THE GENETICS OF COTTON.

PART VII. "CRUMPLED": A NEW DOMINANT IN ASIATIC COTTONS PRODUCED BY COMPLEMENTARY FACTORS.

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(With Plates VIII and IX and One Text-figure.)

CONTENTS.

Introduction.

The object of this paper is to discuss the genetic behaviour of an abnormal sterile or semi-sterile type ("crumpled") which originated in the progeny of a cross between the wild cotton of the Sudan (*G. Nanking* var. *soudanensis*[1]), and a strain of *G. arboreum* var. *sanguinea*. Since cases of interspecific sterility capable of exact genetic analysis are seldom encountered (cf. Hollingshead, 1930), the genetic behaviour of "crumpled" has been investigated in considerable detail, and it will be shown that this type of sterility can be interpreted in terms of genetic factors, the distribution of which differs from strain to strain in Asiatic cottons.

Description of "crumpled."

"Crumpled" appeared first in a cross between a plant of the Sudan cotton known as "Abu Hareira" and a variety of *G. arboreum* var. *sanguinea*. The F_1 was normal, but in a back-cross to a closely related *sanguinea*, half the plants were "crumpled." The intensity of the development of the "crumpled" character varied very greatly from plant to plant. The most normal-looking "crumpled" were characterised by

[1] Specific and varietal names are as far as possible those given by Watt (1907).

irregular development of the vegetative buds, and young leaves, causing abortion of many shoots and distortion, crimping and brittleness of the leaves. A large proportion of the buds which would normally have remained dormant developed into short, distorted shoots, giving a "witch broom" appearance to the plant (see Fig. 1). A few of the most normal crumpled produced three or four flowers per plant. The buds were small, and the bracts and petals so disproportionately short that the stigmas were exposed in the bud stage. Nevertheless a few bolls were set and produced mature seed.

Certain other crosses which it was shown should give rogues in F_1 failed to give viable seed, the embryo being more or less shrivelled or completely aborted. Between these extremes a complete range of intermediates was obtained, a representative series of which is shown in Plate VIII, Fig. 5.

Previous work.

Harland (1915), working on New World cottons, stated that the F_1 of the cross Sea Island × St Croix Native (*G. barbadense* L. × *G. purpurascens* Poir.) was characterised by a peculiar stunted appearance due to excessively short internodes, and by a scaly, corky covering of the stems. The leaf colour was green, but with dots and splashes of lighter yellowish green irregularly distributed over the surface, giving the leaf a variegated appearance.

In F_2 some plants showed the F_1 habit and leaf variegation in a more extreme form, and complete sterility was often present.

Experimental work.

A single plant (A.H. 1–9) of Abu Hareira obtained from the Sudan was crossed with two plants (G.S. 1 and G.S. 2) of a strain of *G. arboreum* var. *sanguinea* obtained from India. G.S. 2 × A.H. 1–9 was planted, and 17 normal F_1 plants were grown. G.S. 1 × A.H. 1–9 was kept as a reserve, but was planted later when it became of interest to know the constitution of G.S. 1. Germination was poor, but 4 seedlings were obtained, and these were sterile "crumpled."

A single F_1 plant of G.S. 2 × A.H. 1–9 was back-crossed to G.S. 1. Of 90 plants grown in the back-cross 46 were "crumpled" and 44 normals. Germination was good and very few seedlings died. The seedlings were transplanted into 6 in. flower pots and looked after as carefully as possible. The normals all grew well and were subsequently planted out in the field. The "crumpled" plants showed varying degrees of abnor-

mality (see Plate VIII, Fig. 2), some shedding all their leaves early and remaining for weeks in a moribund condition, with a few weak almost leafless side shoots. Others held their leaves, and a few even produced a few flowers. Seven "crumpled" plants ripened one boll each.

Fig. 1. *Sanguinea* type of "crumpled" from F_1 of A.H. 1–9 × N 8.

Seven normal plants were selected from the back-cross, and back-crossed again to A.H. 1–9. The seeds obtained were planted in small pots, but owing to a rush of work at the time, they could not be given very close attention. There was consequently a considerable mortality

before the seedlings were big enough to classify. The results are given in Table I.

TABLE I.

Results of back-crossing normal segregates of (G.S. 2 × A.H. 1–9) 9 × G.S. 1 to A.H. 1–9.

