

CHROMOSOME NUMBERS OF MISCELLANEOUS SOUTHWESTERN NORTH AMERICAN ANGIOSPERMS

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ABSTRACT

Fifty-nine original meiotic chromosome counts are presented for 43 angiosperm species across 31 genera in 14 families. Reports are based on observation of pollen mother cells in flower bud collections made from wild populations in New Mexico, western Texas, southeastern Arizona, and north-central Mexico. Of these 59 counts, 10 are first reports, nine belong to assorted genera of Nyctaginaceae (*Acleisanthes*, *Allionia*, *Boerhavia*, *Cyphomeris*, *Mirabilis*) and one pertains to Lamiaceae (*Scutellaria*). New counts and results are compared to chromosome counts and analyses published in earlier studies. Findings highlight specific taxa, populations and areas that would benefit from additional sampling to elucidate patterns of variation related to cytology and biodiversity that could, in turn, strengthen genomic studies, taxonomic classifications and conservation strategies.

RESUMEN

Se reportan un total de 59 conteos cromosómicos de 43 especies que pertenecen a 31 géneros de 14 familias de angiospermas. Los conteos fueron observados en células madre de polen de botones florales recogidos en Nuevo México, el oeste de Texas, el sureste de Arizona y en el norte-centro de México. De los 59 conteos cromosómicos, diez representan nuevos informes, de los cuales nueve corresponden a la familia Nyctaginaceae, específicamente en los géneros *Acleisanthes*, *Allionia*, *Boerhavia*, *Cyphomeris* y *Mirabilis*, así como una especie en el género *Scutellaria* de la familia Lamiaceae. Cada informe se ha comparado con conteos previos publicados en la literatura, proporcionando un contexto histórico y científico a los hallazgos. Para un grupo de especies, se incluyen comentarios sobre su biodiversidad y variación cromosómica en ciertas poblaciones y áreas geográficas que podrían fortalecer futuras investigaciones genómicas, taxonómicas y estrategias de conservación.

INTRODUCTION

There has been a shift away from determining chromosome number in plants in recent decades due to the rise of modern genomic methods (Goldblatt 2007; Soltis et al. 2018; Windham et al. 2020), but such data remains important to research involving plant genetics, evolution, polyploidy, morphology and taxonomy (Stuessy 2009; Stuessy et al. 2014). Documenting variation in the number of plant chromosomes in a group of closely-related species or between populations of a single species often identifies interesting patterns of adaptation and evolution in studies of polyploidy (e.g., *Larrea*, Porter 2016) and for morphologically uniform species (e.g., *Dalea formosa* Torr, Spellenberg 1981). In addition, ascertaining the actual chromosome number of a taxon is necessary to verify results generated via rapid, but still relatively expensive flow cytometry methods (Suda et al. 2007). Despite the continued benefits of counting plant chromosome numbers, little progress has been made since Bennett (1998) estimated that this kind of cytological information was available only for about 25% of angiosperm species worldwide.

The present study continues earlier cytological research emphasizing angiosperm taxa occurring in New Mexico and the surrounding area (e.g., Ward & Spellenberg 1982; Ward 1983a, 1983b, 1984; Ward & Spellenberg 1986, 1988). Most of the taxa covered in the paper belong to desert ecosystems or some other form of drylands in regions that have undergone dramatic historical changes, including alteration from ancient pine forest to present-day desert scrublands (Delcourt & Delcourt 1993, and other chapters in this volume concerning phytogeography).

MATERIALS AND METHODS

Flower buds were collected in the field into vials of a modified Carnoy's solution (4 chloroform: 3 absolute ethanol: 1 acetic acid), which was kept cool and then stained in the lab using Snow's (1963) hydrochloric acid-carmine stain (protocol briefly outlined in Ward 1983b). The buds, now prestained for immediate or later use, were kept in 70% ethanol. To obtain a meiotic spread of chromosomes, anthers were removed from buds and gently macerated on a microscope slide in a drop of 45% acetic acid, or in Hoyer's solution, then gently squashed under a coverslip. All counts are based on microscope observations of pollen mother cells that were present in the anthers and undergoing meiosis. Counts were made from a pollen collection in the population, and not necessarily from buds of the specific plant represented by the voucher specimen. See the appendix for a more detailed explanation of the chromosome count protocol (and cf. methodology of Windham et al. 2020).

Voucher specimens documenting chromosome counts in this paper were collected from wild populations in the American Southwest and northern Mexico. All vouchers are deposited at NMC and are available to view in digital form as images posted on the NMC-NMCR herbarium database (<http://nmc.nmsu.edu/nmc/>). See Thiers (2024) for herbarium acronyms and related institutional information. Every voucher image includes chromosome squash data accompanied by either a hard-copy illustration of the original squash drawn with the aid of a camera lucida (for older observations), or via a series of digital photomicrographs (for newer observations). The NMC barcode number cited for each voucher can be used to view specific squash images and count details using the aforementioned link.

Two comprehensive databases were consulted to identify literature and other sources containing previously published chromosome counts for the taxa treated herein (*The Chromosome Counts Database*, CCDB 2024, see also Rice et al. 2014; and *Tropicos* 2024, data available under the "Chromosome Counts" tab in individual taxon records). Taxonomic circumscriptions, identifications, and geographic distribution statements were based on the most recent revisions and floras as cited in the discussion of the individual chromosome counts listed below.

RESULTS AND DISCUSSION

The meiotic chromosome number was determined for 43 species, including two subspecies and 14 varieties (59 total counts). The species, all eudicots, belong to 31 different genera in 14 families. More than a third of the counts represent a single family, Nyctaginaceae, a group that has been poorly documented cytologically in the past, in part because of the difficulties with preparing and observing their small chromosomes, which have bivalents that tend to clump together in meiotic squashes.

Ten counts are first counts (23%), 30 are corroborating counts (70%), and three are differing counts (7%). The first counts pertain to nine species and one variety, with eight species belonging to Nyctaginaceae and one to Lamiaceae (*Scutellaria potosina*). First counts are also presented for two genera of Nyctaginaceae (*Acleisanthes*, *Cyphomeris*). Reports of $n = 35$ and $n = 40$ are noted for two different species of *Acleisanthes* (a genus of 17 spp.). Both species of the genus *Cyphomeris* were sampled and found dissimilar ($n = 13$ for *C. crassifolia* and $n = 22$ for *C. gypsophiloides*). The three novel counts differing from previous reports are $n = 24$ for *Packeria multilobata* (vs. normally 23), $n = 15$ for *Drymaria leptophylla* (vs. $n = 18$), and $n = ca. 20$ for *Eriogonum polycladon* (vs. $n = 13$).

Geographically, about half of the combined counts correspond to taxa occurring in New Mexico (37

counts, 18 from Doña Ana Co.), with the remainder based on populations located in southeastern Arizona (8, all in Cochise Co.), western Texas (6), and north-central Mexico (8). Most species treated in this paper are poorly sampled cytologically throughout their geographic ranges. For several taxa, it is obvious that much denser cytological sampling, within and between populations, will be necessary to determine the substantial variation in chromosome number and ploidy levels (e.g., see entries for *Eriogonum polycladon*, *Larrea tridentata*, *Nerysirenia camporum*). Additional chromosome data will be useful to address some taxonomic issues encountered during the preparation of the paper (e.g., the *Glandularia bipinnatifida* complex including *G. wrightii*, *Mirabilis linearis* s. lat., *Dalea albiflora* together with the recently synonymized *D. ordiae*, and both species of *Cyphomeris*).

In the following listing of 59 chromosome counts, taxa are arranged alphabetically first by family and then genus. In a few discussions we refer to “the Four Corners” area, meaning the region where the state lines of Utah, Colorado, Arizona, and New Mexico all have a common corner.

