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FLOWER COLOR INHERITANCE IN DIPLOID AND TETRAPLOID ALFALFA - A REEVALUATION

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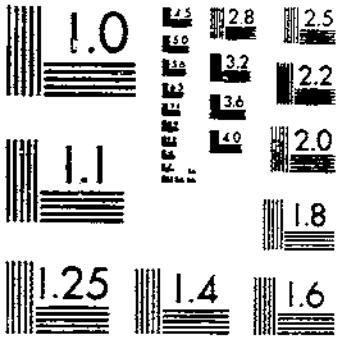

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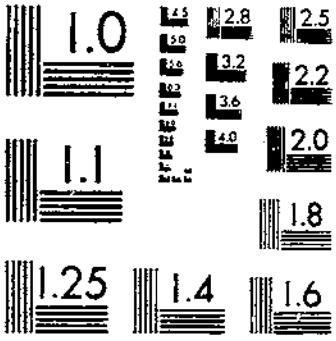

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FLOWER COLOR INHERITANCE IN DIPLOID AND TETRAPLOID ALFALFA: A REEVALUATION

By D. K. Barnes,[1] *research geneticist, Crops Research Division, Agricultural Research Service*

BACKGROUND

For nearly 50 years geneticists and plant breeders have searched for gene markers in order to understand alfalfa inheritance patterns. Flower color inheritance has been studied more intensively than any other qualitative character in alfalfa. More than 20 scientific papers have dealt with the subject. Hagem (*20*)[2] and Moe (*28*) were among the first workers to describe the variability in flower colors found in F_2 progenies from crosses between purple-flowered *Medicago sativa* L. and yellow-flowered *Medicago falcata* L. The many patterns, hues, and intensities of purple, yellow, and variegated flowers (mixtures of purple and yellow pigments produce numerous classes of greens and blues) have caused difficulty in phenotypic classification of plant progenies.

Hagem (*20*) suggested that two disomic genes controlled purple flower color and one disomic gene controlled yellow flower color. The theory of disomic inheritance was also preferred by most later researchers. Waldron (*40*), Korohoda (*21*), Burkart (*8*), Burton (*9, 10*), Lepper and Odland (*22*), Armstrong and Gibson (*4*), Weihing (*41*), Wood (*42*), and Storgaard (*35*)—all suggested that at least two and as many as four disomic genes controlled flower color. The tendency to interpret genetic results in alfalfa on the basis of disomic inheritance was not limited only to flower color genes; it included all qualitative characters studied.

Autotetraploid inheritance in alfalfa was first proposed by Tysdal (*37*) and was later illustrated by Tysdal, Kiesselbach, and Westover (*38*) from a reanalysis of data on leaf shape previously reported by Korohoda (*21*). However, a conclusive case for tetrasomic inheritance in alfalfa was not presented until Stanford (*32*) described the inheritance of a gene controlling white flower color. Since that time Armstrong (*3*), Lesins (*23*), and Stanford and Clement (*34*) have reported cases of cytological devia-

[1] Acknowledgement is made to W. M. Clement, Jr., Vanderbilt University, and R. L. Cooper, University of Minnesota, for their contribution of unpublished data and for their comprehensive review of this bulletin.

[2] Italic numbers in parentheses indicate references listed on page 24.

tions from a true autotetraploid behavior. However, most of cytological evidence reported by Armstrong (*3*), Grun (*19*), and Cleveland and Stanford (*11*) support the presence of autotetraploid-like pairing in tetraploid *M. sativa*. Besides the cytological evidence for autotetraploid behavior, most recent studies of qualitative genes have been interpreted on the basis of tetrasomic inheritance, i.e., inheritance of **elongated hypocotyle,** Davis (*17*); **exposed stigma,** Markus and Wilsie (*26*); **zebra leaf,** Stanford (*33*); and **root-knot nematode resistance,** Goplen and Stanford (*18*).

Because of the increasing genetic and cytogenetic evidence for autotetraploid behavior in cultivated alfalfa, all data available on flower color inheritance have been brought together and the possibility of a tetrasomic interpretation has been considered. The objectives of this publication are to (1) report recent findings on flower color inheritance, (2) consolidate all data on flower color inheritance into a single source, (3) compare the obvious similarity of data among studies, (4) set forth a uniform system of gene designations for researchers to refer to, and (5) compare the interpretations of flower color inheritance in tetraploid alfalfa with those from diploid alfalfa.

The limitations and difficulties of interpreting tetrasomic segregations of characters controlled by two or more genes were discussed by Little (*25*). Some difficulties that have often plagued research workers studying flower color inheritance in tetraploid alfalfa have been: (1) the use of small populations, which is often necessitated by increased self-incompatibility after several generations of inbreeding; (2) the difficulty in making definite phenotypic classification because of the continuous nature in expression of several flower color genotypes; and (3) the failure to grow critical F_3, F_4, or backcross generations, which are often necessary to demonstrate tetrasomic inheritance. Twamley (*36*), Buker and Davis (*7*), and Cooper and Elliott (*13, 14*) tried to circumvent some of these difficulties by studying flower color inheritance on the diploid level rather than on the tetraploid level.

FLOWER COLOR INHERITANCE IN DIPLOID ALFALFA

Purple Pigments

Twamley (*36*), Buker and Davis (*7*), and Cooper and Elliott (*13*) all extracted anthocyanins from purple-flowered diploid alfalfa. Twamley and Buker and Davis did not identify the specific anthocyanins isolated. Cooper identified three anthocyanin pigments from purple alfalfa flowers by means of paper chromatography. The three anthocyanin pigments were identified as malvidin 3–5 diglucoside, petunidin 3–5 diglucoside, and delphinidin 3–5 diglucoside. Cooper observed that in all crosses the three anthocyanin pigments were inherited as a unit. If one pigment was present, all three were present; however, the relative amounts sometimes varied.

These results were in general agreement with similar studies of tetraploid alfalfa. Lesins (*24*) identified the anthocyanins as derivatives of the three aglycones—delphinidin, petunidin, and malvidin. All of the anthocyanin pigments appeared to be inherited as a genetic unit with the possible exception of the independent segregation of the

malvidin derivative in one cross. In another study of tetraploid alfalfa Davies (*16*) identified two of the anthocyanin aglycones as delphinidin and malvidin, but the third aglycone was identified as cyanidin rather than petunidin.

Genetic data based on phenotypic classification of purple vs. nonpurple flowers reported by Buker and Davis (table 1) suggested that a single dominant gene (*P*) controlled the presence of purple color. Genetic data presented by Cooper indicated a similar pattern of inheritance. The majority of Twamley's data indicated segregation of a single gene controlling purple flower color (table 1). But Twamley separated out three F_2 families and one F_3 family, which gave a better fit to a pattern of two complementary genes than to a monofactorial segregation. The possibility of two genes controlling purple pigments cannot be totally discounted. But the basis for separation of the four families is questionable, especially since all four families were very small. Although it is true that the limited data fit a 9:7 ratio better than a 3:1 ratio, none of the four families failed to fit a 3:1 ratio. According to Mather (*27*), 95 plants (94.32) are necessary in order to distinguish between 3:1 and 9:7 ratios with a minimum certainty of 0.025. The published data tend to indicate that a single dominant gene (*P*) controls purple flower color in diploid alfalfa.

