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THE COMPARATIVE GENETICS OF *GOSSYPIUM ANOMALUM* AND THE CULTIVATED ASIATIC COTTONS

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(With Plate 18)

CONTENTS

I. INTRODUCTION

GOSSYPIUM ANOMALUM Wawra & Peyr. is a distinct and truly wild species with a very wide distribution in Africa. As far as is known it is confined

to arid steppe country, in two apparently discontinuous areas. The first of these is on the southern borders of the Great Desert Belt, where the species has been recorded from as far west as the Hombori district south of Timbuktu, from the Damergou region south of Air, from Kordofan in the Anglo-Egyptian Sudan, to as far east as Somaliland and Abyssinia, north to Nubia. In parts of this area it is said to provide valuable fodder for camels. The other area of occurrence is the dry coastal belt in Angola and South-West Africa, from Loanda in the north, southwards as far as Damara Land. Thus its general distribution considerably overlaps that of *G. arboreum* and *herbaceum*, the two cultivated Asiatic cotton species, but localities are not known with any great exactitude. From the marked xerophytic habit of *anomalum* as contrasted with the mesophytic character of the cultivated cottons it appears unlikely that they occupy the same ecological areas, though in country of the steppe type their habitats might not necessarily be far removed from one another.

This species has been variously described under the names *G. microcarpum* Welw., *G. senarense* Fenzl., and *G. herbaceum* var. *Steudneri* Schweinf., and has even been transferred to the genus *Cienfuegosia* by Schumann as *pentaphylla* and by Gürke as *anomala*, on the basis of its small linear bracteoles, well-marked calyx teeth, and tricarpellary ovary. The morphological grounds for its retention in *Gossypium* have been adequately discussed by Chevalier (1933), who collected material near Damergou and sent seed to this and several other experiment stations in 1932. Later, Chevalier (1935) felt justified in his conclusions on receiving information that the species had been crossed with both Old and New World cottons by a Russian worker, and that second generation hybrids were in existence. No further reports on the behaviour of these hybrids have followed this announcement.

G. anomalum is not of direct commercial importance since the hairs on its seeds are only 8–10 mm. long, sparse and dark brown in colour, and non-expansive, remaining compactly adherent to the testa. Its particular interest lies in the fact that it is the only wild species which has given any fertility in hybrids with the cultivated Asiatic cottons, so that it has been possible to analyse its genetic constitution in terms of that of the latter. Like *arboreum* and *herbaceum*, *anomalum* is also a diploid species, with $n=13$ (Skovsted, 1934). The results of this analysis, together with the evolutionary implications, form the subject of the present paper. They give confirmatory evidence of the general validity of Harland's (1936) conclusions on the formation of species in *Gossypium* by divergence of alleles, duplicates, and modifier backgrounds. Harland's

thesis has been established almost entirely upon crosses within the tetraploid New World complex of species, whichform a relatively homogeneous group, so much so that their hybrids are fully fertile, though that they are also good species is shown by the breakdown of viability and fertility in F_2. The two cultivated Asiatic species *arboreum* and *herbaceum* are similarly quite closely related, giving a fully fertile F_1, and breakdown in F_2, but in most characters they show much less modifier segregation than New World interspecific hybrids. On the other hand, *anomalum* has been found to be so distinct from the cultivated Asiatics that its hybrids are almost sterile, and early generations from them give highly complex segregation which completely defies analysis. By repeated backcrossing into the two cultivated species it has been possible to sort out particular factors into different lines on stable backgrounds, and later by synthesis some of the complex types of earlier generations were reestablished and their constitution demonstrated. Relatively little backcrossing to *anomalum* was performed, not only because in any case its whole genotype was unknown, but also because of its slow growth and uncertain cultivation under the highly humid conditions in Trinidad. Moreover, seed when obtained was difficult to germinate on account of its very hard testa. This characteristic was unfortunately carried over to a considerable extent into the cultivated species. *G. herbaceum* is also difficult to deal with under local conditions, and for this reason and also on account of its low content of recessive genes, the greater part of the backcrossing was confined to *arboreum*.

A large number of different strains of *arboreum* and *herbaceum* were used in this investigation. They are listed below in order of their type numbers:

A 1	*C.W.**; Cawnpore white flower, y_a	India
A 7	"*G. cernuum*" pale flower, $Y_a{}^P$	Bengal-Assam
A 8	*B.L.*; Burma laciniated	Burma
A 15	Pale flower, $Y_a{}^P$	Burma
A 16	Ghost spot, $R_a{}^{GS}$	China
N 5	*B.G.*, pale flower, $Y_a{}^P$; ghost spot, $R_a{}^{GS}$	Burma
N 9	*A.H.*; carries Cp_a (crumpling)	Sudan
N 14	White flower, y_a; ghost spot, $R_a{}^{GS}$	India
N 19	Wagale lintless, h_a	Burma
N 24	Petalody heterozygote, *Pdy pdy*	South India
N 25	Pale pollen, p_b	South India
N 44	Chinese pale-flower, $Y_b{}^P$	China
H 10	Spotless, $R_a{}^{dO}$	Afghanistan
H 16	Cream pollen, p_a	Tashkent
O 1	1027 ALF	Surat, India
O 7	Bushveld cotton	Portuguese East Africa
O 8		Transcaucasia

* In the case of a few strains reference by these symbols has been made in previous publications from this Station.

Following the recent revision of the classification of the Asiatic cottons by Hutchinson & Ghose (1937*a*), these strains belong to the taxonomic groups indicated below. The two species were subdivided by these authors primarily on the basis of the perennial or annual habit into varieties, and then by distribution into forms. Some of the more important genetic differences however appear to be associated rather with geographic distribution (Silow, 1939*b*) than with the acquisition of the annual habit, and for this reason it will be more satisfactory when it is necessary to discuss in the text the taxonomic ranking of particular strains, to refer briefly to their formal status only:

G. arboreum var. *typicum* forma *soudanensis*	N 9
G. arboreum var. *neglectum* forma *indica*	N 24, N 25
G. arboreum var. *neglectum* forma *bengalensis*	A 1, N 14
G. arboreum var. *neglectum* forma *burmanica*	A 8, A 15, A 16, N 5, N 19, N 44
G. arboreum var. *cernuum*	A 7
G. herbaceum var. *typicum*	H 10, H 16
G. herbaceum var. *frutescens*	O 1, O 8
G. herbaceum var. *africanum*	O 7

The following synthesized multiple recessive types were also used for particular backcrosses:

T 1	Multiple recessive lintless selection, predominantly *arboreum*, with trace of *herbaceum* in ancestry
T 3	Multiple recessive cream pollen selection, from F_2 of H 16 × T 14
T 4	Multiple recessive pale pollen selection, from F_2 of N 14 × N 25
T 6	Multiple recessive linted h_a selection; same origin as T 1
T 14	Multiple recessive selection, from F_2 of A 1 × N 5 (*1304*)
T 17	Multiple recessive petaloidic selection, from F_2 of N 14 × N 24

All pollinations were performed in insect-proof greenhouses after emasculation. In most cases it was more convenient as a routine procedure to use hybrid material as the seed parent, though reciprocal crosses were frequently performed and their progeny analysed separately.

In an investigation of this nature it is not possible to plan a comprehensive series of tests right from the start, as it is not known beforehand what will segregate. In the early stages especially segregating families must be used as they appear to demonstrate the nature of the parental type. Rather than describe each of the hybrids and their progeny in turn it has therefore been considered more suitable to discuss independently each of the main characters studied, bringing together all related information from various sources. The gene symbols used are based on the revised list of Hutchinson & Silow (1939).

II. Compatibility and fertility

G. anomalum has been crossed with representatives of the chief subdivisions of the genus, and it will be worth summarizing the available information on its behaviour.

(1) *With New World wild diploid species.* Skovsted (1935, 1937) has reported that *anomalum* crosses easily with *aridum* (Rose & Standley) Skovsted, *davidsonii* Kellogg, and *thurberi* Tod., but both he and Webber (1939) found that the hybrids were completely sterile, and their conjugation was as low as 0·2–5·7 bivalents ($2n=26$). Skovsted found that when *anomalum* is crossed with *armourianum* Kearney or *sturtii* F.v.M. bolls set freely, but contain only empty seeds. Similar behaviour with *raimondii* Ulb. has been reported by Hutchinson (1939).

(2) *With New World non-cultivated tetraploid species.* Skovsted (1937) reported that *anomalum* crosses relatively easily with *taitense* Parl., but most of the seeds obtained were empty or contained only partially developed embryos. One unthrifty hybrid was grown. With *darwinii* Watt *anomalum* crossed less easily, giving only a few empty seeds.

(3) *With New World cultivated tetraploid species.* Skovsted (1937) found that *anomalum* crossed with some difficulty with *barbadense* L., giving mostly empty seeds. Compatibility with *hirsutum* L. was not adequately tested. Further information has been collected, and this together with that reported by Skovsted (1937) may be summarized as follows:

♀	♂	Flowers pollinated	Capsules set	Total seeds	Empty seeds	Hybrids raised
anomalum	*barbadense*	84	26	120	All	—
anomalum	*hirsutum*	46	7	32	All	—
barbadense	*anomalum*	313	19	49	All but 4	1
barbadense haploid	*anomalum*	208	2	2	1	1
hirsutum	Mixed*	284	44	142*	None	5*
G 9543	*anomalum*	26	6	42	None	30

* A little own or other compatible pollen carrying a marker gene was mixed with that of *anomalum* to improve setting. Only five of the progeny were the result of the interspecific cross.

When *anomalum* was used as female all seeds obtained were empty. With *barbadense* as female relatively fewer seeds set than in the reciprocal cross, but about 10% of them contained fully developed embryos. The *barbadense* strain used was St Vincent Superfine, V 135. With *hirsutum* as female the seed setting was proportionally even lower, although a little compatible pollen had been mixed with that of *anomalum* to improve boll setting, but all seeds which were the result of the interspecific cross contained normal embryos. The cross-compatibility of plant G 9543 was of interest in this connexion. This plant was one of the selfed progeny of a sixth backcross of *hirsutum* to the same *barbadense* strain as used above (V 135), and therefore predominantly of the latter genotype, yet considerably more compatible with *anomalum* than the pure strain, and all seeds, instead of only 10%, contained normal embryos. Evidently in

such cases as these, which are on the borderline between compatibility and incompatibility, slight differences in constitution may considerably modify crossing behaviour. All the *anomalum*-New World hybrids raised have been completely sterile to their own, New World, *anomalum* and Asiatic pollen. Skovsted & Webber found a mean pairing of only 2·6–10·48 in these hybrids with $2n=39$.

(4) *With wild Asiatic diploid species.* Skovsted (1935) reported that Hutchinson had raised four hybrids of *anomalum* × *stocksii* M.Mast., which died after producing only a few leaves.

(5) *With cultivated Asiatic diploid species.* The majority of *anomalum* flowers crossed in either direction with *arboreum* L. and *herbaceum* L. set bolls which contain a full complement of seeds with fully viable embryos. Skovsted & Webber have found that the mean pairing in these hybrids with $2n=26$ is as high as 10–12; the former author found that approximately 25% of pollen mother cells examined showed complete pairing. Webber found this figure to be as high as 50%, and also stated, without citing any data, that the hybrid between *anomalum* and *sanguineum* (a variety of *arboreum*) is 45% fertile when selfed or backcrossed. Experience at this Station would not, however, lead to the assessment of fertility as anything like as high as this. Actually it is not easy to state fertility figures in simple terms for cotton, as bolls containing a low proportion of pollinated ovules frequently drop before maturity, quite apart from the fact that setting is very sensitive to slight physiological derangements totally distinct from compatibility. Both the percentage of flowers which set capsules, and the proportion of ovules which set seed within these capsules should be taken into consideration. Such data with reference to three *arboreum* and four *herbaceum* hybrids are summarized in Table 1. The full complement of seeds per capsule is about 20 in the *arboreum* and *herbaceum* types, except in the case of *herbaceum* H 10 which usually contains about 32–36 seeds. *G. anomalum* has a complement of about 10–12 seeds. Capsules on all of the hybrid plants contained about 30 ovules. Cotton flowers normally become pollinated autonomously. These hybrids must have borne at least 6000 flowers in the greenhouse, but not a single boll set. To a very slight degree this is due to the fact that their poor pollen does not readily become transferred to their unusually long stigmas, but the selfing data in Table 1, referring to flowers in which pollen was transferred by brush from anthers to stigma, indicates that in any case functional gametes are exceedingly few. In the *arboreum* hybrids approximately only 10% of self-pollinated flowers set bolls, containing only 10–20% of their full

complement of seeds. The *herbaceum* hybrids appear to be of even lower fertility, though this may partly be due to the fact that *herbaceum* types and their hybrids tend to shed very readily for purely physiological reasons under local conditions. In the few bolls which matured the proportion of ovules fertilized was much the same as in *arboreum* hybrids. On backcrossing the hybrids as female (after emasculation) with Asiatic types, fertility was higher. About 50% of the flowers pollinated set, and these capsules contained about 25% of their complement. Boll setting was again lower in *herbaceum* than in *arboreum* hybrids, but the proportion of seeds set per boll much the same. The reciprocal pollinations, in which the hybrids were used as male parents, showed a similar

Table 1. *Fertility of* anomalum-*Asiatic hybrids on selfing and backcrossing to Asiatic types*

Hybrid	Selfing			Backcross to Asiatic types: Hybrid as ♀			Backcross to Asiatic types: Asiatic as ♀		
	Flowers pollinated	% capsules set	Seeds per capsule	Flowers pollinated	% capsules set	Seeds per capsule	Flowers pollinated	% capsules set	Seeds per capsule
anomalum × *arboreum* A 8	150	7	4·1	816	54	5·5	2	(0)	.
anomalum × *arboreum* A 16	48	8	3·0	318	47	5·6	4	(25)	5·0
anomalum × *arboreum* N 14	92	13	2·0	109	68	5·1	39	59	7·0
anomalum × *herbaceum* H 10	128	0	.	360	22	7·5	77	25	5·2
anomalum × *herbaceum* O 1	82	0	.	4	(0)	.	26	8	7·5
anomalum × *herbaceum* O 7	358	1	2·7	14	(0)	.	.	.	.
anomalum × *herbaceum* O 8	209	0	.	86	12	2·4	38	16	7·0

Unreliable percentage estimates based on low numbers are in brackets.

degree of setting. Actually it might have been expected that with the heavy pollinations used there would have been an excess of functional male gametes over the number required to give a full set of seeds on the pure Asiatic seed parents, but this was not so. A further complication in the way of stating fertility of these hybrids in simple terms is introduced at this stage, since many of the seeds obtained contained only imperfectly developed embryos or were quite empty. Some 1500 seeds derived from the hybrid (*anomalum* × *arboreum* A 8) pollinated by *arboreum* N 14 or *herbaceum* H 10 were carefully examined, and usually only 75–85% were fully developed, and in some batches pollinated at different times or from different sib plants, this figure was as low as 50%. Bearing in mind the proportion of flowers which set bolls, the proportion

of seeds within the bolls which set, and the proportion of those seeds which contained good embryos, it may be estimated that the fertility of hybrids between *anomalum* and *arboreum* or *herbaceum* is no greater than 10% on backcrossing, whilst on selfing it is as low as 1%. These figures of course refer to fertility in terms of potential seed-setting capacity and, in view of the multiplicity of factors operating, are not exact estimates of the proportion of functional gametes.

The hybrids have also been backcrossed to a small extent to the *anomalum* parent. The figures are not sufficiently extensive to warrant citation, but the indications are that their fertility with *anomalum* is of comparable degree with that with the cultivated Asiatic species.

Only a very limited number of F_2 progeny of these hybrids have been grown. Not only did a high proportion of the very few seedlings

Table 2. *Ovule fertility of backcrosses to* arboreum *and* herbaceum

	Mean seeds per boll*														
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28
Plants of the 1st backcross to *arboreum*	Several	.	1	2	7	1	.	.	.	.	.	.	.	.	.
Plants of the 2nd backcross to *arboreum*	1	.	1	1	.	2	1	1	1	.	1	.	.	.	.
Plants of the 3rd backcross to *arboreum*	.	.	.	.	.	.	.	4	1	1	.	.	.	.	.
Plants of the 1st backcross to *herbaceum*	Several	.	.	.	.	.	1	1	.	.	.	.	.	.	.
Plants of the 2nd backcross to *herbaceum*	1	.	.	.	.	.	.	.	.	1	3	.	1	.	1

* 20 represents a full set in *arboreum* backcrosses; the *herbaceum* type used for these backcrosses contained up to 28 or 36 seeds per boll.

obtained die early or fail to mature, but in addition the extremely wide modifier segregation precluded the possibility of any useful genetic analysis. Even in the first backcross, with its considerably lower genetic variance, interpretation was in most cases impossible.

The high fertility which was rapidly attained on recurrent backcrossing into the cultivated species is indicated in Table 2. This shows, in the form of a frequency table, the fertility of certain plants backcrossed for genetic purposes in the first backcross and subsequent generations. This table does not indicate segregation for fertility in any way, but only indicates the approach to fertility which was attained. It is not a random sample of the populations since conscious selection was practised in propagating only plants which were setting in the field or showed reasonably good pollen. Only the seed content of the bolls which set is indicated; as fertility is approached this affords a better impression of

fertility than the percentage of flowers which set, as even on a fully fertile non-hybrid plant a full set of capsules is rarely attained, for physiological reasons as already mentioned.

In the first backcross to *arboreum* or *herbaceum* some plants were completely sterile. Dr Skovsted, formerly of this laboratory, examined somatic plates of one group of 49 plants of the backcross (*an.* × A 8) × N 14, and found that 46 of these had the normal diploid number of chromosomes, whilst the remaining three had $2n=27$. The latter plants were quite sterile; one of them was very stunted. All of the others, which were backcrossed, set seed to some extent. The plants included in the frequency array in Table 2 were members of another series which were more extensively backcrossed. Up to 89% of the flowers pollinated set on one of the first backcross plants; others set a lower proportion of flowers. Of the plants in the first backcross to *arboreum* which set, it will be seen from Table 2 that most contained about eight seeds per capsule, which is nearly twice the number set on the F_1, and represents about 40% of the seed complement. In the second backcross there were still some sterile plants present, but on the whole the mean fertility of plants as shown by the seed content of their bolls was higher, and even in this generation some contained the full complement of 20 seeds. The average fertility in the third backcross was even higher, and fewer sterile plants appeared. The same course of increasing fertility is evident from the two backcross generations to *herbaceum* which are shown.

Fourth and fifth backcross progenies have also been grown, and selfed progenies of these later backcrosses. Since most of these are of complex constitution, having been backcrossed to either of the cultivated species at different stages for particular genetic tests, it is not worth citing fertility figures. It is important to note that high fertility can eventually be attained as a result of recurrent backcrossing, whilst retaining at the same time at least one or two genes of *anomalum* origin. In practically all selfed progenies from late backcrosses which have been grown a very slight degree of infertility has been observed—for instance, the occasional appearance of petalody of stamens. In this connexion it must be stated that the primary object of this investigation was genetic; stringent selection for fertility was not practised, but the results obtained suggest that if it had been, absolute fertility could have been attained along with the transference of particular genes from the wild to the cultivated species.

With reference to the compatibility of other wild species with the cultivated Asiatics, Skovsted (1935) reported only empty seeds on

crossing with *armourianum* and *davidsonii*, and a great preponderance of empty seeds on crossing with *sturtii*, though in this case two sterile hybrids were also obtained. Hutchinson (1939) also obtained only empty seeds on crossing with *raimondii*. With *aridum*, *thurberi*, and *stocksii*, Skovsted (1935) and earlier workers obtained only completely sterile hybrids. Thus no wild species other than *anomalum* has given any fertility in hybrids with the cultivated Asiatic cottons. Since no other hybrids involving *anomalum* which have been produced have given any fertility either, the interest attaching to the *anomalum*-Asiatic crosses is readily apparent.

III. Corolla colour

(1) *The situation in the cultivated Asiatic species*

Hutchinson (1931) has shown that the three main corolla colour types encountered in *G. arboreum*, full yellow, pale yellow, and white, are due to a multiple allelomorph series which he designated Y–Yp–y. He confirmed earlier indications of a physiological relationship between corolla colour and size, yellow flowers having long petals and whites short, with pales of intermediate length. For the classification of segregating progenies he made use of a graded series of paintings, in which the deepest yellow was numbered 10, and white was 1. On this scale full yellow types carrying Y grade from 7 to 10, and y types are grade 1. Pale Yp types are very much nearer white than yellow in intensity of colour, usually grading at 2, very occasionally at 1·5 or 3. As far as length is concerned the relationship is the reverse; Hutchinson's petal length estimates within segregating progenies showed white petals about 20–25% shorter than full yellows; but pales were very nearly the same length as full yellows, being only 1–2% shorter, and the difference barely significant. Hutchinson also showed that there are modifiers affecting intensity of corolla grade, and that these affect petal length in the same way as the main genes. Thus in *G. herbaceum*, which almost invariably carries Y, many yellow-flowered strains are both paler and shorter than those in *arboreum*.

A pale yellow-flowered strain of *arboreum* from China, grown under the type number N 44, has recently been studied by the writer. It is very similar to types carrying Yp, but is distinguishable in that whilst the greater part of the lamina of the petal is pale yellow of grade 2, there is a very slight intensification of yellow towards the base of the petal round the margin of the anthocyanin spot, a tendency absent from the more common type of pale. The N 44 strain is complementary with

common pale and white-flowered strains, giving the ordinary full yellow. This demonstration of complementary factors necessitates the addition of a subscript to the symbols used by Hutchinson, and in order to bring the system of nomenclature into conformity with accepted genetic convention, the pale allele should be indicated by a superscript. It is therefore proposed that the series described by Hutchinson, that most frequently encountered in *arboreum*, be designated Y_a–$Y_a{}^P$–y_a, and the pale concerned in the Chinese strain $Y_b{}^P$. On analogy with the Y_a series it seems quite possible that a lower allele may be encountered in the future, and the suggested allocation makes allowance for this eventuality. On this scheme ordinary yellows are regarded as $Y_a\ Y_b$, the common pales and whites as $Y_a{}^P\ Y_b$ and $y_a\ Y_b$ respectively, and the Chinese pale strain as $Y_a\ Y_b{}^P$.

In this investigation it was not found that distinctions between Hutchinson's grades above 7 could be made with any confidence, though no difficulty was found in using the lower grades. It was therefore decided to grade up to class 8 only, reserving this for the most intense yellows. The vast majority of full yellows were graded at 7. The grades are not reproduced here, but may be seen in Hutchinson's 1931 paper. Flowers were collected from the field in the early morning, and graded in the laboratory; three readings were taken from flowers collected on different days. For the estimation of flower size, the length of the longest petal on three flowers was measured from the point of insertion to the extreme tip.

(2) *Hybrids and first backcross progeny*

G. anomalum has a very pale cream, almost white corolla, with a faint tinge of pink (Pl. 18, fig. 4). The flower is of medium size, with petals 30–37 mm. long. It grades approximately at 2, though exact comparison with the scale of yellows is difficult on account of the pink tinge. It will be shown later that this pink is associated with one of the main anthocyanin spot genes, but in this section it is proposed to deal with the inheritance of the yellow flavone only, ignoring as far as possible the complications in corolla colour introduced by the anthocyanin.

G. anomalum was crossed with full yellow, pale and white representatives of *arboreum* and *herbaceum*. In each F_1 from two to twenty plants were examined, and the results of their corolla colour grading are shown in Table 3. Each progeny was quite uniform within itself, but scoring on the individual plants varied slightly from day to day as shown. Grading of yellow intensity was not easy on account of the

intensification of the accompanying pink, especially in the hybrids involving full yellow Asiatic types. The interaction between flavone and anthocyanin will be discussed later. At this stage it is only necessary to state that the development of petal red was later found to be dependent to some extent upon exposure of the bud to sunlight, and it is to differences in this exposure that a great deal of the observed variation in pink, and consequently in yellow intensity, can be attributed. Quite apart from this physiological interaction, the variation in pink naturally introduced a serious subjective error in yellow estimation. The same diffi-

Table 3. *Yellow corolla grade in F_1 hybrids.* G. anomalum = *grade* 2

Asiatic parent			F_1
Type no.	Gene	Grade	Grade
A 8	Y_a	7	4–5 (see Pl. 18, figs. 1, 5)
A 16	,,	7–8	3–4 (see Pl. 18, figs. 3, 7)
N 25	,,	7–8	5
H 10	,,	7	3–5 (see Pl. 18, figs. 2, 6)
H 16	,,	5	5–6
O 1	,,	7–8	3–5
O 7	,,	7	4–5
O 8	,,	8	4–5
N 5	$Y_a{}^P$	2	4–6
N 14	y_a	1	4–5
T 14	,,	1	4–5
N 44	$Y_b{}^P$	2	3–4

culties were encountered in grading the first backcrosses, data for which are summarized in Table 4. The main findings requiring interpretation were as follows:

(i) The F_1 hybrids were all of approximately the same shade of low-grade yellow, no matter whether the Asiatic parent were full yellow, pale, or white (Table 3).

(ii) In backcrosses to Asiatic full yellow there was a very wide modifier segregation, and full yellows reappeared (Table 4, *a*). The modifier segregation was much wider than is encountered in crosses between *arboreum* and *herbaceum*. The two most nearly comparable cases available are Hutchinson's (*arboreum* pale × *herbaceum* full yellow) backcrossed to *herbaceum* full yellow, shown in his 1931 Table 1, and the (*anomalum* pale × *arboreum* full yellow A 8) backcrossed to *herbaceum* full yellow H 10, shown in the first line of Table 4:

	Yellow grades						
	8	7	6	5	4	3	Total
(*arb.* pale × *herb.* yellow) × *herb.* yellow	.	1	29	102	59	.	191
(*an.* pale × *arb.* yellow) × *herb.* yellow	71	116	107	101	97	58	550

Table 4. *Segregation for yellow corolla grade in first backcrosses*

	Yellow grades										Total	
	8	7	6	5	4	3	2·5	2	1·5	1	Full yellow	Pale yellow or white
(*a*) anomalum × *full yellow, backcrossed to full yellow*:												
(*an.* × A 8) × H 10	71	116	107	101	97	58	.	.	.	.	*550*	.
(*an.* × A 8) × A 8	2	7	7	2	8	5	.	.	.	.	*31*	.
(*an.* × A 16) × A 16	31	48	5	11	13	.	.	.	.	.	*108*	.
(*b*) anomalum × *full yellow, backcrossed to* anomalum:												
(*an.* × A 16) × *an.*	.	.	2	2	1	.	6	.	.	.	*5*	*6*
(*an.* × H 10) × *an.*	2	1	6	3	.	.	.	11	.	.	*12*	*11*
(*c*) anomalum × *full yellow, backcrossed to* arboreum *white*:												
(*an.* × A 8) × N 14	7	37	26	37	44	49	.	.	.	.	*200*	.
(*an.* × H 10) × N 14 or T 14	4	10	11	10	11	1	.	.	.	.	*47*	.
(*d*) anomalum × *pale, backcrossed to* arboreum *pale or white*:												
(*an.* × N 5) × N 14, white	.	4	.	.	.	.	.	1	3	.	*4*	*4*
(*an.* × N 5) × N 5, pale	1	2	.	.	2	1	.	2	.	.	*5*	*3*
(*e*) anomalum × *white, backcrossed to* arboreum *pale or white*:												
(*an.* × N 14) × N 14, white	12	26	25	9	12	.	.	.	.	88	*84*	*88*
(*an.* × N 14) × N 5, pale*	.	36	.	14	.	.	.	.	49	.	*50*	*49*

* Progeny not graded in this backcross, but grouped into three classes (i) Y 8–7–6; (ii) Y 5–4; (iii) Y^p 2–1.5–1. (i) and (ii) not distinct from each other; (iii) quite distinct.

The much greater genetic variance in the *anomalum* × *arboreum* cross than in the *arboreum* × *herbaceum* cross is clearly evident in this comparison. This particular cross of Hutchinson's shows the greatest amount of modifier segregation which he encountered in the inter-species crosses within the cultivated Asiatics which he studied.

(iii) *Anomalum* × full yellow backcrosses to *anomalum* (Table 4, *b*) indicated a single main factor difference between the parents.

(iv) The F_1 of *anomalum* × full yellow, when backcrossed to *arboreum* white (Table 4, *c*), gave no progeny of appreciably lower grade of yellow than the F_1, though in the other direction full yellows were again recovered. The absence of pale segregates in these backcrosses indicated that *anomalum* pale and *arboreum* white are not homologous. The evidence, together with that in (i) above, suggests that *anomalum* and pale or white *arboreums* carry complementary factors for yellow corolla. The appearance of full yellows in these backcrosses and in those discussed in (ii) above, and the uniform low grade of all the F_1 hybrids, suggested the presence of one or more yellow depressors in *anomalum*.

(v) The backcrosses from *anomalum* × *arboreum* pale or white to the latter types (Table 4, *d*, *e*) showed a single main factor difference between these parental types.

