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# A CHEMICAL SURVEY OF SEEDS OF THE GENUS *GOSSYPIUM*

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Abstract-The amino acid patterns of the seed proteins and the fatty acid patterns of the seed oils of twenty-five species of ***Gossypium*** are reported. A genome specific pattern was not found for either the amino acids or the fatty acids or for the seed index. G. ***anomalum*** differed markedly from the other species in its seed oil; the kernel contained 74.8 per cent oil and the oil contained about 20 per cent of the fatty acid having an equivalent chain length of 19·6 which was not present in the seed oil of any of the other species. The gossypol content of the defatted seed meals ranged from 0·12 and 0·13 for G. ***stocksii*** and G. ***incanum to*** 9·25 per cent for G. ***klotzschianum*** (var. ***duoidsonii),*** and a multiple range classification of the species in accordance with the gossypol content groups the species into specific genomes, with exception of G. ***gossypioides*** and G. ***longicalyx*** which seem to be out of place. I.r. spectra of the acetone-hexane-water extracts of hexane-defatted meals showed specific differences among the species and led to the grouping of the twenty-five species into their specific genomes; exceptions were G. ***gossypioides, G. longicalyx*** and G. ***tomentosum.***

## INTRODUCTION

COTTON cytologists and geneticists now recognize at least thirty species of the genus *Gossypium,* four of which are cultivated. On the basis of cytological data and geographical distribution, Beasley[1] separated the species into six groups, each with chromosomes that belong to a distinct genome. Each diploid genome (with thirteen pairs of chromosomes) was represented by a single letter from A through E, while each allotetraploid genome was represented by the two letters AD to symbolize its two diploid genomes.

The recognized species, where the species within each genome is identified by subscripts, that were examined in this study are listed in Table 1.

Chemical analyses of seed of several of the species have been reported. Ergle *et al.*,[2] for example, reported on the nucleic acid composition, while Frampton *et al.*[3] and Carter *et al.*[4] reported on amino acid, fatty acid and gossypol patterns of seeds of several of the species. Additional data on seed properties are reported here.

## RESULTS AND DISCUSSION

The seed characteristics of the fourteen species that were available to us as intact seed are reported in Table 2. The seed indices (g per 100 seeds) range from about 1 g (genomes $C_1$, $C_{1-2}$ and $C_3$) to 8·5 g (genome $D_{2-2}$). These indices are smaller than those reported for

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1 J. 0. BEASLEY, *J. Heredity 31, 39* (1940).
2 D. R. ERGLE, F. R. H. KATTERMAN and T. R. RICHMOND, *Plant Physiol. 39,145* (1964).
3 VERNON L. FRAMPTON, WALTER A. PONS, JR. and T. KERR, *Econ. Botany* 14,197 (1960).
4 F.L. CARTER, A. E. CASTILLO, VERNON L. FRAMPTON and T. KERR, *Phytochem.* 5, 1103 (1966).

TABLE 1. *Gossypium* SPECIES USED IN THE ANALYSIS

| Genome | Species | No. of samples | Nature of samples |
|---|---|---|---|
| $A_1$ | ***G. herbaceum*** L. | 2 | HEM |
| $A_2$ | G. ***arboreum*** L. | 1 | HEM |
| $B_1$ | G. ***anomalum*** Wawra and Peyr | | S |
| $B_2$ | G. ***triphyllum*** Hochreutiner | 1 | S |
| $C_1$ | G. ***sturtianum*** Willis | 1 | S |
| $C_{1-2}$ | G. ***sturtianum*** (var. ***nandewerense)*** | 1 | S |
| $C_2$ | ***G. robinsonii*** von Mueller | 1 | S |
| $C_3$ | ***G. australe*** von Meuller | 1 | S |
| $C_4$ | G. ***bickii*** Prokhanov | | S |
| $D_1$ | G. ***thurberi*** Todaro | 1 | HEM |
| $D_{2-1}$ | G. ***armourianum*** Kearney | 4 | HEM |
| $D_{2-2}$ | G. ***harknessii*** Brandegee | 1 | S |
| $D_4$ | G. ***aridum*** (Rose and Standley) Skovsted | 5 | HEM |
| $D_7$ | G. ***lobatum*** Gentry | 1 | S |
| $D_{3-k}$ | G. ***klotzschianum*** Anderson | 2 | HEM |
| $D_{3-d}$ | ***K. klotzschianum*** var. ***dauidsonii*** (Kellogg) | 5 | HEM |
| $D_5$ | G. ***raimondii*** Ulbrich | 5 | HEM |
| $D_6$ | G. ***gossypioides*** (Ulbrich) Standley | 3 | HEM |
| $E_1$ | G. ***stocksii*** Masters | 1 | S |
| $E_2$ | G. ***somalense*** (Gurke) J. Hutch. | 1 | S |
| $E_4$ | G. ***incanum*** (Schwartz) D. Hille | | S |
| $E_5$ | G. ***longicalyx*** Hutch. and Lee | 1 | S |
| $(AD)_1$ | G. ***hirsutum*** L. | 1 | HEM |
| $(AD)_2$ | G. ***barbudense*** L. (Pima S-2) | 1 | HEM |
| $(AD)_3$ | G. ***tomentosum*** Nut. | 1 | S |

S : whole seed ; HEM : hexane extracted meal.

