A Cytological and Morphometric Analysis of a Triploid Apomict, Opuntia xKelvinensis (Subgenus Cylindropuntia, Cactaceae)

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A CYTOLOGICAL AND MORPHOMETRIC ANALYSIS OF A TRIPLOID APOMICT, OPUNTIA ×KELVINENSIS (SUBGENUS CYLINDROPUNTIA, CACTACEAE)

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Baker, Marc A. and Donald J. Pinkava (Department of Botany and Microbiology, Arizona State University, Tempe, AZ 85287). A cytological and morphometric analysis of a triploid apomict, *Opuntia ×kelvinensis* (subgenus *Cylindropuntia,* Cactaceae). Brittonia 39: 387–401. 1987.—A morphometric analysis of *Opuntia spinosior, O. fulgida,* and their putative hybrid, *O. ×kelvinensis* was supplemented with cytogenetic data and pollen stainability for all OTUs. The morphometric analysis supported the hypothesis for the hybrid origin of *O. ×kelvinensis* and indicated that limited backcrossing has occurred between *O. ×kelvinensis* and *O. spinosior.* Almost all individuals investigated of *O. ×kelvinensis* are triploid, with 33 chromosomes, those of *Opuntia fulgida* are mostly diploid, but in part triploid, and all of *O. spinosior* investigated are diploid. The very high percentage of sterile seed produced by triploid *O. ×kelvinensis* is almost certainly a consequence of unequal segregation of chromosomes in pollen mother cells during anaphase I. The ability of *O. ×kelvinensis* to reproduce vegetatively is attributable to its *O. fulgida* parentage. It is hypothesized that the success of *O. ×kelvinensis* is a result of its particularly preadaptive genome isolated from infrequent backcrossing via meiotic irregularities of odd-polyploidy and its ability to reproduce vegetatively.

Although hybridization in *Opuntia* Mill. (Cactaceae) has long been known (Britton & Rose, 1919), it has only recently been thought to be of taxonomic or evolutionary significance (Grant, 1979; Grant & Grant, 1971a, 1979b, 1979c, 1980, 1982; Parfitt, 1980; Pinkava & McLeod, 1971; Pinkava et al., 1985). The present study employs a more objective approach identifying hybrids within the subgenus *Cylindropuntia* Engelm. and for elucidating their origin.

Hybridization between *Opuntia spinosior* (Engelm.) Toumey and *O. fulgida* Engelm. was first cited in the literature by Britton and Rose (1919). Peebles (1936) described a population of the hybrids at Sacaton, Arizona, and, although he regarded them as a nascent species, he simply referred to them as *O. spinosior ×fulgida.* In addition to the Sacaton population, Benson (1969) cited two specimens near Tucson, Arizona. Grant and Grant (1971a) studied the hybrid population at Sacaton and another population near Kelvin, Arizona, in greater detail. Because of the success of the hybrid individuals, Grant and Grant (1971a) considered *O. fulgida* × *O. spinosior* to be an agamospermous microspecies and named it *O. kelvinensis* Grant & Grant. Since *O. kelvinensis* is accepted as a hybrid taxon by most authorities, we consider it hereafter as *O. ×kelvinensis* pro sp. The clonal complex represented by *O. ×kelvinensis* was considered by Grant and Grant (1971a) to be composed of several slightly fertile morphotypes. Their conclusion was that the slightly fertile F_1 hybrids reproduced mostly asexually but that additional morphotypes resulted from occasional backcrossing to *O. spinosior.* Thus, they concluded, it was both the sexual and asexual reproductive modes that led to the development of this clonal hybrid microspecies.

Grant and Grant (1971a) suggested their study of *O. ×kelvinensis* was only a first step in its analysis and that cytotaxonomic and chemotaxonomic evidence was needed. Chemotaxonomic studies were initiated but both Clark et al. (1980), using flavonoids, and Baker (1982), using alkaloids, concluded that their analyses

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were of little use in determining hybrid relationships between *O. fulgida* and *O. spinosior*. Pinkava and McGill (1979) began a cytological study using chromosome number determinations. They discovered that many individuals of *O.* ×*kelvinensis* were triploid, as were some individuals of *O. fulgida*. All individuals of *O. spinosior* investigated were diploid. They classified the hybrids into three major groups and used the following notations. The apparent diploid hybrids were designated as FS, the triploid hybrids more resembling *O. spinosior* than *O. fulgida* as SSF, and the triploid hybrids more resembling *O. fulgida* than *O. spinosior* as FFS. In addition, Pinkava and McGill found three morphotypes of *O. fulgida* near Florence, var. *fulgida,* var. *mammillata* Coulter, and a morphotype with an enlarged yellow-brown spine sheath, here referred to as the "golden-spine" form. They also found both yellow- and purple-flowered *O. spinosior*.

Our present work expands the cytological investigation of Pinkava and McGill (1979) and compares it with a morphometric analysis.

Materials and Methods

Morphometric analysis

Data were collected from mature living specimens in the field. Forty individuals or operational taxonomic units (OTUs) from an area 30 km southwest of Florence, Pinal Co., Arizona (T5S R12E Sects. 21, 27, 28, 34, 35; T6S R12E Sects. 2, 3, 10–14, 24; T6S R13E Sects. 19, 28–30, 33–35) were selected along a 100 km road transect. The transect was chosen because it traversed the Florence Population through an elevational gradient and, with respect to the two parents and their hybrids, *Opuntia fulgida* occurs only at the lower elevations (west) whereas *O. spinosior* grows only at the upper elevations (east). Mutually exclusive parental species populations were sampled. The *O. fulgida* population is located east of Apache Junction in Peralta Canyon, Pinal Co., Arizona (T1N R10E Sects. 31, 32; T1S R9E Sects. 11, 12 & R10E Sect. 6) and the *O. spinosior* population is located just east of Superior at Oak Flat, Pinal Co., Arizona (T1S R13E Sects. 16, 28 & T1S R15E Sect. 16). Twenty individuals each from the Peralta and Oak Flat Populations were selected randomly along a roadside by drawing from a table of random numbers, representing 0.1 km of road length and taking the first individual that was over 20 m from the road's edge.