(3) *A third main locus, Y_c*

The hypothesis that *anomalum* is complementary with pale and white corolla types of *arboreum*, and that it carries one or more yellow depressors, received confirmation from certain segregates in a series of backcrosses to A 8 of the hybrid *an.* × A 8 full yellow. The wide segregation in the first backcross has been shown in Table 4, *a*. Very small second backcross progenies from two first backcross selections of grade 6 were grown, primarily for transference of the leaf-shape gene from the wild species, and in these corolla colour was not scored. From some of the second backcross plants third backcross and selfed progenies were grown in the following season. By that time this material had been superseded for the original purpose of leaf shape transference by other lines and was only planted in the field at the end of the season after more important material had received attention. On that account mortality was high, and only a few plants survived. Amongst these it was noticed that, in addition to full yellows of grades 8–7–6, there were some very clear-cut segregates of grade 4 (fluctuating to grade 5) in one of the backcrosses, and of grade 3 in one of the selfings (Table 5). These two groups of lower grade segregates were actually much more distinct from each

other than would appear from their gradings. As a working hypothesis it was thought that the grade 3 plant (no.'14,868) in the selfed progeny carried the transferred *anomalum* corolla colour gene extracted as a homozygote, and that the grade 4 plants (nos. 14,859 and 14,862) in the backcross progenies might be heterozygotes for this same gene. Accordingly the former plant was selected, and both of the latter and their sibs and some of their half-sibs. The behaviour of selfed progenies from these selections (Table 5, third backcross selfed column) effectively disposed of this hypothesis, since the grade 4 parents gave only grade 8–7 and grade 4 progeny, whilst the grade 3 type appeared in the progeny of

Table 5. *Corolla grade segregation in third backcross of* (an. × *A* 8) *to A* 8 *full yellow, and in selfed progenies of second and third backcross selections*

1st B.C. plant nos.	2nd B.C. plant nos.	3rd B.C. yellow grades				2nd B.C. selfed yellow grades		3rd B.C. selfed yellow grades		
		8	7	6	4	7	3	8–7	5–4	3
P 1169	P 1874	.	1	.	.	2	1 *a*	.	.	.
	P 1875	2	.	1	.	1	.	.	.	.
	P 1876	3	2	.	.	.	.	.	.	.
P 1170	P 1877	5	.	.	.	.	.	28	.	7 *b*
		.	.	.	.	.	.	69	.	21
		.	.	.	.	.	.	11	.	.
		.	.	.	.	.	.	16	.	.
	P 1881	3	.	.	2	.	.	81	.	. *d*
		.	.	.	.	.	.	34	.	. *d*
		.	.	.	.	.	.	41	.	. *d*
		.	.	.	.	.	.	35	49	. *e*
		.	.	.	.	.	.	6	11	. *e*

a This grade 3 segregate was no. 14,868.
b One of these grade 3 segregates was no. 9932.
c These grade 4 segregates were nos. 14,859 and 14,862.
d Parents of these progenies were grade 8.
e Parents of these progenies were grade 4 (nos. 14,859 and 14,862).

some of the full yellow selections. Furthermore, as will be discussed later, no grade 3 segregates appeared in the progeny of 14,868 when crossed with 14,859 and 14,862.

The breeding behaviour of the grade 3 type will be discussed first, but before doing so it is necessary to emphasize that it must not be presumed that it bears any genotypic relationship to the grade 3 phenotype which appeared in the first backcrosses. First, the modifier welter in the latter should preclude any such assumption. Secondly, plants of the grade 3 type in the first backcross all showed a strong development of pink, and it will be demonstrated later that interaction between flavone and anthocyanin may lead to the lowering of grade of yellow. Actually

the early backcross grade 3 types correspond to the grade 4 type here under study, from which complications due to anthocyanin were absent. The extracted grade 3 type appeared only in selfed progenies, and these particular lines had relatively uniform modifier backgrounds. Although pink was present in some of these lines, it was not expressed in the grade 3 segregates.

The original grade 3 segregate, no. 14,868, which appeared in the selfed second backcross family, unfortunately died before any selfed seed could be obtained from it, but as it gave rise to some outcross progeny which will have to be discussed, it is necessary to establish its constitution. It was indicated, in connexion with Table 5, that plants of this phenotype reappeared in the following season in some selfed progenies of third backcross full yellow selections. It will be seen from Table 5 that these and 14,868 trace back to different first backcross plants. That they were of the same genotype as 14,868 is shown by the fact that only grade 3 progeny appeared in crosses between one of them (no. 9992), and certain double recessives extracted from an F_2 involving 14,868 and the *arboreum* pale petal type N 5. This point will be discussed in more detail below, but is important here in indicating that both 14,868 and 9992 may be considered as being of the same constitution.

In the two third backcross selfed families which threw grade 3, the ratios 28 : 7 and 69 : 21 were indicative of a monofactorial 3 : 1 segregation. The grade 3 selection when selfed gave 14 progeny, all grade 3. Either 14,868 or 9992 was crossed with full yellow, pale yellow $Y_a{}^P$, white y_a, and Chinese pale $Y_b{}^P$ representatives of *arboreum*. All progeny in each case were full yellow of grade 8–7:

Cross	No. of progeny observed
14,868 × A 8, full yellow	9
9992 × A 16, full yellow	5
14,868 × A 7, pale yellow $Y_a{}^P$	3
14,868 × N 5, pale yellow $Y_a{}^P$	11
9992 × N 5, pale yellow $Y_a{}^P$	8
14,868 × N 14, white y_a	15
9992 × N 44, Chinese pale $Y_b{}^P$	10

This extracted grade 3 behaved as a complementary type with the *arboreum* recessive corolla colour types in exactly the same way as the original *anomalum* did, except that the latter only gave low grade yellow hybrids on account of the presence of a yellow depressor, as will be evident later.

Two plants from the cross 14,868 × N 5 were backcrossed to *anomalum*, and gave small progenies containing in all 8 plants of low grade yellow

similar to the original *anomalum*-Asiatic hybrids, and 14 plants of exactly the same corolla colour as the wild species itself. This shows that the extracted grade 3 type contains the main pale corolla colour gene derived from *anomalum*. The higher grade of the transferred pale is due to the loss of the yellow depressor. At this stage it will be a convenience for discussion to assign the symbol Y_c^P to this pale corolla gene.

Five F_1 hybrids between pale 9992 and full yellow A 16, when back-crossed to 9992, gave clear-cut segregation into grade 7 and grade 3 as follows:

Plant no.	Grade 7	Grade 3	$\chi^2_{1:1}$	P
P 4098	38	16	9·0	.
P 4126	52	30	5·9	.
P 4127	40	32	0·9	.
P 4128	45	35	1·2	.
P 4129	37	36	0·0	.
Total	212	149	11·0	Very small
Heterogeneity			6·0	0·2

These five families were homogeneous amongst themselves, but gave in all a significant deficiency of recessives on a 1 : 1 basis. Introduced genes were frequently found to be in defect in this study, and this problem will be discussed later. From all the other evidence on corolla colour which has been obtained it appears reasonable to interpret this ratio as a distorted 1 : 1, indicating that the grade 3 type differs from full yellow in one gene.

Two of the full yellow F_1 hybrids between pale 14,868 (Y_a Y_c^P) and pale N 5 (Y_a^P Y_c) gave a remarkably clear complementary factor ratio on selfing. Since both A 8, to which the *anomalum* pale had been transferred, and N 5 are Burmese forma *burmanica* representatives, the absence of any marked modifier segregation was not surprising. Four main classes were observed:

(*a*) Full yellows, ranging 8–7.
(*b*) Pale yellows of grade 3.
(*c*) Pale yellows of grade 2.
(*d*) Very pale yellows, almost white, grade 1+.

After becoming familiar with the type of segregation, scoring of individual plants from day to day was very consistent, and all plants could be assigned without difficulty to one of these four classes, except a very small proportion of the pales which were intermediate between grades 2 and 3. Although no further genotypic testing was performed, except in the case of the class (*d*), the four groups may be taken as

corresponding to the four expected genotypes $Y_a\,Y_c$, $Y_a\,Y_c{}^P$, $Y_a{}^P\,Y_c$, $Y_a{}^P\,Y_c{}^P$ on the following grounds:

(1) Class (*a*) was perfectly distinct from the other three pale yellow classes.

(2) The *anomalum* pale is far more distinctive phenotypically from the common Asiatic pale than the classification into grades 3 and 2 indicates. Part of this distinction is associated with the fact that the $Y_a{}^P$ type is considerably shorter than full yellow or $Y_a{}^P$ types. Petal length measurements were not taken in this family, but this general observation is confirmed by measurement in the $Y_b{}^P$ cross which will be discussed next.

(3) The constitution of the near-white class (*d*) was confirmed genetically as shown below. On this basis the segregation may be taken as conforming to a 9 : 3 : 3 : 1 as follows:

	Yellow grade					
Family	3–7	3	2·5	2	1+	Total
3046	55	11	1	12	3	82
3047	45	14	2	10	3	74
Total	100	25	3	22	6	156
Expected *9 : 3 : 3 : 1*	*87·75*	*29·25*		*29·25*	*9·75*	*156*

The families are sufficiently similar to warrant combination, and the totals accord well with expectation ($\chi^2=4{\cdot}5$, $P=0{\cdot}2$). Two selections from the grade 1+ group, supposed double recessives, were each crossed with both of their parental types 9992 and N 5, and all progeny were pales of grade 3–2. The number of progeny examined in each cross was as follows:

	× 9992	× N 5
Grade 1+ selection no. 15,723	3	6
15,759	4	16

That these selections were not complementary with either of their parental pales confirms the supposition that they were double recessives.

Since the phenomena of complementary alleles at the same locus and of complementary recessive genes in duplicate loci will be demonstrated in connexion with anthocyanin inheritance, it is necessary to indicate that the more normal interpretation of complementary factors applies to this case of corolla colour. If it were a case of complementary alleles at the same locus, the F_2 would have given an allelic 2 : 1 : 1 ratio, which could easily be confused with a 9 : 3 : 3 : 1 if the latter term were incorporated with either of the middle terms giving a 9 : 3 : 4. However, the demonstration of the presence of pale segregates giving no comple-

mentary effect with either parental pale disposes of the possibility that the latter might be due to complementary alleles.

A third plant from the F_1 between 14,868 and N 5, when backcrossed to N 5, segregated 31 full yellows and 33 pales of the N 5 type, in conformity with expectation.

Similar complementary factor ratios were observed in two F_2 families derived from the full yellow hybrids between pale 9992 (Y_b Y_c^P) and N 44 Chinese pale (Y_b^P Y_c)—again forma *burmanica*. There was a satisfactory distinction between full yellows, the grade 3 *anomalum* pales with short petals, the "grade 2 Chinese" type with the characteristic slight intensification of yellow just around the red petal spot, and with petals of practically the same length as the full yellows, and a fourth class with very pale, practically white, corolla showing a very slight intensification of yellow near the spot, and with petals of intermediate length—presumably the double recessives (Y_b^P Y_c^P).

	Yellow grades				
Family	8–7	4 3	2 Ch	1+Ch	Total
3715	34	5	13	3	55
3716	33	1 3	4	3	44
Total	67	9	17	6	99
Expected *9 : 3 : 3 : 1*	*55·6*	*18·6*	*18·6*	*6·2*	*99·0*

Agreement with expectation was not quite as good as in the previous cross considered ($\chi^2 = 7{\cdot}5$. $P = 0{\cdot}1$–$0{\cdot}05$), but most of the discrepancy was due to a deficiency of *anomalum* pale segregates. Nevertheless, it is clear that *anomalum* pale is independent of Chinese pale, as well as of the common Asiatic pale or white locus.

(4) *A yellow depressor, Ydp*

It has already been mentioned, at the beginning of the preceding section, that two grade 4 segregates (nos. 14,859 and 14,862) were observed in the third backcross of *anomalum* to full yellow A 8, and their segregation on selfing was summarized in Table 5. It was thought at first that they might be heterozygous for the grade 3 *anomalum* pale type, but quite apart from the fact that heterozygotes for the latter were later found to be full yellow, 108 progeny derived from their backcross with *anomalum* itself were all yellows of grade 4–5. Furthermore they threw nothing paler than themselves either on selfing or crossing to the grade 3 plant no. 14,868. In each case they gave two reasonably distinct groups, one fluctuating between 7 and 8, and the other from just below grade 4 to just above grade 5. Within these groups it was not felt that

classification was reliable, but there was no hesitation in classifying them into the full yellow and the "depressed yellow" class, as it was termed in order to differentiate from true pales. The fact that none of the latter appeared indicates that 14,859 and 14,862 were both of $Y_c\ Y_c$ constitution. These two plants were also crossed with white petalled N 14 (y_a) and gave two similar clear-cut phenotypes. In all, the segregations from these two plants were as follows:

Progeny	Full yellow	Depressed yellow
14,859 selfed	35	49
14,862 selfed	6	11
14,859 × 14,868	12	12
14,862 × 14,868	12	6
14,859 × N 14	95	101
14,862 × N 14	109	88

The backcross ratios were very suggestive of the presence of a single dominant depressor, but the two selfings did not conform at all well to the 3 depressed : 1 full yellow expected on that basis. Attempts to establish a homozygous depressor line have not so far met with any success. Four low grade yellows from 14,859 selfed were selfed; all threw some full yellows, giving in all 40 full yellows : 51 depressed; a further six plants from one of these selfings were also tested, and again all gave some full yellows, and a ratio of 15 : 29 in total. In each case the proportions were much the same as from the original depressed yellows, 14,859 and 14,862, which were known to be heterozygotes in view of their backcross origin. All the above selfings total to 96 full yellows : 140 depressed. The deficiency of depressed segregates is even too great to explain on any hypothesis of inviability of the homozygote ($\chi^2_{2:1}=5{\cdot}7$). On a normal single gene interpretation the probability of having found at least one homozygote amongst the 10 depressed yellows tested is as high as 0·98. A further five plants will be tested next season; these have been examined by Dr J. Pisk of this laboratory, who has found no abnormality in their male meiosis which might account for the genetic situation. The backcrosses, and those reported below, which also showed normal behaviour, were all from the heterozygote as female, so there is no information on the gametic ratio on the male side. This is under investigation, as additional data are very desirable. There was, within each of the two main classes, a little more variation in selfings than there was in backcrosses, and possibly some of the apparent deficiency may be attributed to errors in scoring, but it is not believed that this can explain the situation, as on the whole there was a perfectly clear distinction between the two groups. As this lowering of yellow intensity was found

to exert a very profound influence on the expression of anthocyanin in the petal, as will appear later, it is proposed, in order to facilitate the discussion, to allocate the symbol *Ydp* to the gene responsible, though in view of the selfing results it is realized that the situation may not be as simple as the backcross data suggest.

Two of the depressed yellow segregates from the cross 14,859 × N 14 mentioned above were backcrossed to N 14 white, and gave three clear classes in very good independent 1 : 1 ratios for both y_a and *Ydp*:

Family	Full yellow	Depressed yellow	White	Total
3408	41	36	78	155
3409	36	47	73	156
Total	77	83	151	311

Evidently *Ydp* is completely independent of the Y_a locus. It could not of course be situated at the Y_c locus in *anomalum*. That *Ydp* is also independent of the Chinese pale locus Y_b was shown by the segregation in two families derived from depressed yellow plants from the cross 14,859 × N 44 (Y_b Y_b, *Ydp ydp* × Y_b^P Y_b^P, *ydp ydp*). These plants were backcrossed to the double recessive N 44; full yellows and depressed yellows appeared in equal numbers, and the influence of the depressor gene could also be seen within the Chinese pale segregates, approximately half of which were distinctly paler than the others:

Family	Full yellow	Depressed yellow	Chinese pale	Depressed pale	Total
3708	40	34	47	43	164
3709	16	22	12	14	64
Total	56	56	59	57	228

Very few backcrosses to *anomalum* have been grown, and as far as corolla colour is concerned only one large progeny. This, the result of selfing a plant from the second backcross of H 10 to *anomalum*, and carrying the Y_c allele from the former type, segregated 53 plants of grades 6–5–4 and 27 of *anomalum* grade 2. The yellows went rather higher than might have been expected on a presumably homozygous *Ydp* background, though there was no evidence that *ydp* from H 10 had not also been carried over into this line. Scoring of exact yellow grade was very difficult on account of the wide range in intensity of pink in the petal. The point of greatest interest in this progeny was that it showed an excess of pales—53 : 27—which though not significant suggests a tendency for a foreign gene to be in defect when introduced into *anomalum* also.

The depressor gene is of sufficient potency to account for practically all of the downward extension of the great range in corolla colour segre-

gation which characterizes *anomalum* hybrids as against those between the two cultivated Asiatic species. As far as modifiers affecting the upper portion of the range in yellow intensity are concerned, *anomalum* is probably not very different from many of the *herbaceum* types which are at a lower level than *arboreum* in this respect.

(5) *The effect of* Y_a^P *and* Ydp *on petal length*

In view of the influence of the Y_a series of alleles on flower size, petal length was measured in certain segregating families, and the results are summarized in Table 6. The first three families all agreed in showing that

Table 6. *The effect of* Y_a^P *and* Ydp *on petal length*

Family	Corolla colour	No. of plants	Mean petal length mm.	S.E.	Diff.	t	P
3rd B.C. A S selfed	Yellow	54	33·14	—	3·37	2·0	0·05–0·02
	anomalum pale	9	29·77	—			
(A 16 × 9993) × 9992	Yellow	206	35·59	—	5·57	16·0	Very small
	anomalum pale	143	30·02	—			
(N 44 × 9992) selfed	Full yellow	63	43·55	0·52	10·89	8·6	Very small
	anomalum pale	9	32·66	1·15			
	Chinese pale	17	44·47	0·80	—	—	—
	Double recessive	6	38·66	1·23	—	—	—
(N 44 × 14,859) × N 44	Full yellow	55	45·78	0·39	0·73	1·4	0·2 –0·1
	Depressed yellow	55	45·05	0·33			
	Chinese pale	57	44·98	0·36	1·16	2·1	0·05–0·02
	Chinese pale depressed	57	43·82	0·39			
14,859 × N 14	Full yellow	56	40·17	—	1·47	3·2	Small
	Depressed yellow	60	38·70	—			

the *anomalum* pale segregates have petals from 10 to 25% shorter than those of their full yellow sibs. In each case the difference was significant, and its magnitude was comparable with that which Hutchinson (1931) found to be associated with white corolla in the series which he studied. From the third and fourth families it will be seen that Chinese pales are more like the common pale which Hutchinson studied, in not being very different in petal length from yellows. In the third family the Chinese pales were 0·92 mm. longer than the yellows, but the standard error of this difference was 0·95. In the fourth family they were 0·80 mm. shorter, with a standard error of 0·54. In each case the differences were non-significant. In the third family the double recessives were found to be intermediate in petal length between *anomalum* pale and Chinese pale. They were 6·00 mm. longer than the former ($t=3·5$, P very small), and 5·81 mm. shorter than the latter ($t=4·0$, P very small), but in view of their small number it is doubtful whether much significance can be attached to their measurements.

In the fourth family the depressor class was shorter than its corresponding full yellow or Chinese pale class in each case, though only in the latter comparison was the difference significant. Comparison of the petal length of the depressed Chinese pales with that of the full yellows showed a more marked difference, the former being 1·96 mm. shorter than the latter ($t=3·5$, P very small). This can no doubt be attributed to the cumulative effects of the Chinese pale and the depressor, which separately are small and not easily demonstrable. In the fifth family the depressed yellows again had slightly shorter petals than the full yellows, and in this case the difference was clearly significant. Evidently *Ydp* is similar in this respect to the petal colour modifiers which Hutchinson studied.

(6) *Linkage tests against* Y_c *and Ydp*

Since markers for these loci have not previously been available in Asiatic cottons, correlations with other gene segregations have been observed whenever possible, and are recorded in Tables 7 and 8. In none of the cases examined was there any evidence of linkage with either Y_c or *Ydp*.

Table 7. *Two-factor selfed segregations involving* Y_c

Factor	Family	Parental constitution	Y_cX	Y_cx	Y_c^PX	Y_c^Px	T	χ^2_L
L	*an.* 3rd B.C. A 8, S	+ + × − −	55	13	10	2	80	0·1
	14,868 × N 5, S	+ − × − +	92	30	18	11	151	2·1
	9992 × N 44, S	+ − × − +	62	21	12	3	98	0·2
Lc_1	14,868 × N 5, S	+ − × − +	90	19	21	4	134	0·1
	9992 × N 44, S	+ − × − +	57	15	7	6	85	3·8
R_c	14,868 × N 5, S	+ − × − +	91	31	25	6	153	0·5
H_a	9992 × T 6, S	+ − × − +	44	16	11	5	76	0·1

Table 8. *Two-factor backcross segregations involving Ydp* (F_1 *used as female*)

Factor	Family	Parental constitution	$YdpX$	$Ydpx$	$ydpX$	$ydpx$	T	χ^2_L
L	(14,859 × N 14) × N 14	+ + × − −	39	43	36	41	159	0·1
	(14,859 × N 44) × N 44	+ + × − −	56	56	54	61	227	0·2
Lc_1	(14,859 × N 14) × N 14	+ + × − −	36	43	41	34	153	1·1
	(14,859 × N 44) × N 44	+ + × − −	56	51	55	47	209	0·1
R_c	(14,859 × N 14) × N 14	+ + × − −	42	41	32	45	160	1·3
Ne	(14,859 × N 14) × N 14	+ + × − −	45	37	31	46	159	3·4

(7) *Summary*

(i) With respect to flower colour, *G. anomalum* is complementary with *arboreum* strains carrying the common pale or white allele in the Y_a locus, or Chinese pale in the Y_b locus. To the gene responsible for the pale corolla of *anomalum* the symbol Y_c^P is assigned. All strains of *arboreum*

and *herbaceum* carry its allele Y_c. The constitution of *anomalum* is $Y_a\ Y_b\ Y_c{}^P$.

(ii) Although the $Y_c{}^P$ pale corolla, when in *arboreum*, is not very different in appearance from the $Y_a{}^P$ pale, it is more like y_a white in being associated with a considerably smaller flower than the full yellow types.

(iii) All *anomalum* hybrids, even those involving full yellow cultivated Asiatic parents, are alike in the low intensity of yellow in the corolla. This is due primarily to a yellow depressor from *anomalum*, to which the symbol *Ydp* is allocated. This gene lowers the intensity of full yellow in *arboreum* from grade 7–8 to grade 4. $Y_c{}^P$ pale is of grade 3 in an *arboreum* genotype (*ydp*), but in *anomalum* (*Ydp*) the expression of this gene is reduced to grade 2 by the depressor.

(iv) The depressor, like other corolla colour modifiers, restricts petal length to a very slight extent.

(v) *Anomalum* hybrid progenies show a very much wider corolla colour modifier segregation than do crosses between the two cultivated Asiatic species. Most of the downward extension in range of yellow intensity is due to the *Ydp* gene.

(vi) The Y_c locus is independent of Y_a, Y_b, R_2, H_a, and the L–Lc_1 linkage group. *Ydp* is independent of Y_a, Y_b, Y_c, R_2, *Ne*, and the L–Lc_1 group.

IV. Anthocyanin

(1) *The situation in the cultivated Asiatic species; symbolization*

In *G. arboreum* and *herbaceum* anthocyanin distribution is controlled by a lengthy series of multiple allelomorphs. Six types were originally described by Hutchinson (1932*b*) and the genes arranged serially:

$$R\text{–}R^L\text{–}R^C\text{–}R^S\text{–}r^g\text{–}r^o.$$

R^S is by far the commonest allele, and the red spot at the base of the petal and the variable intensities of red tinge on the stem which it produces might be described as the typical condition in cotton (Pl. 18, fig. 1). The r^o type is completely spotless (Pl. 18, fig. 2); the red tinge on the stem of the strain in which this gene occurs is slightly paler than that on most R^S types, but this is almost certainly due to modifiers and not to any difference in the main gene in this respect. The r^g type, ghost spot (Pl. 18, fig. 3), has a clear white area at the base of the petal in the position which is occupied by the red spot in R^S types. Ghost spot types however not only lack anthocyanin from the petal spot, but from the entire vegetative part of the plant as well. The two alleles r^o and r^g act as complementaries in that their compound (Pl. 18, fig. 14) has a similar

expression to that of the next higher member, R^S. Hutchinson described this compound as "spot/ghost" on account of the pale whitish margin surrounding the pigmented area, as if the red spot were superposed on a slightly larger ghost. Evidence which will be presented in this paper shows that this expression is not an essential characteristic of the compound, which on certain genetic backgrounds is intensified until it is quite indistinguishable from R^S phenotypically. The higher members of the series, R^C, R^L, and R, are also, like R^S, characterized by a red petal spot, but in addition determine progressive extension of intense anthocyanin pigmentation first to the calyx and bolls, next to the leaves as well, and finally also to the petal lobe. Subsequently Hutchinson & Ghose (1937*b*) reported a seventh member of the allelic series, R^o, similar to R^L vegetatively, but, like r^o, without any petal spot, and in this respect also complementary with r^g. This, together with information obtained in the course of this investigation with *anomalum*, has led to the realization that the anthocyanin alleles may be arranged in two series, similar in vegetative expression but respectively with and without red petal spot. On this basis a new system of nomenclature of the genes has been proposed (Hutchinson & Silow, 1939). Two superscripts are attached to the main *R* symbol; the first indicates distribution of anthocyanin if present, and the second, presence or absence of spot. In the following list the new symbols are indicated, together with the old ones in brackets. As Harland (1935) has established homology of this series with the R_2 series in the tetraploid New World cottons, this series is also given the same numerical subscript.

	Petal spotted	Petal spotless
Red plant body and petal	R_2^{LS} (R)	
Red leaf	R_2^{LS} (R^L)	R_2^{LO} ($R_2{}^o$)
Red calyx	R_2^{CS} (R^C)	
Red tinged stem (i.e. basic anthocyanin present, expression variable but slight)	R_2^{AS} (R^S)	R_2^{AO} (r^o)
Green stem, ghost spot (basic anthocyanin absent)	R_2^{OS} (r^s)	

This scheme of symbolization indicates the basis of the complementary nature of the ghost spot (R_2^{OS}) and spotless (R_2^{AO} or R_2^{LO}) in giving the red spot phenotype. In the course of discussion in this paper it is proposed to refer to the phenotype of R_2^{AS} as "full spot", and to the compound between ghost R_2^{OS} and spotless R_2^{AO} as "compound spot". The latter term will also be used for compounds between ghost and spotless when such genes occur in different loci, as will be shown to be the case in *anomalum*. In cases where there is no immediate evidence as to whether a particular phenotypic grouping is composed of full spot, or compound,

or both, or where the distinction is immaterial to the point under discussion, the class will be referred to as "red spot".

From Hutchinson's earlier work it had been thought likely that the compound spot would usually be distinct in expression from the full spot allele R_2^{4S} in having a clear white marginal area around the spot (see Pl. 18, fig. 14), though he had reported the appearance of a single intensified spot/ghost plant which was hardly distinguishable from a typical full spot, and suggested that it might be possible to develop a spotless type with a complete set of *arboreum* modifiers which in compound with ghost would give a full spot phenotype. A large number of progenies which have been grown from *anomalum* interspecific hybrids have included both the ghost and spotless alleles, so considerable attention was focused on the scoring of the compound as distinct from the full spot, especially in early progenies when *anomalum* was thought to carry a full spot duplicate. The separation was found to be exceedingly difficult, and expected ratios were not attained. Spot/ghost types appeared even in progenies where only *anomalum* and Asiatic full spots had entered. These difficulties of interpretation were eventually explained by the demonstration that the *anomalum* spot is itself due to the complementary reaction between ghost and spotless, though these two genes are in different loci, and that compounds can be intensified by modifiers right up to the expression of full spot. In such cases it is now evident that there would be no point in maintaining the distinction between compound and full spot, and the data have been grouped as "red spot".

It will be shown that *anomalum* carries a duplicate anthocyanin locus. On account of sterility barriers it has not been possible to investigate the relationship of this duplicate locus with the R_1 series of New World cottons. From work in progress at this Station it has been established that the anthocyanin locus of the wild American diploid species *G. armourianum*, *aridum*, and *thurberi* is homologous with R_1 of the tetraploid American species. Skovsted (1934) has suggested that the latter are allopolyploids derived from an Asiatic species and an American wild species, or cytologically similar types. On this basis R_1 would not be expected to occur in an Asiatic genom, and it is therefore proposed tentatively to symbolize the *anomalum* anthocyanin duplicate locus as R_3.

