TAXONOMIC RELATIONSHIP OF *OPUNTIA KLEINIAE* DE CANDOLLE AND *OPUNTIA TETRACANTHA* TOUMEY

by

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INTRODUCTION

In general, a younger plant group, other things being equal, will tend to show more uniformity in its members than an older one which has had a longer time to differentiate, and conversely, it would be expected that the members of an older family would show more distinct limits in its genera and species than a younger one. Thus, Clausen (1957) states that usually, in time, subspecies will tend to differentiate into species, and species into distinct genera. It is found that in many cases the genera and species of the Cactaceae are poorly defined (Stanley in Craig, 1945), and a great amount of variability within the same group is present. This is consistent with the idea that the Cactus family is of recent origin, and according to Craig (1945) probably is still actively evolving. Backeberg (1949) believes that the family was evolved in the Cretaceous, while Marshall (1941) even suggests that it might not be older than 10,000 years.

Clausen (1957) points out that one of the important factors in the differentiation of plants into distinct groups is the change in chromosomes and in the genic make-up of the different groups, thus eventually making the groups so genetically different that they become reproductively isolated. This genetic isolation would be found in older groups of plants. Such changes might consist of differences in chromosome numbers, and (or) different chromosomes in the karyotype. It is found by the different workers (Darlington and Wylie, 1955) that the basic number of chromosomes of the Cactaceae is, except in a few cases, uniformly 11. The somatic number is 22 or sometimes 44, but rarely of higher ploidy. It might be expected that the species of this family are able to hybridize rather readily. Both Benson (1950) and Marshall (1953) consider that in Arizona much of the variability in the genus Opuntia results from hybridization between the species. Due to the above-mentioned poorly differentiated groups, high variability, and hybridization, there is considerable difference of opinion on the classification of the cacti into tribes, subtribes, genera, and species. (Backeberg 1958-61, Benson 1950, Buxbaum 1950, 1958, Marshall 1953).

To add further to the taxonomic difficulties, many of the members of the genus Opuntia easily drop their joints which then readily root (Shreve, 1931), thus forming new plants. In this manner, clones of hybrids may be propagated which under other conditions would not survive, with the result that many "new species" of Opuntia are added to the genus. This method of reproduction is in many cases more common than through sexual means. Since morphological means alone do not give definitive information about this kind of phenomenon, other methods of taxonomical investigation must be used to determine the status of members of this genus.

The chromosomes of the individual in question should be studied for mitotic or meiotic number and, if possible, for the determination of the karyotype. This type of study should be extended to plants which seem to have a possible connection with the taxon being investigated.

Pollen of the various plants should be examined to see if important differences are present. Kurtz (1948) has shown that some differentiation of species is possible in Arizona cacti on the basis of pollen characteristics. Some of the pollen characteristics which together may be helpful in determining the closeness of relationship of the different populations are the size and shape of the pollen grains, the size and amount of pitting or reticulation of the exine, and the shape and nature of the germ pores.

The abundance of pollen in the flower as well as the ratio of sterile to fertile pollen is an important indication of hybridization, since many hybrids are sterile. Stebbins (1950) points out that hybrids often reflect this sterility by having a large percentage of sterile pollen.

An investigation embodying these botanical disciplines was undertaken by the author in order to determine the relationships of *Opuntia kleiniae* De Candolle and *Opuntia tetracantha* Toumey, and also to determine the range of these plants in the Southwest and, specifically, in Arizona.

Opuntia kleiniae was described by De Candolle in 1828 from a collection made by Coulter in Mexico and sent to him in France. As is true of many of the descriptions made in those days, this one is very brief. De Candolle describes the new species as:

> Erect, many branched, ashy green, branches erect, cylindric, not tuberculate, arranged in fascicles which spiral to the left. Areoles velvety. Spines of two kinds, one of bristles innumerable in numbers, white to red. Others large, lower open-deflexed, thin, whitish, Stems one finger thick. Stems of plants reminiscent of Kleiniae. Leaves small, oblong, deciduous. Larger spines' length equal to the thickness of a thumb.

This species has been found throughout much of the Mexican plateau, and has been reported in the United States only in western Texas, extreme southern New Mexico, and southeastern Arizona to Douglas. Although found at lower elevations, its range extends above 5,000 feet in Texas and Mexico.

Opuntia tetracantha was described by Toumey in 1896, who found it five miles east of Tucson, Arizona. His description, unlike that of De Candolle, was quite precise:

> Irregular branching shrub 6 to 15 dm. Primary branches erect or ascending from a stout woody trunk 5 to 8 cm. in diameter and bearing numerous short, lateral branches at irregular intervals; ultimate branches 12 to 15 mm. in diameter; joints cylindrical, 25 to 30 cm. long with a reticulated woody skeleton; tubercles at first prominent, 16 to 22 mm. long, but on old stems more or less inconspicuous; pulvinii sparingly covered with wool and bearing a small crescent shaped tuft of light brown bristles at the upper margin; spines usually four, stout, loosely sheathed, straw colored, strongly deflexed, flattened, 2 to 3.5 cm. long, occasionally one or two smaller ones, not

increasing in size or number after first season's growth; glands conspicuous, a half dozen or more between the spines and bristles; flowers greenish purple, 1.5 to 2 cm. broad; fruit ovate to subglobose, narrowly but deeply umbilicate, 2 to 2.5 cm. long, juicy, scarlet, usually nearly smooth, but sometimes some of the pulvinii bearing one to three strong, deflexed spines; seeds irregular, 3 to 5 mm. in diameter, commissure broad, with conspicuous spongy appearance.

O. tetracantha has been found on the desert in the vicinity of Tucson, in the foothills of the Rincon and Santa Catalina Mountains, and north to Florence; it has also been found in Organ Pipe National Monument, and south of the border in San Luis, Sonora. It has not been reported as growing much above 3,000 feet.

MATERIALS AND METHODS

In order to study the taxonomical relationships of the different populations of the Opuntia kleiniae complex, several lines of investigation were followed. The methods used are explained below.

Distribution of Plants:

The plants native to Arizona are not abundant in any one spot, but occur scattered over a large territory so that it is easy to search a considerable area and find only a few plants. For this reason, and since Arizona is difficult to explore, due to its large size and wilderness, it has not been attempted to determine very accurately all the places where members of this complex occur in the state; rather, the general distribution was inferred from all the collections made and from the literature.

External Gross Morphology:

The morphology of the stem was studied, with special attention given to the diameter of joints, size and appearance of podaria, character of spines and glochids, and appearance of areoles. The flowers were observed and their size, shape, areoles, and floral parts noted. The fruit shape and color were also studied.

Phyllotaxy:

To determine the phyllotaxic ratio, and areole chosen arbitrarily was numbered 0, the next higher areole 1, the next higher 2, etc., up to the final areole (number 8 in Figure 1); this final areole is the first areole immediately above areole number 0. The distance between areole 0 and the final areole, I have called the "internodal distance."