Measured were 119 vegetative and reproductive characters of 79 OTUs. Habit and habitat characters were measurable only once, but the remainder were measured five times. Terminal stem segments were chosen from the top and the south-facing side of each individual. From a set of five terminal stem segments, one set of tubercle measurements was taken from a tubercle from the top and thickest portion of each stem segment. Areole and spine measurements were taken from the same five tubercles. Flowers and fruits were also taken from the top and south-facing side of each individual. Colors were measured using Munsell's (1929) color scheme.

Using the SAS software system (SAS Institute, Inc., Box 8000, Cary, NC), repeatedly measured characters were averaged and natural logarithms of these and the unaveraged characters were incorporated into a SAS data set. The data were then tested for normality using the normal probability plot procedure of BMDP (BMDP Statistical Software, Department of Biomathematics, University of California Los Angeles, Los Angeles, CA). Characters having a skewed distribution within either of the *a priori* defined OTU groups or characters whose means from the Oak Flat and Peralta Canyon populations were not statistically significantly different, were discarded. The remaining 65 characters are listed in Table I.

The normalized SAS data set was analyzed using the following algorithms of

the BMPD and Clustan Computer System (Clustan Project, University College London, 19 Gordon Street, London, England).

1. Principal factor analysis (PFA) of BMPD using eight, nine, and ten factors on the Oak Flat, Peralta Canyon, and Florence populations.
2. Multigroup discriminant analysis of BMPD on the three preselected populations.
3. Agglomerative cluster analysis using six methods of the procedure hierarchy of Clustan. These included nearest neighbor, furthest neighbor, group average, centroid, Gower's method and Ward's method.

These three approaches were selected because their statistical origins and assumptions are varied enough from each other as to enable a comparison of nonhomologous morphometric analyses (Sneath & Sokal, 1973; Pimentel, 1979).

Cytology

Chromosome number determinations were attempted from the first or second meiotic divisions of PMCs or from mitotic divisions of early ovule development. Flower buds were trimmed of excess ovary material and fixed in a modified Carnoy's solution (95 percent ethanol : chloroform : glacial acetic acid, 3:3:1 v/v), transferred to 70 percent ethanol after 24 hours, and refrigerated. The anthers were squashed and stained in 45 percent acetocarmine and mounted in Hoyer's medium (Pinkava & Baker, 1985). Mitotic counts were obtained from certain individuals of the Peralta Canyon population by squashing immature ovules using the same staining and mounting techniques.

Pollen stainability

Percent pollen stainability was determined for 102 individuals for which chromosome numbers had also been determined. Pollen was obtained from herbarium specimens and stained in aniline-blue-lactophenol for 24 hours (Maneval, 1936). A minimum of 500 pollen grains per individual were scored and the following pollen classes were recorded: staining average-size pollen, macropollen (pollen at least 50 percent larger by volume than average), and micropollen (pollen at least 50 percent smaller than average); and nonstaining micropollen.

Results

Morphometric analysis

In PFA, nine factors proved to be the most parsimonious. The first two factors explain 90 percent of the variation among the OTUs. A plot of factor one versus factor two showed no overlap among individuals belonging to either of the parental study populations (Fig. 1), but there was no marked separation of the two taxa. When OTUs from the Florence population were included, those of the putative hybrids were intermediate to the Oak Flat and Peralta Canyon populations.

Better resolution between the putative hybrids and the parental types was obtained with multigroup discriminant analysis. The four characters that best separated the three *a priori* defined groups were characters six (number of branches at second "node"), 15 (number of areoles seen from one side of the terminal stem segment along the shortest spiral), 19 (number of spineless basal areoles), and 23 (spine base color). Some of the OTUs of the putative hybrids fell within the two main groupings of putative parental OTUs and some of the OTUs of the putative parents fell within the main grouping of putative hybrid OTUs (Fig. 2). The mean

