ABSTRACT OF THESIS

****

INHERITANCE OF FLOWER COLOR IN ALFALFA

Submitted by

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In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado

Agricultural and Mechanical College

Fort Collins, Col

May 1949

INTRODUCTION

The genetics of flower color inheritance in alfalfa, Medicago Spp., is not well understood. Studies of the inheritance of flower color are usually secondary to more important economic characters. Because of this fact, parents may not be carefully selected and the population may be too small, due to space limitations, to draw definite conclusions about the inheritance of this character. Self-sterility is also a handicap in studying the genetics of alfalfa.

The problem

What is the mode of inheritance of purple flower color in white x white crosses of alfalfa?

Problem analysis.--To properly answer the major question it is necessary to answer the following questions:

1. Is the inheritance of purple flower color disomic or tetrasomic?

2. How many genes control the inheritance of flower color in each case?

3. How do these genes that control purple flower color interact?

Delimitations.--This study has been limited to the F_2 progeny of 12 crosses between 9 white flowered alfalfa plants.

METHODS AND MATERIALS

This study of the inheritance of flower color in alfalfa was undertaken with 16 F_2 families from 12 white x white crosses between 9 white flowered alfalfa plants. These families were obtained from Doctor Ralph M. Weihing, formerly Associate Agronomist, Colorado Agricultural Experiment Station, in the summer of 1947. Fourteen of these families were the result of self-pollination of purple F_1 plants. Two families were the progeny of self-pollinated white F_1 plants which resulted from the cross white 4 x white 6. The F_1 generation of this cross was all white.

Flower color readings were made in the summers of 1947 and 1948. An attempt was made to classify various shades of purple by using color plates prepared by Robert Ridgway (Color standards and color nomenclature. Washington, D. C. 1912.) It was later decided to group all shades of purple into a purple class and contrast purple and white.

The chi-square test was used to test goodness of fit, heterogeneity and independence.

ANALYSIS OF DATA

Two hypotheses were developed in an attempt to explain the segregations obtained in the F_2 generation.

Disomic segregation

In the first hypothesis it was assumed that complementary factors for the production of purple color were segregated in disomic ratios. One factor pair (Cc) must be present in the dominant condition before color can develop. Later it was found necessary to postulate a duplicate factor pair (C_2c_2) which would also produce color when present in the dominant condition. Another factor pair, Pp, when present in the dominant condition, would act with the basic color factors Cc or C_2c_2 to produce purple color. Three genes of this type, duplicate in mode of expression, were postulated, Pp, P_2p_2, and P_3p_3.

Four ratios were calculated from theoretical F_1 plants of varying genotypes. The ratios of purple to white and the F_1 genotypes were as follows: 2.368:1, $Ccc_2c_2PpP_2p_2p_3p_3$; 2.821:1, $Ccc_2c_2PpP_2p_2P_3p_3$; 7.258:1, $CcC_2c_2PpP_2p_2p_3p_3$; and 11.962:1, $CcC_2c_2PpP_2p_2P_3p_3$. One family was found to fit the 2.368:1 ratio, 10 families the 2.821:1 ratio, 2 families the 7.258:1 ratio, and 1 family, the 11.962:1 ratio. The fit of the observed data to the calculated ratios were determined by use of chi-square tests.

Tetrasomic segregation

Autopolyploid plants segregate in different kinds of ratios than do diploid plants. The reason for this fact is that diploid plants have two homologous chromosomes, whereas autopolyploid plants have 3, 4, 5, etc., homologues. Alfalfa is thought to be an autotetraploid, at least in part. That is, some chromosomes have 4 homologous chromosomes. Julen, a Swedish worker, has observed quadrivalent pairing in alfalfa although the majority of the pairing seemed to be bivalents. It was decided to attempt to fit the observed segregations to calculated autotetraploid ratios. It was assumed that complementary factors that segregate in autotetrasomic ratios controlled purple flower color in alfalfa.

There are two main types of autotetraploid segregations. Random chromosome segregation occurs when the genes are located near the spindle fiber attachment. In this case, they will assort at random, depending upon the way the chromosomes are oriented at metaphase I. However, if the genes are located far enough from the spindle fiber attachment so that crossing over can occur in the quadrivalent, with the resultant exchange of chromatids, random chromatid segregation will occur.

Chromatid and chromosome ratios were calculated for the complementary genes C, P and P_2, but the theoretical ratios deviated distinctly from the observed data. However, when it was assumed that C, the basic factor for the production of color, was assorted by random chromatid segregation, and that the factors P and P_2, which act with C to produce purple color were assorted by random chromosome segregation, several ratios were calculated which more nearly approached the observed segregations.

Four ratios were calculated from theoretical F_1 plants of varying genotypes. The ratio of purple to white and the F_1 genotypes were as follows: 2.009:1, $CcccPpppP_2p_2p_2p_2$; 2.516:1, $CCccPpppp_2p_2p_2p_2$; 8.474:1, $CCccPpppP_2p_2p_2p_2$; and 12.650, CCcc-$PPppP_2p_2p_2p_2$. Three families were found to fit the 2.009:1 ratio, 8 families failed to fit the 2.516:1 ratio, 1 family showed a good fit to the 8.474:1 ratio, and two families were found to fit the 12.650:1 ratio. The fit of the observed data to the calculated ratios were determined by the use of chi-square tests.

