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EXPERIMENTAL HYBRIDIZATION OF NORTHERN CHIHUAHUAN DESERT REGION *OPUNTIA* (CACTACEAE)

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ABSTRACT

Possible natural hybridization among 11 taxa of *Opuntia* sensu stricto was investigated in the northern Chihuahuan Desert region through the use of experimental hybridization. Established plants representing specific taxa growing in the Sul Ross State University *Opuntia* garden were used for all experiments. Reciprocal crosses were made between putative parental taxa of field-observed putative hybrids, and each experimental cross analyzed for fruit and seed set. For each taxon, tests were performed to control for possible apomictic, autogamous, and geitonogamous seed set. Several experimental crosses were found to set seed in amounts expected for natural pollination events. Data gathered from the tests also provided basic information regarding the breeding systems of the taxa investigated. Data presented here provide support for several hypothesized hybridization events among *Opuntia*.

Key words: Chihuahuan Desert, hybridization, *Opuntia*.

INTRODUCTION

Opuntia sensu lato is the largest genus within the subfamily Opuntioideae (Cactaceae), with an estimated 160 or more species (Gibson and Nobel 1986). Ninety-eight taxa of *Opuntia* within 44 species occur within the United States (Benson 1982). The most recent treatments recognize 24 species of *Opuntia* within the state of Texas with 22 taxa occurring in the Trans-Pecos (Powell 1998; Anderson 2001).

Three North American subgenera are traditionally recognized within *Opuntia*. The *Cylindropuntia* (chollas), *Corynopuntia* (club chollas), and *Opuntia* sensu stricto (prickly-pears) are distinguishable by habit and stem shape (Britton and Rose 1919–1923; Benson 1982). Numerous authors have recommended that the subgenera of *Opuntia* should be elevated to generic rank (Robinson 1973; Anderson 1999, 2001; Pinkava 1999). Representative species from each of these three subgenera are found within the Trans-Pecos. The present study concerns itself only with plants of *Opuntia* sensu stricto. The prickly-pears form the bulk of the opuntoid taxa in the Trans-Pecos region, with 13 recognized species (Powell 1998). Numerous prickly-pear populations, which are not easily accommodated within these 13 species, exist within the Trans-Pecos region and adjacent areas. Hybridization between taxa is a common explanation for these populations.

Hybridization has been thought to give rise to new species, varieties, or morphotypes among *Opuntia*

(Gibson and Nobel 1986; Anderson 2001). Examples of putative hybridization abound in the literature. *Opuntia kelvinensis* has been described as a clonal microspecies (Grant and Grant 1971) occurring in southern Arizona derived through hybridization between *O. fulgida* and *O. spinosior*. Numerous examples of putative hybrids have also been documented in Trans-Pecos Texas. Tetraploid *Opuntia* × *spinosibacca* has been described as a hybrid nothospecies derived from natural crossing of diploid *O. aureispina* and hexaploid *O. phaeacantha* (Pinkava and Parfitt 1988). Interploidal hybridization is believed to occur in *Opuntia*, resulting in even-ploid (Pinkava and Parfitt 1988) or odd-ploid (Grant and Grant 1982) progeny. Recent molecular work (Mayer et al. 2000) has elucidated the hybrid origin of *Opuntia* × *prolifera*. In addition to the above documented cases of putative hybridization, many additional workers have observed and collected prickly-pears that exhibit intermediate morphology between described taxa, and were thought to be of hybrid origin. In previous studies, morphological, molecular, cytological and geographical data provided essentially all the evidence of hybridization. In spite of the mountain of references to natural hybrid *Opuntia*, I know of no documentation that transfer of pollen between taxa can result in seed set. Although strong evidence exists for the hybrid origin of certain cacti through the use of artificial hybridizations (Powell et al. 1991; Powell 1995; Powell 1999), putative hybrid *Opuntia* have not been documented through artificial hybridization experiments.