Since it has been possible to transfer the R_3 gene from *anomalum* to the cultivated Asiatics, and obtain normal segregation, it is highly probable that the latter species also carry the R_3 locus. There is of course a possibility that the *arboreum-herbaceum* homologue of the *anomalum* R_3 chromosome has a deletion at this locus, but in the absence

of any positive evidence to this effect, it is more rational to suppose that the locus is present. Within the cultivated Asiatic species there is however no evidence of its activity. The R_2^{OS} type does not carry R_3^{AO}, nor does the R_2^{AO} type carry R_3^{OS}, since, on evidence which will be brought forward in this paper, these combinations of genes would also be complementary in duplicate loci just as they are when in the same allelomorphic series. There is no evidence that these spotless and ghost types carry the same alleles in each of their duplicate loci, since in the F_2 between them they would give 14 spot : 1 ghost : 1 spotless, whereas they actually give 2 : 1 : 1. The duplicate locus in *arboreum* and *herbaceum* must therefore carry an allele which is inactive so far as anthocyanin expression is concerned—r_3^{oo}—a new spotless allele phenotypically similar to R_2^{AO} but lacking even the basal anthocyanin episome and therefore not complementary with R_2^{OS}.

When it became known that *anomalum* carries a duplicate spot mechanism, careful watch was kept for any evidence of duplication for anthocyanin in the cultivated Asiatic species, especially in the African types *G. arboreum* forma *soudanensis* and *G. herbaceum* var. *frutescens* and var. *africanum*. None has been found.

(2) *Evidence of duplication*

With reference to anthocyanin *G. anomalum* (Pl. 18, fig. 4) is phenotypically similar to full spot R_2^{AS} types in the cultivated Asiatic species (Pl. 18, fig. 1), with four of which it has been crossed—A 8 and N 19 from *arboreum*, and O 1 and O 8 from *herbaceum*. The hybrids were backcrossed to several *arboreum* ghost types, and the progeny segregated for petal spot as follows:

Cross	Red spot	Ghost	Total	$\chi^2_{3:1}$	P
(*an.* × A 8) × A 16	8	1	9	—	—
(*an.* × A 8) × N 14	174	28	202	13·4	Very small
(*an.* × A 8) × T 3	103	35	138	0·0	0·95–0·90
(*an.* × A 8) × T 14	6	0	6	—	—
(*an.* × N 19) × T 1	37	22	59	4·8	0·05–0·02
(*an.* × O 1) × N 14	8	3	11	—	—
(*an.* × O 8) × N 5	8	1[3]	9	—	—
(*an.* × O 8) × N 14	15	7	22	—	—

The occurrence of recessive ghosts in a backcross involving two dominant phenotypes clearly indicated duplication. There was considerable heterogeneity amongst the families (χ^2 heterogeneity = 19·1, P = 0·01). Of the three larger families, one was an excellent fit to the 3 : 1 expectation, whilst the other two gave a very significant excess and deficiency of ghosts respectively. In such a wide cross and with so much sterility this was not altogether unexpected. The duplicate gene interpretation

was substantiated by subsequent breeding behaviour of spotted selections from the backcross (*an.* × A 8) to N 14, which were again backcrossed to N 14 ghost.

Plant	Red spot	Ghost	Ratio	$\chi^2_{3:1}$	$\chi^2_{1:1}$
P 1780	285	108	2·64 : 1	1·3	—
P 1781	138	43	3·21 : 1	0·1	—
P 1774	61	58	1·05 : 1	—	0·1
P 1783	94	93	1·01 : 1	—	0·0
P 1795	111	90	1·23 : 1	—	2·2
P 1797	47	52	0·90 : 1	—	0·3

One in three of these would have been expected to be double heterozygotes, and the other two single heterozygotes giving 1 : 1. A seventh plant, P 1772, was selfed and gave 51 red spot : 5 ghost, indicating that it also was a double heterozygote.

Some selections from the third backcross of (*an.* × A 8) to A 8 were also of interest in this connexion. A 8 being homozygous full spot, the backcross progeny were all phenotypically spot. Two selections when selfed gave the following:

Plant no.	Red spot	Ghost
14,854	54	9
14,864	55	3

These two plants evidently were heterozygous in both the A 8 full spot locus and in the duplicate locus, giving approximations to the 15 : 1 ratio. One red spotted segregate, no. 9992, from the first of these selfed progenies, also turned out to be a double heterozygote like its immediate parent. It was crossed with A 16 ghost, and the five progeny which were grown were all red spotted. On the basis of duplicate factors, one-third of these spotted progeny should have been double heterozygotes, and two-thirds single heterozygotes. They were crossed, as mentioned in § III in connexion with corolla colour analysis, back to their 9992 parent, the double heterozygote, with which the double heterozygotes should have given a 15 : 1 ratio, and the single heterozygotes a 7 : 1. Two of the families appeared to be of the former type, and three of the latter:

Cross	Red spot	Ghost spot	Ratio	$\chi^2_{15:1}$	$\chi^2_{7:1}$
P 4098 × 9992	50	4	12·5 : 1	0·1	1·3
P 4126 × 9992	78	4	19·5 : 1	0·3	4·4
P 4127 × 9992	64	8	8·0 : 1	2·9	0·1
P 4128 × 9992	68	12	5·7 : 1	10·5	0·5
P 4129 × 9992	66	7	9·4 : 1	1·4	0·6

Plant 9992 was also crossed with N 5 ghost, and one of its spotted progeny gave further evidence of duplication. On selfing it gave 49 red spotted and 3 ghost spot progeny (15 : 1) and a backcross to ghost gave 52 red spot and 12 ghost (3 : 1). Abundant other evidence of duplication for spot will appear incidentally later in this paper.

(3) *The R_2 allele in* anomalum

When it became known that the *anomalum* spot is a duplicate of that in *arboreum* and *herbaceum*, it was necessary to determine the nature of the allele which *anomalum* carries in the *arboreum* anthocyanin locus (R_2). The fact that ghost segregates appeared in the backcrosses of (*an.* × Asiatic full spot) to ghost R_2^{OS} types indicated that the R_2 allele from *anomalum* must be either ghost or a lower member. Accordingly four of the ghost segregates, nos. P 1791, P 1801, P 1818, and 11,319, from the above (*an.* × A 8) × N 14 backcross, were crossed to H 10 spotless R_2^{AO}. They gave a total of 316 plants all with the red spot phenotype characteristic of the ghost-spotless compound, indicating that their ghost parent must have been homozygous for the ghost allele. That the *anomalum* R_2 contribution is a ghost allele was also confirmed by the following line of evidence. The hybrid (*an.* × A 8) was itself backcrossed to H 10 spotless R_2^{AO}, and all 550 progeny grown were red spotted. This should be contrasted with the 3 red spot : 1 ghost segregation which occurred on backcrossing the same hybrid to ghost types. Two plants, nos. 11,703 and 11,757, were selected from the H 10 backcross for further testing. On again backcrossing they gave 1 : 1 and not 3 : 1 ratios, indicating that the duplicate spot from *anomalum* was not present in these selections. Attention need therefore be directed to the R_2 locus only, at which, in addition to the spotless allele derived from H 10, these selections might have carried full spot from A 8, in which case a backcross to ghost would have given all spotted progeny; actually about 50 % ghost were obtained, so their second R_2 member must have been ghost, as was confirmed by backcrossing these same two selections again to spotless. This ghost could only have been derived from *anomalum*. The actual numbers obtained in these backcrosses of 11,703 and 11,757 (R_2^{AO} R_2^{OS}) were as follows:

Cross	Segregation		$\chi^2_{1:1}$	P
11,703 backcrossed ghost (R_2^{OS})	220 compound spot (R_2^{OS} R_2^{AO})	149 ghost (R_2^{OS} R_2^{OS})	13·7	Very small
11,757 ditto	25 ditto	21 ditto	0·3	0·7–0·5
11,703 backcrossed spotless (R_2^{AO})	52 spotless (R_2^{AO} R_2^{AO})	35 compound (R_2^{AO} R_2^{OS})	3·3	0·1–0·05
11,757 ditto	172 ditto	139 ditto	3·5	0·1–0·05
Total	469 R_2^{AO}	344 R_2^{OS}	20·8	Very small
Heterogeneity			1·6	0·7

In all four progenies plants carrying the introduced ghost gene R_2^{SO} of *anomalum* origin were fewer in number than expected. The families formed a homogeneous group, and although in only one of them was the deficiency actually significant, in total there was a very significant

shortage of plants carrying the introduced gene—a frequent tendency to which attention will be directed again later.

The plants 14,854 and 14,864, to which reference has already been made in the preceding section, also showed that the R_3 allele in *anomalum* is ghost. They were selections from a third backcross of (*an.* × A 8) to A 8, and threw ghosts in their selfed progeny. Since A 8 is homozygous R_2^{AS}, the R_2^{OS} gene must have come from *anomalum*.

(4) *The complementary nature of the duplicate in* R_3, *and its pleiotropic "gold petal" effect*

The knowledge that *anomalum* carries ghost in the R_2 locus raised the possibility that the duplicate might not necessarily be a full spot allele on its own account, but a spotless gene giving the spot phenotype in association with ghost. Critical evidence is available from the backcross of the hybrid (*an.* × H 10 spotless) to H 10. If the duplicate were a full spot, it should have given a 3 : 1 duplicate gene ratio, since the *anomalum* R_2^{OS} would also have acted as a spot phenotype on the H 10 spotless background. Actually 29 red-spotted and 28 spotless plants were obtained, clearly a 1 : 1 ratio, which can only be interpreted on the basis that the *anomalum* spot is due to complementary alleles, respectively ghost and spotless, in duplicate loci, as follows:

Parents	*anomalum* spot $R_2^{OS}R_2^{AO}$		H 10 spotless $R_2^{AO}r_3^{OO}$	
F_1	spot $R_2^{OS}R_2^{AO}R_3^{AO}r_3^{OO}$			
F_1 gametes	$R_2^{OS}R_2^{AO}$	$R_2^{OS}r_3^{OO}$	$R_2^{AO}R_3^{AO}$	$R_2^{AO}r_3^{OO}$
Phenotypes in B.C. to H 10 $R_2^{AO}r_3^{OO}$	Spot	Spot	Spotless	Spotless

It was not practicable to grow a larger backcross family, since many *herbaceum* types, including H 10 which was the only spotless available at the time of these experiments, are not well adapted to the local climate. The difficulty of obtaining a good stand was increased by the high sterility of the hybrid. There is however further very conclusive evidence of the spotless nature of the *anomalum* R_3 allele, and this was derived from the hybrid between *anomalum* and the *arboreum* ghost type A 16. Since *anomalum* also carries ghost in the R_2 locus, this hybrid was homozygous for R_2^{OS}, as was confirmed by a backcross to H 10 spotless, in which all 241 progeny were spotted. On this account all spotted progeny in recurrent backcrosses to A 16 were of the same main genotype for spot as the original (*an.* × A 16) hybrid. This point is of considerable importance in facilitating subsequent analysis.

Although *anomalum* itself (Pl. 18, fig. 4) has only the faintest suggestion of pink on the petal lamina, all of its hybrids were characterized by a distinct tinge of pink overlying the otherwise yellow corolla (Pl. 18,

figs. 5–7), giving a gold or bronze appearance to the petal which had never before been seen in Asiatic cottons. The degree of expression of gold was very different in the several hybrids which have been grown, and was strongest in the A 16 cross (Pl. 18, fig. 7). Practically all red-spotted segregates in each of the successive backcrosses to A 16 were also characterized by gold appearance to some extent, whereas all ghost segregates had a perfectly clear yellow petal as in A 16 itself (Pl. 18, fig. 3). This association of spot with gold petal has been carefully followed through wherever possible. In the early days of this investigation, before the significance of gold petal expression was fully appreciated, a first backcross of (*an.* × A 16) to A 16 was classified as 20 red spot : 30 ghost. All of the latter were absolutely clear yellow petal, and in progeny derived from these and other ghost segregates gold petal has never appeared. Fifteen of the red-spotted plants were noted as having the gold expression in varying degrees from intense to very faint, and only one was scored as clear yellow petal. For the other four there was no definite record of gold, attention having been distracted in the case of two of them to the expression of the yellow depressor. The remaining two had very small abnormal petals on which it was difficult to determine colour exactly, but gold occurred in the selfed progeny of one of them. Three of the red-spotted gold-petalled plants of this first backcross were again backcrossed to A 16, giving 11 red spot : 6 ghost. All of the latter were yellow petal, whilst 8 of the red spot were classified as gold. Of the remaining three not recorded for gold, one had small abnormal petals; the other two in subsequent progeny showed gold petal red spot, so again must have been gold petal genotypically if not phenotypically.

Seven of the red-spotted plants of the second backcross when backcrossed again to A 16 (third backcross) gave 49 red-spotted all with gold, and 60 ghost all clear yellow petal. Three red-spotted gold-petal plants of the third backcross, nos. 15,094, 15,095 and 15,099, were selfed, and gave respectively 46 : 23, 22 : 9, and 14 : 8 red-spotted and ghost-spot segregates. All of the latter were clear yellow, and all except one of the red-spotted plants were gold petalled. This exception which occurred in the progeny of plant no. 15,094 was of the same type as the remainder of the family, but could not be rechecked when its aberrant scoring was noted. However 15,094 was again selfed and backcrossed, giving in the respective families 39 : 4 and 20 : 28 red-spotted and ghost-spot plants. All of the spotted class showed gold petal. It is therefore very likely that the exception noted in the earlier sowing was incorrectly graded. The gold was still variable in intensity in the third and fourth backcrosses,

though present in some degree in every one of the spotted segregates. It may reasonably be concluded that gold petal is constantly associated with the *anomalum* red-spot class, and it has never been observed in ghost segregates.

In this connexion the following evidence is of particular significance. At the time when the above constant association was first suspected, a red-spotted selection, no. 14,544, from the third backcross of (*an.* × H 10) to N 14 was available. N 14 has both white petal and ghost spot; 14,544 also had a white corolla, and its breeding behaviour had shown that it was also homozygous for ghost in the R_2 locus, and heterozygous in the R_3 locus for the spotless allele derived from *anomalum*, to whose interaction with ghost its red-spot phenotype was due. Yet no gold was visible on the petal, as a result of the absence of yellow flavone. It was therefore of interest to determine if gold were really latent in this plant, so it was crossed with A 16 ghost, homozygous for yellow corolla. The progeny consisted of 85 with ghost spot and absolutely clear yellow petals, and 83 with red spot and a distinct though faint tinge of gold. The low intensity of gold was later found to be a result of heterozygosity for white corolla; the important point is that gold was present on all plants which showed the *anomalum* spot, and in that respect this evidence strongly supports the conclusions drawn from the A 16 backcross lines.

Reverting to the latter, a gold-petalled red-spotted plant was selected from the second backcross of (*an.* × A 16) to A 16. This plant must have been homozygous for ghost, since both *anomalum* and A 16 carry this gene in the R_2 locus, and heterozygous for the *anomalum* R_3 spotless allele to which its red-spot phenotype was due. This was crossed with H 10 spotless, and a small progeny grown. As a result of the interaction with ghost, all progeny were spotted, and two with gold petals, P 2381 and P 2386, were selected. These were backcrossed to H 10, when it was found that not only were some of the red-spotted progeny gold-petalled, but some of the spotless segregates also showed gold of varying intensities. The scoring was as follows:

	Red spot		Spotless		$\chi^2_{1:1}$	
Cross	Gold	No gold	Gold	No gold	Spot	Gold
P 2381 × H 10	40	23	39	32	0·5	4·3
P 2386 × H 10	116	140	96	126	2·4	6·1

The families were somewhat dissimilar in their segregation (χ^2 heterogeneity = 11·14, P = 0·01); in the first family the spot segregation was near the 1 : 1 expected (63 : 71), but in the second family there was a fairly large excess of spotted plants (256 : 222). In the first family there was

a significant excess of golds (79 : 55) and in the second a significant deficiency (112 : 266), and to this the heterogeneity between the families was due. No explanation of the aberrant ratios can be brought forward. It can only be mentioned that other obviously monogenic segregations were also frequently distorted in this hybrid material, and in no case has breeding behaviour been found to be conflictory with the genic interpretation suggested. The point of importance is that both of the above families agreed in showing complete independence of spot and gold (χ^2_L for total deviation 0·4, $P = 0{\cdot}5$, for heterogeneity 0·4, $P = 0{\cdot}5$). This independence was in striking contrast to the segregations previously discussed, and nothing like the gold spotless type, which is shown in Pl. 18, fig. 13, had ever previously been seen in the Asiatic cottons. The basal area of the petal, corresponding to the portion occupied by red spot or ghost spot in other cottons, was clear yellow, but the remainder of the petal lamina was of the same gold tinge as had previously been associated with the *anomalum* spotted types in the A 16 backcross lines. The occurrence of gold spotless types, the complete independence of gold from the R_2 spot segregation in this H 10 backcross, and its absolute association with the *anomalum* R_3 spot phenotype in the A 16 backcross lines, can only be explained on the basis that the *anomalum* R_3 allele is itself spotless, gives the complementary spot reaction with ghost, and is responsible for the development of gold petal. This conception is not in any way different from that of the various vegetative red effects which have been shown to be associated with both spot and spotless members of the R_2 series in the cultivated Asiatics. The symbol R_3^{GO} is proposed for this allele—*G* in the superscript signifying the presence of basic anthocyanin with gold petal as its special expression; and *O* signifying that no spot is present. The above segregations would therefore be interpreted as follows:

Parents	*anomalum* spot $R_2^{OS}\ R_3^{GO}$		A 16 ghost $R_2^{OS}\ r_3^{OO}$	
F_1 genotype	$R_2^{OS}\ R_2^{OS}\ R_3^{GO}\ r_3^{OO}$			
Gametes of F_1 (and of spotted selections from recurrent B.C.'s to A 16)	$R_2^{OS}\ R_3^{GO}$		$R_2^{OS}\ r_3^{OO}$	
Phenotypes in 1st B.C. to A 16, $R_2^{OS}\ r_3^{OO}$ (and in recurrent B.C.'s of spotted selections)	Spot, gold petal		Ghost, no gold	
Phenotypes in progeny of spotted, gold petal selections (same genotype as F_1) crossed with H 10, $R_2^{AO}\ r_3^{OO}$	Spot, gold petal		Spot, no gold	
Gametes of spotted, gold petal selections P 2381 and P 2386, $R_2^{AO}\ R_2^{OS}\ R_3^{GO}\ r_3^{OO}$	$R_2^{AO}\ R_3^{GO}$	$R_2^{AO}\ r_3^{OO}$	$R_2^{OS}\ R_3^{GO}$	$R_2^{OS}\ r_3^{OO}$
Phenotypes in B.C. of P 2381 and P 2386 to H 10, $R_2^{AO}\ r_3^{OO}$	Spotless, gold	Spotless, no gold	Spot, gold	Spot, no gold

P 2381 and P 2386 were also selfed. On the above basis expectation would be:

Red spot, gold petal	Red spot, no gold	Ghost spot, no gold	Spotless, gold petal	Spotless, no gold
9	3	1	3	1

Actual figures were

	Red spot			Ghost spot	Spotless			
	Gold petal	No gold	Gold not scored	No gold	Gold petal	No gold	Gold not scored	Total
P 2381 selfed	74	29	(3)	3	43	15	(2)	169
Expected	*95·1*	*31·1*		*10·6*	*31·7*	*10·6*		*169·1*
P 2386 selfed	210	43	(4)	20	64	22	(4)	367
Expected	*206·4*	*45·9*		*22·9*	*68·8*	*22·9*		*366·9*

In the first family the fit was not very good. χ^2 for the three main classes—red spot, ghost and spotless, was high, 13·8, and most of this was due to a deficiency of ghost and an excess of spotless. Within the spotless class the ratio gold : no gold was fairly good, but was somewhat out within the red-spotted class. Expectation was however very closely realized in the second family.

From this material R_3^{GO} has been established in the homozygous condition in association with R_2^{4O}, giving gold spotless. Three of the deepest gold types, one from P 2381 selfed, and two from P 2386 selfed, all scored as intense, were tested for homozygosity. Two of these selections when selfed gave 49 and 43 plants respectively, all of which were spotless, with medium gold to intense gold or intense pink petal. The third plant, no. 6144, although somewhat more intensely gold than its homozygous sib, turned out to be heterozygous for R_3^{GO}. It will be remembered that P 2381 and P 2386 were the result of two backcrosses to A 16 followed by one backcross to H 10. The latter strain will be shown later to be low in content of gold intensifying modifiers relative to A 16, so that wide segregation for the modifiers would be expected, and this would to some extent mask the distinction in gold intensity between R_3^{GO} homozygotes and heterozygotes.

R_3^{GO} has also been established in the homozygous phase as a gold-petalled red-spotted type in association with R_2^{OS} ghost, i.e. of the same main anthocyanin gene constitution as *anomalum* itself, but on a yellow-petalled *arboreum* genotype. The same seven spotted segregates from the second backcross to A 16 ghost, which as reported above were selected for further backcrossing, were also selfed. In all they gave 37 red-spotted and 20 ghost segregates. All of the red-spotted plants had gold petal, ranging from intense to very intense. Six of the latter type were selfed. Four of them were heterozygotes, giving in all 42 red spot, gold : 21

ghost spot, no gold. Two others were homozygotes. One of these, 15,240 (Pl. 18, fig. 9), gave a small selfed progeny of eleven plants, all red-spotted, intense to very intense gold petal. Its homozygosity was further confirmed by backcrosses to ghost, which gave 85 progeny, all red-spotted. The other homozygote, 15,245, did not give any selfed seed, but in crosses with ghost gave 145 progeny, all red spotted. These red-spotted $R_2^{OS}\ R_3^{GO}$ homozygotes were more intensely gold than the spotless $R_2^{AO}\ R_3^{GO}$ ones, since the latter carried part of the low-grade gold genotype of the H 10 strain.

(5) *Variability in gold petal expression*

In presenting the evidence for the constitution of *anomalum* with reference to corolla colour and anthocyanin each particular factor has been discussed separately. Whilst this greatly facilitates analysis, it does not give any idea of the extreme variability within the early segregating progenies, nor of the great differences in range of variability between those derived from different hybrids. Not only was there the wide range in shade of yellow, even in the absence of the main gene segregation, as was shown in Table 4, but superposed on this there was also a wide segregation for gold or pink, together with great variability in size and intensity of the petal spot, ranging from very minute to one which covered about half of the petal surface. Attention will first be directed to the variability in gold expression. Its association with the *anomalum* anthocyanin allele has been illustrated from material derived from the *an.* × A 16 hybrid, of which a small first backcross was cited. Later a larger first backcross was grown, and its classification for yellow corolla grade and for pink is shown in Table 9.

Table 9. *Segregation for yellow corolla grade, and for spot and the associated gold or pink expression, in the first backcross* (an. × *A* 16) *to A* 16

		Yellow corolla grade								
		8	7	6	5	4	In-definite*	Not scored	Total	
			9							
Red spot	Petal very pink or with deep pink edge	.	1	.	1	1	2	.	5	130
	Medium pink	.	2	.	4	4	1	.	11	
	Faint pink	.	.	.	2	5	.	.	7	
	Very int. or int. gold	.	4	.	.	.	12	.	16	
	Medium gold	2	11	2	1	.	6	.	22	
	Faint gold	.	7	1	.	.	8	.	16	
	Very faint gold	.	5	1	.	3	3	.	12	
	Gold not recorded	.	.	.	.	.	.	41	41	
Ghost spot	No gold	29	18	1	3	.	.	17	68	105
	Gold not recorded	.	.	.	.	.	.	37	37	

* Yellow grade obscured by gold or pink.

This table adequately demonstrates the complexity of segregation of early backcrosses. Scoring of pink or gold on the corolla was difficult. Four rather indefinite grades were used—very intense to intense, medium, faint, and very faint. Very intense gold corresponds to fig. 9 in Pl. 18, medium gold corresponds to fig. 10, and very faint gold to fig. 11. Fig. 12 represents the intense pink grade, though in this family many of the pinks were less uniformly coloured over the petal surface, with a strongly marked tendency to deepening towards the petal edge where the petal had been exposed to the sun in the bud stage. Although the scoring for any particular plant was somewhat variable, it was fairly consistent. The greater part of the variability within a plant appeared to be due to differences in exposure of the bud to the direct sun. It was also difficult to score accurately the wide segregation in corolla grade on account of the variation in the overlying pink or gold. Pink as distinct from gold was not merely the same intensity of anthocyanin overlying a paler grade of yellow, but there was a real intensification of anthocyanin pigment in the petal lobe, as may be seen by comparing figs. 10 and 12 in Pl. 18; the plants from which these figures were painted were sibs of known and similar constitution except for the presence in the pink plant of the *Ydp* gene which has been discussed in the corolla colour section, and which will be shown to be responsible for the distinction between gold and pink.

The fact that in the A 16 backcross lines there were only two main anthocyanin genotypes segregating facilitated recognition of the association between gold and the spotted class, though from Table 9 it will be seen that there was great variability in expression of gold within this class. Since so many of the backcrosses involving other *arboreum* and *herbaceum* types did not manifest gold petal to anything like the same extent as did backcrosses to A 16, doubts arose at one time as to whether gold had really come in from *anomalum*, and to make sure that A 16 was not in some obscure way the source of it, A 16 itself was crossed with 14,859, a plant carrying the gene *Ydp* which had been found to accentuate the expression of gold to pink; the thirteen plants derived from this cross showed no sign of pink, confirming that A 16 was not the source of gold. It was eventually realized that A 16 was at a rather higher modifier level than most other types used in this study, but this situation in itself was not sufficient to explain all the observed variability in expression of gold, and an attempt was therefore made to determine what other factors were involved.

The two red-spotted plants 15,240 and 15,245, from the second back-

cross selfed of (*an.* × A 16) to A 16, and which had been found to be homozygous for the gold spotless allele, were used. They themselves were graded as "very intense gold", and the selfed progeny of one of them graded "intense to very intense", mostly the latter. All were on a homozygous yellow corolla background (Y_a Y_a) (Pl. 18, fig. 9). Backcrosses of these two plants to A 8 (81 progeny observed) gave an estimate of the expression of heterozygous gold on homozygous yellow corolla. They graded "medium to intense", mostly the former, as also do red-spotted segregates in backcrosses of heterozygous selections in the A 16 backcross lines (Pl. 18, fig. 10). The indication that gold is not completely dominant therefore accounts for some of the variability of gold in selfed lines carrying R_3^{GO}.

The same two homozygous gold spotless plants were also crossed with N 5 and N 14. N 5 is a ghost spot pale yellow corolla Y_a^P strain, and N 14 is a ghost spot white corolla y_a type. The progeny gave an estimate of heterozygous gold on a heterozygous yellow corolla background. Where N 5 was involved (100 progeny observed), and corolla constitution of the progeny was therefore Y_a Y_a^P, gold was reduced to very faint (Pl. 18, fig. 11), being hardly discernible except against clear yellow petal types for contrast, in which case there was then no doubt as to its presence. Examination of the paintings will show however that it would not always be easy to see this faint expression in a family with a wide segregation for gold and yellow. In progenies involving N 14 (130 plants examined) gold, though discernible, was even fainter. The hybrids (15,240 × N 14), (15,245 × N 14), (15,240 × N 5) and (15,245 × N 5) were extensively selfed in connexion with R_3 linkage studies, and observations were also made on gold expression in these F_2 progenies. The hybrids were of R_2^{OS} R_2^{OS} R_3^{GO} r_3^{OO} constitution, so that all spotted progeny were of necessity compounds between R_2^{OS} and R_3^{GO}, but all-white corolla segregates (y_a y_a) which carried red spot lacked any trace of gold on the petal. As expected, the red-spotted yellow petal segregates showed a range from very intense to very faint gold, since they were either homozygous or heterozygous for both Y_a and R_3^{GO}. Some but not all of the spotted pale corolla (Y_a^P Y_a^P) segregates, when contrasted against ghost segregates, showed an extremely slight indication of gold. The pales ranged from grades 3–1½ in these families, and there was a tendency for the gold to be somewhat more definite on the higher grade backgrounds. All this evidence showed that the expression of gold is very dependent on corolla colour constitution.

The plants 15,240 and 15,245 were also crossed with 14,859, a selection

from a third backcross of (*an.* × A 8) to A 8, lacking R_3^{GO}, homozygous for $Y_a\ Y_a$, and heterozygous for *Ydp*, the independent gene which reduces the expression of yellow corolla from grade 7 or 8 to about grade 4 (see Pl. 18, fig. 8). In outcrosses this gene has been shown to give a perfectly clear 1 : 1 segregation. The progeny of 14,859 with 15,240 and 15,245 would all be heterozygous $R_3^{GO}\ r_3^{OO}$, homozygous $Y_a\ Y_a$, and; if *Ydp* had not been segregating, should all have shown medium to intense gold. However only 50% of the progeny showed this, the remaining 50% showing a very striking rose pink coloration over the entire petal, a further character never before seen in Asiatic cottons. This type is shown in Pl. 18, fig. 12, and one of its gold sibs in Pl. 18, fig. 13. The classes were remarkably distinct, and scoring gave:

Cross	Gold	Pink
14,859 × 15,240	46	49
14,859 × 15,245	35	34

Since 14,859 was known to be heterozygous for *Ydp*, it was thought that this new pink might be the expression of gold on a depressed yellow corolla. To test this hypothesis, two gold and two pink selections were each crossed to A 8 (clear yellow, homozygous), and small progenies grown.