TABLE 2. SEED CHARACTERISTICS OF *Gossypium* SPECIES

| Genome | Species | Seed index | % Kernel | % Oil in Seed | % Oil in Kernel | % Nitrogen | % Protein |
|---|---|---|---|---|---|---|---|
| $B_1$ | ***anomalum*** | ***2.29*** | 31.4 | 23.9 | 74.8 | 9.14 | 57·1 |
| $B_2$ | ***triphyllum*** | ***4.57*** | 38.3 | 8·8 | 24.5 | 9.89 | 61·8 |
| $C_1$ | ***sturtianum*** | 1·04 | 52.1 | 18.6 | 35·7 | 7.41 | 46.3 |
| $C_{1-2}$ | ***sturtianum*** (var. ***nandewarense)*** | ***0.99*** | 45.3 | 15.9 | 35.2 | 7.91 | 49.4 |
| $C_2$ | ***robinsonii*** | 1.51 | ***53.4*** | 18.7 | 35·0 | 8.83 | 55·2 |
| $C_3$ | ***australe*** | 1.02 | 47·0 | 14.4 | 31.4 | 9.00 | 56.3 |
| $C_4$ | ***bickii*** | 1·19 | ***43.4*** | 12.4 | 28.7 | 8.51 | 53.2 |
| $D_7$ | ***lobatum*** | 3.06 | ***67.1*** | 20.7 | 31.7 | 9.62 | 60·1 |
| $D_{2-2}$ | ***harknessii*** | 8.48 | ***66.1*** | 21.8 | 38.0 | 9·81 | 61.3 |
| $E_1$ | ***stocksii*** | 1.99 | ***35.9*** | 8.5 | 23.2 | 8.31 | 51.9 |
| $E_2$ | ***somalense*** | 1.73 | ***45.0*** | 12.1 | 26.9 | 9.37 | 58.6 |
| $E_4$ | ***incanum*** | 1·99 | ***39.2*** | 9·8 | 26.8 | 9.26 | 57.9 |
| $E_5$ | ***longicalyx*** | 3·05 | 57·4 | 18.1 | 31.5 | 8·90 | 55·6 |
| $(AD)_3$ | ***tomentosum*** | 6.14 | ***35.5*** | 9·7 | 27.2 | 9.99 | 62.4 |

the American cultivated species (genomes (AD),) of 10 g for moisture-free seed and 11·1 grams for fuzzy seed containing 10 per cent moisture,[5] or the Egyptian cultivated species (genome (AD),) of 9·1–10·5 g.[6]

It may be seen from the data in Table 2 that the per cent by weight of the kernels in the seeds of the different species ranges from 31·4 for genome $B_1$ to 67·1 for genome $D_7$ and 66·1 for $D_{2-2}$. These data are compared with the weight per cent of the kernels of ***Gossypium hirsutum*** of about ***55*** per cent[5] and 63 per cent for G. *barbadense*.[6]

The data of Table 2 reveal that the sum of the per cent protein and per cent oil is correlated significantly (coefficient of 0·56 for 12 degrees of freedom) with the seed index. This correlation is significant at the 5 per cent level of probability. Table 2 also reveals a substantial variation among the species in the oil content of the kernels. The maximum occurred for $B_1$, where the analysis indicate an oil content of 74·8 per cent while the minimum occurred for $E_1$ at 23.2 per cent.

Carter ***et al.***,[4] had available to them numerous collections for each of several species, and they reported analyses of variance of the data for several of the seed properties. In addition, the oils and hexane defatted meals from their investigation were available for this research, and additional chemical analyses were carried out and the data were subjected to analysis of variance. Error variances estimated from these several studies were used to estimate the least significant differences for the multiple range classifications[7] of the species in accordance with each chemical property measured.

The variations among the several species in gossypol content of the defatted meals was substantial (Table 3). The extremes were about 10 per cent for genome $D_{3-d}$ to about 0·1 per cent for genome $E_1$. The multiple range classification of the species in accordance with the gossypol contents of the hexane-defatted meals is also shown in Table 3. It will be observed that the different diploid genomes are ranged together, with the species belonging to genome D occupying the higher rank and those belonging to genome E the lower rank. Exceptions are observed for G. ***longicalyx*** and G. ***gossypioides,*** which two species seem to be out of place.

The relative areas under the curves for the methyl esters of the component fatty acids of the species as estimated from the GLC tracings are reported in Table 4. The multiple range classification of the species in accordance with the component fatty acids in the seed oils was determined and it was concluded that the distributions of the component fatty acids of the seed oils do not coincide with the cytological and genetic assignments of the species of specific genomes and it is evident that the fatty acid patterns are not genome specific.

Attention is called to the unusually high level of oil in the kernels of G. ***anomalum*** ($B_1$). This species differs also from the other species in the genus in oil composition. A fatty acid component is present in genome $B_1$ the range of 19 per cent that has an equivalent chain length of 19·6. This acid is not present in the hexane extracts of any of the other species. The evidence from TLC and from i.r. studies on fractions collected from the GLC apparatus indicate that this fatty acid is unsaturated; it is not malvalic acid.

