THE ANOMALOUS BEHAVIOR OF THE GHOST SPOT OF *GOSSYPIUM ANOMALUM* IN AMPHIDIPLOID *GOSSYPIUM HIRSUTUM*

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WILD *Gossypium anomalum* Wawrs. and Peyr. among Gossypium species has anomalous petal spot inheritance. Its petal spot involves two independently inherited genes; a ghost spot gene interacts with a spotless gene making a large anthocyanin area at the base of a petal (SILOW 1941). In the two cultivated Asiatic diploid species red petal spot involves three closely linked genes—margin, ghost spot, and spotless (YU and CHANG 1948). In amphidiploid *G. hirsutum* L. and *G. barbadense* L. red petal spot acts as a unit.

Ghost spot of *G. anomalum* is allelic with ghost spot of *G. arboreum* L. Ghost spot is detected as a white spot on a yellow petal but is invisible on a white petal (SILOW 1941). Red petal spot of *G. arboreum* is allelic in amphidiploid *G. hirsutum* with red petal spot (HARLAND 1935). The spotless gene of *G. anomalum* is a flush expression on petal margins (SILOW 1941) and phenotypically it resembles the spotless allele of *G. arboreum*. Both produce red petal spot through interaction with ghost spot of either species. The similarity of flush of the *G. anomalum* spotless and red petal-margin of *G. hirsutum* R_1 was discussed by SILOW (1941), but sterility of his hybrids prevented him from determining allelic relationships.

This paper is concerned with petal spot genes of *G. anomalum* in amphidiploid *G. hirsutum*. Anomalous placement of the ghost spot gene and associated disturbance in lint-color segregation are reported. Methods of transferring these genes are compared.

MATERIALS AND METHODS

Triploid hybrids having $A_hD_hB_1$ genome composition were obtained by repeatedly pollinating spotless *G. hirsutum* ($2A_hD_h$) stigmas with *G. anomalum* ($2B_1$) pollen. These sterile 3x hybrids had large petal spot and a faint flush on petal margins. Seedling 3x plants were treated with colchicine and fertile 6x $2A_hD_hB_1$ flowers were obtained.

A low bivalent frequency of the 3x hybrids (BROWN and MENZEL 1952) and a very low quadrivalent frequency in the 6x hybrids both indicated that chromosome homology was low between genomes. Genomic interchange was low in the 6x hybrid $2A_hD_hB_1$ when GERSTEL and PHILLIPS (1958) scored segregation in the 5x hybrids which they obtained by crossing the 6x flowers with spotless 4x *G. hirsutum*. Similarly, 5x hybrids $(2A_hD_h)B_1$ were obtained by crossing the 6x allohexaploid with spotless 4x *G. hirsutum*. The 5x hybrids had a large petal spot and a faint flush on petal margins.

The 5x hybrids $(2A_hD_h)B_1$ were used as pollen parents on spotless 4x *G. hirsutum*. This pollen

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transfer procedure eliminated a transfer of most B_1 chromosomes, and, as RHYNE (1951) reported, most plants were phenotypically and genotypically equivalent to *G. hirsutum*. The most frequently observed non-*hirsutum* characteristic was a large petal spot lacking petal margin-flush. This petal spot phenotype was studied to determine whether ghost spot, R^{OGO}, of *G. anomalum* was present either as a chromosome addition or as a chromosome segment substitution.