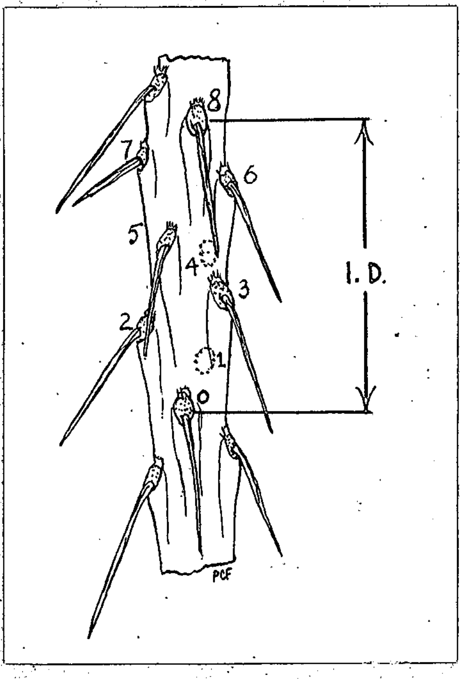


Fig. 1. Phyllotaxic ratio: 3/8

I. D. : "Internodal distance"

◌ : Areoles in back of stem

The final areole number (8) is then equal to the number of areoles present above areole 0 and is given as the denominator of the ratio. The number of times that it is necessary to circle the stem during the determination is given in the numerator (3). Thus, in the example in Figure 1, 3/8 is the phyllotaxic ratio. The above method of determining the phyllotaxy as well as the manner in which Figure 5 was diagrammed, is outlined by Kerner and Oliver (1904, p. 396).

Cytology:

Branches were rooted in water or Perlite, and the resulting root tips were cut, killed, and fixed in Navashin's solution as described in Johansen (1940, p. 45), where they were left 24 to 48 hours. They were then transferred to 50% alcohol and dehydrated by the method outlined in Johansen (1940, p. 130), and then imbedded in paraffin. The time of greatest mitotic activity was determined by killing root tips at different times of the day. The root tips were all cut on a rotary microtome to a thickness of 10 microns. Staining was done according to the Newton crystal violet method (Johansen 1940, p. 159). The sections were viewed under high dry power (43X objectives) and under oil immersion (97X to 100X objectives). In practically all instances, chromosomes were only distinguishable under oil immersion at magnifications of 970X to 2000X. To increase the resolution of the chromosomes, several different filters were tried, of which orange and red (Nos. 22 and 29) were found to be the best.

Pollen Examination:

Mature pollen was collected and imbedded in gelatin on microscope slides according to the method described by Kurtz (1948); only basic Fuschin stain was used. When at all possible, pollen was removed from the anthers of the plant. In many cases, such as dried specimens, this was not possible; pollen already shed had to be picked up from around the dried flower. The size, shape, and nature of the exine of the pollen, as well as the shape of the germ pores, was observed under low power.

Cell Size Determination:

The epidermal layer was removed from the leaves and stems and made into wet mounts; these were examined under low power. The size and shape of the cells and of the stomates was noted. Cells of root tips were also measured.

Internal Anatomy of Root Tips:

Root tips 2 to 3 mm. long were killed in FAA (Johansen 1940, p. 41) and left there 24 hours, then transferred to 50% alcohol, dehydrated as described under cytology, and imbedded in paraffin. The 10 microns thick longitudinal sections were left 24 hours in Safranin and further stained with Fast Green and Orange G. The vascular system as well as the epidermis and crystal inclusions were examined.

Sources of Plant Material Used:

The plants described below will be referred to in tables and in the text by the names and abbreviations written after them in parentheses.

Some of the plants examined were collected by me at the following localities: in Texas, 8 miles south of Kent at about 4,600 feet (Texas A to G); Texas, Indian Lodge, Davis Mountains State Park at 5,500 feet (Texas H). In Arizona, 5 1/2 miles northeast of Globe at 4,400 feet (Globe); at Lower Bear Canyon picnic grounds in the Santa Catalina Mountains at about 2,800 feet (L.B.C.); at the southwest base of Tumamoc Hill, Tucson at 2,450 feet (Tucson); by side of road somewhere between Showlow and Holbrook, between 5,000 and 6,500 feet (Northern Arizona or N.A.); in Sonora by San Carlos Bay near Guaymas at sea level (San Carlos).

Acknowledgments:

I wish to thank the following individuals, who have been kind enough to provide me with living plants:

Dr. Helia Bravo H., Mexico City, who sent me cuttings from Hidalgo, Mexico; Dr. Barton Warnock, Alpine, Texas, who sent me cuttings from Musquiz Canyon, 11 miles north of Alpine at about 4,600 feet (Texas L); Messrs. Hubert Earle and John Weber of Tempe, Arizona, for a plant from Gisela, Arizona, at about 2,500 feet (Gisela); Mr. Palmer Rogers of Tucson for a cutting from the Chiricahua Mountains foothills, Arizona (Chiricahua); Mr. P. G. Nichols of Tucson for a cutting from 10 miles north of Alpine, Texas (Texas M).

I also want to thank the Curators of the Gray Herbarium and of the U. S. National Herbarium for loaning me herbarium material of Opuntia kleiniae and Opuntia tetracantha including the type specimen of the latter species.

I wish to express my appreciation to Dr. Robert Harris, Dr. Edwin Kurtz, Dr. Charles Mason, Dr. Walter Phillips of the Botany Department; Dr. Alice Boyle of the Plant Pathology Department; and Mr. Donald Sayner of the Zoology Department, who have given me much help in carrying out my research and in the preparation of this paper.

DATA AND RESULTS

As a result of the investigations undertaken, the following sets of data were obtained.

Habitat:

The plants collected in Arizona and Sonora, although growing in dry regions, seem to be found in the wetter localities. For instance, the specimen collected at San Carlos Bay, Sonora, in a very dry environment, approximately 50 yards from the waters of the bay, nevertheless, grew within a few feet of a major wash. Some moisture from the sea breeze may also have increased the available water. All except one of the specimens collected near Tucson were in close proximity to washes. The plant received from Gisela reportedly grew near Tonto Creek; the exact distance from the creek is not known. The specimens from Lower Bear Canyon all grew on the edge of a large canyon wash. No habitat information is at hand for the plants from the Chiricahua foothills, nor for those from northern Arizona. The plants from the vicinity of Globe grew at an elevation of 4,400 feet and were conspicuously growing near the tops of hillsides; but at that altitude, in this location, the increased rainfall would probably offset the drier habitat (see Table I).

Table I. Altitude and Average Yearly Rainfall* of Towns Nearest Collection Sites.

Locality		Altitude in feet	Rainfall in inches
Gisela	Arizona	2, 500	16 1/2
Globe	"	3, 450	15 1/2
Hillside	"	3, 850	10
Holbrook	"	5, 080	8
San Simon	"	3, 600	8 1/2
Showlow	"	6, 500	10 1/2
Tucson	"	2, 400	10 1/2
Alpine	Texas	4, 480	15 1/2
Fort Davis	"	4, 930	16 1/2
Kent	"	4, 250	9 1/2

* U. S. Weather Bureau Climatological Data.

