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CHEMICAL COMPOSITION STUDIES 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 were determined for eleven species of the genus Gossypium. A genus specific pattern was not found for either the amino acids or the fatty acids. The greatest difference among the amino acids was noted for lysine which ranged from 4·12 g/16 g of meal nitrogen for *G. hirsutum* L. to 4·82 for *G. klotzschianum* var. *davidsoni* (Kellogg). A preliminary survey was made of the qualitative differences in the free amino acids in the ethyl alcohol extract of the defatted meals; there was a considerable variability in the chromatographic patterns from the different species. Palmitic acid in the seed oils was observed to vary from about 22 per cent for *G. arboreum* L. to about 35 per cent for *G. aridum* (Rose and Standley) Skovsted; oleic acid from about 16 for *G. aridum* to about 36 for *G. armourianum* Kearney; linoleic acid from about 35 for *G. armourianum* Kearney to about 55 for *G. hirsutum* L. Gossypol was noted to vary from 0 per cent for the glandless variety of *G. hirsutum* L. to more than 9 per cent for *G. klotzschianum* var. *davidsoni* (Kellogg). Statistical analyses of the data indicate that genes from the wild species should be of value in the production of commercial genotypes with improved proteins and oils.

INTRODUCTION

COMPREHENSIVE studies on the taxonomy and genetics of the genus Gossypium have been carried out to provide basic information for cotton breeding programs.[1–5] Recently surveys have been made on the chemical composition of the seeds in different species in the genus. Frampton *et al.*,[6] studied the gossypol content and Ergle *et al.*,[7] reported on the nucleic acid composition. In this paper the amino acids of the seed proteins and the fatty acid pattern of the oil are compared in the different species of the genus. Further data on gossypol content are also reported.

Description of species. Botanists now recognize twenty-three species in Gossypium, four of which are cultivated. In the distant past, the four species were domesticated independently by different tropical peoples, two of the species (*G. arboreum* L. and *G. herbaceum* L.) in the eastern hemisphere and the other two (*G. hirsutum* L. and *G. barbadense* L.) in the western

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hemisphere. The old-world cottons are diploids with thirteen pairs of chromosomes; the American cultivated cottons are tetraploids with twenty-six pairs of chromosomes. Beasley[8] demonstrated that the latter are allotetraploids, which must have originated ages ago through the spontaneous doubling of the chromosomes of a sterile hybrid between diploid Asiatic and diploid wild American species. With the exception of the tetraploid Hawaiian species (*G. tomentosum*) the remaining nineteen species are wild diploids found in zerophytic areas in the tropics of both hemispheres.

On the basis of cytological studies and geographical distribution, Beasley[8] classified the species into six groups, each group with chromosomes that belong in a distinct set or genome. Each diploid genome is represented by letters from A to E, and the allotetraploid by the letters (AD) which symbolize its two diploid genomes. The recognized species within each genome are represented by subscripts (see Table 1).

TABLE 1. SPECIES OF GOSSYPIUM USED IN THE ANALYSES

Genome	Species	Number of samples
A_1	*G. herbaceum* L.	2
A_2	*G. arboreum* L.	1
D_1	*G. thurberi* Todaro	3
D_2-1	*G. armourianum* Kearney	6
D_3-k	*G. klotzschianum* Anderson	8
D_3-d	*G. klotzschianum* var. *davidsoni* (Kellogg)	6
D_4	*G. aridum* (Rose and Standley) Skovsted	6
D_5	*G. raimondii* Ulbrich	5
D_6	*G. gossypioides* (Ulbrich) Standley	6
$(AD)_1$	*G. hirsutum* L. (glandless variety)	1
$(AD)_2$	*G. barbadense* L. (Pima S-2 variety)	1

The present study was made on all four commercial species and on seven wild species. Seeds of the wild cottons came from Iguala, Mexico, and represent the only species with an available seed supply at the time the investigations were initiated. Samples of seeds collected in different years (1955–1962) or from different sources were kept separate and analyzed individually. The samples analyzed are listed in Table 1, in accordance with Saunders[9] who recently revised the descriptions of the wild species given by earlier workers.[2, 5, 10]

RESULTS AND DISCUSSION

Amino acid composition of the meal hydrolyzates. The average values for the component amino acids of the seed protein of the several species (24-hr hydrolyzates) are reported in Table 2. The confidence limits of the individual amino acids, at the 5 per cent level of probability, were calculated from the within-species variance in the species for which five or more samples were analyzed and are listed in Table 2. The overall confidence limits in the individual amino acids, and with odds of 20:1, are of the order of 2 parts per hundred.

