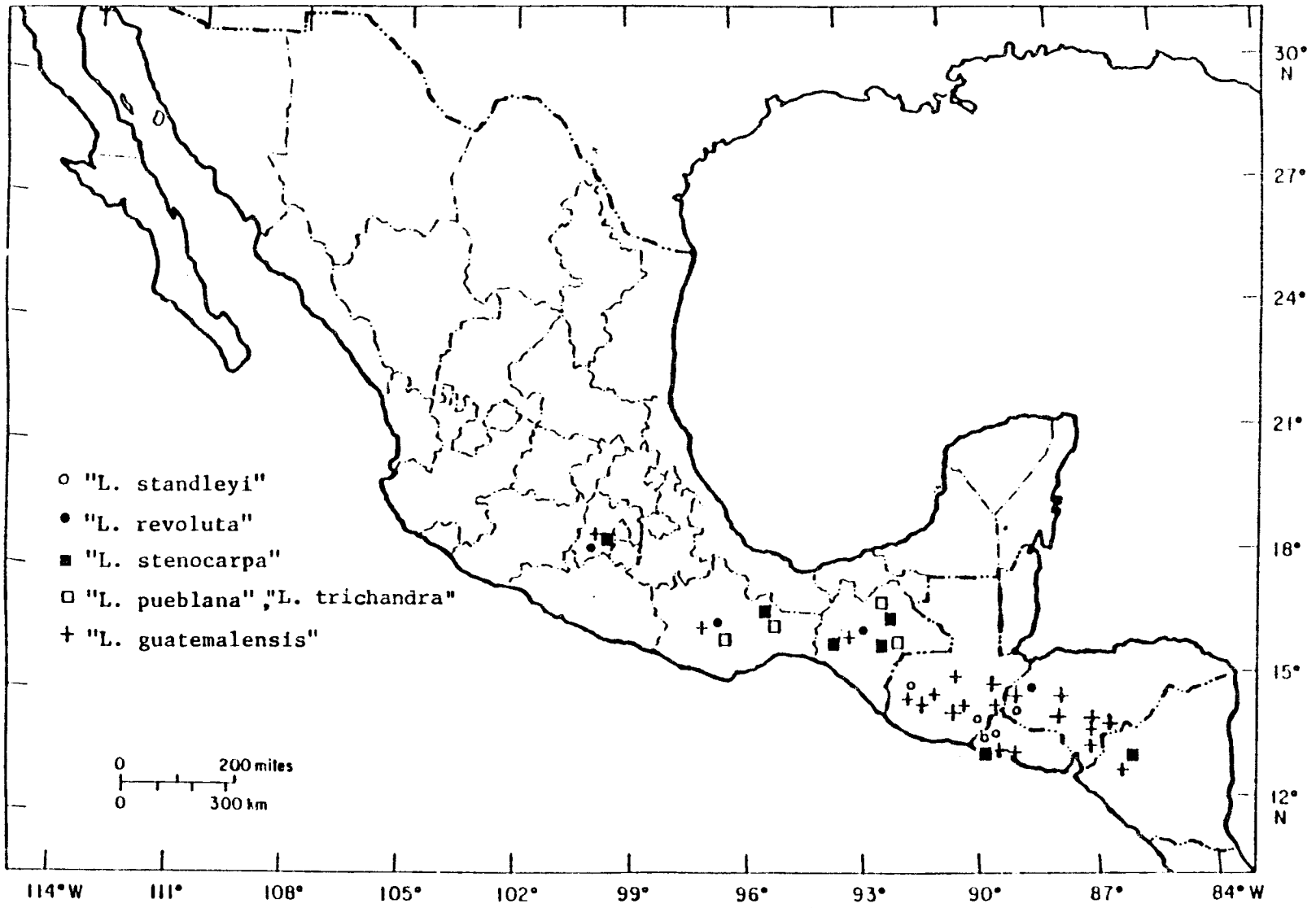


Fig. 39. Distribution of "Leucaena trichandra" and related species.

5.2. Arboretum Analyses

In an effort to understand the basis for the taxonomic treatment of the Leucaena diversifolia complex, a detailed analysis of both qualitative and quantitative morphology was undertaken on accessions planted at the Waimanalo Research Station. Each accession was represented by 5 randomly selected plants, and whenever possible, measurements or observations of each character under analysis were made on the same plant.

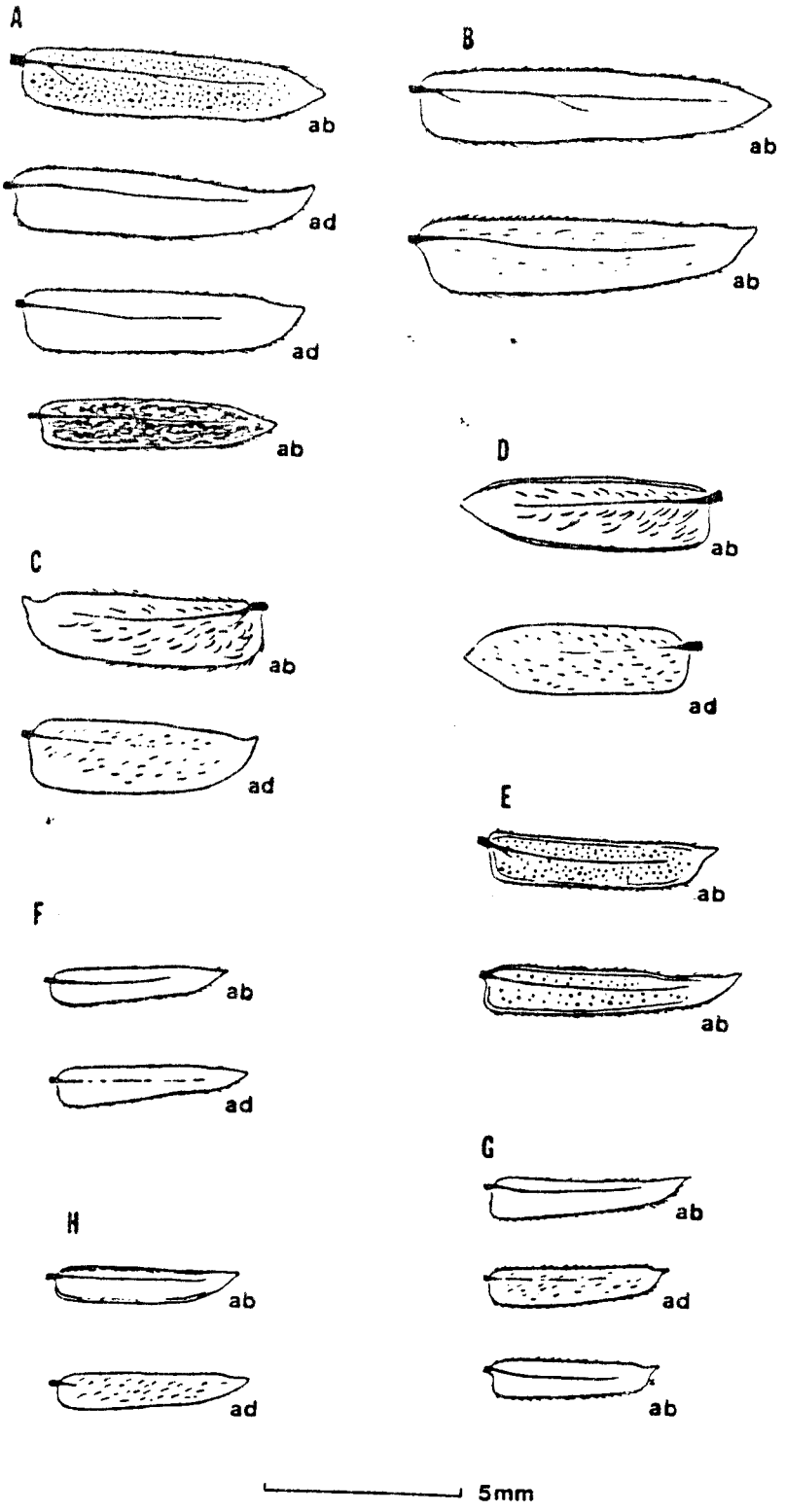
5.2.1. Qualitative Characteristics

a. Leaflet

Leaflets of the Leucaena diversifolia complex are highly variable in shape, size and vesture of the undersurface. Previous authors often used these features to define species. In a comparison of leaflets from accessions planted in the garden, at least eight types could be identified easily (Fig. 40).

Leaflets of DIV4N (Leucaena diversifolia 4N) were glabrous on both sides (Fig. 40A). Leaflet size was large but variable, 4-7 cm long. Leaflet shape was also highly variable, not only from accession to accession but also from leaf to leaf on the same tree. The apex of leaflets varied from acuminate to mucronate. Venation varied from slightly eccentric to strongly eccentric. Usually the lower-surface

Fig. 40. Variation in leaflet morphology in the Leucaena diversifolia complex. (A) K156, K165 (DIV4N), note the plicate-striate surface of the fourth leaflet; (B) K174, K376 (PAL); (C) K407 (DIV2N, pilose type); (D) K480 (DIV, revolute type); (E K454, K465 (DIV2N, spotted type); (F) K409 (DIV glossy type); (G) K412, K413 (DIV2N, glabrous type); (H) K423 (DIV2N, glabrous type). ab = abaxial (lower) surface, ad = adaxial (upper) surface.


A
ab
ad
ad
ab
B
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ab
H
ab
ad
5mm

(abaxial) was sparsely or densely spotted, but a few accessions such as K159 and K160 revealed plicate-striate leaflet surfaces (Fig. 40A). This feature had been used to distinguish Leucaena laxifolia Urban from other species (Urban, 1900, Britton and Rose, 1928).

Leaflets of the accessions of PAL (Leucaena pallida), K174, K177, K178 and K376, were glossy and usually glabrous underneath (Fig. 40B), but one individual of K376 had leaflets pubescent on both surfaces. When dry, leaflets often were plicate-striate, or sometimes had only white spots. Leaflet size was large, 7-10 mm long, and with acuminate apex.

Leaflets of DIV2N (Leucaena diversifolia 2N) showed great variation in size, shape and the nature of the vesture. Several types of leaflets were recognized. The first type was oblong, mucronate (tipped) at the apex, about 4-6 mm long, pubescent above and pilose beneath (Fig. 40C). This type of plant was distributed mainly in Guatemala, although specimens with these features were occasionally found in southern Mexico, Honduras and Nicaragua (Fig. 39). The second type of leaflet was also pubescent above and pilose beneath, 4-6 mm in length, but strongly revolute-margined and acute to mucronate at the apex (Fig. 40D). K478, K480 and K483 from Zamorano, Honduras were of this type. The third type of leaflet was glabrous on both sides and densely dotted beneath, mucronate or acuminate at the apex, 4-6 mm long.

K454 and K465 belong to this type (Fig. 40E). The fourth type of leaflet included leaflets 2-3 mm long, glabrous and glossy on both surfaces (Fig. 40F). The fifth type of leaflet was pubescent above, glabrous beneath, 3-5 mm long; K412, K413 and K423 were of this type (Figs. 40G,H).

The nature of the vesture varies not only from one accession to the nest, but also showed variation within an accession. For example, the accession from Democracia, Guatemala, designated as K409, was composed of two types of individuals. Among the ten trees planted in the arboretum, four had glossy leaflets and six had pubescence on leaflets. More importantly, the character failed to correlate with geography.

b. Petiole and gland

Glands in the Leucaena diversifolia complex were exclusively cupulate (Fig. 41) and borne on rachis at the position of basal and terminal pairs of pinnae. Their presence was generally constant in the same accession. Occasionally, each accession possessed 2 or more gland types. Glands of the complex varied from 1 to 5 mm in length, and were orbicular to obovate in shape. The overview of glands shows shallow to deep holes, which were sometimes only depressed or nearly flat (Fig. 41E-G). DIV2N accessions usually had small, cupulate, orbicular glands, about 1.5 mm broad, borne on slender petioles (Fig. 41A-D). The leaves of DIV4N accessions from Veracruz, Mexico possessed large or

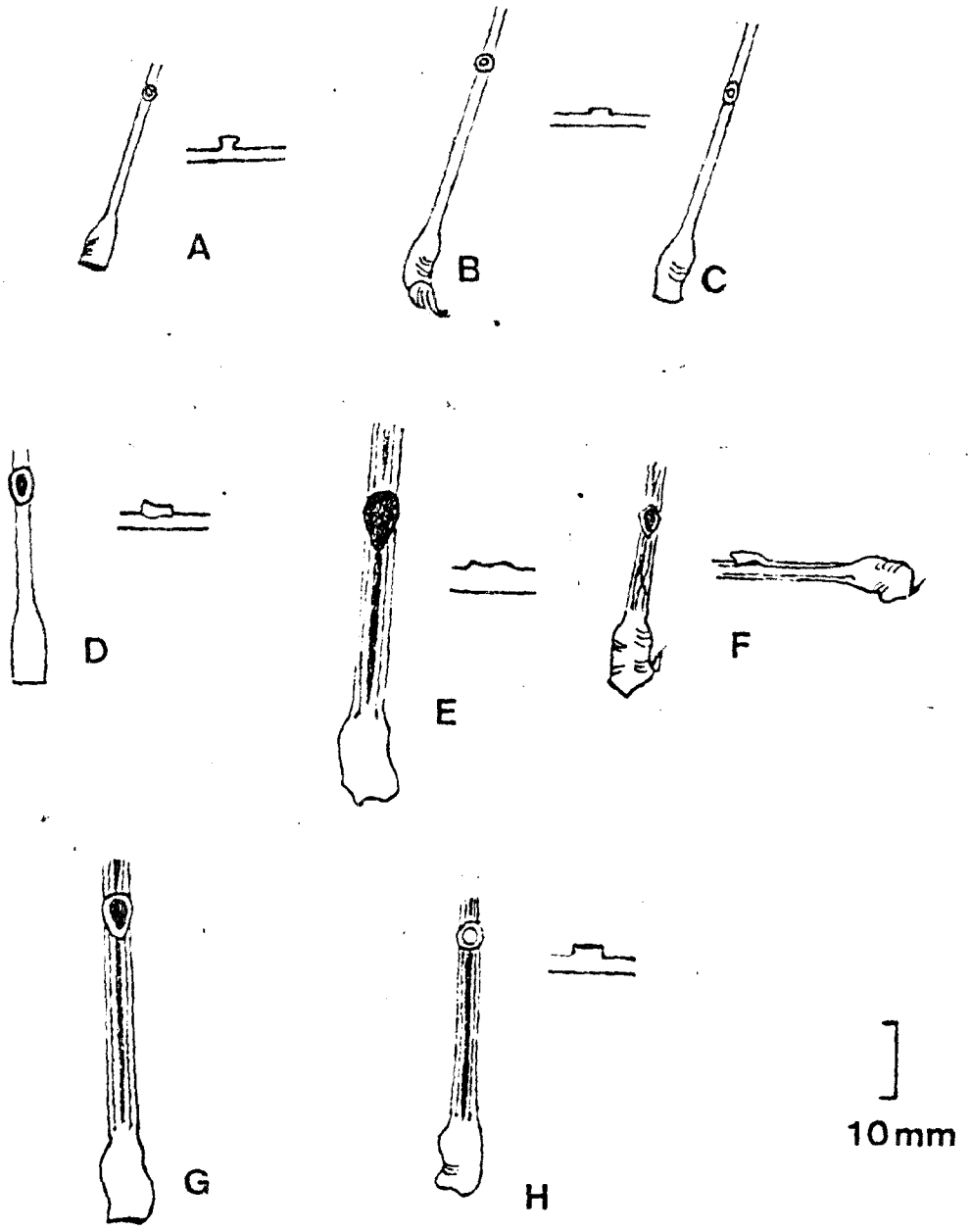

Fig. 41. Variation in petiole and gland shape in the Leucaena diversifolia complex. (A) K406, (B) K411, (C) K480, (D) K423, (E) K164, (F) K156, (G) K165, (H) K178.

sometimes depressed, cupulate, obovate, glands 3-5 mm long that were borne on bulky petioles (Fig. 41E,F,G). PAL accessions also had large cupulate glands, 4-5 mm long, orbicular or suborbicular, with deep holes. Their petioles were stout compared with those other members of the complex (Fig. 41H).

c. Floret color

The pink flower color of the Leucaena diversifolia complex is seen in the anthers, styles, or filaments, or all of these organs (Table 6). The DIV4N accessions had a pink color in both their anthers and styles but not in their filaments. This made the flower heads appear light pink (Fig. 42). Plants of PAL accessions (K174, K178 and K376) showed a pink color only in the anthers while the styles and filaments were white (Fig. 42). In DIV2N accessions, the flower color varied from dark pink to very light pink (Fig. 42). Accessions with the pilose leaflet type (which includes the revolute margin types such as K478, K480 and K483) possessed dark pink flower heads. Under microscope observation, all three organs of these accessions appeared pink. Some accessions such as K408 had only pink anthers and light pink filaments and styles. One accession, K409, was found to be very light pink on all three organs of flower heads.

Table 6. Variation of flower color in accessions of the *Leucaena diversifolia* complex.

Group	Access. No.	Overall color	Anther	Filament	Style
DIV4N	K146	light pink	pink	light pink	pink
	K155	light pink	pink	light pink	pink
	K156	light pink	pink	light pink	pink
	K157	light pink	pink	light pink	pink
	K159	light pink	pink	light pink	pink
	K160	light pink	pink	light pink	pink
	K164	light pink	pink	light pink	pink
	K165	light pink	pink	light pink	pink
	K166	light pink	pink	light pink	pink
	K186	light pink	pink	light pink	pink
PAL	K174	light pink	dark pink	white	white
	K177	light pink	dark pink	white	white
	K178	light pink	dark pink	white	white
	K376	light pink	dark pink	white	white
DIV2N	K406	pink	pink	pink	pink
	K407	pink	pink	pink	pink
	K410	pink	pink	pink	pink
	K411	pink	pink	pink	pink
	K408	light pink	pink	light pink	light pink
	K409	light pink	light pink	light pink	light pink
	K412	light pink	light pink	light pink	light pink
	K413	pink	pink	pink	pink
	K423	pink	pink	pink	pink
	K454	pink	pink	pink	pink
	K465	pink	pink	pink	pink
	K478	pink	dark pink	pink	pink
	K480	pink	dark pink	pink	pink
	K483	pink	dark pink	pink	pink

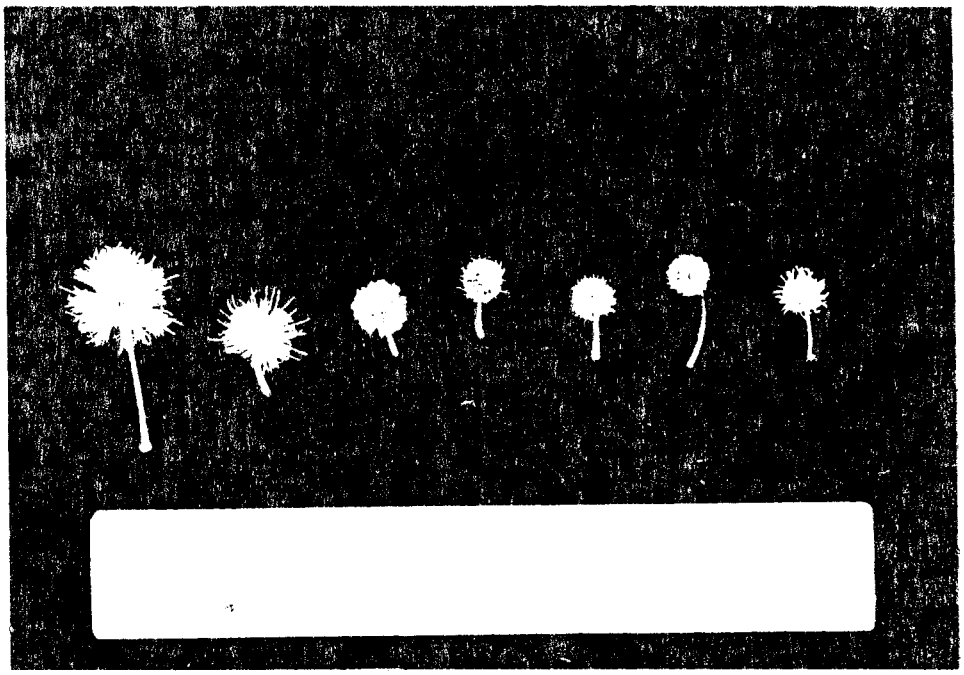

Fig. 42. Variation in inflorescence size, color and peduncle length in the Leucaena diversifolia complex. From left to right: K376 (PAL), K156 (DIV4N), K483 (DIV2N), K480 (DIV2N), K411 (DIV2N), K408 (DIV2N) and K409 (DIV2N). Ruler = 15 cm.

d. Pods

Pods of the Leucaena diversifolia complex varied in shape, size (Fig. 43) and the vesture nature of the surface. The accessions of DIV2N showed variation in both shape and size of pods (e.g. Fig. 43A,B,E,I). Most of them had glabrous pod surfaces, but some accessions such as K410 and K411 had puberulent hairs on the pod surfaces. In DIV4N accesions, the pods were linear, rounded or mucronate at the base and abruptly apiculate at the apex (Fig. 43D, F). The pod surfaces of tetraploid plants observed were glabrous. Among accessions of PAL, pods of K178 were much shorter when compared with the other three accessions (Fig. 43K-M).

e. Inflorescence size and involucre position

Generally speaking, inflorescence size in the Leucaena diversifolia complex is correlated with ploidy level, e.g., DIV4N accessions had much larger inflorescence than the DIV2N accessions (Fig. 44). Inflorescences from twelve accessions of DIV4N were large, about 1.5-2.0 cm in diameter. In four PAL accessions, inflorescences were found to be even larger, ranging from 1.8 to 2.4 cm in diameter. The inflorescences in DIV2N accessions were small, varying from 0.6 to 1.2 cm in diameter.

Involucre position was reported by previous taxonomists to distinguish species of this complex (Britton and Rose, 1928; Standley, 1946). Most arboretum accessions of the complex were observed to have the involucral bracts closely

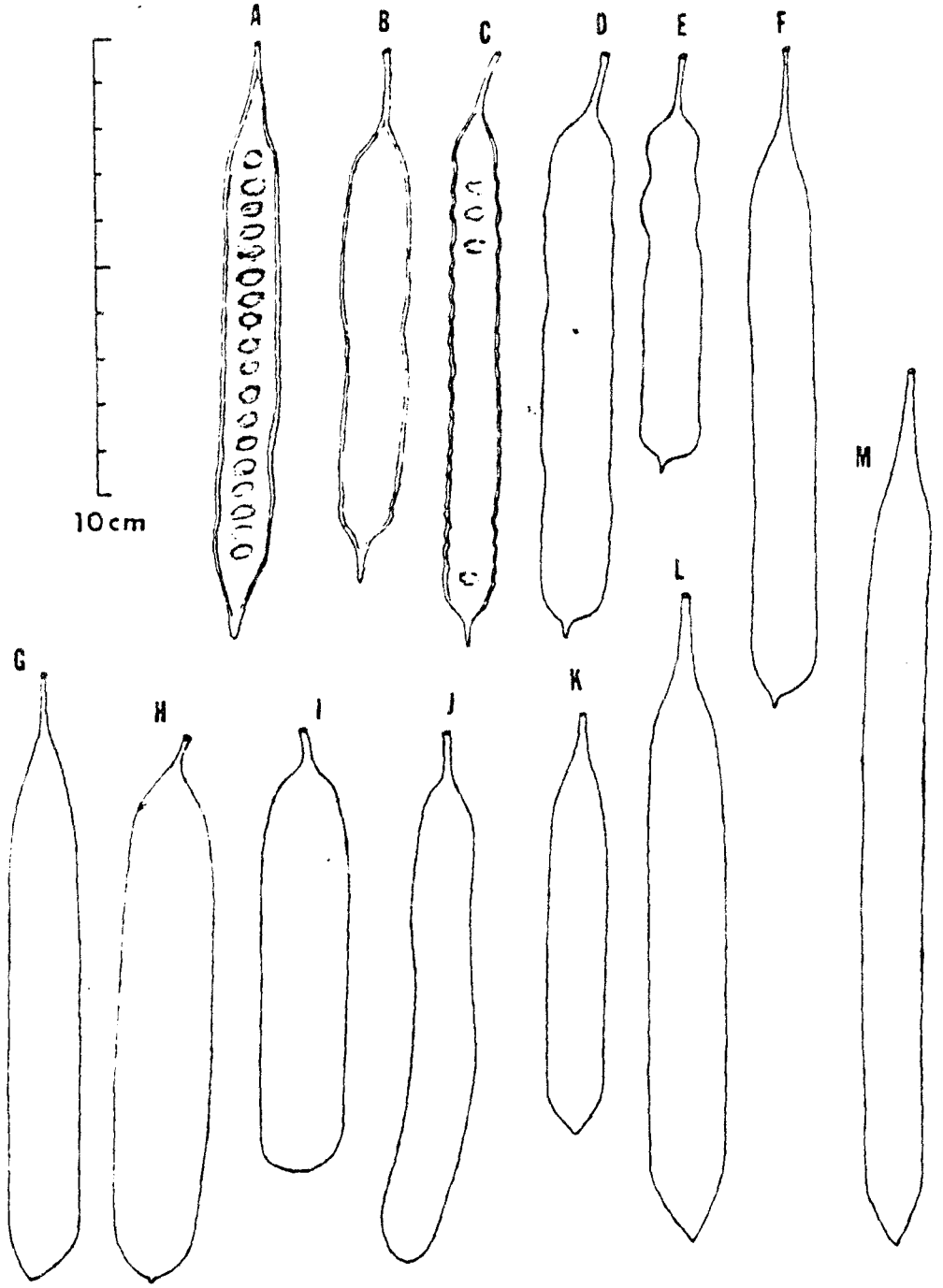

Fig. 43. Variation in pod size and shape in the Leucaena diversifolia complex. (A) K423, (B) K454, (C) K157, (D) K156, (E) K411, (F) K166, (G) K480 and K483, (H) K409, (I-J) K478 and K483, (K) K178, (L) K376, (M) K174.

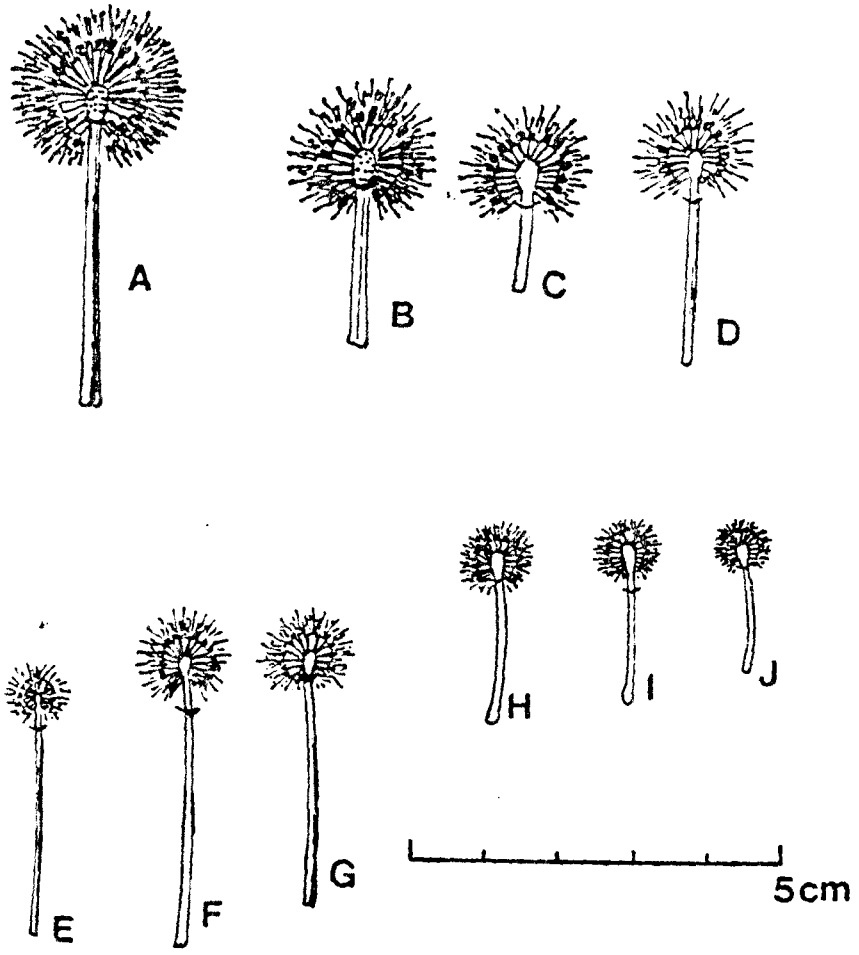

Fig. 44. Variation in involucre position and inflorescence size in the Leucaena diversifolia complex. (A) K174, (B) K156, (C-D) K160, (E-G) K409, (H) K480 and K483, (I) K423, (J) K412. Note three involucre positions in K409 (E-G).

appressed to inflorescences (Fig. 44). Several plants of DIV4N accession such as K160 and two DIV2N accessions K408 and K409, were found to have the involucres spaced 1 to 3 mm proximally from the heads (Fig. 44C-D). However, on close inspection, one could find both types of involucre on the same tree (Fig. 44E-G).

f. Floral parts

Almost all plants in the eleven DIV4N accessions observed had pilose ovaries. The corolla/calyx length ratio was usually more than two (Fig. 45A). The four PAL accessions were found to have glabrous ovaries and a corolla/calyx length ratio in excess of 2 (Fig. 45B). Plants in DIV2N accessions showed variation in ovary pubescence: some accessions were glabrous (Fig. 45D), two accessions were pubescent (Fig. 45C) and many were puberulent (Fig. 45E). However, the corolla/calyx length ratios were all less than 2. The outer surface of the calyx in the complex was usually puberulent, but some accessions such as K478, K480 and K483 had pilose calyces (Fig. 45F).

g. Young branches, rachis and rachilla

The vesture of young branches, rachis and rachilla was investigated in all DIV4N accessions. Four accessions of PAL were glabrous or nearly so on these organs. In DIV2N accessions, pilose hairs were found in K478, K480 and K483, densely pubescent in K406, K407, K411, K423, K454 and K465,

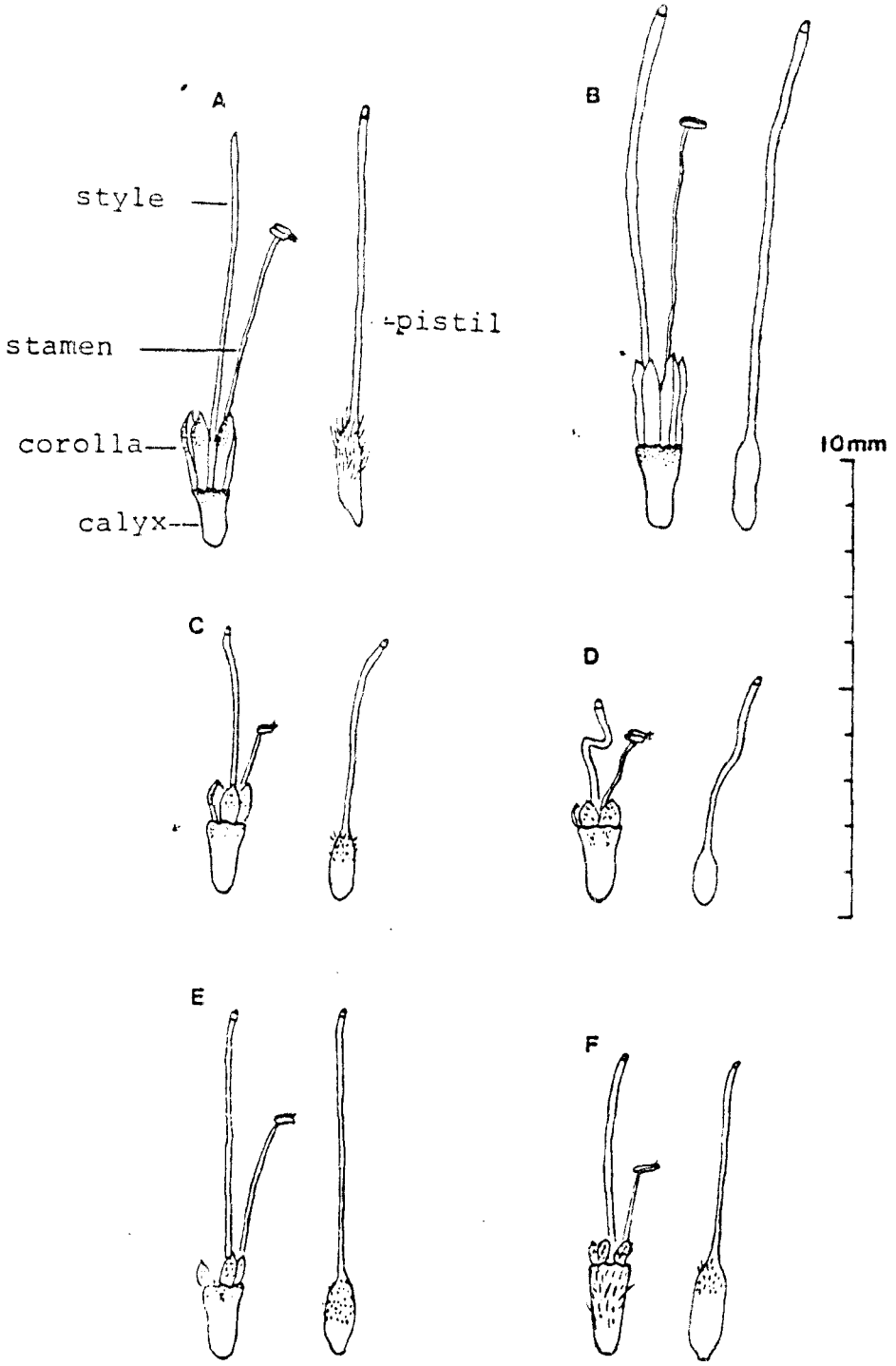

Fig. 45. Variation of floral parts in the Leucaena diversifolia complex. (A) K156(DIV4N), (B) K376(PAL), (C) K409(DIV2N), (D) K423 (DIV2N), (E) K412 (DIV2N), (F) K480(DIV2N). Nine stamens omitted.

sparsely pubescent in K412 and K413. These structures were glabrous in K408 and K409.

