DEOXYRIBONUCLEIC ACID OF COTTON[1]

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The deoxyribonucleic acid (DNA) composition of microorganisms has been studied extensively and the vast data obtained form the basis for the concept of chemical specificity of nucleic acids in general (1). In contrast, the DNA composition of higher plants has received relatively little attention (11, 14, 15, 16). This applies particularly to studies, e.g., those on wheat (11, 16), in which isolation and purification of the DNA have preceded determination of the constituent purine and pyrimidine bases.

In this paper we report the isolation and composition of DNA from nine genotypes of the genus Gossypium, in which two species, four races, and five varieties are represented. Although we give major emphasis to cotton, we also include limited data on the DNA composition of corn, peanut, sunflower, and wheat for comparison.

MATERIALS & METHODS

DESCRIPTION OF COTTON GENOTYPES[2]: Paymaster 54B, Deltapine 15, and Stoneville 2B are cultivated varieties of American Upland cotton, species *Gossypium hirsutum* L. The Red Empire stock used in this study, while qualifying as a variety of *G. hirsutum,* carries a simply inherited gene for red leaves which distinguishes it from the usual green leaf types. Latifolium, punctatum, richmondi, and palmeri are primitive or noncommercial types of *G. hirsutum* which have been identified by a subclassification of the species into geographical races. Only one stock of *G. barbadense,* an agricultural variety named Pima S–1, was used. Characteristically, cultivated vareties of this species have smaller bolls and longer, stronger, and finer fibers than those of *G. hirsutum.*

ISOLATION OF Na-DNA FROM COTTON: All operations, except as noted, were conducted at 0 to 10 C.

1. Freshly-decorticated cotton embryos, 300 g, were homogenized in a high-speed mixer with 500 ml of 95 % ethanol for 5 minutes. After adding another 500 ml of ethanol and 1 liter of ether, the mixture was allowed to stand for 2 hours and then the solvent was removed by suction. The residue was ground to 20-mesh fineness in a small Wiley mill, suspended in 2 liters of acetone, and stored for 24 hours with occasional stirring.

2. The acetone was removed by suction and the residue washed with 100 ml portions of acetone until the washings produced no appreciable precipitate upon the addition of an equal volume of water. Air-dry weight of the defatted residue was approximately 190 g. A suspension of the residue in 1 liter of 0.1 M NaCl was stirred vigorously for 3 hours with a heavy duty stirrer (paddle-type propeller) and centrifuged at 2,520 × *g* for 15 minutes. After discarding the supernatant, the residue was washed and centrifuged 12 times in a similar manner with 0.1 M NaCl.

3. The washed residue was next suspended in 500 ml of 0.9 % NaCl and 45 ml of Duponal (10) solution (5 g of purified Duponal, essentially sodium dodecyl sulfate, in 100 ml of 45 % ethanol) were slowly added while stirring. The mixture was stirred vigorously for 3 hours with a heavy-duty stirrer.

4. The mixture was then centrifuged for 3 hours at 2,520 × *g* and sufficient NaCl was added to the decanted supernatant to bring the concentration of NaCl up to 1 M. An equal volume of 95 % ethanol was added with stirring to this solution and the resultant precipitate of crude Na-DNA, which existed in both fibrous and granular form, was collected by centrifugation and washed once with 80 % ethanol.

5. The crude Na-DNA was dissolved in 300 ml of 0.14 M NaCl by stirring for 2 hours at 25 C. Duponal solution, 27 ml, was added and the mixture was stirred for 1 hour. After sufficient NaCl was added to make the NaCl concentration 1 M, the solution was centrifuged at 21,600 × *g* for 1 hour at 25 C. After adding an equal volume of 95 % ethanol to the decanted supernatant, the Na-DNA, which separated as a fibrous mass, was collected by spooling. The Na-DNA was washed successively with 80 % and 95 % ethanol, acetone, and air-dried. The Na-DNA (approximately 500 mg) at this stage still contained a small amount of protein, some free nucleotides, and

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[2] For detailed information on the origin and classification of the species of Gossypium and the races of *G. hirsutum* refer to: A: J. B. Hutchison, Heredity 5: 161–193 (1951) and B: The Evolution of Gossypium and the Differentiation of the Cultivated Cottons. J. B. Hutchison, R. A. Silow, and S. G. Stephens, Oxford University Press, London.