* indicates probable first published count for the taxon

+ denotes a novel number reported for the taxon

Abbreviations for commonly cited collectors below: DEW (Darrell E. Ward), RJS (Robert J. Soreng), RWS (Richard Spellenberg).

ASTERACEAE

Aphanostephus ramosissimus DC. var. **humilis** (Benth.) B.L. Turner & Birdsong. $n = 4$. USA. New Mexico. Doña Ana Co.: E end of Aden Crater lava bed, 4100 ft [1250 m], 32°05'N, 106°50'W, 6 Apr 1988, DEW & RWS 88-031 (NMC029735).

The count agrees with previous reports for populations observed in USA: New Mexico. Lincoln Co.; Texas, Terrell Co. (Solbrig et al. 1969); Mexico: Chihuahua (Keil & Stuessy 1975; Powell & Powell 1978) and San Luis Potosi (Lane & Li 1993). No published counts were found for this species occurring in southern Arizona, the western edge of the distributional range of *Aphanostephus ramosissimus* var. *humilis*. Other varieties have been similarly reported as $n = 4$ (e.g., Solbrig et al. 1969; Nesom 2006; CCDB 2024).

Conoclinium dissectum A. Gray. $n = 10$. USA. New Mexico. Hidalgo Co.: Playas Valley, between Little Hatchet Mountains and the hills at the N end of the Animas Mountains, 3.8 mi [6.1 km] W of Playas Smelter Road, 4500 ft [1372 m], 31°55'N, 108°40'W, 17 Sep 1989, DEW, RWS & D. Sutherland 89-020 (NMC031790).

This report appears to be the first count for a New Mexico population of *Conoclinium dissectum* [syn: *C. greggii* (A. Gray) Small]. The same number of $n = 10$ was published for plants growing in Texas: Brewster Co. (Watanabe et al. 1995); Mexico: Coahuila (Powell & Powell 1977), Durango (Keil & Stuessy 1977) and San Luis Potosi (Urbatsch 1974). Regarding sampling, no reports have been published for Cochise Co., Arizona, or Duval Co., Texas, the geographic E and W ends of the distribution of the species.

Malacothrix fendleri A. Gray. $n = 7$. USA. New Mexico. Doña Ana Co.: Doña Ana Mountains, 4600 ft [1402 m], 32°25'N, 106°45'W, 10 Apr 1988, DEW 88-034 (NMC036057).

The count $n = 7$ for *Malacothrix fendleri* matches two earlier reports for New Mexico populations located in Doña Ana (Ward 1984) and Socorro counties (Tomb et al. 1978). $N = 7$ has also been reported for several different localities in Apache Co., Arizona (Powell et al. 1974; Tomb 1974; Zhao 1996). The species is well sampled across the core of its distributional range (see USDA Plants 2024).

+Packera multilobata (Torr. & A. Gray ex A. Gray) W.A. Weber & A. Löve. $n = 24$. USA. New Mexico. McKinley Co.: 3 mi [4.8 km] N of Prewitt and IH-40, 6900 ft [2103 m], 3 May 1988, DEW & RWS 88-057 (NMC036462).

The count of $n = 24$ reported here is novel for *Packera multilobata* [syn: *Senecio multilobatus* Torr. & A. Gray ex A. Gray]. Most reports are $n = 23$ for a number of populations, including those from USA: California, Inyo Co.; Colorado, Rio Blanco Co.; Utah, Iron Co. (Ornduff et al. 1967); Wyoming, Uinta Co.; (Hartman 1977); Arizona, Coconino Co.; Colorado, Grand Co. (Keil & Stuessy 1977), and Montrose counties; Utah, San Juan

Co.; Nebraska, Lincoln Co. (Bain 1985). Several additional counts of $n = 46$ were published for New Mexico, Grant Co.; Utah, Iron Co.; Arizona, Gila Co. (Bain 1985); Utah, Salt Lake Co. (Morton 1981), and for a few counties in Arizona including Mohave (Ward & Spellenberg 1982), Yavapai (Keil 1981) and Pima counties (Morton 1981). No apparent correlation with local habitat has been suggested; for the most part tetraploid counts are from more southern populations.

Platyschkuhria integrifolia (A. Gray) Rydb. var. **oblongifolia** (A. Gray) W.L. Ellison. $n = 12$. USA. New Mexico. San Juan Co.: ca. 30 mi [48 km] N of Crownpoint, NM-371 roadside, 5800 ft [1768 m], 36°07'N, 108°14'W, 3 May 1988, DEW & RWS 88-059 (NMC037218, NY01923059).

Four varieties of *Platyschkuhria integrifolia* were recognized in the latest taxonomic revision of this monotypic genus (Ellison 1971). New Mexico populations represent *P. integrifolia* var. *oblongifolia* (Allred & Jercinovic 2020). This variety, geographically isolated from the three other more northern varieties, is centered around the Four Corners area and differs from the other varieties in its tendency to have more leafy stems. Other recent floristic treatments (e.g., Strother 2006; Ackerfield 2022) do not recognize varieties in this species. The new count of $n = 12$ reported here corroborates an earlier count by Ward (1984) for a different San Juan Co., New Mexico, population. See Keil and Pinkava (1976) and Brown (1983) for summaries of variation in ploidy across the distributional range of the species.

Solidago wrightii A. Gray. $n = 9$. USA. New Mexico. Grant Co.: NE side of Bear Mountain, NW of Silver City, 6 Sep 1980, RWS, RJS & DEW 5856 (NMC038702).

The $n = 9$ number reported here agrees with earlier published counts for *Solidago wrightii*, including those cited in literature previously as *S. wrightii* var. *adenophora* S.F. Blake. Populations throughout most of the geographical range have been sampled, i.e., USA: New Mexico, Hidalgo Co. (Ward & Spellenberg 1988); Grant, Lincoln and Luna counties (Ward & Spellenberg 1986); Arizona, Cochise and Greenlee counties (Ward & Spellenberg 1986; Semple & Chmielewski 1987) and Coconino (Keil 1979) and Yavapai counties (Semple et al. 1992); Texas, Culberson and Jeff Davis counties (Ward & Spellenberg 1986; Semple et al. 2001); Mexico: Durango (Turner et al. 1962). In fact, all taxa in *Solidago* subsect. *Thyrsiflorae*, including *S. wrightii*, are reportedly diploid, $2n = 18$ (Semple et al. 2016).

BORAGINACEAE

Lappula occidentalis (S. Watson) Greene var. **occidentalis**. $n = 12$. USA. New Mexico. Doña Ana Co.: NE of Las Cruces, ca. 1 mi [1.6 km] S of County Road D063, SE foothills of the Doña Ana Mountains, 4600 ft [1402 m], 32°30'N, 106°45'W, 17 Mar 1988, DEW 88-002 (NMC041814, RSA0140371).

Two varieties of *Lappula occidentalis* were accepted in the latest flora of New Mexico, with the species noted to show “a bewildering array of inconsistent forms” (Allred & Jercinovic 2020: 250). The voucher from the Doña Ana Mountains fits the autonymic variety best. Löve and Löve (1975) published one report of $2n = 48$ for the species from Manitoba, Canada, with the population mostly likely representing *L. occidentalis* var. *occidentalis*.