Newly synthesized amphidiploids were used to transfer *G. anomalum* petal spot to amphidiploid *G. hirsutum*. These were *G. arboreum-G. thurberi* $2A_2D_1$, *G. anomalum-G. thurberi* $2B_1D_1$, and *G. arboreum-G. anomalum* $2A_2B_1$. For convenience the transfer methods were termed *direct*, when *G. hirsutum* was hybridized directly with either $2B_1D_1$ or $2A_2B_1$ to obtain $F_1(2A_hD_h \times 2B_1D_1)$ and $F_1(2A_hD_h \times 2A_2B_1)$ respectively, and *bridging amphidiploid*, when either of the new amphidiploids were hybridized first with new amphidiploid $2A_2D_1$, making $F_1(2A_2D_1 \times 2B_1D_1)$ and $F_1(2A_2D_1 \times 2A_2B_1)$, and the amphidiploid hybrids then crossed with *G. hirsutum*. In the latter, either the $2A_2$ or the D_1 genome of amphidiploid $2A_2D_1$ served as the bridging genome for gene transfer from *G. anomalum*. All amphidiploid hybrids were backcrossed with spotless *G. hirsutum*.

Attention had to be focused on ghost spot, R^{OGO}, for R_3 of *G. anomalum* was never recovered in any *G. hirsutum* backcross plants.

RESULTS

Ghost spot as a chromosome addition: Approximately 10% of the plants obtained by pollination of 4x spotless *G. hirsutum* with pollen of 5x hybrid $(2A_hD_h)B_1$ had large petal spot lacking petal-margin flush. This phenotype had been described (SILOW 1941) for an interspecific hybrid involving spotless *G. hirsutum*, as the interaction of a ghost spot gene R^{OGO} with an unidentified spotless gene of *G. hirsutum*; this phenotype was reproduced by transfer (RHYNE 1951) of ghost spot of *anomalum* and by transfer (GILES 1962) of *G. arboreum* ghost spot to *G. hirsutum*.

Ghost spot could be expected as a chromosome addition in most petal spot plants, because of low homology between *G. anomalum* $2B_1$ chromosomes and *G. hirsutum* $2A_hD_h$ chromosomes. A chromosome addition genotype, i.e. a 4x plus one-B_1-chromosome composition, should produce the same segregation pattern, a 1:1 ratio, when either self-pollinated or backcrossed with spotless 4x pollen donor plants of *G. hirsutum*. The backcrossing of spotless 4x stigmas with pollen of petal spot plants having the chromosome addition would fail to transmit R^{OGO} as a chromosome addition; no progeny with petal spot, but only spotless progeny, would result, since embryos having a normal chromosome complex survive, but embryos having an imbalance rarely survive in capsules of 4x *G. hirsutum* plants (RHYNE 1951). In contrast, a chromosome substitution genotype, i.e. a 4x chromosome composition, should transmit petal spot (R^{OGO}) when either self-pollinated or used as a pollen parent on spotless 4x stigmas. Seed number of self-pollinated capsules and capsules of 4x plants used as ovular parents would be essentially normal, but seed number of capsules of *G. hirsutum* have approximately 50% of the normal seed number and many aborted embryos (motes) when crossed with pollen from chromosome addition plants having petal spot.

Typical families from three seasons (1949, 1955, 1960) demonstrate that ghost spot, R^{OGO}, was present as a chromosome addition to the 4x *G. hirsutum* complement. In Table 1 the results of both self-pollination and pollination of heterozy-

TABLE 1

Segregation of petal spot in offspring of plants heterozygous for R^{OGO} *of* G. anomalum *as a chromosome addition to the 4x* G. hirsutum *complement*

Plant number	Testing procedure	Petal spot		Observed ratio
		Present	Absent	
49–14–3	F_2, selfed	19	23	1 : 1
	Backcross as female	10	4	1 : 1
55–39–1	F_2, selfed	23	15	1 : 1
	Backcross as female	47	48	1 : 1
60–13–1	F_2, selfed	2	8	1 : 1
	Backcross as female	16	17	1 : 1

None of the ratios deviates significantly from 1:1.

gous petal spot plants with pollen from spotless *G. hirsutum* conform to a ratio of 1 petal spot : 1 spotless. Seed number was much reduced in the self-pollinated capsules. Petal-margin flush continued to be lacking both in plants having and lacking petal spot. The spotless R_3 of *G. anomalum* was not detected.