The correlation between linoleic and oleic acids for the genus was estimated to be −0·79, which is very highly significant. With the exception of G. ***anomalum*** the sum of oleic and linoleic acids are approximately constant at about 70 per cent-the one fatty acid increases at the expense of the other. It was also observed that the level of "total" gossypol of the defatted meals (hexane-extracted) is correlated with the linoleic acid content of the seed oil;

[5] A. E. BAILEY, ***Cottonseed and Cottonseed Products,*** Interscience, New York (1948).
[6] AHMED S. EL NOCKRASHY, S. FIAD and A. M. GAD, unpublished data.
[7] D. B. DUNCAN, ***Biometrics*** **11,** 1 (1955).

the coefficient was estimated to be -0.46, which for 23 degrees of freedom is significant at the 5 per cent level of probability. The correlation between oleic acid and "total" gossypol was not observed to be statistically significant.

The amino acid patterns of the seed proteins of the individual species are reported in Table 5. The weights of all of the amino acids, including the ammonia present in the hydrolyzates, estimated from the tracings from the automatic amino acid analyzer, were summed

TABLE 3. AZEOTROPE EXTRACT OF HEXANE - DEFATTED MEALS

| Genome | *Gossypium* species | % CZE | Gossypol in CZE Free | Gossypol in CZE Total | Gossypol in Meal Free | Gossypol in Meal Total | Multiple range classification of species in terms of gossypol in meals | Formyl absorption at 6·15 $\mu$ |
|---|---|---|---|---|---|---|---|---|
| $A_1$ | ***herbaceum*** | 6·1 | 13.02 | 14.63 | 0.77 | 1·20 | f | 0.456 |
| $A_2$ | ***arboreum*** | 4.4 | 14.49 | 16.92 | 0.62 | 0·81 | f | 0.415 |
| $B_1$ | ***anomalum*** | 12·0 | 10.33 | 10.95 | 1·24 | 1·32 | f | 0·187 |
| $B_2$ | ***triphyllum*** | 17.5 | 11·60 | 12.33 | 2.03 | 2.16 | e | 0.178 |
| $C_1$ | ***sturtianum*** | 13.2 | 3.49 | 3.81 | 0.46 | 0.50 | fg | 0.23 l |
| Cl-2 | ***sturtianum*** (var. *nandewerense*) | 14·0 | 3.60 | 3.66 | 0.50 | 0·51 | fg | 0·233 |
| $C_2$ | ***robinsonii*** | 13·0 | 1·48 | 1·54 | 0·19 | 0.20 | g | 0·104 |
| $C_3$ | ***australe*** | 13·4 | 1·24 | 1·36 | 0·17 | 0.18 | g | 0·132 |
| $C_4$ | ***bickii*** | 10·6 | 3.53 | 3.82 | 0.37 | 0.40 | g | 0·205 |
| $D_1$ | ***thurberi*** | 11.6 | 26.51 | 32·40 | 3.21 | 3.77 | d | 0·639 |
| $D_{2-1}$ | ***armourianum*** | 7·4 | 23.21 | 26.96 | 2·04 | 2.56 | e | 0·447 |
| $D_{2-2}$ | ***harknessii*** | 11·3 | 25·62 | 26·52 | 2·90 | 3.00 | de | 0.508 |
| D4 | ***aridum*** | 11·5 | 33.61 | 3605 | 4.70 | 5·37 | c | 0·672 |
| D7 | ***lobatum*** | 15.6 | 44.16 | 44.91 | 6.89 | 7·01 | b | 0·658 |
| $D_{3-k}$ | ***klotzschianum*** | 12·9 | 46.52 | 54.40 | 6.37 | 7·32 | b | 0.735 |
| $D_{3-d}$ | ***klotzschianum*** (var. *dacidsonii)* | 19·0 | 39.86 | 43.45 | 7.98 | 9.25 | a | 0·651 |
| $D_5$ | ***raimondii*** | 11·4 | 26·86 | 28.94 | 3.08 | 3.47 | d | 0·488 |
| D6 | *gossypioides* | 6·4 | 12.41 | 13.41 | 0·69 | 0.73 | f | 0.379 |
| $E_1$ | ***stocksii*** | 12.0 | 0·96 | 0.96 | 0·12 | 0.12 | g | 0·129 |
| $E_2$ | ***somalense*** | 10.3 | 1.24 | 1.44 | 0·13 | 0·15 | g | 0·126 |
| $E_4$ | *incanum* | 13·4 | 1.01 | 1.01 | 0·13 | 0·13 | g | 0.081 |
| $E_5$ | *longicalyx* | 10·1 | 10·12 | 10·52 | 1.02 | 1·07 | f | 0·302 |
| $(AD)_1$ | ***hirsutum*** | 12.2 | 11.50 | 14.69 | 1·40 | 1·79 | e f | 0·622 |
| $(AD)_2$ | ***barbadense*** | 13·6 | 9.84 | 13.40 | 1·28 | 1·80 | e f | 0.233 |
| $(AD)_3$ | ***tomentosum*** | 30·9 | 11.36 | 11·61 | 3.51 | 3.59 | d | 0·135 |

and the quantity of each amino acid estimated from the tracing was expressed as a weight per cent of this total. The data are not expressed as g per 16 g of nitrogen, as is conventional, because the nonprotein nitrogen of the seed meals is not known.