Several specific cases of possible natural hybridiza-

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tion in Trans-Pecos *Opuntia* were identified and brought under investigation during the current study:

1. *Hybrid status of Opuntia ×rooneyi*.—*Opuntia ×rooneyi* is a taxon known only from Rooney's Place in southern Brewster County, Texas. *Opuntia ×rooneyi* is putatively derived from hybridization between *O. aureispina* and *O. macrocentra* (Griffith 2001). Both putative parents are sympatric with *O. ×rooneyi*.

2. *Interfertility of O. engelmannii var. engelmannii and O. engelmannii var. lindheimeri*.—*Opuntia engelmannii var. engelmannii* occurs over much of the Trans-Pecos and beyond to the west, and may hybridize with *O. engelmannii var. lindheimeri* (Parfitt and Pinkava 1988), which is distributed in the Trans-Pecos from the area of Boquillas Canyon SE through Val Verde County, and is distributed elsewhere in central and S Texas, and in adjacent Mexico.

3. *Interfertility of O. strigil and O. atrispina*.—*Opuntia strigil* occurs on the Stockton Plateau and is thought to intergrade with *O. atrispina* populations farther to the SE in Val Verde and adjacent counties, and possibly in adjacent Mexico.

4. *Interfertility of O. aureispina and O. chisosensis*.—Both *O. aureispina* and *O. chisosensis* are restricted to limited areas of S Brewster County, Texas. Populations of these species are allopatric so far as known. In current distribution they are separated by only a few kilometers. These taxa are similar in habit and morphology (Powell 1998), and both are diploid (Powell and Weedin 2001).

5. *Putative hybrids between O. rufida and O. macrocentra "azurea type"*.—In the Boquillas Canyon area of S Brewster Co., Texas, certain specimens have been collected which exhibit intermediate morphology between *O. rufida* and *O. macrocentra*. The putative hybrids exhibit a betacyanic cast resembling *O. macrocentra*, with much-reduced spination and abundant glochids, characters resembling those of *O. rufida*. The unusual Boquillas Canyon plants might represent an undescribed species, or putative hybrids of *Opuntia rufida* and *O. macrocentra* (Powell pers. comm.). These taxa are traditionally placed in different series within *Opuntia* (Britton and Rose 1919–1923).

6. *Crosses within the O. macrocentra complex*.—Two varieties of *O. macrocentra* are currently recognized, var. *macrocentra* ($2n = 22$) and var. *minor* ($2n = 44$). Another distinct morphotype that is tentatively placed in the *O. macrocentra* complex (Powell et al. in prep.) is readily observable in the Trans-Pecos. The distinct morphotype ($2n = 22$) resembles both *O. macrocentra* and the Mexican taxon *O. azurea*. Here I refer to this morphotype as *Opuntia macrocentra* "azurea type."

7. *Crosses between different ploidy levels*.—Pentaploid taxa of *Opuntia* have been reported (Grant and Grant 1979). The pentaploids were believed to result from natural interspecific hybridization between hexaploids and tetraploids. Interfertility between tetraploid and hexaploid *Opuntia* species has not been established experimentally.

METHODS AND MATERIALS

Artificial crosses.—A total of 11 crosses involving 11 taxa of *Opuntia* were performed during April–June 1999, and April–June 2001 (Table 1). Further tests involving the emasculation of flowers of *Opuntia engelmannii var. engelmannii* and *O. engelmannii var. lindheimeri* were also carried out in May 2000. Plants were selected from stock growing in the *Opuntia* Garden at the Sul Ross Field Plot and Experimental Vineyard. Plants selected were healthy, reproductively mature plants that morphologically represented the typical elements for that taxon. Voucher specimens for the taxa concerned are on file at SRSC (Sul Ross State University herbarium; Table 1). Chromosome numbers are known for all but one of the specimen plants used in crossing experiments.