Nine purple plants were observed in a total population of 339 plants, from 2 F_1 white 4 x white 6 F_1 plants. These nine plants were probably the result of stray pollen effecting fertilization before the selfed flowers were bagged.

The data presented show that the observed ratios could be best explained by assuming that complementary factors were segregated in a disomic manner. When tetrasomic segregation was assumed, 8 families did not fit any calculated ratio.

Data on the theoretical behavior and the observed behavior in the F_1 and F_2 generations are presented in Figures 1 and 2. It was possible to postulate a genotype for every parent assuming that the disomic segregation hypothesis was the logical one.

Parent plants & genotypes	2	3	4	5	6	8	9	10	12
2 ccC_2C_2PP- $P_2P_2P_3P_3$	W	W	-2.821:1-	W	2.821:1 11.962:1	W	W	W	W
3 ccC_2C_2PP- $P_2P_2P_3P_3$		W	-2.821:1-	W	-2.821:1- 11.962:1	W	W	W	W
4 CCc_2c_2pp- $p_2p_2p_3p_3$			W	-2.821:1-	W	-2.821:1-	2.368:1 -2.821:1-	-2.368:1- -2.821:1-	2.368:1 -2.821:1-
5 ccC_2C_2PP- $P_2P_2P_3P_3$				W	-2.821:1- 11.962:1	W	W	W	W
6 CCc_2c_2pp- $p_2p_2p_3p_3$					W	2.821:1 -11.962:1-	2.368:1 2.821:1 -7.258:1- 11.962:1	2.368:1 2.821:1 7.258:1 11.962:1	2.368:1 2.821:1 -7.258:1- 11.962:1
8 ccC_2C_2PP- $P_2P_2P_3P_3$						W	W	W	W
9 ccC_2C_2PP- $P_2P_2P_3p_3$							W	W	W
10 ccC_2C_2PP- $P_2P_2P_3p_3$								W	W
12 ccC_2C_2PP- $P_2P_2P_3p_3$									W

PARENT PLANTS

Figure 2.--The expected F_2 segregations from selfed purple flowered F_1 plants of various white x white flowered alfalfa crosses and the hypothetical genotypes of the parents. Dashes indicate that data fitting the calculated ratio of purples to white were obtained. The letter W indicates that only white F_1 plants would be obtained and no F_2 segregation would be expected.

CONTRASTING GENOTYPES & PARENT PLANTS	2 cP	3 cP	4 Cp	5 cP	6 Cp	8 cP	9 cP	10 cP	12 cP
2 cP	W̲	W̲	-P-	-W-	P̲	-W-	W	W̲	-W-
3 cP		-W-	-P-	-W-	-P-	-W-	-W-	W̲	-W-
4 Cp			-W-	-P-	-W-	-P-	-P-	-P-	-P-
5 cP				W̲	-P-	W̲	W̲	-W-	-W-
6 Cp					W̲	-P-	-P-	P̲	-P-
8 cP						W̲	-W-	-W-	-W-
9 cP							-W-	W̲	-W-
10 cP								W̲	-W-
12 cP									W̲

CONTRASTING GENOTYPES & PARENT PLANTS

Figure 1.--The expected behavior of the F_1 generations of various white x white flowered alfalfa crosses under the complementary factor hypothesis and the contrasting genotypes of the parent plants. The letter W indicates that only white flowered plants would be obtained. Dashes indicate that obtained data were in agreement with the theoretical expectation. Underlined letters indicate that no data were obtained. The letter P indicates that purple flowered plants would be obtained.

Suggestions for further study

Very little information is available on the genetics of alfalfa. More study of this important crop plant is needed, not only from the qualitative standpoint, but from the quantitative standpoint as well.

Cytological investigations to determine the type of pairing that occurs and thus, the types of segregation to be expected, would be a great aid in making genetic studies of the alfalfa plant.

T H E S I S

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY DONALD ROY WOOD

ENTITLED INHERITANCE OF FLOWER COLOR IN ALFALFA

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ACKNOWLEDGMENT

The writer is indebted to his many teachers for their patience and leadership.

Special acknowledgment is extended to Doctors D. W. Robertson, Jess L. Fults, and H. H. Stonaker for their assistance in the preparation of this thesis.

The writer also wishes to thank the Colorado Agricultural Experiment Station for providing funds and materials which made this study possible.

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INTRODUCTION

The genetics of flower color in alfalfa, *Medicago* Spp., is not well understood. There may be several reasons for this fact. Studies of the inheritance of flower color are usually secondary to more important economic characters. Because of this fact, crosses made in the crop breeding program may not be carefully selected, and populations are often too small to draw definite conclusions about the inheritance of this character. Self-sterility is also a handicap in studying the genetics of alfalfa because of the difficulty of obtaining adequate populations.