Cross	Gold	Pink	Full yellow	Low-grade yellow
19,176 = pink, × A 8	5	7	7	2
19,179 = pink, × A 8	4	6	7	5
19,217 = gold, × A 8	10	0	11	0
19,226 = gold, × A 8	6	0	18	0

The pink plants evidently carried *Ydp*, whilst the gold ones did not, confirming that it is the interaction between gold and *Ydp* which accounts for the development of pink. This however only appears to hold on a homozygous yellow corolla background. There is evidence that when yellow corolla Y_a is heterozygous, although *Ydp* is able to lower the expression of yellow, it does not change gold to pink. A particular example is a plant P 1822, a selection from the second backcross of (*an.* × H 10) to N 14; this plant which was spotted, must at least have been heterozygous for R_2^{OS} because of its N 14 parentage; the full spot gene R_2^{AS} not having entered this series, its spot might have been due to either R_2^{AO} from H 10 or to R_3^{GO} from *anomalum*, or to both. R_3^{GO} was definitely present because P 1822 was very faintly gold. When backcrossed again to N 14 it gave 35 spotted : 63 ghost, definitely not a duplicate gene 3 : 1 backcross ratio, thus eliminating the possibility of spotless genes in both loci. P 1822 must therefore have been $R_2^{OS}\ R_2^{OS}\ R_3^{GO}\ r_3^{OO}$. The grading of P 1822 at about 4–5 suggested that it carried

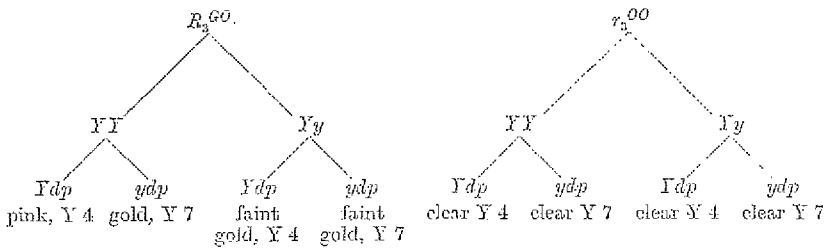
Ydp. There is ample evidence of this from several sources. For instance, P 1822 was crossed with N 44, a Chinese pale petal type of the constitution $Y_a\ Y_a\ Y_b{}^P\ Y_b{}^P$, and the 76 progeny were scored as follows:

13 plants with pink (medium-intense); Y grades 4, 3, or not visible below intense pink.
15 plants with gold (faint—very intense); Y grades 7–3, or not visible below intense gold.
48 plants with no gold or pink; Y grades 7–3.

Amongst the 48 clear yellow, grading was as follows:

Y grades				
7	6	5	4	3
9	8	5	21	5

The pink-gold segregation implied a segregation for *Ydp* acting on $R_3{}^{GO}$ in the presence of a full yellow corolla potentiality. From other crosses it was already known that heterozygosity for $Y_b{}^P$ does not reduce gold expression as does heterozygosity in the Y_a locus. The presence of *Ydp* was confirmed by the downward grading of the clear yellows as low as grades 4 and 3, though the expected 1 : 1 for full yellow : depressed yellow was somewhat obscured by the complex modifier constitution of P 1822, with three species in its pedigree. The actual segregation expected in this progeny was:



or, in a family of 76 plants, 9·5 pink, 9·5 gold, 19 faint gold, 38 no gold. Scoring for gold was not easy in those with only a faint expression, and the deviation observed may have been due either to the scoring as clear yellow of some plants which were genotypically faint gold, or to deficiency of $R_3{}^{GO}$ segregates, which was also observed in the cross P 1822 × N 14, $R_2{}^{OS}\ r_3{}^{OO}$, which gave 35 red spotted : 63 ghost.

It is clear that P 1822 carried both $R_3{}^{GO}$ and *Ydp*, yet it showed only faint gold on depressed yellow, with only the very faintest suggestion of pink in the gold, and that mostly in the petal edge. From its N 14 parentage, P 1822 was heterozygous for y_a, and it is to this fact that the failure of *Ydp* to intensify gold to pink is attributed.

P 1822 ($R_2{}^{OS}\ R_2{}^{OS}\ R_3{}^{GO}\ r_3{}^{OO}$, $Y_a\ y_a$, *Ydp ydp*) was also crossed with 15,240 and 15,245 ($R_2{}^{OS}\ R_2{}^{OS}\ R_3{}^{GO}\ R_3{}^{GO}$, $Y_a\ Y_a$, *ydp ydp*). All of the

progeny would have carried R_3^{GO}. Half of them should have been heterozygous for y_a, and therefore have shown only very faint signs of gold; of the other half homozygous for Y_a, only half should have carried Ydp and therefore shown pink, whilst the other half should have shown medium to very intense gold. Actual scoring was as follows:

	Pink	Gold					Total
		Very intense	Intense	Medium	Faint	Very faint	
	17	1	2	4	6	16	46
Expected	*11·5*		*11·5*			*23*	*46*

Expectation was reasonably well satisfied. The pinks were all of more or less uniform type similar to those in 14,859 × 15,240. The latter were all heterozygous $R_3^{GO}\ r_3^{GO}$, yet in the family here under discussion half of the pinks should have been homozygous $R_3^{GO}\ R_3^{GO}$, implying that the maximum expression of pink is obtainable in the heterozygote. Selfed progeny from nine of the plants from P 1822 × 15,240 were of particular interest in confirming these indications. Their segregation is shown in Table 10.

Table 10. *The influence of corolla colour genotype on gold expression of the R_3^{GO} allele*

Plant no. and characters	Segregation in selfed progeny										Indicated parental genotype
					Pink	Gold					
						Very intense	Intense	Medium	Faint	Very faint	
	R	*r*	Y_a	y_a							
P 2951 Y 4, pink	45	0	45	0	29	2	9	.	5	.	$R_3^{GO}\ R_3^{GO}$, $Y_a Y_a$, *Ydp ydp*
P 2953 Y 4, pink	9	0	9	0	2	2	5	.	.	.	,, ,, ,,
P 2958, —, pink	21	0	21	0	9	2	5	3	2	.	,, ,, ,,
P 2955 Y 5, pink	20	7	27	0	6	2	4	2	5	1	$R_3^{GO}\ r_3^{GO}$, $Y_a Y_a$, *Ydp ydp*
P 2954 Y 6, intense gold	15	0	15	0	.	8	.	3	4	.	$R_3^{GO}\ R_3^{GO}$, $Y_a Y_a$, *ydp ydp*
P 2952 Y 6, faint gold	17	0	12	5	.	.	3	4	4	1	$R_3^{GO}\ R_3^{GO}$, $Y_a y_a$, *ydp ydp*
P 2957 Y 6, faint gold	28	0	23	5	.	.	3	7	9	4	,, ,, ,,
P 2956 Y 4, very faint gold	11	1	8	4	.	.	2	.	1	5	$R_3^{GO}\ r_3^{GO}$, $Y_a y_a$, *Ydp ydp*
P 2959 Y 4, very faint gold	20	6	23	3	1	.	2	7	5	2	,, ,, ,,

P 2951, P 2953, and P 2958 were, from their breeding behaviour, homozygous for R_3^{GO} and Y_a, and also carried Ydp; their origin indicates that they could only have been heterozygous for that gene. They themselves were pink on a low grade yellow as expected (P 2958 was so intensely pink as to obscure yellow grade) and those of their progeny

which carried the depressor should also have been pink. None of these pinks, which incidentally were not very variable, were appreciably deeper than those of 14,859 × 15,240, which were all heterozygous for *Ydp*. Whether any homozygous depressors occurred in these families is not known; it will be noticed that the largest of them gave 29 pink : 16 gold, much the same distorted 3 : 1 as was discussed earlier in connexion with the depressor itself. Those of their progeny lacking the depressor should have been very intense to medium gold. Some however went down to faint, which is not usual on a homozygous $Y_a\ Y_a$ background. This, however, may be accounted for by the presence of other modifiers probably from the original H 10 parent of the *anomalum* hybrid. *Herbaceum* types are known, from Hutchinson's (1932 *b*) work, to lack general anthocyanin intensifiers, and that H 10 lacks gold petal intensifiers in particular was shown both by the fact that the hybrid (*an.* × H 10) (Pl. 18, fig. 6) was considerably less pink than (*an.* × A 16) (Pl. 18, fig. 7), though both were of comparable main gene constitution, and also by the fact that 6144, an $R_3^{GO}\,r_3^{OO}$ plant to which reference has already been made, gave nearly as faint gold when crossed with H 10 $Y_a\ Y_a$ as it did when crossed with N 14 $y_a\ y_a$, though with A 16 $Y_a\ Y_a$ the gold grade was medium. 14,544, to which reference has also been made, gave further evidence of this. It has been shown to be of $R_2^{OS}\,R_2^{OS}\,R_3^{GO}\,r_3^{OO}$ constitution, and had white corolla. On crossing with A 16 (ghost, yellow corolla) it had given 83 red-spotted segregates, with gold faint on account of heterozygosity for y_a, and 85 clear yellow ghost segregates. But on crossing with H 10 (spotless, yellow corolla) it gave 169 red-spotted progeny, on none of which gold was visible, although about half of these must have been carrying the R_3^{GO} allele. Evidently modifiers carried by H 10 further reduced the expression of this allele even beyond the very faint which would have been expected on these $Y_a\ y_a$ heterozygotes. Whether these gold modifiers were the same as those which brought down the corolla grade in the backcross to H 10 by about ½–1 grade below that in the family involving A 16 (Hutchinson, 1931, has shown that *herbaceum* carries yellow reducers), or whether it was the general anthocyanin reducers which were responsible, it is not possible to say. That gold petal is highly susceptible to intensification and reduction is very clear. For instance, in the homozygous $R_2^{AO}\,R_2^{AO}\,R_3^{GO}\,R_3^{GO}$ progenies whose establishment has already been discussed, and from which *Ydp* was absent, the intense golds in several cases ranged over to a definite intense pink, though it was not quite the same as in the more uniformly pigmented *Ydp* type, tending to be more intense at the

petal edge. By bagging flowers on a pink-petalled plant, it was also shown that sunlight is essential to the formation of pink, since these bagged flowers were almost pure yellow.

P 2955 in Table 10 only differed from the above three plants in being heterozygous instead of homozygous for R_3^{GO}, but was as pink as they were. P 2954 was, like the first three plants considered, homozygous for R_3^{GO} and Y_a but, since it lacked *Ydp*, remained intense gold. P 2952 and P 2957 were homozygous R_3^{GO}, but heterozygous $Y_a\ y_a$, and therefore only faint gold. P 2956 and P 2959 were heterozygous $R_3^{GO}\ r_3^{OO}$, $Y_a\ y_a$. That they carried *Ydp* was evident, in the case of P 2959 because one of its progeny was definitely pink and in others with gold the underlying yellow was definitely of the low grade 4 depressor type, and in the case of P 2959, although in its small progeny no pinks appeared, again depressor yellow could be seen underlying some of the golds. P 2956 and P 2959 then, although they carried *Ydp*, were not pink but only very faint gold, because they were heterozygous for y_a.

Heterozygosity in the Chinese pale Y_b locus does not appear to affect gold expression, since in a cross of 15,240 × N 44 (only two progeny examined, of constitution $R_3^{GO}\ r_3^{OO}$, $Y_a\ Y_a$, $Y_b\ Y_b^P$), the gold expression was medium to intense as in the case of heterozygous R_3^{GO} in other homozygous yellow corolla families previously examined. Heterozygosity in the *anomalum* pale Y_c locus does not reduce gold expression either; 14,854 was a selection from the third backcross of (*an.* × A 8) to A 8, which was later found by its breeding behaviour to be heterozygous for both R_3^{GO} and Y_c^P; it showed medium gold on a full yellow background; one of its progeny derived by selfing, no. 9992, a $Y_c^P\ Y_c^P$ segregate of grade 3 yellow, was also shown to carry R_3^{GO}, but was not gold in appearance.

The factorial basis of the interaction between the main corolla colour factors and the *anomalum* R_3^{GO} allele, as deduced from these findings, is summarized in Table 11. In view of its complexity it is not surprising that at one time prospects of analysing the wide colour segregation of early generations seemed remote, especially as the various crosses behaved so differently. First of all it is worth looking back at the (*an.* × A 16) first backcross to A 16 in Table 9. It may now be seen that this should have segregated for corolla colour, in terms of the yellow depressor, into two equal-sized groups, one grouped around grade 7 and the other around grade 4. The interspecific modifier segregation obscured this to a considerable extent. Moreover yellow grading in the red-spotted class was consistently higher than in the ghost class, and in the latter

Table 11. *The interaction between R_3^{GO} and the main factors affecting corolla colour*

Phenotype	Yellow corolla constitution: Genotype				Gold petal expression	
	Y_a locus	Y_b locus	Y_c locus	*Ydp*	R_3^{GO} homozygous	R_3^{GO} heterozygous
Yellow	Homo. $Y\ Y$	(Homo. $Y\ Y$)	(Homo. $Y\ Y$)	(Homo. *ydp ydp*)	Gold; very intense to int.	Gold; intense to medium
Yellow	Het. $Y\ Y^P$	(Homo. $Y\ Y$)	(Homo. $Y\ Y$)	(Homo. *ydp ydp*)	—	Gold; faint
Yellow	Het. $Y\ y$	(Homo. $Y\ Y$)	(Homo. $Y\ Y$)	(Homo. *ydp ydp*)	Gold; very faint	Gold; very faint
Pale	Rec. $Y^P{}_aY^P$	(Homo. $Y\ Y$)	(Homo. $Y\ Y$)	(Homo. *ydp ydp*)	—	Gold; very faint or none
White	Rec. *y y*	(Homo. $Y\ Y$)	(Homo. $Y\ Y$)	(Homo. *ydp ydp*)	—	No gold; occasional faint pink edge to petal
Yellow	(Homo. $Y\ Y$)	Het. $Y\ Y^P$	(Homo. $Y\ Y$)	(Homo. *ydp ydp*)	—	Gold; intense to medium
Yellow	(Homo. $Y\ Y$)	(Homo. $Y\ Y$)	Het. $Y\ Y^P$	(Homo. *ydp ydp*)	—	Gold; intense to medium
Pale	(Homo. $Y\ Y$)	(Homo. $Y\ Y$)	Rec. $Y^P\ Y^P$	(Homo. *ydp ydp*)	—	No gold
Yellow depressed	Homo. $Y\ Y$	(Homo. $Y\ Y$)	(Homo. $Y\ Y$)	Het. *Ydp ydp*	Pink	Pink
Yellow depressed	Het. $Y\ y$	(Homo. $Y\ Y$)	(Homo. $Y\ Y$)	Het. *Ydy ydp*	Gold; very faint	Gold; very faint

there was far less clear distinction into the two expected groups. This was partly due to the fact that the biochemical interaction between flavone and anthocyanin appears to work in two directions—thus although lowering of yellow grade leads to intensification of gold to pink, the development of these two latter colours can also lower yellow grade to some extent, and the levels at which development of yellow and pink are ultimately established are the result of the balance between these two tendencies. The higher grading of the ghosts may also partly be due to a subjective tendency in scoring, yellow appearing brighter in the absence of gold or pink. Within the red-spotted class it will be seen that most of the high grade yellows were gold and the depressed yellows pink. In a few of the high grade yellows gold was intensified to pink by modifiers other than the yellow depressor; such types were usually phenotypically different, most of the pink being concentrated towards the petal edge instead of evenly distributed over the petal as in the case of the depressor type.

The hybrid *an.* × A 8 (Pl. 18, fig. 5) showed considerably less pink than *an.* × A 16 (Pl. 18, fig. 7) being really only medium gold. Since in constitution it was heterozygous for R_3^{GO}, homozygous Y_a, and carried *Ydp*, exactly as the A 16 hybrid, it should have been rather more pink than it was. This can only be attributed to the absence of pink intensifiers in both A 8 and *anomalum*, since plants of the same *main* gene constitution as their F_1 were later synthesized, using 15,240 as the immediate source of the R_3^{GO} allele, and 14,859 as the source of *Ydp*, which gave the pinks which have already been discussed (Pl. 18, fig. 12). In this series intensifiers derived from A 16 (by way of 15,240) came into play, the presence of which accounted for the suitability of backcrosses to A 16 for the study of gold. For this reason A 16 has been regarded as the "standard" basis for discussion of gold expression. Only small backcrosses of (*an.* × A 8) to A 8 were grown, and since there was no specific "tag" such as the spot segregation which occurred in the A 16 backcrosses to assist with gold scoring, the latter was not seriously dealt with in these lines. Large backcrosses of (*an.* × A 8) to N 14 and H 10 were grown, but heterozygosity for y_a in the former, and the absence of intensifiers in the latter, rendered these families also unsuitable for observation of gold.

The hybrid *an.* × H 10 (Pl. 18, fig. 6), showed slightly less gold than *an.* × A 8; this again was comparable in main gene constitution with the hybrid *an.* × A 16, and it can only therefore be assumed that H 10 lacks gold intensifiers. Other indications of this have already been discussed. H 10 cannot however lack intensifiers so completely as does *anomalum*

itself, as in a small first backcross to H 10 those plants which showed gold were of medium grade, more intense than the F_1.

With regard to *anomalum* itself, the corolla of which is very pale cream, almost white, there is only the faintest suggestion of pink over the petal (Pl. 18, fig. 4). This is no doubt particularly due to its low-grade corolla colour. The appearance of gold and pink in crosses between *anomalum* and *arboreum* or *herbaceum* is a most characteristic and unexpected feature of these hybrids. As far as the modifier level of *anomalum* is concerned, there is not a great deal of direct evidence available. The fact that there is a general tendency for gold and pink to become accentuated in passing from the F_1 hybrid to later backcross generations to the cultivated species, suggests that the hybrid must itself have been brought down by the low level of *anomalum*. Only very few moderately large families of backcrosses to *anomalum* have been grown. In one, a selfing of a second backcross of H 10 (yellow corolla), there was a segregation 53 yellow corolla (4–5–6 of depressed type) : 27 cream of *anomalum* type (no doubt = 3 : 1). Amongst the yellows there were no real pinks, only golds ranging from intense to very faint, and some were apparently clear yellow, yet all must have carried R_3^{GO} and some at least the depressor from *anomalum*, and all would have been homozygous $Y_a\ Y_a$; $Y_c\ Y_c^P$ was segregating, but in the heterozygous condition this locus has been shown not to affect gold expression. The inference is therefore that the modifier level is not high enough in *anomalum* to result in the production of pink where it would otherwise be expected, nor was the gold as intense on the average as it would have been in a family of similar constitution in an *arboreum* genotype. That there was also an introduction of pink intensifiers normally absent from *anomalum* was evident from the fact that some of the pale petal segregates showed a definite pinkish tinge, not at all intense, but much more marked than in *anomalum* itself. The evidence on the whole points to *anomalum* lacking the modifiers which intensify gold and pink, as well as the main gene for flavone development essential to the full expression of the R_3^{GO} allele.

(6) *Spot size modifiers*

In New World cottons there is an exceedingly wide range in size of petal spot, from only a few pigmented cells to a large intensely pigmented area. In segregating progenies the range was such that Harland (1929*a*, Pl. IX) found it necessary in scoring to make use of a graduated scale of 22 classes, in which grade 1 was the smallest with only a trace of pigment, and grade 22 the largest. In cultivated Asiatic cottons, on the

other hand, there is much less variation in size and intensity of spot where present, so that Hutchinson (1932*b*) in his detailed study of anthocyanin found a graduated scale quite unnecessary, though in crosses between *arboreum* and *herbaceum* there was some indication that in one *herbaceum* type at least (H 10, spotless) the spot intensity genotype was at a slightly lower level than in most other strains, and size and intensity of spot are to some extent correlated. Practically all *arboreum* and most *herbaceum* types have a spot of about size 17–19 on Harland's scale, though many *herbaceum* strains, especially of varieties *typicum* and *africanum*, have a slightly smaller spot. The A 8 strain used in this study has a rather larger spot, which is quite at the top end of the range of Asiatic spots. The A16 ghost type has a rather small ghost area, which is right at the lower end of the normal range in Asiatic spot sizes. Recently Hutchinson & Ghose (1937*b*) have described an uncommon small spot type from *arboreum*, about one-eighth of the usual size, which they attribute primarily to a petal spot reducer (*Sr*). In the New World cottons Harland did not find it possible to identify particular genes affecting spot size, but different strains and species differed radically in their entire spot size genotype. The discovery by Hutchinson & Ghose of a particular and very rare size reducer does not affect the general situation that the spot size genotype in *arboreum* and *herbaceum* is remarkably uniform in contrast to the position in New World cottons.

The spot of *G. anomalum* is also of the normal size common in the cultivated Asiatic species, but in its interspecific hybrids the spot is very markedly enlarged (Pl. 18, figs. 5–7), and usually has a very characteristic streaked edge, whereas in *anomalum* and the Asiatic cottons it is clearly delimited. In progenies derived from these hybrids there was a very wide range in spot size, extending even beyond that in Harland's scale, and as the latter spots were of rather different shape from those under examination, a new scale of grades was devised. The new series contained fifteen classes, of which 1 was the smallest, similar to Harland's grade 1, and 15 the largest. It has not been thought necessary to reproduce this scale here, but in order to give some indication of the range, the size of some of the spots shown in Pl. 18 is indicated in the legend. Usually three flowers were graded on each plant, and the mean taken.

Hutchinson (1932*b*) has shown that H 10, the *herbaceum* spotless type, lacks anthocyanin intensifiers, so that the red spot area in ghost-spotless compounds is smaller than the underlying "ghost" which appears as a clear white marginal area around the pigmented spot, which is of lower intensity than most full spot types (Pl. 18, fig. 14). In practice it is

not easy to differentiate between spot size genes and spot intensity modifiers. In general larger spots are more deeply pigmented than smaller ones, and large faint spots or small deeply pigmented ones are not encountered, though for each size of spot there is a slight range in intensity. In a small F_2 between H10 and an *arboreum* ghost, Hutchinson found some range in size and intensity of the spot compound. From his description it appears that his extremes would correspond with my grades 3 and 8, and this has been confirmed in a repeat F_2 of similar type. Omitting Hutchinson & Ghose's "small spot type" it may be summarized that Asiatic spots normally range from size 10 to 12, very occasionally to 13; whilst in some interspecific hybrids the spot compound may range down as low as 3. This latter is of course a very exceptional case amongst the cultivated Asiatic cottons. In the *anomalum* interspecific hybrids and their progeny a very much wider segregation was encountered, right from complete extinction of the spot by modifiers up to size 15 and occasionally even slightly above this. Some particular examples will be discussed.

G. anomalum spot is graded as size 12; A16 is size 10; the hybrid has a considerably larger spot than either of its parents, of grade 13–14. In the first backcross to A 16, segregating 1 red spot $R_2^{OS}\,R_2^{OS}\,R_3^{GO}\,r_3^{OO}$: 1 ghost spot $R_2^{OS}\,R_2^{OS}\,r_3^{OO}\,r_3^{OO}$, size was scored in both classes, and the wide range encountered is shown in the following frequency table:

	Spot size														
	15	14	13	12	11	10	9	8	7	6	5	4	3	Not graded	Total	Mean
Red spot	10	31	20	21	20	8	8	4	5	.	.	.	.	3	130	12·08
Ghost	.	5	18	20	23	10	14	5	3	.	.	1	1	5	105	10·91

$d = 1{\cdot}17$; $t = 4{\cdot}47$; P very small.

This very wide range in size segregation shows that the size genotypes of the two parental species must be very differently constituted, though their end effects are much the same. On the average, spot on the red-spotted types was a little more than one grade larger than the ghost on ghost-spotted types, and the difference was highly significant. The tendency persisted into some lines of later backcrosses. In the second backcross to A 16 only small progenies were grown, so sufficiently large classes to make the comparison in size worth while are not available. From two of the second backcross lines seven plants were again back-crossed and selfed. These progenies had very distinct size ranges, as is shown below, giving very definite evidence that size is genotypically controlled.

Plant no. and spot size			Spot size of progeny: B.C. progeny 14	13	12	11	10	9	8	7	6	Selfed progeny 14	13	12	11	10	9	8	7	6	5	4
P 1325	Size 9	Red spot	.	.	.	1	6	2	.	.	.	.	.	.	3	4	4	1	1	.	1	.
		Ghost	.	.	.	.	3	1	3	.	1	.	.	1	1	2	.	2	1	.	.	1
P 1326	Size 9	Red spot	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	1	1	2	.	.
		Ghost	.	.	.	1	.	.	1	.	.	.	.	.	.	1	.	.	.	.	.	.
P 1327	Size 10	Red spot	1	2	3	1	1	2	1	.	.	.	.	.	.	1	.	1	.	1	.	.
		Ghost	.	.	4	1	2	.	4	1	.	.	1	.	.	1	.	.	1	.	.	.
P 1338	Size 11	Red spot	.	3	.	3	1	.	.	.	1	.	1	1	2	.	.	.	.	.	.	.
		Ghost	.	3	2	3	1	4	3	1	.	.	.	.	1	1	.	2	.	.	.	.
P 1339	Size 12	Red spot	1	3	.	2	1	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
		Ghost	.	2	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P 1340	Size 12	Red spot	.	.	.	3	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Ghost	.	.	.	.	5	4	3	2	.	.	.	.	1	1	.	.	.	.	.	.
P 1341	Size 11	Red spot	.	2	2	1	.	.	.	.	.	.	2	1	4	3	.	.	1	.	.	.
		Ghost	.	.	1	3	1	1	.	.	.	.	.	.	1	.	.	1	.	.	.	.

The tendency for red-spotted types to grade larger than ghosts is brought out more clearly when these seven third backcross progenies are considered in total:

	Spot size 14	13	12	11	10	9	8	7	6	Total	Mean
Red spot	2	10	5	11	9	6	2	.	1	46	10·97
Ghost	.	4	7	9	12	10	13	4	1	60	9·71

$d = 1{\cdot}26$; $t = 3{\cdot}66$; P very small.

Three selections from these third backcrosses which were selfed are also interesting in showing the very distinct size genotypes which can be established:

Plant no. and spot size	Spot size of progeny 14	13	12	11	10	9	8	7	6	5	4	3	2	Total
15,094 Size 14	3	13	14	8	8	7	9	3	1	.	.	.	.	66
15,095 Size 8	.	.	.	.	.	.	7	10	9	.	1	2	1	30
15,099 Size 8	.	.	.	.	.	4	5	3	3	3	1	2	.	21

The first of these families ranges considerably higher than an Asiatic family normally does, while both of the other two families contain spot reducers. The latter families are too small to make the size distinction between spot and ghost worth while, but in the large family the mean size for red-spot plants was 11·06, and that for ghosts 10·09, again a significant difference (0·97, $t = 2{\cdot}14$, $P = 0{\cdot}05$–$0{\cdot}02$). The data strongly suggest that R_3^{GO} is associated with a slightly larger size effect.

There is further evidence on this point from the first backcross of the same hybrid (*an.* × A 16) to H 10. All progeny were red-spotted, but some had gold petal and others were clear yellow. The expected segregation was 1 : 1 for the two genotypes $R_2^{OS}\, R_2^{AO}\, R_3^{GO}\, r_3^{OO}$ and $R_2^{OS}\, R_2^{AO}\, r_3^{OO}\, r_3^{OO}$, the former gold and the latter clear yellow. Gold petal was not easy to

score, as a large number were very faint gold on account of the influence of H 10; amongst the plants adequately scored there was a deficiency of gold segregates, and in the absence of any definite evidence as to the cause, the most plausible explanation is that some genotypic golds were scored as clear yellow. Notwithstanding this there was still a significant difference in spot size between the two classes:

	Spot size										
	15	14	13	12	11	10	9	8	7	Total	Mean
Gold	11	13	14	5	3	3	2	.	1	52	13·01
No gold	6	27	17	9	10	7	8	7	5	96	11·87

$d = 1{\cdot}14$; $t = 3{\cdot}04$; P very small.

In this backcross an attempt was made to differentiate full spot from spot compounds, as it was still thought that the *anomalum* duplicate was a full spot. About 50% of the plants up to grade 11, and only 14% of those above, were scored as spot/ghost, the others as full spot. It is now known that this was of no significance to the main gene segregation, and was only an expression of the tendency for large spot to be more diffuse at their edges, thus obscuring any white margin which is more easily seen beneath a smaller fainter pigmentation area. A similar case occurred in the family 14,544 ($R_2^{OS}\,R_2^{OS}\,R_3^{GO}\,r_3^{OO}$) × H 10 ($R_2^{AO}\,r_3^{OO}$), in which it was not possible to see gold at all, as all plants were heterozygous for y_a as well as carrying the H 10 anthocyanin reducing genotype. 85 of the plants were scored as full spot phenotypically, and 84 as indefinite spot/ghost, though there was very little size segregation in the family. The similarity to the last case suggests that the full spot phenotypes probably carried R_3^{GO}, and that the slightly less intense spot types were the result of the $R_2^{OS}\,R_2^{AO}$ compound.