The hairs on these plants, including the hairs on leaflets and young twigs, were all non-glandular, unicellular to multicellular, and had nearly straight, thick and cuticularized walls (Fig. 46). Hairs defined as pubescence were short, about 50 - 80 um in length, and 1 celled. The so-called pilose hairs were much longer, about 200-500 um long, and 2 - 6 celled (Fig. 46). All hairs were cylindrical and straight or slightly bent.

h. Bark color

The trunks of the Leucaena diversifolia complex were usually smooth and with slightly elevated lenticels about 1 to 3 mm in length (Fig. 47). Vertical rows of lenticels frequently occurred opposite the wide vascular rays. Bark of both DIV4N and DIV2N accessions had longitudinal, deep-purple and branched stripes, on which there were no lenticels (Fig. 47). These stripes were narrow in DIV4N accessions and made the bark appear brownish. In DIV2N accessions, these stripes were usually wider, from 1 to several cm wide, and made the bark appear purplish. The stripes were not seen in accessions of PAL in which bark was whitish. In addition, bark lenticels in the latter were fewer and shorter than in the former two groups.

Table 7 lists the differences in vesture nature of

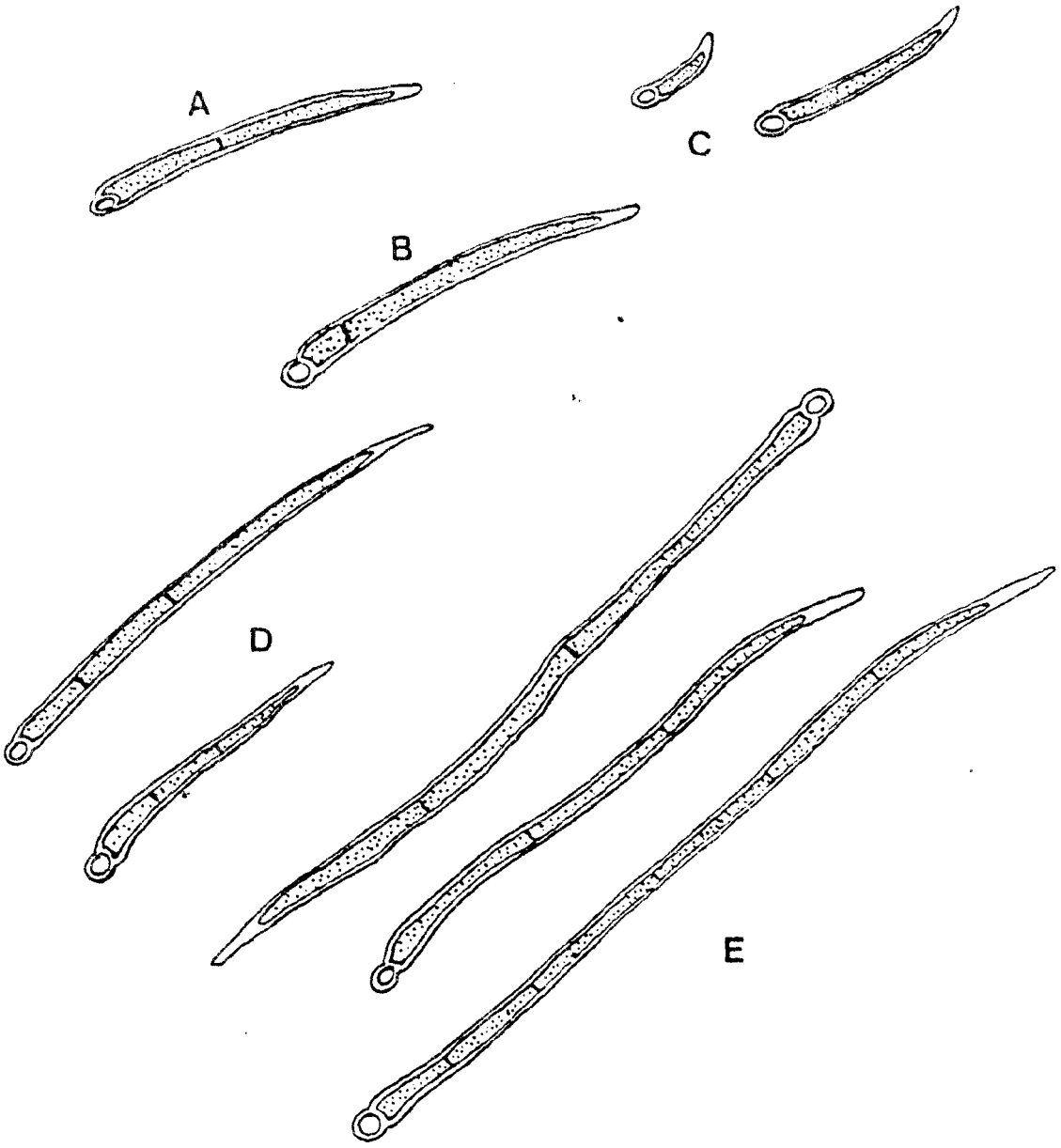

Fig. 46. Trichomes from leaflets and young twigs of the Leucaena diversifolia complex. (A-B) 2-celled hairs from leaflet surface of K423, (C) 1-celled hairs from leaflet margin, (D) 3-celled hairs from leaflets of K480, (E) 3-6-celled hairs of abaxial leaflet surface from K483. (All figures 400x).

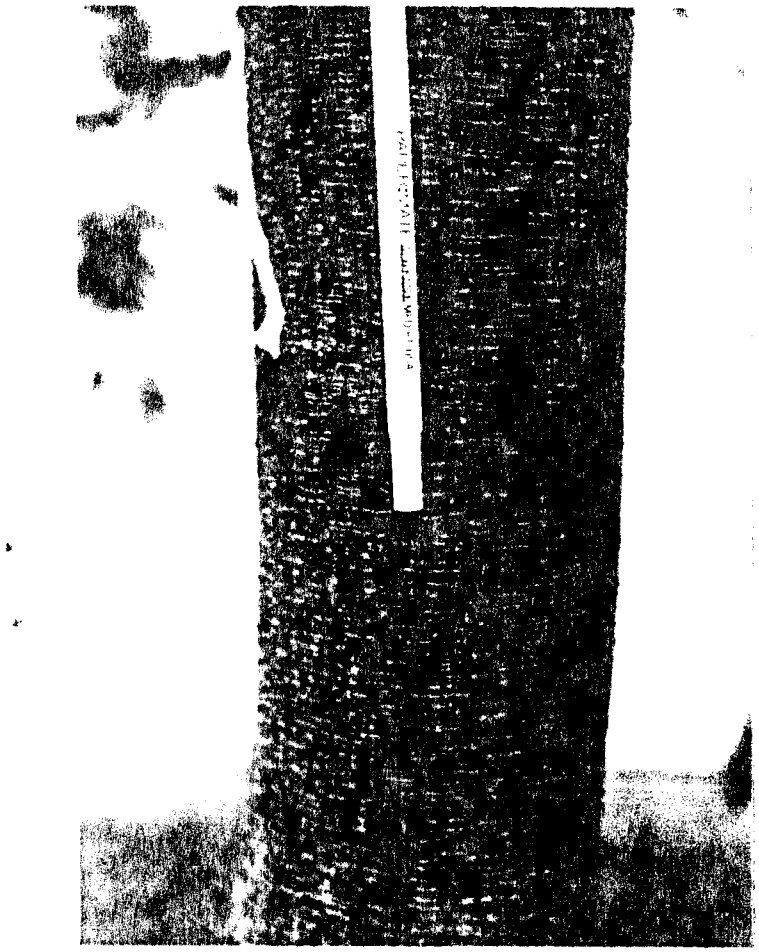

Fig. 47. Bark appearence and lenticel distribution of Leucaena diversifolia (K156, 4N).

Table 7. Summary of vesture differences among 30 accessions of the *Leucaena diversifolia* complex.

Group	K No.	*Vesture twigs, rachis etc.	leaflets	ovaries	pods	Matches the described taxon
	K145	P	G	Pi	G	
	K146	P	G	Pi	G	
	K154	P	G	Pi	G	
	K155	P	G	Pi	G	
	K156	P	G	Pi	G	*L. diversifolia*
DIV4N	K157	P	G	Pi	G	
	K159	P	G	Pi	G	*L. brachycarpa*
	K160	P	G	Pi+G	G	
	K164	P	G	Pi	G	*L. laxifolia*
	K165	P	G	Pi	G	
	K166	P	G	Pi	G	
	K186	P	G	Pi	G	
	K174	Gs	G	G+Pi	G	*L. pallida*
PAL	K177	Gs	G	G	G	*L. dugesiana*
	K178	Gs	G	G	G	*L. paniculata*
	K376	Gs	G+Pi	G	G	*L. oaxacana*
	K406	P	Pi	Pr	G	
	K407	P	Pi	Pr	G	*L. guatemalensis*
	K410	P	Pi	Pr	Pr	
	K411	P	Pi	Pr	Pr	"pilose type"
	K408	P	Gs	P	G	*L. standleyi*
	K409	G+P	Gs	Pr	G	"glossy type"
DIV2N	K412	P	G	G	G	*L. pueblana*
	K413	P	G	G	G	*L. revoluta*
	K423	P	G	G	G	*L. trichandra* "glabrous type"
	K454	P	G	G	G	
	K465	P	G	Pr	G	"spotted type"
	K478	Pi	Pi	Pr	G	*L. stenocarpa*
	K480	Pi	Pi	Pr	G	
	K483	Pi	Pi	Pr	G	"revolute type"

* Vesture: G = glabrous; Gs = glossy; P = pubescent; Pi = pilose; Pr = puberuent.

twigs, rachis, leaflets, ovaries and pods among 30 accessions, which were used as diagnostic characters of the described species of the Leucaena diversifolia complex. Based of these differences, DIV2N can be divided into five types: pilose, glossy, glabrous, spotted and revolute types.

5.2.2. Quantitative characteristics

a. Leaf

The leaves of Leucaena species are bipinnate. Each leaf consists of few to many pairs of pinna and each pinna has many leaflets. For each accession, measurements were made of leaf length, number of pinna pairs per leaf, pinna length, number of leaflet pairs per pinna and leaflet length (Table 8). These measurements, when combined together, easily separated the 30 accessions into three major groups (DIV4N, PAL and DIV2N). With few exceptions, the separation based on these quantitative characteristics was in agreement with the qualitative data.

Both DIV4N and PAL accessions were highly different from DIV2N accessions in leaf length, with almost no overlap (Table 8, Fig. 48). DIV4N accessions had more pinna pairs per leaf than PAL and DIV2N accessions. The latter overlapped in this character. However, the three groups overlapped in pinna length, though in general, DIV4N and PAL accessions had longer pinnae than DIV2N accessions. PAL appeared to have the longest pinnae of the three. DIV4N

Table 8. Measurements of leaf characteristics in 30 accessions of the *Leucaena diversifolia* complex (N = 20).

Group	K No.	Leaf length (cm) range	Leaf length (cm) $\bar{x} \pm$ s.d.	No. of Pinna pairs/leaf range	No. of Pinna pairs/leaf $\bar{x} \pm$ s.d.	Pinna length range	Pinna length $\bar{x} \pm$ s.d.	No. of Leaflet prs/pinna range	No. of Leaflet prs/pinna $\bar{x} \pm$ s.d.	Leaflet length (mm) range	Leaflet length (mm) $\bar{x} \pm$ s.d.
	K145	20.2-32.8	23.6±2.8	17-31	25.1±2.9	4.0-7.9	6.1±0.9	42-70	55.2±7.8	4-7	5.4±0.8
	K146	16.2-26.3	22.3±3.5	15-30	22.7±3.4	4.8-7.8	6.0±0.7	40-66	56.6±5.7	4.5-8	5.7±0.8
	K154	19.7-32.8	24.1±3.2	18-32	26.1±3.9	4.4-8.0	6.1±0.8	45-72	61.5±7.2	5-5.6	5.5±0.4
	K155	18.4-26.6	22.8±2.4	16-27	21.0±2.8	5.4-8.2	6.6±0.8	43-68	53.6±5.5	4-7	6.1±0.8
	K156	18.2-26.0	22.4±2.0	18-33	26.1±3.6	6.0-8.0	7.2±0.6	50-72	59.9±6.1	5-7	6.0±0.5
DIV4N	K157	17.2-30.1	22.7±2.8	12-29	21.1±3.4	4.2-7.2	5.6±0.7	37-59	47.4±5.9	4-7	5.7±0.8
	K159	20.4-30.6	24.8±2.8	21-32	28.3±3.5	5.3-8.2	6.8±0.7	45-66	54.1±5.2	5-6.2	5.8±0.5
	K160	20.0-34.2	23.7±3.1	17-31	25.6±4.2	4.8-7.0	5.9±0.6	42-62	53.0±5.0	4-6	5.2±0.7
	K164	18.6-33.1	23.9±4.2	13-24	18.6±2.3	7.2-9.9	8.4±0.9	43-62	51.5±5.7	5-8	6.7±0.6
	K165	18.5-32.0	23.8±3.3	15-24	19.7±2.4	6.3-9.6	7.5±0.6	42-58	51.8±4.6	5-7	6.2±0.6
	K166	17.6-32.4	21.8±1.6	19-29	23.4±2.3	5.4-8.3	7.2±0.6	45-72	57.5±4.6	5-7	5.7±0.6
	K186	15.1-28.9	19.0±2.0	12-23	18.9±2.4	4.8-8.8	7.0±1.0	35-61	51.9±5.0	5-8	6.5±0.6
	K174	16.0-30.2	22.8±3.7	12-24	17.8±3.3	6.5-10.8	8.9±1.4	31-50	38.1±4.9	7-10	8.0±1.0
PAL	*K177	17.2-26.7	22.5±3.9	14-19	16.4±2.1	8.4-9.7	9.0±0.5	38-46	42.0±3.1	6-8.5	7.3±1.0
	K178	14.2-23.5	18.7±3.1	9-23	15.1±4.1	7.8-12.5	10.4±1.0	37-56	43.5±4.6	7-10	8.5±0.9
	K376	19.7-35.4	26.0±4.3	11-25	18.8±4.0	7.2-14.2	9.4±1.3	30-69	47.2±10.1	7-10	8.1±0.9
	K406	12.1-16.9	14.1±1.3	12-20	15.6±2.0	4.2-7.0	5.3±0.6	14-44	29.9±3.6	3-5	4.2±0.7
	K407	9.8-16.9	13.1±1.1	12-19	16.0±1.6	3.8-6.1	4.9±0.6	19-32	25.7±4.3	4.5.5	4.7±0.6
	K410	9.1-16.5	11.7±1.8	9-18	12.7±2.8	3.3-6.9	5.7±0.9	22-40	33.7±4.7	4-7	5.7±0.7
	K411	9.1-15.2	12.6±1.9	7-23	16.6±4.2	3.5-6.9	5.0±1.0	18-45	34.0±7.9	4-6	5.1±0.7
	K408	10.0-16.4	13.4±1.8	16-31	23.3±3.5	3.8-5.3	4.4±0.4	30-65	50.4±7.8	2-4.5	3.4±0.5
	K409	8.7-14.3	11.8±1.4	9-18	13.3±2.0	4.2-6.7	5.2±0.7	33-63	40.6±5.4	3-5.5	4.8±0.4
DIV2N	K412	8.6-13.8	11.4±1.2	10-20	14.9±3.6	3.2-5.4	4.3±0.5	21-47	33.4±6.3	3.5-6	4.7±0.8
	K413	7.7-12.1	10.1±1.1	7-22	14.9±2.7	3.3-5.0	3.8±0.4	28-44	35.2±4.5	2-6	4.2±0.6
	K423	10.6-16.8	14.1±1.7	10-19	16.3±2.0	3.5-6.1	4.7±0.7	27-43	37.2±3.6	2.5-5	4.4±0.5
	K454	12.2-17.4	15.0±1.4	14-23	18.6±2.8	5.1-7.7	6.0±0.8	32-66	48.2±9.2	4-7	5.1±0.8
	K465	11.4-17.6	15.2±1.8	11-23	17.4±1.4	4.2-6.8	5.7±0.7	29-55	41.6±8.1	2.5-7	5.1±1.3
	K478	12.3-17.8	15.1±1.4	11-24	16.9±3.3	4.7-6.8	5.6±0.6	23-37	32.1±4.0	5-7	6.0±0.5
	K480	12.8-17.7	15.4±1.2	13-23	18.3±24.1	4.8-6.9	5.5±0.6	27-42	34.1±3.0	4-7	5.3±0.6
	K483	12.4-16.8	14.1±1.2	12-20	16.1±1.9	4.6-6.7	5.6±0.6	26-36	30.6±2.5	4.5-7	5.4±0.7

* N = 5, samples from a single tree.

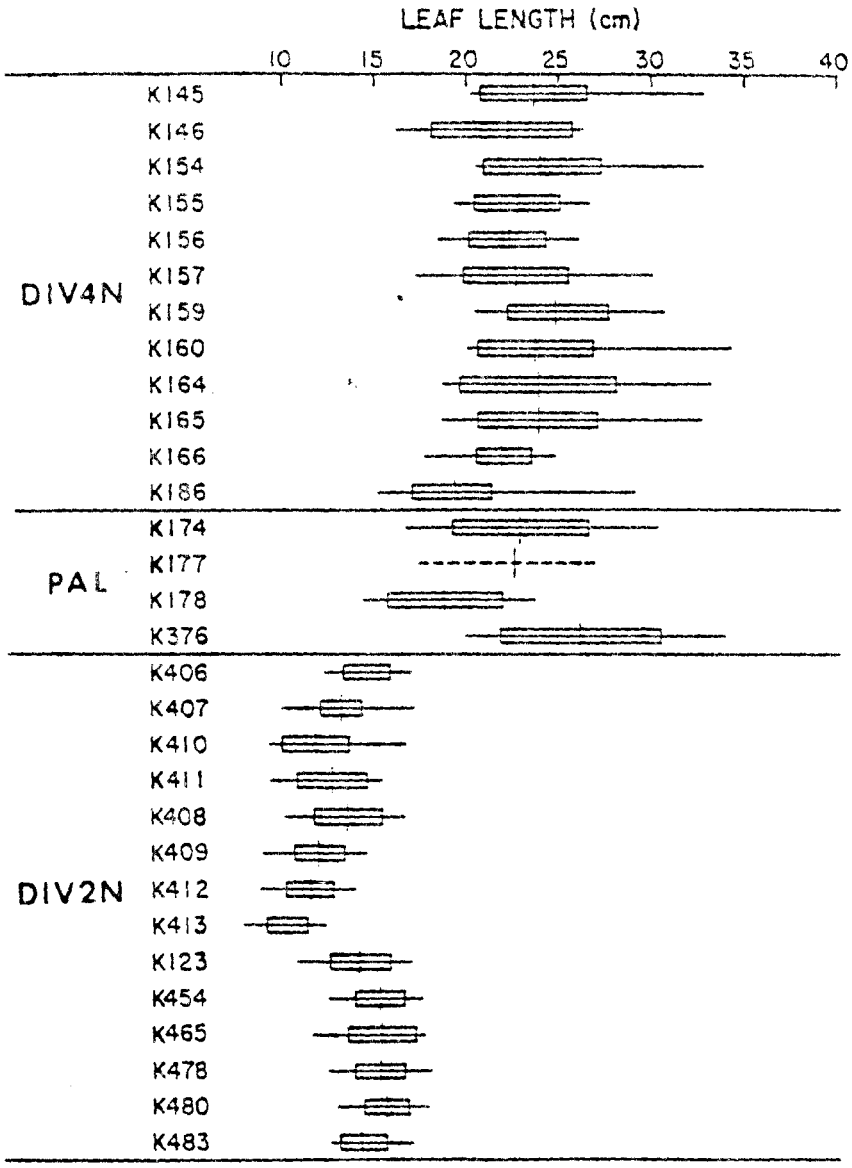

Fig. 48. Comparison of leaf length in 30 accessions of the Leucaena diversifolia complex. Range shown by horizontal line, mean by short vertical line and standard deviation by horizontal bar. Dotted line indicates the range of 5 samples from a single tree.

accessions had more leaflet pairs per pinna than DIV2N accessions (Fig. 49). The leaflet number of PAL was fewer than the other two. Among the DIV2N accessions, K408, K409, K454 and K465 had more leaflets than others, similar to DIV4N (Fig. 49). The leaflets of DIV4N accessions were generally longer than those of DIV2N accessions. The accessions of PAL had much longer leaflets than the others.

Differences in numbers of pinna pairs and leaflet pairs, which seems to be less environmentally modified, were the most useful characters differentiating the three groups in both field and herbarium observations.

b. Inflorescences (flower heads)

Two measurements were taken from in inflorescences--floret number and peduncle length (Table 9). In general, DIV4N accessions had fewer florets than the other two, though there was variation within each entity. Some DIV2N accessions tended to have outstanding floret numbers such as K410, K411 and K483 which were not only different from DIV4N but also from other accessions of DIV2N (Table 9).

Peduncle length was also variable from accession to accession and even from individual to individual within the same accession. There was no difference in peduncle length between DIV2N and DIV4N accessions. However, a conspicuous difference was found in PAL accessions which had much longer peduncle length (Table 9). This character was very useful in

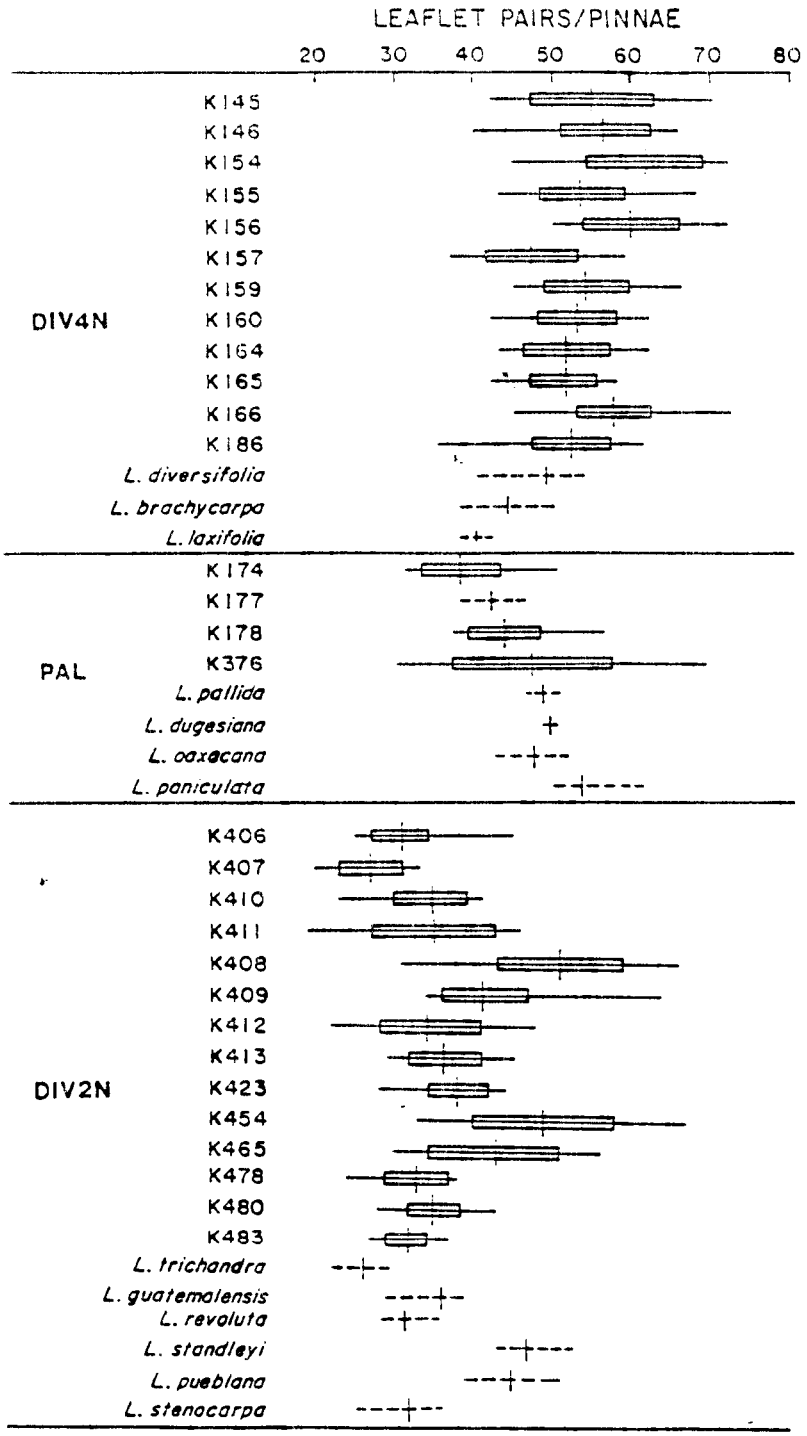

Fig. 49. Comparison of number of leaflet pairs per pinna in living plants of 30 accessions of the Leucaena diversifolia complex and type specimens (dotted lines) of described species. Legends the same as Fig. 48.

Table 9. Inflorescence measurements in 30 accessions of the *Leucaena diversifolia* complex (N = 20).

Group	Accession No.	Floret No./head range	Floret No./head $\bar{x}\pm$s.d.	Peduncle length(cm) range	Peduncle length(cm) $\bar{x}\pm$s.d.
DIV4N	K145	--	--	--	--
	K146	53-73	62.1±5.2	0.6-1.8	1.0±0.3
	K154	--	--	--	--
	K155	41-56	51.1±3.4	0.4-1.5	0.9±0.3
	K156	64-99	82.4±9.5	1.0-2.2	1.6±0.3
	K157	51-71	59.7±6.5	0.5-1.8	1.1±0.3
	K159	55-84	68.3±7.8	1.0-2.0	1.5±0.3
	K160	48-82	68.9±8.9	0.9-1.8	1.3±0.2
	K164	39-60	50.5±5.5	0.9_2.0	1.5±0.3
	K165	42-74	55.5±7.7	0.4-1.2	0.7±0.3
	K166	45-78	62.2±8.3	0.5-1.2	0.8±0.2
	K186	50-68	59.0±5.5	0.5-1.2	0.8±0.2
PAL	K174	59-115	88.0±15.8	1.7-3.6	2.3±0.6
	*K177	82-115	94.4±11.9	2.0-2.6	2.4±0.4
	K178	73-125	105.5±15.8	2.0-3.3	2.6±0.4
	K376	78-115	98.4±11.5	2.2-3.8	2.8±0.5
DIV2N	K406	64-108	81.1±14.3	0.6-1.3	0.9±0.2
	K407	80-141	104.8±18.4	0.4-1.2	0.9±0.2
	K410	91-150	118.8±15.9	0.6-1.5	1.1±0.3
	K411	84-134	115.7±14.9	0.6-2.1	1.4±0.4
	K408	44-71	61.3±9.5	0.8-1.8	1.4±0.3
	K409	57-106	71.8±11.6	1.2-2.6	1.8±0.4
	K412	62-88	74.7±7.5	0.5-1.6	0.9±0.3
	K413	57-92	75.2±11.7	0.7-1.4	1.0±0.2
	K423	78-95	84.3±4.6	0.7-2.1	1.5±0.4
	K454	63-93	78.4±11.2	0.8-1.4	1.1±0.2
	K465	68-106	83.6±12.6	1.3-2.3	1.9±0.3
	K478	52-103	81.6±15.4	0.9-1.8	1.3±0.2
	K480	82-119	96.8±12.2	0.8-2.2	1.3±0.4
	K483	94-149	133.4±16.2	0.7-1.5	1.1±0.2

* N = 5, samples from a single tree.

distinguishing this group from other two.

c. Floral parts

Four measurements were made of floral parts--length of calyx, corolla, stamen and pistil (Table 10). The calyx lengths of PAL accessions were longer than the other two, which were not different in this character (Table 10).

The lengths of corolla, stamen and pistil were useful in recognizing the three groups and showed no overlap at a level of one standard deviation (Table 10, Figs. 50 and 51). PAL accessions had longest corollas, stamens and pistils, DIV4N accessions were intermediate and DIV2N accessions had the shortest length of these organs.

d. Pods

Pod length, pod width and seed number per pod were measured for each accession available (Table 11). No differences were found among the three groups, except two accessions of PAL that tended to have longer pods than the others (Table 11). Within the accessions of PAL, the pod lengths were highly variable, and the means ranged from 11 to 18 cm (Table 11, Fig. 43).