Yellow Pigments

Twamley (*36*) found that the yellow flower color of *M. falcata* was due to two unrelated yellow pigments. One pigment was insoluble in water but soluble in acetone and petroleum ether. It was tentatively identified as the plastid pigment carotene. The other pigment, which was only extractable in hot water, was presumed to be one of the sap-soluble anthoxanthin pigments. Cooper and Elliott (*13*, *14*) identi-

TABLE 1.—*Segregations for purple and nonpurple flower color in* F_2 *and backcross families of diploid alfalfa, and chi-square tests for goodness of fit (P) to genetic ratios*

Author and generation	Number of families	Observed ratio		Author's expected ratio	P
		Purple	Nonpurple		
Buker and Davis (*7*):					
F_2	7	242	86	3:1	0.627
Cooper (*14*):					
F_2	2	81	33	3:1	.340
F_1 × nonpurple	6	50	44	1:1	.547
F_1 × purple	7	101	0	1:0	--------
Nonpurple	5	0	59	0:1	--------
Twamley (*36*):					
F_2	12	229	79	3:1	.795
F_2	1	5	4	[1] 3:1	.184
F_2	1	7	4	[1] 3:1	.403
F_2	1	8	6	[1] 3:1	.131
F_3	1	4	3	[1] 3:1	.279

[1] Previously reported by author as fitting 9:7 ratio.

fied the yellow carotene pigments as xanthophyll esters, which are closely related derivatives of carotene. Chromatographic studies by Buker and Davis (*7*) indicated the presence of four pigments—two fat-soluble pigments with characteristics similar to carotenoids and two water-soluble pigments with characteristics of flavones and flavonols. Traces of yellow pigments were also uncovered at four additional chromatographic locations.

Genetic data presented by Twamley (table 2) suggested that three or more genes were very likely involved in the production of yellow pigment. Only 1 of 342 diploid F_2 plants from crosses of purple and yellow flowered plants was identical to the purple-flowered parent and contained no trace of yellow pigment. Although about one-fourth (85 plants) of the F_2 population was yellow flowered (table 2) none of the plants produced cream flowers or were as deeply pigmented as the *M. falcata* parent.

Unpublished data by Barnes and Cleveland [3] gave additional support to the hypothesis that three or more genes controlled yellow flower color (table 2). All of the F_1 plants from a cream-flowered *M. sativa* plant crossed with an orange-yellow flowered *M. falcata* plant were yellow flowered. Seven F_2 families were produced. Three of the F_2 families failed to segregate for cream-flowered progenies, but a backcross to the cream-flowered parent produced cream-flowered plants in an approximate 15 yellow : 1 cream ratio. The remaining four F_2 families segregated 63 : 1 and backcrosses to the cream-flowered

[3] Barnes, D. K., and Cleveland, R. W., Dept. Agron., Pa. State Univ., University Park, Pa. Unpublished data. 1962.

TABLE 2.—*Segregations for yellow and cream flower color in F_2 and backcross families of diploid alfalfa, and chi-square tests for goodness of fit (P) to genetic ratios*

Author and generation	Number of families	Observed ratio		Author's expected ratio	P
		Yellow	Cream		
Barnes and Cleveland: [1]					
F_1 (cream × yellow)	1	22	0	1:0	-------
F_2	3	90	0	255:1	0.596
BC (F_1 × cream)	1	71	3	15:1	.450
F_2	4	274	8	63:1	.088
BC (F_1 × cream)	1	42	10	7:1	.152
Buker and Davis (*7*): [2]					
F_2 (purple × yellow)	7	72	14	3:1	.066
F_3 (yellow ⊗)	6	49	18	3:1	.729
Cooper and Elliott (*14*): [3]					
F_3 (variegated ⊗)	1	41	2	15:1	.674
Twamley (*86*): [2]					
F_2 (purple × yellow)	1	85	0	-------	-------

[1] Unpublished data. Pa. State Univ., University Park, Pa.; data based on visual phenotypic classification.
[2] Considered only F_2 plants without purple pigments in flowers.
[3] Presence or absence of yellow pigments identified chemically in both purple-flowered and non purple-flowered plants.

parent segregated approximately 7 : 1.

The yellow flower color data presented by Buker and Davis (table 2) were interpreted by them as being controlled by a single gene. However, this interpretation should be viewed with some caution because although the majority of the families gave good fits to a 3 : 1 ratio, several of the F_2 and F_3 families gave as good or better fits to a 15 : 1 ratio. Cooper and Elliott (*14*) did not rely on visual observations of yellow flower color per se, as the other research workers did; they chemically analyzed individual plants for the presence and absence of xanthophyll. The segregations they observed (table 2) definitely established the presence of at least two genes controlling xanthophyll production.

The data indicate that at least three and probably four genes with accumulated effects control yellow flower color in diploid alfalfa. Two of the four genes have been identified as controlling xanthophyll production. It seems appropriate to assign the gene designations Yx_1, Yx_2, Y_3, and Y_4 to the four loci controlling yellow color. The Yx designation refers to the two genes described by Cooper and Elliott (*14*) as controlling xanthophyll production. The other two genes can remain as Y until further studies demonstrate that they are additional xanthophyll genes or that they control some other pigment, at which time a subscript letter can be added.

Basic Color Factor

A white-flowered diploid alfalfa plant (S2128) was found by Dr. J. L. Bolton, Central Experimental Farm, Ottawa, Canada. The phenotype was similar to the *C* gene previously reported in tetraploid alfalfa. The phenotypic expressions and inheritance pattern of the *C* gene in tetraploid alfalfa are summarized on pp. 8, 10–12 of this bulletin. Clement[4] reported that diploid plants which were homozygous recessive for the *C* gene were weak and had poor fertility. Crosses between white-flowered plants and purple-flowered plants produced purple-flowered F_1's. The F_2 segregations were approximately 3 purple to 1 white, and progenies from backcrosses to the white-flowered parent segregated approximately 1 purple to 1 white. A deficiency of white-flowered plants was observed in all crosses. Subsequent data from crosses [(white × yellow, *M. falcata*) × white] closely fit a ratio of 1 white to 1 yellow.

Clement[5] studied critical crosses in diploid alfalfa and concluded that the *C* gene is a basic color factor for both purple- and yellow-flowered plants. The dominant *C* allele had to be present for the production of color. The homozygous recessive genotype masked the usual expression of the primary factors for purple and yellow. However, some pigment factors in *M. falcata* were able to express themselves as a cream flower color. There appeared to be at least two kinds of cream flower color: those *C-ppyy*, and those recessive for the basic color factor and the *p* locus, but dominant for one or more *y* factors. The phenotypic expression for each

[4] Clement, W. M., Jr., Crops Res. Div., U.S. Agr. Res. Serv., Dept. Agron. and Plant Genet., Univ. Minn., St. Paul. Unpublished data. (Personal correspondence dated Sept. 23, 1964.)

[5] See footnote 4.

of the different genotypes was as follows:

*Genotype**	*Phenotype*
C–P–Y–	variegated
C–P–yy	purple
C–ppY–	yellow
C–ppyy	cream
ccP–Y–	cream
ccP–yy	white
ccppY–	cream
ccppyy	white

*Loci controlling yellow flower color of *M. falcata* parent were not specifically indentified, so *Y* is used as general designation for any of Yx_1, Yx_2, Y_1, and Y_2 genes.