BRASSICACEAE

Dimorphocarpa wislizeni (Engelm.) Rollins. $n = 9$ (5 counts). USA. New Mexico. Doña Ana Co.: S foothills of Doña Ana Mountains, Desert Peaks National Monument, 5 mi [8 km] NE of Las Cruces, 4226 ft [1288 m], 32°24.601'N, 106°46.308'W, 11 Mar 2019, DEW 19-016 (NMC098018); W foothills of Doña Ana Mountains, USDA Jornada Experimental Ranch, ca. 1.5 mi [2.4 km] NNE of IH-25 exit 18 (Radium Springs), 4131 ft [1259 m], 32°30.298'N, 106°53.897'W, 11 Mar 2019, DEW 19-017 (NMC098019); Alameda Arroyo, ca. 8 mi [13 km] ENE of Las Cruces, 4114 ft [1254 m], 32°22.466'N, 106°41.293'W, 4 Apr 2019, DEW 19-040 (NMC098020); Crawford Airport, along IH-10 frontage road, W of Las Cruces, 4287 ft [1307 m], 32°16.691'N, 106°53.949'W, 7 May 2019, DEW 19-063 (NMC098021); SE of Las Cruces, W base of Bishop's Cap (mountain) at W end of Bishop's Gap, 4157 ft [1267 m], 32°11.922'N, 106°28.728'W, 3 Apr 2020, DEW 20-003 (NMC098022).

These five new counts of $n = 9$ agree with numerous reports made throughout most of the geographic range of the species, including from USA: New Mexico, Doña Ana Co., SE of Las Cruces (Ward 1983a); Bernalillo Co. (Rollins & Rüdénberg 1977); Texas, Presidio Co. (Rollins 1966); Arizona, Pinal Co. (Rollins 1966), Coconino Co. (Rollins & Rüdénberg 1971), Maricopa Co. (Pinkava et al. 1972); Utah, Washington Co. (Rollins & Rüdénberg 1971). A chromosome number of $2n = 18$ was reported for *Dimorphocarpa wislizeni*, without voucher information (Rollins 1993). No published counts were found for populations representing the NW and N extremes of the geographic distribution, i.e., Nevada: Lincoln Co. and Utah: Emery and Grand counties.

Molecular work by Fuentes-Soriano and colleagues identified a monophyletic Brassicaceae clade in tribe Physarieae formed by five genera, *Dimorphocarpa-Dithyrea-Nerisyrenia-Lyrocarpa-Synthlipsis* (DDNLS clade, Fuentes-Soriano & Al-Shehbaz 2013); the count of $n = 9$ reported here is consistent with earlier determinations of the basic chromosome number of the clade. This number is also notable because *Dimorphocarpa*, unlike other members in the group (cf. *Nerisyrenia*), exhibits one of the most stable chromosome number patterns (Fuentes-Soriano 2010).

Dryopetalon runcinatum A. Gray. $n = 12$. USA. New Mexico. Doña Ana Co.: E-facing slope of the Doña Ana Mountains, 4600 ft [1402 m], 32°29'N, 106°50'W, 24 Mar 1988, DEW 88-013 (NMC043707).

$N = 12$ was reported previously for *Dryopetalon runcinatum* var. *laxiflorum* Rollins based on a population from Sinaloa, Mexico (Rollins & Rüdénberg 1971). Rollins (1993) also noted $n = 12$ for *D. runcinatum* var. *runcinatum*, without providing voucher information. In recent years, workers have abandoned assigning varieties for this highly variable species (e.g., Al-Shehbaz 2007; Allred & Jercinovic 2020).

Lepidium lasiocarpum Nutt. ex Torr. & A. Gray subsp. **lasiocarpum**. $n = 16$. USA. New Mexico. Doña Ana Co.: New Mexico State University campus, 3900 ft [1189 m], 32°17'N, 106°45'W, 17 Mar 1988, DEW 88-001 (NMC044019, NY03178863).

In the US flora, *Lepidium lasiocarpum* includes two subspecies, *L. lasiocarpum* subsp. *lasiocarpum* and *L. lasiocarpum* subsp. *wrightii* (A. Gray) Thell., both of which have overlapping geographic ranges in New Mexico (Allred & Jercinovic 2020). Rollins (1993) cited $n = 16$ for the autonymic subspecies and $2n = 32$ for *L. lasiocarpum* subsp. *wrightii*. Neither count was accompanied by voucher information. Earlier Rollins and Rüdénberg (1971) noted $n = 16$ for a population in Clark Co., Nevada, but the voucher (Rollins 6706) was not attributed to a specific variety in that publication. However, Nevada populations correspond to *L. lasiocarpum* subsp. *lasiocarpum* (Rollins 1993; USDA Plants 2024). Oddly, the specimen labels of both duplicates of Rollins 6706 indicate a discordant number of $n = 14$ (GH01548919, NY03178858). Given the relatively wide distribution of the species, stretching from California to Texas and throughout much of northern Mexico, our understanding of the chromosome variation in the two infraspecific taxa would benefit from broader population sampling.

Nerisyrenia camporum (A. Gray) Greene. $n = 18$ (2 counts). USA. New Mexico. Doña Ana Co.: Robledo Mountains, NW of Las Cruces, 4035 ft [1230 m], 32°23.020'N, 106°52.131'W, 26 Feb 2019, DEW 19-002 (NMC102068); 3 mi [4.8 km] E of Doña Ana (village), S foothills of Doña Ana Mountains, ca. 5 mi [8 km] NE of Las Cruces, Cerro Vista (Bureau of Land Management) Bike Trails, Desert Dance Trail, 4223 ft [1287 m], 32°24.563'N, 106°46.248'W, 6 Apr 2020, DEW 20-008 (NMC098076).

This Chihuahuan Desert species exhibits a large range of chromosome variation, with counts ranging from $n = 7$ (Rollins 1939) to $n = 58$ (Bacon 1975; Rollins 1993). Besides the new reports of $n = 18$ for two Doña Ana Co. populations reported here, counts of $n = 36$ (Ward 1983b) and $2n = 36$ (Bacon, 1978) have been published for plants growing in adjacent Otero County. *Nerisyrenia camporum* displays two main cytotype races: diploids in the Chihuahuan Desert ($n = 18$) and tetraploids ($n = 36$) in the regions surrounding the diploid populations. This distribution pattern aligns with our observations. Further chromosome counts are needed to confirm the types and extent of aneuploidy patterns within both cytotypes. Furthermore, regional research will help

to evaluate the extent of a third cytotype ($n = 16$), which has so far only been reported near Coahuila, Mexico. For a comprehensive discussion of the ploidy and aneuploid variation in *N. camporum* see Bacon (1978).

CARYOPHYLLACEAE

+**Drymaria leptophylla** (Cham. & Schltdl.) Fenzl ex Rohrb. var. **leptophylla**. $n = 15$. USA. New Mexico. Grant Co.: 9 mi [14.5 km] NW of Silver City, 7000 ft [2134 m], 32°50'N, 108°22'W, 15 Sep 1989, DEW, RWS & D. Sutherland 89-013 (NMC046738).

This count of $n = 15$ has not been previously reported for *Drymaria leptophylla*, including under the recently synonymized *D. tenella* A. Gray (Allred & Jercinovic 2020). Ward and Spellenberg (1988) published a first count of $n = 18$ for the species (as *D. tenella*), based on a population from Cochise Co., Arizona. Hartman (2005) noted $2n = 36$ (as *D. leptophylla* var. *leptophylla*) without citing a voucher or providing a reference for the count. The populations in the US belong to the autonymic variety, while the other two recognized varieties are restricted to Mexico (Duke 1961). No earlier chromosome counts were found for either of those varieties.

FABACEAE

Astragalus crassicaarpus Nutt. var. **crassicaarpus**. $n = 11$. USA. New Mexico. Socorro Co.: 7 mi [11.3 km] E of San Antonio, 13 Apr 1988, RWS & DEW 9479 (NMC052553, NY01247916).