5.3. Analysis of Tetraploids and Diploids in Leucaena diversifolia.

DIV4N and DIV2N are much more similar to each other than to any other species, and to another member of the L.

Table 10. Floral measurements in 30 accessions of the *Leucaena diversifolia* complex (N = 20).

Group	Access. No.	Calyx length(mm) range	Calyx length(mm) $\bar{x}\pm$s.d.	Corolla length(mm) range	Corolla length(mm) $\bar{x}\pm$s.d.	Stamen length(mm) range	Stamen length(mm) $\bar{x}\pm$s.d.	Pistil length(mm) range	Pistil length(mm) $\bar{x}\pm$s.d.
DIV4N	K146	1.5-2.0	1.8±0.1	3.4-4.0	3.7±0.2	5.5-6.5	6.2±0.3	8.0-9.0	8.6±0.3
	K154	--	--	--	--	--	--	--	--
	K155	1.2-2.0	1.4±0.3	3.1-4.0	3.6±0.3	5.0-7.0	5.9±0.6	7.0-8.5	8.0±0.4
	K156	1.8-2.5	2.1±0.2	3.8-4.5	4.1±0.2	6.5-7.5	6.9±0.3	8.0-10	8.8±0.6
	K157	1.4-2.2	1.6±0.2	3.0-3,7	3.3±0.2	4.5-6.0	5.0±0.4	6.0-7.5	6.8±0.6
	K159	1.2-2.2	1.7±0.3	3.2-4.5	3.9±0.3	5.0-7.0	5.6±0.7	6.8-8.5	7.5±0.6
	K160	1.2-1.8	1.5±0.1	2.8-4.2	3.4±0.4	3.5-0.6	4.9±0.6	5.5-7.5	6.6±0.6
	K164	1.6-2.2	1.9±0.2	3.2-4.5	3.8±0.3	5.5-7.5	6.3±0.6	8.0-10	8.8±0.6
	K165	1.6-2.2	2.0±0.2	3.5-4.5	4.0±0.3	6.0-7.0	6.6±0.3	8.0-10	9.3±0.5
	K166	1.1-2.0	1.6±0.3	3.4-4.5	3.8±0.4	5.0-6.5	5.6±0.6	6.5-8.0	7.3±0.4
	K186	1.3-1.6	1.5±0.1	3.3-4.2	3.8±0.4	5.0-6.5	5.3±0.4	7.5-9.0	8.3±0.5
PAL	K174	2.2-3.0	2.7±0.3	4.0-5.2	4.6±0.4	7.5-9.0	8.2±0.5	9.0-11	9.9±0.7
	*K177	2.2-3.0	2.5±0.3	4.0-4.5	4.2±0.2	6.5-7.5	6.9±0.4	9.5-10	9.7±0.2
	K178	2.0-2.6	2.3±0.2	3.5-4.7	4.2±0.3	5.5-7.5	6.9±0.6	8.5-11	9.5±0.5
	K376	2.3-3.0	2.7±0.3	4.0-5.0	4.6±0.3	7.5-9.0	8.3±0.6	9.5-12	11 ±0.7
DIV2N	K406	1.5-2.0	1.8±0.1	1.8-2.3	2.1±0.1	2.6-3.3	2.9±0.2	3.8-5.0	4.3±0.4
	K407	1.7-2.0	1.8±0.1	2.0-2.7	2.4±0.2	2.8-3.5	3.1±0.2	4.0-5.0	4.7±0.3
	K410	1.7-2.0	1.8±0.1	2.2-2.6	2.4±0.1	3.0-3.5	3.2±0.2	3.8-4.5	4.2±0.2
	K411	1.7-2.0	1.9±0.1	2.2-2.6	2.4±0.1	3.0-4.0	3.5±0.3	4.5-6.0	5.1±0.5
	K408	1.2-2.0	1.5±0.3	2.2-2.7	2.4±0.1	2.8-3.5	3.2±0.2	3.8-4.5	4.1±0.1
	K409	1.2-2.6	1.9±0.2	2.2-3.2	2.7±0.3	3.5-4.5	4.2±0.3	5.0-6.5	5.8±0.5
	K412	1.2-1.6	1.4±0.1	1.6-2.3	2.0±1.2	2.0-2.5	0.3±0.2	3.0-4.3	3.5±0.4
	K413	1.2-1.5	1.4±0.1	1.8-2.4	2.1±0.2	2.0-2.6	2.3±0.2	3.5-4.2	3.8±0.3
	K423	1.2-1.6	1.5±0.1	2.0-2.5	2.2±0.2	3.0-4.0	3.3±0.3	4.0-5.0	4.4±0.3
	K454	1.5-2.2	1.9±0.2	2.3-3.0	2.6±0.2	3.0-4.0	3.4±0.3	3.5-5.5	4.4±0.4
	K465	1.4-2.4	2.0±0.2	2.3-3.0	2.6±0.2	3.0-4.0	3.3±0.2	4.0-5.5	4.9±0.4
	K478	1.2-1.8	1.5±0.1	1.9-2.6	2.2±0.2	2.5-3.5	3.0±0.3	4.0-5.0	4.4±0.3
	K480	1.8-2.3	2.1±0.1	2.3-3.0	2.6±0.2	3.0-4.2	3.6±0.4	3.8-5.5	4.7±0.5
	K483	1.5-2.2	1.8±0.3	2.0-3.0	2.4±0.2	3.0-3.8	3.4±0.2	3.5-4.5	4.2±0.2

* N = 5, samples from a single tree.

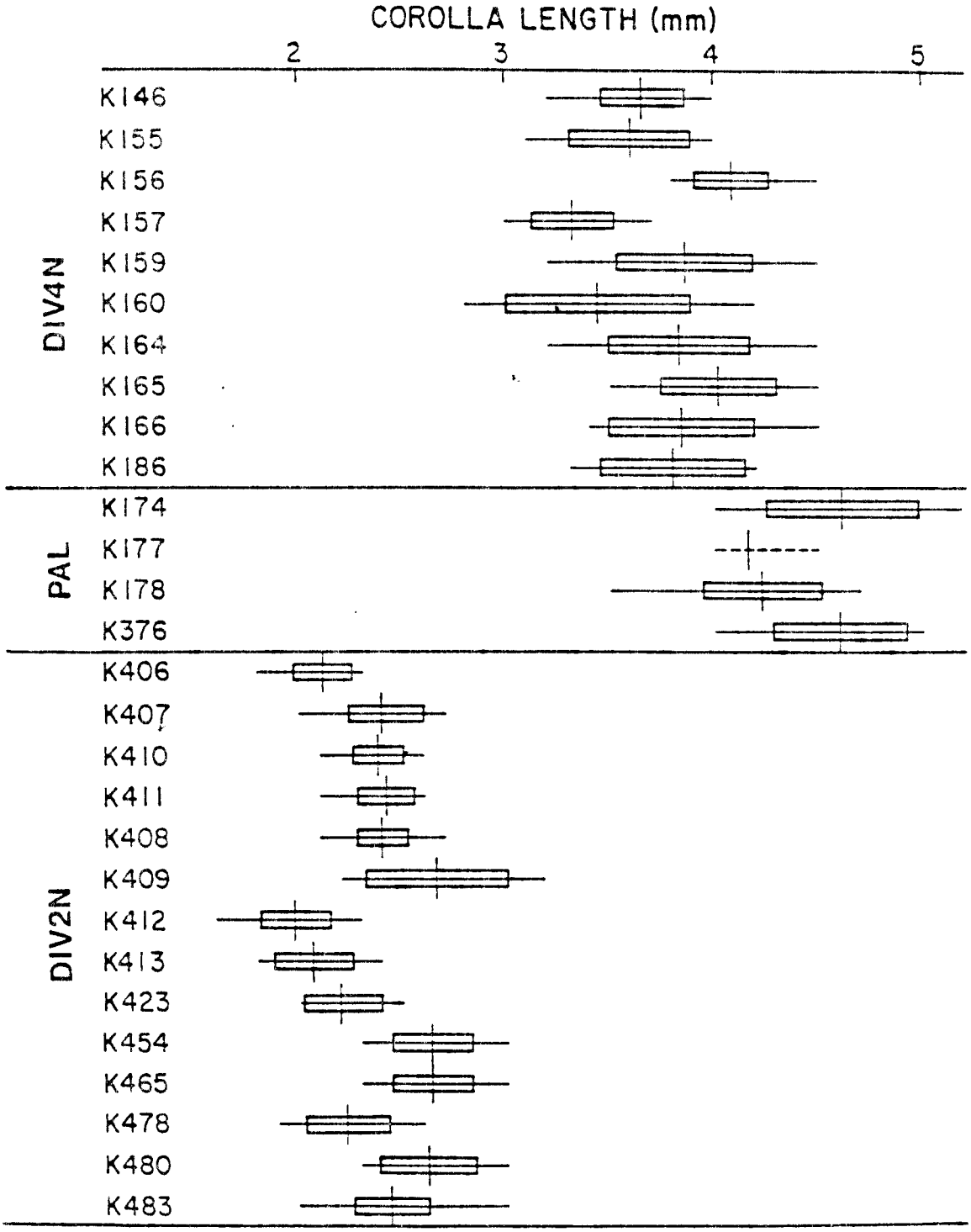

Fig. 50. Comparison of corolla length in 30 accessions of the Leucaena diversifolia complex. Legends the same as Fig. 48.

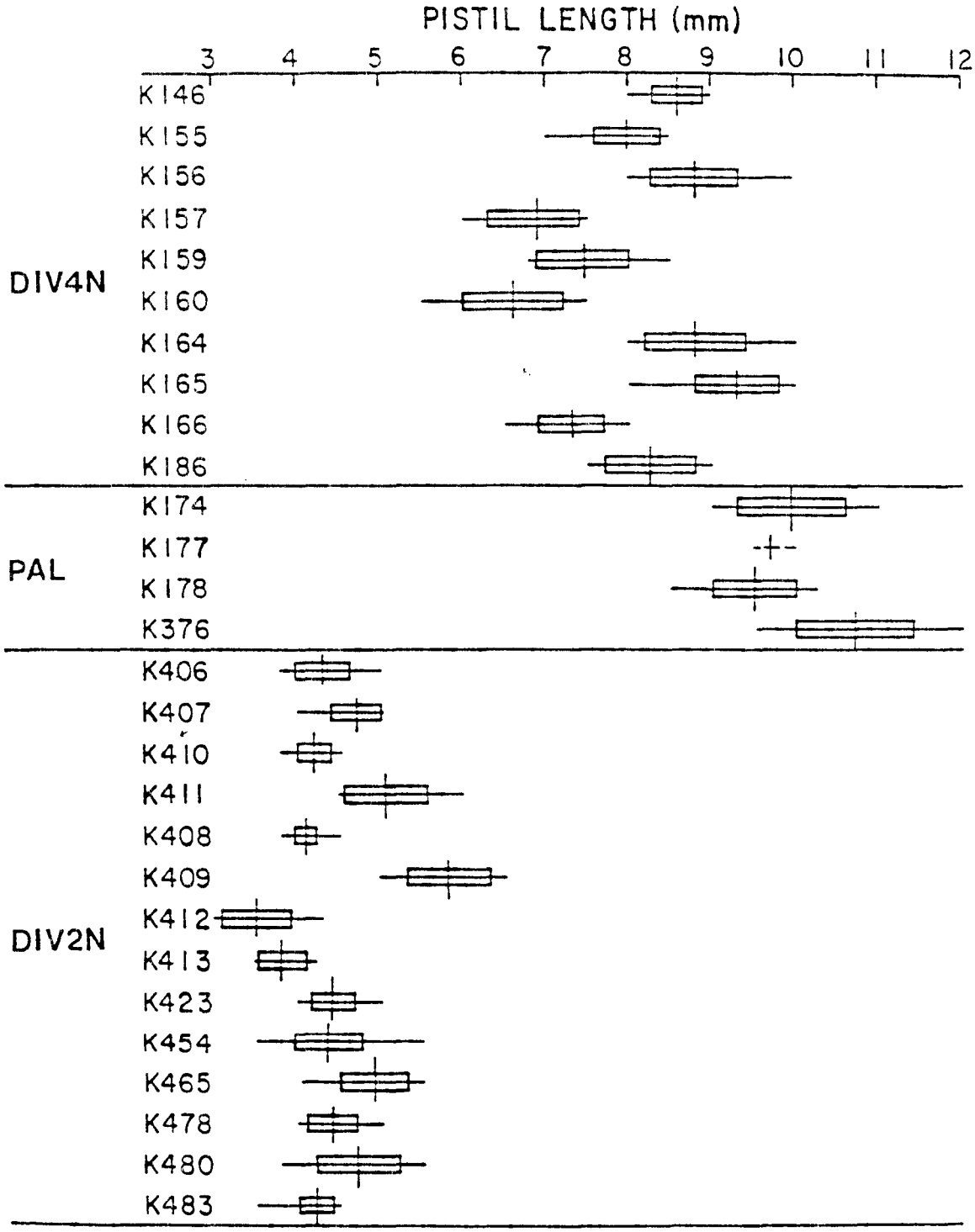

Fig. 51. Comparison of pistil length in 30 accessions of the Leucaena diversifolia complex. Legends the same as Fig. 48.

Table 11. Pod measurements in 30 accessions of the Leucaena diversifolia complex (N = 20).

Group	Access. No.	pod length (cm) range	pod length (cm) $\bar{x}\pm$s.d.	pod width (cm) range	pod width (cm) $\bar{x}\pm$s.d.	seed No. range	seed No. $\bar{x}\pm$s.d.
	K146	9.5-14.3	12.8±1.2	1.2-1.5	1.3±0.1	13-22	19.0±2.4
	K154	*—	—	—	—	—	—
	K155	9.2-13.2	10.6±1.2	1.1-1.4	1.3±0.1	11-19	16.1±2.3
	K156	11.5-14.8	13.1±0.9	1.3-1.8	1.6±0.1	14-22	18.7±2.6
DIV4X	K157	7.9-10.3	8.9±0.7	1.0-1.3	1.1±0.1	9-16	12.8±1.8
	K159	—	—	—	—	—	—
	K160	8.4-13.9	11.1±1.4	1.2-1.6	1.4±0.1	11-19	15.8±2.6
	K164	11.2-15.1	13.1±0.9	1.5-1.8	1.6±0.1	17-24	21.5±1.9
	K165	9.5-13.9	11.2±1.4	1.3-1.7	1.6±0.1	15-20	17.8±1.5
	K166	8.8-14.2	11.4±1.7	1.1-1.5	1.3±0.1	13-22	17.9±2.8
	K186	9.0-13.5	10.9±1.4	1.2-1.6	1.4±0.1	15-19	16.7±1.7
	K174	14.3-23.2	17.8±2.5	1.3-1.6	2.3±0.1	14-23	20.5±2.3
PAL	**K177	14.3-21.0	16.8±1.3	1.3-1.5	1.4±0.1	16-21	18.4±1.6
	K178	8.3-14.0	10.9±1.9	1.0-1.4	1.2±0.1	13-20	16.5±2.1
	K376	11.3-16.7	14.5±1.7	1.0-1.7	1.4±0.2	12-22	17.6±2.3
	K406	9.8-13.8	11.8±1.2	1.1-1.6	1.4±0.2	13-20	16.6±2.1
	K407	—	—	—	—	—	—
	K410	—	—	—	—	—	—
	K411	6.8-10.6	8.8±1.2	1.2-1.9	1.5±0.2	7-18	12.7±3.3
	K408	9.2-12.6	10.7±1.0	1.3-1.7	1.5±0.1	10-17	14.3±1.8
	K409	10.9-16.0	13.2±1.8	1.0-1.9	1.4±0.3	15-24	19.4±3.2
DIV2X	K412	—	—	—	—	—	—
	K413	7.3-12.1	9.34±1.3	0.9-1.4	1.1±0.1	10-21	14.3±2.6
	K423	8.8-14.6	11.5±1.3	1.0-1.4	1.3±0.1	10-16	13.6±1.7
	K454	—	—	—	—	—	—
	K465	—	—	—	—	—	—
	K478	—	—	—	—	—	—
	K480	9.8-13.2	11.3±0.8	1.0-1.6	1.3±0.2	14-20	17.6±1.3
	K483	8.7-14.5	10.9±1.5	1.2-1.6	1.3±0.1	13-21	16.5±1.9

* Data not available.

** N = 5, samples from a single tree.

diversifolia complex, PAL. They have several features in common: both have pink flowers and similar concave glands on the leaf rachises. In experimental plantings, tetraploids always had larger organs and diploids smaller. Figs. 48-51 and Tables 8-11 reveal that tetraploids have longer leaves, more leaflet pairs per pinna, and longer corollas, stamens and pistils than diploids. In addition, they can be distiguished by several other features. These are analyzed in the following sections.

a. Growth Habit

DIV4N accessions differ in growth habit from DIV2N accessions. The former usually have straight stems, while the latter are more highly branching. Their difference in growth habit is indicated by growth rates and the differences in tree height and diameter of breast height (Tables 12 and 13, Appendix 3).

Significant differences were observed in both tree height and diameter between these two cytotypes of Leucaena diversifolia. This indicates that tetraploids have higher growth rates than diploids.

b. Inflorescence Size and Floret Arrangement

The inflorescendes of DIV4N accessions were consistently larger than those of DIV2N accessions. Widely opened inflorescences of the former were 1.5-2.0 cm in diameter

Table 12. Analysis of variance of four year old height (m) in two cytotypes of Leucaena diversifolia.

Source	df	SS	MS	F
Between types	1	76.78	76.78	105.18**
Within types	18	13.14	0.73	
Total	19	89.92		

Table 13. Analysis of variance of four year old dbh (diameter at breast height, cm) of plants of two cytotypes of Leucaena diversifolia.

Source	df	SS	MS	F
Between types	1	69.56	69.56	53.51**
Within types	18	23.65	1.30	
Total	19	92.93		

whereas the inflorescences of the latter were 0.5-1.0 cm. However, there were more florets per inflorescence in the DIV2N accessions than in the DIV4N accessions, averaging 90 (range = 57-150, sample size = 200) vs 62 (range = 41-99, sample size = 280).

There were conspicuous differences between DIV4N and DIV2N accessions in the arrangement of florets, which were loose in tetraploids but compact in diploids. These two characters are very useful in determining ploidy level of species from dry specimens, and, therefore, were used as an indicator in herbaria for distinguishing diploids from tetraploids.

c. Pollen Size

The diameter of pollen grains in the DIV4N accessions was greater than in the DIV2N accessions (Fig. 52). Pollen size can be used as a method for the identification of ploidy levels in Leucaena diversifolia. This technique thus may allow estimation of ploidy level of herbarium specimens, and permit the study of the distribution of cytotypes of the complex. However, due to the difficulty in collecting pollen from specimens, this character was not used in predicting ploidy level of the species.

All Leucaena diversifolia pollen grains observed were 3-colporate monads, circular to rounded-triangular in shape. Two tetraploid accessions, K164 and K165, consisted of both 3-pored and 4-pored grains, while the other tetraploid

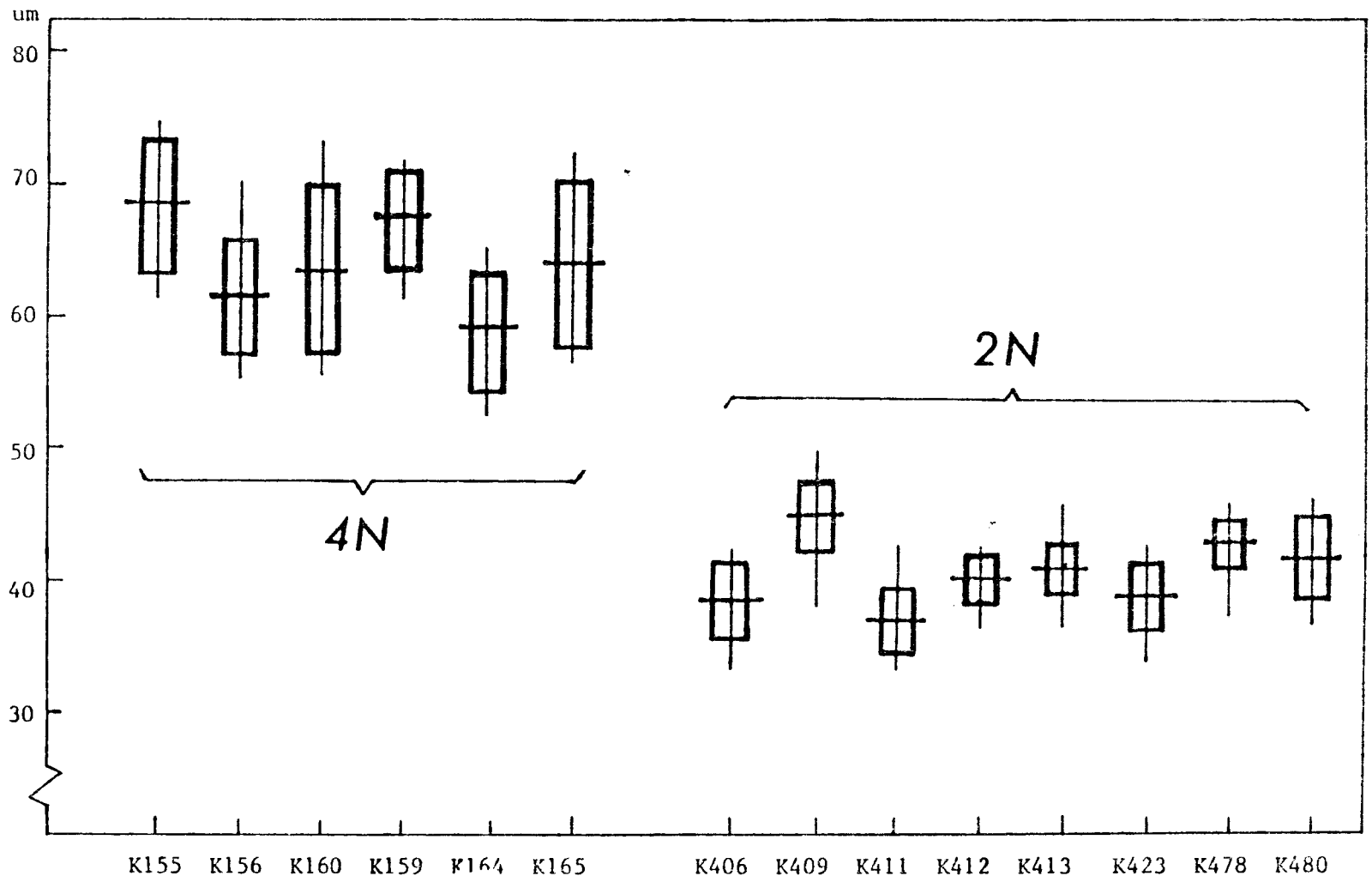

Fig. 52. Pollen diameter in cytologically known tetraploid and diploid accessions of the Leucaena diversifolia complex. Horizontal lines = means, thick bars = standard deviations and vertical lines = ranges.

accessions and all diploid accessions had only 3-pored pollen grains. Little or no difference was found, however, in the size of 3-pored grains as contrasted with 4-pored grains in a given plant.

d. Guard Cells

A correlation was sought between ploidy level and guard cell size in order to make use of herbarium specimens in studying the distribution of the cytotypes over the entire range of the complex. Comparisons of the estimated areas of guard cell pairs (using area of ellipse) showed that diploid and tetraploid plants could be consistently distinguished by using this character (Fig. 53). The values for average guard cell area of tetraploid accessions exceeded 300 um^2 and the corresponding values for diploid accessions was below 250 um^2. Both the lower means and the lower ranges served to differentiate diploids from tetraploids.

Although standard deviations of the four diploid and tetraploid accessions overlapped in value, their means were statistically different, based on a t-test at a 5% level of confidence. Therefore estimates of ploidy level using these data show a high correlation with chromosome count. This supports the validity and efficacy of the guard cell estimates for distinguishing cytotypes.

The ploidy level of the described species of the Leucaena diversifolia complex were determined by measuring guard cell sizes from the type specimens. These data are

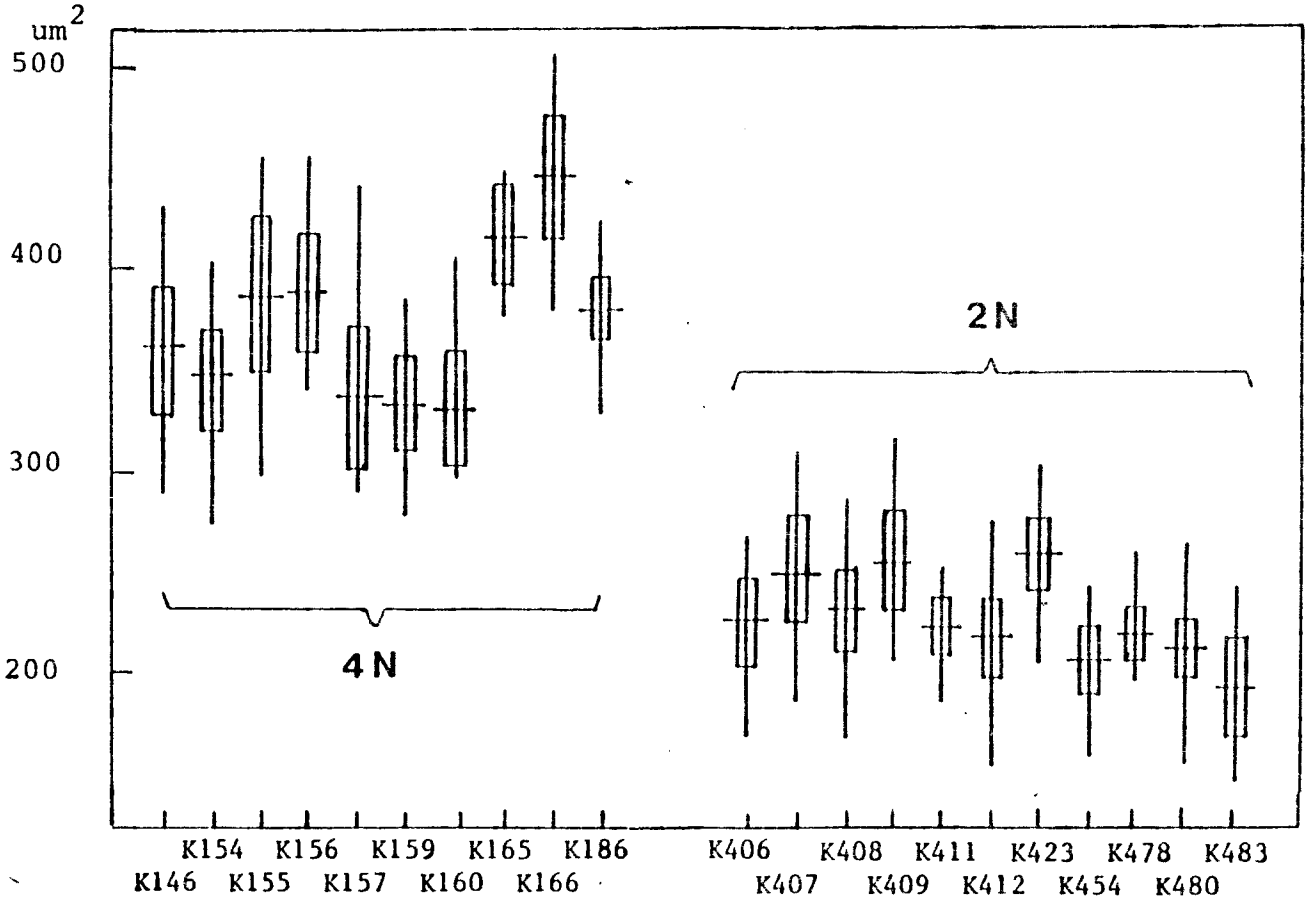

Fig. 53. Guard cell measurements in cytologically known tetraploid and diploid accessions (except K154) of the Leucaena diversifolia complex. Horizontal lines = means, thick bars = standard deviation and vertical lines = ranges.

listed in Appendix 2. Size of stomata of L. diversifolia (Schlech.) Benth. was 313 to 451 mu^2, within the range of tetraploids. The loose heads and stomatal size (325-421 mu^2) of L. brachycarpa Urban and the stomatal size (289-442 mu^2) of L. laxifolia Urban indicated that these two species were also tetraploids. On the other hand, the sizes of guard cells of Leucaena guatemalensis, L. pueblana, L. revoluta, L. standleyi, L. stenocarpa and L. trichandra were within the range of diploids (Appendix 2).

e. Geographical Distribution

The distribution map of DIV2N and DIV4N accessions (Fig. 54) is based largely on data from specimens examined from the four herbaria. In addition, many localities are based on Brewbaker's collections (Brewbaker, unpublished).

In most cases the different cytotypes of the specimens could be separated by noting differences in head size and arrangement of florets. As previously mentioned, tetraploids have larger head size and more compact flower heads than those of diploids. In a few cases, where there was no flower head in the herbarium specimen, stomatal size was measured to help deduce the cytotype.

Tetraploids were much more restricted in distribution than diploids, being confined exclusively to Veracruz, Mexico, except for a few collections known from Jamaica which were evidently due to sporadic introductions (Standley, 1964;

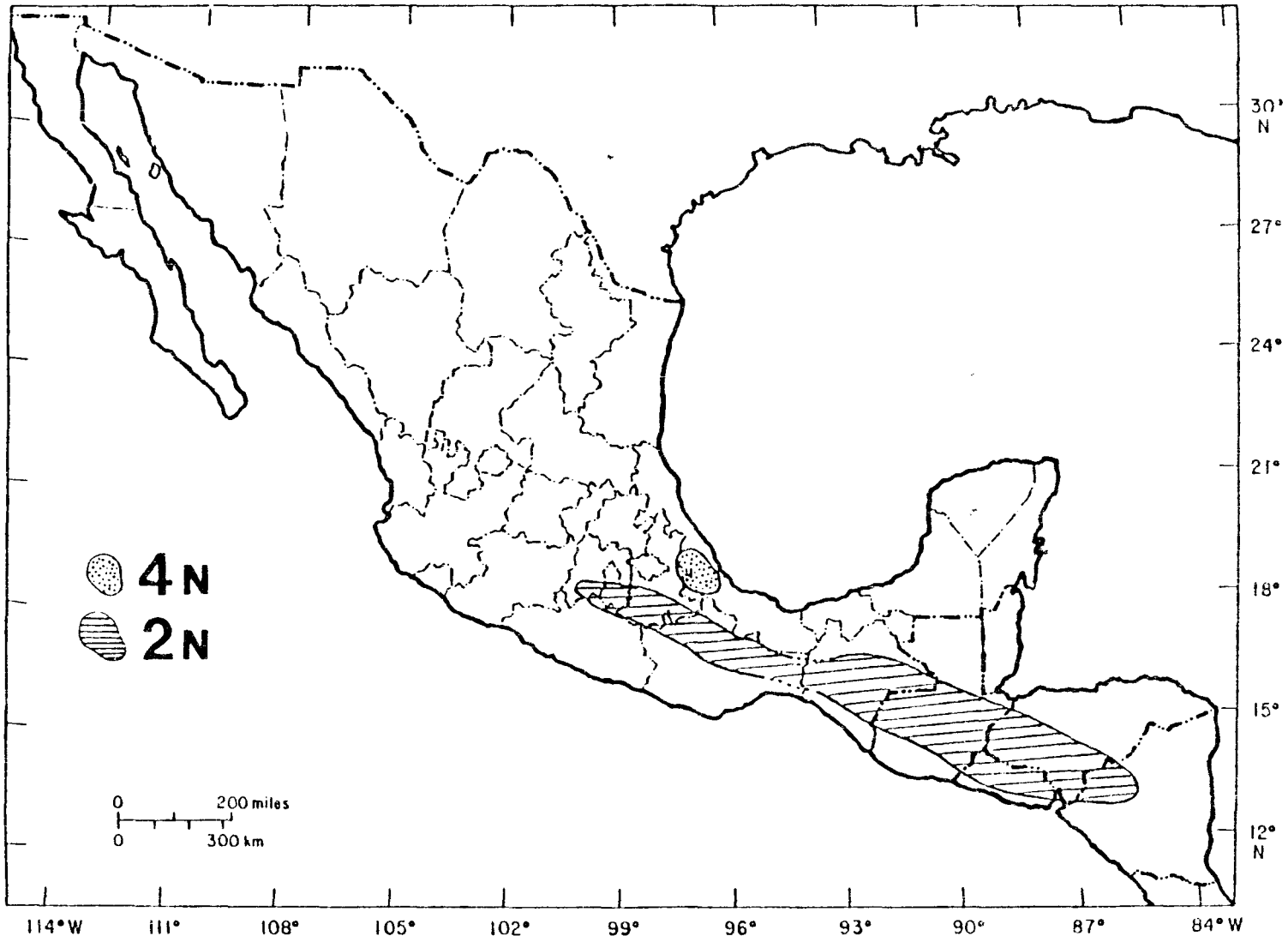

Fig. 54. Distribution of two cytotypes of Leucaena diversifolia.