Modifying Pigments

Little research has been conducted on the nature and identification of pigments other than the primary purple and yellow pigments. Only Cooper and Elliott (*13*) have chemically identified any of the so-called modifying pigments. In addition to the three previously described anthocyanin pigments, Cooper and Elliott isolated nine anthoxanthin pigments from variegated flowered alfalfa. Six of the anthoxanthins (designated Q_1, Q_2, Q_3, Q_4, Q_5, and Q_6) were quercetin glycosides, and three (K_1, K_2, and K_3) were kaempferol glycosides. None of the nine anthoxanthin pigments imparted a phenotypically significant color of its own, but they tended to act either as intensifiers or as modifiers when copigmented with anthocyanin or xanthophyll. Only two of the nine anthoxanthin glycosides exhibited segregation in enough families to permit a genetic analysis.

Kaempferol glycoside K_1 was present in every plant examined; the other two kaempferols, K_2 and K_3, segregated in 3:1 ratios. The inheritance of the production of kaempferol glycoside K_2 and K_3 was shown to be controlled by two independent dominant genes.

Quercetin glycoside Q_1* was present in various concentrations in nearly all plants (*12*). All of the progeny from crosses among plants lacking quercetin glycoside Q_1 also lacked the same glycoside. Therefore, Cooper suggested that the absence of quercetin glycoside Q_1 was controlled by one or more genes in the homozygous recessive condition. The concentration of quercetin glycoside Q_1 appeared to influence the concentration of the other quercetin glycosides. Cooper suggested that the simultaneous increases in intensity of all six quercetin glycosides indicated that an intensifier gene or genes was acting on the aglycone quercetin. Two possible hypotheses that he suggested for the inheritance of quercetin glycosides were: (1) two quantitative genes affecting the intensity of the aglycone quercetin, and segregating for various intensities in a ratio of 1:4:6:4:1; and (2) a single dominant gene controlling the production of quercetin glycoside Q_1 and modified by another single quantitative factor segregating 1:2:1.

Pink bud color was observed and described in floral buds of many white- and yellow-flowered plants from advanced generation hybrids between diploid *M. sativa* and diploid *M. falcata* by both Cooper (*12*) and Twamley (*36*). Both research workers hypothesized that the color was due to an anthocyanin pigment, but unfortunately no analyses were made to substantiate this. The pink color usually faded away soon after the flowers opened. The inheritance of the character was studied by Barnes and Cleveland (*5*). They found no *M. falcata* plants with pink buds. Their

* Listed in Ph. D. thesis (*12*) as quercetin glycoside A, but later referred to by Cooper and Elliott (*13*) as Q.

studies indicated that pink bud color required the presence of two dominant genes with complementary action. They concluded that one dominant gene, *Bs*, came from *M. sativa* and the other dominant gene, *Bf*, came from *M. falcata*.

Floral vein color is also another source of flower pigmentation, because darkly pigmented veins are present in many alfalfa flowers. These veins extend from the throat of the flower toward the forward edge of the standard. Some plants have pigmented veins throughout the standard petal; other plants are devoid of floral vein pigmentation. Floral vein color pigments have not been chemically analyzed. Therefore, they could be the source of one or more of the modifying pigments which were isolated but whose functions were not specifically identified by Buker and Davis (*7*) and Cooper (*12*). Barnes and Cleveland (*5*) reported that the presence of darkly pigmented veins in yellow-flowered diploid alfalfa was controlled by the presence of one or more dominant alleles from either or both of two duplicate genes (Vs_1 and Vs_2). Absence of vein color was due to the homozygous recessive genotype of both genes. An association of wing petal vein pigmentation and standard petal pigmentation was observed but was not studied.

Joint Pigmentation

F_1 progenies from crosses between purple-flowered *M. sativa* and yellow-flowered *M. falcata* have variegated flowers. The buds and freshly opened flowers usually contain more anthocyanins than do flowers several days old. According to Lesins (*23*), purple pigmentation is in the epidermal layer of the flower, which in turn is over a background of yellow. This copigmentation usually gives the variegated flowers from F_1 progenies a green appearance, but as the anthocyanins decrease with age the flower color fades to a dirty yellow. The various dosages of purple, yellow, and modifying pigments found in F_2 and F_3 progenies give additional patterns of variegated flower colors.

Cooper and Elliott (*14*) chemically studied joint segregations in individual plants for: (1) anthocyanin and kaempferol glycoside K_2, (2) anthocyanin and kaempferol glycoside K_3, (3) anthocyanin and xanthophyll, (4) xanthophyll and kaempferol glycoside K_2, and (5) xanthophyll and kaempferol K_3. All joint segregations indicated independent associations between the genes involved.

Buker and Davis (*7*) phenotypically classified F_2 progenies from several crosses of *M. sativa* and *M. falcata* into green, purple, yellow, and cream flower color classes. Data from six of the seven F_2 families and from the pooled data of all seven families fit a 9:3:3:1 ratio. A chi-square test for linkage was not significant for any one individual family, but the pooled data indicated that the one purple gene and the one yellow gene they proposed for controlling flower color may be linked (39 ± 3 units). Buker and Davis listed possible explanations for the inconsistency in the data. They suggested that an error in classification could throw genotypically green-flowered plants into the purple class, and if this was accompanied by a misclassification of cream as yellow, it would give the appearance of linkage. A small number of misclassifications in each family could be responsible for the significant linkage test in the pooled data. Another possibility is that Buker and Davis (*7*) and Cooper and Elliott (*14*) did not study the same yellow flower color genes.

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The chemical investigation of individual plants enabled Cooper and Elliott (*14*) to obtain correlations between various combinations of pigments and modifiers with the phenotypic appearance of the flower. According to Cooper and Elliott the presence of one or more dominant alleles of the *P* gene governs anthocyanin synthesis and produces a light blue to purple color in alfalfa flowers. Anthocyanin intensity ranged from deep purple to very dilute purple to almost white flowers. The phenotypic variability in purple flower color was probably due to blending or copigmentation effects of anthoxanins and background effects of xanthophyll. Kaempferol glycosides produced little or no color when present in white flowers, but had some effect when present in joint pigmentation with anthocyanins. All plants with a maroon phenotype contained at least one factor for xanthophyll production, but none of these plants contained both kaempferol glycosides K_2 and K_3. In plants containing no xanthophyll, a significantly greater number of reddish-blue flowered plants were found in the K_2k_3, k_2K_3, and k_2k_3 genotypes than in the K_2K_3 genotype. However, there was some overlapping of phenotypes between these classes. The blueing effect was assumed to result from copigmentation of glycosides K_2 and K_3 with anthocyanins.

Cooper (*12*) reported that it was difficult to ascertain the exact phenotypic effects of the quercetin glycosides because of the epistatic effect of the yellow xanthophylls. Traces of yellow in some phenotypes void of xanthophyll indicated some phenotypic effect, but the effect was only evident when the concentration of quercetin glycosides was above a certain level.

Xanthophyll was the most important pigment in determining yellow flower color. Xanthophyll was not present in cream flowers; conversely, the intense or orange-yellow flowers from *M. falcata* contained a high intensity of xanthophyll pigments. Indications were that quercetin glycosides produced a phenotypic effect only when low intensities of xanthophyll pigments were present. However, xanthophyll at high intensities may mask the effect of the quercetin pigments.

An inheritance chart for flower color in diploid alfalfa was prepared by Cooper and Elliott (*14*). Because the chart is essential for understanding the inheritance of the many flower color patterns, it is presented in figure 1.

FLOWER COLOR INHERITANCE IN TETRAPLOID ALFALFA

Inheritance of white flower color has undoubtedly caused more problems in genetic interpretations than any other single character in tetraploid alfalfa. This has been due to the presence of two genotypically different types of white flowers. These two types can be designated as white and cream flower color.

