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Botanical Gazette, Volume 145, Issue 2 (Jun., 1984), 240-245.

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Botanical Gazette
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Sat Jun 22 16:55:42 2002

Bot. Gaz. 145(2):240–245. 1984.
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0006-8071/84/4502-0018$02.00

NUCLEAR DNA CONTENTS OF COREOPSIS NUECENSOIDES AND C. NUECENSIS (ASTERACEAE), A PROGENITOR-DERIVATIVE SPECIES PAIR

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Nuclear DNA contents were determined microspectrophotometrically from plants of four *Coreopsis nuecensoides* and three *C. nuecensis* populations representing an aneuploid reduction series (N = 11, 10, 9, 8, 7, 6). Two populations of the putatively derived species, *C. nuecensis* (N = 6, 7), have ca. 12% more DNA than a third population of this species and the populations of the progenitor species, *C. nuecensoides* (N = 11, 10, 9). Therefore, two forms of genome evolution, chromosome repatterning and increase in DNA content, have apparently occurred in the evolution of *C. nuecensis*.

Introduction

Coreopsis nuecensis Heller and *C. nuecensoides* E. B. Smith are annual species that occur in a limited geographic area of southeastern Texas (Smith 1974). Both taxa grow on sandy soils of prairies and plains; their ranges overlap somewhat, but *C. nuecensoides* is more coastal, and *C. nuecensis* occurs inland (Smith 1974).

Both species are similar morphologically; the only consistent contrasting feature is the glabrous inner involucral bract of *C. nuecensoides* and the pubescent bract in *C. nuecensis* (Smith 1974). In addition, *C. nuecensoides* behaves as a weak perennial in the greenhouse, whereas *C. nuecensis* is strictly annual; the stems of the former species tend to be pubescent, while those of the latter are glabrous (Smith 1974; Crawford and Smith 1982).

When describing *C. nuecensoides* as a new species, Smith (1974) noted that it includes plants with $N = 9$ and 10, whereas plants of *C. nuecensis* have $N = 6$, 7, or 8 (figs. 1–5). The former species rarely has a gametic number of $N = 11$ (fig. 6; Crawford and Bayer, unpublished). Smith (1974) and Crawford and Smith (1982) demonstrated that these taxa produce completely sterile F_1 hybrids when crossed in the greenhouse. The hybrids exhibit multivalents at meiosis. In contrast, plants of the same species with different chromosome numbers are highly interfertile.

Smith (1974) hypothesized that *C. nuecensis,* which has the lower chromosome numbers, was derived from *C. nuecensoides* with chromosomal repatterning, i.e., translocations, as the primary isolating mechanism. The morphological similarity of both species led Smith to suggest that divergence has been quite recent. Crawford and Smith (1982) demonstrated lack of differentiation between the species for genes coding soluble enzymes, and they also showed that the presumed derivative species, *C. nuecensis,* is less variable than the putative progenitor, *C. nuecensoides*. These data support the hypothesis of Smith (1974) concerning recent divergence of the two taxa and their progenitor-derivative relationship. Smith and Crawford (1981) detected small, consistent differences in the flavonoids between the taxa.

Our study examined nuclear DNA content among populations of *C. nuecensoides* and *C. nuecensis* with different chromosome numbers. The presumed genome alterations, i.e., loss of centromere and adjacent chromatin in this aneuploid series (N = 11, 10, 9, 8, 7, 6), should have led to a decrease in DNA content, possibly of a magnitude detectable by Feulgen cytophotometric methods.

Manuscript received November 1983; revised manuscript received February 1984.