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TABLE 2. AMINO ACID COMPOSITION OF THE MEAL HYDROLYZATES

Amino acid	Genome (g/16 g N_2)										Confidence limits
	A_1	A_2	D_1	D_2-1	D_3-k	D_3-d	D_4	D_5	D_6	$(AD)_1$	$(AD)_2$	
Lysine	4·12	4·30	4·69	4·58	4·64	4·82	4·43	4·19	4·32	4·30	4·50	±0·09
Histidine	2·59	2·77	2·64	2·69	2·73	2·71	2·55	2·70	2·67	2·78	2·70	±0·06
Ammonia	2·09	2·13	2·02	1·99	1·99	1·95	2·01	2·07	1·95	2·04	2·02	±0·08
Arginine	11·67	11·54	11·02	11·38	10·64	10·46	10·87	11·90	11·05	12·15	11·83	±0·24
Aspartic acid	9·33	10·04	9·51	9·46	9·11	8·92	9·26	9·63	9·23	9·23	9·38	±0·20
Threonine	3·07	3·20	3·37	3·25	3·43	3·54	3·29	3·28	3·19	3·12	3·36	±0·06
Serine	3·97	4·22	4·31	4·34	4·33	4·35	4·26	4·41	4·28	4·28	4·30	±0·06
Glutamic acid	19·46	21·18	19·27	20·32	19·92	19·06	19·66	20·13	20·43	21·27	20·81	±0·05
Proline	3·78	3·79	3·68	3·89	3·89	3·87	3·85	3·98	3·79	3·84	3·78	±0·07
Glycine	4·10	4·23	4·42	4·42	4·54	4·60	4·22	4·39	4·27	4·18	4·24	±0·09
Alanine	3·94	4·06	4·15	4·05	4·24	4·28	4·14	4·09	3·98	3·82	4·03	±0·06
Valine	4·36	4·72	4·45	4·55	4·52	4·50	4·49	4·60	4·43	4·56	4·56	±0·10
Methionine	1·20	1·28	1·54	1·20	1·33	1·34	1·23	1·21	1·31	1·35	1·65	±0·16
Isoleucine	3·25	3·47	3·40	3·43	3·47	3·56	3·37	3·45	3·42	3·17	3·40	±0·05
Leucine	5·58	5·96	6·07	5·99	6·07	6·18	6·13	6·04	5·90	5·73	5·92	±0·06
Tyrosine	2·97	3·03	2·92	2·99	3·06	3·08	2·96	2·91	2·89	3·15	3·11	±0·06
Phenylalanine	5·24	5·53	5·13	5·56	5·32	5·10	5·12	5·51	5·25	5·46	5·32	±0·11

There are several sources of variance in the determinations that were carried out, the most important being those that arise from the analyses of the hydrolyzates and those that arise from the preparation of the hydrolyzates of the defatted flours for analysis. These two major sources of error for each amino acid were assessed in the analyses of variance (Table 3) for

TABLE 3. ANALYSES OF VARIANCE TO ASSESS SOURCES OF ERROR IN CHEMICAL DETERMINATIONS OF AMINO ACIDS IN SEED PROTEINS OF SPECIES OF GOSSYPIUM

Amino acid	Mean square among analyses[a]	Mean square for between hydrolyses[b]	Mean square for combined analytical errors[c]	Mean square for between species[d]
Lysine	0·0105	0·00066	0·0072	0·167
Histidine	0·0028	0·00066	0·0021	0·005
Ammonia	0·0082	0·00367	0·0067	0·026
Arginine	0·0423	0·01467	0·0331	0·725
Aspartic acid	0·0407	0·00200	0·0278	0·007
Threonine	0·0035	0·00066	0·0026	0·058
Serine	0·0055	0·00333	0·0048	0·010
Glutamic acid	0·1998	0·03566	0·145	0·754
Proline	0·0048	0·02300	0·0109	0·0050
Glycine	0·0005	0·00433	0·0018	0·087
Alanine	0·0017	0·00066	0·0013	0·075
Valine	0·0027	0·00267	0·0027	0·009
Methionine	0·0006	0·07847	0·0266	1·3187
Isoleucine	0·0007	0·00033	0·0006	0·073
Leucine	0·0030	0·00400	0·0033	0·119
Tyrosine	0·0027	0·01866	0·0080	0·0400
Phenylalanine	0·0072	0·00867	0·0077	0·018