Note: Altitudes have been rounded off to nearest 10 feet.
Rainfalls have been rounded off to nearest one-half inch.

The plants from Texas all grew at higher elevations: 4, 500 feet up to 5, 500 feet (the highest the author collected them), and usually in locations of higher rainfall (Table I), but often not near any water course or wash. Most of the plants grew in profusion on steep hillsides and made up a conspicuous part of the vegetation, unlike any of the Arizona plants.

In Table I, the rainfall of the towns nearest the collection localities is given, as this information is not available for the actual collection sites. In some cases the elevations of these towns differ markedly with that of the plant localities, with a resulting difference in the amount of rainfall. The plants collected from the Globe region grow at an elevation 1,000 feet above the town, and thus must have somewhat higher rainfall than Globe. The Chiricahua foothills receive more rain than does San Simon, since the latter is in the valley. Kent, Texas, although only 8 miles from the closest collection locality, has a much drier climate than the Davis Mountains at whose base it is located. The rainfall where the Texas A to G plants were collected is much more like that of Alpine or Fort Davis, judging from the altitude (ca. 4,600 feet) and the vegetation.

Growth Habits:

The size and habit of the plants were variable. The Sonoran plant, of which only one specimen was seen, was about 0.60 m. high with thin joints 12 mm. in diameter on the average. The Tucson plants have few branches, and are about 0.55 m. high. The main branch or trunk is quite woody for the size of the plant and is about 30 mm. in diameter at its widest point; the terminal branches are up to 10 mm. in diameter. The plants growing in Bear Canyon represent the largest specimens seen in Arizona and grow to a height of 1.2 m. with a thick trunk 90 mm. in diameter. One specimen, unfortunately a dead plant, measured 1.8 m. and had a trunk 0.11 m. in diameter. The northern Arizona plant was prostrate and small. Plants from Globe grew no higher than 0.40 m. in their habitat and usually less. The plants were prostrate to semi-prostrate, and the largest branches not much more than 25 mm. in diameter. There is no information on size attained by the plants from other localities in Arizona. The majority of plants from Texas exhibit a more robust size and form of growth than those from Arizona. The main trunks are 50 to 60 mm. in diameter or larger in robust specimens; terminal joints are 12 to 13 mm. in diameter and up to 0.40 m. long. Older joints are considerably thicker, and the plants reach a height of from 1 1/2 to 2 m. or more. The spines all are very prominent with showy sheaths (see Figures 9-12).

It is interesting to note that after being transplanted to my garden in Tucson, some of the plants described above did not retain their original size or growth form. This is especially true of the more northerly Arizona plants. The plants cited were all started in the garden as cuttings. The plant from Globe after three years is no longer prostrate, but resembles in habit, length of branches, and height the Texas plant of the same age. They are both about 0.85 m. high. The plant from northern Arizona is no longer prostrate after two years; it now measures 0.35 m. The Texas plant looks much like those in their native habitat, but it is too soon to know how large it will become. The other plants have not been in the garden long enough to show any changes, or are from the vicinity and have not sustained much of a change of habitat.

Possibly some of the variations have resulted from the additional water all have received as garden plants (see Fig. 13).

Morphology:

All measurements and comparisons were made on live material in healthy condition except for the plant from Sonora which died before measurements were made; all data for this particular plant were obtained from dried specimens. The plant when alive looked very much like the Tucson plants. The plant from Gisela was in poor condition when received, and has not recovered to date so that no data could be obtained from it. It, too, looked very similar to the Tucson plant.

Flowers were all observed in the spring and early summer of 1961; these were the blooms of the plants growing in my garden. Although it had been expected that all the plants were healthy and mature enough to flower, most did not. Only the plant from Globe gave satisfactory results; several dozen blooms were observed on this plant. The anthers were full of fertile pollen, and many fruits were set. The Bear Canyon plant had many buds, of which four or five matured and were photographed and pressed immediately; unfortunately, no other buds opened, and measurements had to be made from the pressed flowers and the photographs. The Texas plants only initiated four buds of which only one reached anthesis. The other three developed to full size and color, but never expanded. The open flower was sterile, and an examination of the aborted anthers revealed only five or six pollen grains. One dried flower of Opuntia kleiniae from the Davis Mountains was seen; no pollen was found inside or outside of its anthers. No other plants initiated flowers; consequently, the description of flower morphology is very incomplete.

Figures 2 and 3 are photographs of the flowers of the Globe and Bear Canyon plants. Table II gives a comparison of these flowers with the addition of the one flower from Texas. It also should be mentioned that the petals of the Globe plants are not completely reflexed, while those of the Bear Canyon plants are. As all the flowers and buds of

Fig. 2. Flower of Globe plant.

Fig. 3. Flower of Bear Canyon plant.

Opuntia kleiniae from Texas and central Mexico which I have seen, have been of a violet color, in agreement with the descriptions in the literature, it comes as a surprise to learn from Dr. Warnock (personal communication) that the plants of O. kleiniae which he sent me (Texas L) had greenish yellow flowers.

TABLE II

FLOWER CHARACTERISTICS

		GLOBE	LOWER BEAR CANYON	TEXAS (ONE FLOWER)
PERIANTH	NUMBER	USUALLY 16	USUALLY 16	16
PERIANTH	OUTER	PINKISH BROWN, DARKER ON MID-STRIPE, ACUMINATE	REDDISH BROWN, SOME EDGES LIGHTER, ACUMINATE	PINKISH BROWN IN CENTER TO GREEN AT EDGES, ACUMINATE
PERIANTH	INNER	OBOVATE TO OBCORDATE, CUSPIDATE, SOMETIMES EMARGINATE, CUSPIDATE; LIGHT PINK ON VIOLET SIDE, MID-STRIPE BROWN TO RED AT TIP 17 MM X 13 MM	LINEAR TO RHOMBOID, CUSPIDATE; PINKISH ORANGE TO BROWN WITH DARKER MID-STRIPE 17 MM X 9 MM	OBCORDATE TO OBOVATE, CUSPIDATE; LIGHT TO DEEP VIOLET, GREEN ON MID-STRIPE 22 MM X 12 MM
OVARY	INNER DIMENSIONS	CHAMBER 6 MM LONG OVULES 1.0 MM LONG	OVULE $\frac{1}{2}$ MM LONG	CHAMBER 9 MM LONG OVULES 1.1 MM LONG
OVARY	OUTER DIMENSIONS	20 MM X 12 MM	15 MM LONG (DRY)	24 MM X 16 MM
OVARY	APPEARANCE	TUBERCULATE; AREOLES WITH GLANDS, FEW OR NO SPINES, LEAVES 7 MM LONG	TUBERCULATE; AREOLES WITH GLANDS, 0 TO 5 SPINES, 5 TO 6 MM LONG	TUBERCULATE; AREOLES WITH GLANDS AND SPINES 6 TO 7 MM, LEAVES 10 MM
STYLE		17 MM X 2 MM	12 MM X 1.3 MM (DRY)	21 MM X 2.3 MM
FILAMENTS OF STAMENS		GREEN TO YELLOW	GREEN TO PINK	GREEN TO VIOLET
STIGMAS		5	5	4

In order to gain more information about the flowers of this complex, an attempt was made to induce flowering out of season. The normal flowering time is April to May, when the days are getting longer. This period of flowering does not indicate whether the initiation of flower primordia is influenced by long or short days. To test both periods of day length, some plants were put in boxes in which the duration of exposure to light could be controlled. These plants were kept two to three weeks on eight hours of daylight to simulate winter conditions, and then the day length was increased to 24 hours for several weeks. No flower formation was achieved in either case.