Opuntoid floral morphology early in the flowering season promotes natural outcrossing (Grant 1979). Early flowers were selected to use for the artificial crosses to reduce the possibility of self-pollination. Flower buds were bagged before anthesis with a 900 cm² (30 cm × 30 cm) piece of double-ply cheesecloth to deter pollination by floral visitors. Bagged flowers were checked for anthesis twice daily, at 1000 hrs and 1500 hrs. Appropriate pollen was transferred to open flowers at these times. Pollen transfer was performed with disposable cotton swabs that were broken in half after use to prevent cross-contamination. A set of three controls was designed to check for interfertility. To control for autogamy, flowers were bagged and pollen was transferred from the donor flower to its own gynoecium. To control for geitonogamy, pollen was transferred from several flowers of the plant to stigmas in different flowers on the same plant. To control for apomictic seed set, flowers were bagged, and no pollen transfer was performed. During spring 2000 the flowers of certain species were emasculated. The objectives of the emasculation procedure were to test for apomixis, expected to occur in certain hexaploids such as *O. engelmannii*. Flowers were emasculated prior to anthesis and prior to anther dehiscence, and bagged with cheesecloth immediately following the procedure. Emasculation was carried out by circumcision of the tepals above the attachment point to the pericarpel, followed by careful removal of the androecium with small forceps. Collecting fruits derived from unbagged flowers at the end of the bloom period obtained data

Table 1. Artificial reciprocal crosses involving Trans-Pecos *Opuntia* species; voucher specimens for plants used are housed in SRSC.

Cross performed	Plants used
<i>O. engelmannii</i> Salm-Dyck ex Engelm. var. <i>engelmannii</i>	Texas. Brewster Co., A. M. Powell 6009. 2n = 66
× <i>O. engelmannii</i> var. <i>lindheimeri</i> (Engelm.) B. D. Parfitt & Pinkava	Texas. Uvalde Co., J. F. Weedon 1670. 2n = 66
<i>O. engelmannii</i> var. <i>engelmannii</i>	Texas. Brewster Co., A. M. Powell 6009. 2n = 66
× <i>O. ×spinosibacca</i> M. Anthony	Texas. Brewster Co., Boquillas, B. G. Hughes 801. 2n = 44
<i>O. ×spinosibacca</i>	Texas. Brewster Co., Boquillas, B. G. Hughes 801. 2n = 44
× <i>O. chisosensis</i> (M. Anthony) D. Ferguson	Texas. Brewster Co., Panther Pass, A. M. Powell 5971. 2n = 22
<i>O. aureispina</i> (Brack & Heil) Pinkava & B. D. Parfitt	Texas. Brewster Co., Boquillas, B. G. Hughes 802. 2n = 22
× <i>O. chisosensis</i>	Texas. Brewster Co., Panther Pass, A. M. Powell 5971. 2n = 22
<i>O. aureispina</i>	Texas. Brewster Co., Boquillas, B. G. Hughes 802. 2n = 22
× <i>O. ×spinosibacca</i>	Texas. Brewster Co., Boquillas, B. G. Hughes 801. 2n = 22
<i>O. strigil</i> Engelm.	Texas. Pecos Co., ca. 10 mi W Fort Stockton, A. M. Powell 6008. 2n = 22
× <i>O. atrispina</i> Griffiths	Texas. Uvalde Co., J. F. Weedon 1661. 2n = 22
<i>O. macrocentra</i> Engelm. "azurea type"	Texas. Brewster Co., 2 mi N of Bee Mt., G. G. Raun 94-01. 2n = 22
× <i>O. macrocentra</i> var. <i>macrocentra</i>	Texas. Presidio Co., 30 mi S of Marfa, A. M. Powell 6023. 2n = 22
<i>O. macrocentra</i> var. <i>macrocentra</i>	Texas. Presidio Co., 30 mi S of Marfa, A. M. Powell 6023. 2n = 22
× <i>O. macrocentra</i> var. <i>minor</i> M. Anthony	Texas. Presidio Co., Ruidosa, AMP 6024. 2n = 44
<i>O. rufida</i> Engelm.	Texas. Brewster Co., P. R. Manning s.n. 2n = 22
× <i>O. macrocentra</i> "azurea type"	Texas. Brewster Co., J. F. Weedon 1795. 2n = 22
<i>O. aureispina</i>	Texas. Brewster Co., Rooney's Place, M. P. Griffith 73
× <i>O. macrocentra</i> "azurea type"	Texas. Brewster Co., J. F. Weedon 1795. 2n = 22

relevant to the expected seed set for each individual. These data were used for comparison with the artificial crosses and with the controls. Fruits that developed from open pollinated fruits are hereafter referred to as "native fruits."