Degrees of freedom: [a] 6; [b] 3; [c] 9; and [d] 2.

repeat amino acid determinations of repeat hydrolyses (24 hr) for the three species *G. armourianum*, *G. barbadense*, and *G. hirsutum* (glandless variety). In most instances the mean square for among analyses shown in Table 3 is significantly greater than the mean square for between the preparation of the samples for the amino acid analyzer. The conclusion is that the analytical error due to the automatic analyzer is greater than that due to the preparation (hydrolyses, etc.) of the samples from the defatted flour.

The mean squares for the combined analytical errors and for between species are also included in Table 3. The mean square for between species for each of the amino acids lysine, arginine, threonine, glutamic acid, glycine, alanine, methionine, isoleucine, and tyrosine is significantly greater than the mean square for the combined errors attributable to the chemical

TABLE 4. ANALYSES OF VARIANCE TO DETERMINE EFFECTS OF TIME OF HYDROLYSIS ON LEVELS OF AMINO ACIDS IN HYDROLYZATES

	Average values, g/16 g N, for hydrolyses of		Mean square for		
	24 hr	72 hr	within sample variance[a]	between 24 and 72 hr[b]	F ratio
Lysine	4·47	4·43	0·0477	0·0076	0·16*
Histidine	2·71	2·67	0·0055	0·0062	1·13*
Ammonia	2·04	2·18	0·0052	0·1092	21·10
Arginine	11·40	11·05	0·2468	0·6563	2·66*
Aspartic acid	9·46	9·34	0·0861	0·0756	0 88*
Threonine	3·30	2·96	0·0203	0·6426	31·71
Serine	4·32	3·30	0·0170	5·7017	335·89
Glutamic acid	20·45	19·87	0·5440	1·8793	3·45*
Proline	3·86	3·84	0·0086	0·0035	0·41*
Glycine	4·37	4·38	0·0321	0·0001	0·003*
Alanine	4·11	4·09	0·0246	0·0036	0·15*
Valine	4·61	4·86	0·0120	0·3288	27·46
Methionine	1·36	1·30	0·5680	0·0186	0·03*
Isoleucine	3·42	3·60	0·0126	0·1640	13·02
Leucine	6·02	6·00	0·0307	0·0007	0·02*
Tyrosine	3·03	2·92	0·0062	0·0632	10·23
Phenylalanine	5·42	5·32	0·0224	0 0482	2·15*

[a] 20 degrees of freedom; [b] 1 degree of freedom; * not significant.

analyses. It is concluded that these three species of Gossypium differ among themselves in amino acid patterns of their seed proteins.

It is well known that errors in amino acid analyses accrue because of conditions under which the hydrolyses of the proteins are carried out. One of the most important of these is the duration of the hydrolysis. The contribution of this factor to the chemical analysis for each amino acid in the seed proteins is assessed in the analyses of variance shown in Table 4, where the data were obtained for periods of hydrolysis of 24 and 72 hr. There are 20 degrees of freedom for the analyses of the eleven species, and 1 degree of freedom for between periods of hydrolysis. The data show that the time of hydrolysis influenced the levels of ammonia, threonine, serine, valine, isoleucine, and tyrosine, but it had no influence on lysine, histidine, arginine, aspartic acid, glutamic acid. proline, glycine, alanine, methionine, leucine, or

phenylalanine. Evidently there was some destruction of threonine, serine, and tyrosine, and apparently the increase in ammonia can be attributed to this loss. The increase in valine and isoleucine is due to a more complete hydrolysis of peptides containing these two amino acids at 72 hr than at 24 hr. The levels reported in Table 2 for the five amino acids, threonine, serine, tyrosine, valine, and isoleucine, in cottonseed flour from the eleven species, therefore, are tentative, as they are the values obtained from the 24-hr hydrolyzates.

