TETRAPLOID SEGREGATION IN ANTIRRHINUM MAJUS L.

By

Thomas M. Little

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

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TABLE OF CONTENTS

INTRODUCTION

It is rather surprising, in view of the large number of tetraploids that have been reported, especially since 1937 when the advent of colchicine rendered the artificial induction of polyploidy a simple matter, that so little has been reported on the genetics of tetraploids. For the past six years, the vast majority of papers on tetraploidy have dealt with their cytology, morphology, anatomy and economic importance. All of the important papers on the theoretical aspects of tetraploid genetics appeared before 1937. Since that year the amount of tetraploid plant material available for study has increased many-fold, yet the published data on inheritance in this important group of plants are very meager.

The genetics of autotetraploids is complicated by various factors which are not encountered in the genetics of diploids. The most important of these complicating factors lies in the fact that one of the fundamental concepts underlying diploid genetics, that of "purity of gametes", is meaningless in the discussion of tetraploid genetics. In a tetraploid, the gametes may be hybrid as well as "pure". This leads to a further complication in the zygotes, namely, that instead of having only one type of heterozygote as in the diploid, we have three, which along with the two homozygotes, give five possible genotypes involving any one pair of genetic factors. These five genotypes are known as quadriplex, triplex, duplex, simplex and nulliplex, according to whether there are four, three, two, one or no dominant genes present.

Another complicating factor in the study of tetraploid segregation is the relation between certain cytological phenomena and the genetic ratios obtained. Diploid ratios involving a single gene are affected little or not

at all by such variable factors as pairing, multivalent formation, non-disjunction, chiasmata, and the position of the gene on the chromosome in relation to the centromere, yet all of these factors have a profound influence upon tetraploid ratios. These points will be discussed in more detail later, but it should be pointed out here that while it is well recognized that variations in cytological behavior introduce variables in tetraploid segregation which make a precise mathematical analysis of the ratios difficult or even impossible, still it is felt that certain advantages are to be gained in a study of such ratios. The mere fact that tetraploid ratios are affected by cytological behaviour opens up the possibility of studying the relationships between certain cytological and genetic phenomena, which cannot be studied in diploids where genetic ratios are, in general, more stable. Some one has said of plants, that "their variability is at once the hope and despair of a plant breeder". It can likewise be said of tetraploid segregation, that its variability is at once the hope and despair of the cyto-geneticist.

Even data which cannot be interpreted intelligently in the light of our present knowledge of cytology, may prove valuable at some later time as our cytological and genetic knowledge increases. It is certainly true that the data published by Blakeslee, Belling and Farnham (1923) are much more intelligible and hence more valuable today than at the time they were published. It is therefore hoped that the data presented herein may add to the gradually accumulating mass of data on tetraploid ratios which may someday be pieced together to bring about an intelligent understanding of the genetics of tetraploids.

REVIEW OF LITERATURE

The literature on the segregation of genetic characters in autotetraploids falls into two main categories, those papers dealing with the theoretical aspects of the problem, and those in which actual experimental data are presented and interpreted. While some of the published work deals with both aspects, it has seemed most convenient to discuss these two categories separately, first treating the development of the theories concerning autotetraploid segregation, then reviewing the published data in the light of these theories.

Theoretical. The first report on segregation in autotetraploids appeared when Gregory (1914) presented data on the tetraploid Primula sinensis. He attempted to explain the ratios obtained on the basis of duplicate factor ratios commonly found in diploids. Thus, he assumed that the ratios he obtained on selfing a tetraploid hybrid corresponded to the 15:1 ratios found in diploids and that the backcross ratios represented 3:1. His conclusions were based on the false assumption that chromosome pairing always takes place between a chromosome derived from the maternal parent and one from the paternal parent (allosyndesis). Muller (1914) pointed out this fallacy, and assumed that pairing took place between any two of the four homologous chromosomes at random, independent of their origin. On this basis, he concluded that the ratio obtained from selfing a duplex heterozygote should be 35:1 and that backcrossing such a hybrid to a homozygous recessive tetraploid should give a 5:1 ratio. Moreover, he attempted to show that Gregory's data fit these ratios better than the ones Gregory himself had assumed.