The multiple range classification (at the 1 per cent level of probability) of the species in terms of the individual amino acids was determined. It was concluded the distributions of the amino acids do not coincide with the cytological and genetic assignments of the species to specific genomes and the amino acid patterns are not genome specific.

*Gossypium hirsutum*, the major source of cottonseed meal, ranks low in comparison with other species in concentrations of lysine, threonine, methionine, leucine, and isoleucine. Two of these amino acids, namely lysine and methionine, are frequently in critically low concentrations in rations fed to nonruminant animals.

TABLE 4. FATTY ACID COMPOSITION OF SEED OILS OF *Gossypium* SPECIES

| Genome | Species | $C_{14}$ | $C_{16}$ | $C_{16:1}$ | $C_{18}$ | $C_{18:1}$ | $C_{18:2}$ |
|---|---|---|---|---|---|---|---|
| $A_1$ | *herbaceum** | 0·9 | 23.3 | 1.7 | 3.6 | 19.3 | 51.4 |
| $A_2$ | *arboreum** | o-4 | 21·8 | 1·8 | 4.2 | 32.4 | 40.8 |
| $B_1$ | *anomalum*† | 0·6 | 26.2 | 1.2 | 1·2 | 11·3 | 40.8 |
| $B_2$ | *triphyllum* | 0·8 | 29.3 | 0·9 | 2.4 | 20·4 | 46.2 |
| $C_1$ | *sturtianum* | 0·7 | 21.8 | 0·7 | 3.3 | 11.8 | 61·7 |
| $C_{1-2}$ | *sturtianum* (var. *nandewerense)* | 0·6 | 23.5 | o-3 | 3.4 | 12.0 | 60.3 |
| $C_2$ | *robinsonii* | 0·4 | 22·5 | 0·3 | 2·6 | 8.4 | 65.9 |
| $C_3$ | *australe* | 0·4 | 23.4 | 1.5 | 1·0 | 9.7 | 63.8 |
| $C_4$ | *bickii* | 0·3 | 21.8 | 0·4 | 1.8 | 12·6 | 63.1 |
| $D_1$ | *thurberi** | 0·6 | 26·7 | 0·9 | 2.7 | 17·6 | 51.6 |
| $D_{2-1}$ | *armourianum** | 0·3 | 24.4 | 0·7 | 3.2 | 36·0 | 35.4 |
| $D_{2-2}$ | *harknessii* | 0·3 | 32·2 | 0·5 | 3.2 | 30·8 | 33.1 |
| $D_4$ | *aridum**. | 0·9 | 34.5 | 1·0 | 3·1 | 16·0 | 44·5 |
| $D_7$ | *lobatum* | 0·7 | 36·3 | 0·4 | 3.4 | 11·8 | 47.3 |
| $D_{3-k}$ | *klotzschianum** | 0·9 | 26·5 | 1-8 | 3·0 | 19·5 | 48.3 |
| $D_{3-d}$ | *klotzschianum* (var. *davidsonii*)* | 0·5 | 25·8 | 1.3 | 3·0 | 20.6 | 48.9 |
| $D_5$ | *raimondii** | 0·7 | 24.4 | 1.2 | 3.4 | 23.5 | 46·9 |
| $D_6$ | *gossypioides** | 0·6 | 25·1 | 0·5 | 3.7 | 17.2 | 52.7 |
| $E_1$ | *stocksii* | 0·5 | 23.4 | 0·6 | 1.2 | 8·9 | 65.4 |
| $E_2$ | *somalense* | 0·6 | 26·4 | 0·3 | 3.4 | 8.8 | 60·6 |
| $E_4$ | *incanum* | 0·8 | 26-O | 1·2 | 1·4 | 13.3 | 57·3 |
| $E_5$ | *longicalyx* | 0·2 | 23.7 | 0·4 | 2.3 | 16.9 | 56.6 |
| $(AD)_1$ | *hirsutum** | 0·7 | 24·3 | 1.2 | 2·9 | 16.5 | 54·5 |
| $(AD)_2$ | *barbadense** | 1·0 | 23·6 | 0·8 | 2·9 | 21.2 | 50·7 |
| $(AD)_3$ | *tomentosum* | 0·5 | 32·3 | 1.9 | 1·9 | 11.7 | 51.7 |

* Data reported by Garter *et al.*[4]

† *G. anomalum* contains 19·0 per cent of an unknown $C_{19\cdot6}$ fatty acid.

The acetone-hexane-water azeotrope extracts of hexane defatted meals are of considerable interest. The quantities of materials removed from the defatted meals by the azeotrope ranged from 4·4 per cent for $A_2$ to 30·9 per cent for (AD),. The multiple range classification of the species in accordance with the quantities of materials extracted is shown in Table 6.