P 2381 and P 2386, two plants derived by the intercrossing of a red spot selection from the second backcross to A 16, with H 10, have already been discussed. It has been shown that their constitution must have been $R_2^{OS}\,R_2^{AO}\,R_3^{GO}\,r_3^{OO}$, and that on backcrossing to H 10 $R_2^{AO}\,r_3^{OO}$, four genotypes were obtained, in approximately equal numbers as expected—red spot, gold; red spot, no gold; spotless, gold; spotless, no gold. There was however an amazing difference in the spot-size range in the progenies from these two plants; those derived from P 2381 ranged right up to grade 15, many being of the type with streaked diffuse edges. Derivatives of P 2386 had ordinary Asiatic spot size not above 12–13, with edges fairly clearly defined and not streaked. Unfortunately on account of the less spectacular range in the latter family spot size was not

graded there, but this was carried out in the former family, again clearly showing the association of larger size with R_3^{GO}:

	Spot size						
	15	14	13	12	11	Total	Mean
Gold	7	29	3	.	1	40	14·02
No gold	1	8	12	1	1	23	13·30

$d=0{\cdot}72$; $t=3{\cdot}69$; P very small.

There is no positive evidence that it is possible to separate this particular size effect from the gene R_3^{GO}, and therefore no justification for postulating that it is due to the action of an independent size modifier on the same chromosome.

Other material gave evidence of the association of a size effect with the R_2 locus in *anomalum* also. This effect was somewhat greater than that associated with R_3, and it was possible in certain lines to separate it from the *anomalum* ghost, indicating that it was due to a distinct gene. The F_1 of *anomalum* (spot size 12) × A 8 (spot size 13) showed the usual large spot characteristic of these hybrids, of size 13–15 (Pl. 18, fig. 5). Backcrosses to the ghost types N 14 and T 3, segregating 3 red spot : 1 ghost, showed again the very wide range from rather larger than 15 down to 4. Although the difference in size between the red-spotted types (which included both full spot R_2^{AS} and the compound $R_2^{OS}\,R_3^{GO}$ types) and the ghost types was not significant, it is of some importance to the argument to indicate that in both families the ghost spots tended to grade a little larger than the red-spotted class.

		Spot size													
Cross		15	14	13	12	11	10	9	8	7	6	5	4	Not graded	Total	Mean
(*an.* × A 8) × N 14	Red spot	8	17	24	13	28	16	11	7	3	1	1	.	45	174	11·48
	Ghost	1	5	8	3	4	1	1	.	1	.	.	1	3	28	11·96
		$d=0{\cdot}48$; $t=0{\cdot}99$; $P=0{\cdot}3$.														
(*an.* × A 8) × T 3	Red spot	6	16	25	11	12	10	12	5	1	2	3	.	.	103	11·50
	Ghost	.	11	14	2	.	3	.	4	1	.	.	.	.	35	12·25
		$d=0{\cdot}75$; $t=1{\cdot}62$; $P=0{\cdot}1$.														

The hybrid (*an.* × A 8) was also backcrossed with H 10, again giving a very wide size segregation.

Spot size											
15	14	13	12	11	10	9	8	7	6	5	Total
66	117	134	73	53	51	22	10	3	.	1	530

It has already been demonstrated that two selections from this backcross, nos. 11,703 and 11,757, of size 15 and 13 respectively, must have been of constitution R_2^{OS} (from *anomalum*) R_2^{AO} (from H 10) $r_2^{OO}\,r_3^{OO}$.

These plants when crossed with ghost types (R_2^{OS}) gave 1 : 1 segregations for the compound spot $R_2^{AO}\ R_2^{OS}$ and ghost $R_2^{OS}\ R_2^{OS}$, and the ghosts were found to grade consistently higher than the compounds:

		Spot size																
Cross		16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	Not graded	Total	Mean
11, 703 × N 14	Red spot		1	1	11	9	7	8	2	1	1	1	.	.	.	.	.	42	.
	Ghost	2	4	4	6	2	.	.	.	.	.	.	.	.	.	.	2	20	.
11,703 × T 3	Red spot	.	.	1	12	15	19	15	15	7	5	1	2	.	.	1	.	93	.
	Ghost	3	7	19	17	6	6	1	1	2	.	.	.	.	.	.	.	62	.
11,703 × T 3	Red spot	.	.	1	2	9	13	13	16	15	12	3	1	.	.	.	.	85	.
(2nd sowing)	Ghost	.	2	16	25	13	5	.	4	1	1	.	.	.	.	.	.	67	.
11,703 Total	Red spot	.	1	3	25	33	39	36	33	23	18	5	3	.	.	1	.	220	10·09
	Ghost	5	13	39	48	21	11	1	5	3	1	.	.	.	.	.	2	149	12·95
					$d=2{\cdot}86$; $t=13{\cdot}84$; P very small.														
11,757 × N 14	Red spot	.	1	3	9	3	5	2	1	.	.	.	.	.	.	.	1	25	12·25
	Ghost	.	4	13	3	.	1	.	.	.	.	.	.	.	.	.	.	21	13·90
					$d=1{\cdot}65$; $t=4{\cdot}44$; P very small.														

This is obviously not the result of a subjective tendency in scoring, as was at one time thought might be the case, since in the series previously considered ghosts were found to be smaller than red spots. It will of course be realized that although the phenotypes being contrasted are the same in both series, the genotypes are not. The essential point of significance is that in this series the R_2^{OS} allele under consideration is of *anomalum* origin, and this appears to be associated with large size. Of course in the first two families considered in this series, (*an.* × A 8) backcrossed to N 14 and to T 3, the *anomalum* R_2^{OS} allele was present in *all* the ghost segregates, and in *some* of the red-spotted group. The expected size segregation would have been 1 large compound spot + 2 ordinary-sized full spot (these two genotypes not separable phenotypically) against 1 large ghost. The size distinction tendency was present, but naturally the occurrence of the large-sized class in each of the two main phenotypes tended to obscure the contrast. In the derivatives of 11,703 and 11,757 the two classes compared were ghost containing R_2^{OS}, both from *anomalum* and from the backcross parent, and compound spot in which ghost came from the latter only, so that the size distinction associated with the introduced gene was quite clear.

These same two plants have been crossed with H 10, segregating 1 compound spot : 1 spotless. The former graded as follows:

	Spot size							
Cross	15	14	13	12	11	10	Total	Mean
11,703 × H 10	5	12	14	2	2	.	35	13·46
11,757 × H 10	6	50	66	8	6	3	139	13·24

It will be seen that these spot compounds, which carried R_2^{OS} of *anomalum* origin, graded larger than those in the N 14 and T 3 backcrosses, which contained R_2^{OS} from the latter types. It was also recorded during scoring that they were considerably more intense than those in the latter backcrosses. In size they corresponded with the *ghost* segregates in the N 14 and T 3 backcrosses, as would be expected if large size is associated with the *anomalum* allele. It is not a question of the difference in size of the spot compounds in the two backcrosses being due to the genotype contributed by the *arboreum* and *herbaceum* backcross parents respectively. If that had been the explanation, the compound would have been smaller in the H 10 series, whereas the reverse was the case. This size effect is obviously of considerable magnitude. The striking differences in spot size between sib-progenies which have already been demonstrated in other crosses (e.g. 15,094 progeny *versus* those from 15,095 and 15,099; P 2381 *versus* P 2386) strongly suggested the existence of a single gene of some considerable effect. It is also significant that selections which were known to carry R_2^{OS} from *anomalum*—P 1791, P 1801, P 1818, 11,319, 11,703 and 11,757—all also showed the size effect.

In particular lines however this effect has become dissociated from the *anomalum* allele. Some selections from the third backcross of (*an.* × A 8) to A 8 were selfed. The progenies showed different size ranges, and two which carried R_2^{OS} (15 : 1 segregation), which must have originated from *anomalum* because A 8, the only other type in their progeny, is homozygous R_2^{AS}, were segregating up to size 15. Some of the ghosts in these families however were as low as 7. A red-spot selection, no. 9992, from one of the segregating selfed progenies, of size 13, at the top of the Asiatic range but not beyond, was found to carry R_2^{OS}. It was crossed with the *arboreum* ghost type A 16, and backcrossed to 9992, and all five of the families grown, as previously described, segregated red spot : ghosts in 7 : 1 or 15 : 1 ratios. These ghost segregates must have contained at least one R_2^{OS} derived from 9992 and therefore from *anomalum*, yet on all of them as well as on the red-spotted types the spot was of perfectly normal Asiatic size. It cannot be allowed that the ghosts were small on account of their modifier background, because this was predominantly *arboreum*. Evidence will be presented later to show that dominance is in the direction of large size, so that the *anomalum* spot must have become dissociated in this line from its earlier size effect, which implies that in this case the effect must be due to a fairly closely linked but not inseparable gene.

The F_1 *an.* × H 10 spotless also showed a large spot of size 14–15, and the first backcross to H 10 gave the usual wide range:

Spot size							
14	13	12	11 ... 4		Not graded	Total red spot	Spotless
8	7	10	2	1	1	29	28

Only small second and third backcrosses were grown, and it is not worth discussing the results in detail. The range was similar to that in the first backcross. One of the second backcross plants, P 1898, with red-spot size 14–15, was selfed. From its origin and phenotype it must have carried R_2^{AO} from H 10, and R_2^{OS} from *anomalum*. Its behaviour showed that it did not carry R_3^{GO}. This locus may therefore be ignored. A backcross to A 16 ghost gave:

	Spot size							
	15	14	13	12	11	10	9	8
Red spot	.	.	1	2	1	.	.	1
Ghost	1	3	1	.	.	.	.	.

On backcrossing to H 10 it threw:

Red spot, size			Spotless
15	14	13	
1	1	2	4

Although the progenies were small, the larger size and greater intensity of the red-spot compounds in the H 10 backcross was in striking contrast to the usual situation where comparable compounds in the *herbaceum* genotype usually grade lower than in an *arboreum* genotype. Their only distinction was that whereas the compound in the A16 backcross carried ghost from A 16, that in the H 10 backcross derived its ghost from *anomalum*. The same tendency for ghost spots from *anomalum* to range higher was also observable in a backcross of the same plant P 1898 to 14,350, a ghost genotype which carried minus modifiers so that it appeared spotless, and whose origin and behaviour will be discussed later.

	Spot size							
	15	14	13	12	11	10	9	8
Red spot	.	.	4	3	2	.	.	1
Ghost	3	7	2	.	.		.	.

The fact that the ghost in this series still retained its large size into the third backcrosses into Asiatic types suggests that the accompanying size gene must be fairly closely linked with the R_2 locus.

A large ghost segregate, 15,305, of size 14, selected from a selfed progeny of P 1898, was crossed with the following main spot types:

A 8—red full spot, R_2^{AS}—itself large in the Asiatic range, size 13 (Pl. 18, fig. 1).

A 16—ghost, R_2^{OS}—low in the Asiatic range, size 10 (Pl. 18, fig. 3).

H 10—spotless, R_2^{AO}—A 16 × H 10 gives a small red-spot compound, about size 7 (Pl. 18, fig. 14).

15,240—red-spotted compound, $R_2^{OS}\,R_2^{OS}\,R_3^{GO}\,R_3^{AO}$—the synthesized *anomalum* compound on A 16 background, size 12 (Pl. 18, fig. 9).

The way in which 15,305 raised all these spot types is shown below:

		Spot size					
Cross	Progeny	15	14	13½	13	12	
15,305 × A 8	Red spots	23	17	.	.	.	(Pl. 18, fig. 16)
15,305 × A 16	Ghosts	.	6	6	3	1	(Pl. 18, fig. 17)
15,305 × H 10	Red-spot compounds	.	7	4	1	1	(Pl. 18, fig. 18 is of this type)
15,305 × 15,240	Red-spot compounds	3	25	.	3	1	(Pl. 18, fig. 7 is of similar genotype and phenotype)

15,305, which is homozygous for the ghost allele R_2^{OS} from *anomalum*, is also apparently homozygous for the associated large spot gene, and dominance is obviously strongly in the direction of large size. All these large spot types also had the diffuse edges which are such a characteristic feature of the large spots of the *anomalum* × Asiatic F_1 hybrids. It is evidently to this particular spot size modifier associated with R_2 that this effect is due. The size modifier is obviously not specific to any particular allele, but affects ghost, full spot, and red-spot compounds, both those due to complementary alleles at the same locus, and those due to complementary genes in duplicate loci.

The *an.* × H 10 hybrid was also backcrossed repeatedly to N 14 (ghost spot, white corolla), and a very interesting situation arose in this material, in which it was possible to select in the reverse direction for small-size modifiers. In the first backcross, segregating 3 spot : 1 ghost, size ranged from 15 to 5. One of the ghost segregates was again backcrossed, giving 3 yellow corolla ghosts and 9 white corolla segregates, which presumably also carried ghost. These plants were selfed, but since ghost can only be seen on yellow corolla and not on a white background, attention will be confined to the yellow-petalled progeny of the three yellow-petalled second backcross plants, P 1850, P 1852, and P 1853. Since these plants were ghost spot, they could not have been carrying the spotless allele R_2^{AO} or they would have shown the red spot-compound, but nevertheless some completely spotless types appeared amongst their yellow-petalled progeny. As this result was so unexpected the three parent ghosts were

again selfed the following season, with the same result. The total scoring is summarized below:

	Yellow corolla				White corolla
	Ghost definite, large	Ghost definite, small	Ghost indistinct	Definite spotless	
P 1850 selfed	30	8	2	5	16
P 1852 selfed	4		1	3	3

	Yellow corolla Size of ghost													White corolla
	13	12	11	10	9	8	7	6	5	4	3	2	1	0	
P 1853 selfed	4	14	9	9	10	8	5	2	.	1	.	.	.	6	40

In each of these families ghost spot size ranged right down to the point of extinction without any real break in the distribution. In the progeny of P 1850 two plants were graded as indistinct ghost, and also one in the progeny of P 1852, and two (sizes 6 and 7 respectively) in the progeny of P 1853. These indistinct types need special description. In the true spotless type R_2^{4O} (H 10) there is a tendency for the yellow pigment of the corolla to be slightly more intense right towards the base of the petal, in the area usually occupied by the spot when present. In ghost and red-spot types this basal intensification does not occur. The spotless types in these progenies were similar to the usual type, with yellow slightly intensified at the base of the petal. In the distinct ghost segregates the ghost area was perfectly white, and the upper yellow portion of the corolla uniformly pigmented. The intermediate types scored as "indistinct ghost" had the yellow pigment deepening towards the base of the petal, and a small area right at the base of slightly paler yellow, giving the appearance of a pale yellow small ghost, instead of the usually clear white one.

One of the spotless plants from the progeny of P 1852, no. 14,350, was further investigated. To confirm that it was not a new type of spotless recessive to ghost but not complementary with it, it was crossed with H 10 spotless. All the progeny were red spotted, showing that genotypically 14,350 was an extinguished ghost. In addition 14,350 was crossed with A 16 (ghost, size 10), giving ghosts only, and with A 8 (full spot, size 13), and was also selfed. Spot sizes in these four progenies were as follows:

		Yellow corolla Spot size														White corolla
Cross	Phenotype of progeny	13	12	11	10	9	8	7	6	5	4	3	2	1	0	
14,350 × H 10	Red spot	8	13	13	8	9	7	2	2	2	.	.	.	.	.	.
14,350 × A 16	Ghost	7	8	5	5	3	2	3	.	1	1	1	1	.	.	.
14,350 × A 8	Red spot	14	4	2	.	.	.	.	.	.	.	.	.	.	.	.
14,350 selfed	Ghost	.	.	.	.	1	1	1	7	3	2	1	3	3	28	25

The selfed progeny threw only a few small ghosts, all of which were of the indistinct type, and the majority of the yellow-petalled segregates were completely spotless. A constant extinguished ghost line has not been established, but no doubt it could be from this material. In the family 14,350 × A 16, in which the normal size complement was brought in by the latter parent, the general size level was of course raised. All the larger ghosts were perfectly clear, but all below size 8 were of the indistinct type. In 14,350 × H 10 there was the usual correlation between size and intensity of red pigment. Many of the progeny, both large and small-spotted types, had no sign of any white surround. Some of the fainter ones without white margin were very flecked and indefinite (Pl. 18, fig. 15), and highly suggestive of many New World spot types, which are in general very distinct from the intense well-defined spot characteristic of Asiatic cottons. These families again give evidence on the direction of dominance of large spot modifiers, the majority of spots in the outcross progeny of 14,350 coming well within the Asiatic range. They afford an interesting comparison with the similar outcrosses involving 15,305, the large ghost, which have been discussed above. It seems likely that several modifiers are concerned in 14,350. They apparently do not include the spot size reducer *Sr* of Hutchinson & Ghose (1937*b*) since the latter was dominant in F_1 crosses with normal-sized Asiatic spots[1].

In addition to studying the association of spot size with the main anthocyanin loci, that with the genes L^4, p_a, and *Ne* from *anomalum* has also been observed in some families. The data were not very extensive, and no definite conclusions could be drawn, other than that there were no striking effects associated with these genes. In one series of backcrosses derived from (*an.* × A 8) there was some suggestion of a small difference in spot size associated with the *L* chromosome:

Cross	Class	*n*	Mean spot size		*d*	*t*	*P*
(*an.* × A 8) × H 10	L^L	281	12·36	}	+0·43	2·75	Very small
	L^4	266	12·79	}			
(*an.* × A 8) × N 14	L^L	77	11·62	}	−0·22	0·61	0·5
	L^4	77	11·40	}			
(*an.* × A 8) × T 3	L^L	69	11·73	}	−0·07	0·17	0·9
	L^4	69	11·66	}			

The results were, however, not consistent, though this may be due to a difference in this respect between the *arboreum* and *herbaceum* backcross

[1] That *Sr* is not one of the small size genes concerned in 14,350 has been confirmed since this manuscript was sent to press. Amongst a total of 70 F_2 progeny derived from the cross (reduced spot × 14,350), nine plants had petal spot of full normal size.

parents. Indication of a similar difference between the two cultivated species was also found in lint length genotype (see § XI).

(7) *Linkage tests with* R_3

The discovery of the gold spotless allele provides a new locus for linkage investigation in the cultivated Asiatic species. Information has been collected in the course of the backcrossing reported in this paper, and after R_3^{GO} had been established in the *arboreum* genotype a number of crosses were designed specifically for linkage testing. The available data have been summarized in Table 12. All crosses were of the R_3X–r_3x type except in the case of the Lc_1 test. These figures show that R_3 is independent of the three linkage groups—leaf shape–lint colour, L–Lc_1 (L–K group of Hutchinson, 1934); lintless–lint colour, H_a–Lc_2 (Silow, unpublished); and nectaries–pale pollen, Ne–P_b (Silow, unpublished). Lc_1 and R_3 gave a considerable deviation, but the association was the reverse of that in the parents. The family was fairly small, and no significance can be attached to this observation. χ^2_L for the R_3H_a segregation was only just below the conventional limit of significance, but again the suggested association was the reverse of that in the parents. There was not any evidence of association of R_3 with the other pollen colour locus cream P_a, or with petalody Pdy, or with the corolla colour factor Y_a. R_3 has not been adequately tested against the *anoma-*

Table 12. *Two-factor segregations involving* R_3

Factor	Family	Type	R_3X	R_3x	r_3X	r_3x	T	χ^2_L	P
L	(*an.* × N 14) × N 14	B.C., F_1 ♀	39	45	30	39	153	0·13	0·7
	(*an.* × N 14) × N 5	B.C., F_1 ♂	22	40	10	29	101	1·07	0·3
	(15,099 × P 2774) × T 17	B.C., F_1 ♀	45	46	45	43	179	0·05	0·8
	15,240 × N 5 or N 14	F_2	297	117	84	27	525	0·68	0·5
Lc_1	(P 2406 × T 1) × N 14	B.C., F_1 ♀	34	22	24	42	122	7·20	Very small
H_a	(P 1338 × 429) × T 1	B.C., F_1 ♀	164	188	196	169	717	3·62	0·06
Ne	(*an.* × N 14) × N 14	B.C., F_1 ♀	42	46	33	38	159	0·02	0·9
	(*an.* × N 14) × N 5	B.C., F_1 ♂	40	22	22	18	102	0·92	0·7
	15,240 × N 14 or N 5	F_2	357	98	101	33	589	0·57	0·5
P_b	(15,099 × T 4) × T 4	B.C., F_1 ♀	20	17	24	21	82	0·004	0·95
P_a	(P 1327 × P 2375) × T 3	B.C., F_1 ♀	51	71	50	61	233	0·25	0·7
Pdy	(15,099 × P 2774) × T 17	B.C., F_1 ♀	51	41	43	46	181	0·92	0·3
Y_a	(*an.* × N 14) × N 14	B.C., F_1 ♀	40	48	37	35	160	0·56	0·5
	(*an.* × N 14) × N 5	B.C., F_1 ♂	30	30	20	19	99	0·01	0·9
	(P 1338 × 429) × T 1	B.C., F_1 ♀	164	154	158	162	638	0·31	0·5
	(15,099 × T 4) × T 4	B.C., F_1 ♀	17	20	26	19	82	1·14	0·3
	(15,099 × P 2774) × T 17	B.C., F_1 ♀	51	41	38	51	181	2·94	0·1
	15,240 × N 14 or N 5	F_2	318	105	104	30	557	0·33	0·5
Y_c	(9992 × A 16) × 9992	$R_{1:1:1:1}$ $Y_{1:1}$	83	45	7	1	136	1·73	0·2
	(9992 × A 16) × 9992	$R_{7:1:1}$ $Y_{1:1}$	109	89	13	14	225	0·46	0·5

lum corolla colour factor Y_c, since the latter has not yet been established in *arboreum* in conjunction with either of the recessive anthocyanin types, ghost or spotless. Some little information on the point has been summarized in the table, derived from two small families segregating 15 : 1, and three small families segregating 7 : 1 for R_2 and R_3, all segregating 1 : 1 for Y_c. Although the evidence of complete independence is not conclusive, it is obvious that Y_c is not closely associated with either of the anthocyanin duplicates. The segregation of R_3 in conjunction with yellow corolla grades has been shown in Table 9 earlier. Most of the range in yellow in this cross was associated with the segregation of *Ydp*, and from the table it might appear that R_3 and *Ydp* are linked, since in the ghost class yellow grading was consistently higher than in the spot class, and there was far less clear distinction into the two expected yellow classes. This has already been discussed. Furthermore, delimitation of the depressor genotypes in such a family by simple inspection was impossible, on account of the interspecific modifier segregation which was also present. From examination of similar data from other families it has been concluded that, when due allowance is made for the interaction between gold expression and corolla grade, there is no evidence of linkage between R_3 and *Ydp*, and it has been found easy to separate them into different backcross lines.

(8) *Summary*

(i) Red petal spot in *anomalum* is not due to a single allele as in the cultivated Asiatic species, but is the result of the complementary interaction between a ghost allele in the Asiatic R_2 locus, and a spotless allele in a duplicate anthocyanin locus. This spotless gene is characterized by a pleiotropic gold-petal expression. The symbol R_3^{GO} is allocated to it.

(ii) *G. arboreum* and *G. herbaceum* carry in the duplicate locus a basal recessive allele, lacking both basic anthocyanin expression and the spot characteristic. It is symbolized as r_3^{OO}.

(iii) The *anomalum* duplicate R_3^{GO} was established in the homozygous condition in the cultivated Asiatic genotype, both as a spotless type in conjunction with R_2^{dO} and as a red-spotted type similar to *anomalum* in conjunction with R_2^{OS}. The gold appearance of the petals of these types is something quite new in Asiatic cottons.

(iv) The gold-petal expression of R_3^{GO} is very variable, being especially dependent upon yellow corolla constitution. Only when on a homozygous yellow background is gold developed to an appreciable extent. R_3^{GO} is not completely dominant, since gold is intensified in the homozygote.

In conjunction with *Ydp* on a homozygous yellow background, gold is intensified to pink. Homozygosity for R_3^{GO} does not lead to any appreciable intensification in pink beyond the expression of the heterozygote. Heterozygosity in the Y_a locus for either Y_a^P or y_a is a very potent factor in limiting the expression to a very faint tinge of gold, even when R_3^{GO} is duplex, and not even *Ydp* is able to intensify it. Heterozygosity in the Y_b and Y_c loci does not reduce gold expression. On the recessive pale petal colours and on white, gold is not expressed, except occasionally as a faint pink edge to the petal. In addition other factors such as exposure to sunlight, and other modifiers less easily analysable than the main corolla colour factors, also produce profound effects on gold and pink expression. Strains of the cultivated species, in which the gold-producing allele does not occur, differ considerably in their content of these modifiers. In *anomalum* itself, which is very pale cream, almost white, there is only a faint suggestion of pink on the petal. This is a result of the absence of the main gene for yellow flavone development essential to the full expression of the gold spotless allele, and to the fact that this species also lacks modifiers which intensify gold and pink.

(v) Part of the size of the *anomalum* spot is a subsidiary effect of R_3^{GO}. The more important component of its size constitution is a modifier of considerable magnitude fairly closely linked to the ghost gene. There was no conclusive evidence of the linkage of spot size modifiers with the *L*, P_a, or *Ne* loci.

(vi) As a result of the low level of the *anomalum* spot size genotype, apart from these large-size components, it was possible to develop, from hybrid progeny, lines so low in their content of size modifiers that the expression of the main spot gene was reduced to extinction.

(vii) Dominance of spot modifiers is in the direction of larger size.

(viii) Using the large spot modifier associated with R_2^{OS}, it was shown that spot size modifiers are not specific to any particular allele, but affect all types of spot—ghost, full red spot, and red-spot compounds, both those due to complementary alleles at the same locus and those due to the complementaries in duplicate loci.

(ix) There was no evidence of linkage of R_3 with any one of the three linkage groups *L*–Lc_1, H_a–Lc_2, and *Ne*–P_b, or with the independent genes P_a, Y_a and *Ydp*. Evidence of complete independence from Y_c was not conclusive, but the genes are certainly not closely associated.

V. Pollen colour

In the cultivated Asiatic species pollen is almost invariably deep yellow in colour and there is remarkably little variability in shade. Only two exceptions have been observed. Ramanathan & Balasubramanyan (1933*b*) reported a single occurrence of "cream pollen" in a strain of *G. obtusifolium* Roxb. = *G. arboreum* var. *neglectum* forma *indica*, Hutchinson & Ghose. They found that it differed from full yellow in a single recessive gene, and there was no evidence of modifier segregation even in an interspecific cross involving *G. herbaceum*. Through the courtesy of Mr V. Ramanathan seed of the "cream pollen" strain was sent to this Station in 1934. The writer has grown it under the type number N 25, and has confirmed the above findings. In no cross studied was there any difficulty in classifying segregating progenies. Whilst on a visit to the U.S.S.R. in 1933 Dr S. C. Harland collected seed from a plant of *G. herbaceum* with cream pollen in Uzbekistan, district Tashkent. On his return he handed this material over to the writer for study. The strain, grown under the number H 16, has bred true to a pollen colour grade similar to that which characterizes the cream pollen Uplands and those wild species of *Gossypium* which have cream pollen. This grade is very much whiter than that of Ramanathan's *arboreum* "cream" N 25, and it is proposed to refer to the latter as *pale yellow*, whilst the *herbaceum* H 16 type will be called *cream*. In appearance the common full yellow, pale yellow, and cream pollens correspond very closely to the phenotypes of full yellow, pale and white corolla in the Y_a series—i.e. at grades 8, 3–2 and 1 respectively on Hutchinson's (1931) corolla colour plate. The genes responsible for the recessive cream and pale pollen colours are not however allelomorphic like the Y_a series of corolla colours with which they have been compared phenotypically. The F_1 between N 25 and H 16 has full yellow pollen, and the F_2 gave the dihybrid 9 : 3 : 4 ratio, in which all three terms, representing full yellow, pale and cream could be satisfactorily delimited. Data will be published shortly. The symbol p_a is proposed for the cream pollen gene, and p_b for pale pollen. On this basis the H 16 cream type is regarded as $p_a P_b$, and the N 25 pale as $P_a p_b$.

Although a series of pollen colour grades was not found necessary in studying crosses within the cultivated Asiatic cottons, they were used to some extent in studying the *anomalum* hybrids. In such cases flowers were collected in the field in the early morning and graded at about 10 a.m. in the laboratory, a necessary precaution on account of deepening

of the colour of the pollen during the day. The same scale as Harland (1929*b*) had used in his pollen colour study in New World cottons was employed. The coloured plate in his 1929 paper is not a good reproduction of his grades, but fortunately his standard strains were available. Grade 0 is white, grade 1 pale yellow, and grade 4 the deepest yellow. Practically all the yellow pollen Asiatics grade between 2 and 3, N 25 pale at 1·2, and H 16 cream at 0·3.