TABLE I

LIST OF CHARACTERS USED IN THE MORPHOMETRIC ANALYSES

1. Maximum distance at a right angle from the axis of the branch with the maximum curvature to an imaginary straight line connecting its origin and termination.
2. Length of first internode of first branch of main trunk.
3. Length of spines on trunk 1 dm from ground level.
4. Number of spines per areole on trunk 1 dm from ground level.
5. Percentage of terminal branches of the plant forming a negative angle with the plane of the earth.
6. Number of branches at second "node."
7. Ratio of terminal stem length to its maximum diameter.
8. Ratio of terminal stem length to distance from its maximum diameter to its tip.
9. Ratio of terminal stem length to its diameter at 1/10 of stem length from base.
10. Ratio of terminal stem length to diameter of its detachment scar.
11. Ratio of tubercle length to its width.
12. Ratio of tubercle length to distance from its maximum width to its tip.
13. Ratio of tubercle length to its maximum height.
14. Ratio of areole length to its width.
15. Number of areoles seen from one side of the terminal stem along the shortest spiral.
16. Distance from tip of areole to nadir of sinus between tubercles.
17. Maximum erect spine length.
18. Maximum erect spine length minus basal reflexed spine length.
19. Number of spineless basal areoles.
20. Spine tip color (hue).
21. Spine tip value (lightness).
22. Spine tip chroma (saturation).
23. Spine base color.
24. Spine base value.
25. Spine base chroma.
26. Sheath tip color.
27. Sheath tip chroma.
28. Sheath base value.
29. Sheath base chroma.
30. Number of tepals per flower.
31. Ratio of longest tepal length to distance from its maximum width to its tip.
32. Ratio of longest tepal length to its maximum width.
33. Ratio of longest tepal length to its width at base.
34. Ratio of longest tepal length to longest filament length.
35. Ratio of longest tepal length to style length.
36. Ratio of style length to stigma length.
37. Ratio of style length to stigma width.
38. Ratio of style cavity length to width.
39. Ratio of ovary length to the distance from distal end of the locule to base of style cavity.
40. Tepal color (hue).
41. Tepal value (lightness).
42. Tepal chroma (saturation).
43. Filament color.
44. Filament value.
45. Filament chroma.
46. Style color at tip.
47. Style value at tip.
48. Style chroma at tip.
49. Style color at base.
50. Style value at base.
51. Style chroma at base.
52. Stigma color.
53. Stigma value.
54. Stigma chroma.
55. Maximum number of proliferating fruits persisting in a chain.
56. Ratio of fruit length to diameter 1/10 from base of fruit.
57. Ratio of fruit length to diameter at its tip.
58. Ratio of fruit length to diameter at its middle.
59. Ratio of fruit umbilicus depth to its diameter.

TABLE I
CONTINUED

60. Number of seeds per fruit.
61. Ratio of length of largest fruit tubercle to its height.
62. Ratio of fruit length to tubercle length.
63. Fruit color, sun side.
64. Fruit value.
65. Fruit chroma.

of the hybrid group is closer to the mean of *O. spinosior* than to the mean of *O. fulgida* (Fig. 2).

The results from the six clustering methods of the Clustan package used were in close agreement with one another. There was no overlap between OTUs of the Oak Flat and Peralta Canyon populations and no overlap among OTUs of the putative parents in the Florence population. The *O. spinosior* Oak Flat population formed a tight cluster and only one OTU from the hybrid Florence population identifed as *O. spinosior* was included within it. The *O. fulgida* Peralta Canyon population was not so well defined and all of the OTUs from the Florence population identified as *O. fulgida* were mixed among them (Fig. 3). The two OTUs representing suspected hybrids involving *O. acanthocarpa* Engelm. & Bigel. as one putative parent were clustered at a very low degree of correlation with the other OTUs. A dendrogram resulting from the centroid method was chosen to represent the analyses (Fig. 3). Clearly, as with the discriminant analysis, most of the putative hybrids fall within the range of individuals identified as *O. spinosior.* Only one hybrid, a diploid, falls within the range of *O. fulgida.*

Cytology

The base chromosome number for Cactaceae is $x = 11$ as reported by Lewis (1980) and Pinkava et al. (1985) and as confirmed by this study. All meioses from diploid individuals, including hybrids, revealed only complete bivalents and nearly all meioses from triploid individuals showed only trivalents. In meiosis II of triploid individuals, however, chromosomes often did not segregate properly and formed micronuclei. The chromosome numbers we have determined for 123 individuals of *O. fulgida, O. spinosior,* and *O.* ×*kelvinensis,* as well as 38 unpublished counts by Pinkava and McGill, are summarized, with all previously published counts, in Table II. The number of chromosomes for each OTU was successfully determined. Of the 48 counts for *O.* ×*kelvinensis* (as we previously determined), only four individuals were found to be diploid. Of the three forms of *O. fulgida,* var. *mammillata* was diploid for all six individuals counted, the "golden-spined form" was triploid for all four individuals and var. *fulgida* was diploid for 47 individuals and triploid for 16. The individuals of the Peralta Canyon population, including three individuals of var. *mammillata,* were diploid except for one triploid individual of var. *fulgida.* All *O. spinosior* individuals sampled at the Florence and Oak Flat populations, including yellow-flowered forms, were diploid.

Pollen stainability

The pollen data are summarized in Table III. As expected, the percent pollen stainability was low for all triploid individuals and putative diploid hybrids, while that for diploid parental individuals was high. Note that the SD for diploid *O. fulgida* was much higher than that for *O. spinosior* due to a few diploid *O. fulgida*