A chromosome count for Plant 55–39–1 of Table 1 revealed 26 small bivalent chromosomes typical of *G. hirsutum* and one large univalent typical of the larger $2B_1$ genome chromosomes. (This count was a courtesy of DR. MARGARET MENZEL, then a staff member of Texas A&M College, College Station.) This univalent carrying R^{OGO}, and producing petal spot by interaction with *G. hirsutum* spotless genes, confirmed SILOW (1941) that in *G. anomalum* R^{OGO} and R_3 had independent inheritance. This intact B_1 chromosome in *G. hirsutum* indicated that the two interacting petal spot genes R^{OGO} and R_3 of *G. anomalum* are on separate chromosomes.

Plants having petal spot as a chromosome addition occasionally produced a few petal spot offspring when crossed with spotless females. Also, self-pollination of chromosome addition petal spot plants produced occasional true-breeding petal spot plants. These petal spot plants must be of genotype $R^{OGO}R^{OGO}$, and have a chromosome composition of 27 bivalents. Such 54-chromosome plants were obtained by self-pollination of chromosome addition plants (53 chromosomes) and were reported by BROWN (1949).

Ghost spot as a substitution to the A_h genome: The crossing of spotless 4x *G. hirsutum* with pollen of 5x $(2A_hD_h)B_1$ hybrids produced petal spot by substituting ghost spot, R^{OGO}, for the chromosome segment of the A_h genome marked by r_2 (spotless). The heterozygous petal spot plants were hybridized with S4025, marked with R_2 (Petal Spot) and R_1 (Red Plant and red petal margin), to test for allelism. Two phenotypes were obtained: Type I, having medium size petal-spot, had the genotype $\frac{R_2\ Lc_1\ N}{r_2\ lc_1\ n}\ \frac{R_1}{r_1}$ for the A_h and D_h genome linkage groups respectively; Type I is typical spot × spotless 4x *G. hirsutum*. Type II, having large petal spot, had the genotype $\frac{R_2\ Lc_1\ N}{R^{OGO}}\ \frac{R_1}{r_1}$; Type II is a chromosome-segment substitution genotype that was detected by its discrete larger petal spot size

TABLE 2

Petal spot and lint color segregation in offspring of plants having R^{OGO} *of* G. anomalum *as a chromosome segment of an* A_h *genome chromosome*

Type	Genotype	Testing procedure	Petal spot			Lint color*		
			Large R^{OGO}	Small R_2	None r_2r_2	Brown Lc_1	Tan Lc_x†	White lc_1lc_1
I	$\frac{r_2\ lc_1}{R_2\ Lc_1}$	F_2, selfed	0	39	19	31	12	13
			(0)	(43.5)	(14.5)	(42)	(0)	(14)
II	$\frac{R_2\ Lc_1}{R^{OGO}}$	F_2, selfed	206	65	0	187	74	16
			(203)	(68)	(0)	(208)	(0)	(69)
		Backcross as female	30	26	0	26	7	23
			(28)	(28)	(0)	(28)	(0)	(28)
		Backcross as male	79	67	0	4	33	112
			(73)	(73)	(0)	(74)	(0)	(74)
		Backcross as male (exception)	9	15	1	16	0	10
			(12)	(12)	(0)	(13)	(0)	(13)

Expected values are given in parentheses.
* Plants observed differ for each character since some plants produced no bolls and others produced only a few flowers.
† Probably gene Lc_2 of *G. anomalum*, but its relationship with *hirsutum* Lc_1 and Lc_2 has not been established.

and by its failure to produce spotless plants when both types had been either self-pollinated or reciprocally backcrossed with spotless 4x *G. hirsutum*. (*N* denotes Naked Seed.)