Hexane is not a good solvent for gossypol, whereas the azeotrope is. Most of the gossypol of the seed remains with the residual meal when the oil is removed by hexane, while the major portion of the gossypol is removed when the meal is extracted with the azeotrope, and the total quantity of materials extracted increases as the quantity of gossypol in the meal increases.

The absorption band at 6·15 $\mu$ in the spectrum of the azeotrope extract is attributed to the formyl groups of gossypol-the correlation between the intensity of this band and the "free" gossypol of the extract is very good. The coefficient was estimated to be 0·89, which for 42 degrees of freedom is very highly significant.

A further study of the i.r. spectra of the azeotrope extracts of the defatted meals from the 44 samples belonging to the 25 species studied revealed specific differences among the species (Table 7). The salient features of the spectra are summarized: (1) All of the species show a strong absorption band at 7·25 $\mu$; (2) A strong band at 6·05 is present in the spectra for the four cultivated species, namely, G. ***hirsutum, G. barbadense, G. arboreum*** and G. ***herbaceum*** and, with the exception of the wild allotetraploid G. ***tomentosum*** where the band is weak, absorption is absent from the spectra for all other species; (3) All of the species of genome D,

Table 5. AMINO ACID PATTERNS OF PROTEINS OF THE SPECIES OF THE GENUS *Gossypium*

| Genome | Lys. | His. | $NH_3$ | Arg. | Asp. | Thre. | Ser. | Glu. | Pro. | Gly. | Ala. | Val. | Met. | Ileu. | Leu. | Tyr. | Phen. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $A_1$ | 4.54 | 2.85 | 2.30 | 12.88 | 10·28 | 3.38 | 4.38 | 21.45 | 4.17 | 4.52 | 4.34 | 4·81 | 1·32 | 3.58 | 6·15 | 3·27 | 5.78 |
| $A_2$ | 4·50 | 2·90 | 2.23 | 12·08 | 10·51 | 3.35 | 4·42 | 22.19 | 3.97 | 4.43 | 4.25 | 4.94 | 1.34 | 3.64 | 6.28 | 3·17 | 5·79 |
| $B_1$ | 4·72 | 3.00 | 2.91 | 11.54 | 9.92 | 3·51 | 4.95 | 21·69 | 3.79 | 4.92 | 4.35 | 4·41 | 2.58 | 3.51 | 6.33 | 3.02 | 5.10 |
| $B_2$ | 4·07 | 2.89 | 2·37 | 11·22 | 10·33 | 3.52 | 5.08 | 22.37 | 3.96 | 4.59 | 4.23 | 4.61 | 1·97 | 3·88 | 6.47 | 2.92 | 5.50 |
| $C_1$ | 4·97 | 2·73 | 2.55 | 10·58 | 10.33 | 3-96 | 5.07 | 20·27 | 3.57 | 4.89 | 4·72 | 4.89 | 2.63 | 3.60 | 6.57 | 2·98 | 5·67 |
| $C_{1-2}$ | 5.32 | 2.77 | 2.44 | 10·44 | 10.45 | 3.86 | 4.97 | 19.93 | 3.72 | 5·05 | 4.93 | 4.93 | 2·61 | 3.61 | 6.58 | 2·97 | 5·60 |
| $C_2$ | 4.81 | 2.80 | 2.45 | 11·49 | 10.20 | 3.78 | 4.90 | 20.58 | 3·76 | 4.76 | 4·63 | 5.04 | 2·20 | 4.03 | 6.46 | 2.79 | 5.66 |
| $C_3$ | 4·62 | 2·70 | 2.37 | 11·48 | 9.95 | 3.49 | 4.73 | 21.80 | 3·71 | 4·81 | 4.38 | 4.44 | 2.26 | 4·07 | 6·88 | 3·06 | 5.23 |
| $C_4$ | 4·67 | 2.70 | 2.58 | 11.55 | 10·55 | 3·73 | 4.87 | 21.20 | 3.73 | 4·98 | 4.62 | 4.53 | 2.28 | 3.36 | 6.33 | 2.94 | 5.38 |
| $D_1$ | 5·06 | 2.85 | 2.17 | 11.89 | 10.25 | 3.63 | 4.65 | 20·79 | 3.97 | 4.77 | 4·48 | 4.80 | 1.66 | 3·67 | 6.54 | 3.15 | 5.53 |
| $D_{2\ 1}$ | 4.87 | 2·86 | 2·11 | 12·09 | 10·05 | 3.45 | 4.61 | 21.60 | 4.13 | 4.70 | 4.30 | 4.84 | 1·28 | 3.65 | 6.37 | 3·18 | 5.91 |
| $D_{2-2}$ | 4.81 | 2.78 | 2.23 | 12.33 | 1 0-03 | 3·37 | 4·71 | 22.25 | 3·58 | 4·58 | 4·23 | 4.59 | 2.28 | 3.45 | 6.20 | 2.90 | 5.67 |
| $D_4$ | 4·82 | 2·45 | 2·19 | 11·84 | 10·08 | 3.58 | 4.64 | 21·41 | 4.19 | 4.59 | 4.51 | 4.89 | 1·34 | 3.67 | 6.67 | 3.22 | 5·57 |
| $D_7$ | 4·60 | 2.72 | 1·93 | 11.71 | 10·47 | 3·50 | 4.73 | 21·87 | 3·75 | 4.51 | 4.40 | 4.53 | 2.29 | 4·07 | 6.86 | 2.74 | 5·31 |
| $D_{3\ k}$ | 4.98 | 2.92 | 2·13 | 11.41 | 9.77 | 3·68 | 4.64 | 21.37 | 4·17 | 4.87 | 4.55 | 4·85 | 1·43 | 3.72 | 6·51 | 3.28 | 5·71 |
| $D_{3-d}$ | 5.22 | 2.94 | 2·11 | 11·33 | 9.66 | 3.83 | 471 | 20.65 | 4·19 | 4.98 | 4.64 | 4·87 | 1·45 | 3.86 | 6.69 | 3.34 | 5.52 |
| $D_5$ | 4.43 | 2.86 | 2.19 | 12.59 | 10·19 | 3·74 | 4·67 | 21.30 | 4·21 | 4.65 | 4.33 | 4.87 | 1.28 | 3.65 | 6.39 | 3·08 | 5.83 |
| $D_6$ | 4.67 | 2·89 | 2.11 | 11·95 | 9.98 | 3.44 | 4.63 | 22·10 | 4.10 | 4.62 | | 4·31 | 4·79 | 1·42 | 3·70 | 6.38 | 3·22 | 5·68 |
| $E_1$ | 4·31 | 2·64 | 2·31 | 12.10 | 10·27 | 3.55 | 5·01 | 22·01 | 3.87 | 4.84 | 4.29 | 4·73 | 1·97 | 3.36 | 5·88 | 3·09 | 5·80 |
| $E_2$ | 4.58 | 2.74 | 2.13 | 12·29 | 10.44 | 3.60 | 4.97 | 21·76 | 3.95 | 4.52 | | 4·21 | 4·60 | 2·19 | 3·21 | 5.98 | 3.17 | 5.66 |
| $E_4$ | 4·31 | 2.51 | 2·76 | 12.35 | 10·82 | 3.60 | 4·95 | 21·48 | 3·77 | 4.88 | 4·40 | 3·50 | 2·64 | 3.39 | 6·12 | 2.87 | | 5.73 |
| $E_5$ | 4.05 | 2·73 | 2.42 | 1249 | 10·36 | 3·68 | 4.91 | 18·19 | 5.62 | 4·71 | 4.54 | 4.90 | 1·26 | 3-39 | 6.69 | 2·95 | | 5.85 |
| $(AD)_1$ | 4·55 | 2.94 | 2.16 | 12·87 | 9.77 | 3.30 | 4·53 | 22.52 | 4·07 | 4 4 2 | 4·05 | 4.82 | 1·43 | 3.36 | 6·07 | 3.34 | | 5.78 |
| $(AD)_2$ | 4.74 | 2.84 | 2.13 | 12·45 | 9.88 | 3.54 | 4.53 | 21·91 | 3.98 | 4.46 | 4.24 | 4.80 | 1·74 | 3.50 | 6.23 | 3.27 | | 5·60 |
| $(AD)_3$ | 4·64 | 2.84 | 2·62 | 12·18 | 9.90 | 3·48 | 4·65 | 22.22 | 3·66 | 4-45 | 4·15 | 4.62 | 2·89 | 3.41 | 6.08 | 2.86 | | 5.36 |