Brewbaker, 1983). In contrast to the narrow distribution of tetraploids, diploids were very widely distributed, occurring from Oaxaca in southern Mexico to Nicaragua.

This investigation demonstrates the existences of a distinct gap in the distribution of diploids and tetraploids. This gap coincides with the Eastern Cordillera of Mexico, with mountains to 4500 m. Tetraploids and diploids appear to form distinct ecogeographic taxa, and it is reasonable to assume that gene exchange between them is very limited.

5.4. Discussion

The principal vegetative characteristics used to diagnose thirteen described species of the Leucaena diversifolia complex are given in Table 5. The taxonomy of these species has been based very largely on herbarium materials. To a great extent this was justified by only a few herbarium collections. For example, L. dugesiana Britton & Rose appears to be represented by a single specimen, its Type (US). Similarily, only two specimens of L. paniculata Britton & Rose were located.

When all of the morphological characters were lumped together, the Leucaena diversifolia complex could be easily resolved into three major groups, namely, DIV4N (tetraploids), PAL (L. pallida) and DIV2N (diploids). Due to its diversity in many characters, the third group may be subdivided into five small groups, corresponding to the

observed leaflet differences: glabrous, glossy, pilose, revolute and spotted types (Table 7).

The tetraploids, which were morphologically quite uniform, included three described species, Leucaena diversifolia (Schlecht.) Benth., L. brachycarpa Urban and L. laxifolia Urban. These three were recognized from other members of the complex by the following features: they all had pubescent young branches, rachis, and rachilla, glabrous leaflets and loose flower heads. The critical characters separating these three species were: (1) involucres (bracts) of L. brachycarpa Urban and L. laxifolia were appressed to the head of flowers, whereas involucres of L. diversifolia were distinct from the head of the flowers; (2) leaflets plicate-striate beneath were found in L. laxifolia Urban, but not in the other two species. The diagnostic characters used to distinguish them by Britton and Rose (1928) and Urban (1900), were separate involucral bracts and plicate (striped) leaflets. These features were observed in only one or two of ten arboretum plants in Hawaii. This suggests that these are minor differences resulting from intraspecific gene segregation. Therefore, they are treated here as a single species, L. diversifolia (Schlecht.) Benth.

The diploids were more variable than the tetraploids, as indicated by the standard deviation for most of the characters. A great deal of variation exists with respect to leaflet shape and size. The type specimens representing the

various synonyms of DIV2N differed essentially only in their leaves, especially in vesture which seems to have been exaggerated as a systematic tool. Most of the variability in vesture on leaflets, rachis or young twigs is between, rather than within, accessions. For example, the pilose leaflets were found primarily in the populations of Honduras and in the apparently disjunct populations of Western Guatemala, but they were also scattered through the range of the complex. The experimental hybridization analysis reported in a later chapter showed that vesture on young twigs and leaflets might be controlled only by one gene. Thus, if this is true, it is not a reasonable basis for the recognition of different species. Furthermore, vesture was observed to be variable even in the same population. For example, two types of leaflets were found in K409.

Leucaena pallida, L. dugesiana, L. oaxacana and L. paniculata were four species described by Britton and Rose (1928) in the same publication. L. pallida was characterized by its pubescent leaflets and long pods (12-16 cm long), L. dugesiana by its glandular peduncles, and L. oaxacana and L. paniculata by their panillate or plicate-striate leaflets. However, the accessions planted at the Waimanalo Research Station, designated as K174, K177, K178 and K376, collected from the same area, revealed all the characters mentioned above. Pubescent leaflets were found on one plant of K376 while the rest had glabrous leaflets. K174 had long pods

(12-22 cm long), while K178 had short ones (9-12 cm long). Plicate-striate leaflets were usually observed in the dry specimens of old leaflets. All accessions as well as specimens observed in the herbaria had more or fewer glands on peduncles. Consequently, these four species are here treated as one species, L. pallida Britton and Rose.

Two cytotypes of Leucaena diversifolia occupy different geographical areas, and there is sufficient genetic isolation between them to propose that they are already directed into distinct evolutional pathways which could lead to speciation. Stebbins (1950, 1970) indicated that most successful polyploids result from interspecific hybridization or from crossing between differently adapted populations within a single species. It seems apparent that tetraploids of Leucaena diversifolia arose from diploids having n = 26. De Harlan and De Wet (1975) indicated that almost all polyploids arose by way of unreduced gametes. Environmental stress, perhaps associated with steep climatic gradients, is known to influence the production of unreduced gametes (Grant, 1952). It could be hypothesized that the tetraploids of leucaena diversifolia arose from diploid hybrids in this fashion.

It seems plausible that tetraploids evolved initially at one or several localities in several populations. In conjunction with meiotic normalization, which effectively diploidized the tetraploid types, they could have spread to habitats distinct from the diploids. At first, however, the

two cytotypes might have been fully sympatric in some areas. As will be observed in Chapter 6, the two cytotypes appear to be highly cross-sterile.

CHAPTER 6

EXPERIMENTAL HYBRIDIZATION

An artificial self-pollination and hybridization program was established in an attempt to determine some of the genetic relationships among the species of the Leucaena diversifolia complex and some other species in the genus. Crossability, i.e., the yield of hybrid seed following experimental cross-pollination, fertility of hybrids and extent of chromosome pairing in the hybrids were determined.

As indicated by Rick et al. (1976), determination of genetic isolation should not be based only on tests of crossability as indicated by Rick et al. (1976). Even though seemingly good seeds are produced by the hybridization, other pitfalls to subsequent development and later generations might impede gene exchange between the parent plants (Rick et al, 1976). Seed production by F_1 plants reflects all phases of gamete formation, and should determine the reproductive success of a hybrid more directly than any single component process (Fahselt et al., 1976). Therefore, seed germination rate of F_1 hybrids was also measured.

In order to determine whether DIV4N and DIV2N were separated by reproductive barriers, selfing, backcrossing and sibcrossing of the F_1 hybrid of K409 (DIV2N) x K156 (DIV4N) was conducted.

Some morphological characterisics of F_1 and F_2

hybrids were observed to determine whether these characters can be considered diagnostic of species.

6.1. Floral Biology

The stamens and carpels in plants of the Leucaena diversifolia complex seem to mature at the same time. The anthers mature in the bud, force their way out of the small corolla, and dehisce immediately after stamens have straightened. Dehiscence of the anthers appeares to be affected by temperature. On cool, rainy days, dehiscence took place about one to two hours later than on sunny, hot days. Light yellow pollen is released through a longitudinal slit in each anther lobe. The styles straighten out much earlier than the stamens. The mature styles bear a cup-shaped apical stigma. A mass of pollen could be seen gathered in the cup-shaped stigma when flowers were being pollinated. Under normal field conditions, effective pollination was carried out exclusively by bees in Hawaii. Where native, these Leucaena species might be pollinated by a wide range of insects.

The chronology of flowering in two accessions of the Leucaena diversifolia complex, K156 of the tetraploids (DIV4N) and K409 of the diploids (DIV2N), was studied on November 23, 1984 (a day with about 11 hours and 50 minutes daylength). Tetraploids (Fig. 55) reached anthesis very early in the day, with florets starting to open on the

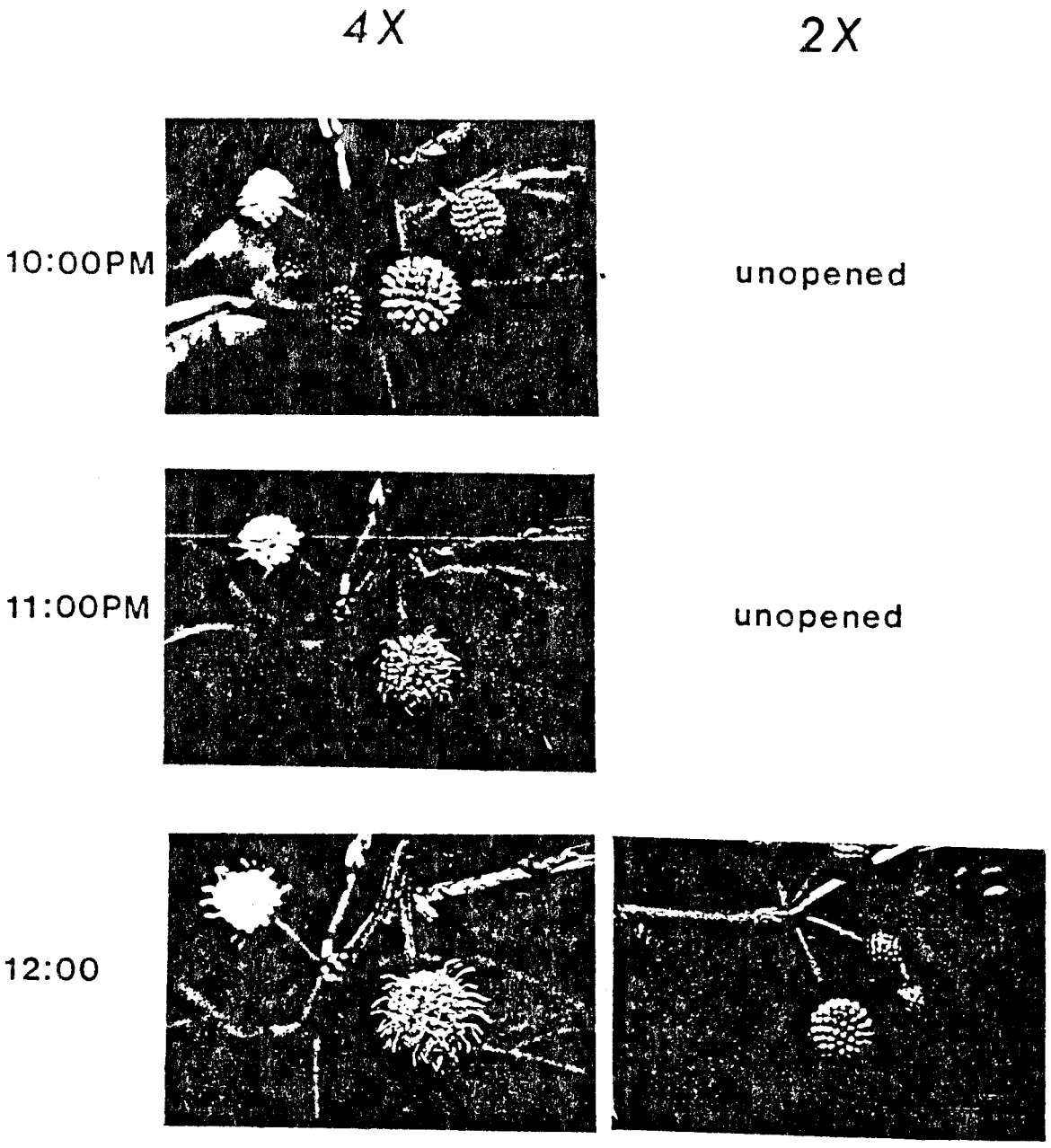

Fig. 55. Comparison of flowering sequences in tetraploid (K156) and diploid (K409) Leucaena diversifolia. Photos taken at November 23, 1984.

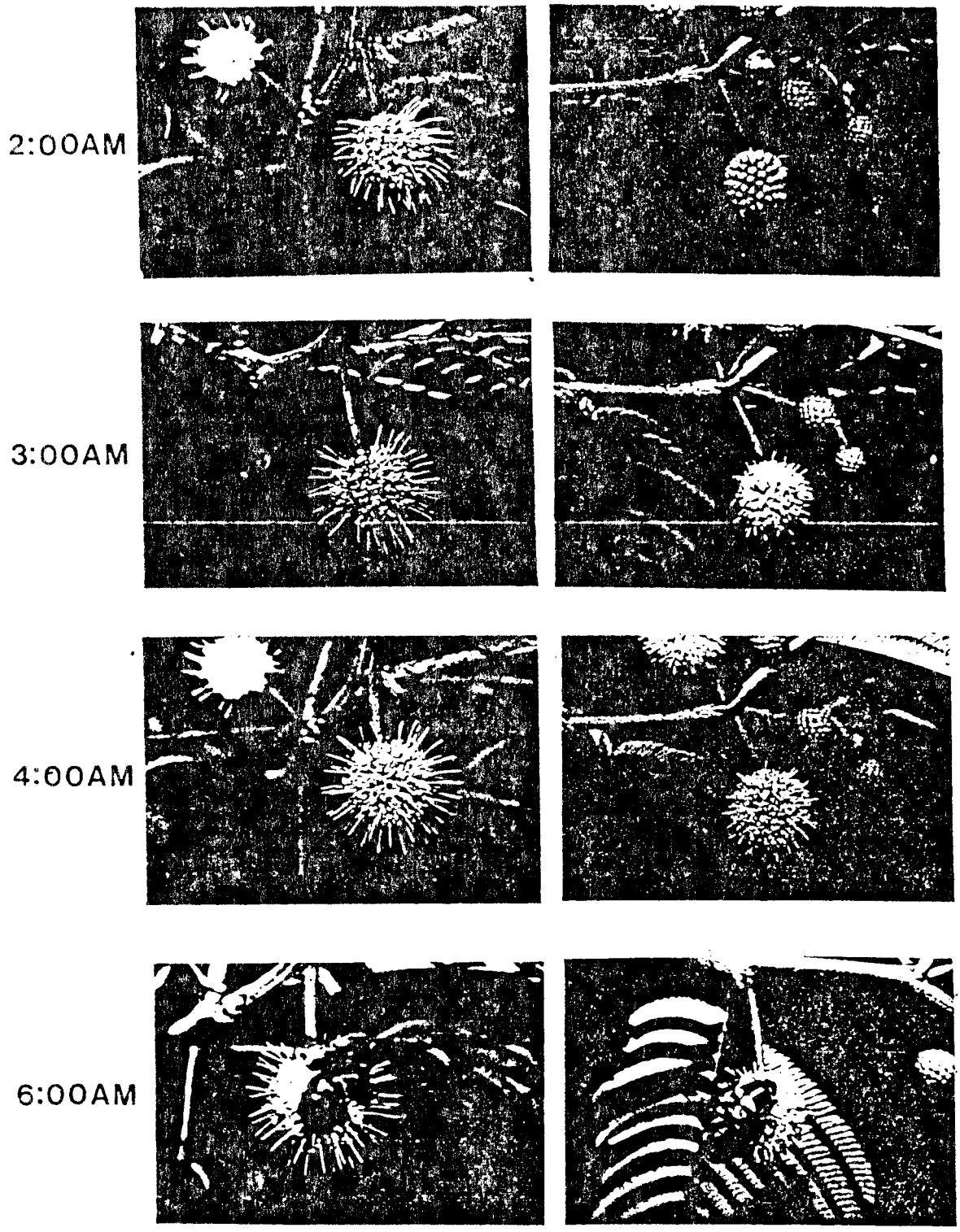

Fig. 55. (cont'd).

preceeding night at about 10:00PM. The first florets to open were the top ones on the head. By about 11:00PM, all florets had opened and some styles were stretched out. By midnight, almost all styles had emerged but were still recurved. One hour later, styles were almost completely straightened. Stamens were straightened out by about 4:00AM. At first, only a few stamens of the top florets were observed. Stamens were all raised and straightened by about 6:00AM. Pollen was shed immediately after the stamens straightened. AT about 6:10AM a swarm of bees were seen flying around the flowering plants (Fig. 55). Diploids started to open their florets two hours behind the tetraploids, at about midnight. Styles stretched out at 2:00AM, four hours later than in the tetraploids. However, emergence of the stamens of diploids proceeded very rapidly. At 4:00AM, the stamens were out, almost at the same time as tetraploids. By 6:00AM, the stamens also had straightened and were ready to shed pollen. Bees were observed to visit flower heads of both diploids and tetraploids at the same time.

The seasonal cycles of flowering of 26 accessions of the Leucaena diversifolia complex were observed from July, 1981 to December, 1983 (Fig. 56). Most DIV4N accessions started to flower in late April or early May and ended in October. Accessions of PAL (L. pallida) had a longer flowering period than DIV4N, and overlapped with the winter-flowering species, L. esculenta, which usually flowered from late November to

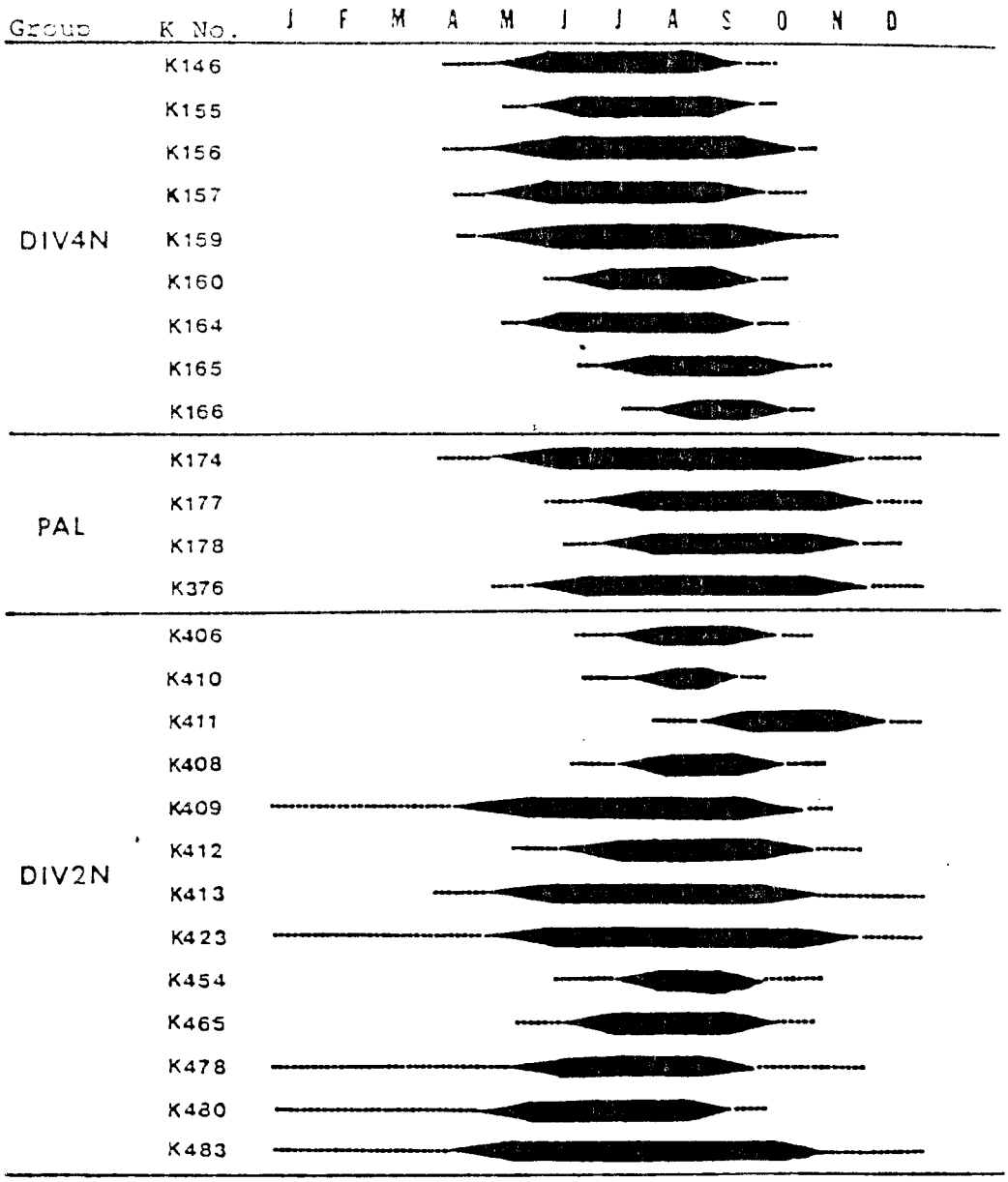

Fig. 56. Flowering cycles of 26 accessions of the Leucaena diversifolia complex observed in Waimanalo Research Station, Hawaii. Data recorded from July, 1981 to December, 1983. For a given bar the width reflects the number of flowers observed for that accessions; dotted lines indicate sproradic flowering.

February. The flowering period of DIV2N was variable, from three months (K410) to all year round (K423, K483). Most of them started to flower in different seasons. The overlapping flowering cycles of some accessions of the species provided an opportunity for them to cross with L. esculenta.

6.2. Self-compatibility

Self-pollinations were completed in 30 accessions of the Leucaena diversifolia complex (Table 14). Artificial selfing in DIV4N accessions resulted in an average seed production ranging from 37.5% to 100%, indicating self-compatibility. On the contrary, of some 226 self pollinations in DIV2N accessions there was no stimulation of pod elongation in any of fourteen accessions tested (Table 14). All plants tested in PAL appeared also to be highly self-sterile. One case of pod development under these conditions in K376 might have resulted from accidental introduction of pollen before bagging.

6.2.1. Mechanism of Self-incompatibility in Diploids of Leucaena diversifolia Complex

The cause of self-incompatibility of diploids whether due to inhibition of pollen-tube growth, or of embryonic development, or of still other stages in embryogeny, need to be investigated.

Table 14. Pod set resulting from self-pollinations in 27 accessions of the *Leucaena diversifolia* complex.

Group	Accession No.	No. heads selfed	No. heads producing pods	Freq. of head producing pods %
DIV4N	K146	16	6	37.5
	K155	6	3	50.0
	K156	31	14	45.1
	K157	2	2	100.0
	K159	2	2	100.0
	K160	24	10	41.7
	K164	10	5	50.0
	K165	15	8	60.0
	K166	2	1	50.0
DIV2N	K406	5	0	0
	K407	10	0	0
	K408	10	0	0
	K409	43	0	0
	K410	10	0	0
	K411	10	0	0
	K412	16	0	0
	K413	10	0	0
	K423	21	0	0
	K454	11	0	0
	K465	5	0	0
	K478	35	0	0
	K480	21	0	0
	K483	10	0	0
PAL	K174	10	0	0
	K177	10	0	0
	K178	10	0	0
	K376	37	1	3.0

The results obtained by intercrossing 20 plants of F_1 progenies of K409 x K480 are shown in Fig. 57. The plants fall into four groups, arbitrarily labelled from A to D. Crosses between plants within each group were unsuccessful. Crosses between plants placed in different groups were generally successful. Most crosses were made successfully in both directions, and there was no evidence of reciprocal differences in compatibility. Four plants, represented from compatibility groups, were selected to cross with one of the parents, K409. The crosses were also successful.

The interpretation of the result is that two parent plants, K409 and K480, differed in both S-alleles at a single incompatibility locus. Assuming parental genotypes to be S_1S_2 and S_3S_4; the four groups of plants established are S_1S_3, S_1S_4, S_2S_3 and S_2S_4 and all plants would be compatible with parents. The result indicates that the self-incompatibility is of gametophytic system.

6.3. Inter-group Crosses within the Complex

A crossing program was initiated in attempt to produce hybrids between the accessions of different groups as described in an earlier chapter . Plants were first divided into three major groups, DIV4N, PAL (L. pallida) and DIV2N. The latter group was subdivided into five types according to leaflet characters. These were designated as "spotted",

Group	F1 No.	A 2	6	23	25	44	B 11	12	27	34	C 10	14	19	21	29	38	D 3	23	26	35	48
	2	-	-	-	-	-	+		+		+			+	+			+		+	
A	6	-	-	-	-	-		+	+	+			+			+	+		+		
	23	-		-	-			+	+		+	+	+		+			+		+	
	25	-	-	-	-	-	+	+		+		+		+	+	+		+			+
	44	-	-	-	-	-			+		+	+		+	+		+		+	+	+
	11	+	+			+	-	-	-				+			+	+	+	+	+	+
B	12			+	+			-		-			+				+	+			+
	27	+	+				-	-	-	-	+	+					+			+	
	34		+		+		-	-	-	-			+			+		+			+
	10		+	+			+	+		+	-	-			-	-	+		+		
	14		+	+		+		+			-	-	-	-		-		+			+
C	19		+		+			+		-		-		-			+		+	+	
	21	+			+	+	+					-		-	-	-	+				
	29	+	+	+		+		+	+		-	-		-		-		+		+	+
	38			+	+					+	-		-	-		-	+	+			+
	3					+		+	+		+		+	+	+		-		-	-	-
	23						+		+			+	+		+	+		-		-	-
D	26	+	+		+	+		+	+	+	+	+	+	+		+	-		-		-
	35		+								+	+		+	+	+		-		-	-
	48	+			+			+		+		+		+		+	-	-	-	-	-
	K409		+				+				+				+				+		

Fig. 57. Results of intercrossing 20 F_1 hybrids of K409 x K480, and backcrossing of selected F_1 individuals to one parent (K409).
+ = compatible; - = incompatible; missing cells = cross not attempted.

"glossy", "pilose", "glabrous" and "revolute" (Table 7,Fig. 58).

The results of intercrosses among the 3 groups are summarized in Table 15 and portrayed graphically in Fig. 58. Five types of DIV2N accessions crossed freely among themselves. Crosses between individuals from a single accession were always successful.

Unidirectional incompatibility was found in crosses between DIV2N and DIV4N accessions. Seeds were set when DIV2N accessions were used as the female parent but no seeds were produced in the reciprocal combinations. DIV2N and PAL accessions did not cross. The latter was thus well isolated from the former by strong incompatibility barriers. However, all attempts to cross DIV4N and PAL accessions were successful (Table 15).

These data indicate that the Leucaena diversifolia complex contains, therefore, at least three groups, DIV2N (L. diversifolia 2N), DIV4N (L. diversifolia 4N) and PAL (L. pallida).

Hybrids of two extreme types of DIV2N, K409 representing glossy type and K480, the revolute type, were planted for further analyses.

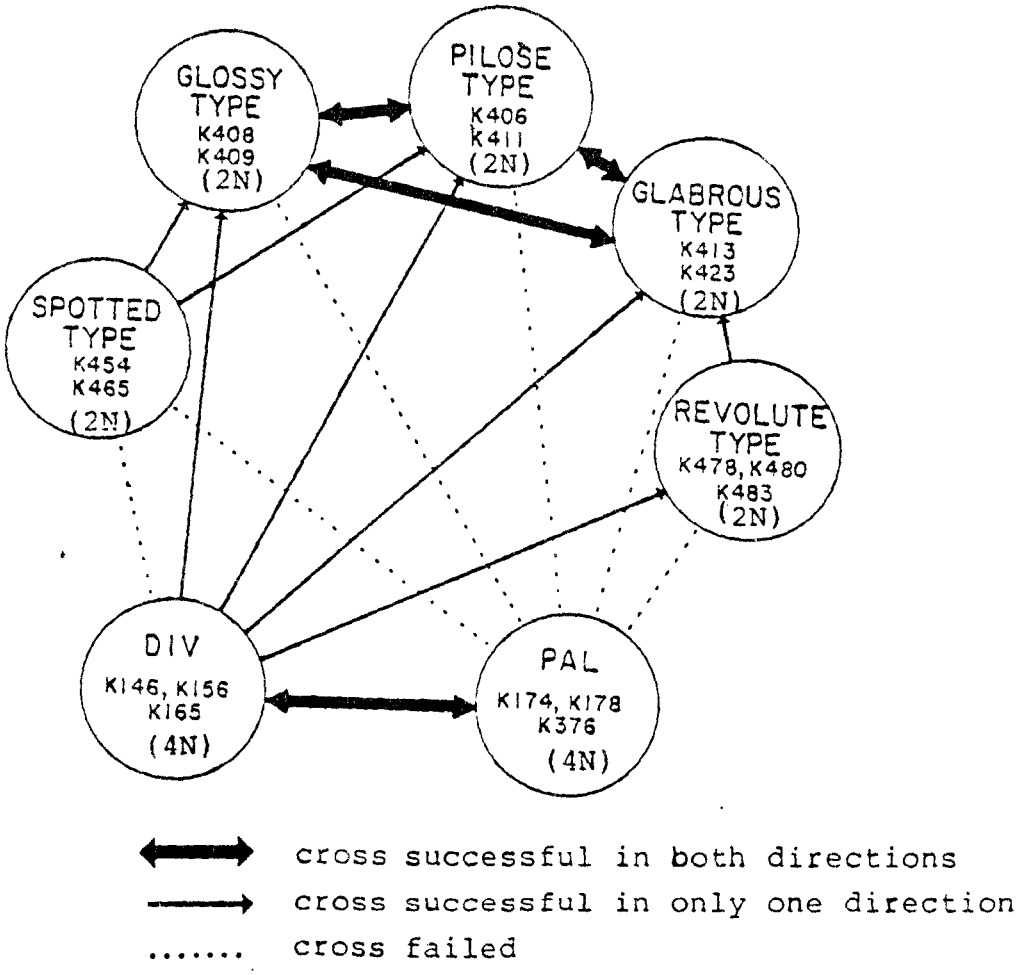

Fig. 58. Crossability based on pod set of inter-group and inter-group combinations of the Leucaena diversifolia complex. DIV = L. diversifolia; PAL = L. pallida.

Table 15. Seed set resulting from crosses between and within groups of the Leucaena diversifolia complex.