Petal spot segregation for Type I in Table 2 was the typical ratio of $3R_2 : 1r_2$ in *hirsutum* F_2 populations. Lint color segregation was disturbed however; plants having Lc_1, (Brown Lint) were present in deficient numbers, and Lc_x [Tan Lint, like that of the 5x hybrid $(2A_hD_h)B_1$] was unexpectedly present in some plants. The F_2 of Type II had no plants lacking petal spot. Allelic R_2 and R^{OGO} segregation therefore indicated substitution of R^{OGO} for r_2 in the 5x hybrid. Lc_1 segregation was again disturbed; too few plants had *hirsutum* Lc_1 and lc_1 and 74 had Lc_x, Tan Lint of the 5x ancestor.

When Type II was used as ovule parent, the allelic $1\ R_2 : 1R^{OGO}$ ratio was obtained in the progeny, but seven of these plants had Lc_x instead of Lc_1 and lc_1. Type II as pollen parent had anomalous segregation. In Table 2 the behavior is grouped into common and exceptional, the latter at the bottom of the table. For the common behavior, no-petal-spot flowers were absent and Lc_1 was unexplainably absent except for four plants. Both Lc_1 and Lc_x were deficient, being unexplainably either not expressed or not transmitted into viable condition. For the exception, one no-petal-spot plant was observed, but Lc_1 to lc_1 allelism was undisturbed. The plant that exhibited spotless flowers perhaps was a R_2r_2 heterozygote, as a faulty expression of petal spot sometimes occurs in this heterozygote. Faulty Lc_1 expression is not a valid explanation for the absence of this reliable gene in the progeny of Type II as a pollen parent.

Segregation of R_1 was normal in all families, but is not shown in Table 2. Similarly N, Naked Seed, segregated normally. N is linked with Lc_1 (STEPHENS 1955) but disturbance in Lc_1 segregation did not noticeably alter N:n segregation. After 14 backcross generations R^{OGO} was retested against R_2–Lc_1; allelic petal-spot segregation occurred without distortion of Lc_1 segregation. After 14 backcrosses to *G. hirsutum*, R^{OGO} also failed to mask cl_1 (cluster), as it had in the earliest generation. However R^{OGO} remained completely linked with Yg (not yellow-green) and with the modifier gene for large sized petal spot (SILOW 1941), as in the *G. anomalum* parent.

Direct amphidiploid substitution of R^{OGO} *to the* A_h *genome:* Ghost spot, R^{OGO}, of $2B_1$ was substituted from the poorly fertile amphidiploid hybrid F_1 ($2A_hD_h \times 2B_1D_1$), and the reciprocal F_1 ($2B_1D_1 \times 2A_hD_h$). Through the courtesy of MRS. VESTA MEYER, Research Associate at the Delta Branch Experiment Station, Stoneville, Mississippi, a plant having large petal spot was obtained. Her initial hybrid had been F_1 ($2B_1D_1 \times$ spotless 4x M8), and three backcrosses to M8 pollen donor stock had ensued. Petal spot might have persisted as R^{OGO} as a chromosome addition to the M8 stock, since this had been ovular transfer from $2B_1D_1$, or as a whole chromosome substitution for r_2 of the M8 stock. The plant was hybridized with the marker stock having R_2^{MGS}, red petal margin and tiny or absent petal spot, using the latter as ovular parent. Petal spot was transmitted to a portion of the plants, indicating R^{OGO} was probably a chromosome substitution. The reciprocal backcrossing of this hybrid to spotless r_2r_2 *G. hirsutum* produced 51 R_2^{MGS}:44 R^{OGO} and 32 R_2^{MGS}:26 R^{OGO} plants from the egg and pollen donating petal spot plants respectively. One small F_2 was grown and it contained 6 R_2^{MGS}:14 R_2^{MGS} R_2^{OGO}:3 R_2^{OGO} R_2^{OGO} plants. Hence R^{OGO} had been substituted for r_2 of the A_h genome.