TABLE 6. MULTIPLE RANGE CLASSIFICATION OF THE SPECIESINACCORDANCEWITHQUANTKIES EXTRACTED BY THE AZEOTROPE FROM DEFATTED MEAL

| Genome | Range |
|---|---|
| $(AD)_3$ | a |
| $D_{3-d}$ | b |
| $B_2$ | bc |
| $D_7$ | bcd |
| $C_{1-2}$ | bcd |
| $D_4$ | bcd |
| $(AD)_2$ | bcde |
| $C_3$ | bcde |
| $E_4$ | bcde |
| $C_1$ | bcde |
| $C_2$ | bcde |
| $D_{3-k}$ | bcdef |
| $(AD)_1$ | cdefg |
| $E_1$ | cdefg |
| $B_1$ | cdefg |
| $D_1$ | cdefg |
| $D_5$ | cdefg |
| $D_{2-2}$ | cdefg |
| $C_4$ | defgh |
| $E_2$ | defgh |
| $E_5$ | defgh |
| $D_{2-1}$ | efgh |
| $D_6$ | fgh |
| $A_1$ | gh |
| $A_2$ | h |

with the exception of G. ***gossypioides*** are characterized by five strong and distinct bands which appear at 6·35, 6·91, 7·05, 7·19 and 7·40 $\mu$. Some of these bands are missing or are weak in the spectrum for G. ***gossypioides,*** $D_6$; (4) Species belonging to genome C showed only three of the bands which characterized genome D, namely bands at 7·05, 7·19 and 7·40 $\mu$; (5) Species belonging to genome B showed weak absorption bands at 7·19 and 7·40 $\mu$ and strong absorption at 7·05 $\mu$; (6) Species belonging to genome E were more diffuse in their differentiation. The identity of the seed constituents which are responsible for these absorptions remains to be established.