As in the case of flowers, few fruits were available on any except the Globe and Bear Canyon plants. The fruits of these two populations are alike except for the presence of some short spines in the case of Bear Canyon plants. They are obovate, slightly tuberculate, and umbilicated rather deeply and convexly; the color is mottled red-orange to yellow; the length is between 25 and 30 mm. on fully mature specimens. Apparently, not all the fruits mature; many remain green and undersized. In many instances the areoles of the fruits of the Bear Canyon plants give rise to new flowers and sometimes new joints in succeeding seasons. Fruit proliferation is also found on Globe plants, but to a lesser extent. The one mature Texas fruit seen was obovate, slightly tuberculate, orange-red, and 32 mm. long.

The width of year-old joints varied but little, but the length of the podaria was variable. As may be seen from Table III, the Texas plants have longer podaria than the other plants. The plants from Globe on the contrary have slightly shorter podaria than the other Arizona plants.

In all plants examined, the spines consist of two kinds: the very prominent sheathed centrals described in Table IV, and a set of very small accessory spines about 4 mm. long, bearing no sheaths, straw colored and somewhat translucent. The number of accessory spines may vary from none in some areoles to as many as eight in others. These accessory spines are found at the lower part of the areoles below the centrals.

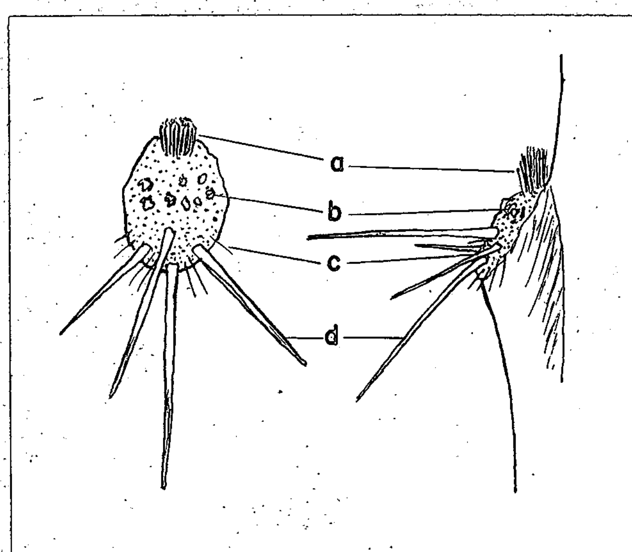


Fig. 4. Typical areole of a plant of the O. kleiniae complex.
a. Glochids
b. Nectar glands
c. Accessory spines
d. Central spines

TABLE III

BRANCH CHARACTERISTICS

		GLOBE	L.B.C.	TUCSON	N. A.	CHIRICAHUA	TEXAS
DIAMETER OF YEAR OLD BRANCHES		11-12 MM	9-11 MM	9-10 MM	c. 10 MM	c. 10 MM	12-13 MM
PODARIA	LENGTH	12-15 MM	14-16 MM	14-15 MM	c. 17 MM	c. 16 MM	18-20 MM
	WIDTH	4-5 MM	4-5 MM	4-5 MM	c. 4 MM	c. 3 MM	5-7 MM
	PROMINENCE	NOT VERY	NOT VERY	NOT VERY	STRONGLY	MODERATELY	VERY STRONGLY

The number of central spines per areole is constant in some plants, and quite variable in others, and may even vary from one joint to the next. The same pattern of variation is found in all populations. The spines range from slightly to strongly deflexed; this effect is usually more marked on the middle lower spine and more often seen on older joints. The northern Arizona plant has conspicuously more porrect spines with the upper middle spine being the longest. The data in Table IV show the uniformity in sizes between the non-Texas plants, and the consistently larger spines in the Texas plants.

All the plants have similar glochids situated at the upper part of the areoles, their color being orange when young and straw color in age. Older joints grow new glochids which tend to be longer than the original ones. These secondary glochids are somewhat longer in plants from Globe than in those from other populations: ca. 3 mm. as compared to 1.5 to 2.5 mm. for the other Arizona plants, and 1.8 to 2.0 mm. for the Texas plants.

In the upper part of the areoles, below the glochids, are located nectar glands which are more prominent on young joints; the number of glands range from three or five to many and are found in plants from all the populations.

The epidermis of all the plants appears very similar: green to grayish green on older joints. Texas plants are usually a little more bluish gray. In cold weather all plants turn purplish below the areoles

TABLE IV

SPINE CHARACTERISTICS

	GLOBE	L.B.C.	TUCSON	NO. ARIZONA	CHIRICAHUA	SAN CARLOS	TEXAS
NUMBER	1 TO 4 SOMETIMES 6	1 TO 4	3 TO 4 OR FEWER	USUALLY 4	1 TO 3	1 TO 3	1 TO 4
LENGTH	8 TO 26 MM	6 TO 13 MM	12 TO 32 MM	12 TO 21 MM	7 TO 14 MM	15 TO 22 MM	10 TO 40 MM
WIDTH	0.4 - 0.5 MM	LIKE GLOBE	LIKE GLOBE	LIKE GLOBE	LIKE GLOBE	LIKE GLOBE	c. 0.80 MM
SHAPE	ACICULAR, FLATTENED ONLY AT BASE	"	"	"	"	ACICULAR, FLATTENED MOST OF THE LENGTH, BUT NOT AT TIP	ACICULAR, FLATTENED MOST OF THE LENGTH, BUT NOT AT TIP
COLOR	WHITE AT BASE TO RED ORANGE AT TIP; OR DARK RED TO ORANGE	WHITE AT TIP TO DARK RED AT BASE; OR WHITE; OR DARK RED ALL OVER	WHITE AT BASE TO DARK RED AT TIP	REDDISH AT BASE TO YELLOWISH AT TIP	WHITISH, ORANGE AT TIP	GRAYISH RED TO YELLOW AT TIP	WHITISH AT BASE TO YELLOWISH ORANGE OR REDDISH AT TIP
SHEATHS	0.5 - 0.8 MM WIDE	LIKE GLOBE	LIKE GLOBE	UP TO 0.9 MM WIDE	NOT SEEN	NOT SEEN	0.8 - 1.3 MM WIDE

or over the whole joint depending on the severity of conditions. In addition to the normal heavy coat of cutin on the epidermis of the stem, there are, on all the plants examined, a large number of circular waxy secretions which seem to be peltate in shape and probably are secreted by glands in the epidermis. These secretions are of the order of 30 microns in diameter.