Lint color was classified in the reciprocal backcrosses and in the F_2. Only the three $R^{OGO}R^{OGO}$ plants had Lc_x (Tan Lint), and the other F_2 plants and the backcrosses had lc_1. This recovery of recessive tan lint in the F_2 in the $R^{OGO}R^{OGO}$ phenotype indicated that a considerable length of B_1 chromosome, containing R^{OGO} and Lc_x at least, had been substituted in the A_h genome. The absence of Lc_1–N in the R_2^{MGS} tester left the question of B_1 linked genes in the Lc_1–N region in doubt.

Bridging amphidiploid substitution of R^{OGO} *to the* A_h *genome:* Amphidiploid *G. arboreum-G. thurberi* $2A_2D_1$ was hybridized with $2B_1D_1$. F_1 ($2A_2D_1 \times 2B_1D_1$) having R^{MGS} and R^{OGO} on the A_2 and B_1 genomes respectively was then hybridized with a spotless 4x stock having the intact A_h genome linkage r_2-yg_2-lc_1-n and the cl_1-R_1-yg_1-dw D_h genome linkage. This procedure at the amphidiploid level paralleled SILOW's (1941) transfer of R^{OGO} from B_1 at the diploid level, both using R^{OGO} and R^{MGS} as alleles. Therefore those plants having large petal spot (R^{OGO}) were backcrossed to *G. hirsutum*. In this first backcross large petal spot again indicated the genotype $\frac{r_2\ yg_2\ lc_1\ n}{R^{OGO}}\ \frac{cl_1\ R_1\ yg_1}{cl_1\ r_1\ yg_1}$, which was used for backcrossing to a recessive marker stock. The segregation in this 2nd backcross progeny is given as the first line of Table 3, which shows that R^{OGO} and R_1 were inde-

TABLE 3

Segregation of petal spot and petal margin color in offspring of heterozygous plants having R^{OGO} *which had been transferred from* G. anomalum *($2B_1$) of the bridging amphidiploid hybrid F_1 ($2A_2D_1 \times 2B_1D_1$)*

Genotype	Testing procedure	Petal margin color: Red, Petal spot: Large R^{OGO}	Red, Petal spot: None R	None, Petal spot: Large R^{OGO}	None, Petal spot: None $r_2r_2r_1r_1$
$\frac{r_2\ yg_2\ lc_1\ n}{R^{OGO}}\ \frac{cl_1\ R_1\ yg_1}{cl_1\ r_1\ yg_1}$	Backcross as female	11	15	14	12
	(Expected, R^{OGO} and R_1 independent)	(13)	(13)	(13)	(13)
$\frac{R_2{}^{MGS}\ yg_2\ Lc_1\ n}{R^{OGO}}\ \frac{cl_1\ r_1\ yg_1}{Cl_1\ r_1\ yg_1}$	F_2, selfed	52	34	28	0
	(Expected, R^{OGO} and R_2 allelic)	(57)	(28.5)	(28.5)	(0)

pendently inherited, as expected, and that R^{OGO} probably was allelic with r_2–yg_2 of the A_h genome.

One plant of the 2nd backcross having large petal spot was hybridized with a *G. hirsutum* tester marked by $R_2{}^{MGS}\ yg_2\ Lc_1$. This set up a test for R_2 allelism and for disturbance of Lc_1 segregation. Self-pollination of the large petal spot phenotype produced the segregation reported in the lower half of Table 3. The observed segregation, a 2:1:1 ratio, closely fits the expected 2:1:1 ratio expected for incompletely dominant alleles. This segregation paralleled SILOW's (1941) observation for allelic R^{OGO} and R^{MGS} segregation in diploid $2A_2$. Cluster (cl_1cl_1) phenotype occurred in $R_2{}^{MGS}R_2{}^{MGS}$ plants in the expected 25% frequency, but only non-cluster expression occurred in $R^{OGO}R^{OGO}$ plants. Yellow-green phenotype was restricted to $R_2{}^{MGS}\ R_2{}^{MGS}$ plants. The substitution of R^{OGO} brought in *Cl* and *Yg* loci of the $2B_1$ diploid parent. However Lc_1 segregation was undisturbed by the presence of the genes of $2B_1$ and recombination between yg_2 and lc_1 was 34.1%, which is within the range of values obtained for *G. hirsutum* hybrids. The Lc_x observed in the previous R^{OGO} substitutions was not present in this B_1 segment.