Parents	No. heads pollinated	No. head set seeds	No. pods set	Total seeds	$\bar{x}$ good seeds/pod
DIV2N (glossy) x DIV2N (glossy)	52	21	20	186	9.3
DIV2N (glossy) x DIV2N (revolute)	28	25	71	861	12.1
DIV2N (glossy) x DIV2N (glabrous)	12	8	12	187	15.6
DIV2N (glossy) x DIV2N (pilose)	34	21	43	556	13.0
DIV2N (glossy) x DIV2N (spotted)	6	3	4	8	2.0
DIV2N (glossy) x DIV4N	66	36	109	816	7.5
DIV2N (glossy) x PAL	40	0	0	0	0
*DIV2N (revolute) x DIV2N (revolute)	5	4	11	--	--
*DIV2N (revolute) x DIV2N (glossy)	10	2	--	--	--
*DIV2N (revolute) x DIV2N (glabrous)	10	1	--	--	--
*DIV2N (revolute) x DIV2N (pilose)	17	7	--	--	--
DIV2N (revolute) x DIV2N (spotted)	2	0	0	0	0
DIV2N (revolute) x DIV4N	15	0	0	0	0
DIV2N (revolute) x PAL	21	0	0	0	0
*DIV2N (glabrous) x DIV2N (glossy)	18	12	11	--	--
*DIV2N (glabrous) x DIV2N (pilose)	22	11	17	--	--
*DIV2N (glabrous) x DIV2N (revolute)	7	6	10	--	--
*DIV2N (glabrous) x DIV4N	19	11	--	--	--
DIV2N (glabrous) x PAL	0	0	0	0	
DIV2N (pilose) x DIV2N (pilose)	15	11	31	279	9.0
DIV2N (pilose) x DIV2N (glossy)	22	21	117	901	7.7
DIV2N (pilose) x DIV2N (revolute)	19	13	57	425	7.6
DIV2N (pilose) x DIV2N (glabrous)	20	15	36	379	10.5
*DIV2N (pilose) x DIV2N (spotted)	6	5	23	--	--
DIV2N (pilose) x DIV4N	17	15	79	206	2.6
DIV2N (pilose) x PAL	30	0	0	0	0
DIV4N x DIV4N	144	92	499	8013	16.0
DIV4N x DIV2N (glossy)	11	0	0	0	0
DIV4N x DIV2N (revolute)	10	0	0	0	0
DIV4N x DIV2N (glabrous)	9	0	0	0	0
DIV4N x DIV2N (pilose)	12	0	0	0	0
DIV4N x DIV2N (spotted)	11	0	0	0	0
DIV4N x PAL	51	25	85	1114	13.1
PAL x PAL	132	60	131	1840	14.0
PAL x DIV2N (glossy)	21	0	0	0	0
PAL x DIV2N (revolute)	11	0	0	0	0
PAL x DIV2N (pilose)	16	0	0	0	0
PAL x DIV2N (spotted)	7	0	0	0	0
PAL x DIV2N (glabrous)	10	0	0	0	0
PAL x DIV4N	60	29	57	884	15.5

* pods broken before mature.

6.4. Interspecific Crosses

DIV2N (n = 26) was crossed with *Leucaena lanceolata* (n = 26), *L. shannoni* (n = 26) and *L. pulverulenta* (n = 28) for studies of F_1 and advanced generations. Some seeds of DIV2N x *L. lanceolata* and DIV2N x *L. shannoni* were from previous crosses of Booman (unpublished). *Leucaena diversifolia* and *L. lanceolata* are morphologically different species, the former with tiny leaflets (3-5 mm long) and numerous pinna pairs (9-18) while the latter has large leaflets (25-50 mm) and fewer pairs of pinna (2-5) (Appendix 5). However, they could be crossed artificially with ease, and produced fertile F_1 and F_2 hybrids. The F_1 yielded nearly as many good seeds as the intraspecific hybrids (Tables 15,16). DIV2N also crossed freely with two other selected species, *L. shannoni* and *L. pulverulenta*, and produced good quantities of seed (Table 16).

Sixteen F_1 hybrids of DIV2N and *L. shannoni*, designated as B46, and 15 F_1 individuals of *L. diversifolia*, designated as B51 and B52, were grown to flowering.

6.4.1. Pollen Stainability in F_1's

The results revealed, as summarized in Table 17, that the hybrids between DIV2N and *L. shannoni* exhibited a high percentage (average of 98%) of pollen fertility, ranging from 95.5% good pollen to 100%. The hybrids between DIV2N and *L.*

Table 16. -- Fertility data based on pod sets of the crosses between the *Leucaena diversifolia* (2N) and three other species.

Hybrid combination	No. individual pollinated	No. heads pollinated	No. good pods	No. good seeds	x good seeds/pod
L. diversifolia(K409) x *L. lanceolata*(K401)	6	4	46	452	9.8
L. diversifolia(K409) x *L. shannoni*(K405)	5	3	28	199	7.1
L. diversifolia(K409) x *L. pulverulenta*(K75)	5	4	20	122	6.1

Table 17. Pollen fertility of F1 hybrids with the Leucaena diversifolia complex and between the L. diversifolia complex and other species.

Cross	No. individual observed	No. heads counted	Pollen stainability % Range	Pollen stainability % x ± s.d.
L. diversifolia(K409) x L. diversifolia(K480)	5	25	84.0-100.0	93.8± 4.9
L. diversifolia(K409) x L. diversifolia(K156)	5	25	1.5- 41.1	16.2±13.0
L. diversifolia(K409) x L. shannoni(K405)	5	25	95.5-100.0	98.0± 1.4
L. diversifolia(K409) x L. lanceolata(K401)	5	25	82.3- 97.1	94.0± 3.6
*L. diversifolia (K409, 2N)	2	10	96.3-100.0	98.2± 2.3
*L. lanceolata(K401)	2	10	95.0-100.0	97.5± 1.7
*L. shannoni(K405)	2	10	93.6- 99.8	96.7± 1.3
*L. diversifolia (K480, 2N)	2	10	94.2- 98.8	95.8± 2.1

*Plants cultivated from the original seeds collected by Dr. Brewbaker.

lanceolata also revealed a high percentage of good pollen (94.0% average) ranging from 82.3% to 97.1%. These results were as high as those intraspecific hybrids which showed mean of 93.8% to 100% good pollen. In general, therefore, the F_1 hybrids were as vigorous reproductively as the parental plants. The triploid hybrids of DIV2N by DIV4N, however, were highly pollen-sterile.

6.4.2. Seed Germination and Seedling Vigor of F_1's

Seeds of three hybrid combinations were collected at maturity and the number of "good" seeds was evaluated. Seeds scored visually as good invariably contained embryos and cotyledons and germinated readily. Crosses within species regularly produced about 10 good seeds per pod and interspecific crosses produced 6-7 good seeds per pod.

From 62 - 82% of the F seeds germinated and grew without difficulty (Table 18). Seeds from crosses between two DIV2N accessions (K409 and K480) had an average germination of 82%, compared with two parents of 93% and 91% (Table 18). Seeds from crosses of K409 and L. shannoni, and from K409 and L. lanceolata showed average germination of 71% and 76%, respectively. Their parents L. shannoni (405) and L. lanceolata (K401) of 98% and 96%, respectively. All of these F_1 hybrids showed highly vigorous growth.

Table 18. Seed germination rate and seedling vigor of F_1 hybrids and their parents. DIV = Leucaena diversifolia; LAN = L. lanceolata; PUL = L. pulverulenta; SHA = L. shannoni.

	Germination rate (N = 200)	*Seedling vigor
**(B85) K409 x K480	82%	vigorous
**(B46) K409 x K405	78%	vigorous
**(B51) K409 x K401	71%	vigorous
K409 x K75	62%	unknown
K409 (DIV,2N)	93%	vigorous
K480 (DIV,2N)	91%	vigorous
K405 (SHA)	98%	vigorous
K401 (LAN)	96%	vigorous
K75 (PUL)	95%	vigorous

* Seedling vigor estimated by subjective valuation based on growth parameters including height, growth rate and canopy development.

** Some seeds from crosses made by James Booman.

6.5. Observation of the F_2 Generation

Diploid species of the genus Leucaena are all self-incompatible (Brewbaker, 1983; Sorensson, 1984) and yield self-incompatible F_1 progenies. F_2 plants were produced by sibcrossing the F_1's in each combination made. In this study, the F_2 generations of K409 x K480 (intraspecific) and of K409 x K405 and K409 x K401 (interspecific) were grown at Waimanalo Research Station. The plants were periodically irrigated. Eighty-eight of 100 seeds of the F_2 of K409 x K480 were germinated. Among these 88 seedlings, 82 persisted for further study. These surviving seedlings were vigorous and uniform in growth habit. In the cross of DIV2N (K409) x L. shannoni (K405), 46 out of 100 seeds were germinated. Of these 46 germinated seeds, only 25 seedlings survived. In the cross of DIV2N (K409) x L. lanceolata (K401), 147 of 200 seeds were germinated and 103 seedlings survived. However, the surviving F_2 plants of the latter two interspecific hybrids were not uniform in development. They ranged from weak to as vigorous as their parents, and the height of six-month-old seedlings ranged from 4 cm to 200 cm. Some of these seedlings would have died under normal or drought conditions.

6.6. Progeny Analysis of Hybrids between DIV2N and DIV4N

K156 is an accession of DIV4N (n = 52), with large

inflorescences and pubescent young stems; K409 is an accession of DIV2N (n = 26), with small inflorescences, and glossy and glabrous young stems. The F_1 generations of these two, designated as B42, was made by Booman in 1981. Twelve plants of the hybrids were grown to flowering. These hybrids appeared to be triploids. These hybrids resembled their tetraploid parent in almost all morphological characteristics such as inflorescence size, pubescence on twigs etc.

No pods were obtained from all the crosses of K409 (DIV2N) x K156 (DIV4N) (Table 19). Thus the diploid and tetraploid were separated by the formation of sterile triploids.

The F_1 hybrids of K409 x K156 produced a high percentage of abortive pollen (Table 17). The mean pollen fertility was 16.2% and ranged from 1.5% to 41.1%. The size of the hybrid pollen grains varied greatly and viability was estimated at 13%.

6.7. Inheritance of Some Morphological Characters

6.7.1. Morphological Characteristics of the F_1 Hybrids

The F_1 hybrids were intermediate between the parental plants in some characteristics such as petiolar gland type, number of pinnae per leaf, number of leaflets per pinna, size of leaflets, floret number per inflorescence and seed shape and size etc. Figs. 59, 60 show the intermediacy of leaves,

Table 19. Crosses involving F_1 plants from K409 (DIV2N, female) x K156 (DIV4N, male).

Crosses	Flower heads pollinated	Pod sets
Bagged	42	0
Selfed	36	0
Sib-cross	54	0
Backcrosses		
to K409 (2N)	46	0
to K156 (4N)	28	0

seeds and petiolar glands of the F_1s, from the crosses of DIV2N (K409) X L. lanceolata (K401) and DIV2N (K409) X L. shannoni (K405). The average numbers of leaf parts for twenty leaves on five individual plants of F_1 and their parental plants are given in appendixes 4 and 5.

Characters such as vesture nature of twigs and leaflets, odor of flower, pendulous inflorescences, vesture nature of ovaries and pods, and growth habit seemed to be under single genetic control. In all organs mentioned, pubescence was dominant and glabrous recessive (Table 20). Flowers of L. shannoni had a strong odor of Johnson's baby powder, while no odor was observed in DIV2N. F_1 hybrids of these two species revealed the strong odor. Pendulous inflorescences were found in K409 (DIV2N). When crossed with common type K480 (DIV2N), with upright inflorescences, the F_1 hybrids showed only pendulous inflorescences. DIV4N (K156) with straight stems crossed with the shrubby, branching DIV2N (K409), and DIV4N (K156) with straight stems crossed with branching PAL (K376) produced straight stem F_1 hybrids. These results suggest that pendulous-inflorescences and straight-stems are dominant.

Flower color varied greatly among DIV2N accessions. In the 45 F_1 hybrids between and K409 and K480, 10 plants were like K409, with very light pink flower heads, 16 were like K480, with dark pink flowers, the others with a mixture of both parental types -- light or dark pink on anthers,

Fig. 59. Leaves, pods and seeds of Leucaena lanceolata (K401) (left), L. diversifolia (K409,2N) (right) and their F_1 hybrid (middle). Ruler in right side of upper figure = 15 cm.

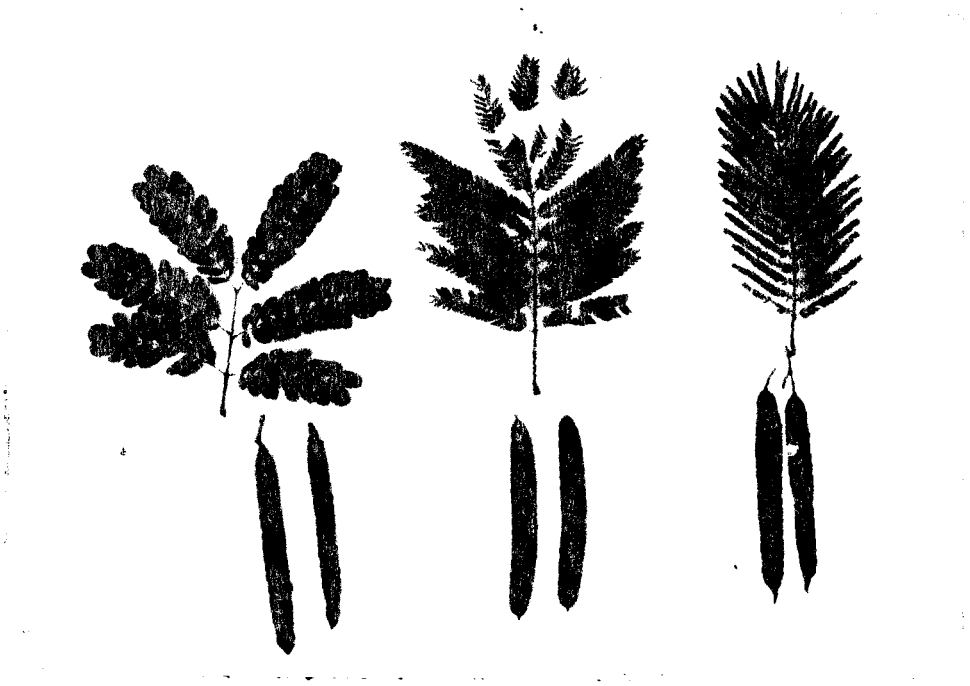

Fig. 60. Leaves and pods of Leucaena shannoni (K405)(left), L. diversifolia (K409,2N)(right) and their F_1 hybrid (middle). Ruler in right side = 15 cm.

Table 20. Morphological characters in Leucaena diversifolia (2N), L. shannoni (K405), L. lanceolata (K401) and selected F_1 hybrids.

Category	Vesture*			Petiolar glands	Leaflet margins	Peduncles	Flower** color
	Leaflet	Twig	Pod				
K409 (L. diversifolia)	G	G	G	concave	flat	pendulous	LP
K409 x K480	Pi	P	G	concave	flat	pendulous	W, LP and P
K480 (L. diversifolia)	Pi	P	G	concave	revolute	upright	P
K405 (L. shannoni)	P	P	P	convex	flat	upright	W
K409 x K405	P	P	P	intermediate	flat	pendulous	W, LP
K401 (L. lanceolata)	P	P	G	convex	flat	upright	W
K409 x K401	P	P	G	intermediate	flat	pendulous	W, LP

* Vesture: G = glabrous; P = pubescent; Pi = pilose.

** Flower color: LP = light pink; P = pink; W = white.

filaments or styles (Appendix 6). This suggests that some individuals in the natural populations are heterozygous for the gene(s) controlling this character.

6.7.2. F_2 Segregation of Some Morphological Characters

The F_2 generation was studied at the age of only six months. Segregation could be observed for the following morphological characters.

a. Leaflet vesture

Plants of K480 (DIV2N) with pilose leaflets were crossed with plants of K409 (DIV2N) with glabrous leaflets. Their F_1 had pilosity on the leaflets. The F_2 segregated 57 trees with pilose leaflets and 25 with glabrous leaflets (Appendix 7). The backcrosses of the F_1 hybrids to the glabrous parent, K409, produced a ratio of 12 pilose to 8 glabrous individuals (Appendix 8). These data suggest that leaflet pubescence is controlled by one gene with 2 alleles, and that pubescence is dominant over glabrousness.

b. Leaflet size

The leaflet is about 24 mm long and 10 to 15 mm wide in Leucaena lanceolata and about 3 - 5 mm long and 0.5 mm wide in K409 (DIV2N) and intermediate in the F_1 hybrids (Table 21). In the F_2 generation of 75 individuals leaflet length ranged from 5 - 17 mm (Table 21, Appendix 9). A smaller F_2

Table 21. Range of leaflet length in parents and F_1s and frequency distribution of F_2 and backcross progenies of *Leucaena diversifolia* (K409,2N) x *L. lanceolata* (K401).

		Leaflet length (mm)																	N
		3-4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	25-50	
P_1	*L. diversifolia*	+																	
P_2	*L. lanceolata*																	+	
F_1									+	+	+	+	+						
F_2			3	3	11	13	11	13	8	6	2	3	0	1	1				75
Backcross to K409				1	6	4	3	3	1										18

population of DIV2N (K409) x L. shannoni (K405), gave similar results (Table 22, Appendix 10). Leaflet size, therefore, appears to be controlled by several gene loci having variable expressivity. Backcrosses of F_1's to both parents revealed typical quantitative inheritance patterns for this feature (Appendixes 11,12).

c. Gland type

The genetic control of petiolar gland type was studied in a cross between plants of K409 (DIV2N) with concave glands and plants of K401 (L. lanceolata) with convex glands (Fig. 61). The F_1 progenies of 17 individuals uniformly possessed glands of intermediate type. The F_2 progeny contained 8 plands with concave glands, 50 with intemediate ones and 17 with convex ones (Appendix 9). The 18 individuals of the backross progeny of the F_1 hybrid to K409 yielded 6 intermediate and 12 parental types (Appendix 11), suggesting that the petiolar gland type is controlled by two or more loci.

d. Vesture of young stems, rachis and rachilla

Vesture of young stem, rachis and rachilla seem to be under the control of the same linked genes. All three organs were observed to be either pubescent or glabrous and other combinations of pubescence on these organs were not found. The genetic control of vesture of these organs was studied in a cross between glabrous type of K409 (DIV2N) and a densely

Table 22. Range of leaflet length inparent and F_1 and frequency distribution of F_2 and backcross progenies ot Leucaena diversifolia (K409, 2N) x L. shannoni (K405):

	Leaflet length (mm)												N
	3-4	5	6	7	8	9	10	11	12	13	14	15-20	
P_1 L. diversifolia	+												
P_2 L. shannoni												+	
F1					+	+	+	+	+				
F2	1	7	9	6	2								25
Backcross to K409		7	7	6									20
Backcross to K405							1	4	3	2	1		10

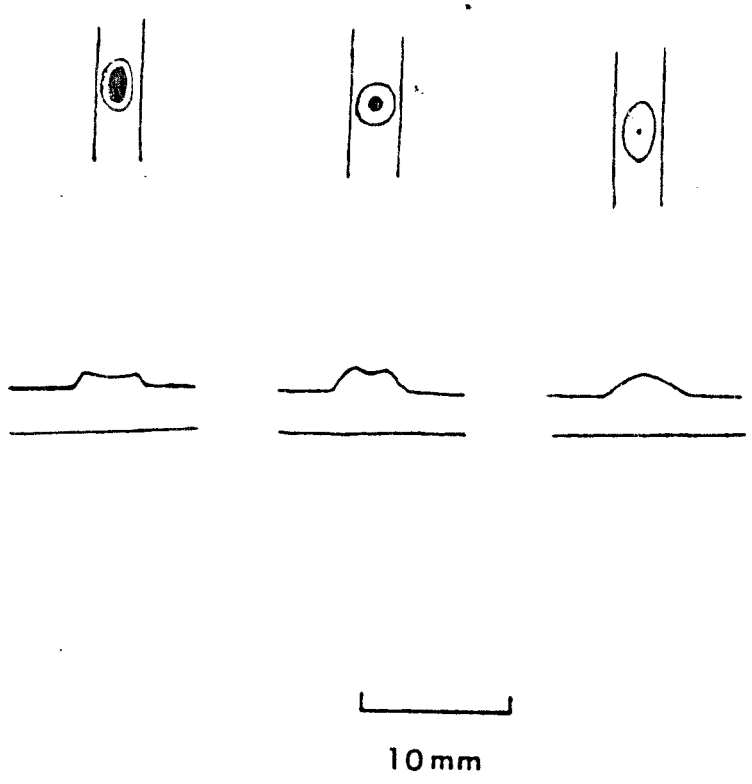

Fig. 61. Petiolar glands of Leucaena diversifolia (K409) (left), L. lanceolata (K401) (right), and their F_1 hybrid (middle).

pilose type of K480 (DIV2N). The 46 F_1 hybrids showed uniform pilosity on these organs. In the F_2 generation of 82 plants, 56 were densely pilose and 26 were glabrous (Appendix 7). The backcross of F_1 hybrids to the glabrous parent showed a 1 : 1 ratio of densely pilose and glabrous types (Appendix 8). The results also indicate that a single gene controls vesture of each of the three organs (Appendix 7).

e. Leaflet number per pinna

K409 (DIV2N) had 33-54 pairs of leaflets per pinna, K401 (Leucaena lanceolata) 4-6 pairs, and K405 (L. shannoni) 7-10 pairs. F_1 hybrids of K409 X K401 and K409 X K405 were intermediate between their parents, with 10 to 18 pairs and 21 to 31 pairs of leaflets, respectively (Tables 23 and 24). The F_2 progenies of both hybrids ranged between their parents (Tables 23 and 24). Thus the numbers of leaflet pairs also seem to be controlled by several gene loci.

f. Pinna number per leaf

The number of pinna pairs per leaf of DIV2N (K409) is 12 to 18, Leucaena lanceolata (K401) is 2 to 5, and L. shannoni (S405) is 3 to 7. The pinna number per leaf were studied in the hybrids of DIV2N x L. lanceolata nd DIV2N x L. shannoni. The F_1 of these crosses were intermediate to the parents (Tables 25, 26). No further investigation of this character was done after the F_1 generation. It is assumed that this character is also controlled by several genes.

Table 23. Range of leaflet pairs per pinna in parents and F_1s and frequency distribution of F_2 and backcross progenies of *Leucaena diversifolia* (K409,2N) x *L. lanceolata* (K401).

		Pairs of leaflet per pinna														N
		4-6	7	8	10	12	14	16	18	20	22	24	26	28	30	33-54	
P_1	*L. diversifolia*															+	
P_2	*L. lanceolata*	+															
F_1					+	+	+	+	+								
F_2		2	4	3	17	13	14	12	4	4	0	0	1	1			75
Backcross to K409									4	5	5	1	2	1			18

Table 24. Range of leaflet pairs per pinna in parents and F_1 s and frequency distribution of F_2 and backcross progenies of *Leucaena diversifolia* (K409,2N) x *L. shannoni* (K405).

		Pairs of leaflet per pinna														N
		7-10	11	12	13	15	17	19	21	23	25	27	29	31	33-54	
P_1	*L. diversifolia*														+	
P_2	*L. shannoni*	+														
F_1									+		+	+	+	+		
F_2		2	4	4	5	7		1		1		7	.			31
Backcross to K409						1	2	4	3	4	3	1	1			20
Backcross to K405		3	5	2												10

6.8. Discussion

It is worth noting that diploid species of Leucaena possess a gametophytic self-incompatibility system which makes them obligate outcrossers. This outbreeding system ensures that gene flow will take place through large populations. Self-incompatibility seems to have been responsible for the maintenance of a high degree of intraspecific variability. It also provides the basis for rapid evolutionary change as suggested by Hawkes (1972) in his study of Solanum.

The genetical differences between the taxa of the Leucaena diversifolia complex were first studied through crossing experiments. The crossing program and the breeding systems revealed that the complex comprises three groups, DIV4N, DIV2N and PAL, in accordance with the result of morphological studies. The five subgroups of the second group, DIV2N, were highly intercrossable. Two morphologically different subgroups, K409 and K480, respresenting glossy and pilose types, respectively, were studied further. Not only did they cross freely, but also produced uniform fertile and vigorous F_1 hybrids. The F_2 progenies were also vigorous and uniform in growth rate, based on the observation of 82 six-month-old seedlings. These data indicate that at least two of the five subgroups

Table 25. Range in the number of pinnae per leaf for Leucaena diversifolia (K409,2N) x L. lanceolata (K401) and their F_1 hybrid.

	Number of pinnae per leaf																
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
P_1 L. diversifolia											+	+	+	+	+	+	+
P_2 L. lanceolata	+	+	+	+													
F_1				+	+	+	+	+									

Table 26. Range in the number of pinnae per leaf for Leucaena diversifolia (K409,2N) x L. shannoni (K405) and their F_1 hybrid.

	Number of pinnae per leaf																
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
P_1 L. diversifolia										+	+	+	+	+	+	+	
P2 L. shannoni	+	+	+	+	+												
Fl						+	+	+	+	+							

of DIV2N are not separated by discernable genetic barriers. All five of the subgroups are here considered to constitute a single subspecies.

Leucaena species are separated spatially by mountains, or are adapted to a rather clearly defined elevational range. However, artificial hybrids were generally very easy to produce. The F_1 plants were quite fertile, with regular bivalent pairing at meiosis and quite abundant seed production. This may be used to argue that such species are not "good" or "valid", since they are not separated by sterility barriers of the usual type. However, another point should be considered.

F_2 hybrids resulting from F_1 sib-crossings of DIV2N x L. lanceolata and DIV2N x L. shannoni in this study, showed genetic breakdown, with seedlings ranging from inviable or poorly developing plants to ones as vigorous as the parents. This seems to be the evidence of a difference in the genetic background of these species, such that blocks of genes connot substitute for each other during the recombination of F_2 segregation to provide vigorous individuals. Moreover, Stebbins (1945) postulated that chromosomes may evolve by cryptic structural changes too small to prevent normal chromosome pairing in F_1 hybrids. Nevertheless, these differences may have serious genetic consequences in the F_2 and later generations. Cryptic interspecific genomic differences which were undetected in F_1 hybrids but which

produced visible effects in F_2 progenies were also demonstrated by Swaminathan (1953). The species of Leucaena investigated are sufficiently close genetically to allow almost free exchange of genes. However, they are also reasonably morphologically differentiated and there are genic and/or cryptic chromosomal differences between them, in addition to definitive morphological distinctions.

Natural hybridization was reported between Leucaena pulverulenta and L. leucocephala, and between L. diversifolia and L. leucocephala (Gonzalez et al., 1967; Brewbaker, 1982). Spontaneous hybridization is very likely between species which are sympatric or contiguous. From recombination between differentiated forms, a wide array of genotypes are expected to arise. Anderson hypothesized (1949) that any natural or man-made change of environment which results in a blurring of the natural habitats may bring together species previously isolated ecologically and allow them to hybridize. With intermediate habitats available, hybrids which previously no apporpriate ecological niche might be able to survive. Such disturbances may be responsible for taxonomic problems in other species of Leucaena as suggested by Brewbaker (1982).

CHAPTER 7

ISOZYME POLYMORPHISM

Of the five emzyme systems examined, only peroxidase exhibited clear and interpretable bands for studies. No isozyme bands of leucine aminopeptidase (LAP) were observed in any seedling tissue or in dry seeds. Acid phosphortase showed faint and blurred bands only in seedling roots. Six to fifteen blurred bands of esterase were seen in dry seeds and seedling roots. They seemed to be unstable in tissues of Leucaena species. Malate dehydrogenase (MDH) showed dense bands in 2-4 day cotyledons but faint bands in other seedling tissues. These bands were blurred in areas of Rf 45-60. Five taxa, including Leucaena leucocephala (K8), L. pulverulenta (K19), L. pallida (K177 and K376), DIV4N and DIV2N, were observed for this emzyme system. The isozymes seemed to be monomorphic in all taxa examined.

7.1. Peroxidase Isozymic Polymorphism

7.1.1. Peroxidase Polymorphism in Leucaena Tissues

The isozymes of peroxidase were temporarily marked with Rf values which were calculated by dividing the distance the enzyme bands migrated by the distance a standard marker dye migrated.

Thirteen peroxidase isozymes were observed in the mature

plants and seedlings of Leucaena leucocephala. Ten moved to the anode and three to the cathode in acrylamide gels (Fig. 62). Eight of the anodal isozymes were observed in mature plants as well as in seedling tissues. The cathodic isozymes were only found in seedlings. These were less consistently well defined than anodal ones. In starch gels, ten isozymes were detected, six anodal and four cathodic bands (Fig. 63).

Rf40 and 36 were intensely stained isozyme bands and were found in all tissues observed. These two bands always appeared together in both acrylamide and starch gels. Rf45 appeared in most tissues, except pods in acrylamide gels, and cotyledons, embryo and pollen in both acrylamide and starch gels. Rf32 was occasionally found in seedling tissues such as leaves and roots in the acrylamide gels, but was not detected in starch gels. Rf28 occurred in cotyledons, embryo, rachilla, bark, stems and old leaves. It was intensely stained in older tissues but lightly stained in young tissues. Rf22 was found only in old tissues, endosperm and pollen, and was a weakly stained band. Rf18 and 12 also occurred in most tissues in acrylamide gels. Rf10 was weakly stained isozyme and observed only in the perianth in acrylamide gels. Rf8 was detected only in rachilla and bark tissues.

Cathodic isozymes were observed more clearly in starch gels in both mature plants and seedling tissues. In acrylamide gels, only seedling roots exhibited cathodic

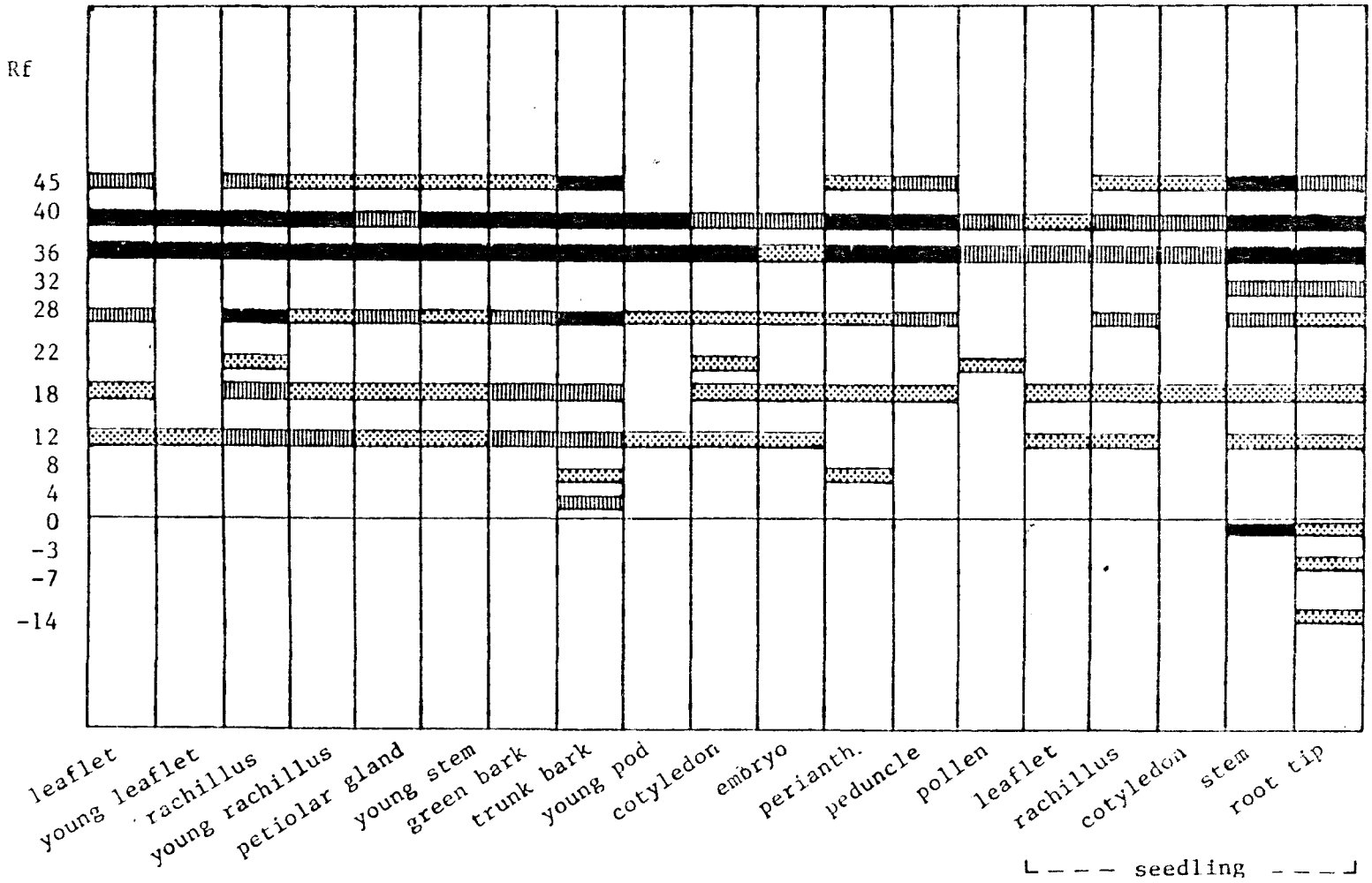

Fig. 62. Peroxidase isozymes of 19 tissues of Leucaena leucocephala (K8) in 6% acrylamide gel at pH 8.1. Intensity rank: strong ▬, medium ▥, weak ▩.

