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A Second Locus for Pollen Color in Pima Cotton, *Gossypium barbadense* L.[1]

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ABSTRACT

Pollen color in the amphidiploid species of *Gossypium* varies from deep golden yellow to cream and is conditioned by one pair of alleles, *P* and *p*, with yellow dominant to cream. A mutation causing orange pollen was found in 'Pima S-1', a normally yellow pollen commercial variety of Pima cotton (*Gossypium barbadense* L.). The mutant, when selfed, showed pleiotropic effects which gave ranker plants that flowered later and fruited higher. Also, the flower buds were not conical and the stigma extruded beyond the scalloped tips of the corolla.

The inheritance of orange pollen was determined from crosses of yellow with mutant orange, yellow with cream, and cream with mutant orange pollen strains. Single gene differences conditioned pollen color in the first two crosses; yellow was dominant. Pollen color in the cross of cream with mutant orange was conditioned by two complementary genes, giving an F_2 segregation of 9 yellow: 3 orange: 4 cream. One of the cream phenotypes in F_2 was found to be conditioned by the homozygous double recessive — a new genotype for cream pollen.

The symbols P_1 or p_1 and P_2 or p_2 are proposed for the genes conditioning pollen color in *G. barbadense*. True breeding yellow and cream pollen strains would have the genotypes $P_1P_1P_2P_2$ and $p_1p_1P_2P_2$, respectively. The orange pollen mutant would have the genotype $P_1P_1p_2p_2$.

POLLEN color in the amphidiploid species of *Gossypium* varies from cream to deep golden yellow. Pollen color of Pima cotton, *Gossypium barbadense,* is predominantly yellow; however, a few experimental strains have cream pollen segregates as a result of introgression of cream pollen from *G. hirsutum* L. A mutation causing orange pollen was found in a commercial planting of Pima S-1 in 1956. Mutant plants, when selfed, had orange pollen and showed pleitropic effects on growth habit, fruiting pattern, and flower bud appearance. Plants with orange pollen are ranker, flower later, and fruit higher. The flower buds are not conical and have stigmas extruding beyond the scalloped tips of the corolla.

Balls (1) and Kearney (3) observed intermediate-colored F_1's in crosses between cream pollen Upland (*G. hirsutum*) and yellow pollen Egyptian (*G. barbadense*) cottons. In the F_2 generation, Balls reported a ratio of 1 yellow : 2 intermediate : 1 cream; and Kearney observed a bimodal distribution in which cream appeared to be a simple recessive. Harland (2), working with strains of *G. hirsutum* and *G. barbadense,* used nine color grades to describe pollen color in parental material and segregating populations. He found that pollen color was conditioned by one pair of alleles, *P* and *p,* with yellow dominant to cream. In the presence of *P,* pollen color varied from deep golden through various shades of yellow, due to modifying genes.

Negi and Aujla (4) made crosses between *G. hirsutum* strains having yellow and cream pollen. They found that all F_1 plants had yellow pollen, and that in the F_2, pollen color segregated 1 deep yellow : 2 yellow : 1 cream, indicating a single gene difference with yellow dominant to cream. In certain crosses they obtained a deficiency of deep yellow plants possibly due to the influence of modifying factors.

The diploid species of cotton, *G. herbaceum* L., *G. arboreum* L., and *G. anomalum* Wawra. and Peyr., contain two complementary genes for pollen color, P_a and P_b (Silow, 5). The dominant genes together, P_a and P_b, condition yellow pollen color; P_a alone conditions pale yellow pollen, and P_b alone results in cream pollen.

Stephens (6), by means of a tri-species hybrid involving *G. hirsutum*, *G. raimondii* Ulbr., and *G. arboreum* as parents, presented evidence that the amphidiploid pollen color locus, *P,* is carried in the A genome of the AD chromosome set, and that *P* probably is homologous with the P_a locus in the diploid species *G. arboreum*. He discussed the possibility of the amphidiploids having two or possibly four pollen color loci, because pollen color in the Asiatic diploids (A genome) is controlled by two complementary genes. The presence of the P_b locus in the amphidiploids was inferred but not located by Stephens.

This study reports the inheritance of an orange pollen mutation found in the commercial variety, Pima S-1. A second locus for pollen color in amphidiploid species of cotton is proposed.