The bridging-amphidiploid procedure, F_1 ($2A_2D_1 \times 2B_1D_1$) $\times$ $2A_hD_h$, was judged to be superior to the direct hybrid procedure, F_1 ($2A_hD_h \times 2B_1D_1$), for transferring R^{OGO} from the $2B_1$ genome. Fertility was sufficient, segregation ratios were less disturbed, and appearance of offspring was more nearly normal during the bridging transfer. Large F_2 populations were readily obtained by self-pollinating F_1 ($2A_2D_1 \times 2B_1D_1$), wherein mostly intergenomic A_2–B_1 exchange ensued. The same chromosomes were present in diploid F_1 ($2A_2 \times 2B_1$), but SILOW (1941) had difficulty in obtaining F_2, and reciprocal backcross fertility was limiting. These same difficulties were present in F_1 ($2A_hD_h \times 2B_1D_1$).

Ghost-spot substitution to the D_h genome: No evidence was obtained to indicate that ghost spot, R^{OGO}, had been transferred to the D_h genome from the 5x ($2A_hD_h$)B_1 hybrid.

The direct hybrid, F_1 ($2A_hD_h \times 2A_2B_1$) (*G. arboreum-G. anomalum*), was exceedingly infertile. A large petal spot was transferred eventually from the F_1 to *G. hirsutum* but no evidence was obtained in support of a D_h genome substitution from F_1 ($2A_hD_h \times 2A_2B_1$).

The bridging amphidiploid hybrid F_1 ($2A_2D_1 \times 2A_2B_1$) permitted a transfer of R^{OGO} of $2B_1$ to the D_h genome. On the assumption that R^{OGO} and R_1 of the D_h genome would be allelic, F_1 ($2A_2D_1 \times 2A_2B_1$) was hybridized with G. *hirsutum* to obtain the desired genotype $\frac{r_2\ yg_2\ lc_1\ n}{R^{OGO}\text{–}Yg\text{-}lc_x\text{-}n}\ \frac{cl_1\ R_1\ yg_1\ dw}{R^{OGO}\text{ etc. of }B_1}$. Its large petal spot involved ghost spot of $2A_2$ as well as ghost spot of $2B_1$, both interacting with *hirsutum* spotless. Plants of this phenotype were backcrossed to a recessive tester, and large petal spot of the probable genotype $\frac{r_2\ yg_2\ lc_1\ n}{r_2\ yg_2\ lc_1\ n}\ \frac{cl_1\ r_1\ yg_1\ dw}{R^{OGO}\text{ etc. of }B_1}$ was recognized. These 1st-backcross plants were hybridized with the *hirsutum* R_1 tester to obtain 2nd-backcross plants having large petal spot and the probable genotype $\frac{r_2\ yg_2\ lc_1\ n}{r_2\ yg_2\ lc_1\ n}\ \frac{cl_1\ R_1\ yg_1\ dw}{R^{OGO}\text{ etc. of }B_1}$. These were self-pollinated. Typical segregation for this genotype is presented at the top of Table 4 (the ratio $1R^{OGO}R^{OGO}$: $2R^{OGO}R_1$:1 R_1R_1).