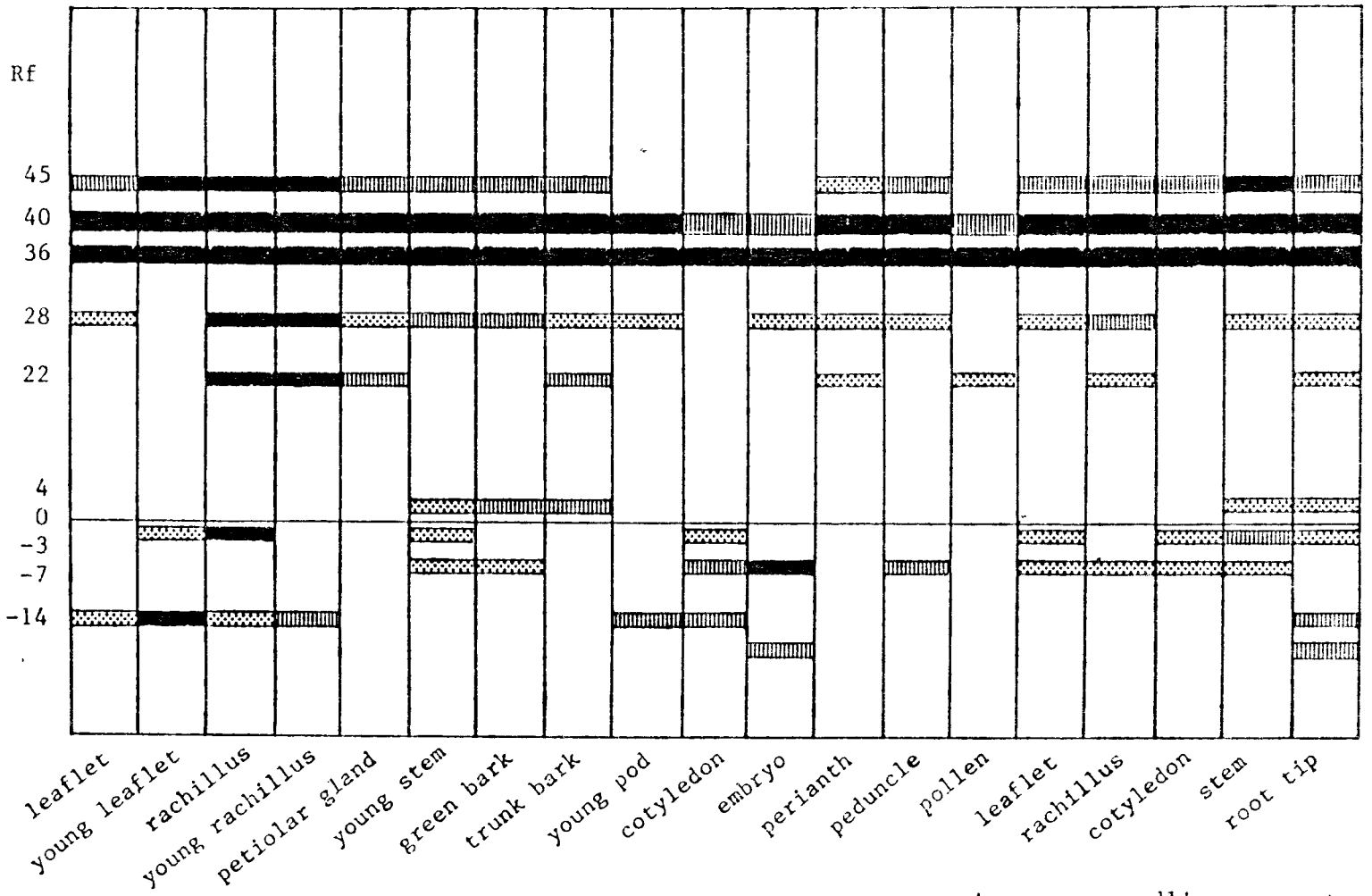

Fig. 63. Peroxidase isozymes of 19 tissues of Leucaena leucocephala (K8) in 12% starch gel. Legend the same as Fig. 62.

bands. Of the bands seen on acrylamide gels, Rf18, Rf12 and Rf8 were not observed in starch gels.

7.1.2. Isozyme Differentiation and Tissue Specifity

Bands Rf40 and 36 were observed in all tissues studied. Most other peroxidase isozymes appeared to be tissue specific. The analysis also showed clearly that some peroxidase isozymes and their intensity in corresponding tissues were specific to maturity or developmental stage. Leaves were analyzed in a sequential manner from base to top of the mature plant to study ontogenetic isozyme differentiation.

Six stages of leaves were obtained from the same tree of Leucaena leucocephala (K8). Middle leaflets of middle pinnae were sampled. The result revealed a gradual increase in peroxidase isozyme polymorphism with increasing maturity (Fig. 64). The same phenomenon was observed in starch gels.

All stem tissues showed almost similar peroxidase isozyme patterns, with slight quantitative changes. The exceptions were bands Rf8 and Rf4, which occurred only in bark. In acrylamide gels, bands Rf45, Rf28, and Rf12 changed intensity from young to old tissues. Rf4 occurred only in stem tissues in starch gels.

The rachilla tissues and gland had isozymes similar to those of stem tissues, except that the rachilla tissues had

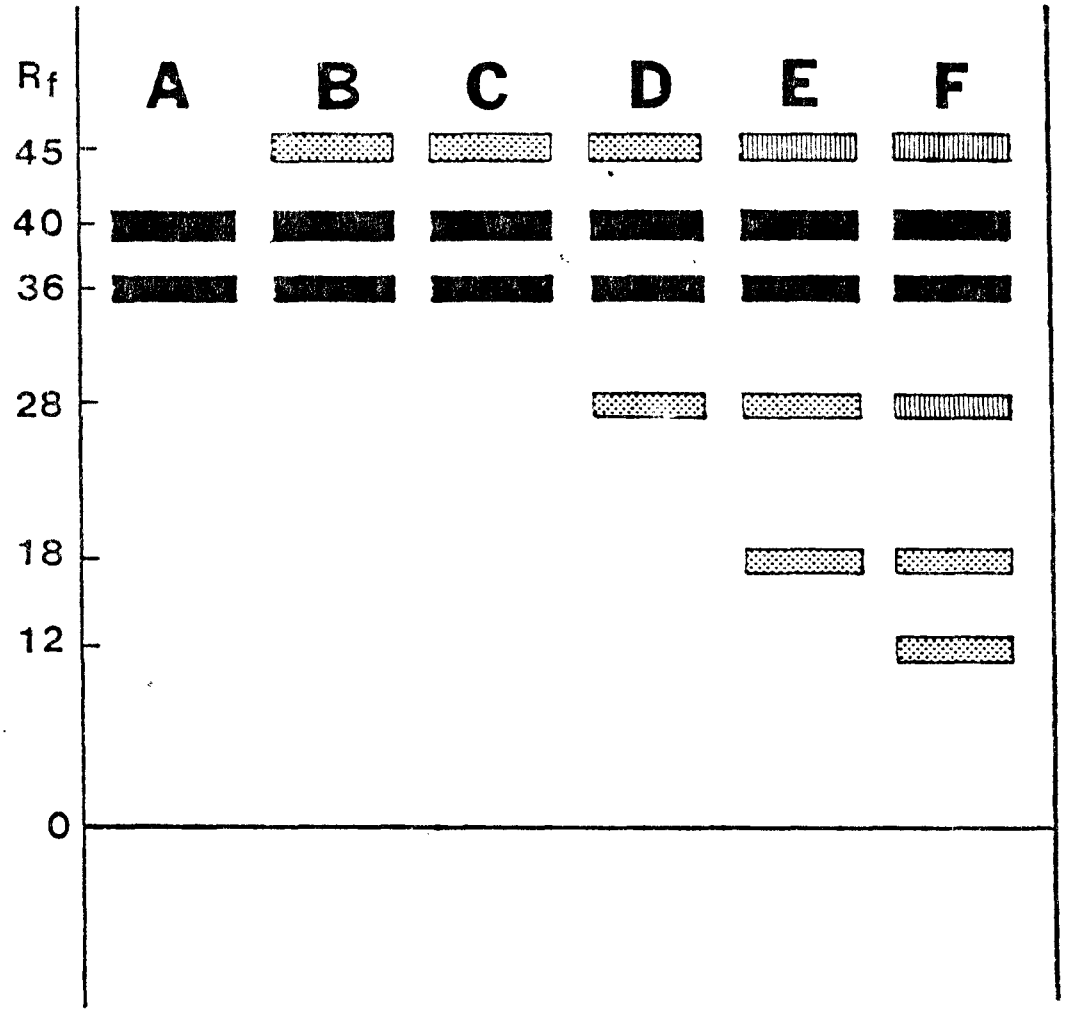

Fig. 64. Peroxidase isozyme patterns of the leaflets of Leucaena leucocephala (K8) taken from top (A) to base (F) of one branch in 6% acrylamide gel at pH 8.1.

band 22. Both quantitative and qualitative changes were observed between young and old tissues.

The cotyledon and embryo of leucana seeds and pollen were the only tissues lacking band Rf45. Flower tissues had similar band patterns.

Seedling roots possessed most bands in both acrylamide and starch gels. Almost all peroxidase isozymes of Leucaena leucocephala detected in acrylamide gels appeared in seedling roots, but faded during maturation.

Based on this study, it can be seen that several peroxidase isozymes of Leucaena species, such as Rf45 and Rf28, have played an important role, possibly in lignification, maturation or senescence activity like peroxidase isozymes in corn shoots and roots reported by Brewbaker and Hasegawa (1975). The same results were also reported in leaf peroxidase isozymes by Racusen and Foote (1966) and in leaf and internode peroxidase isozymes of cottonwood by Gordon (1971).

7.2. Genetic Control of Isozyme Polymorphism

Isozymes of peroxidase were studied in extracts obtained from 5 to 15 day seedling roots. Root tissues were chosen in preference to other tissues because they gave clear, sharp bands and presented no difficulties in interpretation, and because they comprised all the band patterns other tissues had.

At least 8 bands were resolved in acrylamide gels, wich appeared to represent 4 loci when the results of genetic tests were considered (Fig. 65).

Px1 Peroxidases

Bands Rf12 and Rf18 were adjacent on gels (Fig. 62). They were present together in many plants of both K409 (DIV2N) and K480 (DIV2N), but single Rf18 or Rf12 was also observed in plants of these two and other accessions. F_1 progenies of K409 and K480 were examined for genetic segregations (Table 27). Zymograms of F_1 hybrids not only displayed both isozymes, but also the isozyme at either Rf18 or Rf12. The ratio of Rf18: Rf16/Rf12 : Rf12 was 1:2:1 (Table 27), suggesting that these two bands are controlled by the same gene. The locus was designated Px with alleles Px-1 for Rf18 and Px-2 for Rf12.

Px2 Peroxidases

Band Rf-3 was present in roots of K409 (DIV2N), but absent from K405 (*L. shannoni*). All F_1 plants of these two different phenotypes displayed the band. The F_2 generation segregated in approximately a 2:1 ratio (presence to absence) (Table 28). The data indicated simple monogenic control for band Rf-3, with presence being dominant. The locus was designated Px2 with the allele Px2-1 for presence and allele Px2-null for absence.

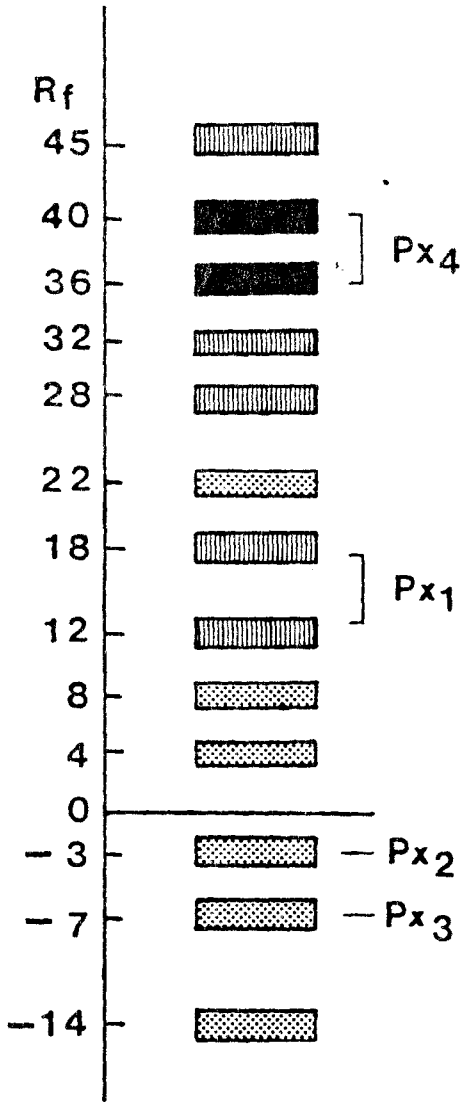

Fig. 65. Composite drawing of the 13 peroxidase isozymes observed in Leucaena species and suggested genetic loci.

Table 27. Segregations of Px Peroxidases in progenies of K409 x K480.

Progeny	Plant with phenotype (Rf)		
	18	18/12	12
K409 x K480	8	16	6

Expected ratio 1 : 2 : 1, x^2 = 0.6, P = 0.75-0.90.

Table 28. Segregations of Px2 Peroxidases in progenies of K409 x K405.

Progeny	Plant with phenotype (Rf)	
	-3	null
K409	20	0
K405	0	20
K409 x K405	50	0
(K409xK405) x (K409xK405)*	33	17

* Expected ratio 3 : 1, x^2 = 2.14, P = 0.50-0.75.

Px3 Peroxidases

Crosses were made of K405 (L. shannoni), which displayed band Rf-7, and K409 (DIV2N), which lacked the band. Band Rf-7 was present in all F_1 hybrids, while the F_2 generation segregated in approximately a 2:1 ratio and the backcross to K409 in a 1:2 ratio (Table 29). The data were interpretable on a monogenic model for control of band Rf-7. The locus was designated Px3 with the allele Px-1 for presence and allele Px3-null for absence.

Px4 Peroxidases

The study of genetic control of Px4 was complicated because most diploids under analysis had variable combinations of alleles within accession, i.e. each accession had either band Rf40, Rf36 or both. The plants of one accession of L. shannoni, K405, possessed only band Rf36. When crossed with K409 (DIV2N), phenotype unknown, the F_1 progenies had both bands. The F2 generation from sibcrosses of the F_1 displayed 1:2:1 ratio of Rf40: Rf40/Rf36: Rf36 (Table 30), suggesting the phenotype of the unknown parent, K409, was Rf40. The data were also interpretable on a monogenic model for control of bands Rf36 and Rf40. The locus was designated Px4, with alleles Px4-1 for Rf40 and Px4-2 for Rf36.

Bands Rf45 and Rf-14 appeared in many tissues of most accessions tested but sometimes they did not show up in

Table 29. Segregation of Px3 Peroxidases in Progenies of K409 x K405.

Progeny	Plant with phenotype (Rf)	
	-7	null
K405	20	0
K409	0	20
K409 x K405	50	0
(K409xK405) x (K409xK405)*	31	19
K409 x (K409xK405)**	7	13

* Expected ratio 3 : 1, x^2 = 4.51, P = 0.10-0.50.

** Expected ratio 1 : 1, x^2 = 1.80, P = 0.10-0.50.

Table 30. Segregation of Px4 Peroxidases in progenies of K409 x K405.

Progeny	Plant with phenotype (Rf)		
	40	40/36	36
K405	0	0	20
K409 x K405	0	20	0
(K409xK405) x (K409xK405)*	12	17	11

* Expected ratio 1 : 2 : 1, x2 = 0.95, P = 0.50-0.90.

plants of the same accession. Bands of Rf32, Rf28, Rf22, Rf8 and Rf4 were rare bands. They seemed to be unstable in Leucaena species. Genetic control of these bands were difficult to determine.

7.3 Comparison of Peroxidases in Leucaena diversifolia Complex and Related Species

Leucaena diversifolia (DIV2N) - It may be noted from Table 31 that all the accessions observed of the taxa were polymorphic at all loci examined except for K478, K480 and K483 which were monomorphic in two peroxidase isozyme loci (Px2 and Px3). K478 was also monomorphic at Px1 and Px4.

Leucaena diversifolia (DIV4N) - No variation was detected with each of the four accessions examined (Table 31). Plants of tetraploid L. diversifolia were fixed at four loci.

Leucaena pallida - Only one accession was available for isozyme analysis, and it was found to be monomorphic at all the loci examined except for Px4 (Table 31). According to the peroxidase zymogram, L. pallida appeared closer to L. diversifolia than to the other suspected parental species, L. esculenta. L.pallida differed from L. esculenta in that it had Px1 and Px2 while the latter did not. However, they both had high frequency of Px4-1/Px4-2 alleles in Px4.

L. esculenta - Three accessions were observed. Only

Table 31. Peroxidase polymorphism of root from Leucaena species (N = 25).

Species	Access. No.	Frequecy %									
		Px1			Px2		Px3		Px4		
		18	18/12	12	-3	null	-7	null	40	40/36	36
DIN2N	K406	0	0	100	50	50	50	50	40	50	10
	K407	0	0	100	50	50	50	50	50	50	0
	K408	0	0	100	50	50	50	50	0	100	0
	K409	30	70	0	100	0	0	100	20	40	40
	K411	20	20	60	40	60	50	50	0	70	30
	K412	50	0	50	40	60	80	20	10	30	60
	K413	0	0	100	50	50	70	30	20	30	50
	K454	30	0	70	40	60	80	20	0	40	60
	K478	0	0	100	100	0	0	100	100	0	0
	K480	10	10	80	100	0	0	100	60	20	20
	K483	10	20	70	100	0	0	100	50	50	0
DIV4N	K146	0	0	100	100	0	100	0	0	100	0
	K155	0	0	100	100	0	100	0	0	100	0
	K156	0	0	100	100	0	100	0	0	100	0
	K164	0	0	100	100	0	100	0	0	100	0
PAL	K376	0	0	100	100	0	0	100	0	90	10
ESC	K342	0	0	0	0	100	0	100	0	100	0
	K459	0	0	0	0	100	0	100	0	100	0
	K546	0	0	0	0	100	0	100	20	80	0
LAN	K401	50	0	50	100	0	0	100	0	0	100
LEU	K8	0	0	100	100	0	0	100	0	100	0
SHA	K405	30	50	20	0	100	100	0	0	0	100

one isozyme, Px4, segregated in this species (Table 31).

L. shannonii - The species was polymorphic in Px1, but monomorphic for other loci.

L. lanceolata - Four loci were dectected in this species, three of them were monomorphic. Locus Px was polymorphic, with equal frequency of Px-1 and Px-2 alleles. Px2 was present, Px3 null and Px4 was represented by Px4-2 allele.

L. leucocephala - Most isozyme patterns of this species were identical to those of L. diversifolia (tetraploids) and were non-variable.

7.4. Discussion

Isozyme diversity in DIV2N corresponds to the morphological variation of the taxon. The rich variability in both morphology and isozyme in these plants is no doubt due to the wide range of climatic and other selection pressures which have acted on these plants over periods of perhaps millions of years. The frequencies of peroxidase isozymes can serve to identify different types of DIV2N. From Table 31, it can be seen that almost all DIV2N accessions collected from the same area, such as K478, K480 and K483 which are from Mt. Uyuca, Honduras, have similar peroxidase isozyme patterns.

One object of this study was to use electrophoretic data for quickly and positively identifying hybrid species in the

genus Leucaena. Due to only one peroxidase locus being detected in L. esculenta (Table 31), the data do not confirm that L. pallida is a hybrid derivative species of L. esculenta and L. diversifolia even though many morphological characters of the former are intermediate between the latters. However, all peroxidase isozymes detected in L. pallida were observed in L. diversifolia (Table 31).

Three tetraploid taxa, Leucaena diversifolia (DIV4N), L. pallida and L. leucocephala were fixed at all 4 peroxidase isozyme loci (Table 31). It suggests that these taxa may be relatively stable at the enzymic level.

CHAPTER 8

ANALYSES OF SOME TAXA OF SUSPECTED HYBRID ORIGIN

Many morphological characteristics of taxa of Leucaena, L. pallida Britton & Rose, K740 (an accession from Guatemala) and L. greggii L. Watson, were observed to be intermediate between certain species. It was suspected that these taxa were of hybrid origin. Morphology and geographical distribution (and cytology, experimental hybridization, if possible) of these taxa and their putative parents were studied in order to obtain evidence to support this hypothesis.

8.1. Leucaena pallida Britton & Rose

Leucaena pallida (PAL) is intermediate with respect to geography and morphology between L. diversifolia (Schlech.) Benth. and L. esculenta (Moc. & Sesse) Benth. Most herbarium specimens of this species observed were identified as either L. diversifolia or L. esculenta rather than as one of the synonyms. Brewbaker (unpublished) put this species, together with three other synonyms, L. dugesiana Britton & Rose, L. oaxacana Britton & Rose and L. paniculata (Britton & Rose), under the species L. diversifolia.

8.1.1. Morphological Comparisons of *Leucaena pallida* and the Putative Parents, *L. diversifolia* and *L. esculenta*

Leucaena esculenta (Moc. & Sesse) Benth. is a species, with fine-leaflets, shrubby stems and corky bark (Fig. 66). Its leaves are usually large (up to 40 cm long), with 30-25 pairs pinnae. Leaflets are very tiny (3-4 mm long) and numerous (30-100 pairs per pinna), the apex is acute or rounded. Inflorescences are white, large (2.5-3 cm in diameter), with 90-150 florets. Peduncles are 1-2.5 cm long. Pods are large, 2-3 X 10-25 cm, reddish. Seeds are ovate to rounded, about 8 mm in diameter.

Plants of *Leucaena pallida* closely resemble *L. esculenta* in diameter and morphology of inflorescences, the glossy nature of twigs, and the number of floret per inflorescence (Fig. 67). Young unopened leaves of both species are held together by sticky material. However, the color of anthers and numbers of leaf parts resemble those of *L. diversifolia*. Other features of *L. pallida* are intermediate between *L. diversifolia* and *L. esculenta* (Figs. 66, 67).

In this study, characters by which *Leucaena esculenta* and *L. diversifolia* differ significantly were used in scoring. Each of the selected characters was subjectively evaluated according to Anderson (1949). A value of 2 was assigned when the feature was like *L. esculenta*, 0 when like *L. diversifolia* and 1 when intermediate (Table 32). Ten

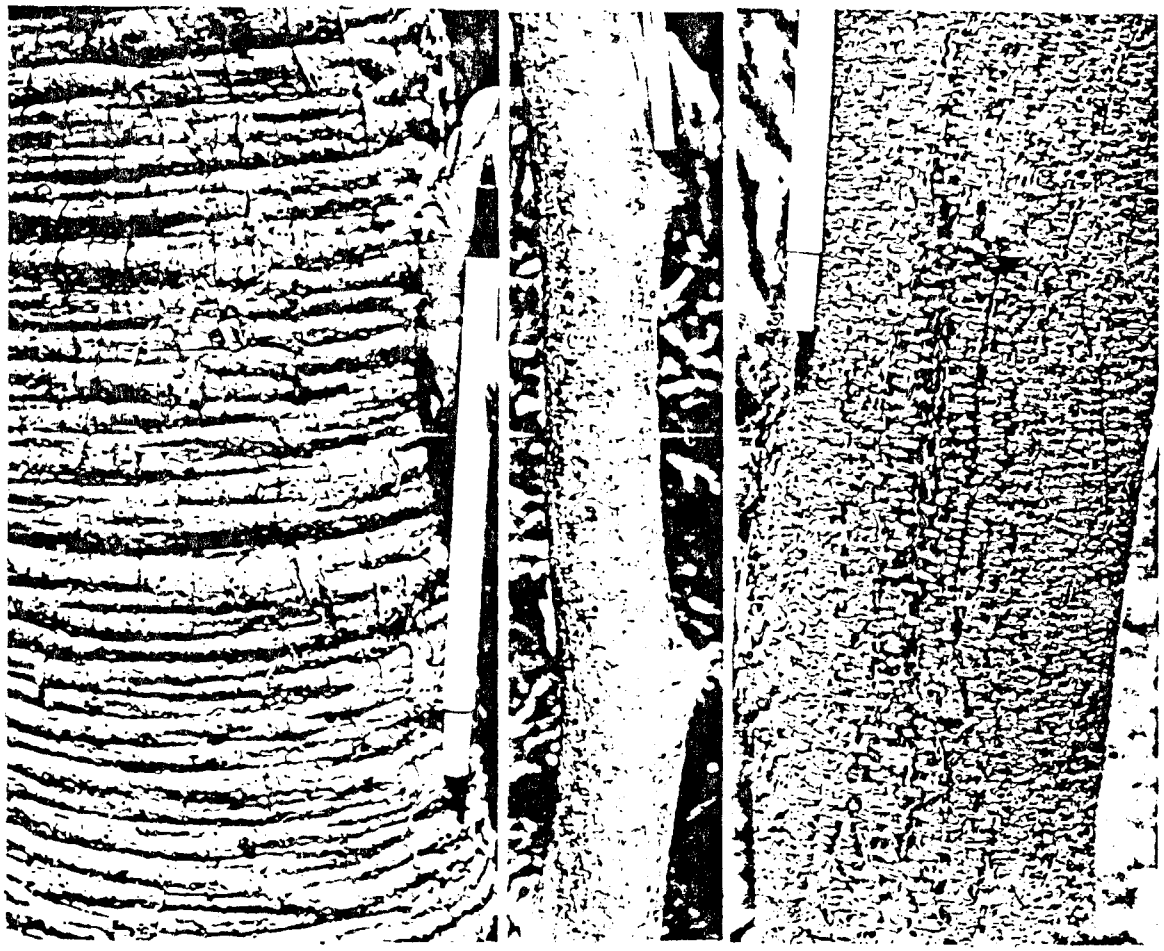

Fig. 66. Bark morphology, left to right, Leucaena esculenta (K138), L. pallida (K376) and L. diversifolia (K480, 2N).

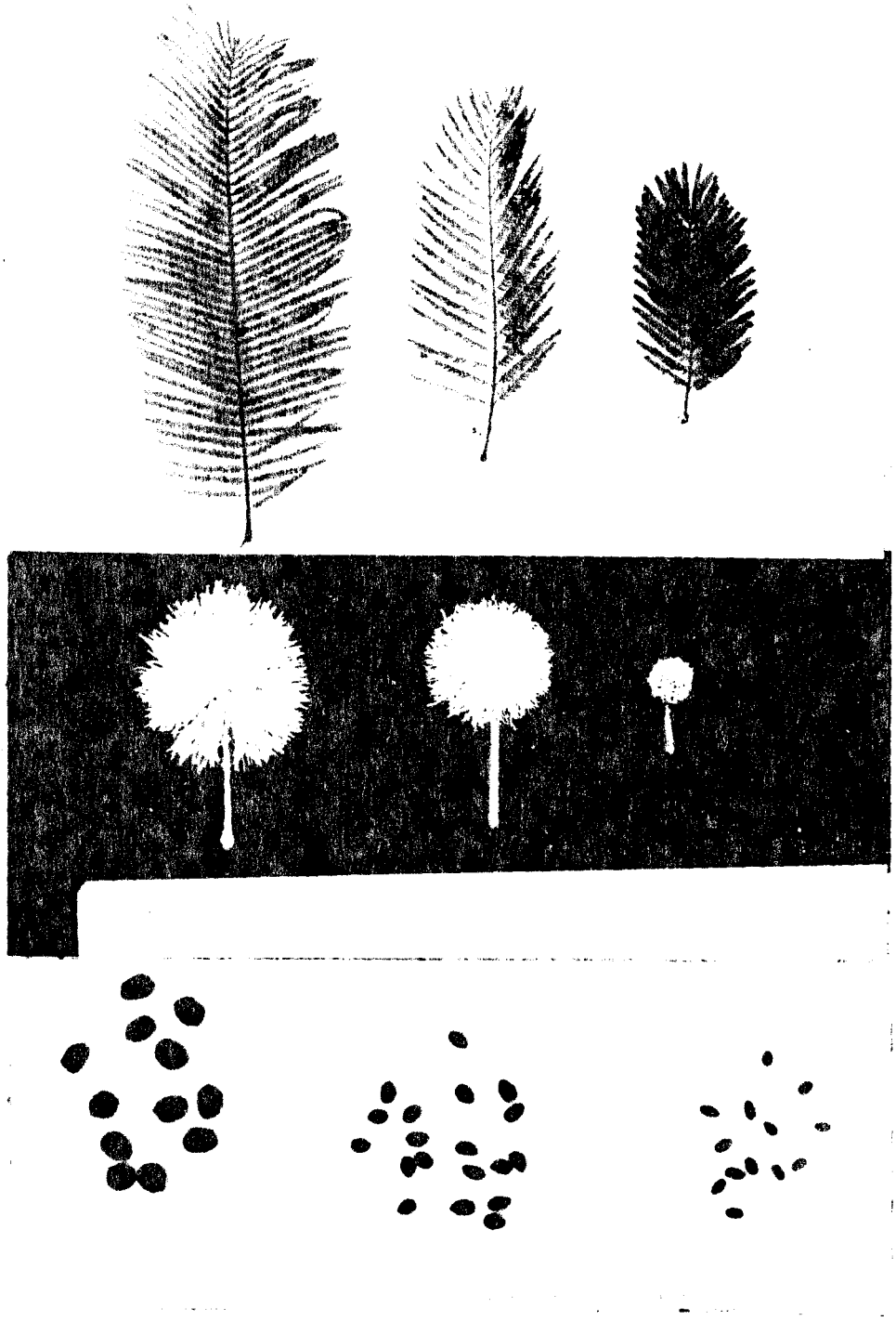

Fig. 67. Leaves, inflorescences and seeds of Leucaena esculenta (K138)(left), L. pallida (K376)(middle) and L. diversifolia (K413, 2N)(right).

characters were selected, all from live materials planted at the Waimanalo Research Station. A hypothetical L. esculenta score would thus be 20 and a perfect L. diversifolia score would be 0.