The effect of age of seed on the amino acid patterns was ascertained from the correlation between the concentration of several amino acids in the seed protein and age of seed. The amino acids selected for the determinations were lysine, histidine, and arginine, and the level of ammonia. Seeds from six species (genomes D_2-1, D_3-k, D_3-d, D_4, D_5, and D_6), for which five or more samples had been collected over a period of years, were used for the analyses. The average of the coefficients of correlation between age of seed and lysine for the six species was −0·34. The average correlation coefficient was −0·49 for histidine, −0·17 for arginine, and −0·03 for ammonia. These coefficients are not significant, and it is concluded that the age of the seed did not influence the amino acid patterns of the seed proteins. The amino acid data can therefore be used in a multiple range test analysis to group the several species in terms of the amino acid composition of the seed proteins.

Duncan's multiple range test is shown in Table 5, where the least significant difference was calculated for the 5 per cent level where the shortest significant range was determined from data where five or more samples were available. It is obvious that the amino acid patterns of the seed proteins vary from species to species. The data for $(AD)_1$ are in line with those determined in analyses of many samples from this species.

The concentration of lysine in the meal proteins is a factor that limits the usefulness of the meals as protein supplements for non-ruminants. It is noted that the level of lysine in the seed proteins of *G. klotzschianum* var. *davidsoni* is about 20 per cent greater than that noted for *G. hirsutum*. It may be noted, also, that *G. klotzschianum* var. *davidsoni* seed proteins also rank high in the essential amino acids (for man) threonine, leucine, isoleucine, and tyrosine, and moderately high in valine and phenylalanine. In comparison, *G. hirsutum* ranks low in lysine, threonine, leucine, and isoleucine and high in valine and phenylalanine.

Amino acids of alcohol extracts of defatted meals. The chromatogram patterns of the free amino acids and peptides extracted from the meals with 80% aq. ethyl alcohol differed significantly from the patterns of the amino acids found in the HCl hydrolyzates of the meal proteins. A comparison of the chromatograms for the basic amino acids (15-cm column) showed that the peak areas for lysine and histidine were relatively small for all of the species, and that the peaks for ammonia, arginine and three unidentified constituents varied considerably from species to species.

The peak for arginine was most prominent on the chromatograms for *G. hirsutum* and *G. barbadense* and least prominent for *G. aridum* and *G. klotzschianum*. The peak for the unidentified constituent which is eluted just prior to lysine was the tallest peak on the chromatogram for *G. arboreum* and was also prominent for *G. armourianum*. This unidentified constituent was also noted in all species.

An additional unidentified constituent produced a shoulder on the afterside of the lysine peak. Only in the case of *G. arboreum* was this peak larger than those for lysine and histidine. It was noted on the chromatogram for *G. raimondii* and appeared only as a trace for the remaining species. An additional unidentified constituent was eluted just before ammonia and was not completely resolved from the ammonia peak. This constituent was prominent

TABLE 5. SEPARATION OF AMINO ACID LEVEL OF EACH SPECIES BY DUNCAN MULTIPLE RANGE TEST*

Lysine		Histidine		Ammonia		Arginine		Aspartic acid		Threonine	
Genome	Range	Genome	Range	Genome	Range	Genome	Range	Genome	Range	Genome	Range
D_3-d	a	$(AD)_1$	a	A_2	a	$(AD)_1$	a	A_2	a	D_3-d	a
D_1	b	A_2	a	A_1	a	D_5	a	D_5	b	D_3-k	b
D_3-k	b	D_3-k	a	D_5	a	$(AD)_2$	a	D_1	b	D_1	b
D_2-1	bc	D_3-d	a	$(AD)_1$	a	A_1	ab	D_2-1	bc	$(AD)_2$	b
$(AD)_2$	c	$(AD)_2$	ab	D_1	ab	A_2	ab	$(AD)_2$	bc	D_4	bc
D_4	c	D_5	ab	$(AD)_2$	ab	D_2-1	b	A_1	bc	D_5	bc
D_6	cd	D_2-1	ab	D_4	ab	D_6	c	D_4	bc	D_2-1	c
A_2	d	D_6	ab	D_2-1	ab	D_1	c	D_6	c	A_2	c
$(AD)_1$	d	D_1	ab	D_3-k	ab	D_4	c	$(AD)_1$	c	D_6	c
D_5	d	A_1	b	D_3-d	ab	D_3-k	cd	D_3-k	c	$(AD)_1$	cd
A_1	de	D_4	bc	D_6	ab	D_3-d	d	D_3-d	cd	A_1	d
4·82–4·12		2·78–2·55		2·13–1·95		12·15–10·46		10·04–8·92		3·54–3·07	