White vs. Purple Flower Color

Waldron (*39*) reported that in 1914 he received a small quantity of "albino seed." The seed was produced on a plant with "pure white flowers, which lacked even the violet veining in the petals of the young flowers usually found on so-called white-flowered alfalfa." The seed itself also lacked the pigment of normal alfalfa seed. Seed from self-pollinated plants bred true for the albino flower-seed character. Open-pollinated seeds from the white-flowered plant produced all purple-flowered F_1 plants; this in-

FIGURE 1.—Genetic hypothesis for flower color inheritance in diploid alfalfa.* (Source: By permission Cooper and Elliott (*14*).

Genotype				Phenotype
YxYx, YxYx	*P*	K_2	K_3	variegated (dark purple)
	p	K_2	K_3	orange yellow
YxYx, Yxyx	*P*	K_2	K_3	variegated (purple)
	p	K_2	K_3	bright yellow
YxYx, yxyx	*P*	K_2	K_3	variegated (purple)
			k_3	variegated (purple to maroon)
		k_2	K_3	variegated (purple to maroon)
			k_3	variegated (maroon)
	p	K_2	K_3	yellow
Yxyx, yxyx	*P*	K_2	K_3	purple
			k_3	purple to maroon
		k_2	K_3	purple to maroon
			k_3	maroon
	p	K_2	K_3	light yellow
yxyx, yxyx	*P*	K_2	K_3	blue
			k_3	blue to reddish blue
		k_2	K_3	blue to reddish blue
			k_3	reddish blue
	p	K_2	K_3	white (cream)

Yx=factors for xanthophyll production and intensity. *P*=factor for anthocyanin production. K_2=factor for kaempferol glycoside K_2 production. K_3=factor for kaempferol glycoside K_3 production.

*This inheritance chart has been used to show the interaction of the various color factors in alfalfa flowers and the relationship between genotype and phenotype. To determine the genotype of any given phenotype start at the name and follow the line to the left and upward until all genes are accounted for. The multiple parallel lines indicate no phenotypic effect of the underlined genes.

dicated that a natural cross had occurred with ordinary purple-flowered alfalfa. The F_2 progeny segregated for purple-flowered plants and white-flowered plants.

Most research workers who have used white-flowered parents in tetraploid flower color studies have failed to distinguish between white- and cream-flowered plants. However, through careful inspection of the data, it usually has been possible to establish which type of flower color was used in each study. The factor(s) controlling white flower color can be generally described as epistatic to the *P* gene, which is responsible for anthocyanin production in the flowers. The action of the color-conditioning factor blocks not only the formation of purple flower color pigments, but also the normal anthocyanin production in the seedcoat and vegetative stems. Clement[7] reported that the anthocyanins produced by the *Rd* gene, which controls red roots in alfalfa, are also affected. Burkart (*8*), Covas and Fernández (*15*), and Stanford (*32*) reported that white-flowered plants had green hypocotyls in the seedling stage, whereas some anthocyanin pigments were found in the hypocotyls of purple-flowered plants. The white flower factor described by Waldron (*39*) was apparently present in some of the parental material used in studies by Burkart (*8*), Lepper and Odland (*22*), Stanford (*32*), Storgaard (*35*), Twamley (*36*), Weihing (*41*), and Wood (*42*).

All of the early research workers (*8*, *22*, *39*) interpreted the inheritance of white flower color as being controlled by two or more disomic genes. However, Stanford (*32*) grew the critical generations necessary to differentiate between disomic and tetrasomic inheritance and established that a factor controlling purple vs. white flower color in alfalfa was inherited in a tetrasomic manner. Stanford indicated that he had been studying a factor for purple flower color, but it was more precisely the study of a basic color factor. Besides the study by Stanford, only Twamley (*36*) interpreted the inheritance of white flower color on a tetrasomic basis. The F_2 data from studies by Waldron (*39*) and Lepper and Odland (*22*) fit a disomic scheme of inheritance better than the tetrasomic schemes. However, Lepper and Odland's data represented combined progenies from eight different crosses and no assurance was given that all crosses represented the same genotypes. The results from the study by Burkart (*8*) fit either disomic or tetrasomic inheritance. A summary of the results from papers presenting data on crosses between purple-flowered plants and plants homozygous for white flower color are presented in table 3. When interpreting many of these studies, it should be remembered that the critical F_3, F_4, and backcross generations that were needed to positively differentiate between disomic and tetrasomic inheritance were not grown.

After considering the evidence for tetrasomic inheritance presented by Stanford (*32*) and in view of the lack of concrete evidence for disomic inheritance from advanced generations, it appears reasonable to assume that tetrasomic inheritance is the normal inheritance pattern for the basic color factor that produces white flower color. Stanford (*32*) did not specifically assign a letter designation to the gene, but he used the symbol *c* in a table describing expected tetrasomic segregations.

[7] Clement, W. M., Jr., Crops Res. Div., U.S. Agr. Res. Serv. and Dept. Agron. and Plant Genet., Univ. Minn., St. Paul. Unpublished data. (Personal correspondence dated December 14, 1962.)

TABLE 3.—*Segregations for purple and white flower color in tetraploid alfalfa from crosses among white-flowered and purple-flowered plants, and chi-square tests for goodness of fit (P) to both disomic and tetrasomic ratios*

Author and generation	Number of families	Observed ratio		Expected disomic ratio	P	Expected tetrasomic ratio [1]			
		Purple	White			Random chromatid	P	Random chromosome	P
Burkart (*8*):									
F_1 (purple × white)	--------	All	0	--------	--------	--------	--------	--------	--------
F_2	--------	223	4	63:1	0.806	20.8:1	0.044	35:1	0.367
BC (F_1 × white)	--------	12	2	7:1	.848	3.7:1	.530	5:1	.817
Lepper and Odland (*22*):									
F_1 (purple × white)	8	All	0	--------	--------	--------	--------	--------	--------
F_2	8	411	29	15:1	.773	20.8:1	.049	35:1	.001
Stanford (*32*):									
F_1 (white × purple)	1	All	0	--------	--------	--------	--------	--------	--------
F_2	3	209	62	3:1	.437	2.5:1	.041	3:1	.437
	5	302	15	15:1	.270	20.8:1	.900	35:1	.040
	4	422	11	63:1	.101	20.8:1	.047	35:1	.700
Combined data	9	724	26	--------	--------	20.8:1	.152	35:1	.257
F_3 [2]	1	Raw data not presented.		1:00	--------	783:1	--------	1:0	--------
	8	do		Not possible.	--------	20.8:1	--------	35:1	--------
	26	do		3:1	--------	2.5:1	--------	3:1	--------
	11	do		0:1	--------	0:1	--------	0:1	--------
Waldron (*39*):									
P_1 (white ⊗)	--------	0	All	--------	--------	--------	--------	--------	--------
F_1 (white × purple)	1	All	0	--------	--------	--------	--------	--------	--------
F_2	1	1,943	20	63.1	.051	20.8:1	.000	35:1	.001

[1] Expected ratios calculated on the basis of segregation limits presented by Allard (*1*).

[2] Progenies from F_2 family segregating 3:1, self-pollinated. Fit of F_3 families to a tetrasomic pattern of inheritance, expected segregation (calculated with double reduction=0.144)=0.8 (1:0): 10.8 (35:1): 22.1 (3:1): 12.3 (0.1), X^2=1.601, P=0.562.