Hutchinson *et al.*[8] grouped G. ***anomalum*** and G. *triphyllum* together in the section ***Anomala*** (genome B) on the basis of morphological grounds. Cytological investigations later confirmed their close relationship[9] and the i.r. data reported here fit in with this concept. ***G. barbosanum*** was assigned to this genome[10] but this specimen was not available for study.

Genome C (all of which are endemic to Australia) traditionally contains G. ***sturtianum*** and G. ***robinsonii,*** and more recently Saunders" and Fryxell[9] assigned ***Notoxylinum australe*** to the genus ***Gossypium*** and placed it in genome C. The i.r. absorption spectrum of the azeotrope extract from this species is in conformation with this assignment.

[8] J. B. HUTCHINSON, R. A. SILOW and S. G. STEPHENS, ***The Evolution of Gossypium,*** Oxford University Press, London (1947).
[9] P. A. FRYXELL, *J. Botany* 13, 71 (1965).
[10] L. L. PHILLIPS, *Am. J. Botany 53, 328 (1966).*
[11] J. H. SAUNDERS, *The Wild Species of Gossypium and their Evolutionary History* Oxford University Press, London (1961).

TABLE 7. INFRARED ABSORPTION CHARACTERISTICS OF THE AZEOTROPE EXTRACT OF THE HEXANE DEFATTED MEAL OF SEEDS BELONGING TO GENUS *Gossypium*

| Genome | Species | No. of sample | Wave-length (μ) 6·05 | 6.35 | 6.91 | 7·05 | 7.19 | 7.40 |
|---|---|---|---|---|---|---|---|---|
| Al | *herbaceum* | 2 | + | | — | — | — | — | | — |
| A2 | *arboreum* | 1 | + | | — | — | — | — | — | — |
| $B_1$ | *anornalum* | 1 | — | — | | — | + | w | w |
| $B_2$ | *triphyllum* | 1 | — | — | | — | + | w | w |
| $C_1$ | *sturtianum* | 1 | — | — | | — | + | + | + |
| $C_{1-2}$ | *sturtianum* (var. *nandewerense*) | 1 | — | — | | – | + | + | + |
| $C_2$ | *robinsonii* | 1 | — | — | | — | + | + | + |
| $C_3$ | *australe* | 1 | – | — | | – | + | + | + |
| $C_4$ | *bickii* | 1 | — | — | | — | + | + | + |
| $D_1$ | *thurberi* | 1 | — | + | | + | + | + | + |
| $D_{2-1}$ | *armourianum* | 4 | — | + | | + | + | + | + |
| $D_{2-2}$ | *harknessii* | 1 | — | + | | + | + | + | + |
| D4 | *aridum* | 5 | – | + | | i - | + | + | + |
| D7 | *lobatum* | 1 | — | + | | + | + | + | + |
| $D_{3-k}$ | *klotzschianum* | 2 | – | + | | + | + | + | + |
| $D_{3-d}$ | *klotzschianum* (var. *davidsonii*) | 5 | — | + | | + | + | + | + |
| $D_5$ | *raimondii* | 5 | – | + | | + | + | + | — |
| $D_6$ | *gossypioides* | 3 | – | w | | – | w | w | + |
| $E_1$ | *stocksii* | 1 | — | – | | w | + | + | + |
| $E_2$ | *somalense* | 1 | — | – | | w | + | + | w |
| E4 | *incanum* | 1 | — | + | | w | + | + | + |
| $E_5$ | *longicalyx* | 1 | — | — | | w | w | w | w |
| $(AD)_1$ | *hirsutum* | 1 | + | w | | + | — | w | – |
| $(AD)_2$ | *barbadense* | 1 | + | w | | – | + | — | + |
| $(AD)_3$ | *tomentosum* | 1 | w | — | | — | w | w | w |

+, present; —, absent; w, weak.

***Gossypium areysianum*** (not available for this study), ***G. stocksii*** and ***G. somalense*** are well established members of genome E,[12] and to this has been added also G. *incanum* and G. ***longicalyx.*** While G. ***incanum*** closely resembles G. ***somalense*** and G. ***areysianum*** in many respects, ***G.*** *longicalyx* does not. It is readily seen from Table 7 that the spectrum of the azeotrope extract for G. ***longicalyx*** does not seem to fit in with the other species of this genome. G. *longicalyx* apparently is not related cytologically to other species of genome E, and it has been suggested that it is a monotypic representative of a sixth diploid genome.[10,13] Other data reported here seem to support the suggestion that G. ***longicalyx*** should be assigned to a different genome, as may be seen from the comparisons made in Table 8.

Species belonging to genome D are found in the western parts of the New World. This is the largest and most variable genome of the genus. G. ***gossypioides*** was assigned to this genome, but it varied from other species in the genome in several characteristics. For example, the gossypol content of the seed is of the order of 0·7 per cent while the gossypol content of seeds of the other members of the genome vary from about 2·5 per cent to nearly 10 per cent. It also differs in the i.r. absorption characteristics of the azeotrope extract of the hexane defatted meal, cf. Table 7. According to Brown and Menzel[14] G. ***gossypioides*** ($D_6$) shows

[12] H. J. DOUWES, *Genetics* **51** 611 (1953).
[13] L. L. PHILLIPS and M. A. STRICKLAND, *Can. J. Genet. Cytol.* **8**, 91 (1966).
[14] M. E. BROWN and M. Y. MENZEL, *Bull. Torry Botany Club 79,285* (1952).