Parent plant	Crumpled	Normals	Total
239	22	38	60
240	11	7	18
247	6	4	10
250	13	9	22
252	29	36	65
267	15	23	38
272	11	18	29
Total	107	135	242

All plants behaved alike and gave in all 107 "crumpled" and 135 normals. The deviation from equal numbers is probably due to excessive mortality in the "crumpled" class.

The seed obtained from "crumpled" selfed was planted in pots and looked after very carefully. Apart from obviously immature seeds, germination was complete and all seedlings were raised successfully until they were large enough to classify.

The results are given in Table II.

TABLE II.

Progeny of back-cross "crumpled" selfed.

Parent plant	Crumpled	Normals	Total
283	1	1	2
285	4	4	8
293	4	3	7
298	7	3	10
303	6	3	9
315	1	4	5
327	7	4	11
Total	30	22	52
Expected (on 9 : 7 ratio)	*29·2*	*22·8*	*52·0*

The individual families are very small, but they agree well together, and on the totals give a very close approximation to 9 "crumpled" : 7 normal.

These results fit very well the theory of two complementary factors A and B, producing "crumpled" when they come together. The constitution of the parents is then:

A.H. 1–9 ... ... **AAbb**
G.S. 1 ... ... **aaBB**
G.S. 2 ... ... **aabb**

and the constitution of the filial generations:

F_1:	G.S. 2 × A.H. 1–9	**Aabb** (normal)
	G.S. 1 × A.H. 1–9	**AaBb** ("crumpled")
Back-crosses:		
	(G.S. 2 × A.H. 1–9) 9 × G.S. 1 ...	1 **AaBb** "crumpled": 1 **aaBb** (normal)
	(**aaBb** plants) × A.H. 1–9 ...	1 **AaBb** "crumpled": 1 **Aabb** (normal)
First back-cross "crumpled" selfed:		9 **AB** "crumpled": 7 (**Ab** + **aB** + **ab**) (normals)

In the progeny of "crumpled" selfed were a number of "crumpled" much more nearly normal in appearance than any of their parents. None were more abnormal than the most abnormal of the original progeny, even though homozygous "crumpled" should occur among them. This was no doubt the result of very rigorous selection in a highly heterozygous stock, since only the seven least deranged plants produced seed. In Plate IX, fig. 3 is a photograph of the most normal-looking "crumpled" dried and amounted. Eleven of these produced seed, which was planted in small pots and grown until large enough to classify.

The results are given in Table III.

TABLE III.

Progeny of second generation crumpled selfed.

Parent plant	Crumpled	Normals	Casualties	Total
457	3	4	0	7
464	4	8	5	17
474	22	20	13	55
478	6	7	3	16
479	9	8	5	22
483	17	11	0	28
496	5	5	0	10
497	2	3	7	12
501	5	4	3	12
503	5	1	0	6
506	4	5	3	12
Total	82	76	39	197
Expected (on 9 : 7 ratio)	*88·9*	*69·1*	—	*158*

The individual families are again very small, but they form a homogeneous group giving approximately 9 "crumpled" : 7 normals. All the "crumpled" which produced seed, therefore, must have been double heterozygotes (**AaBb**).

Casualties include seeds which failed to germinate and plants which died in the cotyledon stage. The defect of "crumpled" is no doubt due to higher casualty rate among "crumpled" than among normals. The four families in which there were no casualties gave 30 crumpled : 21 normal, rather more than 9 : 7.

Since there were only 30 "crumpled" in the F_3, of which 11 produced seed, and since only 4/9 of the "crumpled" should be double heterozygotes, probably almost all the double heterozygotes produced

seed. In the back-cross family, in which all "crumpled" were double heterozygotes, only 7 out of 46 "crumpled" produced seed. A single generation of selection has therefore had an enormous effect on the fertility of the double heterozygote.

Twenty-seven other types of Asiatic cotton have been crossed with Abu Hareira. The results of four crosses representative of the types of behaviour observed are described in detail below, and the rest are summarised.

A form of *G. arboreum* var. *neglecta* known as Cawnpore White gave an F_1 of 17 plants with A.H. 1–9, all "crumpled." These "crumpled" plants were much more abnormal than those from the G.S. crosses, the cotyledons were thick and somewhat abnormal. Plant 6 in Plate VIII, fig. 5 is a good example of this type of "crumpled."

A form of *G. herbaceum* received from Turkestan under the number N 289 gave only normal F_1 seedlings in crosses with both Abu Hareira and Cawnpore White, and so cannot carry either of the factors for "crumpled."