Lepper and Odland (*22*), Weihing (*41*), and Wood (*42*) all used *c* to designate a factor for the production of color; Twamley (*36*) used *c* to describe white flower color; and Oldemeyer (*29*) used *c* to describe one of the factors he proposed for controlling white seed color. Since all of these papers have dealt with effects of what is apparently the same gene, it seems appropriate to maintain the gene symbol *c* as the designation for the basic color gene controlling white flower color. Available evidence indicates that white flower color is expressed by the homozygous recessive genotype of the *c* gene (*cccc*). The presence of one or more doses of the dominant allele (*C*– – –) permits the expression of anthocyanins.

Cream vs. Purple Flower Color

Cream-colored flowers usually have pigmented floral veins and appear practically white, but when they are compared directly with truly white flowers they will have a slight ivory or off-white hue. Plants with cream-colored flowers have some anthocyanins in the stems and have yellow or tan seeds. It is therefore possible to differentiate between cream-flowered and white-flowered plants.

Research on the inheritance of cream vs. purple flower color has been reported by Armstrong and Gibson (*4*), Barnes and Cleveland,[8] Soudah (*31*), Storgaard (*35*), and Weihing (*41*). The data from these studies have been summarized in table 4. Data from some of the F_2 families presented by Armstrong and Gibson fit either a tetrasomic or disomic pattern of inheritance, but this work should be interpreted cautiously because it deals with crosses between *M. media* Pers. and *M. glutinosa* Bieb. At the present time, little is known about the genetics of *M. glutinosa* and its relation to *M. sativa*.

F_2 and backcross data presented in the study by Weihing (*41*) fit either a disomic or tetrasomic pattern of inheritance. If the tetrasomic model is used, the data from all six F_2 families can be pooled and the data from both backcross families consolidated because all of the F_1 plants appeared to have the same genotype. If the disomic model is used, the F_1 plants need to be separated into two genotypes. The results of this study are inconclusive because the F_3 generation was not grown.

Almódovar (*2*) conducted a quantitative study of cream vs. purple flower color, but drew only general conclusions from it because of small population sizes. Soudah (*31*) used parental material obtained from Almódovar and studied in somewhat greater detail the inheritance patterns of cream vs. purple flower color. Soudah observed the segregation from crosses among plants that were nulliplex, simplex, and duplex for the gene controlling purple flower color. The evidence obtained from these crosses (table 4) did not fit expected disomic segregations, but it did fit a tetrasomic pattern of inheritance. Soudah also presented data on the relation of plant genotypes and flower color intensity. He proposed the idea that the effects of the gene controlling purple flower color were accumulative and could be used as criteria for phenotypic classification of various genotypes (i.e., nulliplex=cream, simplex=light purple, duplex=purple, triplex=deep purple, and quadriplex=very deep purple). The data

[8] Barnes, D. K., and Cleveland, R. W., Dept. Agron., Pa. State Univ., University Park, Pa. Unpublished data. 1962.

as presented suggested this interpretation, but no progeny tests were grown to verify or disprove the theory.

The F_2 and backcross data presented from the study by Barnes and Cleveland[9] fit either a disomic or tetrasomic pattern of inheritance much the same as did the data by Weihing (*41*). However, F_3 segregations gave conclusive evidence that cream flower color was due to the homozygous recessive condition of a tetrasomically inherited dominant gene. It was ascertained that cream vs. purple flower color was studied without the presence of the recessive allele of the *C* gene. This was checked by crossing the true-breeding cream parent with a white-flowered plant of the same genotype used by Stanford (*32*). All F_1 plants were purple flowered; this indicated that the cream parent carried at least three doses of the dominant allele of the *C* gene. The presence of the dominant *C* allele in the F_1 plants allowed expression of the allele for anthocyanin production that was carried in the white-flowered parent.

When considered in total, all available evidence indicates cream flower color is due to the homozygous recessive condition of a single gene. This gene is inherited in a tetrasomic manner and produces purple flower color when one or more doses of the dominant allele are present. The most appropriate gene designation for this character would be *P*. The *P* symbol was first used by Lepper and Odland (*22*) to designate a gene controlling purple flower color. Since that time *P* has been used by most workers when referring to purple flower color inheritance. Cream and purple flower colors can be expressed genotypically as cream=*C*– – –*pppp* and purple=*C*– – –*P*– – –.

Cream vs. White Flower Color

The criteria for phenotypically differentiating cream-flowered and white-flowered plants were described on pages 10 and 12. The small and often subtle phenotypic differences between cream and white flowers were not always recognized. Nevertheless, from genetic evidence, some research workers became aware of the presence of at least two genotypically different types of white-flowered plants. Risius (*30*), Storgaard (*35*), Twamley (*36*), Weihing (*41*), and Wood (*42*) all presented data demonstrating that crosses between cream- and white-flowered plants sometimes produced purple-flowered F_1 progeny (table 5). All workers except Risius and Twamley interpreted the various segregations for purple vs. white flower color on the basis of disomic inheritance. Since conclusive evidence was presented in tables 3 and 4 for tetrasomic inheritance of the *C* and *P* genes, and since several of the proposed models for disomic inheritance were very complex, only expected ratios for tetrasomic inheritance are presented in table 5. However, several proposed models for disomic inheritance are discussed below.

Weihing (*41*) suggested that a color factor *Cc* was present, which was complementary to purple. Purple flower color was expressed only in the presence of one or more doses of the dominant *C* allele and one or more doses of the allele for purple. Wood (*42*), continuing the studies begun by Weihing, produced an F_2 generation and suggested a disomic hypothesis similar to Weihing's based on the idea that

[9] Barnes, D. K., and Cleveland, R. W., Dept. Agron., Pa. State Univ., University Park, Pa. Unpublished data. 1962.

TABLE 4.—*Segregations for purple and cream flower color in tetraploid alfalfa, and chi-square tests for goodness of fit (P) to both disomic and tetrasomic ratios*

Author and generation	Number of families	Observed ratio		Expected disomic ratio	P	Expected tetrasomic ratio [1]			
		Purple	Cream			Random chromatid	P	Random chromosome	P
Armstrong and Gibson (*4*):									
P_1 (purple ⊗)	1	90	0						
P_2 (cream ⊗)[2]		0	All						
F_1 ($P_2 \times P_1$)	1	All	0						
F_2	5	365	102	3:1	0.121	2.5:1	0.001	3:1	0.121
	2	239	9	15:1	.096	20.8:1	.479	35:1	.430
	6	702	5	63:1	.071	20.8:1	.000	35:1	.001
Combined data	8	941	14			20.8:1	.000	35:1	.015
Barnes and Cleveland: [3]									
P_3 (cream ⊗)	1	0	14						
P_4 (purple ⊗)	1	34	0						
F_1 ($P_3 \times P_4$)	1	56	0						
F_2	7	1,000	23	63:1	.081	20.8:1	.000	35:1	.305
BC (F_1 × cream)	1	16	3	7:1	.675	3.7:1	.575	5:1	.921
BC (F_1 × purple)	1	30	0	1:0		1:0		1:0	
F_3 [4]	2	0	37	0:1		0:1		0:1	
	13	274	85	3:1	.578	2.5:1	.045	3:1	.578
	23	837	36	15:1+63:1		20.8:1	.518	35:1	.016
	17	531	0	1:0		783:1		1:0	
Soudah (*31*): [5]									
P_5 (cream ⊗)	1	0	9	0:1		0:1			
P_6 (purple ⊗)	1	13	1	15:1	.897	2.5:1	.080	3:1	.131
P_7 (purple ⊗)	1	55	4	15:1	.875	20.8:1	.439	35:1	.065
F_1 ($P_5 \times P_6$)	4	52	39	3:1	.001	0.87:1	.047	1:1	.180
F_1 ($P_5 \times P_7$)	4	66	25	3:1	.603	3.7:1	.158	5:1	.001
F_1 ($P_6 \times P_7$)	4	122	20	15:1	.001	7.7:1	.343	11:1	.014