Three of the five varieties recognized for *Astragalus crassicaarpus* occur in New Mexico (Allred & Jercinovic 2020). There are two chromosome number counts known for this variety, this New Mexico count of $n = 11$ and another of $2n = 22$, based on material from Manitoba, Canada, near the northern extreme of the geographic distribution of the species (Löve & Löve 1982). Welsh (2023) recorded $2n = 22$ for three other varieties of *A. crassicaarpus*. There is no record of a count for the Texas endemic, *A. crassicaarpus* var. *berlandieri* Barneby.

Astragalus mollissimus Torr. var. **bigelovii** (A. Gray) Barneby. $n = 11$. USA. New Mexico. Grant Co.: NW of Deming, City of Rocks State Park, 5200 ft [1585 m], 28 Mar 1981, DEW, R. Develice, J. Hansen & R. Steeb 81-040 (NMC052809, NY01265934).

The same count has been reported for several populations of *Astragalus mollissimus* var. *bigelovii* in New Mexico in Doña Ana and Luna counties (Spellenberg 1976), and in Texas in Hudspeth Co. (Powell & Turner 2005). Six varieties are currently accepted for the species in the New Mexico flora (Allred & Jercinovic 2020). All varieties counted are consistently $n = 11$ (see CCDB 2024).

Astragalus wootonii E. Sheld. var. **wootonii**. $n = 11$. USA. New Mexico. Doña Ana Co.: NW Doña Ana Mountains, in an arroyo ca. 2 mi [3.2 km] E of Fort Selden, 4200 ft [1280 m], 32°30'N, 106°53'W, 19 Mar 1988, DEW 88-007 (NMC052443, NY01283796).

The identification of this voucher follows several recent treatments (Estrada-Castillón et al. 2023; Welsh 2023). In others, such as Allred and Jercinovic (2020), the specimen would be treated as *Astragalus allochrous* A. Gray var. *playanus* (M.E. Jones) Isely. Nonetheless, the two species, *A. allochrous* and *A. wootonii*, are similar morphologically, largely sympatric, and share the same $2n = 22$ chromosome number (Spellenberg 1976; Ward 1983a).

Dalea albiflora A. Gray. $n = 14$ (3 counts). USA. Arizona. Cochise Co.: Chiricahua Mountains, Coronado National Forest, 3 air-mi [4.8 km] W of Portal, 6400 ft [1951 m], 8 Sep 1986, DEW 86-023 (NMC054664, NY010733348); same general locality, Turkey Creek Canyon, near Onion Saddle, 7 air-mi [11.3 km] W of Portal, 6800–7000 ft [2073–2134 m], 9 Sep 1986, DEW 86-039 (NMC054665, NY010733349, RSA0207412). New Mexico. Grant Co.: Burro Mountains, 10.9 mi [17.5 km] SW of US Highway 180, 6.2 mi [10 km] W of Forest Service Road 851, 16 Sep 1989, Sutherland, RWS & DEW 6864 (NMC054281, NY01073378).

The three new counts of $n = 14$ reported here for *Dalea albiflora* [syn: *D. ordiae* A. Gray] match four of the five previous counts made for populations in Sierra Co., New Mexico, and Cochise Co., Arizona (Ward & Spellenberg 1988; Ward et al. 1993). The exception was a report of $n = 7$ for a different Cochise Co. population, located near Bisbee, Arizona (Ward et al. 1993). Sutherland (2023) noted the complex and variable nature of

D. albiflora and stressed the importance of additional cytological population sampling across the entire geographic range of the species that extends from Mohave Co. in northwestern Arizona to Sierra Co., New Mexico (USDA Plants 2024).

Dalea leporina (Aiton) Bullock. $n = 7$. USA. Arizona. Cochise Co.: Chiricahua Mountains, Coronado National Forest, Rustler Park, 8000 ft [2438 m], 16 Sep 1989, Sutherland, RWS & DEW 6873 (NMC054854, NY01270145).

Dalea leporina is the most broadly distributed species in the genus, ranging throughout most of the United States through Costa Rica, and then disjunct in the Andes of South America (Sutherland 2023). $N = 7$ has been reported for numerous populations in several parts of the US and Mexico (Ward et al. 1993 references therein).

Dalea polygonoides A. Gray. $n = 7$. USA. Arizona. Cochise Co.: Chiricahua Mountains, Coronado National Forest, Rustler Park, 8000 ft [2438 m], 16 Sep 1989, Sutherland, RWS & DEW 6872 (NMC054954, NY01277232).

Other reports of $n = 7$ have been published for individuals in New Mexico in Sierra and Grant counties (Ward et al. 1993). Barneby (1977) cited a count of $2n = 7_{II}$ made by Mosquin, without providing a specific locality.

Sophora nuttalliana B.L. Turner. $n = 18$. USA. New Mexico. Sierra Co.: ca. 7 mi [11.3 km] E of San Antonio, barren Dakota sandstone, 5000 ft [1524 m], 33°50'N, 106°45'W, 13 Apr 1988, DEW 88-046 (NMC057760).

The report of $n = 18$ for *Sophora nuttalliana* [syn: *Vexibia nuttalliana* (B.L. Turner) Yakovlev] is the first known count for populations occurring in New Mexico. The species is found in much of the American West, and well into Chihuahua, Mexico. Vincent and Kearns (2024) also recorded $2n = 36$.

LAMIACEAE

Monarda punctata L. var. **occidentalis** (Epling) E.J. Palmer & Steyer. $n = 12$. USA. New Mexico. Doña Ana Co.: Organ Mountains, Aguirre Springs Recreation Area, Pine Tree loop, 1993 m, 32.357947°N, 106.561283°W, 12 Nov 2017, A. Abair 355 (NMC099197).

$N = 12$ was reported previously for this variety based on a population located in Cibola Co., New Mexico (Ward & Spellenberg 1988). Five other varieties have all been noted as $n = 11$ (see references in CCDB 2024).

***Scutellaria potosina** Brandege var. **tessellata** (Epling) B.L. Turner. $n = 26$. USA. New Mexico. Sierra Co.: roadside of NM-27, 14 mi [22.5 km] N of Nutt, 5100 ft [1554 m], 32°43'N, 107°35'W, 15 Sep 1989, DEW, RWS & D. Sutherland 89-007 (NMC064952).

This report of $n = 26$ marks the first known count for *Scutellaria potosina*. Six infraspecific varieties are recognized for the species (Turner 1994), with two occurring in New Mexico (Allred & Jercinovic 2020). *Scutellaria* is a diverse, cosmopolitan genus of ca. 360 species. Counts ranging from $n = 7$ to $n = 44$ have been noted for various species of *Scutellaria* (see CCDB 2024). The only other *Scutellaria* species reported with $n = 26$ is *S. longituba* Koidz., a species from Ogasawara Islands in the Japanese archipelago (Goldblatt 1981; Iwatsuki et al. 1993). For a cytological review of the American species see Harley and Heywood (1992).

NYCTAGINACEAE

***Acleisanthes lanceolata** (Wooton) R.A. Levin var. **lanceolata**. $n = 35$. USA. Texas. Culberson Co.: state highway TX-54, N of Van Horn, 15 mi [24 km] S of junction with US Highway 62-180, 3500 ft [1067 m], 5 Sep 1988, RWS & DEW 9711 (NMC068595, NY03371016).

The counts presented here for *Acleisanthes lanceolata* (Wooton) R.A. Levin and *A. longiflora* A. Gray (next entry) appear to be the first published reports for the genus *Acleisanthes* (17 species – sensu Poole 2003). The autonymic variety occurs throughout central New Mexico and reaches western Texas and parts of Chihuahua, Mexico. For a discussion of *A. lanceolata*, an obligate gypsophilous species complex, see Spellenberg and Poole (2003).