Six plants of the wild *G. obtusifolia* var. *africana* grown from seed obtained from Rustenberg, South Africa, were crossed with A.H. 1–9. Plants 2, 3 and 4, gave "crumpled" progeny, some of which grew 4 ft. high, but were very abnormal and entirely sterile. Plants 1, 5 and 6, gave seeds which failed to germinate.

A form of *G. obtusifolium* var. *Wightiana* known as 1027 when crossed with A.H. 1–9 gave seeds which were normal in external appearance, but were invariably empty. The cross was made both ways, and about 100 seed coats were cut open and all were empty.

In order to find out whether the "crumpled" factors were the cause of the empty seeds obtained from 1027 × A.H. 1–9, an F_1 plant of Cawnpore White × 1027 was crossed with A.H. 1–9. One hundred and twenty-nine seeds were obtained, 62 of these were cut open and the embryos examined. Thirty-six embryos appeared normal and filled the seed coats. Twenty-three were more or less shrunken, failing to fill the seed coat, and 3 were empty as in 1027 × A.H. 1–9. There was no sharp demarcation into groups, but a continuous gradation from normal to empty seeds. The remaining 67 seeds were germinated on moist blotting paper. Fifty-four germinated and 13 rotted. Those which germinated were planted out in small pots. A few failed to expand the cotyledons. About half of those that pushed off the seed coat had markedly abnormal cotyledons (see Plate VIII, fig. 5, plant 8) and died without the plumule showing any signs of further development. Twenty-

three plants had normal cotyledons and some of these continued to grow for several weeks. All were "crumpled" but they varied enormously in degree of abnormality. Eight specimens typical of the range of types found were pressed and mounted, and a photograph is reproduced in Plate VIII, fig. 5. Plant 1 was the most normal-looking in the family, and showed no signs of the crumpled character until the fourth leaf had expanded.

Four F_1 plants of *G. obtusifolium* var. *africana* (plant 2) × 1027 were pollinated with A.H. 1–9. The seeds were germinated on blotting paper and planted out in boxes.

Results are given in Table IV.

TABLE IV.

Progeny of (G. obtusifolium *var.* africana × 1027) × *A.H.* 1–9.

F_1 plant	No. of seeds obtained	Failed to germinate on blotting paper	Abnormal cotyledons	Normal cotyledons
2	204	106	98	—
3	193	184	8	1*
4	205	168	36	1*
5	258	151	70	37

* Accidental "selfs."

From F_1 plants 3 and 4 only a very small proportion of the seeds obtained germinated. With the exception of one accidental normal "self" in each family, all the seedlings obtained had abnormal cotyledons, and died without further development. From F_1 plant 2 nearly half the seeds obtained germinated, but all had abnormal cotyledons and died in a few days. From F_1 plant 5 about 40 per cent. of the seeds obtained germinated, and of these 70 were of the abnormal cotyledon type and soon died, and 37 had normal cotyledons, and developed into typical "crumpled" such as were obtained from *G. obtusifolium* var. *africana* × A.H. 1–9.

The wild lintless cotton *G. Stocksii* was crossed with three F_1 plants of G.S. 2 × A.H. 1–9. The seeds obtained were soaked and germinated on blotting paper and planted out in small pots. In each family approximately half the seedlings obtained were crumpled with abnormal cotyledons, and failed to develop any further.

The direct cross *G. Stocksii* × A.H. 1–9 gave 9 seedlings. All were extremely crumpled with abnormal cotyledons, and died without further development.

There were two distinct types of Abu Hareira obtained from the Sudan. Several plants of each strain, grown from the seed received from

the Sudan were crossed by G.S. 1, and all gave crumpled in F_1, so that both strains must be homozygous for factor **A**. No other strains carrying **A** have yet been found.