Storgaard (*35*):[6]									
P_8 (cream ⊗)	3	0	28	0:1	--------	0:1	--------	0:1	--------
P_9 (purple ⊗)	1	165	1	63:1	.324	20.8:1	.015	35:1	.091
F_1 (P_8 × P_9)	2	63	8	7:1	.760	3.7:1	.042	5:1	.227
Weihing (*41*):									
F_1 (purple × cream)	1	All	0	--------	--------	--------	--------	--------	--------
F_2	3	219	16	15:1	.729	20.8:1	.105	35:1	.000
BC (F_1 × cream)	1	22	5	3:1	.452	3.7:1	.732	5:1	.796
F_2	3	197	3	63:1	.945	20.8:1	.040	35:1	.276
BC (F_1 × cream)	1	44	7	7:1	.793	3.7:1	.191	5:1	.589
F_2, combined data	6	416	19	--------	--------	20.8:1	.835	35:1	.045
BC, combined data	2	66	12	--------	--------	3.7:1	.205	5:1	.767

[1] Expected ratios calculated on the basis of segregation limits presented by Allard (*1*).

[2] Cream parent = *Medicago glutinosa*.

[3] Unpublished data, Pa. State Univ., University Park, Pa.

[4] Fit of F_3 families to a model of tetrasomic, random chromosome inheritance, expected segregation=1.5 (0:1): 12.2 (3:1): 27.5 (35:1): 13.8 (1:0), X^2=1.697, P=0.642.

Fit of F_3 families to a model of 3 genes with disomic inheritance, expected segregation=0.9 (0:1): 5.2 (3:1): 17.2 (15:1+63:1): 31.7 (1:0), X^2=21.817, P=0.000.

[5] P_5 represents combined data from 2 cream-flowered plants; P_6 and P_7 each represent combined data from 2 purple-flowered plants. Author's proposed genotypes are *pppp*, *Pppp*, and *PPpp*, respectively.

[6] Author lists cream plants as clones 8(8), 207(10) and 77(3), purple plant as clone C–194.

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TABLE 5.—*Segregations for purple and for white and cream flower color in tetraploid alfalfa from crosses among white-flowered and cream-flowered plants, and chi-square tests for goodness of fit (P) to genetic ratios* [1]

Author and generation	Assumed genotype	Number of families	Observed ratio		Expected tetrasomic ratio [2]			
			Purple	White and cream	Random chromatid	P	Random chromosome	P
Risius (*30*): [3]								
P_1 (white ⊗)	*ccccPPP–*		0	All				
P_2 (cream ⊗)	*CCC–pppp*		0	All				
F_1 ($P_1 \times P_2$)			All	0				
F_2		9	3, 890	206	10. 2:1	0. 001	17. 3:1	0. 225
BC ($P_1 \times F_1$)		10	2, 462	465	3. 7:1	. 001	5:1	. 264
BC ($P_2 \times F_1$)		10	3, 041	490	3. 7:1	. 001	5:1	. 001
Storgaard (*35*): [4]								
P_3 (white ⊗)	*ccccPPP–*				0:1		0:1	
P_4 (cream ⊗)	*Ccccpppp*		0	19	0:1		0:1	
P_5 (cream ⊗)	*CCcccpppp*		0	9	0:1		0:1	
F_1 ($P_3 \times P_4$)			3	4	0. 87:1	. 854	1:1	. 707
F_1 ($P_3 \times P_5$)			8	0	3. 7:1	. 151	5:1	. 207
F_1 ($P_4 \times P_5$)			0	23	0:1		0:1	

Twamley (*56*):								
P_6 (cream ⊗)	*CCC-pppp*	2	0	All	0:1	----------	0:1	----------
P_7 (white ⊗)	*ccccPPP-*	2	0	All	0:1	----------	0:1	----------
F_1 ($P_6 \times P_7$)	----------	2	All	0	1:0	----------	1:0	----------
F_2	----------	3	405	22	10.2:1	.001	17.3:1	.781
BC ($F_1 \times P_7$)	----------	2	206	34	3.7:1	.001	5:1	.299
Weihing (*41*):[5]								
P_8 (cream ⊗)	*CCC-pppp*	6	0	All	0:1	----------	0:1	----------
P_9 (white ⊗)	*cccc-PPpp*	2	0	All	0:1	----------	0:1	----------
F_1 ($P_9 \times P_8$)	----------	5	72	14	3.7:1	.263	5:1	.929
Wood (*42*):								
F_2[6]	{--	2	618	51	10.2:1	.242	17.3:1	.015
	{----------	10	1,904	816	2.1:1	.012	2.7:1	.001

[1] Parental genotypes and expected ratios calculated by reviewer on basis of inheritance patterns indicated for *C* and *P* genes in tables 3 and 4, respectively.

[2] Expected ratios calculated on basis of segregation limits presented by Allard (*1*).

[3] Original clone designations J and N assigned P and P_2 by reviewer.

[4] Original clone numbers 77(3), 8(9), and combined data from clones 8(8) and 207(10) assigned P_3, P_4, and P_5 designations by reviewer for brevity and clarity.

[5] Interpreted from original data that parental clones 2, 3, 8, 9, 10, and 12 (listed in table as P_8) were cream and probably all of the *CCC-pppp* genotype. Both parental clones 4 and 6 (listed in table as P_9) appeared to be *ccccPPpp*.

[6] Assuming genotype of parents *CCC-pppp* × *ccccPPpp*, then F_2 families from purple-flowered F_1 plants should segregate for random chromosome-tetrasomic inheritance in a ratio of 1 (17.3:1): 4 (2.7:1). $X^2=0.000$, $P=1.000$.

flower color was controlled by two sets of duplicate genes (C_1c_1, C_2c_2, and P_1p_1, P_2p_2, P_3p_3). A detailed study of the data from the combined studies of Wood and Weihing indicated that eight parental clones appeared to represent only two different genotypes and that they fit a tetrasomic pattern of inheritance better than the previously proposed model of five disomic genes. For this reason, the data from several families with similar genotypes were pooled. The consolidated data (table 5) gave a good fit to the proposed model for tetrasomic inheritance. It should be pointed out that, according to the probability values for the F_2 data presented by Wood (*42*), neither family gave close fits to the expected segregations for tetrasomic inheritance. However, the actual ratios of purple to white were 12.1:1 and 2.3:1, which are near the midpoint between random chromosome and random chromatid segregations. Even though fits to both extremes are poor, both families fall within the expected limits of tetrasomic segregations for two independent loci.

Risius (*30*) and Twamley (*36*) presented F_2 and backcross data from crosses between cream- and white-flowered plants. All of their data presented in table 5 could be explained on the basis of two tetrasomic genes, except for one backcross family from the study by Risius. However, those backcross data represented a total of 10 families, 7 of which gave satisfactory fits to the expected tetrasomic ratios. Even though it is only academic and would not alter the expected segregations or interpretations of the data, it should be pointed out that the parental genotypes as presented by Twamley should be reversed if they are to be in agreement with the findings of other workers and the pattern of inheritance previously described in this review for the *C* and *P* genes. Plants with white seed should be of the *ccccP*– – – type instead of *C*– – –*pppp*.