***Acleisanthes longiflora** A. Gray. $n = 40$. USA. Texas. Hudspeth Co.: Texas Road 192, between Quitman Mountains and the Rio Grande, 3300 ft [1006 m], 2 Sep 1988, RWS & DEW 9687 (NMC068642).

Acleisanthes longiflora occurs in dry regions ranging from southern California through Texas and is also found in several states across most of northern Mexico (Poole 2003).

***Allionia choisyi** Standl. $n = 22$. USA. New Mexico. Otero Co.: at junction of NM Highway 506 N of Dell City, Texas, 1130 m, 5 Sep 1988, RWS & DEW 9712 (NMC068714, NY04268601).

No previously published reports were found for *Allionia choisyi*, a widespread species distributed from the SE corner of Utah southward to Argentina. Within *Allionia*, a different number of $n = 20$ was published for the partially sympatric *A. incarnata* L., based on a plant growing near Las Cruces, Doña Ana Co., New Mexico (Ward 1983b; Spellenberg 2003a); a Peruvian population of *A. incarnata* was reportedly $n = ca. 58$ (Fedorov 1969).

***Boerhavia gracillima** Heimerl $n = 26$. USA. Texas. Brewster Co.: Study Butte, 2600 ft [792 m], 3 Sep 1988, RWS & DEW 9700 (NMC069512, NY03371693).

This report is the first known count for *Boerhavia gracillima*, a species that is distributed in the southern portions of Arizona and New Mexico, the Big Bend region of Texas and southward to Oaxaca, Mexico (Tropicos 2024; USDA Plants 2024). The most common number encountered in taxa of *Boerhavia* is $n = 26$, but published counts range from $n = 12$ for *B. coccinea* Mill. to $n = 52$ for *B. procumbens* Banks ex Roxb. and *B. repens* L. (see CCDB 2024).

***Boerhavia purpurascens** A. Gray. $n = 27$. USA. New Mexico. Grant Co.: E side of Burro Mountains, roadside of US Forest Service Road 851, 6 mi [9.7 km] W of the junction with the road connecting US Highway 180 and Tyrone, 6100 ft [1859 m], 32°40'N, 108°30'W, 16 Sep 1989, DEW, RWS & D. Sutherland 89-014 (NMC069010).

Boerhavia purpurascens occurs in open, dry grasslands on sandy soils and pinyon-juniper woodlands from central Arizona through southern New Mexico and into the Mexican states of Chihuahua and Sonora (Spellenberg 2003b; Allred & Jercinovic 2020). This report is the first known count of the species. One other *Boerhavia* species, *B. wrightii* A. Gray, is often found growing in the same area as *B. purpurascens* and reportedly has a haploid chromosome number of $n = 27$ (CCDB 2024).

Boerhavia triquetra S. Watson var. **intermedia** (M.E. Jones) Spellenb. $n = 26$. USA. Texas. Presidio Co.: 7 mi [11.3 km] E of Presidio, 2 Sep 1988, RWS & DEW 9697 (NMC069770).

This species includes two intergrading varieties, with the New Mexico populations clearly corresponding to *Boerhavia triquetra* var. *intermedia* (Spellenberg 2007). Chromosome data were recorded for this variety as " $2n = 52$, ca. 54" under the synonym *B. intermedia* M.E. Jones (Spellenberg 2003b).

***Cyphomeris crassifolia** (Standl.) Standl. $n = 13$ (5 counts). MEXICO: Coahuila, Mexico Highway 57, 37 km N of central Saltillo, roadside 1.6 km N of La Florida bridge, 20 Oct 1980, RWS, RJS, DEW & J. Zimmerman 5935 (NMC069998); Nuevo Leon, Huasteca Canyon, NW of Monterey, 20 Oct 1980, RWS, RJS, DEW & J. Zimmerman 5936 (NMC069999); 11 km S of Montemorelos, S of crossing of Arroyo Encanedada, 21 Oct 1980, RWS, RJS, DEW & J. Zimmerman 5937 (NMC070000); Tamaulipas, Mexico Highway 101, 7 mi [11.3 km] W of Victoria, 21 Oct 1980, RWS, RJS & DEW 5939 (NMC070002). USA. Texas. Pecos Co.: 26 km W of Sheffield, S of IH-10, 17 Oct 1980, RWS, RJS, DEW & J. Zimmerman 5923 (BRIT375034, NMC070044, NY03372074, TEX00282214).

Cyphomeris is composed of two intergrading species, *C. crassifolia* and *C. gypsophiloides* (M. Martens & Galeotti) Standl. (next entry). Both species inhabit dry regions of central North America (Mahrt & Spellenberg 1995). The counts reported here are the first for the genus and suggest that the present taxonomy for *Cyphomeris* would benefit from reevaluation. Mahrt and Spellenberg (1995) discussed the morphological variation of the two species, noting that *C. crassifolia* occurs generally east of the Sierra Madre Oriental in northeastern Mexico, while *C. gypsophiloides* occurs west of those same mountains and across most of the

Chihuahuan Desert. The authors showed intergrading plants occurring throughout much of the ranges of both species. Species are distinguished by overlapping variation in the length and wartiness of the fruits, leaf blade proportions, degree of sinuousness of blade margins, and general degree of pubescence of the leaf blade. *Cyphomeris crassifolia* usually has shorter, warty fruits, broadly ovate or ovate-deltoid leaf blades with sinuous margins (particularly on those leaves in the basal half of the plant), and an abaxial pubescence composed of curved trichomes. Conversely, *C. gypsophiloides* has slightly longer, less warty fruits or fruits lacking warts, and broadly to narrowly lanceolate leaf blades with entire margins, the abaxial surface of the blades glabrous or nearly so. Mahrt and Spellenberg (1995) also noted that the morphological intergradation between the two taxa is particularly evident in south-central Texas and adjacent Mexico. Their 1995 article was the basis for the genus treatment that appeared in the *Flora of North America* (Mahrt & Spellenberg 2003), where *C. crassifolia* was restricted to the southern tip of Texas and apparently influenced the subsequent mapping of *C. crassifolia* by Turner et al. (2003). Moreover, this evidence may have served as the source for Powell and Worthington's (2018) treatment of the genus west of the Pecos River. In that publication, reference to *C. crassifolia* was not made; it is noted here that the location of Spellenberg et al. 5923, the fifth and final specimen voucher cited in this entry for *C. crassifolia*, is within the eastern extreme of the region covered by Powell and Worthington (2018). The Spellenberg et al. 5923 voucher was originally identified as *C. gypsophiloides* and was distributed widely to BRIT, F, GH, MEXU, MO, MT, NMC, NY, TEX and UC. This determination was apparently accepted by Turner, who annotated the TEX duplicate of 5923 as *C. gypsophiloides* in agreement with Spellenberg's original identification. However, Spellenberg and Rogers re-identified the NMC sheet of 5923 as *C. aff. crassifolia* during the preparation of this paper. The chromosome number of $n = 13$ is more or less consistent with the morphology, the specimen strongly resembling *C. crassifolia* (broad leaf blades about as wide as long with slightly sinuous margins, the abaxial surface with a few curved trichomes, and fruits somewhat warty). If treated as *C. crassifolia*, the 5923 voucher extends the geographic range of that species approximately 600 km to the northwest.

On the other hand, Spellenberg et al. 5927, the fourth and final voucher cited for *Cyphomeris gypsophiloides* in the next species entry, was collected 100 km southeast of Spellenberg et al. 5923 and has been re-identified recently by Spellenberg as *C. aff. gypsophiloides*, based on evidence of a chromosome number of $n = 22$. The duplicate specimens of collection 5927 were distributed broadly to ARIZ, MEXU, NMC, NY, RSA, TEX, UC, UTEP and WTC. The specimen at NMC has ovate-deltoid leaf blades, but they are proportionally narrower, ca. 1.5 times as long as wide, and possess more or less sinuous margins. The abaxial surface of the blade has very sparse curved trichomes and the fruit is faintly warty. Our new cytological information for the two constituent species of *Cyphomeris* signal the need for further investigation into the current taxonomy of the genus.