Serine		Glutamic acid		Proline		Glycine		Alanine		Valine	
Genome	Range	Genome	Range	Genome	Range	Genome	Range	Genome	Range	Genome	Range
D_5	a	$(AD)_1$	a	D_5	a	D_3-d	a	D_3-d	a	A_2	a
D_3-d	a	A_2	a	D_2-1	a	D_3-k	a	D_3-k	a	D_5	a
D_2-1	a	$(AD)_2$	a	D_3-k	a	D_2-1	ab	D_1	b	$(AD)_2$	a
D_3-k	a	D_6	ab	D_3-d	ab	D_1	ab	D_4	b	$(AD)_1$	a
D_1	ab	D_2-1	ab	D_4	ab	D_5	b	D_5	b	D_2-1	a
$(AD)_2$	ab	D_5	ab	$(AD)_1$	ab	D_6	b	A_2	bc	D_3-k	ab
D_6	ab	D_3-k	ab	D_6	b	$(AD)_2$	bc	D_2-1	bc	D_3-d	ab
$(AD)_1$	ab	D_4	b	A_2	b	A_2	bc	$(AD)_2$	bc	D_4	ab
D_4	ab	A_1	b	A_1	b	D_4	bc	D_6	c	D_1	ab
A_2	b	D_1	b	$(AD)_2$	b	$(AD)_1$	bc	A_1	c	D_6	ab
A_1	c	D_3-d	b	D_1	c	A_1	c	$(AD)_1$	d	A_1	b
4·41–3·97		21·27–19·06		3·98–3·68		4·60–4·10		4·28–3·82		4·72–4·36	

Methionine		Isoleucine		Leucine		Tyrosine		Phenylalanine	
Genome	Range	Genome	Range	Genome	Range	Genome	Range	Genome	Range
$(AD)_2$	a	D_3-d	a	D_3-d	a	$(AD)_1$	a	D_2-1	a
D_1	a	D_3-k	b	D_4	a	$(AD)_2$	a	A_2	a
$(AD)_1$	ab	A_2	b	D_3-k	ab	D_3-d	a	D_5	a
D_3-d	ab	D_5	b	D_1	ab	D_3-k	ab	$(AD)_1$	a
D_3-k	ab	D_2-1	b	D_5	b	A_2	ab	D_3-k	ab
D_6	b	D_6	b	D_2-1	b	D_2-1	b	$(AD)_2$	ab
A_2	b	D_1	bc	A_2	b	A_1	b	D_6	b
D_4	b	$(AD)_2$	bc	$(AD)_2$	b	D_4	b	A_1	b
D_5	b	D_4	c	D_6	bc	D_1	b	D_1	b
D_2-1	b	A_1	c	$(AD)_1$	d	D_5	b	D_4	bc
A_1	b	$(AD)_1$	d	A_1	e	D_6	b	D_3-d	bc
1·65–1·20		3·56–3·17		6·18–5·58		3·15–2·89		5·56–5·10	

* a represents highest and e lowest of the ranges.

in *G. thurberi*, *G. aridum*, *G. gossypioides*, and *G. klotzschianum*. The peaks due to ammonia and this unidentified constituent appeared to be the same size in the chromatograms from *G. arboreum*, *G. thurberi*, and *G. davidsoni*.

Very large peaks at the elution time for aspartic acid, serine, and glutamic acid were obtained on the chromatograms from the 150-cm column analyses. The serine peak undoubtedly contained the amides glutamine and asparagine, which are converted to glutamic acid and aspartic acid during acid hydrolysis and thus do not appear in the acid hydrolyzates. For most of the species peaks of significant size were obtained for glycine and alanine, but in general, the peaks for the other amino acids normally found in acid hydrolyzates of proteins, were relatively small. Six peaks for unidentified constituents appeared on the chromatograms prior to the elution of aspartic acid. Some of the pigment in the extracts was eluted in this early portion of the chromatogram and undoubtedly exerted considerable effect on the shape and elution time of some of these peaks. For about one-half of the chromatograms, an asymmetric and double peak occurred in the position of methionine sulfoxide, just prior to the aspartic acid peak. On most of the chromatograms it was of significant size.