TABLE 8. COMPARISON OF PROPERTIES OF *Gossypium longicalyx* WITH OTHER MEMBERS OF GENOME E

| Property | G. *longicalyx* | Other species in genome E |
|---|---|---|
| Seed index | 3.1 | 1·7–2·0 |
| % Kernel | 57.4 | 35·9–45·0 |
| % Oil in kernel | 31.5 | 23·2–26·9 |
| Proline | 5·6 | 3·7–4·3 |
| Gossypol | 1·1 | 0·1 |

gene differences from other species in the genome. For example, they concluded that a complementary lethal mechanism prevents successful hybridization with species of the other diploid genome groups.

It is now accepted that a ***Gossypium*** ancestral to genome D hybridized with a ***Gossypium*** ancestral to genome A and subsequent chromosome doubling gave rise to the modern amphidiploid genome (AD). The synthesis of an amphidiploid from a diploid D and a diploid A by Beasley[15] and by Harland[16] resembling the natural tetraploids and compatible with them gave additional support to this concept. The data from Table 7 seem also to support this concept for G. ***hirsutum*** and possibly G. ***barbadense,*** but it seems possible that G. ***tomentosum*** may have arisen from hybridization of other ancestral genomes.

## EXPERIMENTAL

The seed index (weight per 100 seeds) was determined for the fourteen species of intact seed (collected 1967, Iguala, Mexico) available for this investigation.

Kernels were separated from the hulls (although some of the seeds are very small) and the weight per cent of the kernels was determined for each species. The weighed kernels were ground and extracted twice with hexane in an Omni mixer and marc and miscella were separated on a sintered glass filter of fine porosity. The combined extracts for each species were evaporated under reduced pressure at 40°, and the residual oils were stored in $N_2$ in the deep freeze. The meal from each species was then re-extracted with the azeotropic mixture of acetone-hexane and water.[17] The azetotropic extracts, designated here as CZE, were each evaporated to dryness under reduced pressure at 40° and the residual lipid materials were stored in $N_2$ in the deep freeze. The meals were air dried at room temp., ground in a Wiley mill to pass through a 40-mesh screen. and these were also stored in the deep freeze.

An aliquot of oil from each species was taken and the component fatty acids were converted to their methyl esters by the procedure proposed by Luddy *et al.*[18] Gas-liquid chromatographic analyses of the mixed methyl esters for each species were carried out in triplicate in the manner described by Pons *et al.*,[19] expecting that the pressure head of the developing gas was maintained at 30 lb/in$^2$.

Total N of the meals was determined for each species by the micro-Kjeldahl procedure described by Clark20 and the protein content of each meal was expressed as the product of the per cent N and the factor 6·25.

Meal samples were hydrolyzed with constant boiling aqueous HCl in sealed ampoules (20 mg per 10 ml of acid solution) at 110” for 24 hr. The amino acids for each hydrolyzate were then determined as described by Moore *et al.*,[21] through the use of an automatic amino acid analyzer constructed essentially as described by Spackman *et al.*[22]

[15] J. O. BEASLEY, *Am. Nat. 74,285* (1940).
[16] S. C. HARLAND, *Trop. Agric. 17, 53* (1940).
[17] WILLIAM H. KING, JAMES C. KUCK and VERNON L. FRAMPTON, *J. Am. Oil Chem. Soc.* 38, 19 (1961).
[18] F. E. LUDDY, R. A. BARFORD and R. W. RIEMENSCHNIDER, *J. Am. Oil Chem. Soc. 37,447* (1960).
[19] WALTER A. PONS, JR. and VERNON L. FRAMPTON, *J. Am. Oil Chem. Soc. 42,786* (1965).
[20] E. P. CLARK, *Semimicro Quantitative Organic Analysis*, Academic Press, New York (1943).
[21] S. MOORE, D. H. SPACKMAN and W. H. STEIN, *Anal. Chem.* 30, 1185 (1958).
[22] H. D. SPACKMAN, W. H. STEM and S. MOORE, *Anal. Chem.* 30, 1190 (1958).

The hexane defatted meals from the previous investigation[4] and those prepared in this research were extracted with the acetone-hexane-water azeotrope, and the extracts were analyzed for "total" gossypol in accordance with the procedure proposed by Pons et *al.*[23] and the "free" gossypol was determined in accordance with the method of the American Oil Chemists' Society.*[24]

I.r. spectra were determined with a Perkin Elmer Jnfracord, Model 237-B.

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[23] WALTER **A. PONS, JR., R.** A. PITTMAN and C. L. HOFFPAUIR, *J. Am. Oil Chern. Soc. 35, 93* (1958).
[24] *Official and* ***Tentative Methods*** *of Analysis* (2nd ed. rev. to 1964) American Oil Chemists' Society, Chicago (1964).