****Cyphomeris gypsophiloides*** (M. Martens & Galeotti) Standl. $n = 22$ (4 counts). MEXICO: Coahuila, Mexico Highway 57, just N of Colonia Menonita, 19 Oct 1980, RWS, RJS & DEW 5932 (NMC070050); Durango, Sierra el Rosario, ca. 20 air-km SW of Mapimi, ca. 25°44'N, 103°57'W, 25 Oct 1980, RWS, J. Zimmerman, RJS & DEW 5947 (ASU0045428, NMC070056); Tamaulipas, Mexico Highway 101, 22 km SW of Jaumave, 21 Oct 1980, RWS, RJS, DEW & J. Zimmerman 5940 (NMC070052). USA. Texas. Val Verde Co.: crossing of Texas Highway 163 at Devil's River, S of Juno, 18 Oct 1980, RWS, RJS, DEW & J. Zimmerman 5927 (NMC070047, NY03372053, TEX00299379).

Cyphomeris gypsophiloides is much more widespread than *C. crassifolia*, ranging from New Mexico and western Texas and southwards into the Mexican states of Puebla and Oaxaca (Mahrt & Spellenberg 1995). The four sampled populations of *C. gypsophiloides* are distant from one another and represent much of the geographic range of the distribution of the species (trans-Pecos Texas, southern New Mexico, and the Mexican states of Chihuahua, Coahuila and Durango).

Mirabilis albida (Walt.) Heimerl $n = 26$ (2 counts). USA. New Mexico. Grant Co.: ca. 5 air-mi [8 km] NW of Silver City, on SW side of Bear Mountain, 6 Sep 1980, RWS, RJS & DEW 5882 (NMC070093, NY03375830); 9.1 road mi [14.6 km] NW of Silver City, on road to Bear Mountain, 15 Sep 1989, RWS, D. Sutherland & DEW 9989 (NMC070099).

Mirabilis albida s. lat. is an extremely widespread species that occurs throughout Canada, the United States and Mexico. The two counts of $n = 26$ documented here were made for two populations near one another in Grant Co., New Mexico. Despite the extensive geographical distribution of the species, there is only a single previously published report, that being $2n = 58$, based on material from Manitoba, Canada [Löve & Löve 1982, under the synonym *Oxybaphus hirsutus* (Pursh) Sweet]. The 1982 report was probably used as the unspecified source for the same number cited in the Flora of North America treatment for *M. albida* (Spellenberg 2003c). Clearly more sampling is needed throughout the full geographic extent of the species and its considerable range of morphological variation.

***Mirabilis linearis** (Pursh) Heimerl var. **decipiens** (Standl.) S.L. Welsh. $n = 27$. USA. Arizona. Cochise Co.: 1 mi [1.6 km] N of Bisbee, E of Mule Pass, 17 Sep 1989, RWS, D. Sutherland & DEW 10003 (NMC070806).

Mirabilis linearis is a widespread, morphologically variable species that intergrades into *M. albida* s. lat. ($n = 26$, preceding entry). Besides *M. linearis* var. *decipiens*, two other varieties are recognized for the species, the autonymic variety (reported as $n = 26$, see next entry), and *M. linearis* var. *subhispidata* Heimerl. The $n = 27$ count for *M. linearis* var. *decipiens* is the first report published for the variety; the subhispid variety remains unknown cytologically. Spellenberg (2003c) noted that *M. linearis* var. *decipiens* probably represents a wide range of intergrades between *M. linearis* var. *linearis* and *M. melanotricha* (Standl.) Spellenb. (reported as $n = 26$ in a subsequent entry).

Mirabilis linearis (Pursh) Heimerl var. **linearis**. $n = 26$. USA. Arizona. Cochise Co.: Peloncillo Mountains, US Forest Service Road 63 (Geronimo Trail Road), 0.7 mi [1.1 km] W of the AZ-NM state boundary, 6200 ft [1890 m], 31°30'N, 109°04'W, 17 Sep 1989, DEW, RWS & D. Sutherland 89-018 (NMC070850).

Of the three varieties recognized for *Mirabilis linearis*, the autonymic variety is the most broadly distributed, with a range that extends from southern Canada to northern Mexico (Spellenberg 2003c). Populations of all three varieties become sympatric, or nearly so, in central New Mexico (see maps and discussion in Allred & Jercinovic 2020). Reports indicate substantial variation in chromosome number across populations of *M. linearis* var. *linearis*. Taylor and Brockman (1966) noted $2n = 26_{II}$ and proposed a base number of $x = 13$ for material collected from Alberta, Canada. In that same study, the authors referenced four older publications all noting $n = 29$. More recently, Ward and Spellenberg (1986) observed $n = 20$ in a plant of *M. linearis* var. *linearis* that was collected near La Plata in San Juan Co., New Mexico (Spellenberg et al. 6169, NMC070224, count originally published without attribution to a specific variety). For a discussion of the general difficulties associated with counting chromosomes in the Nyctaginaceae and particularly with respect to this difficult *Mirabilis* species complex see Ward and Spellenberg (1986).

***Mirabilis melanotricha** (Standl.) Spellenb. $n = 26$. USA. Arizona. Cochise Co.: E side of Chiricahua Mountains, 9.1 mi [14.6 km] W of US Highway 180 near Rodeo, New Mexico, 4850 ft [1478 m], 31°53'N, 109°10'W, 16 Sep 1989, RWS, D. Sutherland & DEW 9997 (NMC070911).

Mirabilis melanotricha is yet another species in the genus to show nebulous species limits and morphological variation that intergrades with several other taxa (e.g., *M. albida*, *M. linearis* var. *decipiens*, *M. nyctaginea* (Michx.) MacMill.) in zones where the geographic ranges overlap (Spellenberg 2003c). More cytological observations are needed for plants representing these taxa and their intergrades.

Mirabilis oxybaphoides (A. Gray) A. Gray. $n = 30$. USA. New Mexico. Grant Co.: Bear Mountain, ca. 5 air-mi [8 km] NW of Silver City, 6 Sep 1980, RWS & RJS 5858 (NMC070407, NY03378371).

Mirabilis oxybaphoides ranges from southern Nevada through western Texas and into several states in northern Mexico (Spellenberg 2003c; USDA Plants 2024). The species is very common throughout most of New Mexico except for the SE portion of the state, with populations usually associated with shrubby, mesic, rocky habitat (Allred & Jercinovic 2020).

Mirabilis viscosa Cav. $n = 27$. MEXICO: Querétaro, extreme NW part of the state, ca. 32 air-km SW of Jalpan, ca. 1700 m, 21°03'00"N, 99°44'30"W, 8 Oct 1985, RWS, J. Zimmerman & N. Zucker 8323 (NMC071049).

Mirabilis viscosa is a widespread, more or less weedy species distributed throughout most of Mexico from Sonora through Chiapas, with disjunct populations occurring much further south in Ecuador, Peru and Bolivia. The present count of $n = 27$ from central Mexico appears to be the second known report for the species. Darlington (1955) cited $2n = 58$ for the species based on material from Peru.

PLANTAGINACEAE

Penstemon ophianthus Pennell. $n = 8$. USA. New Mexico. Catron Co.: 4 mi [6.4 km] E of NM-117 on NM-36, N of Pietown, 3 Jun 1988, RWS & DEW 9513 (NMC075021).