A small peak following leucine appeared in chromatograms for *G. armourianum*, *G. klotzschianum*, *G. raimondii*, and *G. davidsoni*. In a few cases, a peak for methionine was not apparent.

Gossypol determinations. Gossypol analyses were also carried out with several samples from the eleven species. The data are reported in Table 6. There is a very large range in the quantities of gossypol that occur in these different species, and the data are in good agreement with the earlier report by Frampton, Pons and Kerr.[6] The levels of gossypol in the samples were not altered with age of seed as is obvious from the data reported in Table 6.

It is interesting that the level of total gossypol in the seeds is correlated significantly with lysine. A coefficient of correlation of 0·71 was calculated (*G. hirsutum* datum was omitted because this glandless variety is an artifact). This coefficient for 9 degrees of freedom is significant at the 2 per cent level of probability.

Fatty acid composition. The average values for the component fatty acids in the seed oils are reported in Table 7. It may be noted that the GLC analyses did not differentiate malvalic acid or other cyclopropene acids from other unsaturated acids.

Analysis of variance of repeat chromatographic determinations of methyl esters prepared from eighteen different cottonseed oils indicate that completely reliable results may be expected on duplicate GLC analyses of a given ester. The sum of squares for among esters was found to be 40·35, with 34 degrees of freedom, while that for between GLC determinations 27·91 with 1 degree of freedom. Moreover, extensive experience in this laboratory with the GLC has established a coefficient of variation of the order of 1–2 per cent for fatty acid analyses and a precision in analyses with mixtures of methyl esters of known composition approaching that obtainable in conventional spectrophotometric determinations.

As noted earlier, the seeds used were collected in different years (1955–1962). The influence of age of the seed on the fatty acid pattern of the seed oils was investigated by determining the coefficients of correlation between the concentration of the several fatty acids and age of seed. The coefficients of correlation were not found to be significant, and there is no evidence that the age of the seed used in these experiments influenced the fatty acid patterns of the seed oil. The average values of the coefficients for correlation of fatty acids with age of seed were for myristic, −0·1; for palmitic, +0·2; for palmitoleic, −0·4; for stearic, −0·3; for oleic, −0·4 and for linoleic, 0·3.

Accordingly, Duncan's multiple range test for the component fatty acids for the different

TABLE 6. COMPARISON OF PERCENT FREE AND TOTAL GOSSYPOL CONTENT

Species and year	Free	Total
G. herbaccum		
1962	0·73	1·06
1959	0·81	1·33
G. arboreum		
. .	0·62	0·81
G. thurberi		
. . .	3·21	3·77
1957	3·73	4·22
1957	3·81	4·15
G. armourianum		
1962	1·87	2·29
1962	2·41	2·85
1961	1·95	2·38
1958	1·83	2·31
1959	2·04	2·49
1959	1·97	2·71
G. klotzschianum		
1962	6·71	7·88
1961	7·09	8·19
1961	6·59	7·14
1960	6·91	8·50
1959	6·13	7·19
1959	6·82	7·64
1959	6·61	7·45
1959	7·23	8·55
G. klotzschianum var. *davidsoni*		
1962	8·26	9·70
1960	9·03	10·45
1959	7·57	8·73
G. klotzschianum var. *davidsoni*—continued		
1958	7·46	8·67
1956	7·59	8·72
1955	9·30	10·30
G. aridum		
1962	4·63	5·23
1962	4·28	4·98
1961	5·46	6·38
1960	4·53	5·19
1958	4·76	5·32
1957	4·32	4·87
G. raimondii		
1962	2·85	3·15
1960	3·08	3·47
1960	3·28	3·59
1960	3·45	3·89
1959	2·74	3·25
G. gossypioides		
1962	0·70	0·76
1961	0·72	0·72
1959	0·67	0·73
1958	0·69	0·74
1956	0·73	0·77
1955	0·67	0·71
G. hirsutum (glandless)		
. . .	0·0	0·0
G. barbadense		
. . .	1·28	1·80