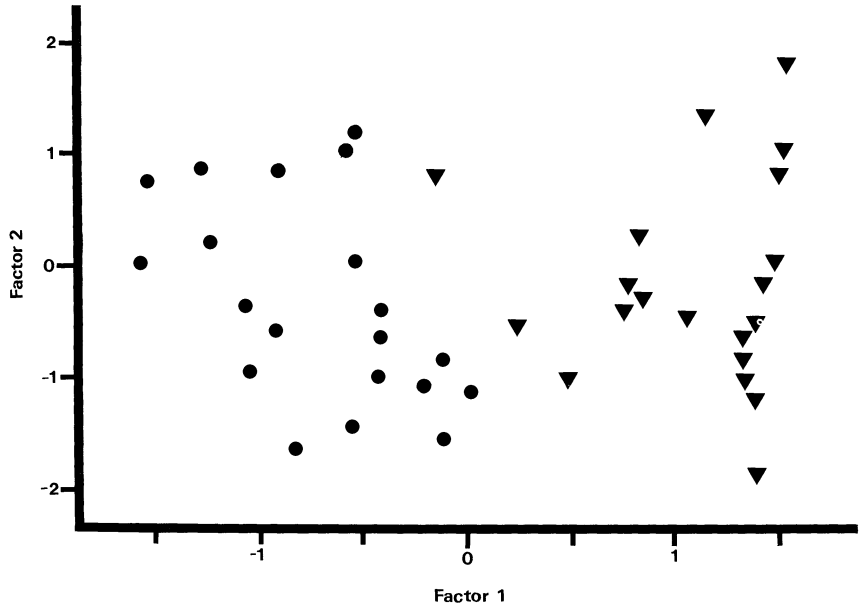

FIG. 1. PFA plot of factor one versus factor two including OTUs from Oak Flat and Peralta Canyon populations. Circles = *Opuntia spinosior,* triangles = *O. fulgida.*

with low pollen stainability. Micropollen grains (Table III) were more common in triploid than in diploid individuals ($p < 0.001$).

Discussion

The Florence population is similar to many already well documented vascular plant hybrid populations because it falls in an area of sympatry between the mostly allopatric or parapatric parent species (Levin, 1979). It is not surprising, therefore, that the habitat of the hybrids is intermediate between the two parental habitats (Anderson, 1948). West of the population is flatland Sonoran Desert (about 700 m elevation) with *Opuntia fulgida, O. leptocaulis* DC., and *O. acanthocarpa* as the *Cylindropuntia* components. East of the population is a gently rolling grassland (above 1200 m) with only *O. spinosior.* The Florence population habitat is intermediate not only in elevation but also with respect to associated species. Disturbance from overgrazing might be a contributing factor in the success of the hybrids here (Anderson, 1949). Kinraide (1978), for instance, found *O. imbricata* (Haw.) DC., a close relative of *O. spinosior,* to be negatively associated with disturbed grassland. If this were true for *O. spinosior,* then overgrazing in the Florence area could reduce the competition of *O. spinosior* versus its hybrid progeny. Figure 4 shows the distribution of the taxa and their ploidal levels along the study transect. There are no individuals of *O. spinosior* or hybrids known to the west of the area represented by the map for twenty miles and no individuals of *O. fulgida* or hybrids known to the east for about 15 miles where the elevation drops below 1000 m. Grant and Grant (1971a) discuss the distributions and habitats of *O. fulgida* and *O. spinosior* and their hybrids, as a whole, in greater detail.

Grant and Grant (1971a) labeled *O.* ×*kelvinensis* in the Florence area as Type W, a hybrid with "cone-shaped" tuberculate fruits. Type W differed from their Type K only in having a smaller stature and slightly more tuberculate fruits. These

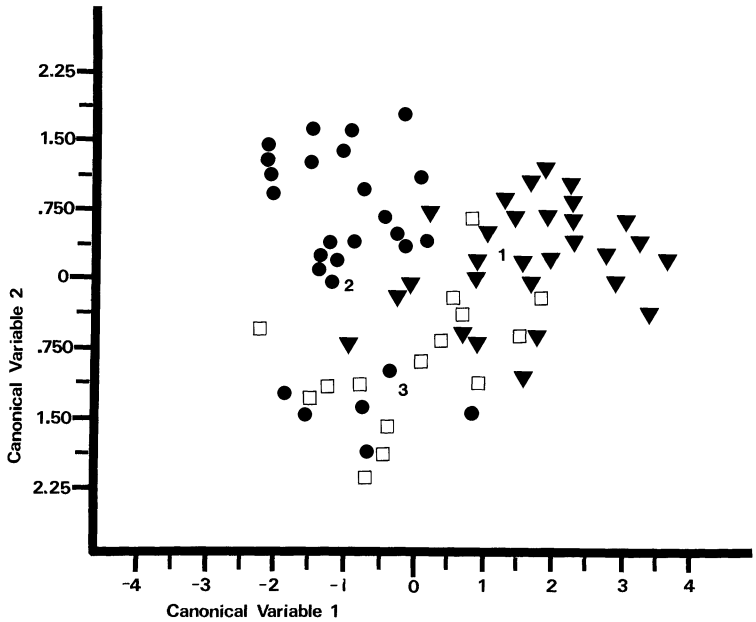

FIG. 2. Discriminant analysis plot including OTUs from Oak Flat, Peralta Canyon, and Florence populations. Circles = *Opuntia spinosior,* triangles = *O. fulgida,* boxes = *O.* ×*kelvinensis.*

two types made up the majority of individuals circumscribed by their *O.* ×*kelvinensis,* because their Types C, R, S, T, and V each consisted of only one to few individuals. Only Types W and K (holotype) apparently were vouchered.

In an attempt to reevaluate and coordinate the studies of Grant and Grant (1971a) and Pinkava and McGill (1979), we have reviewed all the applicable herbarium material and visited all the cited localities of *O.* ×*kelvinensis.* After several trips to Sacaton, an area described by Grant and Grant (1971a), and Peebles (1936), only two plants were located. Both individuals approached *O. spinosior* in morphology. We visited the type locality of *O.* ×*kelvinensis* at Kelvin attempting to establish the identity of Type K. The individuals we suspected as Type K and that represented the type specimen were triploid (Pinkava et al., 1985). After examining the area from the Florence population to Kelvin, we felt the Type K of Grant and Grant (1979a) was the Type FFS of Pinkava and McGill (1979) and individuals of Type W of Grant and Grant (1979a) were only minor variants of Type K.