MATERIALS AND METHODS

Open-pollinated seed was harvested from the orange-pollen mutant and planted in a progeny row in the field at Tempe, Arizona, in 1957. Plants in the 1957 progeny row segregated for yellow and orange pollen. The plants with yellow pollen were rogued, leaving a stand of orange pollen plants which were self pollinated and later bulk harvested. This bulk self-pollinated seed lot was the source of the mutant gene in subsequent genetic studies.

Three strains homozygous for pollen color were used to study the inheritance of orange pollen. These were the orange-pollen mutant, yellow-pollen Pima S-1 or 'Pima S-2', and a cream pollen *barbadense* derived from an advanced strain of complex ancestry, including *G. hirsutum*.

Crosses were made between strains with yellow and orange pollen, yellow and cream pollen, and cream and orange pollen. As the study progressed, a new genotype for cream pollen was isolated and crossed with the yellow and cream pollen strains. F_1, F_2, and F_3 populations were grown in the field or in 6-inch pots in the greenhouse. No difference in segregation ratios was noted in comparing field grown and greenhouse grown populations.

Pollen color was scored on the day of anthesis, after the anthers had fully dehisced. Small variations in yellow and orange color were noted, but classification for these colors was not subdivided further, because progeny tests showed that classification into more than two classes was unnecessary.

RESULTS AND DISCUSSION

The numbers of plants with yellow, orange, or cream pollen in the F_1 and F_2 generations of the crosses yellow with orange and yellow with cream pollen are shown in Table 1. Probability values for goodness-of-fit to a 3:1 ratio in F_2 are also shown.

All F_1 plants from the cross yellow with orange pollen had yellow pollen. Thirteen F_2 families from this cross each segregated 3 yellow to 1 orange pollen.

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Table 1. Classification of *G. barbadense* plants from F_1 and F_2 populations involving yellow, orange, and cream pollen strains.

Population	Proposed parental gametic genotypes	Number of plants			X^2 (3:1)	P
		Yellow pollen	Orange pollen	Cream pollen		
F_1 yellow × orange	$P_1P_2 \times P_1p_2$	34	0	0		
F_2 yellow × orange						
(13 families pooled)	$P_1P_2 \times P_1p_2$	1005	349	0	0.43	.7 -.5
F_1 yellow × cream	$P_1P_2 \times p_1P_2$	11	0	0		
F_2 yellow × cream						
(4 families pooled)	$P_1P_2 \times p_1P_2$	521	0	172	0.01	.95-.9

The data were combined because a Chi-square test for heterogeneity of the F_2 families was not significant. F_3 lines were scored, and they confirmed the F_2 classifications. These data show that pollen color in this cross is conditioned by one gene pair, with yellow dominant to orange.

All F_1 plants from the cross of yellow with cream pollen also had yellow pollen. Four F_2 families from this cross were scored, and each family segregated 3 yellow to 1 cream pollen. A Chi-square test for heterogeneity of the F_2 families was not significant, and the data were pooled. These data show that pollen color in this cross is also conditioned by one gene pair, with yellow dominant to cream.

Data from the F_1 and F_2 generations of the cross cream with orange pollen are presented in Table 2. All F_1 plants from this cross had yellow pollen. Five F_2 families were scored, and each family segregated 9 yellow: 3 orange: 4 cream. These color classes were distinct, with no overlap between yellow and orange. The pooled F_2 data from the five families were significantly different from a 9:3:4 ratio due to a deficiency of orange-pollen plants in each family. The heterogeneity Chi-square value from these data, however, was not significant. Twelve F_2 plants with orange pollen were progeny tested. Eight segregated for orange and cream pollen, and four bred true for orange pollen.

The data indicate that pollen color in the cross cream with orange has a different genetic basis from that in the cross yellow with orange or yellow with cream. F_1 pollen color different from either parent could result from the action of two complementary genes or be the result of a multiple allelic series. The possibility of pollen color being conditioned by a multiple allelic series can be ruled out, since some of the orange pollen phenotypes recovered in F_2 segregated orange and cream in F_3.