The problem

What is the mode of inheritance of purple flower color in alfalfa in white x white crosses?

Problem analysis.--To answer the major question, it is necessary to answer the following questions:

1. Are the factors that control the inheritance of purple flower color segregated in a disomic or tetrasomic manner?

2. How many genes in each case control the inheritance of purple flower color?

3. How do these genes which control purple flower color interact?

<u>Delimitations</u>.--This study has been limited to the F_2 progeny of 12 crosses between 9 white flowered alfalfa plants.

Chapter II

REVIEW OF LITERATURE

Meagre information is available in the literature on the nature of flower color inheritance in alfalfa. There is no record of any white flower x white flower crosses which yielded purple flowered progeny, except those progeny discussed by Weihing (10,11), 1946 and 1948, which are the subject of this study.

The studies most frequently reported are those which involve yellow flowered x purple flowered parents. Waldron (9), in a study reported in 1929, found dominance entirely lacking in a cross between Medicago sativa L. (purple) and Medicago falcata L. (yellow). He found it impossible to classify the wide range of variegated colors in the F_2 generation, but by setting the proportion of yellow segregates against all other classes, he obtained a ratio of 1:63, which indicated 3 factors. Korohoda (4), 1933, reported that the supposition that 2 or 3 factors are involved in determining the color of flowers in alfalfa was inadequate. The results of his studies indicated at least 4 factors, one for each fundamental coloration--cream, blue, and violet, and one or two that intensify these colors. Burton (2), 1937, reported difficulty in classifying the F_2 population in a purple x yellow cross, but suggested that 3 independent factors control flower color inheritance in alfalfa.

More detailed studies involving purple x white, white x yellow, and variegated x white, were reported by Lepper and Odland (5) in 1939. They suggested that 2 dominant supplementary factors influenced the production of color. The absence of both of these factors resulted in white flowers, whether or not the individual plant carried factors for color. Purple was regarded as epistatic to yellow. The following genetic makeup was hypothesized, letting P represent the factor for purple, Y the factor for yellow, and C and A the supplementary factors for the production of color:

Purple PPCCAAyy

Yellow ppCCaaYY

White PPccaayy

Armstrong and Gibson (1), 1941, reported the inheritance of flower color in a cross *Medicago media* and *M. glutinosa*. The flowers of the *M. glutinosa* selection were cream colored, while the *M. media* selection possessed medium dark purple flowers. Thirteen F_2 families segregated as follows: 5 families, 3:1; 2 families, 15:1; and 3 families, 63:1. In 3 families, no cream colored segregates occurred. From this information, they assumed that at least 3 factors controlled purple, and that the factor for cream color was an allelomorph of one of the purple factors.

Weihing (11) presented data in 1948 obtained from segregations of purple x white, yellow x white, and white x white crosses. Segregations of purple and white in the purple x white cross indicated that 3 independent factors were involved. Three independent factors were found to govern the segregation of yellow and white progenies in the yellow x white crosses. In studying the F_1 progeny of the white x white crosses, which, when carried into the F_2, are the subject of this thesis, he found that some families were all purple, some segregated for purple and white, and other families were all white. From these results, he suggested that purple was governed by at least 3 factors which he designated P_1p_1, P_2p_2, and P_3p_3. Three factors for yellow were assumed and designated Y_1y_1, Y_2y_2, and Y_3y_3. In addition, a complementary factor, Cc, for the formation of color was suggested. This factor must be present in the dominant condition, and at least one of the factors for purple or yellow must be dominant before there could be an expression of color.

In discussing the genetics and cytology of alfalfa, Tysdal, Kiesselbach, and Westover (8), 1942, stated:

> Since many Medicago species have 16 chromosomes, it appears not improbable that those with 32 are tetraploid. Moreover, a review of the inheritance studies indicates that, in many cases at least, the genetic ratios obtained fit the expected segregation in an autotetraploid better than the complicated disomic ratios reported, and tetrasomic ratios often explain those cases for which no satisfactory disomic ratios were found. (8:8)

Julen (3), 1944, made a cytological investigation of alfalfa. He found that meiotic fixations were such that he could not make a detailed analysis of the course of meiosis. Marked disturbances were observed. Instead of only bivalents, there often occurred a number of univalents and, in a few cases, multivalents occurred. Trivalents were more often observed than quadrivalents. He concluded, on the basis of his cytological studies, that *M. sativa* is a tetraploid.

Chapter III

METHODS AND MATERIALS

The study of the inheritance of flower color in alfalfa was undertaken with 16 F_2 families from 12 white x white crosses between 9 white flowered alfalfa plants. These families were obtained from Doctor Ralph M. Weihing, formerly Associate Agronomist, Colorado Agricultural Experiment Station, in the summer of 1947. The source of the parent white plants, and the method of making the crosses was reported by Weihing (11), 1948. The origin and the designations of the parent plants are shown in Table 1.

Table 1.--DESIGNATIONS AND ORIGINS OF PARENTAL WHITE ALFALFA PLANTS.