Although there has been little dispute over the hybrid nature of *O.* ×*kelvinensis,* no morphometric methods have been applied to any of the populations. Clearly the present morphometric analyses document the intermediacy of the hybrids and that the morphotypes SSF, FS, and FFS are not well-defined. The small number of FS individuals makes their group difficult to analyze statistically. Also, the placement by the cluster procedure of some of the OTUs *a priori* identified as *O. spinosior* with OTUs identified as *O.* ×*kelvinensis* suggests there has been some backcrossing. It is difficult to determine whether some specimens should be considered *O. spinosior* or *O.* ×*kelvinensis.* As the cluster procedure would indicate, there is rarely a problem identifying an individual as *O. fulgida* vs *O.* ×*kelvinensis,* supporting the hypothesis that there is more backcrossing toward *O. spinosior* than toward *O. fulgida.* Thus, although diploid hybrids that are apparently F_1 are rare, they could play a major role in introgression. Such unilateral backcrossing

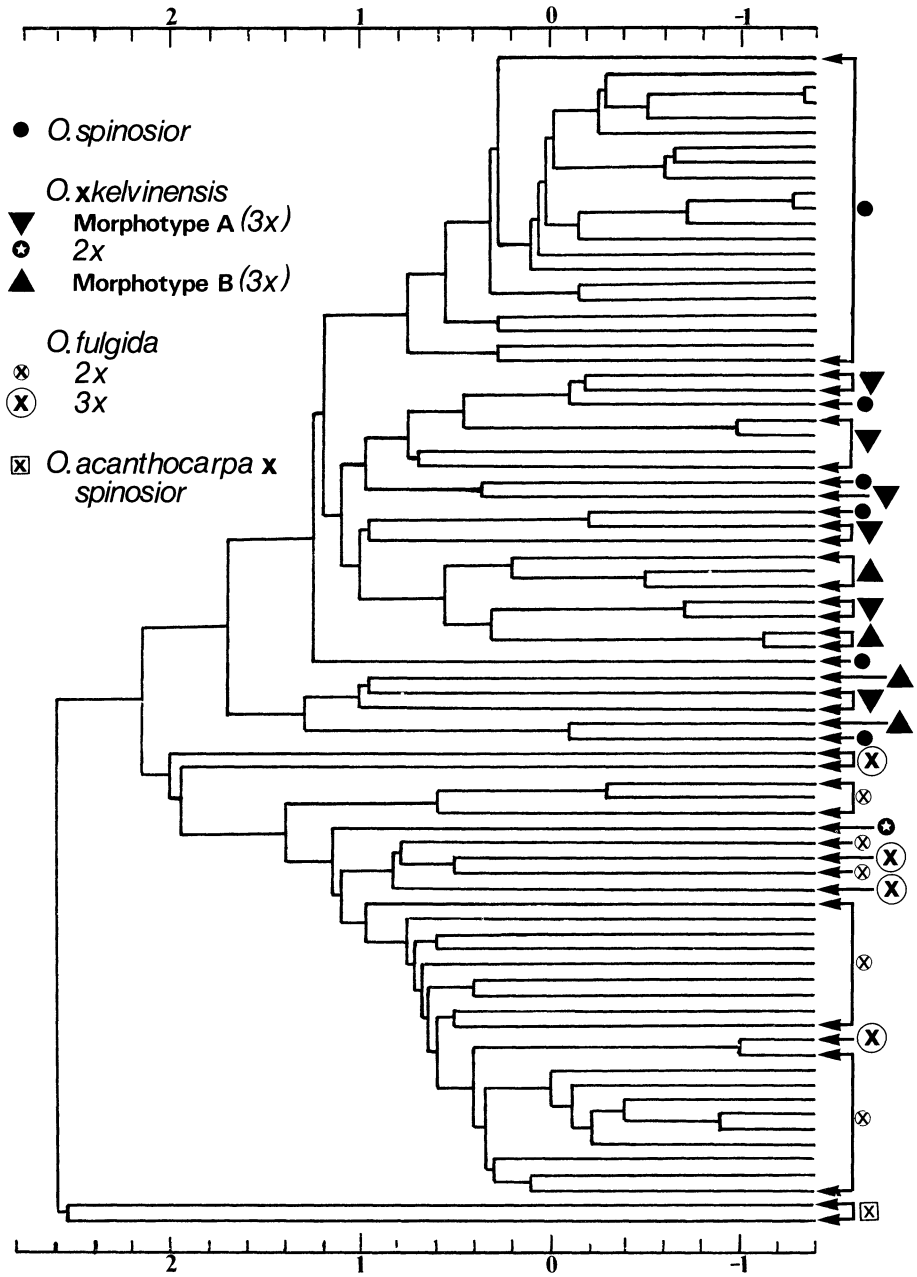

FIG. 3. Correlation phenogram representing the results from the centriod method of cluster analysis. Scale represents the natural logarithm of the distance coefficient.

of *O.* ×*kelvinensis* was proposed by Grant and Grant (1971a). Also according to the morphometric analysis, both triploid morphotypes more closely resemble *O. spinosior,* but SSF more so than the FFS. These results are in disagreement with Pinkava and McGill (1979) who considered the FFS morphotype to be more similar to *O. fulgida* than to *O. spinosior* and in agreement with Grant and Grant