The alternate hypothesis of two complementary genes conditioning pollen color was postulated by Stephens (6). On this basis a genetic model involving two loci is proposed to explain the inheritance of pollen color in this study. The strain with cream pollen would have the genotype $p_1p_1P_2P_2$, and the orange pollen mutant would have the genotype $P_1P_1p_2p_2$. By this hypothesis, yellow-pollen strains would have the genotype $P_1P_1P_2P_2$. F_1 plants from the cross of cream with orange would have yellow pollen conditioned by the genotype $P_1p_1P_2p_2$. F_2 plants from this cross would have three pollen-color phenotypes representing eight genotypes.

F_2 plants with the genotype $p_1p_1p_2p_2$ could conceivably have yellow, orange, or cream pollen. The

Table 2. Classification of *G. barbadense* plants from F_1 and F_2 populations involving cream with orange and yellow with neocream pollen strains.

Population	Proposed parental gametic genotypes	Number of plants			X^2 (9:3:4)	P
		Yellow pollen	Orange pollen	Cream pollen		
F_1 cream × orange	$p_1P_2 \times P_1p_2$	11	0	0		
F_2 cream × orange (Family 1)	$p_1P_2 \times P_1p_2$	92	20	42	3.38	.2 -.1
F_2 cream × orange (Family 2)	$p_1P_2 \times P_1p_2$	86	23	39	1.01	.7 -.5
F_2 cream × orange (Family 3)	$p_1P_2 \times P_1p_2$	46	11	23	1.54	.5 -.3
F_2 cream × orange (Family 4)	$p_1P_2 \times P_1p_2$	49	10	18	2.19	.5 -.3
F_2 cream × orange (Family 5)	$p_1P_2 \times P_1p_2$	80	24	43	1.61	.5 -.3
Pooled F_2		353	88	165	7.41	.05-.02
Heterogeneity					2.32	.7 -.5
F_1 yellow × neocream	$P_1P_2 \times p_1p_2$	18	0	0		
F_2 yellow × neocream(1 family)	$P_1P_2 \times p_1p_2$	94	35	42	0.33	.9 -.8

$p_1p_1p_2p_2$ genotype probably conditioned cream pollen, since the F_2 segregated 9 yellow: 3 orange: 4 cream (Table 2). To confirm this hypothesis, plants with cream pollen were derived from segregating F_3 lines grown from orange pollen F_2 plants, with the genotype $P_1p_1p_2p_2$. These cream-pollen plants, hereafter designated neocream, were progeny tested and found to be true breeding, thus they should have the genotype $p_1p_1p_2p_2$. Neocream was crossed with yellow. Data from the F_1 and F_2 generations of this cross are shown in Table 2. All F_1 plants had yellow pollen. One F_2 family was scored, and it segregated 9 yellow : 3 orange : 4 cream pollen. The results from the F_2 of this cross, in "coupling phase," did not show a deficiency of plants with orange pollen as did the results from the F_2 of the "repulsion phase" cross of cream with orange. No reason is suggested for this discrepancy. Neocream also was crossed with the cream-pollen strain and with cream-pollen *G. hirsutum*. All F_1 plants from these two crosses had cream pollen. These data show that the $p_1p_1p_2p_2$ genotype conditions neocream pollen and is a new genotype for pollen color in amphidiploid cottons. Phenotypically, neocream and cream pollen cannot be distinguished. These data confirm the two-factor genetic model for inheritance of pollen color.

The results obtained in this study verify the existence of a second pollen color locus in *G. barbadense*. No modifying factors or residual genotype influences on the expression of pollen color were observed. The gene symbols, *P* or *p*, have been used for pollen color in amphidiploid species of cotton (Harland, 2). We propose that the symbol *P* be changed to P_1 and that the new locus be assigned the gene symbol P_2. Thus, amphidiploid cottons which breed true for yellow pollen would have the genotype $P_1P_1P_2P_2$, and true-breeding cream-pollen strains would be $p_1p_1P_2P_2$ or $p_1p_1p_2p_2$, the latter being neocream. The orange-pollen mutant would have the genotype $P_1P_1p_2p_2$.

No information was obtained on the homology of the P_2 locus with the P_b locus in the diploid species with A or B genomes, or with the pollen-color locus in D genome diploid species.

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