For several unavoidable reasons, very small populations were used in a study by Storgaard (*35*). Nevertheless, the data indicate the same pattern of inheritance as the other studies. The expected segregation patterns for two sets of disomically inherited genes (as suggested by Weihing, Wood, and later Storgaard) and the expected segregation patterns for two independently inherited tetrasomic genes are similar in many respects. However, the two types of F_2 segregations obtained from the study by Woods and the backcross data reported by Twamley only support the hypothesis of two tetrasomic genes (*C* and *P*) proposed by Twamley as well as by Risius and previously described in this review.

When discussing the gene action between the two types of white-flowered plants most research workers have referred to the purple color as being conditioned by two complementary genes. In all cases this assumption has been based on data for two color classes—purple vs. white. However, the two types of white-flowered plants can be phenotypically differentiated as white and cream. Differentiation between the two white-flower types would change the expected disomic ratios from 9 purple:7 white to 9 purple:3 cream:4 white. The expected ratios for tetrasomic random chromosome inheritance similarly would be changed from 1,225 purple:71 white to 1,225 purple:35 cream:36 white.

Yellow vs. Purple Flower Color

Many earlier alfalfa studies dealt with crosses between purple-

flowered *M. sativa* and yellow-flowered *M. falcata*. Observations of flower color segregations were often reported. Hagem (*20*), Korohoda (*21*), Moe (*28*), and Waldron (*40*) described F_1 plants from crosses between *M. sativa* and *M. falcata* as lacking dominance of either the purple or yellow genes; this resulted in a variegated phenotype. According to Lesins (*23*), the variegated appearance was due to purple pigmentation in the epidermal layer of the flower, which was over a background of yellow. Flower colors in the F_2 generations varied widely with few clear-cut groups. The parental phenotypes seldom appeared. Several research workers tried to catalog the various colors, but were largely unsuccessful because of variation in vein pigmentation, keel color, bud color, and fading characteristics of the variegated flowers.

Burton (*9*, *10*), Korohoda (*21*), Lepper and Odland (*22*), Storgaard (*35*), and Waldron (*40*) all suggested that three factors with disomic inheritance controlled purple vs. yellow flower color. Data from these studies are presented in table 6. None of the original investigators attempted to interpret the data on a tetrasomic basis. A reinterpretation of the results on a tetrasomic basis has been included in the table. Segregations were reported on the basis of flowers with purple pigments vs. flowers without purple pigments (yellow and white flower colors). It was felt that no other general presentation of the data would be valid because different research workers used different systems for classifying flower colors and it has been noted in diploid alfalfa that some purple phenotypes often mask the presence of small amounts of yellow pigments. This means that, without chemical analyses for floral pigments, crosses of purple and yellow flowers will provide accurate information only on the inheritance of the factors controlling purple flower color.

All the data concerning purple flower color inheritance presented in table 6 agree with the conclusive evidence presented in table 4. Purple flower color appeared to be controlled by a single gene, *P*, with tetrasomic inheritance.

Yellow vs. Cream Flower Color

Little work has been published on the inheritance of yellow flower color in tetraploid alfalfa. To be accurately analyzed, yellow flower color inheritance should be studied either phenotypically in the absence of purple pigments or chemically. Up to the present time, all the chemical studies of yellow flower color have dealt only with diploid alfalfa. Those studies were discussed on pages 3–5. In tetraploid alfalfa only three limited phenotypic inheritance studies of crosses between yellow-flowered and cream-flowered plants have been reported. The data from two of these studies (Storgaard (*35*) and Weihing (*41*)) are presented in table 7. Both Storgaard and Weihing interpreted their data on the basis of disomic inheritance of two or three additive genes. The data from these studies were reinterpreted in this review on the basis of one dominant gene with tetrasomic inheritance controlling yellow flower color. With either interpretation, the data are inconclusive because they fit both disomic and tetrasomic patterns of inheritance and because the critical F_3 and F_4 generations were not grown.

There are several reasons for favoring the tetrasomic model of inheritance. Neither Storgaard nor Weihing used parent plants that were of the dark yellow phenotype usually associated with *M. falcata*.

TABLE 6.—*Segregations for purple and nonpurple flower color in tetraploid alfalfa from crosses among purple-flowered and yellow-flowered plants, and chi-square tests for goodness of fit (P) to genetic ratios* [1]

Author and generation	Number of families	Observed ratio		Expected tetrasomic ratio [3]			
		Purple	Non-purple [2]	Random chromotid	P	Random chromosome	P
Burton (*9*):							
F_2 (*M. falcata* × *M. sativa*)	1	100	3	20.8:1	0.435	35:1	0.937
Burton (*10*):							
F_2 (*M. falcata* × *M. sativa*)	1	461	10	20.8:1	.011	35:1	.406
Korohoda (*21*):							
F_2 (*M. sativa* × *M. falcata*)	1	513	22	20.8:1	.616	35:1	.064
Lepper and Odland (*22*):							
F_2 (purple × yellow)	1	38	11	2.5:1	.356	3:1	.685
Storgaard (*35*):							
F_1 (yellow × purple) [4]	3	42	12	3.7:1	.873	5:1	.278
Waldron (*40*):							
F_2 (*M. sativa* × *M. falcata*)	1	225	3	20.8:1	.019	35:1	.185

[1] Expected ratios calculated by reviewer on basis of tetrasomic inheritance patterns indicated for the *P* gene as presented in table 4.

[2] Nonpurple includes both cream- and yellow-flowered plants.

[3] Expected ratios calculated on the basis of segregation limits presented by Allard (*1*).

[4] Genotype of purple parent (C–194) is *PPpp*, according to data in table 4.

TABLE 7.—*Segregations for yellow and cream flower color in tetraploid alfalfa from crosses among yellow-flowered and cream-flowered plants, and chi-square tests for goodness of fit (P) to both disomic and tetrasomic ratios*

Author and generation	Number of families	Observed ratio		Expected disomic ratio	P	Expected tetrasomic ratio [1]			
		Yellow	Cream			Random chromatid	P	Random chromosome	P
Storgaard (*35*): [2]									
P_1 (light yellow ⊗)	1	29	13	3:1	0.391	2.5:1	0.738	3:1	0.391
P_2 (light yellow ⊗)	1	7	0	15:1 or 63:1	.496 or .743	20.8:1	.579	35:1	.662
P_3 (dark yellow ⊗)	1	--------	--------	1:0	----------	783:1	--------	1:0	--------
P_4 (cream ⊗)	2	0	28	0:1	----------	0:1	--------	0:1	--------
F_1 ($P_1 \times P_2$)	1	15	0	7:1 or 15:1	.153 or .324	7.7:1	.172	11:1	.197
F_1 ($P_1 \times P_3$)	1	5	0	1:0	----------	51.3:1	--------	1:0	--------
F_1 ($P_1 \times P_4$)	2	15	14	1:1	.863	.87:1	.591	1:1	.863
F_1 ($P_2 \times P_3$)	1	5	0	1:0	----------	130:1	--------	1:0	--------
F_1 ($P_2 \times P_4$)	2	25	5	3:1 or 7:1	.293 or .493	3.7:1	.550	5:1	1.000
F_1 ($P_3 \times P_4$)	2	14	0	1:0	--------	27:1	--------	1:0	--------
Weihing (*41*): [3]									
P_5 (yellow ⊗)	--------	--------	--------	15:1	----------	783:1	--------	1:0	--------
P_6 (cream ⊗)	--------	--------	--------	0:1	----------	0:1	--------	0:1	--------
F_1 ($P_5 \times P_6$)	--------	All	0	1:0	----------	27:1	--------	1:0	--------
F_2	2	74	24	3:1	.910	2.5:1	.389	3:1	.910
BC ($F_1 \times P_6$)	1	3	2	1:1	.666	.87:1	.559	1:1	.666
F_2	4	163	12	15:1	.745	20.8:1	.161	35:1	.000
F_2	1	58	1	63:1	.944	20.8:1	.290	35:1	.629
BC ($F_1 \times P_6$)	1	34	5	7:1	.950	3.7:1	.198	5:1	.526

[1] Expected ratios calculated on the basis of segregation limits presented by Allard (*1*).