TABLE II
SUMMARY OF CHROMOSOME COUNTS IN *Opuntia*

	fulgida		*×kelvinensis*		*spinosior*	
	2×	3×	2×	3×	2×	3×
Present study	40	13	3	27	40	0
Pinkava & McGill (unpublished)	7	3	1	14	13	0
Pinkava et al. (1973)	1	0	0	0	3	0
Pinkava & Parfitt (1982)	1	0	0	0	2	0
Pinkava et al. (1985)	13	2	0	3	19	0
Yuasa et al. (1973)	9	0	0	0	0	0
Total	71	18	4	44	77	0

(1979a) who considered the FFS (Type W) to be more similar to *O. spinosior* than to *O. fulgida.* Pinkava and McGill (1979) reasoned that since *O. fulgida* is known as an autotriploid and *O. spinosior* is known only as a diploid, then *O. fulgida* probably contributed the unreduced gametes in the formation of FFS individuals. The present data suggest that there was hybridization first between diploid individuals, and morphotype A, the presumed FFS, received the unreduced gamete(s) from diploid *O. ×kelvinensis.* Morphotype B, the presumed SSF, was probably formed in the same manner, but with *O. ×kelvinensis* that had been a result of yet further backcrossing with *O. spinosior.* Similarity among individuals represented by either *O. ×kelvinensis* triploid morphotype could be easily explained by cloning, especially when the individuals are found close together.

Because no triploid individuals of *O. spinosior* were found, there is no reason to believe it has ever contributed unreduced gametes toward the origin of *O. ×kelvinensis.* Considering 18 of the total 88 individuals of *O. fulgida* counted to date are triploid, are from at least four isolated areas (Pinkava et al., 1985), and are of more than one distinct morphotype, diploid *O. fulgida* must be capable of producing unreduced gametes. Thus, *O. fulgida* could have supplied the unreduced gametes for the FFS of Pinkava and McGill, or it could have provided diploid *O. ×kelvinensis* with the genes necessary to produce its own unreduced gametes as supported by our morphometric analyses.

Pollen stainability data supported the hybrid nature of *O. ×kelvinensis* (Table III). Both suspected FS individuals whose percent pollen stainability was determined had reduced pollen stainability and increased micropollen and macropollen production. There were some individuals of *O. fulgida* with these same characteristics but the instability of microsporogenesis in these could be explained by the apomictic nature of the species, as discussed below. The irregular pollen sizes and sometimes lower percentages of pollen stainability in *O. fulgida* could also mean that the species itself is of hybrid origin. Because most individuals of *O. fulgida* show normal meiosis during microsporogenesis, however, the sporadic indication of meiotic abnormality might be related to the interaction of abnormal, possibly unbalanced gametes from triploid individuals and normal gametes from diploid individuals. Punyasingh (1947), for instance, found in maize that a large number of F_1 progenies from intercrosses between diploid and triploid individuals were aneuploid. Since no aneuploid individuals were found in this study, it may be that unbalanced gametophytes are not viable or that unbalanced gametes are particularly lethal in individuals of the *O. fulgida-spinosior* complex.

The very low pollen stainability of the triploid individuals comes as no surprise because of their inherent meiotic problems. Since there was little overlap in percent pollen stainability between diploid and triploid individuals, ploidy levels could be predicted on the basis of pollen stainability with little error ($p < 0.001$).

TABLE III

SUMMARY OF POLLEN STAINABILITY DATA OF *Opuntia fulgida, O. spinosior,* AND THEIR HYBRIDS[a]

	O fulgida		*O ×kelvinensis*		*O spinosior*	
Pollen class	2×	3×	Morphotype A	2×	Morphotype B	2×
Regular, stained	81.6 ± 18.8 45.9–98.6	37.4 ± 8.6 23.0–51.6	22.5 ± 7.8 5.1–30.1	51.4 ± 9.5 44.6–58.1	29.4 ± 12.8 12.8–59.1	91.7 ± 7.0 66.6–98.0
Macropollen, stained	0.1 ± 0.2 0.0–1.0	3.8 ± 1.2 2.2–5.6	2.9 ± 1.5 1.0–5.6	0.9 ± 0.1 0.1–1.0	3.9 ± 1.8 0.7–6.6	0.2 ± 0.4 0.0–1.6
Micropollen, stained	0.3 ± 0.8 0.0–3.1	0.9 ± 1.4 0.0–4.5	1.0 ± 1.0 0.0–2.9	1.2 ± 1.6 0.0–2.3	1.5 ± 2.1 0.0–7.2	0.4 ± 1.6 0.0–5.7
Micropollen, nonstained	1.4 ± 2.7 0.0–11.0	8.9 ± 9.6 0.0–22.9	22.7 ± 9.6 11.4–40.8	4.9 ± 0.6 4.5–5.3	22.7 ± 9.5 13.5–45.7	1.7 ± 2.4 0.0–7.7

[a] All data given as percentage of total pollen, mean ± SD above, range below.