Plant No.	Origin
2	Open-pollinated selection in 1937.
3	Open-pollinated selection in 1937.
4	Open-pollinated selection in 1937.
5	Open-pollinated selection in 1937.
6	Open-pollinated selection in 1937.
8	An S_2 plant from the 1940 breeding nursery from the variety Ladak.
9	Stray plant close to the 1937 breeding nursery.
10	An S_3 plant from the 1938 breeding nursery from the variety Vale.
12	Stray plant selected in the fall of 1940.

Emasculation of several to 25 unfertilized flowers per parent plant was accomplished by means of suction, carried from the windshield wiper of a car through a rubber hose. The hose was tipped with a drawn glass tube just large enough to surround an exposed stigma. The standard was cut away before emasculation to prevent the contamination of foreign pollen which it might have collected. Pollination was accomplished by rubbing the stigma of the emasculated flower in pollen collected from the male parent. The pollinated flowers were immediately enclosed in glassine bags and the raceme tagged with the name of the parents, date of cross, and number of flowers crossed.

Selfed seed was obtained by selecting racemes with a large number of untripped flowers, cutting away all tripped flowers and buds, tripping the remaining flowers artificially, and covering the raceme with a glassine bag.

The seed from cross- and self-pollinations was planted in the spring in the greenhouse. The seedling plants were transplanted to the field the same spring in rows 3 feet apart with about 4 inches between transplants.

Color readings were made in the year of transplanting. All gradations of purple were classified as purple in the F_1 because of the difficulty of accurately identifying different shades.

In the summer of 1947, an attempt was made to classify the various shades of purple in the F_2 according to standards published by Ridgway (6) in 1912. It was noticed that flowers tended to fade as they aged, and since there were a large number of different intensities of color, all shades of purple were grouped in the analysis of data.

The F_2 families used were those obtained by self-pollinating purple F_1's. Two F_2 families, however, that resulted from the self-pollination of white F_1's were grown to determine if segregation would occur.

Statistical analysis

The chi-square tests for goodness of fit and interaction, or heterogeneity, were applied to the obtained data. The methods and terminology of Snedecor (7), published in 1946, were used throughout the study. The general formula for X^2 (chi-square) is as follows:

$$X^2 = \frac{(O-C)^2}{C},$$

where O equals the observed frequency for a particular class, and C the calculated number for the same class on the basis of the selected hypothesis.

Location of original data and seed stocks.

The original data from which this thesis was written are on file in the vault of the Agronomy Section, Colorado Agricultural Experiment Station, Fort Collins, Colorado. The remaining seed stocks and plant materials are being maintained on the Agronomy Farm.

Chapter IV

ANALYSIS OF DATA

The inheritance of flower color in alfalfa was studied by crossing 9 white flowered alfalfa plants in 12 combinations. The F_1 generations of these crosses varied. Some crosses had all white F_1's, some all purple F_1's, and others segregated for purple and white in the F_1. These results were reported by Weihing (11), 1948, who suggested that complimentary factors were involved in the inheritance of flower color in alfalfa. To secure more complete data, purple F_1's from all crosses with purple progeny were selfed and the resulting progeny observed for flower color in the F_2 generation. Two hypotheses were developed in an attempt to explain the segregations obtained in the F_2 generation.

Disomic segregation

In the first hypothesis it was assumed there were complementary factors for production of purple color and that these factors segregated in disomic ratios. One factor, Cc, must be present in the dominant condition before color can develop. Later it was found necessary to postulate a duplicate gene, C_2c_2, which would also produce color when present in the dominant condition. Another factor, Pp, when present in the dominant condition, would act with the basic color factors Cc or C_2c_2, to produce purple flower color. Three genes of this type, duplicate in mode of expression, were postulated, Pp, P_2p_2, and P_3p_3.

If the parents were of the genetic constitution $CCc_2c_2ppp_2p_2p_3p_3$ (white) and $ccc_2c_2PPP_2P_2p_3p_3$ (white), the F_1 would be purple and its genotype would be $Ccc_2c_2PpP_2p_2p_3p_3$. However, as reported by Weihing (11), 1948, few families were all purple in the F_1 generation. Although it is beyond the scope of this thesis to explain in detail the F_1 segregations reported by Weihing (11), there should be some basis for the explanation of these obtained segregations. If, in the above cross, the first parent had been heterozygous for C, a segregation for purple and white would have occurred in the F_1 generation in the ratio of 1:1. Assuming that the factors P and P_2 were heterozygous in the second parent in the above cross, the ratio of purples to white in the F_1 generation would have been 3:1. If the first parent had been heterozygous for C and the second parent heterozygous for P and P_2, the ratio in the above cross would have been 3 purples to 5 whites in the F_1