[2] Clone numbers assigned by Storgaard as 140(6), 113(5), and 208(2) referred to in table as P_1, P_2, and P_3. P_4 represents a combination of data from two similar clones which were listed by Storgaard as 8(8) and 77(3).

[3] Yellow and cream parents listed in table as P_5 and P_6 represent plants listed as 5 and 3 by Weihing.

Therefore the parent plants were probably segregates of crosses with *M. sativa* and were not the genotype of unadulterated *M. falcata*. Data discussed on page 4 showed that at least three genes control yellow flower color in diploid alfalfa. The observed segregations presented by Storgaard and Weihing would fit a pattern of three genes with disomic inheritance, but in no instance did they describe the recovery of plants with the dark yellow phenotype of *M. falcata*.

An example of the difficulty in obtaining parental types from F_2 segregates of a tetraploid cross between cream-flowered plants and a dark-yellow-flowered plant was described by Twamley (*36*). Both parent plants were self-pollinated and yielded uniform-appearing progenies, as did the F_1 population. The hybrid plants were intermediate in depth of color between the white and the yellow parents. The F_2 population consisted of 257 plants and was divided into 13 classes. No segregates were found that approximated either of the parents, although parental types were recovered in the F_3. The modal F_2 class corresponded to the F_1 in depth of yellow pigmentation. This segregation pattern required at least five or more genes with disomic inheritance or at least two genes with tetrasomic inheritance. Tetrasomic inheritance appears more likely because of the pattern of inheritance of yellow flower color in diploid alfalfa and because of the predominantly autotetraploid behavior described for the *P* and *C* flower color genes and for the many morphologic traits reviewed by Barnes and Hanson.[10]

[10] An illustrated summary of genetic traits in tetraploid and diploid alfalfa is now being prepared in the Agricultural Research Service.

Available diploid and tetraploid data suggest that yellow flower color is probably due to the accumulative effects of two or more duplicate genes (the greater the number of dominant alleles, the darker the shade of yellow flower color). The gene symbol *Y* was first suggested by Lepper and Odland (*22*) for designating the gene controlling yellow flower color. Since that time, the *Y* designation has been accepted by most research workers. Twamley's data suggest the possibility that two tetrasomically inherited genes control yellow flower color. These two genes could be designated as Y_1 and Y_2 until the yellow pigments have been identified, at which time the symbols could be appropriately changed. It is very likely that the Y_1 and Y_2 genes in tetraploid alfalfa could be the same as the Yx_1 and Yx_2 genes described by Cooper (*14*) in diploid alfalfa.

Yellow vs. White Flower Color

Only three reports of crosses between yellow-flowered and white-flowered plants have been uncovered. Lepper and Odland (*22*) and Storgaard (*35*) described the F_1 hybrids from such a cross as having purple flowers. In both studies, the genotype of the white-flowered parent was probably *ccccPPP–*. The action of the *P* allele was suppressed by the epistatic action of the *cccc* genotype. When the white-flowered plant was crossed with a yellow-flowered plant, supposedly of the *CCC–ppppYY—* genotype, the action of the *P* allele was no longer suppressed; so that the F_1 plants had purple or variegated flowers. No attempt was made to analyze the data from these studies because there was no way to determine the genotype of the parents and because the F_2 populations were small.

No tetraploid evidence has been reported as to whether the *cccc* genotype is epistatic to the *Y* gene, but diploid data (pages 5–6) indicated that the *ccPPYyYy* is cream colored. Assuming that the *C*, *P*, and *Y* genes in tetraploid alfalfa are identical to the *C*, *P*, and *Yx* genes previously described in diploid alfalfa, it can be postulated that: the *C* gene is a basic color factor for yellow pigments as well as for anthocyanins. Therefore, assuming tetrasomic inheritance with random chromosome segregation, the F_2 generation of the cross *cccc PPPPyyyy* × *CCCCpppp YYYY* should theoretically segregate for flower color in a ratio of 1,225 purple: 42,875 variegated: 1,225 yellow: 1,295 cream: 36 white.

SUMMARY

1. Published data from diploid and tetraploid alfalfa flower color studies and unpublished data from studies conducted by the author and others were summarized and reevaluated. The flower color data from tetraploid alfalfa were reinterpreted according to a tetrasomic pattern of inheritance. It was intended that this paper should consolidate all data on flower color inheritance into a single source, compare the obvious similarity of data among studies, set forth a uniform system of gene designations, and compare the interpretations from tetraploid studies with recent studies using diploid *M. sativa* and *M. falcata*.

2. Chemical analyses of diploid alfalfa indicated that purple flower color is due to three anthocyanin pigments inherited as a unit and controlled by one dominant gene (*P*).

3. Yellow flower color pigments in diploid alfalfa were identified as being primarily xanthophyll, with a small amount of *B* carotene present. Genetic data suggested that yellow flower color was controlled by at least three and probably four genes with additive effects. Two of the genes controlling xanthophyll were designated Yx_1 and Yx_2. The other two genes were not identified with any specific pigment and were designed Y_3 and Y_4. The homozygous recessive condition of the four *Y* genes and the *P* gene produces a cream flower color.

4. Nine anthoxanthin pigments have been chemically identified as flower-color modifying pigments in diploid alfalfa. Six of the anthoxanthins were quercetin glycosides, and three were kaempferol glycosides. None of the pigments appeared to impart a phenotypically significant color of its own, but they tended to act as modifying genes when copigmented with an anthocyanin or xanthophyll color gene. The inheritance of the production of two kaempferol glycosides was shown to be controlled by two dominant independent genes, which were designated K_2 and K_3.

5. Two additional modifying flower-color characteristics in diploid alfalfa were purple bud color and floral vein color. Purple bud color required the presence of two dominant genes (*Bs* and *Bf*) with complementary action. Floral vein color in yellow-flowered diploid alfalfa was controlled by the presence of one or more dominant alleles of two duplicate genes (Vs_1 and Vs_2).

6. No association or linkage was shown between the *P*, Yx_1, Yx_2, K_2, and K_3 genes in diploid alfalfa.

7. White- and cream-flowered plants were described in both diploid and tetraploid alfalfa. White-

flowered plants were completely devoid of all anthocyanin pigmentation in flowers, seeds, stems, leaves, and roots. The white-flowered phenotype was produced by the homozygous recessive condition of the *c* gene. Cream flower color, was due to the homozygous recessive condition of the *P* gene, which controls purple-flower color and was inherited tetrasomically. The genotype of cream-flowered plants did not alter color production of any other organs of the plant except the flower. F_1 hybrids of crosses between cream- and white-flowered plants usually had purple flowers.

8. Data available on the inheritance of yellow flower color in tetraploid alfalfa were interpreted on a pattern of either disomic or tetrasomic inheritance. However, a pattern of tetrasomic inheritance controlled by several genes with accumulative effects was favored.

9. Results of genetic studies have demonstrated a close relationship between the pattern of inheritance for purple and yellow flower color in diploid and tetraploid alfalfa.

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