The following is a complete list of the Asiatic Gossypiums which have been tested for crumpled factors and their constitution:

Species	Variety	Type	Constitution	Remarks on crumpled obtained
G. arboreum	var. *sanguinea*	G.S. 1	aaBB	See text
		G.S. 2	aabb	
		A 6	aaBB	Cotyledons normal
	neglecta	A 10	aaBB	Cotyledons extremely abnormal
		A 11	aabb	
		Bengal 5	aaBB	Cotyledons slightly abnormal
		Bengal Khaki	aabb	
		Burma Laciniated	aaBB	Cotyledons extremely abnormal
	rosea	Cawnpore White	aaBB	See text
	assamica	Cernuum	aabb	
G. Nanking		Million Dollar (two strains)	aaBB aabb	Empty seeds as in *obtusifolium* crosses
		Burma Spotless	aabb	
		5 F	aabb	
		N 14	aaBB	Cotyledons normal
		Kiangyin Black	aaBB	Cotyledons extremely abnormal
		Yenching Fuzzy	aabb	
		Chinese Naked Seeded	aaBB	Cotyledons abnormal
	rubicunda	N 8	aaBB	Cotyledons normal
	soudanensis	A.H. 1–9	AAbb	See text
		A.H. 1–6	AAbb	
G. herbaceum		N 289	aabb	
		H 8	aabb	
		H 9	aabb	
		H 10	aabb	
		H 12	aaBB	Empty seeds as in *obtusifolium* crosses
		H 13	aaBB	
G. obtusifolium	*Wightiana*	1027	aaBB	Empty seeds
		Wagad	aaBB	Embryos shrivelled
		Gambia	aaBB	Empty seeds
	africana	Rustenberg	aaBB	See text

Nearly every strain tested has been self-pollinated for several generations, and this no doubt accounts for the fact that no heterozygotes were discovered.

TABLE V.

Progeny of G. Stocksii × (*G.S.* 2 × *A.H.* 1–9).

F_1 plant	Crumpled	Normals	Failed to germinate	Total
1	6	2	—	8
4	12	11	2	25
9	3	6	—	9
Total	21	19	2	42

DISCUSSION.

The action of two main factors, **A** and **B**, in causing the appearance of "crumpled," is perfectly clear. The very great range in expression of the "crumpled" character is of greater interest. It has been shown by the back-crossing of F_1's to A.H. 1–9 that the same main factors cause the abortion of embryos in hybrid seeds of Abu Hareira × 1027, the production of seedlings which never progress beyond the cotyledon stage in Abu Hareira × Cawnpore White, and the production of crumpled plants with a sufficiently regular metabolism to permit the production of a small quantity of seed in Abu Hareira × *G. arboreum* var. *sanguinea*.

The very rapid increase in fertility as a result of selection, the appearance of "crumpled" plants more vigorous than those obtained from the corresponding direct crosses, in the back-cross of (Cawnpore White × 1927) × A.H. 1–9, and the different behaviour of different F_1 plants in the back-cross of (1027 × *G. obtusifolium* var. *africana*) × A.H. 1–9 all point to the existence of modifying factors which improve the viability of "crumpled," scattered throughout the Asiatic cottons and even occurring in 1027, which in the direct cross gives empty seeds. The distribution of these modifying factors appears to be correlated with the systematic affinities of the varieties carrying **B**. Three *sanguinea* varieties have been shown to carry **B**, viz. G.S. 1 and A 6, in *G. arboreum* and N 8 in *G. Nanking*. All three gave crumpled F_1's which grew to some size, and bore normal cotyledons. Three varieties of *G. arboreum* var. *neglecta*, and one of the closely related *G. arboreum* var. *rosea* all gave extreme crumpled F_1's, with abnormal cotyledons. In three of these F_1's the plants died without further development. The crumpled F_1 from Bengal 5 developed in a similar manner to crumpled F_1's from *sanguinea* varieties. Three of the *G. Nanking* types carrying **B**, viz. Million Dollar, Chinese Naked Seeded and Kiangyin Black, were obtained from a single ecological area in China. The first named gave empty seeds, and the other two gave extremely crumpled F_1's. The cotyledons were abnormal, and the seedlings made no further growth. N 14, the fourth *G. Nanking* carrying **B** is of hybrid origin. Only two varieties of *G. herbaceum* tested carried **B**, and both gave empty seeds. All types of *G. obtusifolium* tested carried **B**, and all except *G. obtusifolium* var. *africana* gave empty seeds. *G. obtusifolium* var. *africana* gave crumpled plants in F_1, which, though they grew taller than those from *sanguinea* varieties, were of about the same degree of abnormality.

If the only crosses investigated had been *G. Nanking* var. *soudanensis* × *G. obtusifolium* var. *Wightiana* the production of empty seeds would have been ascribed to interspecific sterility. The similarity between the crumpled plants in F_1's from related aB types × Abu Hareira shows that the intensity of expression of the crumpled character is a function of the genotype as a whole, and may be used to some extent as an index of relationship between *G. Nanking* var. *soudanensis* and other types.