generation. In all cases it would have been possible to obtain a purple F_1 plant of the genotype Ccc_2c_2-$Pp P_2 p_2 P_3 p_3$. Upon selfing this plant, a segregation of purples to whites in the F_2 generation would give a ratio of 2.368:1. Table 2 gives the result of fitting this ratio to 9 segregating F_2 families. Individual chi-square values indicated a good fit of the observed data to the calculated ratio. In all cases, the probability of obtaining as large or a larger deviation from the calculated ratio due to chance alone, as shown by the chi-square test, was less than 19:1. However, the pooled chi-square value was found to have a P value less than 0.05. The heterogeneity chi-square value indicated that the data from these families were homogeneous. An examination of the data revealed a rather uniform tendency for the calculated numbers of purples to be too low and the whites to be too high. Families 117-4 and 118-2 were found to be exceptions to this tendency. The segregation obtained in family 117-4 was found to be statistically different from the other segregations by the use of the chi-square test for independence. It was assumed, therefore, that this family did fit the ratio, 2.368:1. The remaining 8 families were found to fit the calculated ratio 2.821:1, as shown in Table 3. Since the pooled chi-square value in Table 2 indicated that, for the most part, the tested

Table 2.--THE FIT OF THE OBSERVED DATA TO A CALCULATED 2.368:1 RATIO OF PURPLES TO WHITE, ASSUMING DISOMIC SEGREGATION OF GENES C, P, AND P_2, IN 9 F_2 FAMILIES FROM SELF-FERTILIZED PURPLE F_1 PLANTS.

Cross	Family	Number of Observed Purple	Number of Observed White	Number of Calculated Purple	Number of Calculated White	df	Chi Square	P Value
3 x 6	108-1	279	106	270.69	114.31	1	0.810	.50-.30
4 x 2	110-4	247	87	234.83	99.17	1	2.124	.20-.10
4 x 3	111-4	313	117	302.33	127.67	1	1.269	.30-.20
4 x 5	113-5	207	74	197.57	83.43	1	1.517	.30-.20
4 x 8	115-3	72	27	69.60	29.40	1	0.277	.70-.50
4 x 9	116-7	338	121	322.72	136.28	1	2.438	.20-.10
4 x 10	117-4	173	93	187.02	78.98	1	3.540	.10-.05
4 x 12	118-2	41	17	40.78	17.22	1	0.004	.95
6 x 5	121-1	154	56	147.65	62.35	1	0.920	.50-.30
TOTAL		1824	698	1864.59	787.41	9	12.899	.20-.10
Pooled X^2						1	4.904	.05-.02
Heterogeneity X^2						8	7.995	.50-.30

families did not belong in the 2.368:1 population, it was assumed that the remaining 8 families should be classified under the 2.821:1 ratio.

The ratio, 2.821:1, was calculated by assuming that the selfed F_1 was $Ccc_2c_2PpP_2p_2P_3p_3$. This F_1 would be obtained if the genotypes of the parents were as follows: $CCc_2c_2ppp_2p_2p_3p_3$ and $ccc_2c_2PPP_2P_2P_3P_3$. Segregations in the F_1 would be obtained when these parents were assumed to be heterozygous. Individual chi-square values, shown in Table 3, for ten F_2 families were calculated on the basis of this ratio and were found to show a good fit of the observed data to the calculated segregation. Total chi-square, pooled chi-square, and heterogeneity chi-square also were found to show a good fit of the data to this ratio.

Other disomic ratios were obtained when the F_1 purple plants were assumed to be of the CcC_2c_2Pp-$P_2p_2p_3p_3$ and the $CcC_2c_2PpP_2p_2P_3p_3$ genotypes. In the case of the $CcC_2c_2PpP_2p_2p_3p_3$ genotype, a ratio of 7.258:1, purples to white, would be obtained. The $CcC_2c_2PpP_2p_2P_3p_3$ genotype would segregate in the ratio of 11.962 purples to 1 white. Results of fitting observed data for various segregating families in the F_2 generation to these ratios are given in Table 4. Chi-square tests indicated a good fit for 2 families to the first ratio and for 1 family to the second ratio.

Table 3.--THE FIT OF THE OBSERVED DATA TO A CALCULATED 2.821:1 RATIO OF PURPLES TO WHITE, ASSUMING DISOMIC SEGREGATION OF GENES C, P, P_2 AND P_3, IN 10 F_2 FAMILIES FROM SELF-FERTILIZED PURPLE F_1 PLANTS.

Cross	Family	Number of Observed Purple	Number of Observed White	Number of Calculated Purple	Number of Calculated White	df	Chi Square	P Value
3 x 6	108-1	279	106	284.24	100.76	1	0.369	.70-.50
4 x 2	110-4	247	87	246.59	87.41	1	0.003	.98-.95
4 x 3	111-4	313	117	317.47	112.53	1	0.240	.70-.50
4 x 5	113-5	207	74	207.46	73.54	1	0.004	.95
4 x 8	115-3	72	27	73.09	25.91	1	0.062	.90-.80
4 x 9	116-7	338	121	338.87	120.13	1	0.009	.95-.90
4 x 10	117-1	102	28	95.98	34.02	1	1.444	.30-.20
4 x 12	118-2	41	17	42.82	15.18	1	0.296	.70-.50
6 x 5	121-1	154	56	155.04	54.96	1	0.027	.90-.80
12 x 6	141-3	80	18	72.35	25.65	1	3.089	.10-.05
TOTAL		1833	651	1833.91	650.09	10	5.543	.90-.80
Pooled X^2						1	0.002	.98-.95
Heterogeneity X^2						9	5.541	.80-.70

Table 4.--THE FIT OF THE OBSERVED DATA TO VARIOUS CALCULATED RATIOS OF PURPLES TO WHITE, ASSUMING DISOMIC SEGREGATION, IN SEVERAL F_2 FAMILIES FROM SELF-FERTILIZED PURPLE F_1 PLANTS. THE ASSUMED F_1 GENOTYPE IS SHOWN IN BRACKETS BESIDE THE CALCULATED RATIO.