No further theoretical treatment of the problem appeared until

Haldane (1930) published generalized formulae covering all types of autopolyploids, and showed specifically that if one assumed random assortment of chromatids rather than of chromosomes, a tetraploid carrying two dominants and two recessives would give a ratio of 187:9 (or approximately 21:1) on selfing, and 11:3 on backcrossing to a homozygous recessive. These ratios have attracted considerable attention by subsequent authors, and data which seem to fit them are usually referred to as displaying "chromatid segregation". Along with this term, the term "chromosome segregation" has come into general use, referring to ratios which seem to fit more closely those given earlier by Muller. The unsoundness of considering these two types of segregation as separate and distinct fixed ratios, and of assigning observed data to one or the other, was brought out by Mather (1936), but the practice has persisted to the present time. This point will be discussed later in more detail.

Mather published three papers (1935a, 1935b, and 1936) dealing in part with segregation in autotetraploids. These papers discussed the subject in the light of cytological facts, many of which were not well understood at the time the previous papers were published. In referring to the terms "random chromatid segregation" and "random chromosome segregation", Mather (1936) states:

> It has been recognized by all later workers that these segregation....expectancies are in the nature of limiting types, and that the true segregations to be expected from autotetraploid organisms will actually lie somewhere between them....

Further on in the same paper, he made this somewhat contradictory but probably sounder statement:

> A new segregation was established (1935a) for the case of completely equational separation, and random chromatid segregation was shown to be the result of a combination of the two types in the random proportions of 1/7 reductional and 6/7 equational separation.

Thus the first statement refers to random chromatid segregation as a limiting type, while the second statement represents it as an intermediate type. The second statement brings out an interesting contrast between the phenomenon of linkage of genes in diploids and linkage with the centromere in tetraploids, which has never been emphasized. In diploids, the upper limit of crossing over between two linked genes is 50%, and this limit is the equivalent of independent assortment or random segregation. In tetraploids, the upper limit of crossing over between a gene and the centromere is likewise 50%, but this is not equivalent to random chromatid segregation. Instead, the ratios calculated on the basis of random chromatid segregation are equivalent to those which would be obtained if the amount of crossing over between the gene and centromere were approximately 43%.

One of Mather's most important contributions was the formulation of methods for calculating parameters which could be used to characterize an observed ratio in terms of cytological behavior. These parameters are based on the products of the amount of genetical non-disjunction in a quadrivalent (a) times the amount of equational separation of chromatids (e). The latter variable is in turn dependent upon the frequency of chiasmata, and hence the percent of crossing over, between the centromere and the gene in question. He pointed out that the values for these variables should be the same in triplex and simplex hybrids, but would be different in duplex hybrids where change of pairing partners may alter their value. Therefore the parameter calculated from simplex and triplex data was designated as α, while the one based on duplex data was called β. Theoretically the limits of both these parameters should be zero (when there is no crossing over between the gene and the centromere) and 1/3 (when

there is 50% crossing over between the gene and the centromere and random disjunction of chromosomes).

The expected ratios based on the four hypotheses of Gregory, Muller, Haldane, and Mather are summarized in Table I, the last column giving the ratios expected on the basis of Mather's hypothesis when the parameters α and β have their limiting values of 1/3. It will be noticed that there are nine types of crosses in tetraploids capable of giving segregating progenies, in contrast to diploids where there are only two such crosses, namely, the heterozygote times itself and the heterozygote times the homozygous recessive.

The literature on polyploid genetics in general was reviewed briefly by Lindstrom (1936).

Experimental. The first experimental data on tetraploid segregation were published by Gregory (1914), reporting on work with Primula sinensis. One of the pairs of factors upon which he reported was the Thrum (short styled) versus Pin (long styled) pair, designated by him as A:a but referred to in the later literature as S:s. Table II shows the data he obtained with different mating combinations in comparison with the expectancies based on his own hypothesis and on those propounded by Muller (1914) and Haldane (1930). The values of χ^2 obtained with each of these expectancies show that, due to the small populations involved, the data did not differ significantly from any of the three theoretical ratios. Thus, much of the discussion as to which ratios these data represented was statistically meaningless. The other pair of factors studied by Gregory was green versus red stigmas, designated by G-g. It can also be shown that the data on this character did not deviate significantly from any of the three theoretical ratios.