G. anomalum has cream pollen, grading at 0·1, definitely whiter than that of H 16. An F_1 between them, of eleven plants, also had cream pollen, of an intermediate shade grading at 0·2. When *anomalum* was crossed with full yellow pollen types from *arboreum* or *herbaceum*, the F_1 was brought down in grade to an intermediate shade of yellow. This was in strong contrast to crosses between H 16 cream and *arboreum* full yellows, which were all as intense yellow as the dominant parent. Some representative examples were as follows:

		Pollen colour grade of hybrid with	
Type no.	Yellow grade	*G. anomalum* (cream)	*G. herbaceum* H 16 (cream)
A 8	2·2–2·5	1·8–2·0	2·0–3·0
A 16	2·4–2·6	1·9–2·5	—
H 10	2·5	1·0	—
O 1	2·5	1·1–1·6	—
O 8	3·0	1·3	—

There was considerable breakdown in dominance in the *anomalum* hybrids, and this was more marked in crosses involving the *herbaceum* types H 10, 01, 08, than in those into which the *arboreum* types A 8 and A 16 entered. A backcross of *an.* × A 8 to T 3 (a synthesized multiple recessive cream pollen segregate selected from an F_2 of *arboreum* yellow pollen × *herbaceum* H 16) gave yellows ranging from about grade 3 down to intermediate yellow of about grade 1, and a more uniform group of creams ranging slightly round 0·3. Unfortunately there was no opportunity at the time to grade each plant individually, but in spite of the variation within each group it can be said that the two main classes were quite distinct from one another. When contrasted against the creams even the low grade yellows were distinct. This backcross gave 68 yellows and 70 creams, indicating that the *anomalum* cream pollen gene is allelomorphic and probably identical with that in H 16, p_a.

The pale pollen type N 35 was also crossed with *anomalum* and with the F_1 of (*an.* × H 16 cream). The first progeny consisted of 11 plants all low grade yellow, about 1·5–2, definitely yellow when contrasted against N 25 pale itself. The second progeny of 19 plants ranged from definite full

yellow down to light yellow, but the lightest was somewhat deeper than pale itself. This complementary reaction shows that *anomalum* carries the normal allelomorph of pale.

VI. Nectaries

Extra-floral nectaries are common in cotton, but there is very little published information on their inheritance, largely because of the extremely tedious nature of the observations and the great variability in expression of the character. They occur on the undersides of the leaves, on one or more of the main veins, and at the base of the bracts. If inside the bracts they occur just between them, at the outer base of the calyx tube. If outside the bracts, they occur opposite and immediately below them. On some types nectaries are completely absent from both leaves and bracts. All types of *G. arboreum* which have leaf nectaries have internal bract nectaries also; those types without leaf nectaries have no bract nectaries either. All types of *G. herbaceum* which have been observed at this Station have nectaries on the leaves, and most have internal bract nectaries as well. A few strains of var. *frutescens* lack bract nectaries, even though they have leaf nectaries. External bract nectaries, although common in New World cottons, are extremely rare in the cultivated Asiatics, having only been described from a single type of *herbaceum* from Malta (Watt, 1926).

Leake (1911) studied the inheritance of leaf nectaries in a cross between two *arboreum* types, and demonstrated that presence and absence was controlled by a single pair of allelomorphs. Subsequent work at this Station, which will shortly be published, has confirmed that within *arboreum* presence of leaf nectaries (*Ne*) is almost dominant over their absence (*ne*), but it has been found that in interspecific crosses involving *herbaceum* this dominance may break down completely. The same main gene controls leaf nectaries in both these species, and is also responsible for the formation of internal bract nectaries in *arboreum*. Since it has been found that in interspecific crosses the expression of the main gene may be limited by modifier action to the leaves only, it is highly probable that those occasional types of *herbaceum* which have leaf nectaries but no bract nectaries also carry the common main nectary gene, together with a particular genotypic complex which does not permit its full expression.

G. anomalum is characterized by the presence of leaf nectaries. In hybrids involving the *arboreum* no-nectary types N 5, N 14, and N 25, leaf nectaries were as distinct as in the wild species. In a backcross of (*an.* × N 14) to N 14 leaf nectaries were somewhat indistinct on some

plants, but with careful observation it was possible to classify with confidence, and a clear single-factor segregation of 81 plants with leaf nectaries, and 86 without, was obtained. Evidently the minor gene constitution of *anomalum*, so far as leaf nectaries are concerned, is very similar to that of *arboreum*, and not at all like that of *herbaceum*, which leads to dominance breakdown in crosses with *arboreum*. That the same main locus is concerned as in these species was shown by the backcross of (*an.* × *arb.* A 8 *Ne*) to the *ne* types N 14 and T 3, which gave a total of 301 plants all with leaf nectaries.

The situation with regard to bract nectaries is much more distinctive. There are no internal bract nectaries in *G. anomalum*, as is usual in types with leaf nectaries in the cultivated Asiatics, but external bract nectaries, which are extremely rare in the latter, are present. In hybrids with the *arboreum* no-nectary types N 5, N 14, and N 25, no external bract nectaries were present except on occasional flowers, on which they were indistinct. Apart from the difference in position, this situation is more like that in *arb. ne* × *herb. Ne* than in *arb. ne* × *arb. Ne* hybrids. This indicates that the minor bract nectary genotype of *anomalum* is nearer to that of *herbaceum* than *arboreum*, though of course it is quite distinctive in that *anomalum* only shows externals and not internals. On analogy with the situation in the cultivated Asiatics, it seems likely that these external *bract* nectaries are due to the same *Ne* allele as controls the presence of *leaf* nectaries, together with a particular specific genotype. On the other hand, in the absence of further data, it cannot definitely be concluded that the identical allele is concerned.

VII. Fuzz and lint characters

In cultivated Asiatic cottons the hairs on the seed are differentiated into two distinct coats, one consisting of long fairly easily detached lint hairs, and the other of short strongly adherent fuzz hairs. In a few so-called "tufted" types the latter coat is represented only by a few hairs at the chalazal end of the seed. Hutchinson (1935) found tufted to be due to a partially dominant gene T, whose expression was considerably influenced by modifiers. He made use of a series of grades for classification of segregating progeny, in which grade 1 was the most highly tufted type (naked) and grade 6 almost fully covered except for small naked areas. Fully covered segregates were classified as "fuzzy". Homozygous tufted types grade 1–4, and heterozygotes usually 3–6. The symbol *Fz* has been assigned to this gene in Hutchinson & Silow's (1939) revised list of gene symbols.

In *G. anomalum* the hairs on the seed are not separated into two distinct layers, but all appear to form a single coat of short hairs up to 10 mm. in length. These are very firmly adherent to the testa, so that when pulled off many break along the length of the hairs instead of at or very near to the point of attachment to the testa, as in commercial cottons. Nevertheless *anomalum* carries the genetic basis for fuzz, since fully fuzzy segregates have appeared in progeny derived from *an.* × tufted hybrids. In the F_1 between *anomalum* and *arboreum* A 16 tufted (grade 2–4) the hairs were also very adherent, so that when pulled they broke along their length. It was therefore not possible to obtain any estimate of the dominance of *Fz* in this hybrid. In the first backcross to A 16 approximately 60 % of plants set seed, and on them normal ginning was possible. The following segregation was observed:

Tufted grades					Fuzzy
2	3	4	5	6	
.	.	1	15	29	64

A comparable (*arboreum* fuzzy A 15 × A 16) first backcross to A 16, gave:

Tufted grades					Fuzzy
2	3	4	5	6	
9	16	8	.	.	

In this intra-*arboreum* cross, dominance of *Fz* was almost complete, and the entire segregation was at a very different level from that in the interspecific cross. No direct comparison with the level in *herbaceum* is available, but information on the point may be gathered from the following F_2 progenies, one of the same intra-*arboreum* as cited above, and the other of *arboreum* A 16 × *herbaceum* H 10 fuzzy:

		F_2, percentage class frequencies								
	F_1 Tufted grade	Tufted grades						Fuzzy		Actual no. of F_2 plants scored
Cross		2	3	4	5	6	6+		Total	
A 15 × A 16	3	.	17	34	14	14	.	21	100	29
H 10 × A 16	6+	.	7	12	13	16	6	46	100	162

In the intra-*arboreum* F_2 the expected 25 % of recessive fuzzy segregates was recovered, but in the *arboreum-herbaceum* cross 46 % of the segregates were graded as fuzzy. From the high grading of the F_1 plant, the excess of fuzzy segregates may be attributed to the inclusion of a number of heterozygotes in this class. The general displacement of the segregation was in the same direction as in the *anomalum* hybrid progeny, but to a less extreme degree than in the latter, where even the homozygous tufted class was fairly extensively covered. A selfed progeny derived from an F_1

(P 2386) between H 10 and a selection from the second backcross of (*an.* × A 16) to A 16 gave the following:

F_2, percentage class frequencies							
Tufted grades					Fuzzy	Total	Actual no. of F_2 plants scored
2	3	4	5	6			
.	.	8	9	21	62	100	135

By a second backcross it would be expected that a certain proportion of the *anomalum* genotype might be lost, yet the displacement was still more extreme than in the H 10 × A 16 F_2. That we are justified in considering this a modifier displacement and not the result of the introduction of a fuzzy allele dominant over tufted *Fz* is shown by the fact that a particular (*an.* × A 16) third backcross to A 16 selection (15,094) when selfed, gave:

Tufted grades					Fuzzy
2	3	4	5	6	
.	.	19	27	8	7

In this progeny the fuzzy segregates were clearly recessive. That an allele in the *Fz* locus is concerned is confirmed by the following evidence. A selection from the second backcross of (*an.* × A 16) to A 16 was selfed, giving:

Tufted grades			Fuzzy
5	6	Intermediate	
2	3	1	6

The plant classified as "intermediate" (15,245) had seeds uniformly covered with an extremely thin fuzz layer. Its subsequent behaviour showed that it was a heterozygote; heterozygotes within *arboreum* are usually patchy, with completely naked areas of variable size. Heterozygotes of this uniformly thinly covered type have been observed in *arboreum* × *herbaceum* crosses. The plant 15,245 was crossed with N 5 and N 14, two *arboreum* fuzzy types, and two plants from each F_1 were selfed. Two families segregated for *Fz*, whilst two others were homozygous fuzzy. One of the fuzzy sibs of 15,245 was also crossed with N 5 and N 14, and four F_1 plants selfed gave over 300 progeny all of which were fuzzy. In these non-segregating families *Fz* from A 16 was clearly absent, and its place must have been taken by its allele *fz* from *anomalum*. The results cited previously show that this is not by any means the only fuzz gene of importance in this species. *G. anomalum* has an intensely fuzzy genotype, and whether it carries the genetic basis for lint at all merits further discussion.

The most significant evidence bearing on this point is that, in crosses involving normal fully linted strains of cultivated cottons, segregation of lint characters is not discontinuous but appears to be similar to that which occurs within the cultivated species, where differences have been found to be due to typical quantitative genes of relatively small individual effect. The most satisfactory means of identifying such genes is to trace their association with main genes affecting more easily observable characters. This type of analysis is necessarily restricted to early generations, since in later generations it is impossible to be sure that crossing-over has not occurred. Unfortunately in early generations of this hybrid material only a few families gave a separation of main gene classes sufficiently distinct to be of use in this connexion, and in addition their general low fertility made observation of seed characters particularly difficult. There is therefore practically no information available on inheritance of ginning percentage, since for reliable estimates of this character about 100 seeds are required from each plant. All that can be said is that the amount of hairs on the seeds of F_1 hybrids was intermediate between that on *anomalum* and on the cultivated parents. The quantity is expressed as the percentage weight of hairs on the total weight of seed + hairs. The firm adherence of the hairs made normal ginning impossible, so the hairs were cut off close to the base with scissors. Typical "ginning percentage" results were as follows:

anomalum = 8 %		
A 16	= 41 %	*an.* × A 16 = 28 %
H 10	= 26 %	*an.* × H 10 = 18 %

Rather more satisfactory information is available on the inheritance of hair length, since for the determination of this character only five seeds from each plant are required. On the F_1 hybrids hair length (mean maximum halo length) was intermediate but rather nearer that of the cultivated parent, e.g.

anomalum = 8–10 mm.		
A 16	= 25–26 mm.	*an.* × A 16 = 20 mm.
H 10	= 27–29 mm.	*an.* × H 10 = 23 mm.

Even in the first backcross the mean hair length very closely approached that of the cultivated parent. Data are summarized, as mean "lint" length for contrasting pairs of main gene classes, in Table 13. In each case the main gene derived from *anomalum* is listed after that from the cultivated species.

The first three families, all first backcrosses from the same F_1 *anomalum* × *arboreum* hybrid, show the association of lint length with leaf

shape segregation. In the first family, a backcross to *herbaceum* H 10, the *anomalum* leaf shape class had lint significantly shorter, by about $\frac{1}{2}$ mm., than the laciniated class. There was no significant difference in lint length between the leaf shape classes in the other two families, which were respectively backcrosses of the same F_1 to *arboreum* N 14, and to T 3, a multiple recessive selection derived from an *arboreum* × *herbaceum* F_2, but predominantly of *arboreum* genotype. The second group of three families shows the association with R_2. These families are derived from two sib-plants from the first backcross of (*an.* × A 8) to H 10. In each of the progenies derived by backcrossing again to H 10, the class carrying

Table 13. *Lint length association with main gene segregation*

Family	Class	*n*	Mean lint length mm.	*d*	*t*	*P*
(*an.* × A 8) × H 10	L^L	209	27·59	−0·49	2·16	0·05–0·02
	L^A	167	27·10			
(*an.* × A 8) × N 14	L^L	61	23·27	+0·05	0·10	0·9
	L^A	37	23·32			
(*an.* × A 8) × T 3	L^L	36	23·66	+0·11	0·16	0·9
	L^A	27	23·77			
11,757 × H 10	R_2^{AO}	70	27·37	−1·41	4·32	Very small
	R_2^{OS}	61	25·96			
11,703 × H 10	R_2^{AO}	22	28·68	−1·76	2·21	0·05–0·02
	R_2^{OS}	12	26·92			
11,703 × T 3	R_2^{AO}	79	28·13	+0·26	0·52	0·6
	R_2^{OS}	41	28·39			
(*an.* × A 16) × A 16	r_3^{OO}	42	25·23	−1·02	2·19	0·05–0·02
	R_3^{GO}	64	24·21			
(*an.* × N 14) × N 14	r_3^{OO}	18	24·22	−0·84	0·77	0·5
	R_3^{GO}	18	23·38			
(*an.* × N 14) × N 5	r_3^{OO}	16	25·12	−0·69	0·84	0·4
	R_3^{GO}	23	24·43			

R_2^{OS} from *anomalum* had lint about $1\frac{1}{2}$ mm. shorter than that of the spotless class, and this difference was significant. In the third family, a backcross of one of the sibs to T 3, there was no significant difference in lint length between the two genotypes. There is thus evidence of lint length genes on both the R_2 and L chromosomes, and an indication, from the differences in the backcrosses to *arboreum* and *herbaceum*, that the lint length alleles carried by these two species on these chromosomes are not the same. A similar difference between these two species in spot size genes on the L chromosome has already been mentioned. The third group of families, showing association of lint length with R_3, were all backcrosses of (*anomalum* × *arboreum*) to *arboreum*. In the first of these the class carrying the *anomalum* gold allele had lint about 1 mm. shorter

than the non-gold class. This difference was significant. In the other two families the lint length differences were of much the same magnitude, but in the small numbers available did not reach the level of significance. Lint length genes are evidently located on the R_3 chromosome also. Other small families, not indicated in the table, gave no evidence of association of lint length with pollen colour and leaf nectary segregations, but could not be considered conclusive in this respect. The lint length effects associated with the L, R_2 and R_3 genes from *anomalum* were of just the same degree of magnitude as Hutchinson (1932*b*, 1934) found to be associated with the first two of these loci within the cultivated species. Exactly the same type of gene appears to be responsible for the difference between *anomalum* and the cultivated cottons as is responsible for differences within the cultivated species themselves. Their lint was not acquired at a single step, but was probably built up gradually, either by the development of a morphologically new structure, or by a process of differentiation in a single coat of hairs such as occurs on the seed of *anomalum*. In that case such types as *anomalum* are not likely to be of any value as a source of lint length or ginning out-turn genes. In view of the strength and fineness of its seed hairs, however, it might well be worth paying attention to this species as a source of lint fineness.

VIII. Lint colour

Very little information on the inheritance of lint colour in Asiatic cottons has been published. In view of the fact that most of the few studies which have been reported were interspecific (Kottur, 1923; Ramanathan & Balasubramanyan, 1933*a*), it is not surprising that they have yielded no clear conception of the situation. Experience at this Station has indicated that some of the main lint colour genes are extremely susceptible to modifier action, and that there are great differences between strains in their modifier content. There are two classes of main genes. In one class are those whose expression is a dark brown or khaki, and whose potency is of such magnitude that they are almost fully dominant even in interspecific crosses. In the other class are the light brown genes whose expression is very dependent upon the minor genotype; their presence in certain very near-white cottons would not be suspected, and it has only been possible to confirm it by the intensification which occurs in certain crosses as a result of the introduction of modifiers, and with the help of a close linkage with the lintless h_a gene which facilitates analysis enormously. Without this assistance it would not have been possible

to arrive at any definite conclusions with reference to gene homologies within the light brown class.

Within *arboreum* two independent khakis are known. One of these Hutchinson (1934) has located on the leaf shape chromosome, at a distance of 30 units from the latter gene. For this khaki gene, which Hutchinson termed K, the symbol Lc_1^K is proposed. The other khaki has been found to be closely linked with h_a, and will be termed Lc_2^K. Two light brown lint genes are known; one of them, which is allelomorphic with the second khaki gene, is widespread in *herbaceum* and is occasionally encountered in *arboreum*. This will be symbolized as Lc_2^B. The other light brown gene, Lc_3^B, is independent of the former two lint colour loci, and has been identified only very rarely and in association with Lc_2^B in *herbaceum*, when the homozygous combination is somewhat darker in expression than the khaki genes. It should be mentioned that on certain relatively stable modifier backgrounds, when dealing with the two light browns, an approximation to the expected duplicate gene segregation is sometimes encountered. This is especially so within *arboreum*. Hutchinson (1935) has reported such a case. In general *herbaceum* is at a much lower lint colour modifier level than *arboreum*. For the classification of segregating progeny a series of lint colour grades has been established; grade 4 is the darkest brown; the khakis grade at 2; whilst 0 is white. In modifier studies it has been necessary to interpolate observations in between the primary colour standards numbered 0, 1, 2, 3 and 4.

The lint of *G. anomalum* is a medium brown of grade 3. The lint of F_1 hybrids involving white-linted *arboreums* grades at about 1·6. The most useful information is derived from backcrosses to *arboreum* white (N 14) of the hybrid *an.* × A 8, which is a dark brown Lc_1^K strain. Data are also available from a backcross to T 3, a synthesized multiple recessive from an *arboreum* × *herbaceum* cross, with white lint. The results show that it has a predominantly *arboreum* lint-colour genotype, so they will be considered along with those from the backcross to N 14. A wide range in lint colour segregation occurred, intergrading from dark brown to light brown, together with some whites which formed a distinct class. The families were scored as follows:

	Lint colour grades								Total		
Cross	4	3	2·6	2	1·6	1	0·5	0	Brown	White	Ratio
(*an.* × A 8) × N 14	13	25	15	18	14	15	1	16	101	16	6·3 : 1
(*an.* × A 8) × T 3	.	40	.	.	7	10	.	8	57	8	7·1 : 1

The presence of whites indicates that *anomalum* cannot carry Lc_1^K, which

is known to be almost fully dominant. The 7 : 1 backcross ratios are suggestive of a three factor segregation. There is further evidence that in addition to the dark brown gene introduced by A 8, two other light brown genes were present. An attempt was made to determine if Lc_2^B, the light brown linked with lintless, were present, by crossing *anomalum* with the lintless strain N 19 and backcrossing to lintless, but none of the progeny grown set seed. A light brown of grade 1 was therefore selected from the backcross of (*an.* × A 8) to N 14, and crossed with N 19 lintless, and a brown-linted segregate again backcrossed to a lintless strain. Ordinarily lintless segregates cannot be scored for lint colour; this is a serious disadvantage from the standpoint of linkage observation, but a strain carrying h_a was available, which, by reason of its modifier content, had some lint on its seed. This strain, T 6, has been described elsewhere under the number P 2417 (Silow, 1939*b*). h_a segregates in backcrosses to this strain can be recognized by the glabrousness of their stems, a pleiotropic expression of the main gene not affected by the modifiers which induce sparse lint on the otherwise lintless seed. The following segregation in this backcross was observed:

	Lint colour grades		
	1·8–1·5	1	0
H_a	9	7	.
h_a	.	10	13

Lc_1^K from A 8 had clearly been lost from this material, in view of the absence of segregates of grade 2. The absence of whites amongst the fully linted H_a segregates indicated the presence of the linked Lc_2^B, which must have been derived from *anomalum*. On the other hand if only this gene had been present, there should have been no browns in the h_a class, in view of the closeness of the linkage concerned. That approximately 50% of the h_a segregates were brown indicates the presence of a second brown lint gene independent of h_a. This must also have come from *anomalum*. By way of contrast, a sib selection from the backcross to N 14, similarly treated, gave:

	Lint colour grades	
	2	0
H_a	17	23
h_a	15	28

In this line the Lc_1^K gene from A 8 only was present, giving a very different segregation from the previous family in which the two light brown genes from *anomalum* were present. The evidence indicates that

no more than these two main genes are responsible for the lint colour of *anomalum*. Whether the second of them is homologous with Lc_3^B is not known. The reasonably clear demarcation between browns and whites in the backcross of the *anomalum* × *arboreum* A 8 hybrid suggests that the minor genotype of these two species is very similar. From experience with *herbaceum* × *arboreum* hybrids, it can be stated that there would have been far more intergrading between browns and whites if the modifier complex of *anomalum* had been at all like that of *herbaceum*.

IX. THE COMPLEMENTARY FACTOR LETHAL "CRUMPLED"

In certain interstrain crosses Hutchinson (1932*a*) reported the appearance of a peculiar semi-lethal or even lethal type of abnormality which he described as "crumpled". He showed that this was due to the interaction of two complementary factors which he named A and B, and which have since been assigned the symbols Cp_a and Cp_b (Hutchinson & Silow, 1939). Cp_a was found in only one strain of *arboreum* var. *soudanensis*, grown under the type number N 9, but Cp_b was present in approximately half of the 29 strains of the several varieties and forms of *arboreum* and *herbaceum* which he tested. There were absolutely no phenotypic indications of the presence of Cp_a or Cp_b, but when strains carrying these genes were crossed the resultant progeny exhibited the crumpled characteristic, its expression being dependent upon the modifier background. In the least extreme case, involving a strain of *arboreum* forma *bengalensis*, the plants were stunted in growth, with shortened internodes, abnormal development of vegetative buds giving a witch-broom appearance, and irregular crumpled brittle leaves, and they set only a very few small bolls. In the majority of crosses involving other *arboreum* types the seedlings were much more abnormal and completely sterile, developing little if at all beyond the cotyledon stage, and the cotyledons themselves in most cases were very thickened and crumpled. This may be regarded as the typical expression of crumpled within the *arboreum* complex, but an even more extreme expression occurred in crosses involving five of the six *herbaceum* strains which carried Cp_b; in crosses between these and the Cp_a strain only empty seeds resulted. Such extreme behaviour only occurred in the case of one Chinese strain amongst the eleven *arboreum* types found to carry Cp_b, and the formation of empty seeds is to be regarded as a typical expression of this complementary lethal in crosses involving *herbaceum*.

Since 1932 a large number of other crosses has been performed at this

Station, giving further evidence on the distribution of these genes. Cp_a has been found again in a second accession of material similar to N 9 from the same general locality in the Anglo-Egyptian Sudan, in the Blue Nile district bordering on Abyssinia, but has not been encountered elsewhere. Altogether 128 different strains representing all the geographic subdivisions of both *arboreum* and *herbaceum* have been crossed with types known to carry the Cp_b gene, and all have given normal progeny. Cp_b has been found in 25 of 41 representative strains tested; both phases of this gene have been found in all the geographic groups which have been investigated, except the Indo-African *herbaceum* varieties *frutescens* and *africanum*, all five tested strains of which have been found to carry Cp_b. Only the *arboreum indica* and *soudanensis* groups have not yet been adequately examined.

G. anomalum has been crossed with several strains carrying Cp_b, such as A 8, N 19, and O1, and with all of these has given normal progeny. The wild species evidently does not carry Cp_a. The Cp_a strain N 9 was crossed with *anomalum*, and all 43 seedlings which were obtained had crumpled cotyledons and died before the exsertion of any plumular leaves. In appearance these crumpled seedlings were similar to those which are characteristic of crosses within *arboreum*. The F_1 (*an.* × A 8) was crossed with N 9; 74 seeds were obtained on the F_1 as female, and 46 on N 9 as female; 49% of the former and 37% of the latter were empty or contained imperfectly developed embryos, ranging from very minute about one-twentieth normal size to nearly full size. Examination of some 1500 seeds derived by backcrossing the same hybrid to ten other *arboreum* and *herbaceum* types which did not carry Cp_a also showed from 15 to 50% of empty or imperfectly developed seeds. Thus although Hutchinson showed that in crosses involving *herbaceum* the development of empty seeds was due to the complementary lethal mechanism, their occurrence in the cross of (*an.* × A 8) to N 9 can only be considered an expression of general hybrid incompatibility. From the fully developed seeds 46 seedlings were established, and all were crumpled, almost exactly as in the direct cross of *anomalum* to N 9, except that three of them exserted from 1 to 5 minute rudimentary leaves before dying. That no normal seedlings appeared indicates that the crumpling factor carried by *anomalum* is at the same locus as in *arboreum*, and it is evident that these two species are very similar in their minor crumpling genotype.

X. LEAF SHAPE

It has already been shown (Silow, 1939*a*) that the leaf shape of *anomalum* is controlled by a member of the *arboreum-herbaceum* leaf shape multiple allelomorph series. This gene, L^A, which has not been recorded from the cultivated Asiatic species, is accompanied in *anomalum* by a lobe-broadening genotype similar to that which distinguishes *herbaceum* from *arboreum*.

XI. DISCUSSION

(1) *Genetic aspects; flower pigmentation interactions*

The genetic information and the observational records of interaction now available suggest that the genus *Gossypium* would form very suitable material for a biochemical study of the developmental relationships between anthoxanthins and anthocyanins, which would be the more interesting in that some of these pigments show very striking specific distributions. Within *arboreum* all three dominants Y_a, Y_b, and Y_c are required for the production of full yellow corolla. From such flowers Perkin (1916) was able to isolate the glucosides gossypitrin and isoquercitrin. In the same species y_a leads to the development of white flowers with petals some 25% shorter than yellows. From these Perkin isolated only a small quantity of a substance resembling apigenin in its general properties, a flavone known to occur in several other ivory-white flowered plants. Like these, the white flower of *arboreum* gives the typical flavone reaction on fuming with ammonia. The intermediate pale yellow $Y_a{}^P$ at the same locus is much nearer white than full yellow in its low intensity of colour, but much nearer yellow than white in its very slight reductive effect on length. At each of the other two loci only one recessive allele is known, and both are phenotypically pales. They can be distinguished from one another with reasonable confidence when on a common stable background. In view of the possibility that lower alleles at these loci may eventually be found, like white at the Y_a locus, these pales have also been designated as $\mathring{Y}^P$. The Chinese pale $Y_b{}^P$ is much like the Burmese pale $Y_a{}^P$, being very slightly paler in shade than the latter, and only very slightly shorter than full yellow. $Y_c{}^P$, which is confined to and characteristic of *anomalum*, is definitely slightly yellower than the other two pales, yet shows much the same degree of shortening of the petal as does white. Corolla size appears to be physiologically associated with the main colour classes, since no recombinations which could be attributed to crossing-over between linked genes have ever been

observed. The indication, from the small number of observations available, that the double recessive $Y_b^P\ Y_c^P$ is intermediate in petal length rather than as short as Y_c^P, if substantiated, would imply that the mechanism by which shortening of the petal is effected is not the same in all cases. It seems very likely that the three pale corolla types differ chemically, and full yellow and white certainly do, so that an investigation of these substances might throw interesting light on the relation of certain organic radicals to growth processes.