Cross	Family	Number of Observed Purple	Number of Observed White	Number of Calculated Purple	Number of Calculated White	df	Chi Square	P Value
		(Ratio = 7.258:1) ($CcC_2c_2PpP_2p_2P_3p_3$)						
9 x 6	131-1	165	20	162.60	22.40	1	0.293	.70-.50
12 x 6	141-3	80	18	86.13	11.87	1	3.606	.10-.05
TOTAL		245	38	248.73	34.27	2	3.899	.20-.10
Pooled X^2						1	.462	.50-.30
Heterogeneity X^2						1	3.437	.20-.10
		(Ratio = 11.962:1) ($CcC_2c_2PpP_2p_2P_3p_3$)						
8 x 6	125-1	453	31	446.66	37.34	1	1.166	.30-.20

Tetrasomic segregation

Since common alfalfa has a chromosome number of 32 in the diploid condition, while some related species are known to have a chromosome number of 16, Tysdal, Kiesselbach, and Westover (8), 1942, suggested that autotetraploid ratios might explain the obtained segregations better than complicated disomic ratios. The second hypothesis, therefore, was the assumption that complimentary factors that segregate in autotetrasomic ratios control purple flower color in alfalfa.

Euploid plants have chromosome numbers which are multiples of a basic number. Autopolyploids are polyploids in which the sets of chromosomes or genomes are from the same source. Autotetraploids are autopolyploids which arise by doubling the original chromosome complement. Therefore, instead of having a pair of homologous chromosomes, an autotetraploid has four homologous chromosomes in the 2n condition. Quadrivalents are formed when these chromosomes pair during prophase I in contrast to the bivalents which are formed during the reduction division in ordinary diploid organisms. When not complicated by such factors as non-disjunction, two homologues are assorted to each gamete in an autotetraploid. Because of this fact, autotetraploid segregations differ from diploid segregations. There are two main types of autotetraploid segregations. Random chromosome segregation is thought to occur when the genes are located next to the spindle fiber attachment. In this case, the genes will assort at random depending upon the way the chromosomes are oriented at metaphase I. However, if the genes are located far enough from the spindle fiber so that crossing-over could occur, there would be a random exchange of chromatids in the quadrivalent. This is termed random chromatid segregation.

Chromatid ratios and chromosome ratios were calculated for complementary factors Cc and PpP_2p_2, but the theoretical ratios deviated distinctly from the observed data. However, when it was assumed that Cc, the basic factor for the production of color, was assorted by random chromatid segregation, and that the factors Pp and P_2p_2, which act with Cc to produce purple color, were assorted by random chromosome segregation, several ratios were calculated which more nearly approached the observed segregations.

Assuming C, P, and P_2 in the simplex condition ($CcccPpppP_2p_2p_2p_2$) in the F_1, a segregation in the F_2 of 2.009 purples to 1 white would be obtained. Chi-square values were calculated for 3 families on the basis of this ratio and are shown in Table 5. A good fit of the observed data to the calculated ratio was obtained.

Table 6 gives the results of fitting 10 families to the calculated ratio of 2.516 purples to 1 white. This ratio was obtained when it was assumed that C was in the duplex condition, P in the simplex condition, and P_2 in the nulliplex condition ($CCccPppp p_2p_2p_2p_2$). Individual chi-square values were found to show a good fit of the observed data to the calculated ratio except in the case of family 141-3. The total chi-square value indicated that individual variations may have been due to chance deviations. However, the pooled chi-square value was found to have a P value between 0.02 and 0.01. This indicated that the tested population tended to deviate from the 2.516:1 ratio. Heterogeneity chi-square indicated that there was an uniform tendency in the population to deviate from this ratio. An examination of the data revealed that this tendency was an underestimation of the number of purple segregates and an overestimation of the number of white segregates by the calculated ratio. Unless other undetermined factors, such as linkage or modifying genes, were acting in these crosses, this ratio would not explain the obtained segregations.

Two families, 115-5 and 118-2, were found to fit both of the above ratios. This may have been due to the small size of these families, and the inability of chi-square to differentiate between similar ratios when small numbers are used.

Table 5.--THE FIT OF THE OBSERVED DATA TO VARIOUS CALCULATED RATIOS OF PURPLES TO WHITE, ASSUMING TETRASOMIC SEGREGATION, IN SEVERAL F_2 FAMILIES FROM SELF-FERTILIZED PURPLE F_1 PLANTS. THE ASSUMED F_1 GENOTYPE IS SHOWN BESIDE THE CALCULATED RATIO.