Further data on Primula sinensis appeared in an extensive report

TABLE I

Autotetraploid ratios expected on the basis of various hypotheses.

Mating	Gregory	Muller	Haldane	Mather	Mather's ratios when a & b = 1/3
AAAa selfed	inf.	inf.	783	$64-a^2$	575
	0	0	1	a^2	1
AAAa x Aaaa	inf.	inf.	389	$48-a-ab$	107
	0	0	3	$a+ab$	1
AAAa x Aaaa	inf.	inf.	769	$64-4a-a^2$	563
	0	0	15	$4a+a^2$	13
AAAa x aaaa	inf.	inf.	27	$8-a$	23
	0	0	1	a	1
AAaa selfed	15	35	187*	$35-2b-b^2$	77
	1	1	9	$1+2b+b^2$	4
AAaa x Aaaa	7	11	347	$44-4b-a-ab$	95
	1	1	45	$4+4b+a+ab$	13
AAaa x aaaa	3	5	11	$5-b$	7
	1	1	3	$1+b$	2
Aaaa selfed	3	3	559	$48-8a-a^2$	407
	1	1	225	$16+8a+a^2$	169
Aaaa x aaaa	1	1	13	$4-a$	11
	1	1	15	$4+a$	13

*Frequently called a 31:1 ratio.

by Sömme (1930). This paper reported on the inheritance of seven pairs of factors, including the two discussed by Gregory. The conclusion was reached that none of the ratios obtained differed significantly from those postulated by Muller.

Darlington and Haldane (1931) published results on three pairs of factors in Primula, including the S and G factors discussed by the two earlier authors and the B-b factor pair for magenta vs red flower color. They paid particular attention to linkage among these factors, since it had been shown in diploids that all three are located on the same chromosome. They concluded that all of these factors were located near the centromere, so that double reduction was disregarded. While there was a rather consistent excess of recessives in their data, they attempted to explain part of these excesses by assuming that some of the parents were pentasomic for the chromosome involved. However, in a later report (1935) on linkage in diploids, they used data from tetraploid segregation to locate the position of the centromere on the linkage map of this chromosome. They stated that the data indicated that the centromere was close to the loci of the S and B factors but further distant from the G locus. Table III summarizes the data from the three reports on segregation in tetraploid Primula, including only the mating combinations found in all three papers.

Blakeslee, Belling and Farnham (1923) published a paper on segregation of two factor pairs in Datura. The value of these data, which lies in the extremely large populations involved, has apparently never been fully appreciated. Since no one has ever published a statistical analysis of the data, a table is included here (Table IV) in which the observed ratios are compared with the expectancies according to the Muller hypothesis (which the authors accepted), the Haldane random chromatid ratios, and the Mather ratios

TABLE II

Comparison of Gregory's data on the S-s factor pair with expectancies based on various hypotheses.

Mating	Observed	Expected					
		Gregory	d^2/m	Muller	d^2/m	Haldane	d^2/m
SSss	44 S	43.125	.01775	44.722	.01166	43.888	.00029
selfed	2 s	2.875	.26640	1.278	.40790	2.112	.00594
SSss x	67 S	63.750	.16569	70.833	.30742	66.786	.00069
ssss	18 s	21.250	.49706	14.167	1.03705	18.214	.00251
Sess	257 S	270.750	.69829	270.750	.69829	257.396	.00061
selfed	104 s	90.250	2.09488	90.250	2.09488	103.604	.00151
Ssss x	292 S	278.000	.70504	278.000	.70504	258.142	4.44083
ssss	264 s	278.000	.70504	278.000	.70504	297.858	3.84869
Chi square ----		5.15005		5.86728		8.30107	
P ----		.27		.21		.08	

TABLE III

Comparison of data from Gregory, Somme, and DeWinton and Haldane on tetraploid segregation in *Primula sinensis*