TABLE 7. RELATIVE CONCENTRATIONS (AVERAGE) OF COMPONENT FATTY ACIDS IN SEED OILS OF SPECIES OF THE GENUS GOSSYPIUM

	Component fatty acids*					
Genome	Myristic (%)	Palmitic (%)	Palmitoleic (%)	Stearic (%)	Oleic (%)	Linoleic (%)
A_1	0·9	23·3	1·7	3·6	19·3	51·4
A_2	0·4	21·8	1·8	4·2	32·4	40·8
D_1	0·6	26·7	0·9	2·7	17·6	51·6
D_2-1	0·3	24·4	0·7	3·2	36·0	35·4
D_3-k	0·9	26·5	1·8	3·0	19·5	48·3
D_3-d	0·5	25·8	1·3	3·0	20·6	48·9
D_4	0·9	34·5	1·0	3·1	16·0	44·5
D_5	0·7	24·4	1·2	3·4	23·5	46·9
D_6	0·6	25·1	0·5	3·7	17·2	52·7
$(AD)_1$	0·7	24·3	1·2	2·9	16·5	54·5
$(AD)_2$	1·0	23·6	0·8	2·9	21·2	50·7

* As the methyl esters.

genomes was carried out, and the data are reported in Table 8. The least significant difference was calculated for the 5 per cent level of probability.

Rather dramatic differences between some of the species are noted in the fatty acid patterns. For example, the linoleic acid concentration ranges from about 35 for *G. armourianum* to about 54 per cent for *G. hirsutum*, while that for oleic acid varies from about 16 for *G. hirsutum* to about 36 per cent for *G. armourianum*. It is of further interest to note that the sum of the oleic and linoleic acids in the seed oils of all of the genomes, with the exception of D_4, is about 70 per cent. This observation suggests that a fundamental interrelationship may exist between the two acids in their biosynthesis.

The seed oil of the genome D_4 is of added interest, since the palmitic acid level is out of line with that noted for the remaining species. The value noted for this genome is about 35 per cent, while the values for the other species range from about 23 to 26 per cent. This higher level of palmitic acid was noted in all six of the different seed samples which were

TABLE 8. SEPARATION OF THE FATTY ACIDS OF THE SEED OILS OF EACH SPECIES BY THE DUNCAN MULTIPLE RANGE TEST*

Myristic acid		Palmitic acid		Palmitoleic		Stearic acid		Oleic acid		Linoleic acid	
Genome	Range	Genome	Range	Genome	Range	Genome	Range	Genome	Range	Genome	Range
$(AD)_2$	a	D_4	a	D_3-k	a	A_2	a	D_2-1	a	$(AD)_1$	a
D_3-k	a	D_1	b	A_2	a	D_6	a	A_2	a	D_6	a
D_4	a	D_3-k	b	A_1	a	A_1	ab	D_5	b	D_1	a
A_1	a	D_3-d	b	D_3-d	b	D_5	b	$(AD)_2$	b	A_1	a
$(AD)_1$	ab	D_6	bc	D_5	b	D_2-1	b	D_3-d	bc	$(AD)_2$	a
D_5	ab	D_1-1	c	$(AD)_1$	b	D_4	b	D_3-k	bc	D_3-d	ab
D_1	b	D_5	c	D_4	bc	D_3-k	b	A_1	bc	D_3-k	ab
D_6	b	$(AD)_1$	c	D_1	bc	D_3-d	b	D_1	c	D_5	b
D_3-d	b	$(AD)_2$	c	$(AD)_2$	c	$(AD)_1$	b	D_6	c	D_4	b
A_2	b	A_1	c	D_2-1	c	$(AD)_2$	b	$(AD)_1$	c	A_2	c
D_2-1	bc	A_2	d	D_6	c	D_1	b	D_4	c	D_2-1	d
1·0%–0·3%		34·5%–21·8%		1·8%–0·5%		4·2%–2·7%		36·0%–16·0%		54·5%–35·4%	

* See Table 5.

collected over a period of 5 years. This seed oil should have some interesting physical properties, especially in its melting behavior.

Evidently there is no fatty acid pattern that is characteristic of the genus, and there should exist the possibility that the composition of the seed oils of commercial cottonseed may be modified genetically to emphasize characteristics that are of interest to end users of processed cottonseed oil.*

EXPERIMENTAL

Preparation of samples. The seeds were weighed, cracked in a Bauer mill,† and then passed through a series of screens to facilitate the separation of kernel and hull particles.