Fruit and seed set evaluation.—Floral products were collected as they matured, and evaluated visually for fruit set. Fruit set was scored as either positive or negative. Seeds were dissected out of the fruits and counted. Mean and standard deviation of seed number were calculated for each test.

Analysis of hybrid embryos.—Any live embryos derived from experimental crosses were determined to be hybrid embryos if two conditions were met: (1) if normal seed set was observed in native fruits of the same plant; and (2) if virtually no seed set was observed in the apomictic, autogamous, and geitonogamous tests on the same plant.

RESULTS

Fruit and seed set scoring.—Native fruit set occurred as expected in all 13 taxa of *Opuntia* in the current study (Table 2). The results show apomictic seed set in only three taxa: *O. macrocentra* var. *macrocentra*, *O. engelmannii* var. *engelmannii*, and *O. engelmannii* var. *lindheimeri*. Autogamous and geitonogamous fruit set was only observed for the hexaploids *O. engelmannii* var. *engelmannii* and var. *lindheimeri*. Fruit set was observed in all reciprocal crosses except between

O. aureispina and *O. ×spinosibacca*, and between *O. chisosensis* and *O. ×spinosibacca*. Healthy mature fruits were always present when seed set numbers were above zero. Conversely, all floral products that did not develop into healthy fruits invariably lacked seeds. Seed counts for all tests performed are listed in Table 2.

Evaluation of hybrid embryos.—Hybrid embryos resulted from the following experimental crosses: *Opuntia aureispina* and *O. macrocentra* "azurea type;" *O. strigil* and *O. atrispina*; *O. aureispina* and *O. chisosensis*; *O. rufida* and *O. macrocentra* "azurea type;" *O. engelmannii* var. *engelmannii* and *O. ×spinosibacca*; and *O. macrocentra* var. *macrocentra* and *O. macrocentra* "azurea type." Hybrid embryos were also derived from the cross between *O. macrocentra* var. *macrocentra* (staminate parent) and *O. macrocentra* var. *minor* (ovulate parent), but the reciprocal cross did not produce seed. Artificial hybridization was not successful in the crosses between *O. aureispina* and *O. ×spinosibacca* or between *O. chisosensis* and *O. ×spinosibacca*, as the reciprocal cross set no seed. Successful artificial hybridization could not be determined with certainty in the crosses involving *O. engelmannii* as the ovulate parent, because seed set was abundant for the self-pollination controls performed.

DISCUSSION

Fruit and seed set evaluation.—Fruit set was in all cases a good indicator of seed set resulting from the

Table 2. Seed set per fruit for all crosses and experimental tests performed during the current study of *Opuntia* in the northern Chihuahuan Desert region.