A plant having $R^{OGO}R^{OGO}$, of the 2nd-backcross F_2 reported in Table 4, was hybridized with a stock having dark brown lint, Dw^B, which previously had been inherited independently of any *hirsutum* brown-lint markers and had showed independent inheritance with R_2 and R_1. The resulting F_1 (the 3rd backcross to hirsutum) $\frac{cl_1\ R_1\ yg_1\ Dw^B}{R^{OGO}\text{ etc. of }B_1}$ was self-pollinated. This F_2 (Table 4 bottom) repeated the 1:2:1 segregation for R_1, proving that R^{OGO} had been substituted on the D_h chromosome of *G. hirsutum*. Total linkage of the $cl_1\ R_1\ yg_1$ loci occurred and 2%

TABLE 4

Petal spot segregation in offspring of plants having R^{OGO} *of* G. anomalum *($2B_1$) which had been obtained from the bridging amphidiploid hybrid $F_1(2A_2D_1 \times 2A_2B_1)$*

Genotype	Testing procedure	Petal spot		No petal spot	
		Petal margin		Petal margin	
		Colorless $R^{OGO}\ R^{OGO}$	Red $R_1\ R^{OGO}$	Colorless $r_1\ r_1$	Red $R_1\ R_1$
$\frac{cl_1\ R_1\ yg_1\ dw}{R^{OGO}}$	2nd backcross F_2, selfed	6	17	0	4
	(Expected, R_1 and R^{OGO} allelic)	(6.75)	(13.5)	(0)	(6.75)
$\frac{cl_1\ R_1\ yg_1\ Dw^B}{R^{OGO}}$	3rd backcross F_2, selfed	46*	114†	0	47
	(Expected, R_1 and R^{OGO} allelic and $R_1\ Dw^B$ linked)	(51.75)	(103.50)	(0)	(51.75)

* All had white lint.
† All red plants except two of this phenotype had Dw^B brown lint.

recombination in the R_1–Dw^B region was detected, despite the fact that cl_1 and yg_1 are usually 30 map units apart, and R_1 and Dw^B had always shown no evidence of being linked. This linkage of R_1 and Dw^B confirmed HARLAND (1939) that his K^B (Dw^B) and K^H (Lc_1) were duplicates, and supported RHYNE (1955) that Dw^B was a member of the cl_1 R_1 yg_1 dw linkage group.

The low recombination in the cl_1–Dw^B region of the D_h linkage group seemed to indicate that little homology existed in the chromosome arms associated with the cl_1–dw linkage group and with R^{OGO} respectively, and an asymmetric bivalent composed of a large B_1 chromosome arm and a small D_h chromosome might be expected at metaphase I. Flower buds of the three genotypes of the 3rd-backcross F_2 of Table 4 were examined for chromosome pairing by DR. JOHN ENDRIZZI, (Department of Plant Breeding, University of Arizona, Tucson). He observed only small symmetrical bivalents in these genotypes (personal communication).

DISCUSSION

Large petal spot in *G. hirsutum* resulted from the interaction of ghost spot, R^{OGO}, and its completely linked modifier for large size, with an unidentified spotless gene of undetermined genome location. This phenotype was reproducible regardless of the location of *G. anomalum* R^{OGO} in the two *G. hirsutum* genomes.

Large petal spot occurred as an addition of an intact *G. anomalum* chromosome to the *G. hirsutum* complement and as a chromosome segment substitution to the A_h and D_h genomes of *G. hirsutum* respectively. The addition was according to expectation, but substituting R^{OGO} of $2B_1$ was anomalous because of the low homology and the infrequent intergenomic exchange.

When the direct amphidiploid F_1 hybrid ($2A_hD_h \times 2B_1D_1$) was used, tan lint was detected only in the $R^{OGO}R^{OGO}$ plants. A large chromosome segment of B_1 having marker genes R^{OGO} and Lc_2 of B_1 had replaced r_2 and possibly lc_1 of the A_h genome. However, if B_1 Lc_2 were a counterpart of *G. hirsutum* Lc_1, the long map distance between Lc_1 and N would require explanation. For as intergenomic exchange between B_1 and A_h chromosomes is most frequent in the Lc_1–N interval (GERSTEL and PHILLIPS 1958), and R^{OGO} substitution restricts recombination in much of the R_2–Lc_1 region, the short B_1 Lc_2–*ha* (*ha* is glabrous lintless) map distance seems incongruous. Yet, in the absence of a genetic test, the facile inference of an inversion shortening Lc_2–*ha* can explain the anomaly.