Although few actual measurements were made, it was noted that the new roots formed in water were consistently thicker in the case of Texas plants. The root of one of the Texas plants was 450 microns in diameter, while the root of a Globe plant of the same age measured 335 microns at equal distance from the tip.

Phyllotaxy:

By the method described on page 7, the phyllotaxy of the different populations was determined. In all but one case the phyllotaxic ratio was found to be 3/8; the one different plant, from Tucson, showed a 3/7 ratio. The determination of "internodal distances" points out the existence of a definite difference between the Texas plants and those from Arizona and Sonora. As can be seen in Table V, all the Arizona populations are similar to each other, while the Texas and Mexican highlands plants all have larger "internodal distances." This size difference is important since, according to Anthony (1956), the distance between nodes in Opuntias is a valid criterion for taxonomical differentiation.

Table V. "Internodal Distances" in Millimeters.

Plant	Measurement
Chiricahua	25 to 32
Gisela	no data
Globe	24 to 30
L.B.C.	26 to 30
San Carlos	ca. 25
No. Arizona	24 to 26
Tucson	20 to 30
Texas and central Mexico	36 to 55

The phyllotaxy of the Arizona and Texas populations have been diagrammed respectively in Figures 5A and 5B. Both diagrams have been drawn to the same scale. The "internodal distances" are estimated average values of 45 mm. for the Texas plants, and 25 mm. for the Arizona plants. It can be seen that many more nodes are present per unit length of stem in Arizona than in Texas plants, giving the former a more crowded appearance.

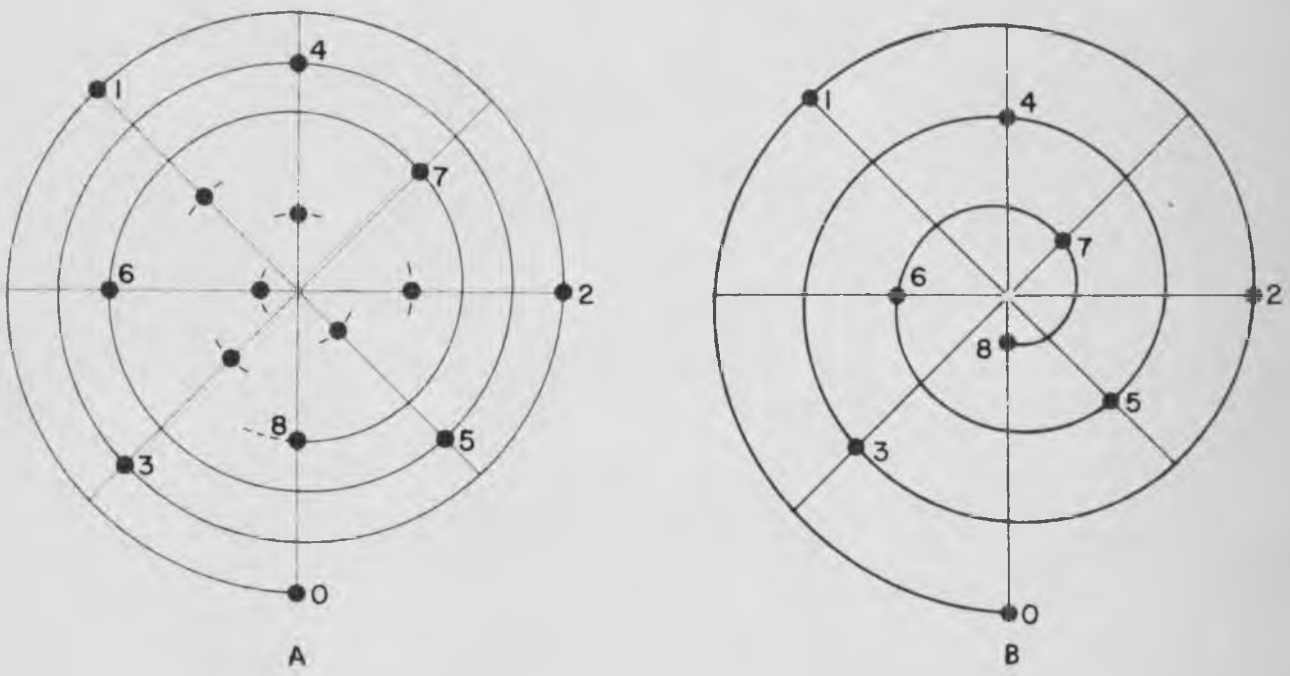


Fig.5 Phyllotaxy in the Opuntia Kleiniae Complex.
A. Arizona & Sonora Plants B. Texas Plants
Each circle represents a node.

Cytology:

During the Spring and Summer, mitotic activity was high between 8 and 11 AM, but beginning in September none could be observed, nor would cuttings root. By exposing the cuttings to 14 hours of continuous light and warmth, roots appeared, and renewed mitotic activity was established, showing the dependence of growth and the mitotic cycle on day length and temperature.

Due to the very small size of the chromosomes involved (1 to 2 microns long), it was not possible to determine more than the somatic number; and even this turned out to be difficult as the resolution of the oil immersion objectives was barely sufficient to separate the chromosomes in some cases. On some plants, in spite of all attempts, no mitotic figures could be obtained. Such cases were the plants from Gisela and Texas (B and D). The plant from northern Arizona was not examined, and the one from Sonora died before any attempts could be made. All the Arizona material examined showed a somatic number of 22, that of Texas 44 except for plants A, C, and G which had 33 chromosomes; plants A, B, C, and D looked very sickly and dwarfed, while plant G looked fairly normal.

Fig. 6. Diploid nucleus at metaphase of mitosis.

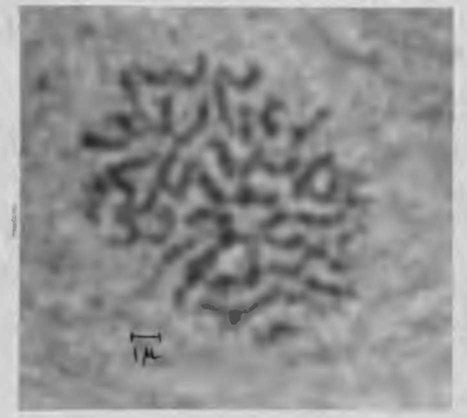


Fig. 7. Tetraploid nucleus at metaphase of mitosis.

Table VI. Somatic Chromosome Numbers.

Texas A	33	Globe, Arizona	22
" B	no count	L.B.C. "	22
" C	33	Tucson "	22
" D	no count	Gisela "	no count
" E	44	Northern "	no count
" F	44	Chiricahua "	22
" G	33	San Carlos, Sonora	no count
" H	44	Opuntia leptocaulis (Texas)	44
" J	44	O. kleiniae X O. leptocaulis	33
" K	44		
" L	44		
" M	44		

Note: For an explanation of the letters in the table, see "Sources of Plant Material Used on pages 9 and 10.