RNA (ribonucleic acid), the major contaminant. The next step removed all but the RNA impurity.

6. The Na-DNA from step 5 was dissolved in 300 ml of 25 % ethanol by stirring for 2 hours at 25 C. The solution was made 1 M with NaCl and centrifuged for 1 hour at 25,000 × *g*. The Na-DNA was precipitated from the supernatant with one-half volume of 95 % ethanol, washed, and dried as in step 5. Air-dried weight of the fibers was about 350 mg.

7. The Na-DNA from step 6 was dissolved in 300 ml of 0.14 M NaCl and stirred for 2 hours with 15 ml of washed Norit A, which selectively adsorbs RNA (17). The solution was then clarified by centrifuging for 2 hours at 28,700 × *g*, brought to 1 M with NaCl, and the Na-DNA precipitated by adding 1 volume of 95 % ethanol. The yield of fibers, washed with alcohol and acetone until free of chlorides, and air-dried, was approximately 150 mg. This weight represents about 50 % of the total DNA content of the embryos (7).

ISOLATION OF Na-DNA FROM THE OTHER GENERA: Na-DNA of corn (germ), peanut (embryo), and sunflower (embryo) was isolated and purified by the method described for cotton. Na-DNA of wheat (germ) was obtained by the method of Lipshitz and Chargaff (11).

ANALYTICAL: Hydrolysis of the Na-DNA isolates to the free purine and pyrimidine bases and their chromatographic separation and determination followed closely the procedure outlined by Lipshitz and Chargaff (11). Four hydrolysates of each isolate were used for determining the bases, which are expressed as moles per 100 gram-atoms of phosphorus. Using four spottings of 0.06 ml per hydrolysate, the standard deviation of each free base ranged from 0.10 to 0.22 mole per 100 gram-atoms of phosphorus. Phosphorus was determined by the Fiske-Subbarow methed (8).

RESULTS & DISCUSSION

The method used for isolating DNA from cotton, which differs in certain essentials from that used by Lipshitz and Chargaff (11) on wheat germ, resulted from unsuccessful attempts to apply their method to cotton. The method for cotton was applicable also to corn, peanut, and sunflower, but it was not tested on wheat germ and the method of Lipshitz and Chargaff was not tried on the other species.

In general all the Na-DNA isolates met the following requirements for satisfactory products (4): A: absence of proteins (biuret) and ribonucleic acid (uracil); B: a phosphorus content close to 9.2 %; C: a maximum absorption in the ultraviolet at 257 to 261

TABLE I

COMPOSITION OF TOTAL DEOXYRIBONUCLEATES OF COTTON*

	Moles/100g-atoms P**						Molar ratios		
	A	T	5MC	C	G	Actual recovery	A/T	Pu/Py	G/C+5MC
G. hirsutum									
Race:									
Latifolium	32.7 (0.05)***	33.0 (0.07)	4.6 (0.06)	12.8 (0.05)	16.9 (0.11)	94.7	0.99	0.98	0.97
Punctatum	32.8 (0.04)	33.0 (0.08)	4.6 (0.08)	12.7 (0.05)	16.9 (0.08)	93.3	0.99	0.99	0.98
Richmondi	32.8 (0.08)	33.0 (0.25)	4.6 (0.08)	12.6 (0.03)	16.9 (0.05)	95.4	0.99	0.99	0.98
Palmeri	32.7 (0.13)	32.8 (0.05)	4.7 (0.06)	12.8 (0.08)	17.1 (0.11)	91.4	1.00	0.99	0.98
Varieties:									
Red Empire	32.8 (0.14)	33.0 (0.06)	4.6 (0.05)	12.7 (0.05)	16.8 (0.25)	96.3	0.99	0.99	0.97
Paymaster 54B	32.8 (0.06)	32.8 (0.05)	4.6 (0.05)	12.8 (0.03)	17.1 (0.07)	98.4	1.00	0.99	0.98
Deltapine 15†	32.8 (0.05)	33.0 (0.10)	4.5 (0.05)	12.8 (0.03)	16.9 (0.06)	92.6	0.99	0.99	0.98
Stoneville 2B†	32.7 (0.03)	32.8 (0.05)	4.4 (0.03)	12.9 (0.03)	17.1 (0.03)	90.5	1.00	0.99	0.97
G. barbadense									
Pima S-1 (Var.)	32.9 (0.03)	33.1 (0.04)	4.6 (0.03)	12.5 (0.03)	16.8 (0.05)	94.9	0.99	0.99	0.98