Taxon	Fruit set	Test	Seed set per fruit \pm SD ^a
<i>O. macrocentra</i> var. <i>macrocentra</i> (2n = 22)	3 of 3	Native Fruit	60.00 \pm 11.53
	3 of 5	Apomixis	6.20 \pm 6.87
	1 of 2	Autogamy	0 \pm 0
	2 of 2	Geitonogamy	5.67 \pm 6.03
	4 of 4	\times "azurea type"	87.50 \pm 65.47
	7 of 7	\times var. <i>minor</i>	61.50 \pm 21.95
	<i>O. macrocentra</i> var. <i>minor</i> (2n = 44)	4 of 4	Native Fruit
0 of 7		Apomixis	0 \pm 0
0 of 5		Autogamy	0 \pm 0
0 of 8		Geitonogamy	0 \pm 0
1 of 5		\times "azurea type"	3.50 \pm 7.00
0 of 8		\times var. <i>macrocentra</i>	0 \pm 0
<i>O. macrocentra</i> "azurea type" (2n = 22)		5 of 5	Native Fruits
	0 of 8	Apomixis	0 \pm 0
	0 of 5	Autogamy	0 \pm 0
	0 of 8	Geitonogamy	0 \pm 0
	5 of 5	\times var. <i>macrocentra</i>	82.00 \pm 47.07
	1 of 5	\times <i>O. rufida</i>	26.50 \pm 10.6
	3 of 3	\times <i>O. aureispina</i>	57.62 \pm 15.35
<i>O. rufida</i> (2n = 22)	3 of 3	Native Fruit	34.67 \pm 6.51
	0 of 2	Apomixis	0 \pm 0
	0 of 2	Autogamy	0 \pm 0
	0 of 2	Geitonogamy	0 \pm 0
	1 of 1	\times "azurea type"	42.00
<i>O. engelmannii</i> var. <i>engelmannii</i> (2n = 66)	5 of 5	Native Fruits	685.00 \pm 78.31
	2 of 2	Apomixis	494.50 \pm 38.89
<i>O. engelmannii</i> var. <i>lindheimeri</i> (2n = 66)	2 of 2	Native Fruits	502.00 \pm 148.49
	2 of 2	Apomixis	443.50 \pm 92.63
<i>O. Xspinosibacca</i> (2n = 44)	4 of 4	Native Fruits	43.33 \pm 3.65
	0 of 5	Apomixis	0 \pm 0
	0 of 3	Autogamy	0 \pm 0
	0 of 4	Geitonogamy	0 \pm 0
	2 of 2	\times <i>O. engelmannii</i> var. <i>engelmannii</i>	14.75 \pm 6.23
	0 of 4	\times <i>O. aureispina</i>	0 \pm 0
	0 of 3	\times <i>O. chisosensis</i>	0 \pm 0
<i>O. aureispina</i> (2n = 22)	2 of 2	Native Fruits	49.00 \pm 2.83
	0 of 3	Apomixis	0 \pm 0
	0 of 2	Autogamy	0 \pm 0
	0 of 2	Geitonogamy	0 \pm 0
	0 of 2	\times <i>O. Xspinosibacca</i>	0 \pm 0
	3 of 3	\times <i>O. chisosensis</i>	41.00 \pm 19.97
	3 of 3	\times <i>O. macrocentra</i> "azurea type"	32.66 \pm 3.25
<i>O. chisosensis</i> (2n = 22)	5 of 5	Native Fruits	45.20 \pm 26.49
	0 of 4	Apomixis	0 \pm 0
	0 of 5	Autogamy	0 \pm 0
	0 of 4	Geitonogamy	0 \pm 0
	0 of 4	\times <i>O. aureispina</i>	51.40 \pm 11.57
	0 of 3	\times <i>O. Xspinosibacca</i>	0 \pm 0
	<i>O. strigil</i> (2n = 22)	5 of 5	Native Fruits
0 of 6		Apomixis	0 \pm 0
0 of 6		Autogamy	0 \pm 0
0 of 6		Geitonogamy	0 \pm 0
3 of 3		\times <i>O. atrispina</i>	63.00 \pm 25.33
<i>O. atrispina</i> (2n = 22)	4 of 4	Native Fruits	62.00 \pm 24.78
	0 of 2	Apomixis	0 \pm 0
	0 of 2	Autogamy	0 \pm 0
	0 of 2	Geitonogamy	0 \pm 0
	2 of 2	\times <i>O. strigil</i>	71.50 \pm 31.82

^a SD = Standard Deviation.

specific test. Fully developed fruits always contained numbers of viable seeds that were consistent with expected seed numbers in natural populations. Shriveled pericarpels, i.e., undeveloped fruits, invariably did not contain any seeds.

The experimentally demonstrated interfertility between *O. aureispina* and *O. macrocentra* "azurea type" supports the hypothesis (Griffith 2001) that *O. ×rooneyi* is the result of hybridization between these parental taxa.