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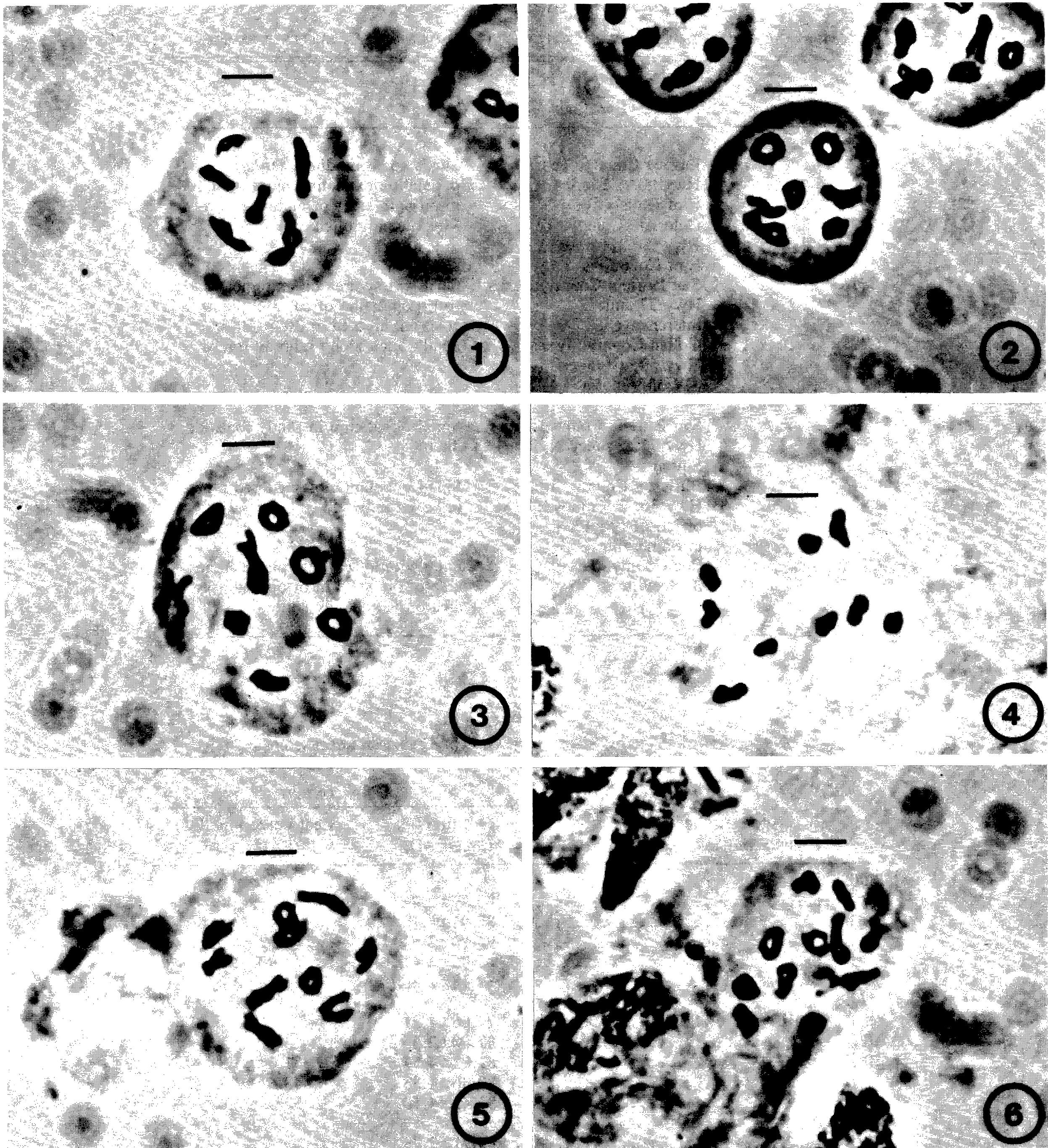
Material and methods

Achenes of wild populations were collected by D. J. Crawford in the spring of 1979 and 1980 (table 1). *Coreopsis basalis* (*C1186* and *C1199*) was used as a control species for all DNA content determinations. This close relative of *C. nuecensis* and *C. nuecensoides* is chromosomally stable (N = 13) and free from supernumerary chromosomes (Smith 1975, 1976; Crawford, unpublished).

Achenes were sown in 0.42 liter soil (4:2:1:3, peat, vermiculite, perlite, and sand) in 7.5-cm diameter plastic cups, placed in a cold (5 C) room in the dark for 1 mo, and germinated in a growth chamber with a day/night regime of 13/11 h, 20/13 C. Pots with young seedlings were transferred to a growth chamber with a day/night regime of 12/12 h, 30/20 C. The pots were saturated with distilled H_2O three times weekly and fertilized every 14 days by saturation with a solution of 3.6 g 10-10-10 soluble fertilizer per liter of distilled H_2O.