* Abbreviations: A, adenine; T, thymine; 5MC, 5-methyl cytosine; C, cytosine; G, guanine; Pu, purines; Py, pyrimidines.

** Mean proportion of each constituent corrected for a 100 % recovery. Actual recovery is given in the seventh column.

*** The standard error of four hydrolysates.

† Double haploid.

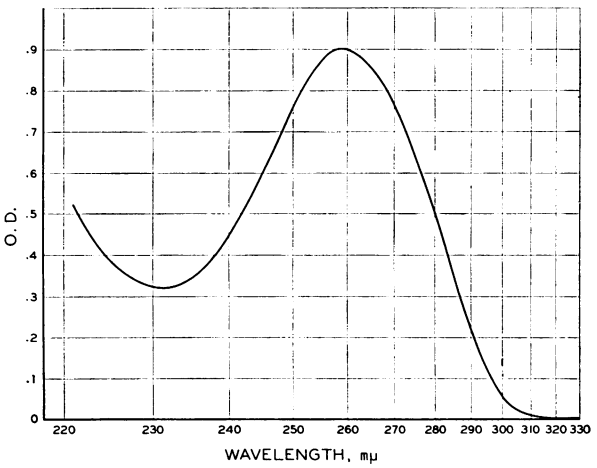

FIG. 1. Ultraviolet absorption curve of cotton Na-DNA (0.0046 %). Curve reproduced from that drawn by a DK-2 spectrophotometer.

mμ (fig 1); D: molar adsorptivity, relative to phosphorus, around 6,600 at 260 mμ, and E: a fibrous character (fig 2).

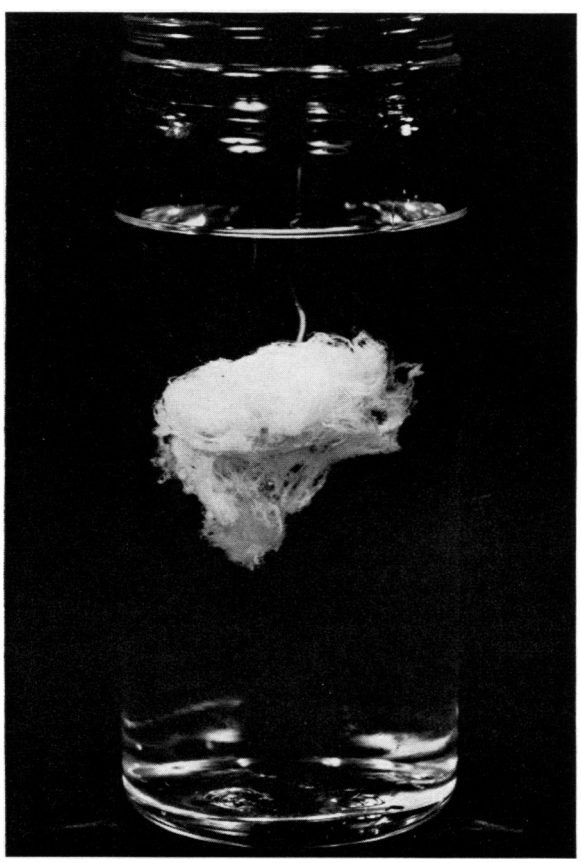

FIG. 2. Suspension of cotton Na-DNA in 95 % ethanol, showing characteristic fibrous nature of all DNA's.