The evidence from the intensity of expression of the crumpled character is consistent with the taxonomic evidence in placing *G. Nanking* var. *soudanensis* nearer to *G. arboreum* and *G. Nanking* than to *G. herbaceum* and *G. obtusifolium*.

It may be suggested that interspecific sterility is in some cases at least a more or less accidental phenomenon, depending on the spread through a species of a gene causing abnormal development in the presence of a complementary gene carried by a related species (cf. *Crepis*, Hollingshead, 1930). If the difference between the genotypes of the two species is great enough, the abnormality will be intensified to the point of complete sterility.

The demonstration of the presence of B in *G. Stocksii* may be emphasised. Since the normal hybrids of *G. Stocksii* with cultivated Asiatic Gossypiums are sterile, it has not hitherto been possible to discover whether there is any genic similarity between them. This is the first occasion on which a gene present in cultivated Asiatic Gossypiums has been shown to exist in *G. Stocksii*.

A close comparison of progeny rows of G.S. 1 and G.S. 2 was made to see whether the effect of B could be detected in the plant in the absence of A. No difference whatever could be found. Attempts to discover an effect of A in the absence of B were also unsuccessful.

Hollingshead (1930) reports crosses between *Crepis tectorum* and *C. capillaris* in which non-viable seedlings were obtained. A single *tectorum* plant gave 50 per cent. of viable hybrids, and was shown to be heterozygous for a factor causing the death of hybrids. A similar factorial scheme is proposed. The non-viable seedlings seem to have behaved in a similar manner to fairly extreme "crumpled" in cotton. Hollingshead reports abnormal differentiation and degeneration of tissue in the young shoot. A "crumpled" seedling from (Cawnpore White × 1027) × Abu Hareira was sectioned, but no marked abnormality could be discovered.

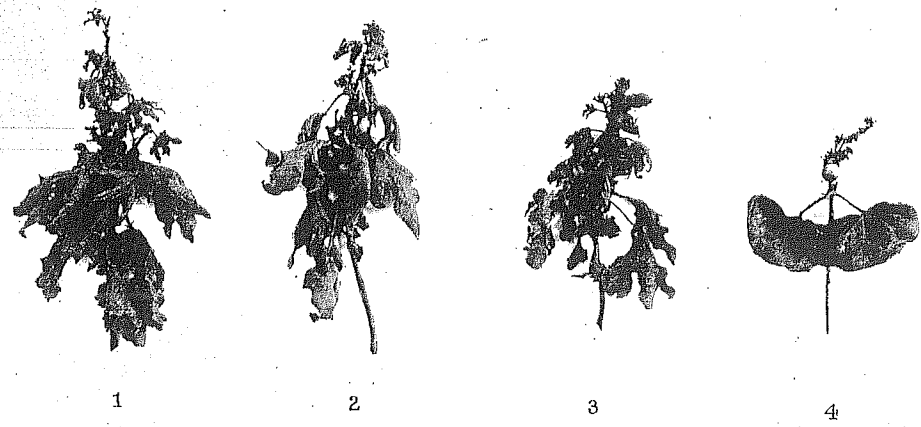

Fig. 2.

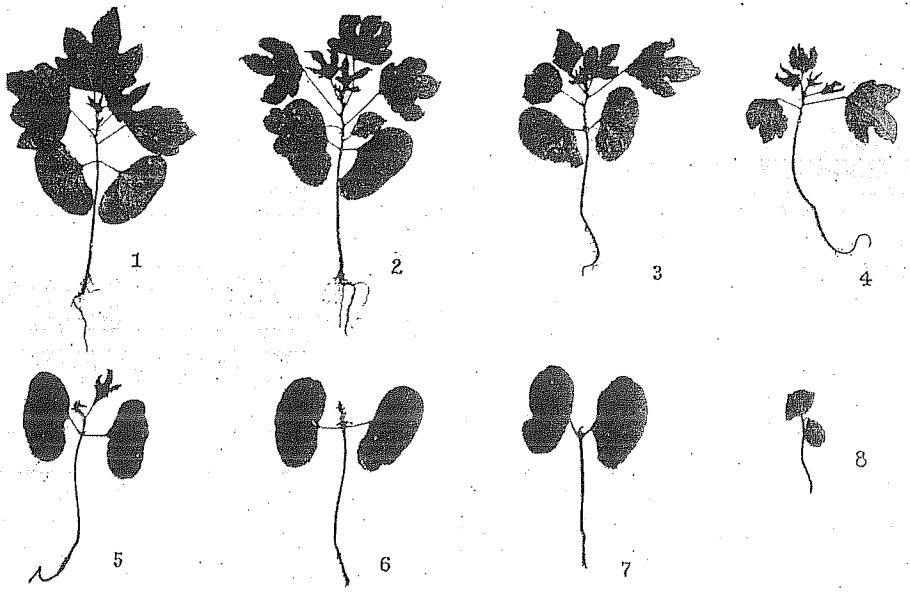

Fig. 5.