Chromosome counts

Immature buds were fixed in cold 3:1 absolute alcohol/glacial acetic acid and transferred to cold 70% alcohol after 24 h. Anthers were dissected,



FIGS. 1–6.—Meiotic chromosomes showing the range of numbers in *Coreopsis nuecensis* (figs. 1–3) and *C. nuecensoides* (figs. 4–6). Bar = 10 μm. Fig. 1, *C1158, N* = 6; fig. 2, *C1175-1, N* = 7; fig. 3, *C1175, N* = 8; fig. 4, *C1198-3, N* = 9; fig. 5, *C1205-1, N* = 10; fig. 6, *C1140-2, N* = 11. All collection numbers are those of D. J. CRAWFORD.

stained in a 1% solution of acetocarmine, and squashed on microscope slides. Chromosome counts were made from meiotic figures at diplotene through metaphase I.

DNA CONTENT DETERMINATION

DNA content was determined from interphase nuclei of leaf epidermal cells, which are arrested at the G_1 stage (2C) of the cell cycle. The lower epidermis was peeled from the middle third of a newly expanded healthy leaf or leaflet differentiated immediately after the appearance of the first flower bud, fixed for 24 h in ice-cold 3:1 absolute ethanol/glacial acetic acid, and stored in cold 70% ethanol. The material was mounted on slides, hydrolyzed in 5N HCl for 35 min, and Feulgen

TABLE 1

LOCATION AND HABITAT DESCRIPTION OF COREOPSIS NUECENSOIDES AND C. NUECENSIS SAMPLED

Species and acquisition no.	Location	Habitat
C. nuecensoides:		
C1200	Lavaca County, Texas Hwy 90A, 2 miles E of Halletsville	Sandy roadside ditch, large population extending more than 100 m on both sides of highway
C1140	Colorado County, Texas Hwy 90, 10 miles E of Columbus	Moist, sandy ditch
C1185	San Patricia County, Texas Hwy 37 and 77 interchange	Open grassy area on extremely sandy soil, population of ca. 300 plants covering an area of 30 × 50 m
C1192	Goliad County, Texas Hwy 59, 10 miles SW of Victoria	Sandy ditch along railroad tracks
C. nuecensis:		
C1148	San Patricia County, Texas Hwy 77, 2 miles N of Sinton	Small population in sandy roadside ditch
C1163	Burleson County, Texas Hwy 36, 2.5 miles E of jct. of FM 1361 in Somerville	Small population of scattered plants in very sandy soil
C1189	Bee County, Texas in Beeville	Population of ca. 700 plants in an area of 60 × 80 m of open grassy area on very sandy soil

TABLE 2

DNA CONTENT AND CHROMOSOME ASSOCIATION OF COREOPSIS NUECENSOIDES

Acquisition no. and chromosome association	DNA content	
	Root tip (4C) (FAU±SD)	Leaf epidermis (2C) (FAU±SD)
C1200:		
10 II	83.57±2.24	...
10 II	82.91±4.18	...
10 II	84.70±3.82	54.33±4.52
10 II	83.85±4.41	51.90±2.99
10 II	82.18±2.80	51.08±2.82
C1140:		
9 II + 1 III + 1 I or 11 II	79.66±4.21	...
10 II	82.80±2.55	...
10 II	81.93±4.54	51.64±3.81
10 II	82.36±4.33	48.41±2.77
11 II	83.45±4.10	52.05±2.63
C1185:		
10 II	83.71±2.78	...
9 II	86.09±4.84	...
10 II	85.59±6.30	48.59±3.84
9 II or 7 II + 1 IV	86.13±2.77	49.54±2.61
9 II	82.62±3.83	49.22±3.22
C1192:		
[a]	79.78±4.28	...
[a]	80.23±4.12	52.39±4.87
9 II	75.92±5.56	44.20±3.40
[a]	80.23±4.12	52.39±4.87
10 II	83.32±4.79	51.27±2.89
10 II or 8 II + 1 IV	82.44±4.24	49.32±2.24
$\bar{X}$	82.66±2.41	50.30±2.51

[a] No chromosome count was obtained.