Pollen Morphology:

Kurtz (1948) states that pollen of cylindropuntia has pitted exine; in all the pollen examined in the present study, this was found to be so. The pitting was coarse in all cases, with some variation in the degree of coarseness.

Except for the material from the Mexican Plateau, the size of the pollen was relatively uniform (Table VII). The shape of all the pollen examined was spherical, and the germ pores of all, except those of two plants, showed a slight convexity. The type specimen of *Opuntia tetracantha* had pollen with concave pores; this was also true of one plant from Texas. The pollen of this last plant did not seem to be completely mature (Texas L).

In addition to the type specimen mentioned, other herbarium material seen were the three plants from Coahuila, Ixmiquilpan, and Zacatecas. As the pollen on these sheets had to be picked off the surface of the dried flowers, I am not completely sure that it all originated from the plant on the herbarium sheet.

Table VII. Pollen Grain Characteristics.

Site	Size in μ	Pores
Globe	72-76	convex
L.B.C.	71-76	convex
O. tetracantha (type specimen)	74-86	concave
Tucson	62-73	convex
Texas K	74-77	convex
Texas L	64-77	concave
Coahuila	81, 86	convex
Ixmiquilpan	96, 100, 105	convex
Zacatecas	60-76	convex

Internal Anatomy of the Root Tip and Cell Size Determination:

The longitudinal stained sections of root tips showed the presence in all cases of crystals in the epidermal layer, probably calcium oxalate. No differences were noted in the internal anatomy of the roots. The plants all had secondary spiral thickening of the vessels.

The cells visible in cross-sections of the roots were on the average 15% to 25% larger in the Texas plants than in the Arizona plants.

No difference in size between the epidermal cells of the stems and leaves of Texas and Arizona plants could be found; the stomates in these plants were of nearly identical size and shape. The guard cells and accessory cells were similar in number and morphology.

CONCLUSIONS

The *Opuntia kleiniae* complex, as shown by the data presented above, is made up of several populations with many similarities and a few differences. These similarities and differences can be assessed as follows.

The general growth habits are similar and differ mainly in the size and height attained by the plants. It was seen, however, that under similar environments these different population plants tend to be more uniform in their habits and sizes. Since these plants have not been growing in the same environment for a long enough time to reach their maximum sizes, it is not possible to know whether they would all grow to similar dimensions, but it is certain that they would be more uniform than in their normal habitats. The diameter of branches is nearly identical except for Texas and Globe plants; these last two having slightly thicker joints.

Although the length of the podaria varies in the Texas and Globe plants, the shape and general morphology of the podaria is quite constant. The morphology of the areoles is the same in all plants, with similar glands and glochids; the length of the glochids is constant, with the exception of the Globe plants which exhibit slightly longer glochids on older joints.

The number, color, and length of spines are quite variable, but roughly the same amount of variation is found in all populations. Accordingly, this variation cannot be used to distinguish between populations.

The phyllotaxic ratio does not vary; only the "internodal distance" varies, but this distance is only a measurement of size, not of phyllotaxy.

The little that has been seen of the flowers indicates a close relationship between the different plants. There are, however, some very striking differences: the shape of the petals of the Globe plants, and the color variation of the perianth in the three populations observed (Table II). It is a well-known fact that flower color in the genus Opuntia is extremely variable within a species, and is a poor criterion for taxonomical differentiation. In view of this, the variation in color of the flowers examined did not seem to be excessive.

Pollen grains show minor variations within some populations, but their characteristics are fairly uniform over the whole complex.

Finally, the one major difference is that of chromosome numbers. The fact that plants from Texas have twice the number of chromosomes found in the Arizona plants, and are consistently larger in their proportions, makes it evident that there is a taxonomical difference present.

From the above discussion it has been shown that the similarities between the various Arizona and Sonora plants are significant, while the differences are small and are more typical of variation within a species than between species. It, therefore, seems likely that all the populations of Arizona and Sonora plants fit best under the description of Opuntia tetracantha; the Texas plants and those from the Mexican Plateau have been described as Opuntia kleiniae. It remains to be determined whether these two taxa are distinct at the species level.

Toumey when he described O. tetracantha mentioned that the differences that set this species apart were the habit of having two kinds of joints: long ones and very small ones, not bigger than fruits; deflexed spines; bright scarlet fruits; and cork-like margins of the seeds. Although Arizona plants examined do have long and short joints, no joints as small as fruits were seen; to a lesser extent this difference in joint size has also been noted in Texas plants, but there the disparity in size is less marked. The spines of O. kleiniae from Texas and Mexico also are deflexed. According to Britton and Rose (1919), O. kleiniae also has scarlet fruits; the one mature fruit that I have seen was bright red-orange. As far as a corky margin of the seeds is concerned, this character is common to several species of Opuntia.

It seems, therefore, that the characters described by Toumey as differentiating O. tetracantha from O. kleiniae are really nonexistent. This is in agreement with the results of this research. Nevertheless, as mentioned above, there are differences between the Texas plants and those from Arizona. I do not believe that these differences are sufficient to recognize two species, but rather that O. tetracantha and O. kleiniae represent two subspecies within a species complex. Evidence indicates that the Texas plants are triploids and tetraploids, whereas Arizona and Sonora plants are the diploid form.

Both Lyman Benson (personal communication) and Marshall (1953) have reached the conclusion that O. tetracantha is only a variety of O. kleiniae, albeit without knowledge of the cytological data.

There are some additional factors besides the difference in chromosome numbers and larger size which support polyploidy in O. kleiniae. The cells of the roots are larger in the Texas material than in the Arizona plants. In Texas, O. kleiniae occupies a much wider range of habitats than the Arizona diploids, because it is found both on dry hillsides and in proximity of water, in the same locality; it also occurs at higher altitudes, and its geographical range is many times greater. According to Stebbins (1950), polyploids are usually better adapted than the corresponding diploids to a wide ecological and geographical habitat.

Stebbins states that very often the cells of the epidermis, and especially the stomates of polyploids, are larger; this was not found to be so in this case.

Stebbins also states that pollen of polyploids is usually larger, but that in autopolyploids the pollen is often poorly represented and much of it sterile. In the case of O. kleiniae from Texas very little pollen was found, and in some of the flowers, none. This pollen was no larger than that of diploid plants from Arizona and may have been immature due to some form of sterility; as a matter of fact Dr. Edwin Kurtz, who examined some of the Texas pollen, was of this opinion. The pollen from the Mexican material (Table VII) was much larger and consistent in size with what might be expected in a polyploid of this species.

Since O. kleiniae does not differ appreciably from O. tetracantha except in size, and does exhibit strong pollen sterility, there is good reason to believe that it is an autotetraploid.