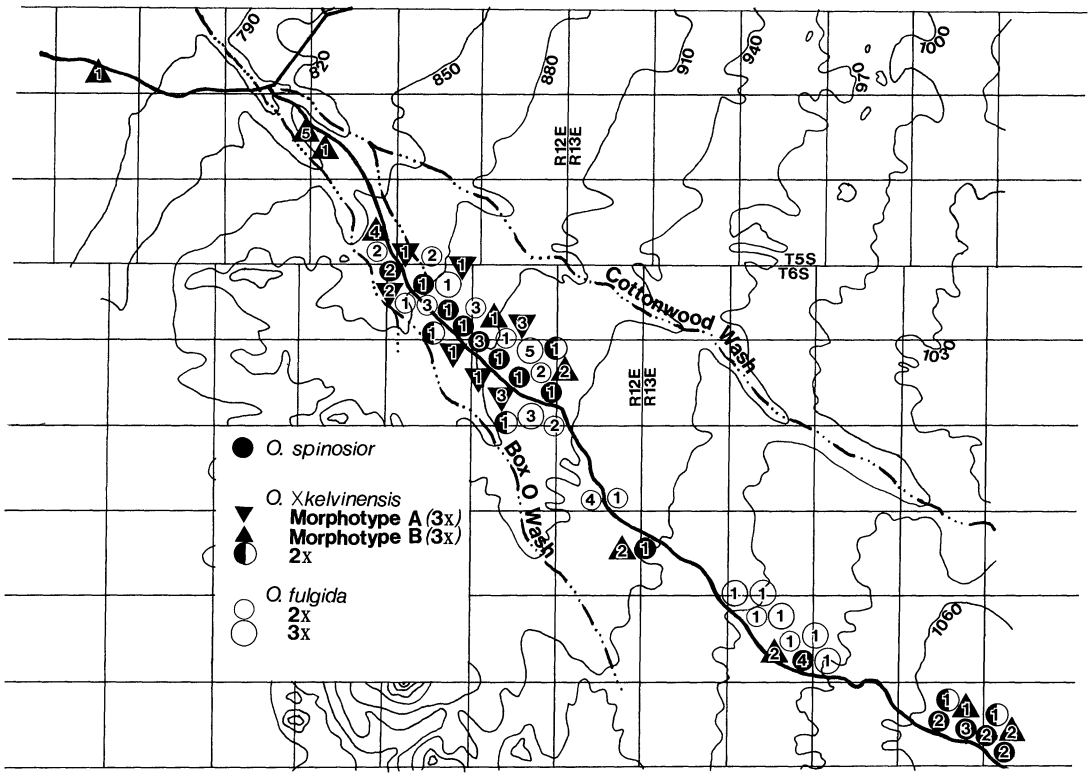

FIG. 4. Distribution map of individuals of the Florence hybrid population for which chromosome numbers have been determined. Numbers within symbols indicate numbers of individuals. Elevation is in meters.

The high pollen stainability of *O. spinosior,* as well as its normal megasporogenesis, megagametogenesis, and embryogenesis (Baker & Pinkava, 1983), suggests that it is a normally sexually reproducing species. Because the study of megagametogenesis of *O. fulgida* was incomplete, it is unknown whether it produces normal embryos or if it can produce them agamospermously or both. The existence of triploid individuals and hybrids with *O. spinosior,* however, indicates that at least some sexual reproduction occurs.

Hybridization between *O. fulgida* and *O. spinosior* is not surprising because taxa of the subgenus *Cylindropuntia,* section *Cylindropuntia,* commonly hybridize and apparently have few isolating mechanisms (Grant and Grant, 1971b; Pinkava et al., 1985). Using the classification of isolation mechanisms of Stebbins (1950), we feel that in the section *Cylindropuntia* ecogeographical isolation is the most important barrier to gene flow, while temporal isolation with respect to flowering phenology is usually only a partial barrier. Temporal isolation probably serves as a barrier to gene flow only among species with nonoverlapping flowering times, as between *O. fulgida* and both *O. acanthocarpa* and *O. leptocaulis.* Hybrids are unknown between *O. fulgida* and either species (Pinkava et al., 1985). The low fertility of *O. fulgida* (Johnson, 1918) may also be a contributing factor. Hybrid inviability or weakness is a minimal barrier in the section *Cylindropuntia,* although it may reduce hybridization between distantly related species (Pinkava et al., 1985). Because chromosomes were always found to pair in all individuals, pairing

plays little or no role in sterility, although sterility could be caused by cryptic structural hybridity (Stebbins, 1945; Stebbins et al., 1946). Mechanical isolation does not appear at all important in the section *Cylindropuntia.* Pollination studies of *Opuntia* indicate that the pollinators of certain *Opuntia* species, with flower morphology typical for the genus, are rather numerous and nonspecific (Grant & Grant, 1979b; Grant et al., 1979; Grant & Hurd, 1979; Parfitt & Pickett, 1980).

Baker (1984) recently discussed the importance of triploidy in the evolution and systematics of section *Cylindropuntia,* especially with regard to the widespread but mostly triploid *O. bigelovii* Engelm. Benson (1982) infers that it is of hybrid origin because it reproduces by vegetative propagules. If Benson's assumption is correct, *O. bigelovii* is probably not of recent hybrid origin because it is not intermediate between any two known extant species. *Opuntia* ×*kelvinensis* is similar to *O. bigelovii* for it reproduces by vegetative propagules and is mostly triploid but differs in its obvious hybrid origin from two extant species. If *O.* ×*kelvinensis* is a nascent species, as Peebles (1936) suspected, then it is possibly evolving in a similar manner as did *O. bigelovii. Opuntia fulgida* also reproduces by vegetative propagules. Although mostly a diploid species, it shares affinities with both *O.* ×*kelvinensis* and *O. bigelovii* (Benson, 1969). Both *O. fulgida* and *O. bigelovii* are widespread and abundant within their ranges. The sterile *O.* ×*kelvinensis* must also be considered successful in view of its abundance.