Cross	Family	Number of Observed Purple	Number of Observed White	Number of Calculated Purple	Number of Calculated White	df	Chi Square	P Value
		(Ratio = 2.009:1) ($CcccPpppP_2p_2p_2p_2$)						
4 x 8	115-5	72	27	66.10	32.90	1	1.585	.30-.20
4 x 12	118-2	41	17	38.72	19.28	1	0.402	.70-.50
4 x 10	117-4	173	93	177.60	88.40	1	0.350	.70-.50
TOTAL		286	137	282.42	140.58	3	2.337	.70-.50
Pooled X^2						1	0.136	.80-.70
Heterogeneity X^2						2	2.201	.70-.50
		(Ratio = 8.474:1) ($CCccPpppP_2p_2p_2p_2$)						
9 x 6	131-1	165	20	165.47	19.53	1	0.013	.95-.90
		(Ratio = 12.650:1) ($CCccPPppp_2p_2p_2p_2$)						
8 x 6	125-1	453	31	448.54	35.46	1	0.605	.50-.30
9 x 6	131-1	165	20	171.45	13.55	1	3.309	.10-.05
TOTAL		618	51	619.99	49.01	2	3.914	.30-.20
Pooled X^2						1	0.087	.80-.70
Heterogeneity X^2						1	3.827	.10-.05

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Table 6.--THE FIT OF THE OBSERVED DATA TO A CALCULATED 2.516:1 RATIO OF PURPLES TO WHITE, ASSUMING TETRASOMIC SEGREGATION, IN 10 F_2 FAMILIES FROM SELF-FERTILIZED PURPLE F_1 PLANTS. THE ASSUMED F_1 GENOTYPE IS $CCccPpppp_2p_2p_2p_2$.

Cross	Family	Number of Observed Purple	Number of Observed White	Number of Calculated Purple	Number of Calculated White	df	Chi Square	P Value
3 x 6	108-1	279	106	275.50	109.50	1	0.156	.70-.50
4 x 2	110-4	247	87	239.	95.	1	0.940	.50-.30
4 x 3	111-4	313	117	307.70	122.30	1	0.321	.70-.50
4 x 5	113-5	207	74	201.08	79.92	1	0.613	.50-.30
4 x 8	115-5	72	27	70.84	28.16	1	0.066	.80-.70
4 x 9	116-7	338	121	328.45	130.55	1	0.975	.50-.30
4 x 10	117-1	102	28	93.03	36.97	1	3.044	.10-.05
4 x 12	118-2	41	17	41.50	16.50	1	0.022	.90-.80
6 x 5	121-1	154	56	150.27	59.73	1	0.325	.70-.50
12 x 6	141-3	80	18	70.13	27.87	1	4.887	.05-.02
TOTAL		1833	651	1777.50	706.50	10	11.349	.50-.30
Pooled X^2						1	6.089	.02-.01
Heterogeneity X^2						9	5.260	.90-.80

A segregation of 8.474:1 purples to white was obtained when it was assumed that C was in the duplex condition, and P and P_2 were in the simplex condition ($CCccPpppP_2p_2p_2p_2$). One family gave a good fit to this ratio as shown in Table 5.

Good fits for the observed and calculated ratios were obtained for two families to the autotetrasomic F_2 ratio of 12.650 purples to 1 white, as shown in Table 5. This ratio was calculated upon the assumption that C and P were in the duplex condition and P_2 in the nulliplex condition ($CCccPPppp_2p_2p_2p_2$). Family 131-1 was found to fit the calculated ratios 8.474:1 and 12.650:1. Since the P-values were not statistically significant in either case, the proper ratio was not determined.

When it was assumed that complementary factors for color were assorted by the above autotetrasomic segregations, 8 families could not be explained. The hypothesis that complementary factors, segregating in autotetrasomic ratios, controlled purple flower color in alfalfa was rejected.

To determine the breeding behavior of plants which were all white flowered in the F_1 generation, 2 F_1's from the cross white 4 x white 6 were selfed and the progeny observed in the F_2 generation. It was found that 9 purple plants occurred in an observed population of 339 plants. These plants were thought to have been the result of natural crossing before the plants were bagged.

Data on the theoretical behavior and the observed behavior in the F_1 and F_2 generations are graphically presented in Figures 1 and 2. It was possible to postulate a genotype for every parent from the obtained data. This strengthens the hypothesis that purple flower color is controlled by complementary factors which are assorted in a disomic manner.

The following genotypes of the parent plants were hypothecated: white flower 2, $ccc_2c_2PPP_2P_2P_3P_3$; white flower 3, $ccc2c_2PPP_2P_2P_3P_3$; white flower 4, $CCc_2c_2ppp_2p_2p_3p_3$; white flower 5, $ccc_2c_2PPP_2P_2P_3P_3$; white flower 6, $CCC_2c_2ppp_2p_2p_3p_3$; white flower 8, $ccc_2c_2PPP_2P_2P_3P_3$; white flower 9, $ccc_2c_2PPP_2P_2P_3p_3$; white flower 10, $ccc_2c_2PPP_2P_2P_3p_3$; and white flower 12, $ccc_2c_2PPP_2P_2P_3p_3$.