TABLE 3

DNA CONTENT AND CHROMOSOME ASSOCIATION OF COREOPSIS NUENCENSIS

Acquisition no. and chromosome association	DNA content: Root tip (4C) (FAU±SD)	DNA content: Leaf epidermis (2C) (FAU±SD)
C1148:		
7 II	88.49±3.19	...
[a]	83.30±4.19	...
6 II	82.62±3.40	48.91±3.53
6 II	82.19±3.60	50.09±1.91
6 II	83.02±3.59	49.05±2.85
$\bar{X}$	83.92±2.59	50.17±1.39
C1163:		
[a]	89.27±4.73	...
6 II + 1 I or 1B	86.19±4.10	58.98±5.86
6 II	84.84±2.71	53.33±2.05
$\bar{X}$	86.77±2.27	56.16±1.47
C1189:		
[a]	104.43±5.88	...
6 II	94.15±6.88	...
7 II	100.08±7.88	58.73±4.63
6 II	95.97±4.62	56.05±3.00
6 II	93.67±5.73	54.92±4.43
$\bar{X}$	97.66±4.55	56.56±1.95

[a] No chromosome count was obtained.

stained (PRICE et al. 1980). After staining and rinsing, the slides were placed in a solution of 2% cellulysin (Calbiochem), 0.5% macerase (Calbiochem), and .001 M EDTA, pH 5.6, for 30 min, rinsed in distilled H_2O, and squashed under cover glasses in a drop of 45% acetic acid on microscope slides. Slides were made permanent with Permount (PRICE et al. 1980).

The DNA content of individual plants was measured microspectrophotometrically with a Zeiss Universal II scanning microscope (PRICE et al. 1980) using Feulgen-stained 2C nuclei with *C. basalis* as the internal standard on each slide. Values of individual nuclei on each slide were adjusted appropriately by multiplying the amount needed to increase or decrease the mean of the internal standard (*C. basalis*) to 50.92 Feulgen absorbance units (FAU). Each value represents the mean of 30 nuclei, 10 from each of three slides (tables 2, 3). Each staining batch consisted of four to six plants (12–18 slides), which included combinations of plants from the same and different populations. The slides of each batch were randomly measured after the labels were covered.

The DNA content was also determined from midprophase nuclei (4C) of root tips and included all plants that had DNA determined from leaf epidermal nuclei. Root tips were fixed and stored, hydrolyzed, and stained as above, except they were not mounted on slides until after Feulgen staining; therefore, root tips of different plants were cut to different lengths to identify them. After staining and rinsing, root tips were treated in a solution of 2% cellulysin (Calbiochem), 0.5% macerase (Calbiochem), and .001 M EDTA, pH 5.6, for 30 min, rinsed in distilled H_2O, macerated, and squashed under cover glasses in a drop of 45% acetic acid on microscope slides. The cover glasses were removed after freezing over dry ice, and the slides were air-dried. Cover glasses no. 1 were mounted over Permount.

The relative DNA content of 10 nuclei at midprophase from each slide was measured microspectrophotometrically (PRICE et al. 1980). The values of 30 nuclei of each plant were adjusted appropriately by multiplying the amount needed to increase or decrease the in-batch standard, *C. basalis,* to 83.62 FAU. Although these lacked the precision of an internal standard on each slide, they provided a second tissue and nuclear state for determination and comparisons of relative DNA content.

Results

Primarily 9 or 10 pairs of chromosome were observed from plants of *Coreopsis nuecensoides*. One plant with 11 pairs and three plants with apparent multivalent associations were observed (table 2). In *C. nuecensis,* mostly 6 pairs were observed. Two plants had 7 pairs, and a supernumerary chromosome was detected in one plant (table 3). Chromosomes from different cytotypes are shown (figs. 1–6).

The mean DNA content of root tip and of epidermal nuclei of *C. nuecensoides* (82.66 ± 2.41; 50.30 ± 2.51; table 2) is significantly different at

the 1% and 5% levels, respectively, from those of *C. nuecensis* (89.86 ± 7.24; 53.76 ± 4.10). Partitioning of the values of *C. nuecensis* showed that the increased DNA content is primarily attributed to plants of populations *C1163* and *C1189* (table 3). The mean DNA value from plants of these pooled populations (93.58 ± 6.71; 56.40 ± 2.44) is significantly different from *C. nuecensis C1148* (5% level for root tips; 1% level for epidermis) and from the mean DNA values of *C. nuecensoides* (1% level for both root tip and epidermal values). The mean DNA amount from either root tip or epidermal nuclei of *C1148* is not significantly different from the mean value of *C. nuecensoides*.