What contributes to the success of *O. bigelovii, O. fulgida,* and *O.* ×*kelvinensis*? If it were simply their vegetative propagagule mode of reproduction, then how have the triploid individuals of *O. bigelovii* and *O.* ×*kelvinensis* become more successful than the diploid individuals of their species? Perhaps the success of triploid apogamy lies in its ability to fix and maintain particularly adaptive genomes. The success of apomictic hybrids has been shown in *Poa* L. by Clausen et al. (1945). If we assumed both *O. bigelovii* and *O. fulgida* originated via hybridization, then this could be a plausible answer. Another hypothesis is that polyploidy, in this case, triploidy, is somehow at a selective advantage for physiological reasons. Although we know of no studies directly connecting polyploidy with the ability of plants to adapt to xeric conditions, it is known for several species (Stebbins, 1950; Lewis, 1980) that polyploidy is correlated with increased cell size. Since increased cell size or succulence is known as a common adaptation to xeric conditions, it follows that polyploidy might also lead to such adaptation.

Whatever the reason, the triploid population of *O.* ×*kelvinensis* is successful with respect to distribution and abundance. What is in store for it in terms of evolutionary success? Grant (1981) considers the combination of sexual and vegetative reproduction of *O.* ×*kelvinensis* as advantageous in that "it permits the formation of an array of new types and the perpetuation of any new types that are adaptively valuable." With respect to *O.* ×*kelvinensis,* this statement would be reasonable, if most of the individuals were not triploid. Because of the inability of a triploid individual to freely exchange genes and thereby maintain its ability to adapt to changing environments, the success of *O.* ×*kelvinensis* in the distant future depends upon the likelihood of rediploidization or redoubling.

Although speciation via allopolyploidy has long been well documented (Stebbins, 1950), the ability for polyploids, especially odd-number polyploids, to rediploidize has not. Recent evidence from studies with European *Taraxacum* Weber (Nijs & Sterk, 1980; Jenniskens et al., 1984), however, documents the occurrence of rediploidization in apogamous species. The situation seems analagous to that in *O.* ×*kelvinensis* and perhaps to that of *O. bigelovii* and *O. fulgida* as well. Baker (1984) postulated that triploid *O. bigelovii* reproduces sexually to a limited extent via infrequent viable 1× and 2× gametes. It is probable that these rare gametes derived from triploid plants, fusing with viable gametes from normally sexual

species, gave rise to the four or more diploid and triploid putative hybrids between *O. bigelovii* and other species (Baker, 1984).

Based on preliminary viability studies, seven seeds of triploid *O.* ×*kelvinensis* germinated out of 332 (2.1% viability). Since there is one seed for about every 50 aborted ovules (data from raw morphometric data set), the number of seeds maturing from young ovules is one seed per 50 ovules times 0.021 or one viable seed from 2386 immature ovules. We do not know if the viable seeds result from sexual reproduction. Sexuality can be tested, however, by the search for diploid seedlings. The presence of diploid seedlings would show that they were produced sexually with *O.* ×*kelvinensis* contributing a 1× gamete, except for the exceedingly improbable production by triploids of a partially reduced gamete (2×) followed by parthenogenesis.

Speculation about *O.* ×*kelvinensis* as a potential evolutionary species must be regarded with great caution. Stebbins (1980) reminds us that polyploidy is almost always unidirectional. Even if triploid *O.* ×*kelvinensis* produces viable gametes, the taxon's evolutionary history cannot be predicted (deWet, 1980).

Conclusions

The morphometric analyses of *Opuntia* ×*kelvinensis* document that it is a hybrid between *O. fulgida* and *O. spinosior.* The individuals of *O. fulgida* within the Florence population are representative of the species in that they very closely resemble those of the Peralta Canyon population. In comparison, the individuals of *O. spinosior* within the Florence population do not closely resemble those of the Oak Flat population. In addition, the individuals of *O. spinosior* within the Florence population more closely resemble the individuals of *O.* ×*kelvinensis* than do the individuals of *O. fulgida* within the Florence population. This suggests that much of the *O. spinosior* at Florence has had some gene exchange with *O. fulgida* through backcrossing with *O.* ×*kelvinensis.* The individuals of *O.* ×*kelvinensis* fall mostly within two morphological groups, both triploid. The designations of SSF and FFS for these are not perfectly suitable, since the FFS individuals appear morphologically closer to *O. spinosior* than to *O. fulgida,* although they are not as similar to *O. spinosior* as are the SSF individuals.

The large percentage of triploid *O. fulgida* (25 percent vs zero percent for *O. spinosior*) indicates that it is the donor of the unreduced gametes in the origin of *O.* ×*kelvinensis.* Since the morphometric analyses indicated, however, that both triploid morphotypes of *O.* ×*kelvinensis* had greater morphological affinities to *O. spinosior,* it is more likely that *O. fulgida* is the donor of genes which increase the likelihood of unreduced gametes and that it was one or more diploid individuals of *O.* ×*kelvinensis* that contributed the unreduced gametes to the triploid hybrids.

The much lower average percent pollen stainability and the higher average percent of micropollen of the two living putative F_1 diploid individuals of *O.* ×*kelvinensis* compared with the diploid individuals of the putative diploid parents adds additional support to the hybrid origin of *O.* ×*kelvinensis.*

Opuntia ×*kelvinensis* probably owes its establishment, large distribution, and abundance to a particularly adaptive genome created by hybridization that was fixed by triploidy and allowed to multiply through vegetative reproduction. Its population structure may represent an initial stage of evolution driven by a process involving the interaction of triploidy, hybridization, and vegetative reproduction. An evolution perhaps similar to that undergone by both *O. bigelovii* and *O. fulgida.* Whether or not *O.* ×*kelvinensis* will become an evolutionary species, either by rediploidization or redoubling of its chromosomes cannot be known.

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Conference on Alternatives to Deforestation

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