It remains to try to explain the presence of Texas plants having a somatic chromosome number of 33. These plants could only arise in the present situation by either the crossing of a tetraploid plant with its diploid, or by hybridization with another species having a somatic number equal to the diploid (in this case 22). Since no diploid plants of O. kleiniae have been found in Texas, it seems most likely that the second explanation is the correct one, although it is entirely possible that sometime a diploid may be found there. Opuntia leptocaulis grows with O. kleiniae in that region of Texas and appears similar enough to it that hybridization seems possible. Such a hybrid between these two species has been reported by Anthony (1956), and I have a plant growing in my garden which is intermediate between the two species in its vegetative and floral characteristics. The somatic chromosome number of this hybrid is 33, but its putative parent O. leptocaulis does not have the expected 22 chromosomes (only one plant examined); like O. kleiniae it has 44 chromosomes. As there were many plants of O. leptocaulis in the Davis Mountains region, it is possible that there are plants of this species with a chromosome number of 22 which could be one of the parents of the hybrid, but at this time the parentage of this hybrid cannot be determined. Nevertheless, it seems very likely that the other plants of O. kleiniae which have a chromosome number of 33 are also hybrids of the same parentage as the hybrid discussed above.

There are, therefore, two populations of O. kleiniae: a tetraploid growing from the Davis Mountains of Texas south into Mexico, and a diploid spread over much of eastern Arizona and perhaps western Arizona (see Map, Fig. 8). It has generally been reported in the literature that O. kleiniae occurs as far west as Douglas, Arizona; the preserved material of O. kleiniae collected by Dr. Lyman Benson east of Douglas and deposited at the University of Arizona Herbarium was examined: the dimensions of its joints including the "internodal distance", do not fit those of the tetraploid population. If the plants growing east of Douglas are diploid, then the ranges of the diploid and tetraploid populations do not come in contact, unless in a very restricted region of extreme southern New Mexico and northern Chihuahua. It is possible that further southwest in Mexico these two populations do meet.

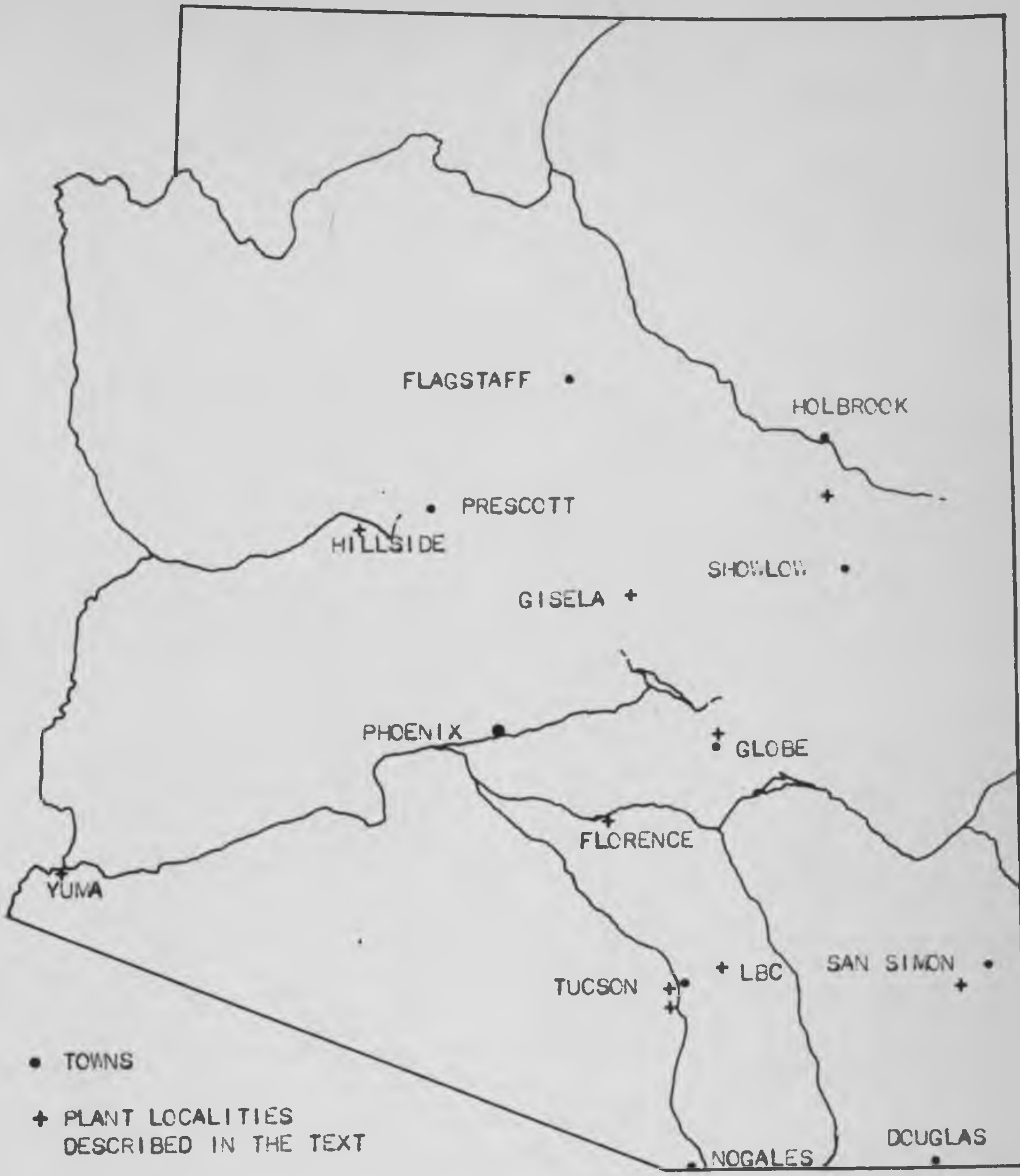


Fig. 8. ARIZONA

In 1909, Griffiths (1909) described a new species of cholla from Hillside, Arizona, which he named Opuntia congesta. He stated in his description that this cactus resembled somewhat O. whipplei, O. arbuscula, and O. kleiniae, but in his opinion differed from all these enough to call for a new specific status. This taxon has been reduced to synonymy under O. arbuscula by Britton and Rose (1919) and others. After reading Griffiths' description of this plant, and looking at the photographs taken by him, it is my opinion that O. congesta resembles most closely the Globe population of the diploid group of O. kleiniae. No actual specimens of this plant have been seen and, consequently, no final decision can be made on its status at this time.

Baxter (1933) reports that some plants which seem to be identical to O. congesta were found near Yuma, Arizona. If the identification of this species is correct and it belongs in this complex, then the range of the diploid does indeed extend throughout the southern half of Arizona, south of the 35th parallel, and south to Guaymas, Sonora.

The distributional pattern of the diploid and tetraploid populations differ. The diploids are scattered in small disconnected colonies throughout the southern half of Arizona and Sonora, and minor morphological differences can be detected between some of these colonies, whereas the tetraploids seem to cover a large area which is not discontinuous at least in the Davis Mountains.