Results from the artificial crosses between *O. engelmannii* var. *engelmannii* ($2n = 66$) and *O. engelmannii* var. *lindheimeri* ($2n = 66$) are inconclusive regarding the interfertility of these two taxa. Both taxa exhibited abundant seed set for every test performed. No test involving var. *engelmannii* or var. *lindheimeri* resulted in complete prevention of seed set. Apomixis trials show that these taxa do set seed in large numbers through apomixis (Table 2). The seeds resulting from artificial reciprocal crosses between the two varieties are likely the result of apomixis as well.

I expect the partially sympatric taxa *O. atrispina* and *O. strigil* to be fully interfertile in the field. The experimentally demonstrated interfertility supports the hypothesis that plants observed in areas of sympatry exhibiting intermediate morphology between *O. atrispina* and *O. strigil* are hybrids between these two taxa. Interfertility may also support the hypothesis that *O. atrispina* and *O. strigil* represent two ends of a morphological cline within one variable species.

Opuntia aureispina and *O. chisosensis* appear to be fully interfertile. The only barrier to the hybridization of *O. aureispina* and *O. chisosensis* in the field appears to be the distance between the populations. The experimentally demonstrated interfertility suggests a close relationship between these two taxa.

The documented interfertility (Table 2) between *O. macrocentra* "azurea type" and *O. rufida* suggests an explanation for a natural population of plants exhibiting intermediate morphology between these taxa. These results support the hypothesis that the natural intermediate population could result from interspecific hybridization of these taxa.

Seed set data suggest that artificial hybridization was successful and that natural hybridization is possible among members of the *Opuntia macrocentra* complex. The artificial crosses between *O. macrocentra* var. *macrocentra* ($2n = 22$) and *O. macrocentra* "azurea type" ($2n = 22$) document the interfertility of these plants (Table 2). I expect *O. macrocentra* var. *macrocentra* and *O. macrocentra* "azurea type" to be fully interfertile under natural sympatric conditions. Complete interfertility was not observed in crosses between *O. macrocentra* var. *minor* ($2n = 44$) and *O. macrocentra* var. *macrocentra*, or between *O. macrocentra* var. *minor* and *O. macrocentra* "azurea type."

Experimental crosses using *O. macrocentra* var. *minor* as the female parent were not found to set seed. The seed set data for *O. macrocentra* var. *minor*, *O. macrocentra* var. *macrocentra*, and *O. macrocentra* "azurea type" suggest a directional barrier to hybridization related to ploidy level. Previous tests (Lewis 1979) demonstrated a similar directional barrier to reproduction among *Pyrus* (Rosaceae: Amygdaloideae) specimens of different ploidy levels. As *O. macrocentra* var. *minor* is sympatric or peripatric with both *O. macrocentra* var. *macrocentra* and *O. macrocentra* "azurea type," it is possible that hybrid triploid plants exist in the field. These data support previously reported odd-ploid chromosome counts (Grant and Grant 1982).

Crosses between *Opuntia engelmannii* var. *engelmannii* (staminate parent) and *O. ×spinosibacca* (ovulate parent) resulted in abundant seed set (Table 2). Because *O. ×spinosibacca* was not observed to set seed by apomixis or self-pollination, the seeds resulting from *O. ×spinosibacca* ($2n = 44$) flowers receiving *O. engelmannii* var. *engelmannii* ($2n = 66$) pollen were determined to contain hybrid embryos. Hybrids between *O. engelmannii* var. *engelmannii* and *O. ×spinosibacca* would most likely be pentaploids.

In both the crosses between *O. ×spinosibacca* ($2n = 44$) and *O. engelmannii* var. *engelmannii* ($2n = 66$), and the crosses between *O. macrocentra* var. *minor* ($2n = 44$) and *O. macrocentra* "azurea type" ($2n = 22$) or *O. macrocentra* var. *macrocentra* ($2n = 22$), hybrids survive only if the male parent is of the higher ploidy level. Lewis (1979) reported a similar unidirectional barrier to compatibility in *Pyrus*. Two diploid taxa, *Opuntia aureispina* and *O. chisosensis*, produced no seed when crossed with tetraploid *O. ×spinosibacca*. *Opuntia ×spinosibacca* occurs in areas of overlap with *O. aureispina*, but these two taxa do not appear to be interfertile.

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