Various strains and species of cotton are characterized by a red corolla. As judged by differences in their appearance and in their reaction with the flavones, at least two different types of anthocyanins are concerned. As an example of one of these types, the *anomalum* R_3 gold-petal allele may be taken. It has been shown (Table 11) that its expression is very dependent upon yellow corolla constitution, changes in genotype at the Y_a and *Ydp* loci especially being associated with variations in intensity of anthocyanin on the petal lobe. In the absence of the gold allele Y_a is almost though not fully dominant as far as yellow intensity is concerned, but heterozygosity for pale or white at this locus lowers the expression of R_3^{GO} very considerably. This is interpreted as indicating a correlation between production of anthocyanin and yellow flavone, such as Lawrence & Scott-Moncrieff (1935) demonstrated in *Dahlia*. Thus although Y_a appears to be of high phenotypic dominance, it is actually of low dominance as far as quantitative production of pigment is concerned. Hutchinson (1931) has shown that this gene is not fully dominant in the petal length effect either. Heterozygosity for pale in the Y_b or Y_c loci was not found to affect gold expression, suggesting either that the physiological dominance relationships at these loci is different from that at the Y_a locus, or that these loci control the development of different types of yellow or pale flavone. In the presence of duplex recessives at any one of the three *Y* loci gold hardly attains expression at all. Interaction with the yellow depressor, *Ydp*, which is restricted to *anomalum* in its natural distribution, is particularly instructive. Since this gene diminishes the intensity of pales as well as of full yellow, it seems likely that it restricts the quantity rather than that it affects the type of flavone, i.e. it acts as a general and not a specific anthoxanthin suppressor. When on a homozygous yellow background the yellow depressor intensifies the expression of R_3^{GO} from gold to pink. That this is a real and not an apparent intensification due to the lowering of intensity of the yellow background is supported by the fact that *Ydp* does not intensify gold on heterozygous yellow to the slightest extent,

though the yellow background is depressed. This incidentally affords further evidence of the physiologically cumulative effect of Y_a dosage. The antagonistic interaction between anthocyanin and flavone which is indicated by the concurrent suppression of yellow and intensification of gold to pink, gives strong support to the theory of Lawrence & Scott-Moncrieff (1935) of a limited pigment source common in part to both anthoxanthins and anthocyanins. That this is not the whole of the story, however, is evident from the fact that, in the absence of the depressor, cyanic intensity is positively associated with Y_a dosage. Lawrence & Scott-Moncrieff described a yellow inhibitor in *Dahlia* which appears to be similar to *Ydp*, and which also increases cyanic intensity. In view of their opinion that the production of specific pigments is simultaneous rather than successive, they suggested that the yellow inhibitor reduced the productive and competitive power of the yellow flavone gene at the source. They found that the cyanic intensification was due to a reduction in the proportion of pelargonin to cyanin. Whether the intensification in the case of R_2^{GO} is likewise qualitative, or whether it is quantitative in nature is not known, and in view of the complications which they observed it would be futile at this stage to attempt an interpretation of the mode of action of the several genes concerned in *Gossypium*. It does however seem clear that *Ydp* must act after the inception of the pigment source. The fact that the depressor inhibits yellow flavone production in both simplex and duplex Y_a, but that this results in cyanic intensification in the latter genotype only suggests that a threshold level is involved and that at least some pigment production reactions of this type are not necessarily simultaneous but may be sequential. Confirmatory support of this hypothesis is available from a totally different source. The typical cultivated Asiatic cotton flower has a yellow corolla, and a red spot due to the gene R_2^{AS}. In the fresh flower anthocyanin is restricted to the red spot at the base of the petal, the petal lobe being totally devoid of any red pigment. As the flower wilts towards the end of the first day of anthesis the petal lobe acquires a faint cyanic tinge, and by the following morning the anthocyanin intensity is very much as in the *anomalum* type of "medium to intense" gold (Pl. 18, figs. 9, 10). The petal lobe of Chinese pale-yellow corolla plants carrying the spot allele is also devoid of anthocyanin when fresh, but as the flowers age they acquire a much more intense coloration than do full-yellow spotted plants, going even beyond the intensity of the "pink" shown in Pl. 18, fig. 12. Here also then there are indications of antagonistic interaction between flavone and anthocyanin production, and in this case there can be no doubt that the pro-

duction of anthocyanin is subsequent to that of flavone. The situation suggests that, as far as their control of anthocyanin production in the petal lobe is concerned, the distinction between the gold petal gene, which initiates anthocyanin production early in the life of the flower, and the ordinary spot gene, which induces anthocyanin formation in the petal lobe only as the flower fades, lies entirely in their reaction rates.

As an example of the second of the anthocyanin types in *Gossypium* the so-called "*sanguineum*" type, which occurs in *arboreum*, may be taken. This has an intense wine-red petal lobe, very different from gold both in appearance and in its interactions, which have been described by Hutchinson (1932*b*). Perhaps the most striking difference is that variability in its expression is almost entirely in terms of distribution on the petal, and to a much lesser extent in intensity, as may be seen from Hutchinson (1932*b*, Pl. XXVI). His grades 1–11 represent increasing degrees of extension of red from the margin to cover the entire surface of the petal. Most $Y_a\,Y_a$ types with red grade at 11, $Y_a{}^P\,Y_a{}^P$ types at 2–4, and $y_a\,y_a$ types at 1–2. Heterozygosity of yellow for pale or white in the Y_a locus has only a slight effect in restricting expression of red to grade 10, whereas gold under the same conditions is reduced from intense-medium to faint or very faint. There is not a great deal of information on the effect of heterozygosity in the Y_b or Y_c loci, but in some families heterozygous both for y_a and $Y_b{}^P$ or $Y_c{}^P$ there was no degradation below that involved in the case of $Y_a\,y_a$ alone. In a family heterozygous for $Y_a\,y_a$ and for the depressor however reds ranged only from 7 to 9, i.e. expression of red was reduced in the same general direction as when yellow grade is lowered by main gene constitution. Unfortunately no data are available for this red on duplex Y_a with *Ydp*. Taking into account the evidence available on interaction with flavone genotype, and the very different colour of the *sanguineum* $R_2{}^{RS}$ red as opposed to the $R_3{}^{GO}$ gold, it appears very likely that these two anthocyanin types differ qualitatively. From phenotypic appearance it is probable that the New World tetraploid *hirsutum* $\mathbf{R}_1$ red petal is similar in nature to the *arboreum* $R_2{}^{RS}$. When this varies in expression it also does so chiefly in distribution. However in its most extreme variants it has never been observed to cover anything like so great a proportion of the petal as does the R_2 red either in *arboreum* or when transferred to *hirsutum* (Harland, 1935). In this case then, with respect to their petal red effects as distinct from their spot effects[1], it appears plausible that the *hirsutum* and *sanguineum* reds represent only *quantitative* variants of the same basic type.

[1] The *arboreum* red is spotted, whilst the *hirsutum* red is spotless.

The diploid American *aridum* has a pink petal, and from work in progress it has been established that the allele responsible is homologous with $\mathbf{R}_1$ of the tetraploid species. It has been observed that the petal colour of many of the segregants derived from backcrosses of *aridum* to the tetraploid species is very suggestive of *anomalum* gold, and as in the latter, variation is primarily in intensity rather than in distribution on the petal. It must be remembered that there is no genetic evidence that $\mathbf{R}_1$ and R_3 are not homologous, and that the latter symbol subscript was adopted because it is unlikely that they are of "recent" homology in view of Skovsted's theory that the New World tetraploids are allopolyploids derived from an Asiatic and an American diploid species or cytologically similar types. Quite apart from this, if the assumption is correct that the *hirsutum* and "gold" reds differ qualitatively, the demonstration that *aridum* red, similar to gold, is located at the *hirsutum* $\mathbf{R}_1$ locus implies that qualitative variants may occur at a single anthocyanin locus. This is quite plausible in view of the fact that alleles which control the production of full yellow and pale yellow flavone, which are almost certainly different, are known to occur at the same locus along with one which determines ivory flavone. Such a qualitative anthocyanin series has been described in *Callistemma* by Wit (1937), who attributed the formation of pelargonidin, cyanidin and delphinidin derivatives to different members of a triple allelomorph series. On the other hand Lawrence & Scott-Moncrieff attributed the formation of specific anthocyanins, not to individul genes, but to a general balance between all anthocyanin and anthoxanthin factorial contributions. It is interesting to note that they consider this to be an uncommon situation resulting from the competition of homologous factors in an allo-octoploid. It is highly probable that such a derivative complication may occur in *Gossypium* also, superposed upon the possibility of specific factorial control. Skovsted (1933) considers that in this genus even the so-called diploid species with $n=13$ are secondary polyploids. He based this opinion on the occurrence of secondary pairing, which by itself is not necessarily a valid indication of homology (Heilborn, 1936). Support is however given to his interpretation by the occurrence in the diploid species of genetic replication, some types of which of late years have come to be regarded as evidence of cytological replication also. Thus in *arboreum-herbaceum* there is triplication of lint colour loci (this paper, § VIII):

$Lc_1{}^K$	$Lc_2{}^K$		(Khaki)
	$Lc_2{}^B$	$Lc_3{}^B$	(Light brown)
lc_1	lc_2	lc_3	(White)

Genetic duplication for anthocyanin has also been demonstrated in *anomalum*. This is an unusual type and its interpretation as such will be further substantiated below. It is probable that in cotton certain complementaries also may be regarded as replicated loci. Though argument on these lines could very easily be carried too far, it is to some extent justified in *Gossypium* by the parallelism in mutant steps which is exhibited in some of these loci, such as those affecting corolla colour and pollen colour:

Corolla colour	Y_a	Y_b	Y_d	(Yellow)
	Y_a^pP	Y_b^pP	Y_d^pP	(Pale)
	y_a	.	.	(White)
Pollen colour	P_a	P_b	.	(Yellow)
	.	p_b	.	(Pale)
	p_a	.	.	(Cream)

If these cases of complementaries be regarded as replication, then there are two cases of triplication. Skovsted (1937) has suggested that the basic 13 in this genus represents five chromosomes duplicated and only one triplicated, so that at first sight it appears that there are too many cases of genetic replication to fit in with the suggestion that they indicate cytological replication, there being no evidence of linkage between any of the lint colour and corolla colour loci. Yet the suggestion cannot be turned down on these grounds alone at this stage of our knowledge. In other genera various cytological mechanisms have been involved in speciation, and some of these, such as replication of parts of chromosomes, may also have played their part in evolution within *Gossypium*. Winge (1938) has extended the underlying idea to its logical conclusion in his hypothesis of the taxonomic importance of polymery, pointing out the stabilizing influence, in units of higher taxonomic rank, of the frequent replication of particular factors throughout the genom as a result of polyploidy, duplication and translocation.

The interpretation of the *anomalum* anthocyanin locus as a duplicate of that in *arboreum* and *herbaceum* requires special explanation. The most common anthocyanin expression in the latter, red-tinged stem and red-petal spot, is due to a single allele in the R_2 series. Two lower members of this allelomorphic series are known, though they are relatively uncommon. They are respectively ghost spot, which lacks the capacity to produce red colouring matter, and spotless, which is similar to red petal spot in vegetative expression. In compound these two lower alleles resemble the common red petal spot phenotype. Of course this compound type never breeds true. *G. anomalum* also has a slightly red-tinged stem and red

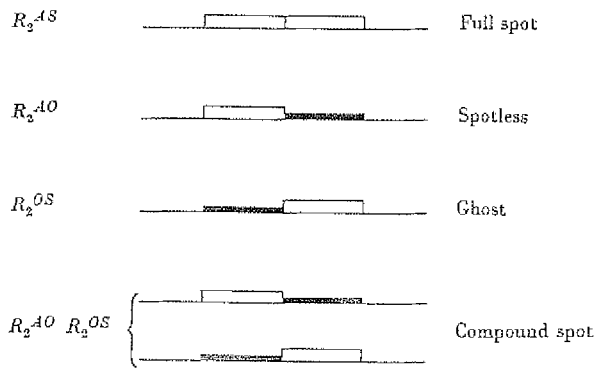
petal spot, similar in size and appearance to that typical of most of the cultivated Asiatic cottons, but genetically it is constructed in a totally different way. It is not, as in the latter, due to a single allele, but is the result of the complementary interaction between a ghost gene in the Asiatic R_2 locus, and a spotless gene in another locus, R_3. This type of compound spot is naturally true breeding. Although these particular R_2 and R_3 genes act as complementaries, the loci are regarded as duplicates for two reasons:

(i) The R_3 allele is comparable in type and behaviour with known members of the R_2 series in *arboreum-herbaceum*.

(ii) If spotless were not known in the latter series, it would not have been possible to identify the presence of a spotless gene in the R_3 locus, and the *anomalum* spot would have behaved as if it were due to an identical duplicate of the Asiatic full spot allele.

Just as in the cultivated species other members of the R_2 series are known which have intense red pigmentation of either petal lobe, plant body, leaves, or calyx, as pleiotropic effects of the main anthocyanin spotted or spotless allele, so the *anomalum* R_3 spotless gene is characterized by a gold-petal lobe. Here again, as in the leaf shape series of allelomorphs which have recently been discussed (Silow, 1939*a*) it looks as if there are at least two independently variable systems within the anthocyanin series, one involving presence and absence of spot, the other presence and distribution of anthocyanin. Thus in *arboreum* red-leaf types and red-tinged stem types, both with and without spot, are known. Hutchinson (1934) in discussing the organization of the gene, envisaged the anthocyanin locus as embodying a protosome with two gene centres on which a series of episomes are attached, with one centre controlling presence or absence of basal anthocyanin, and the second centre determining distribution of anthocyanin in the plant. If this basic concept be extended to include the further information on anthocyanin inheritance in cotton now available, the two gene centres may be better conceived as controlling respectively presence and distribution of anthocyanin, and presence and absence of petal spot. The arrangement of the R_2 series of alleles in *arboreum*, as listed at the beginning of § IV of this paper, conforms to this scheme. It seems highly probable that spotless equivalents of the other spotted alleles R_2^{ES}, R_2^{CS} and R_2^{OS} may be found in the future. It is likely that the spotless equivalent of the latter, r^{OO}, exists in the R_3 locus in *arboreum* (see § IV (2)). This scheme takes into account

the complementary nature of ghost and spotless types in giving the spot phenotype, e.g.



This conception may also be extended to the R_3 locus. It is very likely that, with the discovery of further allelomorphs in the future, it may be found that the anthocyanin possibilities in the petal lobe form a third system varying independently of vegetative anthocyanin and petal spot.

It is tempting to speculate on the possibility that New World spots are not in fact true full spots in the Asiatic sense, but perhaps ghosts segregating on a uniformly spotless background. All New World types without red spot are spotless. The ghost phenotype is not known. It is of interest in this connexion that the progeny of Upland spotless (*hirsutum*) × tetraploid N 14 ghost[1] (*arboreum*) show red spot. At present there is no indication as to the function of red petal spot in the economy of the plant, though that it may be of some significance is suggested both by its very frequent presence not only in *Gossypium* but in several related genera as well, and by the complicated and unusual genetic mechanism by which it is attained in *anomalum*. Yet from some of Wright's (1931) mathematical treatments of the problems involved in variability and differentiation in natural populations it is clear that many specific distinctions may be without any adaptive value whatsoever.

(2) *Speciation in the genus* Gossypium

This study of inheritance in interspecific crosses has clearly demonstrated the genetic processes involved when the plant breeder makes wide crosses in order to "break the type", completely new characters being built up by the combination of genes which do not normally occur together in nature. Similar studies in several other plants and animals

[1] Doubled by colchicine treatment by Mr S. G. Stephens of this Station.

have led to certain deductions as to the mechanisms by which species become differentiated. These have recently been reviewed by Harland (1936), who pointed out that in all cases the process of gene substitution was involved, and he illustrated this from the results of his own experiments in New World cottons. The genus *Gossypium* is particularly favourable material for this type of investigation in that it incorporates a number of well-defined species, several of which yield highly fertile hybrids. Indeed this situation in early days led to considerable difficulty in interpretation of genetic results in this genus, since contrasting pairs of characters were frequently only available in different species. Harland's realization that this involved segregation in many minor genes or modifiers as well as in the main ones under study quickly cleared up the situation, and he has had considerable success in interpreting results following "transference" to a uniform background stabilized by recurrent backcrossing. The application of this method has made possible the genetic analysis of many characters in cotton, and on the basis of the extensive type collection available at this Station it has been possible to formulate a reasonably comprehensive picture of the make-up of several species. However, in this genus, as in others, genetic comparisons have so far been limited to a restricted portion of the wide range of specific differentiation exhibited. Thus Harland's information on differences amongst the New World species, and that of Hutchinson in the Old World cottons, has referred to groups of species so closely related amongst themselves that their hybrids are fully fertile in the first generation, and only show some degree of breakdown in the second and subsequent generations—obviously the minimal degree of distinction which can be regarded as of specific status. The first generation hybrids of *anomalum* and the cultivated Asiatic cottons are only 1% fertile on selfing, and 10% fertile on backcrossing, so that the examination may now be extended to cover a much wider range of specific divergence—the widest in fact which can be subjected to analysis before complete sterility imposes an insurmountable obstacle to genetic study. Admittedly, differences in fertility by themselves must be accepted with caution as indications of relative magnitude of specific differentiation, but in this case, that the distinction involved between *anomalum* and the two cultivated Asiatic species is much greater than that between the latter themselves is supported by morphological differences which have led some taxonomists to consider the wild African species as even generically distinct.

Several evolutionary processes have been at work in *Gossypium*. Skovsted (1934) has shown that the diploid species may be separated

broadly into an Asiatic and an American complex, between which there is cytologically practically no homology. The American tetraploids appear to be allopolyploids containing both the Asiatic and American diploid complements. Interspecific hybridization followed by amphidiploidy has therefore played an important part in the evolutionary history of *Gossypium*, but this Lotsyan type of evolution has undoubtedly been both preceded and followed by the more gradual evolutionary process of gene substitution. The most significant information bearing on this process is likely to be derived from a study of the situation within any one of the basic groups of species, though in view of Skovsted's (1937) hypothesis that even the diploids are secondary polyploids, it will be impossible to be sure which of the differences found are the result of gene substitution and which the result of polyphylesis.

The lowest grade of speciation which it is possible to recognize as such, that in which the first appearance of sterility and breakdown in viability is delayed to the second hybrid generation, is well exemplified by the two cultivated Asiatic diploid species, *G. arboreum* and *herbaceum*. Their differences in genetic structure with reference to the main and minor gene constitution of eight different characters have been assembled in Table 14 from Hutchinson's publications and subsequent work by the author, some of which is in course of publication. Side by side with this information that from *anomalum* is also tabulated for comparison. It is important to point out that in the *anomalum* crosses every character was investigated which was at all amenable to analysis, particularly with reference to the genes so far known in the cultivated cottons, so that the results presented in Table 14 are as complete as is reasonably possible. The only characters on which adequate observations were not made were on such quantitative variations as in intensity of hairiness, and breadth and laciniation of bracts, in which it was felt that the only outcome of laborious observations was likely to be an addition to the already lengthy series of vague statements that "the character was controlled by multiple factors". In Table 14 the symbol + indicates the genotype of the one and only accession of *anomalum* which has been available for study. Herbarium collections do not indicate any great variability within this species. In the two cultivated species, of which a type collection of some 200 lines is available, together with much herbarium and published information, there is considerable variability. In these the relative frequency of occurrence of different alleles is indicated. In estimating the modifier status of a species the terms high and low have been used in a strictly conventional sense, being termed "high" if the modifiers are

such as to enhance the expression of the character in the same direction as the dominance of the main gene. Thus in the case of the leaf shape series where dominance is in the direction of narrower lobed phenotypes, the modifier background of *arboreum* is such that the expression of a particular main gene is narrower than in *herbaceum*, and so is considered relatively "high".

From Table 14 it will be seen that *arboreum* and *herbaceum* are characterized by the same main gene in thirteen of the fifteen loci tabulated. The only tendency to divergence in these loci lies in the strikingly different frequencies with which certain of the less common alleles occur in the two species, as in the Y_a, Y_b, R_2, P_a, P_b, Lc_1 and Lc_3 series. Some of the rarer of these alleles have been recorded in only one or other of the two species. In the L series *herbaceum* is characterized by an allele which is also very common in *arboreum*. In the latter species other alleles, one of which is equally common, occur in addition; these have not been recorded at all in *herbaceum*. In Lc_2, the remaining one of the fifteen loci, is the only case where there does appear to be a fairly constant difference between these two species, though even here the distinction is by no means absolute. Lc_2^B is the most frequent allele at this locus in *herbaceum*, but lc_2 is sometimes present; and although the latter is characteristic of *arboreum*, the higher member does occasionally occur. Thus we may summarize that there is not any striking difference between the two cultivated Asiatic species as far as their main gene constitution is concerned. Practically all of the difference lies in the modifier systems, which are quite distinctive though usually nothing like so different as in the case of *anomalum*. Nevertheless in at least seven of the eight characters studied there is wider modifier segregation in interspecific than in most interstrain crosses, or the species may even be characterized by an obvious displacement in expression of the main allele, as in the case of leaf shape and lint colour.

Much greater divergence in genetic constitution is apparent in *anomalum*. In this species, in only nine of the fifteen loci examined are the same genes present as are common in or typical of the cultivated species. There is no point in trying to make out of this a proportionate estimate of similarity. The number of genes in which *anomalum* is similar to the other two species must be very high, since this figure must include all those genes which might be described as familial or generic, as opposed to specific. There can be no doubt that much the same normal alleles or ones so similar as to be almost indistinguishable must occur in *anomalum*, as in *arboreum* and *herbaceum*, at the loci which occasionally carry the

Table 14. *A comparison of the genetic constitution of three diploid species of* Gossypium

Character	Constitution	*arboreum*	*herbaceum*	*anomalum*
Corolla colour	Y_a yellow	**Typical form**	**Typical form**	+
	$Y_a{}^p$ pale	Restricted to Assam, Bengal and Burma, where it is fairly frequent	Very rare; only once recorded in var. *frutescens*	
	y_a white	Restricted to northern India, Burma and China, where it is fairly frequent	Not recorded	
	Y_b yellow	**Typical form**	**Prevailing form**	+
	$Y_b{}^p$ pale	Restricted to Burma and China, rare	Not recorded	
	Y_c yellow	**Prevailing form**	**Prevailing form**	+
	$Y_c{}^p$ pale	Not recorded	Not recorded	
	Yellow intensity modifier status	—	Somewhat below that of *arb.* especially in var. *typicum*. Much nearer *arb.* than *an.*	**Distinctive;** in aggregate low, primarily on account of the specific member *Ydp*
Anthocyanin	$R_2{}^{RS}$ full red	Occurs in Africa, northern India, Burma and Java; uncommon	Not recorded	+
	$R_2{}^{LS}$ red leaf	Restricted to China, where it is uncommon	Very rare; only once recorded, in *typicum*	
	$R_2{}^{CS}$ red calyx	Not recorded	Rare, in *typicum* only	
	$R_2{}^{AS}$ red spot	**Typical form**	**Typical form**	
	$R_2{}^{OS}$ ghost	Restricted to Burma and China, where it is uncommon	Not recorded	
	$R_2{}^{LO}$ red spotless	Very rare; only once recorded, from China	Not recorded	
	$R_2{}^{AO}$ spotless	Very rare; only one or two records from China	Very rare, in *typicum* only	
	$R_2{}^{GO}$ gold spotless	Not recorded	Not recorded	+
	$r_2{}^{OO}$ spotless	**Prevailing form**	**Prevailing form**	
	Spot size modifier status	—	Somewhat below that of *arb.*, especially in var. *typicum*. Much nearer *arb.* than *an.*	**Very distinctive;** in aggregate the level is much as in *arb.* and *herb.*; since there is one very potent large size gene on the R_2 chromosome, the balance of the modifier system must be at a relatively low level
Pollen colour	P_a yellow	**Prevailing form**	**Typical form**	+
	p_a cream	Not recorded	Very rare; only once recorded, in *typicum*	
	P_b yellow	**Typical form**	**Prevailing form**	+
	p_b pale	Very rare; only once recorded, in *indica*	Not recorded	
	Yellow intensity modifier status	—	Variable; some strains similar to *arb.*, others slightly lower	**Distinctive**

Nectaries	*Ne* present *ne* absent	**Typical form** Common	**Prevailing form** Not recorded	+
	Leaf nectary modifier status	—	**Distinct** from that of *arb.* and *an.*	Intermediate, but very near *arb.*
	Bract nectary modifier status	—	Distinct from that of *arb.*, but much nearer *arb.* than *an.*	**Very distinctive** in that external bract nectaries are present
Seed fuzz	*Fz* tufted	Uncommon except in China	Very rare; not known if the occasional tufted types which occur are really due to this gene	
	fz fuzzy	**Typical form**	**Typical form**	+
	Fuzz intensity modifier status	—	Lower than that of *arb.*	Much lower than in *herb.*
Lint colour	Lc_1^K khaki lc_1 white	Infrequent **Typical form**	Not recorded **Prevailing form**	+
	Lc_2^K khaki Lc_2^B light brown lc_2 white	Infrequent Infrequent **Typical form**	Not recorded **Typical form** Probably not common	+
	Lc_3^B light brown lc_3	Not recorded **Prevailing form**	Occasional **Typical form**	+?
	Brown intensifier status	High	Low	As in *arb.*
Crumpled lethal	Cp_a	Very rare; only once recorded	Not recorded	
	cp_a	**Typical form**	**Typical form**	+
	Cp_b cp_b	Common Common	Common Common	+
	Modifiers	Crumpleds usually lethal in cotyledon stage	Empty seeds	As in *arb.*
Leaf shape	L^L	Very rare, only once recorded	Not recorded	
	L^N	Fairly frequent, though probably confined to *cernuum*	Not recorded	
	L	**Common**, especially in Bengal	Not recorded	
	L^A l	Not recorded **Common**, especially in China and southern India	Not recorded **Prevailing form**	+
	Leaf narrowing modifiers	High	Low	Low as in *herb.*

rare and frequently deleterious recessive mutants such as the five types of lintless, chlorophyll deficiency, petalody, curly leaf, etc. It is the relatively few main loci, six in number, in which these species differ, which are of significance. *G. anomalum* is characterized by an allele at R_2 which is uncommon in *arboreum* and unknown in *herbaceum*, by a brown lint gene which is uncommon in *herbaceum* and unknown in *arboreum*, by a cream pollen gene at P_a which in spite of much search for this type of gene has only once been encountered in *herbaceum* and not at all in *arboreum*, and by alleles which are quite unknown outside this species at the Y_c, R_3, and L loci. In modifier constitution *anomalum* is also strikingly distinct from the two cultivated species, the hybrid progenies showing very much wider segregation than do crosses between the latter species. In modifiers of corolla colour, anthocyanin intensity, spot size, pollen colour, bract nectaries, and seed fuzziness the wild species is very distinct from both of the cultivated ones; in leaf shape modifiers it is much nearer *herbaceum* than *arboreum*, and in leaf nectaries, lint colour, and crumpled there was no evidence that it differed appreciably from *arboreum*, though in modifiers of all four latter characters the two cultivated species are markedly distinct from one another. In Table 15 an attempt has been made to give a diagrammatic representation of the extent to which *anomalum* is considered to differ in modifier constitution from the other two species, by the horizontal displacement of the symbol × from the two dotted lines which are intended to give a conventionalized picture of the relative modifier level in the cultivated species.

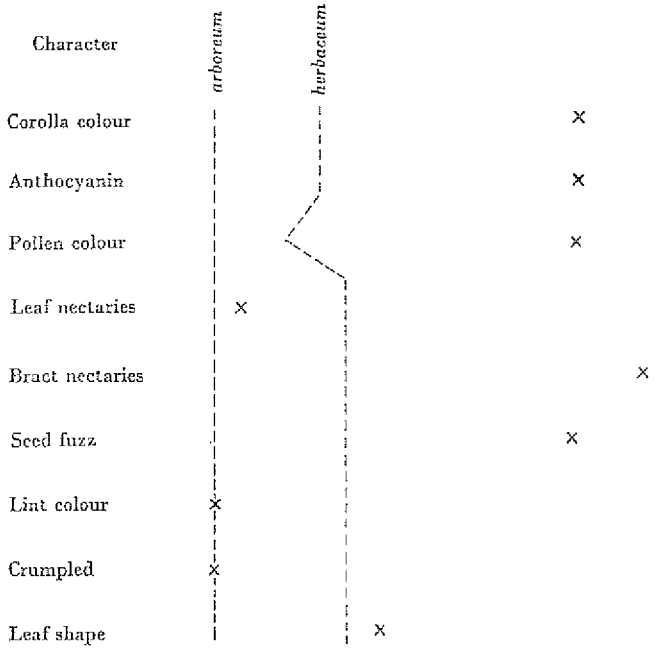
In this table the modifier constitution is depicted in relation to each of the characters studied, and not in relation to each of the independent genes controlling a particular character. This is justified by the fact that the interaction between genes is not direct but by way of their somatic manifestations (Silow, 1939*a*) so that in general it is unlikely that a set of modifiers will be restricted in their action to particular genes. The demonstration (§ IV (6) of this paper) that the spot size modifiers extracted from *anomalum* affect all types of spot, including ghost, full red spot, and those due to complementary alleles either at the same or at duplicate loci, affords an excellent example of the general non-specificity of modifiers as far as the alleles and loci affecting any particular character are concerned.