Since it is assumed in this paper that the tetraploid is derived from the diploid, it is of interest to note that the two populations are at present discontinuous or nearly so. The tetraploid must have arisen sometime in the past with the eventual separation of the two populations. It is not possible, of course, to determine where the tetraploid originated nor when this happened. Nevertheless, from the very discontinuous distribution of the diploid in Arizona, it seems likely that it is a relic population from a time when conditions were more favorable; that is, somewhat wetter. This discontinuous distribution is usually characteristic of declining populations. It is also known that the climate of the Southwest has become drier in the last few million years. In contrast, the tetraploid has been able to spread and become well established over the Mexican Plateau and in Texas.

In order to determine more thoroughly the actual relationships of the different members of the O. kleiniae complex, not only in the United States but throughout Mexico, more studies need by undertaken. Longer range investigations involving a program of self and cross pollination should be undertaken with an analysis of the characters of the F_1 generation. The determination of the ability of certain populations to cross, and the hybrid nature of the parents could be expected from such a program. Some of the special techniques, such as scatter diagrams and hybrid indices, etc., mentioned by Anderson (1949, p. 83) and applied to large samples of each population, would also prove very valuable.

In conclusion, I believe that the Opuntia kleiniae complex is best expressed as a single species with two subspecies and two varieties. The following key can be used to separate the four taxa:

1a. Plants 2 m. or more tall. "Internodal distance" 36-55 mm. Podaria to 20 mm. long, extremely prominent on young joints. spines to 40 mm. long and 0.8 mm. wide. Flowers violet. Polyploid (44). . . . . 1. O. kleiniae subspecies kleiniae

1b. Plants prostrate to 1 m. tall, very occasionally to 1.8 m. in plants from L.B.C. "Internodal distance" 20-32 mm. Podaria to 17 mm. long, not very prominent to prominent on young joints. Spines to 27 mm. long, occasionally to 32 mm., and 0.5 mm. wide. Flowers reddish to brownish violet, not pure violet when fresh. Diploid (22). 2. O. kleiniae subspecies tetracantha

2a. Year old joints 9-10 mm. in diameter. Podaria 14-17 mm. long. Glochids on older joints 1 1/2 - 2 1/2 mm. long, not prominent. Inner perianth segments linear to rhomboid, ca. 9 mm. wide. . . . . 2a. subspecies tetracantha var. tetracantha

2b. Year old joints 11-12 mm. in diameter. Podaria 12-15 mm. long. Glochids on older joints to 3 mm. long, prominent. Inner perianth segments obovate, obcordate, ca. 13 mm. wide. . . . . 2b. subspecies tetracantha var. gilensis

1. Opuntia kleiniae D.C. subspecies kleiniae

Mem. Mus. Hist. Nat. Paris 17:118 1828

Opuntia wrightii Engelmann, Proc. Amer. Acad. 3:308 1856

Opuntia caerulescens Griffiths, Rep. Mo. Bot. Gard. 20:86 1909

Type: Mexico, Coulter (Paris?) not seen

Range: From the Davis Mountains, Texas, south to much of the Mexican Plateau.

Representative specimens: Texas. Jeff Davis Co.: Davis Mountains, Dodd (US), 20 miles north of Ft. Davis Leding S.C. 1003-A (ARIZ), Indian Lodge, Davis Mountains, Fischer 1010 (ARIZ). Brewster Co.: Alpine, Sperry in 1932 (ARIZ). Mexico. Hidalgo: Ixmiquilpan, Rose in 1905 (US).

2. Opuntia kleiniae D.C. subspecies tetracantha comb. nova

Opuntia tetracantha Toumey, Gard. and For. 9:432 1896

Type: 5 miles east of Tucson, Arizona, Toumey in 1895 (US) seen by author

Range: North of the Mogollon Rim in eastern Arizona to most of southeastern Arizona, and south to Guaymas, Sonora, Mexico.

2a. subspecies tetracantha variety tetracantha

Representative specimens: Arizona. Pima Co.: Tucson, Toumey in 1895 (type and isotype US), north of Tucson, Rose 11768 (US), East of Tucson, MacDougal in 1910 (US), Lower Bear Canyon, Santa Catalina Mountains, Fischer 217, 899, 900 (ARIZ), southwest of Tucson, Fischer 315 (ARIZ). Cochise Co.: N.W. of State corner, Benson 10278 (ARIZ). Mexico. Sonora: San Carlos Bay, Fischer 507 (ARIZ).

2b. subspecies *tetracantha* variety *gilensis* var. nova

Opuntia congesta Griffiths, Rep. Mo. Bot. Gard. 20:88 1909

Differs from variety *tetracantha* by thicker joints, wider obcordate petals, longer glochids on old joints, and shorter podaria.

Differens ab varietate *tetracantha* crassioribus artibus, latioribus obcordatis petalis, saetis longioribus in artibus senioribus brevioribus tuberculis.

Type: 5 miles northeast of Globe, Gila Co., Arizona. At 4,400 feet on hillsides with grass and scattered oaks, Fischer 803, deposited at herbarium of the University of Arizona.

Range: known only from type locality.

Representative specimens: Arizona. Gila Co.: 5 miles northeast of Globe, Fischer 803 (ARIZ).

The plants collected at Hillside, Arizona, by Griffiths, and near Yuma, Arizona, by Akin and Morley may belong here.

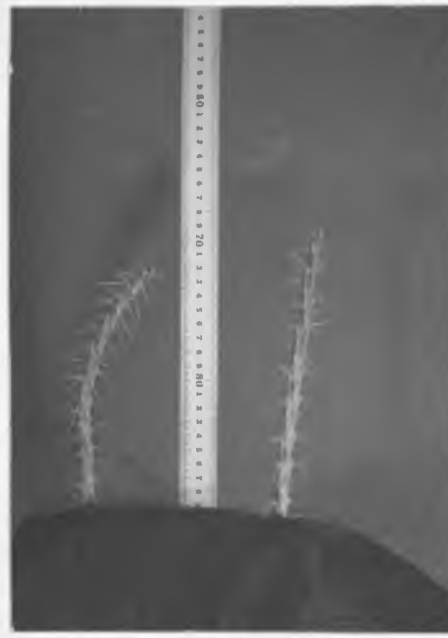
Fig. 9. Type locality of Opuntia kleiniae subsp. tetracantha var. gilensis.

Fig. 10. Opuntia kleiniae subsp. tetracantha var. gilensis in its habitat.

Fig. 11. Opuntia kleiniae subsp. tetracantha var. tetracantha in Lower Bear Canyon.

Fig. 12. Branches and fruits of Opuntia kleiniae subsp. kleiniae.

A.



B.

C.

D.

Fig. 13. Photographic close-ups of representatives of the Opuntia kleiniae complex. A. Subspecies tetracantha var. gilensis. B. Subspecies tetracantha var. tetracantha: at left from Tucson, at right from northern Arizona. C. Subspecies tetracantha var. tetracantha from Lower Bear Canyon. D. Subspecies kleiniae from Texas.

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