The count of $n = 8$ agrees with two earlier reports for *Penstemon ophianthus* that were based on wild-collected material from San Miguel Co., Colorado (Freeman & Brooks 1988), and on samples purchased from the Rocky Mountain Rare Plants Nursery that originated from an unspecified locality (Broderick et al. 2011). The species is geographically centered in the Four Corners region (Freeman 2019). *Penstemon ophianthus* is in a species complex with two closely related sympatric species, *P. jamesii* Benth. and *P. breviculus* (D.D. Keck) G.T. Nisbet & R.C. Jacks., both of which have also been reported as $n = 8$ (Nisbet & Jackson 1960; Freeman 1983).

Schistophragma intermedium (A. Gray) Pennell. $n = 20$. USA. Arizona. Cochise Co.: Peloncillo Mountains, road leading to Guadalupe Canyon, 20 mi [32 km] E of Douglas, 4100 ft [1250 m], 31°20'N, 109°14'W, 17 Sep 1989, DEW, RWS & D. Sutherland 89-017 (NMC075752).

$N = 20$ was also published for a population of *Schistophragma intermedium* from Skeleton Canyon, in the Peloncillo Mountains in Hidalgo Co., New Mexico (Ward 1983b). The species occurs in Arizona, New Mexico, Chihuahua and Sonora (Barringer 2019).

POLEMONIACEAE

Ipomopsis longiflora (Torr.) V.E. Grant subsp. **neomexicana** Wilken. $n = 7$. USA. New Mexico. Doña Ana Co.: W foothills of Doña Ana Mountains, USDA Jornada Experimental Ranch, ca. 1.5 mi [2.4 km] NNE of IH-25 exit 18 (Radium Springs), 4176 ft [1273 m], 32°30.455'N, 106°53.361'W, 27 Apr 2019, DEW 19-051 (NMC102067).

In the U.S., *Ipomopsis longiflora* ranges from Utah to South Dakota and northern Mexico (Wilken 2001). Three allopatric subspecies are recognized, with all of them present in New Mexico (Allred & Jercinovic 2020). $N = 7$ has been reported consistently for all three subspecies (e.g., Wilken 1986, 2001). New Mexico populations of *I. longiflora* subsp. *neomexicana* are particularly well sampled, with reports based on material collected from the counties of Doña Ana (Ward 1983a, 1983b), Sierra (Wilken 2001), Socorro (Grant 1959), Lincoln and San Juan (Ward 1984), and Cibola (Ward & Spellenberg 1988).

POLYGONACEAE

+**Eriogonum polycladon** Benth. $n = ca. 20$. USA. New Mexico. Grant Co.: NM-61, 7.2 mi [11.6 km] N of US-180 on NM Highway 61, 3.6 mi [5.8 km] S of Faywood, 5 Oct 1980, RWS & DEW 5913 (ASU0122533, GH01986878, NMC086216, NY03124510).

This species is widely distributed across the American Southwest and northern Mexico, sometimes becoming locally abundant and even weedy (Reveal 2005). In New Mexico, *Eriogonum polycladon* is mostly confined to the western half of the state (Allred & Jercinovic 2020). Ward and Spellenberg (1988) published a disparate chromosome number of $n = 13$ for the species, based on a population growing in Doña Ana Co., New Mexico, near the town of Organ, at a site located ca. 120 air-km to the ESE of the cited Faywood population.

The floral bud material used to determine the new count of $n = ca. 20$ showed abnormal meiosis. In the post-meiotic mother cells, instead of the expected post-meiotic tetrads of similar immature daughter cells, numerous (usually more than 4) odd-sized chambers of cytoplasm resulted from the attempted meiosis and distribution of cytoplasm within each mother cell.

RANUNCULACEAE

Ranunculus aquatilis L. var. **diffusus** With. $n = 8$. USA. New Mexico. McKinley Co.: NE corner of Zuni Indian Reservation, Nutria Lake, 6800 ft [2073 m], 3 Jun 1988, DEW, RWS, J. *Enote* & S. Davis 88-101 (NMC088421, NY02785157).

Ranunculus aquatilis, with two varieties, is a highly variable, widely dispersed species that grows in aquatic or mesic environments throughout much of North America, Eurasia and Australia (Whittemore 1997). The New Mexico populations represent the more widespread *R. aquatilis* var. *diffusus* (Allred & Jercinovic 2020). Apparently this count of $n = 8$ is the first report for the state. Three different chromosome numbers have been reported frequently in the literature for both varieties, i.e. $2n = 16, 32$ and 48 (see references in CCDB 2024). In addition, cytological sampling of the two varieties has been relatively thorough across the Northern Hemisphere (e.g., Austria, Germany, Spain, NE Asia, Russia, Yukon Plateau).

SOLANACEAE

Nicotiana obtusifolia M. Martens & Galeotti. $n = 12$. USA. New Mexico. Doña Ana Co.: 2 mi [3.2 km] NNE of Doña Ana (village), SW of Doña Ana Mountains, 4092 ft [1247 m], $32^{\circ}35.467'N$, $106^{\circ}49.571'W$, 9 Sep 2020, DEW 20-011 (NMC101486).

Nicotiana obtusifolia is found in the American Southwest (USDA Plants 2024) and throughout most of Mexico (Martínez et al. 2017; Tropicos 2024). In New Mexico, *N. obtusifolia* is recorded for most counties except for those along the state's northern border (Allred & Jercinovic 2020). This is the first count for this species in New Mexico. Other studies have also reported $n = 12$ or $2n = 24$ without citing vouchers or precise localities (e.g., Clarkson et al. 2005; Kitamura et al. 2005; Leitch et al. 2008; Knapp 2023).

Physalis hederifolia A. Gray. $n = 12$. USA. New Mexico. Doña Ana Co.: S foothills of Doña Ana Mountains, Desert Peaks National Monument, Cerro Vista (Bureau of Land Management) Bike Trails, 4244 ft [1294 m], $32^{\circ}24.743'N$, $106^{\circ}46.180'W$, 16 May 2019, DEW 19-068 (NMC102066).

Physalis hederifolia is widespread across the western half of the US and northern Mexico and tolerates habitat disturbance (Sullivan 2023). This $n = 12$ count agrees with those reported for another population sampled in Doña Ana Co., New Mexico (Ward 1983a), as well as for two populations collected from Brewster Co., Texas, and Durango (state), Mexico (Averett & Powell 1972). In addition to two $2n = 24$ counts for plants from the Mexican states of Oaxaca and Tamaulipas, Zhao and Turner (1993) noted $2n = 24$ and $2n = 48$ for material taken from an undisclosed Texas locality (voucher not found on relevant online specimen databases, e.g., SEINet, <https://swbiodiversity.org/seinet/>).

While viewing the slides made from the bud material of the Ward 19-068 collection, a possible quadrivalent was seen during late diakinesis.

VERBENACEAE

Glandularia wrightii (A. Gray) Umber. $n = 10$. USA. New Mexico. Socorro Co.: N end of San Mateo Mountains, US Forest Service Road 476, 13.5 mi [21.7 km] S of US Highway 60, 6700 ft [2042 m], 4 Jun 1988, RWS & DEW 9516 (NMC094082, NY03547317).

Glandularia wrightii is one of six species forming the *G. bipinnatifida* complex (Nesom 2010). Until recently, many New Mexico specimens, including the voucher cited here, were assigned to a broadly delimited *G. bipinnatifida*, a species that is now confined to the Great Plains (Allred & Jercinovic 2020). Plants identified as *G. wrightii* and belonging to 17 different populations located in four counties in western Texas (El Paso, Brewster, Jeff Davis, Presidio) were all found to be $n = 10_{II}$ (Turner & Powell 2005). Referring to Turner and Powell's (2005) paper, Nesom (2010) noted that *G. wrightii* is consistently hexaploid ($2n = 30$, based on 18 populations sampled from seven undisclosed counties). Powell et al. (2017) corroborated the chromosome number of *G. wrightii* as $2n = 15_{II}$ or $2n = ca. 15_{II}$, citing six additional populations sampled from western Texas, and noting $2n = 10_{II}$ for *G. pubera*, based on three new counts from western Texas and adjacent New Mexico.