The final stage in differentiation is seen in those species which are so distinct that their hybrids, if obtainable at all, are completely sterile. In *Gossypium* such sterility is not confined to crosses between species

with different chromosome complements, but may also occur between species within any one cytological grouping. Although the geneticist cannot do a great deal with such material, the differences are in many cases so distinct that there can be no doubt that many of the main genes as well as their modifiers are dissimilar.

We thus arrive at the generalization that the more widely species within this genus are separated, as estimated by the level of fertility of their hybrids and by the number of morphological characters by which they are distinguished, the greater the magnitude and the number of the

Table 15. *The modifier situation—a diagrammatic representation of the extent to which* anomalum (*indicated by* ×) *differs from* arboreum *and* herbaceum *in modifier constitution*



genes in which they differ. It is quite impossible to give any absolute estimate of the magnitude of specific differences in terms of genes, but on the assumption that there is only one modifier affecting practically every main gene, it may be hazarded that the minimum degree of speciation recognizable as such is associated with a difference in at least 50% of the loci involved—bearing in mind in this connexion that there is also a necessarily unknown proportion of the genotype which must be

common to all species in the genus. Actually the number of minor gene differences is probably somewhat greater than this rather conservative estimate, though perhaps not much greater. There is a general impression that modifiers are very numerous and of almost infinitesimally small effect, but it does not seem that the situation is necessarily quite so extreme as this. It has been striking that in favourable material in which it has been possible to identify individual modifiers, four at least have quite considerable potency, although of course not comparable with that of the main gene. It may well be asked whether those particular modifiers have only been identified on account of the magnitude of their effect, but this is definitely not the case, since three of the four were identified almost entirely on account of particularly favourable genetic circumstances. Thus the spot size modifier carried by *anomalum* was identified on account of its linkage with the ghost allele, and by itself is sufficient to account for all of the upward extension in range in spot size in the hybrid progenies. The two lintless modifiers (Silow, 1939*b*) were identified because they affected only one of the two pleiotropic effects of the main lintless gene, so that it has been possible, in a wide segregation, accurately to delimit the main gene phases, a very necessary proviso in the analysis of any interaction system. These two genes were of surprisingly high potency, and in recent work it has been possible to follow them through with considerable confidence. They alone are sufficient to account for practically all of the overlap between two main classes which in narrow crosses are absolutely distinct from each other. The fourth large modifier, yellow depressor *Ydp*, was the only one identified solely on account of the magnitude of its effect, after separation from other interfering factors by backcrossing. This gene was sufficient by itself to account for practically all the downward extension of corolla colour in the interspecific crosses. Although two of these four modifiers were isolated from wide crosses between species which, as has already been shown, tend to differ in genes of relatively large effect, two of them, the lintless modifiers, were found in different varieties of one species. It is evident that a very limited number of such modifiers, with slight variability in expression, would simulate a typical "multiple factor" segregation, and be sufficient to give the apparently complete continuity which characterizes interspecific segregations.

The genic situation in the two cultivated New World allotetraploid species *hirsutum* and *barbadense* is somewhat intermediate between that in the cultivated Asiatics on the one hand and in *anomalum* on the other, although on the basis of morphological criteria of taxonomic importance

and the high fertility of their hybrids they would appear to be hardly more distinct than the two cultivated Asiatics. Harland (1936), in a review of his work in the New World cottons, has tabulated the main gene constitution of *barbadense* and *hirsutum*, and this may be summarized on the following lines. The genes controlling fuzz characters will be ignored, as their relationships in the two species are not fully understood. The S series and the R^B series should not be cited separately, as they have been found to be allelomorphic (Harland, 1932). Contorta has since been found to be allelomorphic with crinkled (Hutchinson, unpublished data). This leaves some 13 main loci; in the revised terminology of Hutchinson & Silow (1939)[1] they are $\mathbf{R_1}$, R_2, $\mathbf{Y_1}$, $\mathbf{Y_2}$, **P**, $\mathbf{Lc_1}$, $\mathbf{Lc_2}$, **Lg**, **Cr**, **L**, **V**, $\mathbf{Chl_1}$, and $\mathbf{Chl_2}$. At seven of these loci the two species are characterized by the same main allele; in most of these the less common alleles are quite rare and have usually been recorded in only one or other of the species. In four other loci ($\mathbf{Y_1}$, **P**, $\mathbf{Chl_1}$, $\mathbf{Chl_2}$) the two species are characterized by different alleles, but these are not confined entirely to the species in which they are most common. In the remaining two loci (R_2 and **L**), both of which contain multiple series, each of the two species is characterized by alleles which are either absent from or extremely rare in the other species. Thus in their main loci these two species show a slightly greater degree of differentiation than the two cultivated Asiatic cottons. Of course their amphidiploid nature itself gives greater scope for divergence, there being no proof that they are monophyletic in origin. Modifier segregation also appears to be somewhat wider in crosses between *hirsutum* and *barbadense* than in those between *arboreum* and *herbaceum*, in that it is usually more difficult to separate the main gene phases; in some cases, as in that of certain members of the spot series, this may be due to the lesser distinction between the alleles themselves, but it is improbable that this is the general situation. On the other hand, it must be remembered that the New World cultivated cottons, being tetraploids, have a greater potentiality for variability than the diploids, and in the absence of a common basis of comparison it cannot be said that the apparently greater modifier segregation necessarily indicates a wider differentiation.

It must not be presumed that each of the species of *Gossypium* is characterized by a uniform modifier constitution. Within each of the four widely-distributed cultivated species there is very considerable

[1] In this scheme genes in the Asiatic cultivated species are indicated by symbols in italics; those in New World cultivated cottons by symbols in bold-faced type, or in italics if proven homologous with loci in the Asiatic species.

diversification in both main and minor genes, so that the geographic forms and many local strains have come to be characterized by markedly distinct complexes with regard to particular characters. Considerable information on this phase of subspecific divergence is available from the two cultivated Asiatic species. Hutchinson & Ghose (1937*a*) have recently revised the taxonomy of these species, and the trends within them may best be discussed on the basis of their classification. Within *arboreum* they established three varieties, two of which were based on the distinction between the annual and perennial habit. Each of these two varieties, the perennial *typicums* and the annual *neglectums*, were separated into the same four geographic forms. Actually from a purely genetic viewpoint it would have been preferable to give the more fundamental geographic trends the higher varietal status; the acquisition of the annual habit is a recent tendency superposed upon these, occurring independently in most parts of the distribution area of the species. As, however, the geographic trends are not associated with any distinctive tags of value to the morphologist, Hutchinson & Ghose did not feel justified in giving them varietal status and relegated them to the subordinate position. Of the four geographic forms, probably the southern Indian *indica* includes the largest proportion of primitive types.—Practically all members of this group have broad leaves (*l*) and yellow flowers (Y_a). They have medium long and moderately fine lint, but are low ginners (Hutchinson & Govande, 1938). This group is characterized by the presence of modifiers of lintlessness, which are members of the genotype controlling the density of lint hairs on the seed; elsewhere in *arboreum* these particular genes only occur in China (Silow, 1939*b*). The African *soudanensis* group is very similar but slightly more variable in simple morphological characters like leaf shape and anthocyanin, narrow leaf (*L*) and the rare full red (R_2^{RS}) occurring occasionally. The central, northern and eastern Indian *bengalensis* types tend to be more hairy than other *arboreums*; narrow leaf is more common here than elsewhere, almost completely supplanting broad in some areas; in some localities full red is fairly common, and both pale ($Y_a{}^P$) and white (y_a) flowers as well as the more common yellow occur. Lint tends to be shorter in this group than in any other, and as a result of its coarseness these types are heavy ginners. Lintless modifiers have not been found amongst them. The fourth geographic section in the classification of Hutchinson & Ghose, *burmanica*, is not by any means a homogeneous natural grouping in that the Burmese cottons appear to be quite distinct from those in China. The Burmese types are a variable lot. Pale and white flowers, and ghost spot

occur. Narrow leaf, rare in China, is common in some parts; and brown lint is far more frequent than in China. Some of the Burmese cottons are fairly long and reasonably fine, in contradistinction to the *bengalensis* types. As in the latter group, however, the lintless modifiers are absent. Further, there is a well-marked tendency for the Burmese group to develop rather large bracts. Burma and Manipur are parts of or a direct extension of the natural area of distribution of *arboreum*, but cottons are not endemic in China, having been introduced from various distinct sources and, in keeping with this fact, they are a somewhat heterogeneous assemblage. From historical records it appears that the two primary routes of importation were overland from Bengal-Assam to the Yellow river basin (presumably *bengalensis* types) and by sea from Indo-China to the Yangtse valley (presumably *burmanica* types, Hutchinson, 1938). The majority of present-day *bengalensis* and Burmese *burmanica* strains appear to lack the lintless epistatics, yet types carrying them are common in both the Yellow river and Yangtse basins. This may indicate that some of the Chinese cottons have originated from sources other than those so far suggested, such as the southern Indian *indica*, or the present distribution of epistatics may be the result of local selection trends from the original prototype. Red leaf (R_2^{LS}, R_2^{LO}) and spotless (R_2^{AO}) types, not recorded elsewhere in *arboreum*, occur in China, and ghost spot (R_2^{OS}) is not uncommon. Though white flower occurs, the common pale does not appear to do so, but another pale type, carrying Y_b^P, is fairly common in certain localities. Tufted seeded types (Fz) are also fairly frequent in China, but have not been recorded elsewhere except rarely in *indica*. Chinese cottons are characteristically broad-leaved, and narrow, though it occurs, is quite rare. In addition, a number of unusual forms such as curly leaf, virescent bud, yellow seedling, red margin petal, and "tinged" ghost spot, not recorded elsewhere in the Asiatic cottons, have recently been reported by Yu (1940*a*, *b*, and personal communications). China now appears to be an important secondary centre of variability in this species.

The third of the varieties of *arboreum* is a group of cottons confined to a limited tract of hill country in Assam and Bengal, characterized by high frequencies of the otherwise uncommon alleles Y_a^P and L^N, and by an unusual elongation of parts of foliar origin such as leaves, bracts, petals and bolls. This group was sufficiently distinctive in morphological characters to be given varietal status, but it appears to be only a specialized offshoot either from or closely related to forma *bengalensis*, and like the latter group has short coarse lint and a high ginning capacity. It

will be seen that genetically the three varieties of *arboreum* are not by any means equal in ranking, the forms of the first two varieties being equivalent to the third variety in status. They are also of much the same status as the three varieties in *herbaceum*, but in this species geographic separation is associated with morphological distinction. Thus the southern African *africanum* cottons are strongly monopodial, with small thin leaves and short coarse lint. The African and western Indian *frutescens* types have larger more rugose leaves, and are intensely hairy. Amongst them occur some of the longest and finest of the Asiatic cottons. The Levant-Turkestan *typicum* group is composed of very early sympodial cottons, many of which are low in their content of yellow corolla and spot size modifiers, and they are very variable in simple morphological characters—for example, the rare alleles R_2^{CS}, R_2^{LS} and R_2^{AO} occur here. Eastwards within this area there appears to be a strong tendency to develop a further distinctive group, with bolls which hardly open when ripe (Chernyakovskaya, 1930; Bordakov & Ivanova, 1935). In this species the three main geographic subdivisions, which Hutchinson and Ghose regarded as varieties, appear genetically to be more or less intermediate in status between the varietal and formal groups in *arboreum*. The situation within the two cultivated Asiatic species admirably illustrates the difficulties underlying efforts to harmonize morphological and biological classifications.

These examples of genetic divergence show that the differences which distinguish geographic groups within a species are of exactly the same kind as those which separate related species within a genus, though they do not attain the level at which even delayed fertility breakdown occurs. It is therefore reasonable to believe that, in some cases at least, the species themselves must have developed by an accumulation of just such small changes in emphasis in particular directions, and in geographic forms we evidently see the beginnings of the dynamic tendency which culminates in the totally different genetic structure of some homologous characters in related species. If species have developed by such an accumulation of certain tendencies, it would not be surprising to find that in some respects they had not diverged at all. In this connexion it is significant that whilst *anomalum* was found to be very distinct in genotype from both *arboreum* and *herbaceum* in many respects, in some characters it was more like one of them, whilst in others it was nearer the second species. Whether the changes in gene content are always adaptive in the Darwinian sense, as Harland (1936) implied, or whether some of them are merely fortuitous, as Wright's calculations show is possible, it is difficult to

decide. Harland has pointed out that in *barbadense* the presence of dominant main genes is frequently associated with the presence of plus modifiers, whilst in *hirsutum* the presence of the recessive allele is associated with a low modifier level. The same situation also exists to a limited extent in the Asiatic species, but is not so well marked as there is a less clear-cut allocation of a series of dominants to one species and of a series of recessives to another. One such instance is afforded by leaf shape, to which attention has been directed elsewhere (Silow, 1939*a*). In *arboreum*, in which narrow alleles occur, their expression is accentuated by narrowing modifiers, whilst in *herbaceum* and *anomalum*, in which only broad alleles occur, the modifier complex enhances the expression of the main gene in the direction of greater broadness. Such cases suggest that with respect to these characters a definite selection pressure has been in operation, to which the genotype as a whole, both main and minor genes, has responded. In the case of lint colour however the situation is the reverse of this. In *arboreum* light brown lint genes are rare, but this species is at a high modifier level, whilst *herbaceum*, which is characterized by main brown lint genes, has on the whole a background which minimizes their expression. *Anomalum* is characterized by brown lint genes and high modifier level. Such a situation suggests that here the fixation of the genotype may have been purely fortuitous. There are also instances, as in the case of nectaries and fuzz, where there are considerable differences in minor genotype amongst the three Asiatic species, in the absence of any main gene distinction. It is possible that the fuzz situation is to some extent a reflexion of the action of artificial selection under cultivation. Neither leaf nor bract nectaries however have any obvious significance in the economy of the plant, nor does the location of the latter either inside or outside the bracts appear to be of consequence, so that the variations in the genotype affecting these characters appear to be, following Wright's terminology, "accidents of sampling", just as in lint colour. Yet caution in applying such an interpretation is necessary. Many of the less common alleles are very localized in their occurrence in *arboreum* and *herbaceum* (Table 14). As in the case of nectaries, they do not appear to have any direct adaptive value, but the possibility that they have been selected on account of certain less obvious pleiotropic effects cannot be ignored, and the danger in assuming that such characters are neutral from the viewpoint of selective value may be illustrated from Hutchinson's (1936) survey of crop populations. In certain contiguous areas in central India he found striking differences in the frequencies of particular leaf-shape and flower-colour alleles, and these

characters, which are known to be independent in their inheritance, showed an unexpected and strong association. The implication is that certain combinations of genes under particular circumstances have a selective advantage over other combinations, though for what reasons is not clear.

Some reference to the isolation mechanisms for the maintenance of species distinctions in *Gossypium* is necessary. Here as elsewhere there cannot be much doubt that physiological and geographic barriers are of paramount importance, but there are also other mechanisms involved. At first sight the most important of these might appear to be the complementary lethal (crumpled) system. A similar system has been described in *Crepis* by Hollingshead (1930). Some but not all strains of *C. tectorum* were found to carry a gene which acted as a lethal in hybrids with certain other species. Whether it was the whole genotype of the latter, or only a single gene as in cotton, which reacted with the *tectorum* gene, it was not possible to demonstrate. Hollingshead concluded that since the lethal was effective only in interspecific crosses, and these were almost sterile anyway, it was unlikely that the gene had played any part in the late evolutionary history of these species, though it may have been of some importance in the early stages of differentiation. In *Gossypium* the system is effective even within a single species. One of the two genes concerned is widespread though not universal in both *arboreum* and *herbaceum*; it was also found in the strain of *anomalum* tested, but whether it is prevalent in that species is not known. The other gene has however only been found in one locality in *arboreum* and not at all in *herbaceum*. It is true that that one locality is within the general distribution area of *anomalum*, but it is very improbable that the two species concerned occur in the same ecological area (see introduction). The system may have been of importance as an isolation barrier at some past time, and might easily become so again under particular local conditions, but, as in *Crepis*, it does not appear to have any great significance at the present time. The whole situation in *Gossypium* points to lack of harmony as the fundamental cause of interspecific incompatibility in this genus as it exists now, and, as has been shown, geographic separation appears to be a potent influence in the accumulation of many small differences into a clear distinction. The figures cited in § II show how even a slight change in genetic constitution can be responsible for the difference between success and complete failure in species hybridization. Further evidence on these lines is available. In discussing the difficulty in hybridizing *G. davidsonii* with other members of the American diploid

section, Skovsted (1937) suggested that the complementary lethal mechanism might be involved, and pointed out that the fertile progeny of hybrids between Asiatic cottons and *anomalum* would be satisfactory material for testing the hypothesis. He had already (1935) reported a striking difference in the relative success of *davidsonii* pollinations on *anomalum* and the cultivated Asiatic species. On *anomalum* he obtained a full set of perfectly good viable seeds; on *arboreum* and *herbaceum* he also obtained a full set, but of some 1200 seeds all were completely empty. The writer has pollinated *davidsonii* on the various derivatives of *anomalum* hybrids with *arboreum* and *herbaceum*, and the results are shown

Table 16. *The result of* davidsonii *pollinations on derivatives of* anomalum *hybrids with* arboreum *or* herbaceum

Plant no.	Description	Total no. of seeds	Percentage		
			Empty	Imperfectly developed	Good*
P 237	F_1 (*an.* × *arb.* A 8)	266	42	52	6
14,859	(*an.* × A 8) 3rd B.C. to *arb.* A 8; only obvious *an.* gene = Fdp	209	64	36	0
14,350	(*an.* × H 10) 2nd B.C. to *arb.* N 14 selfed; only obvious *an.* genes spot size modifiers	684	38	62	0
14,280	(*an.* × H 10) 2nd B.C. to *arb.* N 14. selfed: only obvious *an.* gene = L^4	589	37	53	10
P 1265	(*an.* × H 10) 2nd B.C. to *an.*; only obvious *herb.* gene = F_e	52	0	48	52
14,875	(*an.* × A 8) 3rd B.C. to *an.*; only obvious *arb.* genes = L^L and F_a	123	23	46	31

* These seeds germinated and gave rise to vigorous hybrids which flowered freely. Thus by using *anomalum* as a bridging species it has been possible to secure combinations between *davidsonii* and the cultivated Asiatic cottons which cannot be obtained directly. Unfortunately all plants were completely sterile.

in Table 16. These figures, though difficult of interpretation, do not lend any support to the hypothesis that the difference between *anomalum* and the cultivated Asiatic cottons in their compatibility with *davidsonii* is dependent on any simple gene mechanism such as is concerned in the complementary lethal system, but on the contrary points rather to the importance of general genotypic balance influencing ability to hybridize.

Genotypic disharmony must also constitute an important barrier between species even after successful hybridization. Quite apart from those disharmonies so extreme as to lead to abortion of the developing embryo in the early stages, obviously unsuccessful genic combinations were frequent in *anomalum*-Asiatic hybrid progeny. Harland has also reported them within the New World section of the genus. In parts of

India mixed crops of *arboreum* and *herbaceum* are grown, and although these species intercross freely and their first generation hybrid is vigorous and productive, Hutchinson (1938) has stated that within crop populations "hybrids of later generations are rare, and the integrity of the two species is maintained unimpaired". In the course of this report attention has frequently been directed to the deficiency of genes introduced from *anomalum* into the cultivated Asiatic species. Deficiencies were not observed with all genes, nor always with the same gene. Very few first backcrosses grown which gave clear monogenic segregation were sufficiently large to be of significance, but those which were gave on the whole fairly good segregation—e.g. leaf shape, corolla colour, pollen colour, leaf nectaries. Some of the most striking aberrations were in later generations, and amongst them a deficiency of the introduced gene was far more common than an excess. As particular examples of the deficiency of the *anomalum* gene the following may be cited:

(*a*) The five sib-backcrosses from (9992 × A 16) (§ III (3)). 9992 was a selfed derivative of a third backcross of *anomalum* to *arboreum*. The five backcrosses from (9992 × A 16) to A 16, using the hybrid derivatives as seed parent, were homogeneous amongst themselves, and gave 212 Y_c : 149 Y_c^P (from *anomalum*).

(*b*) Two selections from a first backcross to the cultivated species, 11,703 and 11,757, when again backcrossed (§ IV (3)), gave four homogeneous progenies with 469 R_2^{aO} : 344 R_2^{OS} (from *anomalum*). Here again the hybrid derivatives were used as seed parent.

(*c*) P 1822, a selection from a second backcross to *arboreum*, gave even more striking deviations. When backcrossed as seed parent it gave 25 R_2^{GO} (from *anomalum*) : 43 r_3^{OO}, and as pollen parent 10 : 20. When selfed it gave only 12 : 23 where a 3 : 1 ratio had been expected. If the deficiency is due to any disturbance in gametogenesis it is not confined to the male or female side alone. Even more striking deficiencies of the same type have been found by Hiorth (1933) in backcrosses of interspecific hybrids in *Collinsia*, and Skovsted (unpublished data) has also observed a deficiency of an anthocyanin allele in backcrosses of *G. aridum* to New World cultivated cottons. Clearly there can be under certain circumstances a severe selective elimination of foreign genes, which occurs either in gametogenesis, or, in view of the frequent poorly developed seeds found in *anomalum* derivatives, in the early zygotic stages. Apparently the disadvantage is associated with those genoms which contain the greater proportion of foreign material, again pointing to the importance of a harmonic balance within the genotype. Whatever

the mechanism involved, it must act as a potent stabilizing influence against contamination between related species.

XII. Summary

Very different degrees of specific divergence are represented within the Asiatic diploid section of the genus *Gossypium*. Hybrids between the cultivated species *arboreum* and *herbaceum* are fully fertile in the first generation, and only show breakdown in viability and fertility in the second, but hybrids between those species and the wild African *G. anomalum* are almost sterile, though fortunately not completely so. This paper deals with the inheritance in the latter hybrids of eight characters. The genetic structure of the three species is compared in terms of the fifteen main loci involved, and their associated minor genes. The same main loci are represented in all three species, but different alleles may enter into the construction of homologous characters. A particular and unusual instance is afforded by the anthocyanin petal spot common in this genus. In the cultivated Asiatic cottons its characteristics are determined by a single allele; in *anomalum* they are the result of genes, situated in duplicate loci, which act as complementaries—a conception not to be confused with that involved in the more usual interpretation of complementary factors. Genetic support is given to the contention, hitherto based on cytological grounds, that the diploid species are themselves derived polyploids. The closely related species *arboreum* and *herbaceum* differ hardly at all in their main loci, but their modifier systems are quite distinct. *Anomalum*, which is taxonomically considerably further removed from these two species than they are from one another, shows a much wider divergence in main loci, and whilst in some characters the minor genotype is near that of either one or other of the cultivated species, in others it is very distinct from both. The genetic situation in the three species seems to be a further development of the type of "incipient speciation" which is seen in the geographic differentiation occurring within the species. Some of the differences between species, where both main and minor genes are working in the same direction, appear to be adaptive. Others give no positive indication of being anything other than purely fortuitous.

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EXPLANATION OF PLATE 18

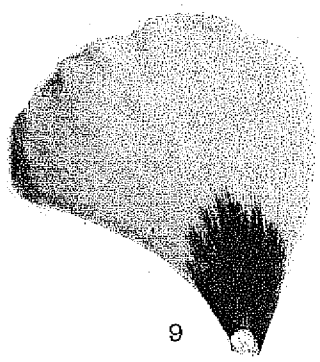
Anthocyanin and corolla colour in derivatives of *anomalum* hybrids.

Fig. 1. *G. arboreum* type A 8. Full petal spot ($R_2^{AS}\ r_3^{OO}$).
Fig. 2. *G. herbaceum* type H 10. Spotless ($R_2^{AO}\ r_3^{OO}$).
Fig. 3. *G. arboreum* type A 16. Ghost ($R_2^{OS}\ r_3^{OO}$).
Fig. 4. *G. anomalum*.
Fig. 5. *G. anomalum* × A 8.
Fig. 6. *G. anomalum* × H 10.
Fig. 7. *G. anomalum* × A 16.
Fig. 8. Corolla colour: homozygous yellow as in fig. 1, heterozygous for yellow depresser *Ydp* (14,859).
Fig. 9. The petal colour effect of R_3^{GO}; very intense gold ($R_2^{OS}\ R_2^{OS}\ R_3^{GO}\ R_3^{GO}$, homozygous Y_a) (15,240).
Fig. 10. The petal colour effect of R_3^{GO}; medium gold (heterozygous R_3^{GO}, homozygous Y_a) (15,240 × 14,859).
Fig. 11. The petal colour effect of R_3^{GO}; very faint gold (heterozygous R_3^{GO}, heterozygous $Y_a{}^P$) (15,240 × N 5 $Y_a{}^P$).
Fig. 12. The petal colour effect of R_3^{GO}; pink (heterozygous R_3^{GO}, homozygous Y_a, + *Ydp*) (15,240 × 14,859).
Fig. 13. The petal colour effect of R_3^{GO}; gold spotless ($R_2^{AO}\ R_2^{AO}\ R_3^{GO}\ r_3^{OO}$, homozygous Y_a) (6144).
Fig. 14. Compound spot, F_1 of A 16 × H 10 ($R_2^{OS}\ R_2^{AO}\ r_3^{OO}\ r_3^{OO}$).
Fig. 15. Low grade compound spot, same constitution as fig. 14, from 14,350 × H 10 (5143).
Fig. 16. Large spot, from 15,305 × A 8 (19,018).
Fig. 17. Large ghost, from 15,305 × A 16 (18,947).
Fig. 18. Large compound spot, same constitution as figs. 14 and 15, from (*an.* × A 8) × H 10 (11,757).

Petal colour grades: A 8, H 10 and A 16 are full yellow, grade 7. Fig. 8, *Ydp*, is grade 4.

Spot size grades: To obviate the necessity for printing a separate plate showing spot size grades, the range may be indicated as follows:

Spot size	Figure
15+	16
15	5
14	7
13	1
12	4, 9
10	3
6–7	14
4	15



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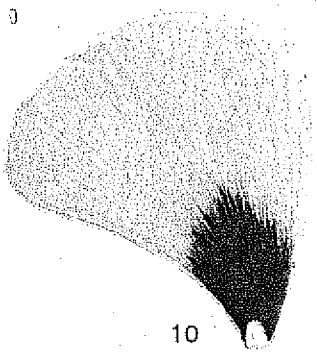

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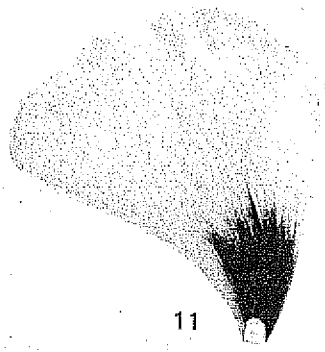

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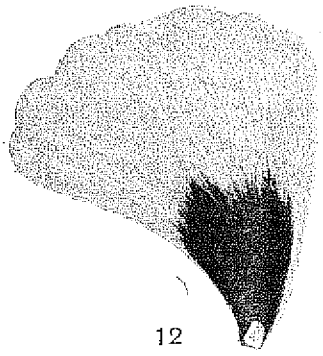

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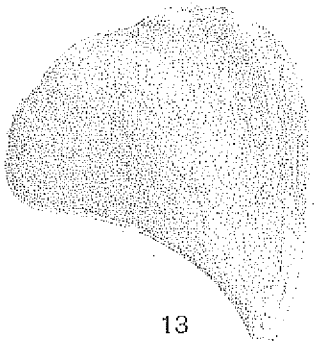

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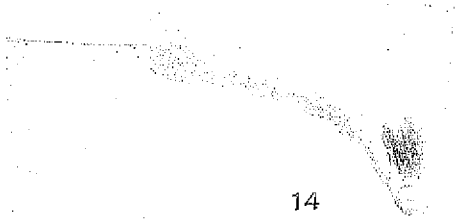

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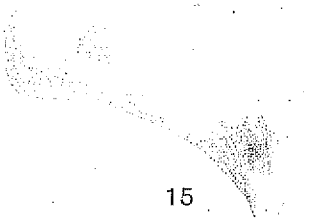

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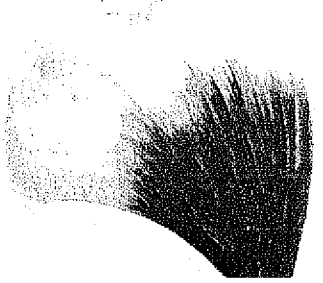

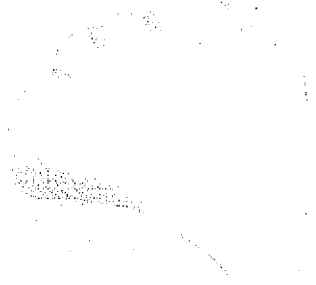

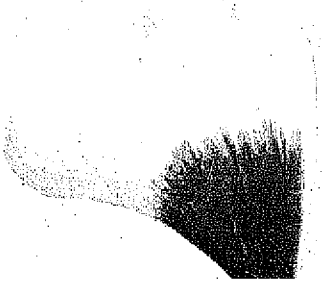


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