CONTRASTING GENOTYPES & PARENT PLANTS	2 cP	3 cP	4 Cp	5 cP	6 Cp	8 cP	9 cP	10 cP	12 cP
2 cP	<u>W</u>	<u>W</u>	-P-	-W-	<u>P</u>	-W-	W	<u>W</u>	-W-
3 cP		-W-	-P-	-W-	-P-	-W-	-W-	<u>W</u>	-W-
4 Cp			-W-	-P-	-W-	-P-	-P-	-P-	-P-
5 cP				<u>W</u>	-P-	<u>W</u>	<u>W</u>	-W-	-W-
6 Cp					<u>W</u>	-P-	-P-	<u>P</u>	-P-
8 cP						<u>W</u>	-W-	-W-	-W-
9 cP							-W-	<u>W</u>	-W-
10 cP								<u>W</u>	-W-
12 cP									<u>W</u>

Figure 1.--The expected behavior of the F_1 generations of various white x white flowered alfalfa crosses under the complementary factor hypothesis and the contrasting genotypes of the parent plants. The letter W indicates that only white flowered plants would be obtained. Dashes indicate that obtained data were in agreement with the theoretical expectation. Underlined letters indicate that no data were obtained. The letter P indicates that purple flowered plants would be obtained.

PARENT PLANTS & GENOTYPES	2	3	4	5	6	8	9	10	12
2 ccC_2C_2PP- $P_2P_2P_3P_3$	W	W	-2.821:1-	W	2.821:1 11.962:1	W	W	W	W
3 ccC_2C_2PP- $P_2P_2P_3P_3$		W	-2.821:1-	W	-2.821:1- 11.962:1	W	W	W	W
4 CCC_2C_2pp- $p_2p_2p_3p_3$			W	-2.821:1-	W	-2.821:1-	2.368:1 -2.821:1-	-2.368:1- -2.821:1-	2.368:1 -2.821:1-
5 ccC_2C_2PP- $P_2P_2P_3P_3$				W	-2.821:1- 11.962:1	W	W	W	W
6 CCC_2C_2pp- $p_2p_2p_3p_3$					W	2.821:1 -11.962:1-	2.368:1 2.821:1 -7.258:1- 11.962:1	2.368:1 2.821:1 7.258:1 11.962:1	2.368:1 2.821:1 -7.258:1- 11.962:1
8 ccC_2C_2PP- $P_2P_2P_3P_3$						W	W	W	W
9 ccC_2C_2PP- $P_2P_2P_3p_3$							W	W	W
10 ccC_2C_2PP- $P_2P_2P_3p_3$								W	W
12 ccC_2C_2PP- $P_2P_2P_3p_3$									W

PARENT PLANTS

Figure 2.--The expected F_2 segregations from selfed purple flowered F_1 plants of various white x white flowered alfalfa crosses and the hypothetical genotypes of the parents. Dashes indicate that data fitting the calculated ratio of purples to white were obtained. The letter W indicates that only white F_1 plants would be obtained and no F_2 segregation would be expected.

Chapter V

DISCUSSION

The data presented in this study of flower color inheritance in white x white crosses of alfalfa, show that complementary factors were involved in the production of purple flower color. Fits of data to calculated ratios indicate that these factors were segregated in a disomic manner rather than by tetraploid segregation, as eight families cannot be explained by this hypothesis.

If it were assumed that the genes which acted on the basic color factors CcC_2c_2 to produce purple, also controlled the intensity of purple, this study in general is in agreement with previous studies.

Julen (3), 1944, pointed out that it was difficult to obtain good meiotic fixations in alfalfa. His studies, however, indicated that although quadrivalents occurred in alfalfa, bivalents were more common. Cytological studies would aid in determining the frequency of quadrivalent pairing in the white flowered parents and the resulting hybrids. If bivalents were predominant, disomic segregations would be expected.

Chapter VI

SUMMARY

1. The inheritance of purple and white flower color in alfalfa was studied in white x white crosses.

2. Purple flowered F_1 plants were obtained from crosses of white flowered plants 4 or 6 x white flowered plants 2, 3, 5, 8, 9, 10, or 12. These purple flowered F_1 plants segregated for purple and white when selfed and carried into the F_2 generation.

3. The F_2 segregations from white x white flowered alfalfa were found to occur in a disomic manner.

4. Complementary factors were found to be involved in the inheritance of purple and white flower color in white x white crosses in alfalfa. One of the factors Cc or C_2c_2 must be present in the dominant condition before color can develop. The factors Pp, P_2p_2, and P_3p_3, duplicate in nature, act with the basic color factors to produce purple color.

5. Theoretical genotypes of each of the 9 white flowered parent plants were hypothecated. Observed data were in agreement with theoretical segregations.

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