In the bridging amphidiploid hybrid ($2A_2D_1 \times 2B_1D_1$) $\times 2A_hD_h$ few difficulties were encountered and tan lint failed to appear in the $R^{OGO}R^{OGO}$ substitution genotype. The ample fertility of F_1 ($2A_2D_1 \times 2B_1D_1$), which is unexpected after considerable A_2–B_1 intergenomic exchange, requires some elucidation. An influence of the $2D_1$ genome cannot be eliminated in the amphidiploid hybrid, since Diploid F_1 ($2A_2 \times 2B_1$) was exceedingly sterile when it had been self-pollinated. The enhancement of chiasmata formation in the amphidiploid hybrid ought to be investigated, since MENZEL (1964) unexpectedly found chiasmata to be higher in allotetraploid *Lycopersicon esculentum-Solanum lycopersicoides* than in the diploid F_1 hybrid and in one parental diploid, *Lycopersicon esculentum.*

The successful substitution of R^{OGO} on the D_h genome using bridging amphidiploid ($2A_2D_1 \times 2A_2B_1$) may be related apart from the A_2 genome influence to D_1–B_1 chromosome homology. BROWN and MENZEL (1952) reported considerable D_1–B_1 homology but little D_h–B_1 homology. The difficulty in the direct amphidiploid hybrid ($2A_hD_h \times 2A_2B_1$) may be attributed to low D_h–B_1 homology. The resulting low frequency of euploid gametes would be associated with low fertility in backcrosses to *G. hirsutum*, which has a low tolerance of aneuploid embryos in its capsules.

SUMMARY

In amphidiploid *Gossypium hirsutum* the behavior of the ghost spot gene of wild diploid *G. anomalum* was anomalous but a second gene, spotless, was not detected. Ghost spot, R^{OGO}, proved to be a petal spot marker gene located on a chromosome addition to the *G. hirsutum* complement; a chromosome addition was expected because of low homology between *G. hirsutum* $2A_hD_h$ and *G. anomalum* $2B_1$ chromosomes. Ghost spot also proved to be a marker gene of a *G. anomalum* chromosome segment which had been substituted in a chromosome of both the A_h and D_h genomes of *G. hirsutum*. In the A_h chromosome, R^{OGO} was allelic with R_2 (red petal spot) and completely linked with *Yg* (not yellow-green). When R^{OGO} was substituted by means of a direct exchange of A_h and B_1 chromatin, an associated appearance of Lc_x (Tan Lint) was observed. Lc_x and Lc_1 (Brown Lint) of the R_2-Lc_1-*N* linkage group were not straightforwardly determined to be alleles of a common locus, for Lc_1, on an unaltered *G. hirsutum* chromosome and intercalated between R_2 and *N*, failed to appear in offspring of a hybrid in which R^{OGO} and Lc_x were present on the altered A_h homologue. Lc_x was not observed when R^{OGO} substitution was by means of bridging genome exchange, i.e., B_1–A_2 to A_h in the bridging amphidiploid hybrid ($2A_2D_1 \times 2B_1D_1$) $\times$ $2A_hD_h$. The bridging amphidiploid exchange was accomplished with fewer fertility disturbances than the direct genome exchange. Also, by means of another bridging amphidiploid hybrid, F_1 ($2A_1D_1 \times 2A_2B_1$), R^{OGO} was intercalated into the D_h chromosome marked by the R_1 (Red Plant) Dw^B (Brown Lint) linkage group. R^{OGO} and R_1 acted as alleles, but recombination between R_1 and Dw^B was reduced from 50% to 2%. R^{OGO} of *G. anomalum* therefore was anomalously shown to be a replacement for both R_2 and R_1 of the two *G. hirsutum* genomes.

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