The composition of the DNA's from the nine cotton genotypes, together with a summary of some of the molar relationships, is given in table I. The ratios A/T, Pu/Py, and G/C+5MC satisfactorily reflect the unity relationships, first noted by Chargaff (5), which are characteristic of all DNA's. Note, however, that the DNA's of the nine types of cotton differed little from one another in base content. Adenine varied from 32.7 to 32.9 mole percent, thymine 32.8 to 33.1, 5-methyl-cytosine 4.4 to 4.7, cytosine 12.5 to 12.9, and guanine 16.8 to 17.1. In addition to the ratios of table I, the DNA bases of each genotype also were expressed as ratios of A: A+T/G+C+5MC and B: C/5MC, and the differences within each were evaluated statistically. The former ratio, while adequate in showing the overall composition, does not reflect differences that may exist in the degree of 5-methylcytosine substitution for cytosine; hence, use of the C/5MC ratio.

TABLE IIA

STATISTICAL ANALYSIS OF C/5MC RATIOS IN COTTON DNA ISOLATES. ANALYSIS OF VARIANCE OF C/5MC RATIOS*

VARIANCE	° OF FREEDOM	MEAN SQUARE	F VALUE
Total	35		
Isolates	8	0.01685	4.39**
Error	27	0.00384	

* Four for each of the nine DNA isolates.
** 3.26 needed at 0.01 level.

Preliminary to statistical evaluation, application of the Bartlett test (13) indicated that the variance within each of the ratios A+T/G+C+5MC and C/5MC from the nine cottons could be pooled for an analysis of variance. Results from the latter showed that differences in the DNA's exhibited by the A+T/G+C+5MC ratios were insignificant whereas significance was found among the C/5MC ratios (table II).

TABLE IIB

SEPARATION OF C/5MC MEANS FROM EACH ISOLATE BY DUNCAN MULTIPLE RANGE TEST

ISOLATES	MEANS			
Stoneville 2B	2.91	a**		
Deltapine 15	2.84	a	b*	
Punctatum	2.78		b	c
Latifolium	2.78		b	c
Richmondi	2.74			c
Paymaster 54B	2.74			c
Red Empire	2.73			c
Pima S-1	2.73			c
Palmeri	2.71			c

* P 0.05
** P 0.01

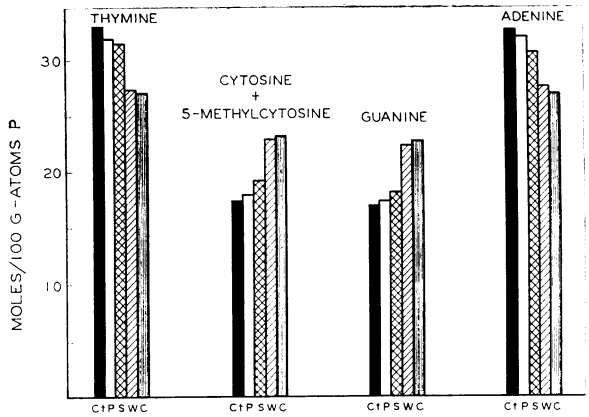

FIG. 3. Proportions of purine and pyrimidine bases in the DNA's of cotton (Ct.), peanut (P), sunflower (S). wheat (W), and corn (C).

The C/5MC ratios of Stoneville 2B and Deltapine 15 varieties (both double haploids), while not differing from each other, were significantly higher than those of the other genotypes except that Deltapine 15 did not differ significantly from the races latifolium and punctatum. Stated otherwise, the DNA of Stoneville 2B and, to a lesser extent, that of Deltapine 15, appeared to contain fewer 5-methylcytosine-guanine combinations than that of the other genotypes. What bearing, if any, the double haploid factor could have on this finding is not apparent at this time.