A correlation analysis using DNA contents from the plants that had nuclei from both root and leaf epidermal cells measured showed a correlation ($r = .72$) between values obtained from these two tissues. However, the 4C values from the mid-prophase nuclei of root tips are not double the 2C DNA contents of the interphase leaf epidermal nuclei as expected (table 2, 3). There is ca. 35% decrease in the expected values from the mitotic chromatin. These results probably are low because of the nature of compacted chromatin, which is less amenable to normal hydrolysis (DUIJNDAM and VAN DUIJN 1975) and also presents optical problems that lead to an underestimate of DNA content (MITTWOCH 1969; BENNETT and SMITH 1976).

Discussion

Coreopsis nuecensoides and *C. nuecensis* apparently represent a recently diverged progenitor-derivative species pair (SMITH 1974, 1982; CRAWFORD and SMITH 1982). Although few morphological and allozymic differences have accumulated between these species, a considerable amount of chromosome repatterning has occurred. The repatterning is evident in an aneuploid reduction series from $N = 11$ to $N = 6$ (SMITH 1975; CRAWFORD and BAYER, unpublished).

The hypothesis that the DNA lost during chromosome repatterning may be detectable by Feulgen cytophotometric methods could not be substantiated, since higher nuclear DNA amounts were observed in two of the three populations of *C. nuecensis* that had $N = 6, 7$. Therefore, any loss of DNA in the origin of this aneuploid series has been overshadowed by the other processes that have generated the DNA content differences. Similar results were reported for 24 diploid species of *Crepis* representing an aneuploid reduction series ($N = 9$, 6, 5, 4, and 3) and an 8.5-fold range in DNA content (JONES and BROWN 1976). Significant differences in genome size among *Crepis* species in each basic chromosome number grouping were also detected (JONES and BROWN 1976). There was no significant correlation between chromosome number and DNA content ($r = -.12$) among these *Crepis* species.

CRAWFORD and SMITH (1982) studied allozyme variation in *C. nuecensoides* and *C. nuecensis* and concluded that speciation had occurred with little genetic divergence and no changes in the number of gene loci coding for the tested soluble enzymes. Since their study included some of the low and high DNA content populations used in our study, we can conclude that the 12% difference in DNA content did not involve the genes coding for these allozymes.

ROOSE and GOTTLIEB (1978) detected no differences in the number of gene loci among *Crepis* species representing more than a sevenfold range in DNA content. This is not unexpected, since large (>two- to threefold) variation in DNA content commonly occurs among congeneric species (PRICE 1976), and only a small percentage of the DNA in plants is considered to be involved in coding for proteins (FLAVELL 1980). Variation in DNA amount generally involves repetitive, or derivatives of repetitive, sequences of noncoding function (FLAVELL et al. 1974; FLAVELL 1980; MURRAY et al. 1981).

The evolutionary and/or adaptative significance, if any, of the DNA content variation in the *Coreopsis* of this study is not known. However, the higher DNA amounts detected from plants of some *C. nuecensis* populations are apparently derived and suggest an origin by amplification of DNA sequences. In other studies of diploid plant species, the amount of nuclear DNA has been positively correlated with several parameters, including nuclear volume (BAETCKE et al. 1967), cell volume (PRICE et al. 1973), mitotic cycle time (VAN'T HOF and SPARROW 1963; VAN'T HOF 1965; EVANS and REES 1971), the duration of meiosis (BENNETT 1971), and minimum generation time (BENNETT 1972). The role of smaller differences of DNA content on plant adaptation is not resolved.

The differences in mean DNA content among populations of both *Microseris bigelovii* and *M. douglasii* may have resulted from selection in different habitats related to water availability, soil development, and environmental stress (PRICE et al. 1981*a*, 1981*b*). For *Coreopsis*, more collecting and studies of populations are needed before any geographical or ecological patterns in DNA content that might exist are detected.

Acknowledgments

Technical article no. 19303 from the Texas Agricultural Experiment Station. Supported in part by National Science Foundation grants DEB 80-11655 and DEB 82-04073 to D. J. CRAWFORD and 80-09427 to H. J. PRICE. We thank Mrs. JENNIFER RIGGS for excellent technical assistance.

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