* Duncan's multiple range test carried out with data published by Stansbury, Hoffpauir and Hopper[11] in their studies of the influence of variety and environment on cottonseed quality, indicated no difference in the iodine value of oils among eight varieties studied. The varieties studied were Acala 4-42, Acala 1517W, Rowden 41B, Stoneville 2B, Deltapine 15, Coker 100W, Coker Wilds 18 and Mebane.

† It is not the policy of the Department to recommend the products of one company over those of any others engaged in the same business.

[11] M. F. STANSBURY, C. L. HOFFPAUIR and T. H. HOPPER, *J. Am. Oil Chemists' Soc.* 30, 120 (1953).

The hulls were discarded. Since some of the seeds were very small, considerable difficulty was encountered in the attempt to obtain quantitative separation of kernels and hulls.

The decorticated samples were ground and extracted at room temperature with a low boiling fraction (< 50°) of purified and redistilled petroleum ether. The solvent was removed from the oil at a temperature under 40° with the aid of a stream of nitrogen which was passed through the oil. The oil was stored under nitrogen in the deep freeze. Similarly, the extracted meals were air dried, ground in a Wiley mill to pass through a 40-mesh screen, and stored under deep-freeze conditions.

Amino acid analysis. Meal samples (250–500 mg) were hydrolyzed with 200 ml of constant-boiling glass-distilled HCl for 24 and 72 hr. The amino acids were determined[12] with an automatic analyzer constructed essentially as described by Spackman, Stein, and Moore,[13] or with a Phoenix Precision Amino Acid Analyzer. The results were calculated on the basis of grams of amino acid per 16 g of meal nitrogen. Nitrogen contents of the hydrolyzates were determined by a modification of a micro-Kjeldahl method.[14]

A preliminary survey of qualitative differences in the free amino acids extracted from the meals with 80% aq. ethyl alcohol was made. Fifteen and 150 cm ion-exchange columns were used under the same conditions employed for the hydrolyzates described above. Only one sample of each species was used in this comparison. One-gram samples of the meals were extracted batchwise with the aq. alcohol to give approximately 180 ml of extract. The extracts were concentrated under reduced pressure in a rotary evaporator at temperatures which did not exceed 35°. The concentrates were then made up to 25 ml with pH 2·2 buffer and 2-ml aliquots were applied to the columns in the amino acid analyzer for analysis.

Gossypol. "Free" gossypol in the meals was determined by the official method of the American Oil Chemists' Society.[15] The "total" gossypol was determined by the procedure described by Pons, Pittman, and Hoffpauir.[16]

Fatty acid determinations. Transesterification of the glycerides in the oils to their methyl esters was carried out according to the procedure of Luddy *et al.*[17] The methyl esters were found to be suitable for chromatography without further purification. The determinations were made with a Beckman Gas Chromatograph 2A equipped with a thermal conductivity detector and a 6-ft copper column, $\frac{1}{4}$ in. O.D. The column was packed with Gas Chrom A, 80–100 mesh, which was mixed with diethylene glycol succinate polyester in a ratio of 4:1. The chromatographs were developed at 182° with helium, under a pressure head of 30 lb, as the carrier gas. The detector current was maintained at 250 mA, and the inlet port temperature maintained at 325°. The GLC data were corrected in accordance with the procedure reported by Pons and Frampton.[18]

[12] S. MOORE, D. H. SPACKMAN and H. STEIN, *Anal. Chem.* **30**, 1185 (1958).
[13] D. H. SPACKMAN, W. H. STEIN and S. MOORE, *Anal. Chem.* **30**, 1190 (1958).
[14] E. P. CLARK, *Semimicro-quantitative Organic Analysis.* Academic Press, New York (1943).
[15] *Official and Tentative Methods of Analysis* (2nd Ed., rev. to 1964). American Oil Chemists' Society, Chicago (1964).
[16] W. A. PONS, JR., R. A. PITTMAN and C. L. HOFFPAUIR, *J. Am. Oil Chemists' Soc.* **35**, 93 (1958).
[17] F. E. LUDDY, R. A. BARFORD and R. W. RIEMENSCHNEIDER, *J. Am. Oil Chemists' Soc.* **37**, 447 (1960).
[18] W. A. PONS, JR. and V. L. FRAMPTON, *J. Am. Oil Chemists' Soc.* **42**, 786 (1965).