As shown in Table 33, Leucaena esculenta and L. diversifolia received total index values of 20 and 0 while L. pallida had indexes of 11-13. This strengthens the view that L. pallida is a hybrid derivative of the parental species L. diversifolia and L. esculenta.

8.1.2. Geographic Distribution of Leucaena pallida and Related Species

The approximate ranges of distribution of Leucaena pallida and related species assigned here to this taxon are illustrated in Fig. 68. The distribution of L. pallida extends southward from southern Zacatecas and North Jalisco through Guanajuato, Guerrero, Morelos and Puebla to central Oaxaca, Mexico. The discontinuities of the distribution of L. pallida between Guanajuato and Morelos, including Michoacan and Queretaro, may reflect incomplete collections of specimens or the lack of favorable habitats for L. pallida.

In most localities, Leucaena pallida and L. esculenta were sympatric. The southern parts of the ranges of these species also overlap with the northern distributional area of

Table 32. Scores assigned for computing the hybrid index of *Leucaena diversifolia* (2N), *L. esculenta* and their putative hybrid *L. pallida*.

Character	Score 0	1	2
Twig nature	terete	intermediate	angular
Glossy leaves	none	intermediate	yes
Flower color	pink	light pink	white
Pod length	<15cm	16-20cm	>20cm
Seed length	<5mm	5-8mm	>8mm
Seed shape	oblong	oblong-ovate	ovate
Bark nature	smooth with conspicuous lenticels	intermediate	corky with few lenticels
Floret No./head	40-80	70-120	120-150
Leaflet pairs/ pinna	20-40	30-70	70-90
Stem forking	no	intermediate	yes
	0	10	20

Table 33. Hybrid indexes in accessions of *Leucaena diversifolia*, *L. esculenta* and *L. pallida*.

Species	Access.No.	Hybrid index
L. diversifolia	K423	0
L. pallida	K174	13
	K177	13
	K178	11
	K376	13
L. esculenta	K138	20
	K342	20

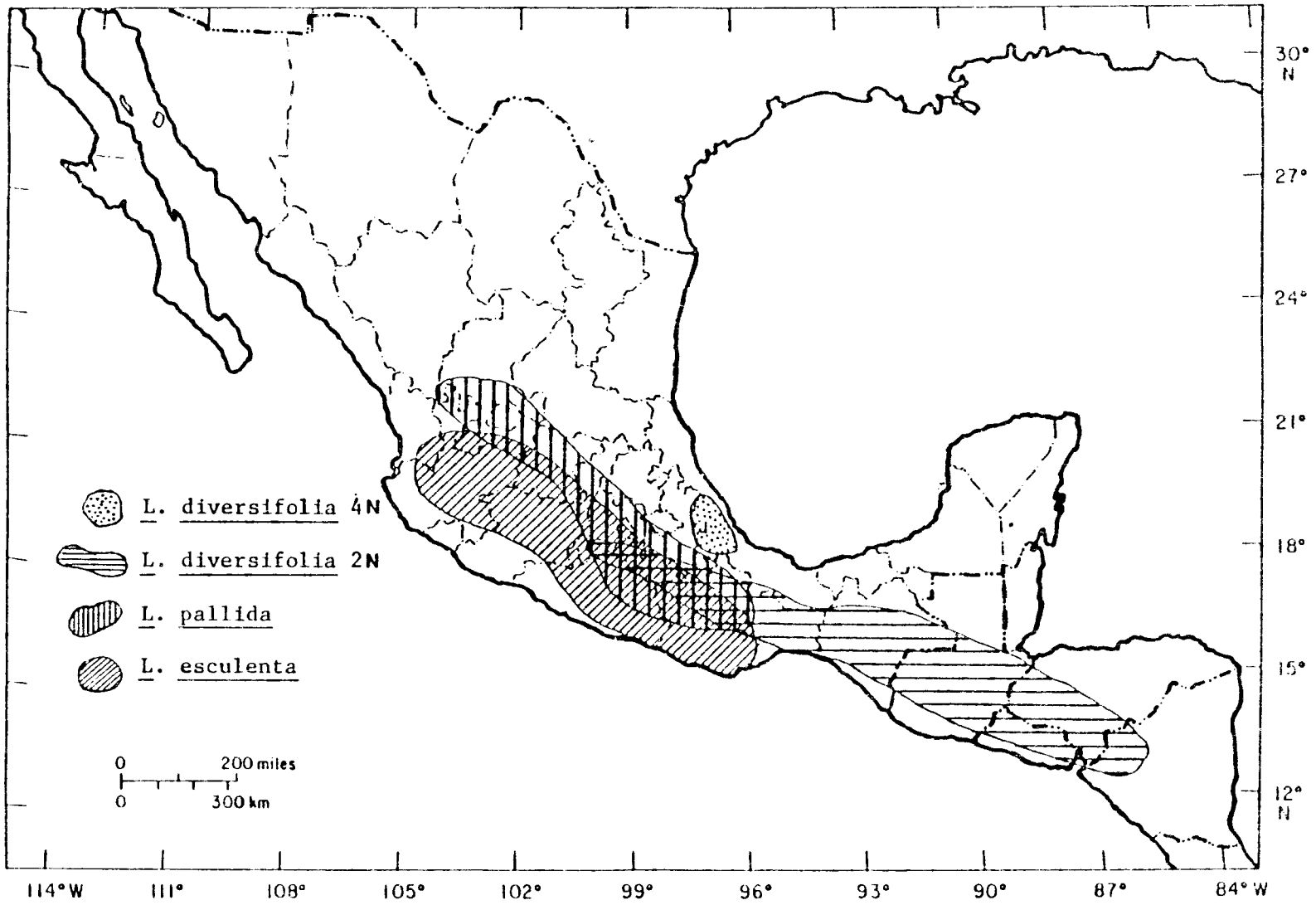

Fig. 68. Distribution of Leucaena pallida and related species.

diploid L. diversifolia (DIV2N). However, L. pallida and tetraploid L. diversifolia (DIV4N) were allopatric in all cases. They are separated by mountain ranges from 1800 to 4500 m in elevation. This makes tetraploid L. diversifolia (DIV4N) an unlikely candidate as one parent of L. pallida.

8.1.3. Cytology of Leucaena pallida

All four accessions (K174, K177, K178 and K376) of Leucaena pallida examined were tetraploid, with n = 52 chromosomes (Table 2). No meiotic irregularities were noted in 12 plants observed, with 52 bivalents at metaphase I, followed by normal meiosis in subsequent stages (Figs. 9-12). The pollen appeared to be well-developed and exhibited a viability of 98-100%.

8.1.4. Artificial Hybridization of Leucaena pallida and Related Species

Leucaena pallida crossed freely with DIV4N, but all attempts to cross it with DIV2N failed (Table 15 and Fig. 58). F_1 hybrids of reciprocal crosses of L. pallida and DIV4N were planted in the garden.

Leucaena pallida and L. diversifolia usually flower in summer, whereas L. esculenta flowers in winter. Some accessions of L. diversifolia species were seen flowering all year round (Fig. 56) and some individuals of L. esculenta

flowered in late summer, providing the opportunity for them to be hybridized with each other. In October 1983, 10 crosses between L. pallida and L. esculenta, and 4 crosses between DIV4N and L. esculenta were made and observed to set seeds. Unfortuntely, all of these seeds were attacked by insects the following winter and no healthy seeds remained for further study.

Leucaena pallida is self-incompatible as mentioned in Chapter 6 (see Table 14). Crosses were made among four different accessions (K174, K177, K178 and K376) of the same species, and among the individuals of K376. Twenty plants of each combination of these crosses were also planted in the garden for observation. Six combinations of F_1 hybrids within Leucaena pallida and sibcrosses within the accession K376 were observed for features such as branching stems, pink flowers, and subterete twigs (Fig. 69). The observations indicate that the putative hybrid derivative species has become stable in at least all observed morphological characters.

Pollen stainability of F_1 plants of K376 x K376, K376 x K174 , K376 x K177, K376 x K178 and K174 x K376 also revealed high pollen viability (averaged 97.4%, ranged 94-100% and sample size = 10 for each cross). Meiotic chromosome pairing of F_1 hybrids of K376 x K174 and K376 x K177 were observed to be regular, with 52 pairs of chromosomes.

The branhcning stem or low-forking habit of Leucaena

Fig. 69. Nine month old seedlings of K376 x K178 showing uniformity in morphological features. Note the forking stems.

pallida seems to have been inherited from L. esculenta. Both L. pallida and L. esculenta are species with stems of the branching type, with 2-5 branches initiated at early growth stages, while L. diversifolia is a species with 1-2 shrubby stems. Ten F_1 hybrids of each of K156 (DIV4N) x K376 (PAL), K165 (DIV4N) x K376 (PAL), and reciprocal crosses were planted in the garden to observe this feature. Branching stem appeared recessive, since all the F_1 hybrids of these crosses produced only 1-2 straight stems.

8.2. Analysis of K740, an Accession from Guatemala

An accession designated as K740 was sent by Mr. Colin Hughes of the Commonwealth Forestry Institute at Oxford University, who collected the seeds from the valleys of central Guatemala at an elevation of 200 to 700 m in 1983. Ten seeds of each of 25 trees from the same locality were planted at the Waimanalo Research Station in November, 1983. The study was based on these plants as well as specimens also sent to Hawaii by Mr. Hughes.

8.2.1. Morphological Characteristics

The plants of the accession are shrubby trees, with tiny leaflets that look like DIV2N (Leucaena diversifolia 2N). Some specimens of the plants were also observed in four herbaria visited in which they were identified as Leucaena

guatemalensis, a taxon treated as L. diversifolia by most botanists. However, upon careful investigation, these plants appear quite different from all the described species, including members of the L. diversifolia complex and other species in the genus. The description of the plants are as follows: leaf length 5-8 cm, 5-7 pairs pinnae and 20-40 pairs leaflets, leaflets about 12 mm long, 1.5 mm wide; petiolar glands convex; flowers white; pods about 15 cm long, 1.5 cm wide; seeds ovate, about 3-5 mm long 2-3 mm wide. Most of these features were indistinguishable from the artificial F_1 hybrids grown at Waimanalo of DIV2N (K409) x L. shannoni (K405), especially the shape and size of the leaflets and the seeds. The plants possess the characters such as glandless anthers, ten free stamens and thin legume pods with transverse seeds which define them as a member of the genus Leucaena.

8.2.2. Distribution of K740 and Putative Parental Species

The distribution of K740 is based on collection data of Mr. Hughes (unpublished) and specimen records obtained from herbaria (Fig. 70). Estimated distributions of Leucaena shannoni and DIV2N indicate that K740 occurs in areas of overlap of these two species. According to morphological and distribution data, these unique collections probably represent hybridization between Leucaena shannoni and DIV2N.

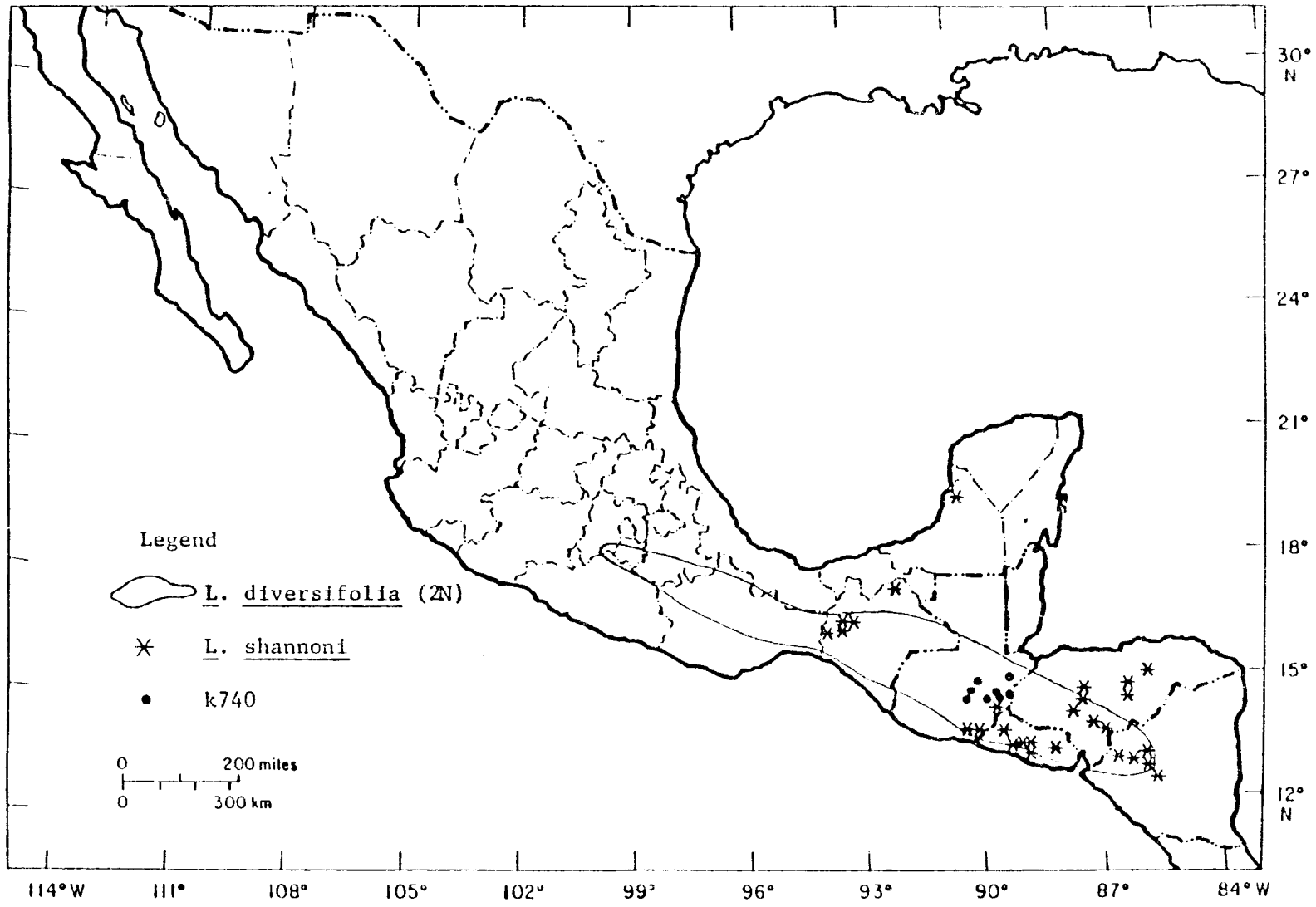

Fig. 70. Distribution of K740, Leucaena shannoni and L. diversifolia 2N.

8.2.3. Chromosome Number of K740

Meiotic chromosome counts based on arboretum plants were attempted. Unfortunately, since few flowers were available, no resolvable cells were obtained. However, several cells from the observation showed the chromosome number of K740 was more than 45, probably n = 52. If this is the case, the origin of K740 may have resulted from hybridization of Leucaena shannoni and DIV2N, followed by a doubling of chromosomes as in the suspected origin of L. pallida.

8.3. Leucaena greggii L. Watson

Leucaena greggii is a species with leaflets similar to L. leucocephala, with yellow flower heads and slim pods like L. retusa. All specimens of the species observed are from regions of overlap of L. retusa and L. pulverulenta (Fig. 71). L. greggii is also very probably an amphiploid between L. retusa and L. pulverulenta.

8.4. Discussion

It is hypothesized here that Leucaena pallida is of hybrid origin, and L. esculenta and DIV2N (L. diversifolia 2N) as parents. This is based on evidence from morphology and geographic distribution of the taxa. In view of the morphological and chromosomal data, it seems reasonable to

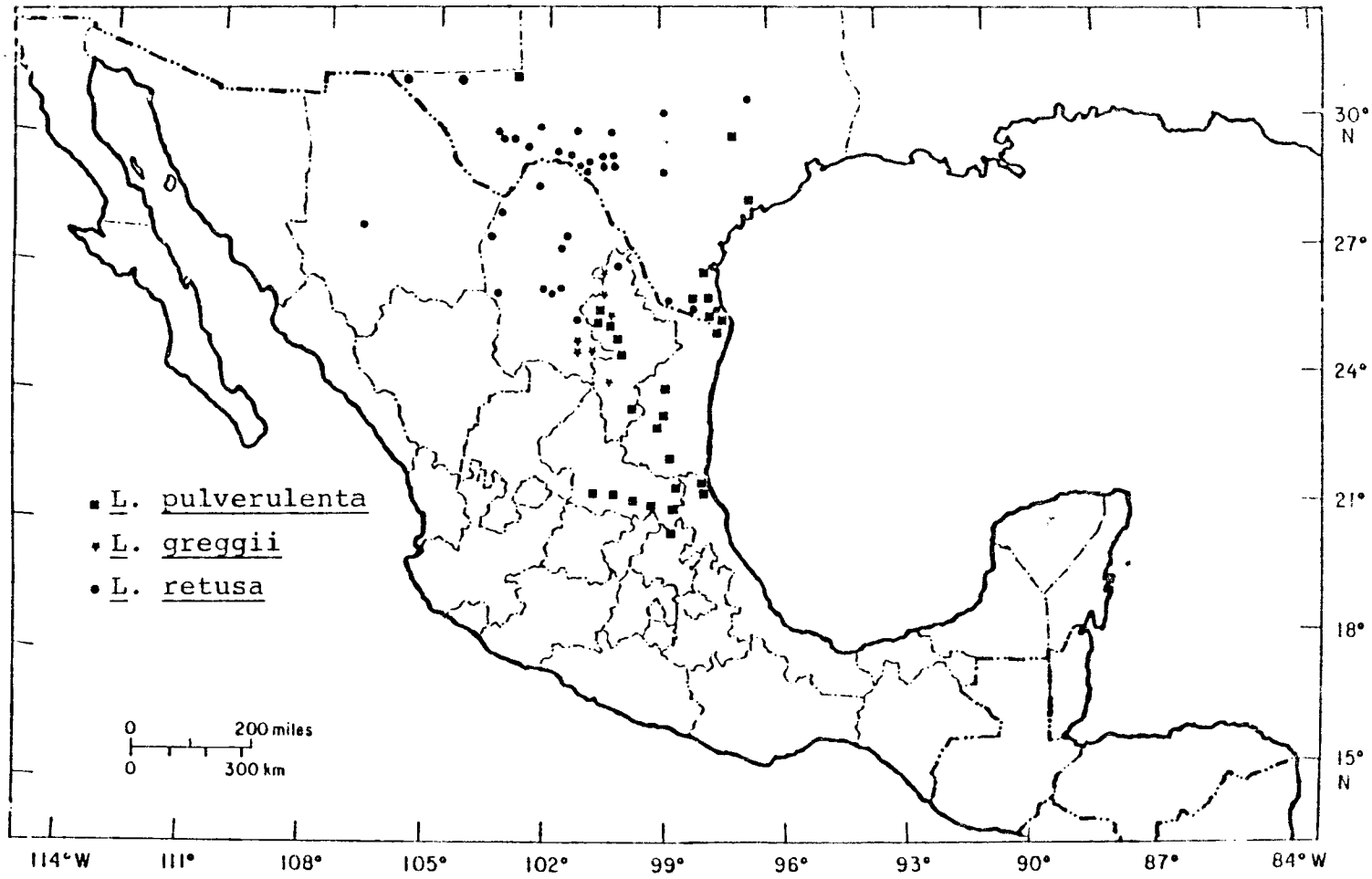

Fig. 71. Distribution of Leucaena greggii and related species.

regard L. pallida as the amphiploid derivative of L. esculenta and DIV2N. Repeated morphological observations on these species indicate that introgressive hybridization with L. esculenta might have taken place before these hybrids became polyploids. This is based on the overlapping distribution of L. pallida and L.esculenta, as well as on the fact that L. pallida has more features similar to L. esculenta than to L. diversifolia (Table 33).

K740 and Leucaena greggii could also reprent amphiploid taxa of genus Leucaena. The former was suggested to be derived from hybridization of L. diversifolia (2N) and L. shannoni, while the latter from L. pulverulenta and L. retusa.

Since Leucaena pallida is sympatric with both parental there must be some sort of isolation mechanisms keeping them distinct. It is reasonable to consider that triploid hybrids between tetraploid L. pallida and its diploid parental species are sterile, just as would be the case with hybrids between DIV4N and DIV2N. This may be the same mechanism in K740 and L. greggii.

It is evident from the foregoing account that hybridization together with polyploidy has played a very important role in the origin of some Leucaena species. Lack of efficient genetic barriers between species and reasonably high fertility in hybrids has apparently promoted this mode of evolution.

CHAPTER 9

TAXONOMY

It has been suggested as a consequence of these studies that the Leucaena diversifolia complex includes two species, Leucaena pallida and L. diversifolia. An autopolyploid may be separated as an infraspecific taxon, usually as a subspecies, if it meets the requisites of the biological species concept (Love, 1964; Vida, 1972). Observation of cytology, experimental hybridization and geographical distribution of L. diversifolia show that the diploids and tetrapoids are well separated by strong pre- and post-zygotic isolation mechanisms. They could be considered at the initial stage in a process of gradual divergence. Therefore, the two cytotypes are here regarded as two subspecies; the tetraploids are assigned to the subspecies diversifolia, and the diploids to the subspecies trichandra. The voucher specimens of these taxa are deposited in Herbarium of Bishop Museum (BISH).

Keys to the Leucaena diversifolia complex and one related species

A. Anthers yellow, twigs sharply angulate, leaflets of central pinnae more than 70, bark corky, usually winter flowering ---------- L. esculenta

A. Anthers pink, twigs terete or subterete, leaflets of central pinnae less than 60, bark smooth, usually summer flowering.

B. Leaflets 8-11 mm long, peduncles 2.5-4 cm long, bark with sparse lenticels ---------- L. pallida

B. Leaflets less than 7 mm long, peduncles less than 2 cm long, bark with dense lenticels.

C. Inflorescences more than 1.5 cm in diameter at anthesis, florets loose in heads, pistils 6-10 mm long. ---------- L. diversifolia spp. diversifolia

C. Inflorescences less than 1.2 cm in diameter at anthesis, florets compact in heads, pistils less than 5 mm long. ---------- L. diversifolia spp. trichandra

1. Leucaena diversifolia (Schlecht.) Benth. in Journ. Bot. Hook. 4:417. 1842.

1a. Leucaena diversifolia (Schlecht.) Benth. ssp. diversifolia

Acacia diversifolia Schlecht. in Linnaea 12:570-571. 1838. Type: MEXICO. Veracruz: Jalapa. Schiede 693 (HOLOTYPE: L; ISOTYPE: GH!)

Leucaena brachycarpa Urban in Symb. Ant. 2:265. 1900. Type: JAMAICA. Hope: 200 m. E. Campbell 6425 (HOLOTYPE: J; ISOTYPE: NY!)

Leucaena laxifolia Urban in Symb. Ant. 2:266. 1900.

Type: MEXICO. No locality data. Sommerschuh s. n. 1833. (HOLOTYPE: Herb. Berol, fragments at US and NY!)

A tree, up to 20 m high. Twigs, rachis and rachilla puberulent. Bark brownish with slightly elevated lenticels which are about 1 to 3 mm in length; vertical rows of lenticels frequently occur opposite the wide vascular rays. Petiolar gland suborbicular, cupulate or sometimes flattened, 1.5 to 2 mm broad and 3 to 5 mm long, borne just below the lowest pair of pinna or sometimes on both terminals and base of rachis. Petioles 1.5-2.0 cm long, pubescent. Pinnae 12-33 pairs. Leaflets 37-72 pairs, linear, 4-6 mm long, glabrous or sparsely puberulent on both sides sometimes dotted or plicate underneath, acutish or obtuse, the venation prominent beneath, margins pilose. Peduncles 1-2 cm long; involucre borne about 1-2 mm below the inflorescences or sometimes attached to the inflorescences, pubescent. Inflorescences solitary or 2-4, 1.5-2.2 cm in diameter when opened, slightly pink, the pink color from anthers, pistils and filaments; florets 50-80 per inflorescence; calyx 1.5-2.2 mm long, five lobes, lobes obtuse, pubescent on outside surface. Corolla 3-4 mm long, 5-lobed, free, tips of lobes acutish; corolla/calyx length ratio usually more than 2; stamens 4-7 mm long, anthers slightly pilose on both terminals, slightly pink, filaments whitish; pistils

(including ovary) 7-9.5 mm long, styles slightly pink; stigmas cup-shaped; ovary pilose or rare glabrous, about 1.5 mm in length. Pods glabrous on surfaces, 9-15 cm long, 1.0-1.8 cm wide, with 10-24 seeds, brownish red when young, dark brown when mature, seed number 11-24; seeds obovate to oblong, 3-4 mm long, brown, shiny.

POLLEN SIZE: 50-75 um in diameter.

STOMATAL SIZE: 250-520 um^2 in area.

CHROMOSOME NUMBER: n = 52 with 0-8 extra chromosomes.

BREEDING SYSTEM: self-compatible.

PHENOLOGY: flowering April to October.

DISTRIBUTION: restricted to Veracruz, Mexico in forests above 800 m.

REPRESENTATIVE SPECIMENS: MEXICO: Veracruz: C.A. Purpus 2337 (US, GH, NY), C. G. Pringle 8183 (US, GH, NY), A. Lot & Colaboradores 2042 (F), Liebmann 4355 (F), J.N. Rose 4267 (US, NY). JAMAICA: Hope: E. Campbell 6425 (NY), Wm. Harris 12451 (F, US, NY, GH), J.R. Perkins 1028 (GH).

1b. Leucaena diversifolia (Schlecht.) Benth. ssp. trichandra (Zuccarini) Pan et Brewbaker, stat. nov.

Leucaena trichandra (Zuccarini) Urban in Symb. Ant. 2:267, 1900.

Acacia trichandra Zuccarini in Abhandl. Akad. Wiss. Munich. 2:349. 1838. Type: grown from Mexican seeds

in the Munich Garden, origin not recorded. L. Monai s.n. 1825 (HOLOTYPE: M, fragment and drawing picture in NY!).

Leucaena guatemalensis Britton & Rose in N. Amer. Fl. 23:126. 1928. Type: GUATEMALA. Dept. Guatemala: Plains near Guatemala City. S. Hays 23 (HOLOTYPE: US!; ISOTYPE: NY!)

Leucaena pueblana Britton & Rose in N. Amer. Fl. 23:126. 1928. Type: MEXICO. Oaxaca: Valley of Cuicatlan, 1300m. E.W. Nelson 1886 (HOLOTYPE: US!, Isotype: NY!)

Leucaena revoluta Britton & Rose in N. Amer. Fl. 23:127. 1928. Type: MEXICO. Chiapas: Mountain slopes near Fenix. C.A. Purpus 10158 (HOLOTYPE: US! ISOTYPE: NY!)

Leucaena standleyi Britton & Rose in N. Amer. Fl. 23:128. 1928. Type: EL SALVADOR. Dept. Santa Ana: vicinity of Santa Ana, in pine forest, 655-900 m. P.C. Standley 20409 (HOLOTYPE: US!; ISOTYPE: NY!)

Leucaena stenocarpa Urban in Symb. Ant. 2:266. 1900. Type: MEXICO. Oaxaca: Foothills of Sierra de San Felipe, 2000 m. C.G. Pringle 4656 (HOLOTYPE: US!; ISOTYPE: GH! NY!, M!)

A shrub, stems usually branched, up to 15 m high. Highly variable in most morphological features. Twigs, rachis and rachilla glabrous, sparsely to densely pubescent,

or pilose. Bark as Leucaena diversifolia ssp. diversifolia. Petiolar glands suborbicular to orbicular, cupulate, 1 to 2 mm in diameter, borne just below the lowest pair of pinna, sometimes also on terminal pairs of pinnae. Pinnae 7-25 pairs. Leaflets 20 to 60 pairs, linear or oblong, glabrous, or pilose on upper side, or if pilose on both sides, hairs of undersurface usually much longer than the upper side; apex acute, obtuse, rounded or mucronate; margins often flat or sometimes revolute, pilose. Peduncles short, 0.5-1.5 cm or rare 2-3 cm long, upright or rare pendulous; involucre usually attached to the inflorescences or borne 1-3 mm separated from the inflorescences, different types of involucre type may be found on the same plant, pubescent or glabrous. Inflorescences 0.5-1.2 cm in diameter when opened, light to dark pink; pink color varies by the different combinations of colored anthers, filaments and styles; florets 60-150, calyx 1.2 to 2.2 mm long, five-lobed, lobes obtuse, pubescent or pilose; corolla 1.6-3.0 mm long, 5-lobed, free, apex obtuse or mucronate; corolla/calyx length ratio less than 1.5; stamens 2-4 mm long, anthers pilose, light to dark pink; pistils 3.5-6.0 mm long; styles straight or curved when flowers widely opened, sightly to dark pink; stigmas cup-shaped; ovary glabrous, puberulent or pubescent, about 1 mm in length. Pods glabrous or puberulent on surfaces, 9-15 cm long, 0.9 to 2.0 cm wide, brownish to reddish, seeds 7-24; seeds oblong, less than 2 mm in length, brown, shiny.

POLLEN SIZE: 30-45 um in diameter.

STOMATAL SIZE: 100 to 250 um^2 in area.

CHROMOSOME NUMBER: n = 26 with 0-6 extrachromosomes.

BREEDING SYSTEM: self-incompatible.

PHENOLOGY: flowering June to September, some populations all year round.

DISBRIBUTION: widely distributed from northern Nicaragua to Oaxaca and southern Mexico, in elevations from 600 to 2200 m.

REPRESENTATIVE SPECIMENS: GUATEMALA: Dept. of Quezaltenango. J.A. Steyermark 31320 (F). Dept. of Chiquimula: A. Molina R. & A. R. Molina 25227 (F) (NY). Dept. of Jutiapa: P.C. Standley 75475 (F). Dept. of Xacapa: J. A. Steyermark 29191. Dept. of Guatemala: A. Molina R et al 15962 (F, US, NY,). Dept. of Sacatepequez: P.C. Standley 59893 (F, GH,). Dept. of Ouiche: A. Molina R. (F). SALVADOR: Dept. of Izalco: P.H. Allen & M. L. V. Severen 6922 (F, US, GH, NY). Dept. of Santa Ana: P.H. Allen & R. Armour 7015 (GH). Dept. of Sonsonate: A. Molina R. et E. Montalvo 21711 (F). Dept. of Libertad: S. Calderon 1359 (US, GH). HONDURAS: Dept of Ocotepeque: A. Molina R. 22544 (F, NY). Dept. of Morazan: L.O. Williams and A. Molina R. 13429 (F). Dept. of Copan: A. Molina R. and A. R. Molina 24647 (F), L.O. Williams et al. 42961 (F, US). Dept. of Comayagua: A. Molina R. 10796 (F, NY). Dept. of La Paz: A. Molina R. and A.R. Molina 24322 (F,

NY). Dept. of Intibuca: P. C. Standley 25446 (F). Dept. of Choluteca: A. Molina R. 13068 (F, US, NY). MEXICO: Oaxaca: J.N. Rose & W. Hough 4567 (US, NY). Solano S.C. & Vara M.A. 427 (F, NY), Chiapas: D.E. Breedlove 7432 (US), 10497 (US). A.S. Ton 1491 (US), 3229 (US). Mexico: G.B. Hinton 3970 (NY), 6843 (GH, NY), 7993 (US).