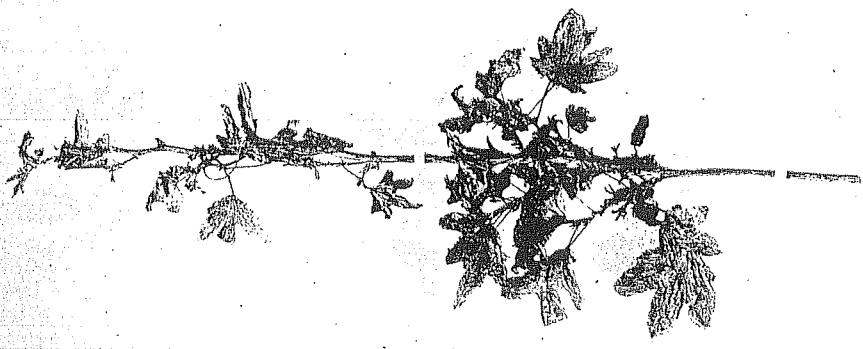

Fig. 4.

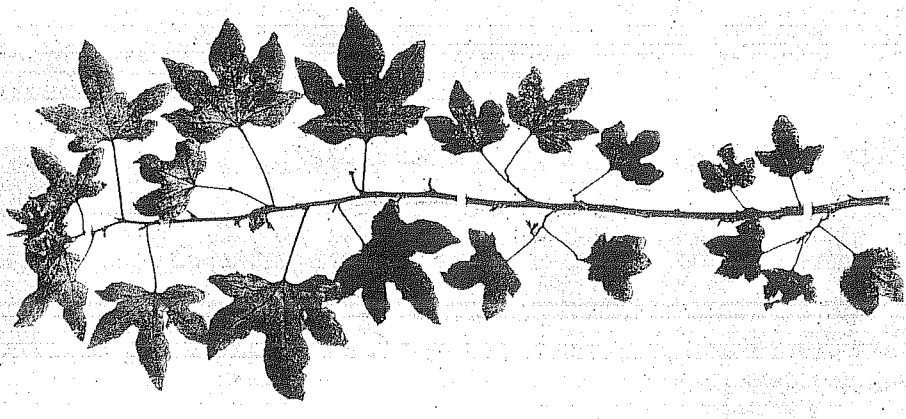

Fig. 3.

SUMMARY.

1. Two complementary factors, **A** and **B**, causing abnormal development have been demonstrated in Asiatic cottons.

2. **A** has been found in two strains of *G. Nanking* var. *soudanensis* obtained from the Sudan.

3. **B** has been found in seventeen varieties of *G. arboreum*, *G. Nanking*, *G. herbaceum* and *G. obtusifolium*, and in a strain of *G. Stocksii* from Sind.

4. The existence of a number of modifying factors affecting the degree of expression of the "crumpled" character has been demonstrated.

5. It was not possible to correlate either **A** or **B** with any character in normal plants.

6. Examination of sections from near the growing point of a "crumpled" seedling revealed no marked abnormalities.

ACKNOWLEDGMENT.

The researches here reported have been carried out under Dr S. C. Harland, to whom I am indebted for much valued criticism and advice.

The first crumpled observed in *G. Stocksii* hybrids were found in material of Dr Harland's, and I am indebted to him for the use of it.

I am indebted to Miss O. M. Atteck for the drawing reproduced in Fig. 1.

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EXPLANATION OF PLATES VIII AND IX.

Fig. 2. Four "crumpled" plants from *G. arboreum* var. *sanguinea* × A.H. 1–9, showing range of expression of "crumpled" character.

Figs. 3 and 4. Two "crumpled" plants which produced seed.

Fig. 5. Representative series of "crumpled" seedlings from (Cawnpore White × 1027) × A.H. 1–9.