Turning from the cotton genotypes, among which there was no significant difference in the overall DNA compositions, to the other genera, certain species differences are indicated (table III). The DNA composition index, A+T/C+5MC+G, ranges from 1.17 for corn to 1.92 for cotton (average of all values in table I). Moreover, on the basis of plant classification, the dicots (cotton, peanut, & sunflower) have a higher index than the monocots (corn & wheat). A comparison of the proportions of purine and pyrimidine bases in the individual DNA's (fig 3) shows that adenine and thymine contents decrease and cytosine+5—methylcytosine and guanine increase in the order: cotton, peanut, sunflower, wheat, and corn. Examination of the data in table III shows that the percentage contribution of 5-methylcytosine to its sum with cytosine was substantial; it varied from 27 % in cotton to 37 % in sunflower. All the DNA's are of the AT type (adenine & thymine predominating).

After the present study was started, Russian workers (14, 15) published data on the DNA compositions (determined directly in the tissue without prior isolation) of some 26 plant species, including corn, peanut, sunflower, and wheat. Analytical agreement between the two sets of data is only fair, and of greater interest is that they found only small differences between representatives of widely different systematic groups. The composition index of the 26 species varied within the narrow range of 1.03 to 1.61. In some instances, like our data on corn and wheat (table III) and the two species of cotton (table I), almost identical compositions were found. In contrast, the DNA composition index of microorganisms has been found A: to vary over the wide range of 0.35 to 2.7, depending upon the species to which they belong, and B: to be species specific (1). However, it would appear that current data on the higher plants are not yet sufficiently numerous for any conclusion on the relationship between species and DNA composition.

Although the main interest of the results discussed herein centered on the overall composition of DNA from several higher plant sources, it is generally recognized, as expressed by Hotchkiss (9): "that different arrangements of bases in a nucleic acid chain —with or without a difference in overall composition —provide the means by which different specific molecules are constructed".

SUMMARY

I. We isolated the DNA's of nine cotton geno-

TABLE III

COMPOSITION OF TOTAL DEOXYRIBONUCLEATES OF COTTON, CORN, PEANUT, SUNFLOWER, & WHEAT*

	MOLES/100 g-ATOMS P**						MOLAR RATIOS			
	A	T	5MC	C	G	ACTUAL RECOVERY	A/T	Pu/Py	G/C+5MC	A+T / C+5MC+G
Cotton***	32.8	32.9	4.6	12.7	16.9	94.2	1.00	0.99	0.98	1.92
Corn	26.8	27.2	6.2	17.0	22.8	89.3	0.98	0.98	0.98	1.17
Peanut	32.1	32.2	5.7	12.3	17.6	93.2	1.00	0.99	0.98	1.81
Sunflower	30.8	31.6	7.1	12.0	18.5	87.8	0.97	0.97	0.97	1.63
Wheat	27.2	27.4	6.2	16.6	22.6	97.0	0.99	0.99	0.99	1.20

* Abbreviations: A, adenine; T, thymine; 5MC, 5-methylcytosine; C, cytosine; G, guanine; Pu, purines; Py, pyrimidines.

** Mean proportions of each constituent corrected for a 100 % recovery. Actual recovery given in the seventh column.

*** Average of all values in table I.

types and of corn, peanut, sunflower, and wheat, and determined and compared the base constituents.

II. The overall DNA compositions of the various cottons did not differ significantly from one another. However, the DNA's of the two double haploid varieties, as indicated by their cytosine/5-methylcytosine ratios, appeared to contain fewer 5-methylcytosine-guanine combinations than those of the ordinary genetic types.

III. Adenine and thymine contents decreased and cytosine plus 5-methylcytosine and guanine increased in the order: cotton, peanut, sunflower, wheat, and corn. All of the DNA's were of the adenine-thymine type.

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