2. Leucaena pallida Britton & Rose in N. Amer. Fl 23:126-7. 1928. Type: MEXICO. Jalisco: Road between Huehuquilla and Mezquitic, near Huejuquilla. J.N. Rose 2569 (HOLOTYPE: US!; ISOTYPE: NY!).

   Leucaena dugesiana Britton & Rose in N. Amer. Fl. 23:127. 1928. (HOLOTYPE: US!) Type: MEXICO. Guanajuato: Guanajuato. J.N. Rose and W. Hough 4841.
   Leucaena oaxacana Britton & Rose in N. Amer. Fl. 23:127. 1928. Type: MEXICO. Oaxaca. J. N. Rose & W. Hough 4648 (HOLOTYPE: US!; ISOTYPE: NY!)
   Leucaena paniculata Britton & Rose in N. Amer. Fl. 23:128. Type: MEXICO. Morelos: Near Cuernavaca. J.N. Rose and J. S. Rose 11090 (HOLOTYPE: US!: ISOTYPE: NY!)

Branching shrubs, up to 15 m high. Twigs subterete, glossy. Young branches, rachis and rachilla glabrous. Young leaves covered with glossy wax which stick young leaflets and pinnae together. Bark whitish purple, with very sparse and tiny (less than 1 mm in length) lenticels. Petiolar glands

orbicular, cupulate, 3-5 mm in diameter, 1 to 2 either on basal or on basal and terminal pinnae. Pinnae 9-25 pairs. Leaflets 30 to 70 pairs, linear, 6-10 mm long, pubescent on upper or both surfaces, dotted or plicate underneath, apex acute to obtuse, the venation prominent beneath, margins pilose, flat. Peduncles 2-4 cm long; involucre attached to the inflorescences. Inflorescences 1-4 in cluster, 2-2.5 cm in diameter when opened; florets 60-125; calyx 2-3 mm long, sparsely pubescent, 5-lobed, lobes obtuse; corolla 3.5 mm long, apex acute; corolla/calyx length ratio more than 2; stamens 6-9 cm long, anthers dark pink, glabrous or slightly pilose, filaments white; pistils 9-12 mm long; styles white; stigmas cup-shaped; ovaries glabrous or rare pilose, 1.5-2 mm long. Pods glabrous 8-24 cm long and 1-1.7 cm wide, brownish red, seeds 12-23. Seeds oblong, 4-5 mm in length, reddish brown.

POLLEN SIZE: 50-70 um in diameter.

STOMATAL SIZE: 300-700 um^2 in area.

CHROMOSOME NUMBER: n = 52 with 0 to several extra chromosomes.

BREEDING SYSTEM: self-incompatible.

PHENOLOGY: flowering April to October.

DISTRIBUTION: from Jalisco through Guanajuato, Morelos to Oaxaca, Mexico, at elevations of 800-1500 m.

REPRESENTATIVE SPECIMENS: MEXICO: Oaxaca: M. Sousa et al. 6500 (F), W. R. Ernst 2408 (F,), C. Conzatti 2526, 2527 (F).

Hough 4961 (US), E. W. Holway 5408 (US), L. C. Smith 116 (US), E. W. Nelson 1835 (US), C. Delgadillo M. 194 (US), S. C. Solano and M. A. Vara 460 (F), C. A. Purpus 3192 (F). Jalisco: J. N. Rose 2569 (US, GH, NY), J. N. Rose & J. H. Painter 7448 (GH). Morelos: S. Zarate 72 (NY). Puebla: C. A. Purpus 3192 (US, NY, GH), Gentry, Barelay and Arguelles 20288 (US), E. W. D. Halway 5349 (US), J. N. Rose et al. 9931 (US), J. N. Rose 9931 (NY). Guerrero: D. E. Breedlove 35999 (NY). Zacatecas: John and C. Taylor 6169 (NY).

It would be desirable from a taxonomic standpoint to be able to segregate the array of variation in Leucaena diversifolia ssp. trichandra into a convenient number of usable categories, and name each group of populations, according to their unique morpholgy such as revolute race, glossy race, spotted race and so on. However, the best arrangement may be to simply recognize that these are genetic variations existing in the subspecies without assigning of Latin names to individual recombinant types.

CHAPTER 10

CONCLUSION

For this study, a species is defined as a set of individuals that interact reproductively as a discrete interbreeding system. The species exhibits genetic differentiation from other discrete interbreeding systems as a result of several kinds of isolation mechanisms. According to this concept, the Leucaena diversifolia complex can be divided into two species and one subspecies: L. pallida, L. diversifolia ssp. diversifolia and L. diversifolia ssp. trichandra. The former two taxa are separated from each other by high mountain ranges, and from the latter by reproductive isolation. These three taxa are easily identified by certain morphological characters such as twig nature, leaf size, leaflet size and number per pinna, and head size etc. They are also different in breeding systems and peroxidase isozyme patterns.

In considering the evolution of Leucaena diversifolia, it is suggested that the diploid was the progenitor of the tetraploid. The tetraploid, L. diversifolia ssp. diversifolia, is suggested to be an autotetraploid based on the evidence that it is very similar morphologically to the diploid, that multivalents formed in diakinesis in the tetraploid, and that the tetraploid is self-compatible while diploid is self-incompatible. It is hypothesized that the tetraploid was formed, possibly from unreduced gametes,

during the last glacial period. At first, the two cytotypes might have been fully sympatric in some areas. However, perhaps after the glacier retreated, the tetraploid spread to habitats distinct from the diploid, and occupied the contemporary distribution area in Veracruz, Mexico, mainly above 1000 m elevations.

Based on the evidence from morphological characters and geographical distribution, Leucaena pallida is suggested to be an amphiploid hybrid derivative, with L. esculenta and L. diversifolia ssp. trichandra as parents. Introgressive hybridization of L. pallida toward L. esculenta might have taken place before its ancestor became polyploid, due to the fact that L. pallida has more features similar to L. esculenta than to L. diversifolia ssp. trichandra. These features include glossy leaflets and young twigs, branching stems and drought tolerance. The original hybrid might have occurred in northern Oaxaca where the two parental species overlapped. Climatic changes in recent millenia or other changes such as man's disturbance in recent centuries could then have favored the expansion of this hybrid derivative species.

Natural hybridization and artificial hybridization were reported among species of Leucaena (Gonzalez et al., 1967; Hutton, 1981; Brewbaker, 1982). In this study, the two interspecific hybrid combinations, L. diversifolia ssp. trichandra x L. lanceolata and L. diversifolia ssp.

trichandra x *L. shannoni*, also show high seed set, and the seeds are viable and generally produce normally developed and vigorous F_1 plants. Therefore, barriers to most interspecific hybridization often seem to be lacking, in at least the F_1 generation. Gene transfer among the species of *Leucaena* may be relative easy and might permit the introduction of desirable genes from one species to another. Also, the lack of internal barriers could allow free gene flow and recombination among species that could lead to further speciation, assuming they are sympatric. However, recombinational speciation at the diploid level has not been detected in the genus *Leucaena*.

The lack of internal barriers between most species of the genus *Leucaena* suggests that geographical and ecological barriers are essential in maintaining species of the genus. A similar circumstance was reported by Gillett and Lim (1970) in *Bidens*, which also exhibits great morphological and ecological diversity without genetic barriers. These authors attributed the forces for maintaining the species of the genus to two isolation mechanisms -- ecology and hybridization. This explanation appears to apply equally well to the situation in the genus *Leucaena*.

Polyploidy as a mechanism of species formation in plants is well understood (Stebbins, 1947; de Wet, 1971; Grant, 1981). Both autoploidy and amphiploidy were found in the genus *Leucaena*. *L. diversifolia* ssp. *diversifolia* appears to

represent an autoploid which is separated genetically from its diploid progenitor by the formation of sterile triploids, and by allopatry. Leucaena pallida, and perhaps L. greggii, represents amphiploids. It is believed that amphiploids are able to evolve more rapidly than autoploids (sensu Stebbins, 1950), and successfully colonize new habitats because they often have wider ecological adaptability and their chromosome systems have the advantage of being well buffered to compensate for alien genetic material. The studies of morphology, experimental hybridization, cytology and isozyme pattern strongly suggest that Leucaena diversifolia ssp diversifolia and L. pallida are stable taxa. Therefore, polyploidy and hybridization have played very important roles in the speciation of the genus Leucaena.

Three lines of evidence can be considered in constructing the phylogeny of the Leucaena species: (1) morphological similarity, (2) geographical distribution, and (3) cytological data. Vavilov (1926) determined the center of origin of a plant by identifying the center of diversity and comparing that with the distribution of related species. The method leads us to consider that Central America is the center of origin of Leucaena. Leucaena diversifolia ssp. trichandra perhaps is the oldest taxon of the genus based on its diversity in morphological characters and on its wide distribution. Brewbaker (1982) also suggested it was the complex central to the evolution of the genus. Since

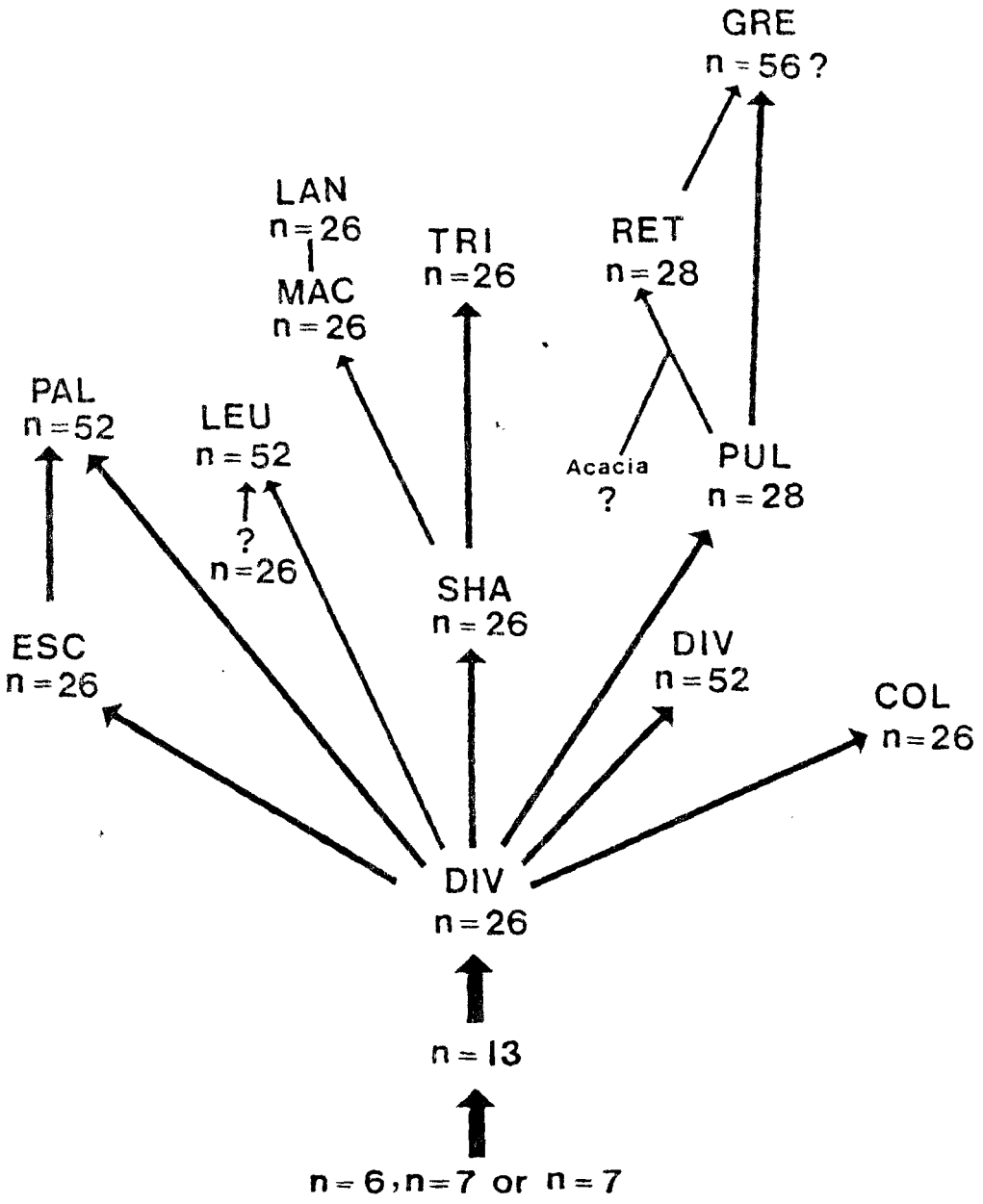

Fig. 72. Phylogenetic relationships of the Leucaena species. COL = L. collinsii; DIV = L. diversifolia; ESC = L. esculenta; GRE = L. greggii; LAN = L. lanceolata; LEU = L. leucacephala; MAC = L. macrophylla; PAL = L. pallida; PUL = L. pulverulenta; RET = L. retusa; SHA = L. shannoni; TRI = L. trichodes.

barriers to gene exchange in the species of the genus are very weak, it is suggested that the species are a monophyletic group. Based on these facts, the phylogenetic relationships of the species are suggested as shown in Fig. 72. L. diversifolia ssp. trichandra is viewed as derived from ancient plants with n = 6 and n = 7, or n = 7 through n = 13. The species with n = 26 might have differentiated directly from L. diversifolia ssp. trichandra, including L. esculenta, L. shannoni, and L. collinsii. Three species, L. lanceolata, L. macrophylla and L. trichodes might be from L. shannoni since they all have large leaflets and convex glands. Two species, L. pulverulenta and L. retusa, with n = 28 could be from L. diversifolia and L. shannoni respectively, by obtaining two extra chromosome pairs. L. retusa might obtain some characteristics from Acacia, since it has yellow flower heads. L. diversifolia ssp. diversifolia is considered an autoploid of L. diversifolia ssp. trichandra, and L. pallida an amphiploid of L. diversifolia ssp. trichandra and L. esculenta as mentioned earlier. The origin of L. leucocephala is not certain. Brewbaker (1982) suggested it was an amphiploid of L. shannoni and L. diversifolia. However, the discovery of K740 which is postulated to be the amphiploid of L. shannoni and L. diversifolia in this study suggests there could be another species which might be involved in the parentage of L. leucocephala.

Appendix 1. Localities of *Leucaena* species used in analyses.

Species	Access. No.	P.I.* number	Area of origin
L. collinsii	K180	324347	Tuxtla Gutierrez, Chiapas, Mexico.
	K450	443514	3 km west of Tuxtla, Chiapas, Mexico.
L. esculenta	K138	324377	Tecamachalco, Puebla, Mexico.
	K342	--	from Mexico via Dr. R. Hamilton.
	K459c**	--	3 km east of Tuxtla, Chiapas, Mexico.
	K546c	443534	Teloloapan, Guerrero, Mexico.
L. lanceolata	K264	324389	Mazatlan (at 100 m), Sinaloa, Mexico.
	K401	--	60 km north of Zihuatenejo, Guerrero, Mexico.
L. leucocephala	K8	263695	Moyahua, Zacatecas, Mexico.
L. macrophylla	K379	--	160 km southeast of Oaxaca City, Mexico.
L. pulverulenta	K19	286223	Texas, USA.
	K75	794093	Sierra San Miguel, San Luis Potosi, Mexico.
L. retusa	K280	321631	Sonora, Texas, USA.
	K502	435920	Dagger Flats, Big Bend National Park, Texas, USA.
L. shannoni	K405	--	Waimanalo Farm, Hawaii, source unknown.
	K487	443486	Mercedes Umana, about km 100 from Salvador, El Salvador.
L. trichodes	K90	317914	Caracas, Venezuela.
	K738	--	about 6 km northeast of Bosconia, Cesar, Colombia.

* P.I. number = the USDA plant introduction number.
** "c" represents seeds from a composite of several trees.

Appendix 2. Ploidy level of selected specimens of L. diversifolia complex as indicated by guard cell size.

Collectors and number (or type specimen)	Locality	Herbarium where deposited	Measurement(μm2) range	Measurement(μm2) mean	Predicted * ploidy level
A.Molina R. 15360	Guatemala	NY	90.3-144.5	93.3	2N
H.S.Mckee 10931	Veracruz	US2564987	210.7-270.9	239.1	2N
P.H.Allen 7415	Salvador	NY	126.4-168.6	149.8	2N
L.Muller 1021	Veracruz	NY	255.8-343.1	301.3	4N
C.A.Purpus 2337	Veracruz	US840450	289.0-421.4	353.7	4N
C.A.Purpus 10795	Veracruz	US1266070	307.0-400.3	332.4	4N
C.A.Purpus 10674	Veracruz	US1265355	313.0-428.9	359.5	4N
W.Harris 12451	Jamaica	NY	421.4-511.7	473.2	4N
P.H.Allen & R.Armour 7015	Salvador	US2366453	108.4-225.8	167.8	2N
A.Molina R. & A.R.Molina 25227	Guatemala	US2619239	75.3-108.4	93.6	2N
S.Calderon 1359	Salvador	US1152356	72.2-162.5	127.6	2N
J.N.Rose & W.Hough 4324	Veracruz	US346274	307.0-379.3	348.7	4N
Type specimens:					
L. brachycarpa (Holotype)		NY	252.8-379.3	311.2	4N
L. diversifolia (Isotype)		GH	313.0-451.5	377.6	4N
L. guatemalensis (Holotype)		US	105.4-162.5	140.0	2N
L. laxifolia (Holotype)		NY	289.0-442.5	349.3	4N
L. revoluta (Holotype)		US	126.4-180.6	144.8	2N
L. standleyi (Holotype)		US	99.3-180.6	127.5	2N
L. stenocarpa (Holotype)		US	90.3-149.0	137.7	2N
L. trichandra (Holotype)		M	90.3-168.6	139.1	2N

* Based on comparison with stomatal guard cell sizes of cytologically known materials (Text and Table 53).

Appendix 3. Height (ht) and diameter at breast height (dbh) of DIV4N and DIV2N. (mean of five trees for each accession, 4 year old).

Cytotype	Accession	ht(m)	dbh(cm)
DIV4N	K146	9.1	6.7
	K154	9.4	5.5
	K155	9.5	7.0
	K156	11.4	7.5
	K157	8.8	5.5
	K159	10.3	5.5
	K160	9.4	5.9
	K164	10.1	9.7
	K165	9.7	7.7
	K166	8.9	5.1
DIV2N	K11	5.9	3.7
	K406	5.0	2.8
	K408	7.1	4.3
	K409	5.7	2.9
	K410	4.6	2.6
	K411	5.8	2.9
	K412	5.5	2.8
	K413	4.2	1.5
	K422	7.0	3.1
	K423	5.4	2.2

Appendix 4. Quantitative characters of Leucaena diversifolia (K409), L. shannoni (K405) and their F_1 hybrid (N = 20).

Character	K409		F1		K405	
	range	$\bar{x}\pm$s.d.	range	$\bar{x}\pm$s.d.	range	$\bar{x}\pm$s.d.
Pinna No./leaf	9-18	13.3±2.0	8-12	9.5±0.9	3-7	5.8±1.2
Leaflet No./pinna	33-63	40.6±5.4	21-32	27.2±2.9	7-10	8.6±2.0
Leaflet length(mm)	3.5-5	4.8±0.4	7.5-11	8.8±1.0	15-20	17.6±2.6
Pod length(cm)	10.9-16	13.2±1.8	11-16.7	14.0±1.7	12-17	15.6±2.0
Pod width(cm)	1.0-1.9	1.4±0.3	1.4-1.9	1.6±0.1	1.2-1.5	1.3±0.1
Seed No./pod	15-24	19.4±3.2	14-24	18.7±2.2	14-21	16.8±3.2
Good seeds/pod	8-24	20.3±2.6	9-13	11.5±1.5	10-21	14.6±1.8
Floret No./head	57-106	71.8±11.6	74-125	97.2±14.3	82-127	101.8±11.8

Appendix 5. Quantitative characters of *Leucaena diversifolia* (K409), *L. lancecolata* (K401) and their F_1 hybrid (N = 20).

Character	K409		F1		K401	
	range	$\bar{X}\pm$s.d.	range	$\bar{X}\pm$s.d.	range	$\bar{X}\pm$s.d.
Pinna No./leaf	9-18	13.3±2.0	6-10	8.0±1.2	2-5	3.7±0.6
Leaflet No./pinna	33-63	40.6±5.4	12-19	16.6±1.9	4-6	5.5±0.6
Leaflet length(mm)	3-5.5	4.8±0.4	11-15	12.9±1.1	25-50	39.3±7.5
Pod length(cm)	10.9-16	13.2±1.8	14.2-22	17.2±2.1	14-21	16.2±3.2
Pod width(cm)	1.0-1.9	1.4±0.3	1.6-2.3	2.0±0.2	1.5-1.8	1.7±0.4
Seed No./pod	15-24	19.4±3.2	15-23	19.9±3.3	15-22	17.6±3.5
Good seeds/pod	8-24	20.3±2.6	8-15	11.2±1.8	11-21	18.2±2.6
Floret No./head	57-106	71.8±11.6	129-209	167.6±23.2	376-586	463.5±56.0

Appendix 6. Flower colors of K409, K480 and their F_1 hybrids. DP = dark pink; LP = light pink; P = pink; W = white.

Plant No.	Anther	Filament	Style	Plant No.	Anther	Filament	Style
K409	P	LP	LP	26	DP	W	DP
K480	DP	P	P	27	P	LP	P
1	*--	--	--	28	--	--	--
2	DP	W	P	29	DP	P	P
3	DP	LP	LP	30	DP	P	P
4	--	--	--	31	DP	P	P
5	--	--	--	32	DP	P	P
6	DP	LP	LP	33	DP	P	P
7	DP	LP	LP	34	DP	DP	P
8	--	--	--	35	DP	P	DP
9	P	LP	LP	36	P	LP	P
10	DP	LP	LP	37	DP	P	DP
11	DP	P	P	38	P	W	W
12	DP	P	P	39	DP	P	P
13	P	W	W	40	P	W	LP
14	DP	P	P	41	DP	LP	P
15	P	LP	P	42	P	LP	LP
16	--	--	--	43	P	P	LP
17	--	--	--	44	DP	P	P
18	DP	P	P	45	--	--	--
19	DP	LP	P	46	P	LP	LP
20	DP	LP	P	47	DP	DP	DP
21	P	W	LP	48	P	LP	W
22	DP	W	LP	49	--	--	--
23	DP	W	LP	50	--	--	--
24	LP	W	W				
25	DP	DP	DP				

* data not available.

Appendix 7. Vesture nature of leaflet and twigs in (K409 x K480) x (K409 x K480). G = glabrous; P = pubescent; Pi = pilose.

Plant No.	Leaflet pilosity	Twig pubescence	Plant No.	Leaflet pilosity	Twig pubescence
1	Pi	P	42	Pi	P
2	Pi	G	43	Pi	G
3	Pi	G	44	Pi	P
4	G	G	45	Pi	G
5	G	P	46	Pi	G
6	Pi	G	47	Pi	P
7	G	G	48	Pi	P
8	G	P	49	Pi	P
9	G	P	50	G	G
10	Pi	G	51	Pi	P
11	Pi	G	52	Pi	P
12	Pi	G	53	Pi	P
13	Pi	P	54	Pi	P
14	Pi	P	55	G	P
15	Pi	P	56	Pi	P
16	Pi	P	57	Pi	P
17	G	G	58	Pi	P
18	G	P	59	Pi	G
19	Pi	P	60	Pi	G
20	G	P	61	G	P
21	Pi	P	62	Pi	G
22	G	P	63	Pi	P
23	Pi	G	64	Pi	P
24	G	P	65	G	P
25	Pi	P	66	Pi	P
26	Pi	G	67	Pi	G
27	Pi	P	68	Pi	P
28	G	P	69	Pi	P
29	G	P	70	Pi	P
30	Pi	P	71	Pi	P
31	G	G	72	G	G
32	Pi	P	73	Pi	P
33	G	G	74	Pi	P
34	G	P	75	Pi	P
35	Pi	G	76	Pi	G
36	G	P	77	G	P
37	G	P	78	G	G
38	Pi	P	79	Pi	P
39	Pi	G	80	Pi	P
40	Pi	P	81	Pi	P
41	Pi	P	82	G	G

Pilose leaflets : glabrous leaflets = 57 : 25, expected ratio = 2 : 1, x^2 = 0.29, p = 0.95-0.99.
Pubescent twigs : glabrous twigs = 56:26, expected ratio = 2 : 1, x^2 = 0.06, p = 0.95-0.99.

Appendix 8. Vesture of leaflets and twigs in backcross of K409 x (K409 x K480). G = glabrous; P = pubescent.

Plant No.	Leaflet pubescence	Twig pubescence
1	G	P
2	G	P
3	G	G
4	P	G
5	P	P
6	P	P
7	P	G
8	G	G
9	G	P
10	P	P
11	P	P
12	G	G
13	G	G
14	P	G
15	P	G
16	P	G
17	P	G
18	G	P
19	P	G
20	P	P
Total	P : G = 12 : 8	P : G = 9 : 11

Appendix 9. Morphological characters in 75 plants of (K409 x K401) x (K409 x K401).

Plant No.	*Gland type	Pinna No.	Leaflet No.	Leaflet length (mm)	Plant No.	*Gland type	Pinna No.	Leaflet No.	Leaflet length (mm)
1	P_1	3	28	6.5	39	F_1	3	7	10
2	P_1	3	6	4	40	F_1	4	15	7
3	P_1	2	9	8	41	F_1	4	11	14
4	F_1	2	9	4	42	F_1	4	9	8
5	F_1	4	12	11	43	P_1	8	15	9
6	P_2	5	5	6	44	F_1	7	17	7
7	F_1	8	16	9	45	P_2	3	11	8
8	F_1	4	10	12	46	F_1	3	14	12
9	F_1	5	14	13	47	P_2	4	13	7
10	F_1	3	12	7	48	F_1	3	10	11
11	F_1	6	14	11	49	P_1	6	19	12
12	F_1	5	15	9	50	F_1	6	16	11
13	F_1	4	7	8	51	F_1	5	13	17
14	F_1	3	8	7	52	F_1	7	14	10
15	P_1	3	15	10	53	F_1	3	10	14
16	F_1	2	10	5.5	54	P_2	4	13	12
17	P_1	4	13	9	55	F_1	4	15	10
18	F_1	2	10	6.5	56	F_1	3	15	8
19	P_2	3	16	7	57	F_1	3	10	9
20	P_2	3	9	8	58	P_2	7	16	9
21	F_1	6	15	10	59	F_1	6	15	12
22	F_1	2	9	8	60	F_1	4	10	10
23	P_2	2	11	9	61	F_1	4	10	8
24	F_1	4	12	13	62	F_1	7	20	8
25	P_2	2	8	10	63	F_1	3	12	11
26	F_1	4	12	12	64	P_2	7	18	11
27	--	1	7	6	65	P_2	3	12	8
28	P_2	6	13	8	66	P_2	6	14	14
29	F_1	1	7	7	67	F_1	4	11	9
30	F_1	4	20	7	68	F_1	6	14	10
31	F_1	3	10	9	69	P_1	3	9	16
32	F_1	6	17	10	70	F_1	6	12	8
33	F_1	2	9	8	71	F_1	8	25	9
34	P_2	4	10	9	72	P_2	4	8	10
35	F_1	3	13	7	73	F_1	5	12	11
36	F_1	2	12	11	74	P_2	5	14	10
37	F_1	1	17	10	75	P_2	4	13	10
38	P_1	4	19	5					

*Gland type: P_1 = concave type as K409; F_1 = intermediate type as F_1 hybrids; P_2 = convex type as K401.

Appendix 10. Segregations of some characters in (K409 x K405) x (K409 x K405).

Plant No.	*Stem Vesture	**Gland type	No. pinna	No. leaflet	Leaflet length(mm)
1	P	F1	6	18	6
2	G	F1	3	14	7
3	G	F1	3	13	9
4	P	P1	6	14	8
5	P	F1	7	22	6
6	G	F1	3	11	7
7	P	F1	3	15	8
8	P	F1	3	13	8
9	P	F1	4	11	8
10	P	F1	5	12	7
11	G	F1	6	15	7
12	P	F1	4	10	6.5
13	P	F1	3	13	6
14	P	F1	4	11	6
15	P	P1	8	15	7
16	P	F1	4	12	8
17	P	F1	4	13	6
18	P	F1	3	13	6.5
19	G	F1	4	14	7
20	P	P1	10	27	5
21	G	F1	5	11	8
22	P	F1	5	12	6
23	G	F1	4	9	9
24	P	F1	7	15	6
25	P	F1	7	12	7

Totals P : G = 18 : 7; P1 : F1 = 3 : 22.

*Stem vesture: G = glabrous; P = pubescent.

**Gland type: F1 = intermediate type as F1 hybrids;
P1 = concave type as K409.

Appendix 11. Morphological characters of K409 x (K409 x K401).

Plant No.	*Gland	Leaflet No./pinna	Leaflet lenght(mm)
1	F1	20	7.0
2	--	--	--
3	F1	21	9.0
4	F1	23	6.5
5	P1	17	10.0
6	P1	19	6.5
7	F1	19	7.5
8	P1	19	8.0
9	P1	18	9.0
10	F1	22	6.0
11	--	--	--
12	P1	25	8.0
13	P1	26	6.5
14	P1	27	11.0
15	F1	22	7.0
16	P1	22	9.0
17	P1	18	8.0
18	P1	17	10.0
19	P1	21	7.0
20	P1	20	10.0

* Gland: F1 = intermediate as F1 hybrids; P1 = concave type as K409.

Appendix 12. Morphological characters of K409 x (K409 x K405).

Plant number	*Gland type	**Stem vesture	Leaflet number	Leaflet length (mm)
1	P1	P	29	6
2	P1	G	25	7
3	P1	P	26	7
4	F1	G	23	6
5	P1	P	26	8
6	P1	G	24	6
7	P1	P	27	7
8	F1	G	24	8
9	P1	G	28	7
10	P1	G	23	8
11	P1	G	25	6
12	P1	P	22	7
13	P1	P	25	7
14	P1	G	28	8
15	F1	G	26	8
16	F1	P	24	8
17	P1	P	30	6
18	F1	G	31	7
19	F1	G	30	6
20	P1	P	24	6
Total	P1 : F1 = 14 : 6; P : G = 9 : 11.			

* Gland type: P_1 = concave type as K409; F_1 = intermediate type as F_1 hybrids.

** Stem vesture: G = glabrous; P = pubescent.

Appendix 13. Morphological charcters of K405 x (K409 x K405).

Plant No	*Gland type	Leaflet vesture	Stem vesture	Leaflet No.	Leaflet length(mm)
1	P_1	glabrous	pubescent	10	11
2	F_1	glabrous	pubescent	10	11
3	F_1	glabrous	pubescent	10	13
4	P_1	glabrous	pubescent	11	12
5	P_1	glabrous	pubescent	12	13
6	P_1	glabrous	pubescent	10	11
7	F_1	glabrous	pubescent	11	10
8	F_1	glabrous	pubescent	10	14
9	P_1	glabrous	pubescent	12	12
10	P_1	glabrous	pubescent	9	12

*Gland type: P_1 = concave type as K409; F_1 = intermediate as F_1 hybrids.

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