The new $n = 10$ count for *Glandularia wrightii* reported here is likely the first authentic count of the species for New Mexico. Our count differs from those of *G. wrightii* reported previously for western Texas populations ($n = 15$). No other counts beyond the one presented here and those documented in Powell et al. (2017) have been found. Multiple attempts were made to confirm the identity of the *Spellenberg & Ward 9516* voucher using three recent *Glandularia* treatments (Nesom 2010; Powell & Worthington 2018; Allred & Jercinovic 2020); in all cases the specimen keyed to *G. wrightii* but we found the current classification of the *G. bipinnatifida* complex challenging to follow. Further counts, supported by well-prepared voucher specimens that possess the characters used to define these species, are needed to determine the degree to which chromosome number correlates with morphology.

ZYGOPHYLLACEAE

Larrea tridentata (Sesse & Moc. ex DC.) Coville. $n = 13$. USA. New Mexico. Doña Ana Co.: NW of Las Cruces, E base of Robledo Mountains, ca. 2 mi W of the Shalem County Road bridge over the Rio Grande, 4010 ft [1222 m], 32°22.372'N, 106°51.957'W, 9 Apr 2019, DEW 19-046 (NMC101574).

Larrea tridentata is the most common lower-elevation shrub in the Chihuahuan, Sonoran and Mojave deserts. The new count of $n = 13$ presented here is consistent with the diploid, $2n = 26$ race that occurs in the Chihuahuan Desert. For a summary of the three chromosome races of *L. tridentata*, i.e., diploid ($2n = 2x = 26$), tetraploid ($2n = 4x = 52$) and hexaploid ($2n = 6x = 78$), and their corresponding geographic distributions, see Laport et al. (2012) and Porter (2016).

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APPENDIX

The detailed chromosome count protocol used in this study (expanded from MATERIALS AND METHODS section).

I. Collecting material in the field

Flower buds appearing immature were collected into vials containing a modified Carnoy's solution (4 chloroform: 3 absolute ethanol: 1 acetic acid). The buds were gathered in as many different sizes as available from the sampled plants. The 4:3:1 solution arrested chromosomal movement during meiotic cell division of the pollen mother cells (PMCs) in the developing anthers. Each vial was then immediately introduced into a container of iced water to aid in the fixation. After no more than 24 hours, the fixative was decanted out of each vial, and enough 70% ethanol was added such that all of the bud material was submerged (DEW has rendered successful chromosome counts from buds that had been in such storage for over fifteen years).

II. Staining the bud material in the laboratory

During preparation of bud material in the lab, the 70% ethanol in the relevant vial was decanted. The buds were gently blotted to remove excess ethanol. They were returned to the empty vial and an amount of stain solution was added that totally submerged the buds (step helps reduce dilution of the stain). Note that a large stock of hydrochloric acid-carmin stain solution had been prepared previously using the procedure described in Snow (1963). Depending on the density of the plant tissue that surrounded the bud's internal organs, the stain solution was left in the vial for a period of 3 to 7 days. Following that, the stain solution was decanted out of the vial and retained, and new 70% ethanol was introduced into the vial for temporary storage of the stained bud material. After filtering, the recovered stain solution was returned to the stock bottle for future reuse.

III. Evaluating the Pollen Mother Cells (PMCs)

Different sizes of stained flower buds were sampled to make temporary microscope slides. The first bud was selected that seemed to have potential to contain dividing pollen mother cells, i.e. a bud not close to floral anthesis but also not among the youngest in the inflorescence. If this bud did not yield useful material, then either one younger or older bud was selected, depending on the stage observed in the original selected bud. Buds were dissected under a stereoscope, and at least one anther from each bud was squashed under a cover slip with a drop of 70% ethanol on the slide. Then, using a compound microscope, the slide was searched for the presence of any PMCs undergoing meiosis. If no appropriate meiotic stage was found, the slide was scanned for the overall state of development of any PMCs that were present. If the PMCs appeared to be too immature, then the slide and cover slip were both wiped clean and re-used for the processing of another bud that was slightly larger. If the PMCs were overly developed, then another slightly smaller bud

was analyzed. This procedure was repeated until the proper size of bud was encountered, or until it was deduced that none of the buds had PMCs of the proper stage of development for further analysis.

When usable anthers were encountered, further microscope analysis was needed to capture the actual chromosome count with supporting evidence.

IV.a. Analysis and documentation of the chromosome counts (before 2000)

This procedure applies to 48 older chromosome counts that were based on cited vouchers collected prior to 2000.

Residual anthers from the usable bud analyzed during the evaluation of the PMCs step were introduced into a drop of 45% acetic acid on a microscope slide and then were macerated with dissecting probes. A cover slip was slowly lowered into place over the area, and the anther material was further squashed by slight pressure from the blunt end of the dissecting probe. Then, the slide was searched using a trinocular Zeiss microscope that had a camera lucida partial mirror device affixed to the viewing tube. When a PMC displaying a meiotic configuration of chromosomes was found, and if the display was deemed to be usable for determining the number of chromosomes, then the camera lucida was used to make a tracing of the outlines of the chromatin. This tracing was later added to the herbarium voucher's specimen label as supporting evidence for the squash. Digitized versions of these illustrations are also visible in the digitized images of the herbarium sheets available via NMC's online database (<http://nmc.nmsu.edu/nmc/>).

IV.b. Analysis and documentation of the chromosome counts (2019–onwards)

The following procedure applies to the 11 chromosome counts made in 2019 and 2020 (indicated by voucher citations with collection numbers including “DEW 19-” or “DEW 20-” prefixes).

The remaining anthers belonging to the usable bud analyzed during the evaluation of the PMCs were introduced into a drop of 45% acetic acid on a clean microscope slide. Then, anthers were slightly macerated with dissecting probes. The acetic acid was allowed to evaporate as extensively as possible without desiccating the anther material. Three minute drops of Hoyer's mounting medium were added to that area, and a clean cover slip was slowly lowered into place. Slight pressure on the cover slip from the blunt end of a probe or other such object(s) further macerated the anther material. After one hour, a ring of clear nail polish was applied around the edges of the cover slip. After hardening, this resulted in a semi-permanent microscope slide that permitted weeks of investigation.

Using a trinocular Olympus BH2 phase contrast microscope with an Amscope MU1803 digital camera affixed on its viewing tube, the slide was scanned until a suitable PMC was located. While the Amscope software was in “capture” recording mode, the PMC was scanned completely through its height. After “capture” was terminated, about five consecutive sets of the adjacent raw images were combined using the software's “EDF” (Extended Depth of Focus) image-fusion function. These fused images provided 3-D renderings of the chromosomes in the relevant regions of the cell. Fused images were edited into evidence sheets that included supporting meta-data and discussion about the different observed chromosomes.

The above recording of information was repeated for other PMCs showing meiotic configurations on the slide, including adding new evidence sheet areas. These sets of evidence sheets were added to envelopes, which are affixed to their respective herbarium specimen sheets. Digitized versions of the sets of evidence sheets are also visible in the digitized images of the herbarium sheets in NMC's online database (<http://nmc.nmsu.edu/nmc/>).

The microscope slide was retained for continued viewing, if needed, to provide sets of images of other PMCs or to study complex regions that required more in-depth interpretation. After a few months, we found that the integrity of a slide had often failed.

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