	Gregory	Somme	DeWinton and Haldane
Ssss selfed	257:104	1000:342	1282:395
Ssss x ssss	292:264	1007:995	1001:1009
Alpha	.033 ±.133	.005 ±.070	-.054 ±.062
GGgg selfed	75: 2	1659:52	1204:44
GGgg x gggg	114: 30	1175:223	643:125
Beta	.182 ±.171	-.006 ±.046	.052 ±.059
Gggg selfed	99: 24	172:59	265:91
Alpha	.045 ±.299	.043 ±.227	.045 ±.183

TABLE IV

Data from Bridges, Belling and Farnham (1933) compared with three theoretical ratios.

Mating	Observed	Calculated on the basis of:					
		"Chromosome Segregation"		"Chromatid Segregation"		Mather's parameters*	
		χ_x.	d^2/n	χ_x.	d^2/n	χ_x.	d^2/n
PPPp selfed	1380	1380.00		1378.37		1379.97	
	0	0.00		1.63		.04	
PPPp x pppp	160	161.00		159.25		160.16	
	1	0.00		5.75		.85	
PPpp selfed	9830	9868.00	.2435	9684.88	1.8851	9834.54	.0021
	281	281.97	0.0035	466.13	39.1089	376.46	.0851
PPpp x Pppp	394	390.67	.0304	385.95	.1679	389.50	.0157
	42	36.34	.3849	50.06	1.7947	39.51	.1670
PPpp x pppp	1400	1434.17	.1400	1382.21	3.3985	1416.37	.0413
	301	286.83	.7020	368.79	12.4610	308.63	.1898
Pppp selfed	9430	9018.25	.8183	9048.81	16.2680	9430.83	.0469
	3261	3172.75	2.4547	3642.19	79.8952	3340.17	.1339
Pppp x pppp	938	968.00	.4132	898.36	8.8400	957.94	.9499
	948	968.00	.4132	1037.14	7.6014	977.16	.9502
		χ^2 14.6736		100.8307		2.5295	
		df 5		5		3	
		P .012		<.01		.475	
AAaa selfed	1106	1105.00		1103.59		1108.60	
	0	0.00		1.41		2.21	
AAaa x AAaa	439	439.00		435.64		439.20	
	0	0.00		3.36		3.80	
AAAa x Aaaa	680	687.00		673.86		669.85	
	7	0.00		13.14		17.15	
AAAa x aaaa	337	365.00		333.61		330.97	
	6	0.00		9.39		13.03	
AAaa selfed	7385	7403.75	.1368	7340.34	.5474	7378.52	.0178
	118	97.25	4.4474	160.76	11.3776	125.68	.4083
AAaa x Aaaa	819	821.75	.0918	810.71	.3918	814.71	.0586
	22	39.25	.2585	40.29	1.7386	26.29	.3670
AAaa x aaaa	818	840.82	1.4190	814.62	.0828	819.03	.0154
	137	102.17	7.0948	140.38	.3814	134.10	1.3429
Aaaa selfed	1487	1490.25	6.2884	1308.71	.8468	1339.02	.0681
	379	476.75	8.3413	347.29	.8613	367.37	.1773
Aaaa x aaaa	144	144.00	.0000	133.71	.7919	150.83	1.0358
	144	144.00	.0000	154.29	.8868	137.17	1.1330
		χ^2 27.8710		10.6476		5.1464	
		df 5		5		2	
		P <.01		<.01		.15	

*For factor P; α=.[illegible] ±.[illegible], β=.[illegible] ±.[illegible]
For factor A: α=.[illegible] ±.[illegible] β= .157 ±.044

obtained by calculating the parameters α and β. The values of χ^2 are given for each set of expectancies, and it will be noted that two degrees of freedom are lost in the Mather ratios as a result of calculating α and β. Data from crosses involving triplex plants are excluded from the calculation of χ^2 because the ratios are too small, and the mere occurrence of recessives gives an infinite value to χ^2 when applied to the Muller ratios. Application of the χ^2 test brings out the fact that the segregations of both the factor pairs show deviations from both the Muller and Haldane ratios which are highly significant.

In recent years, the evidence indicating that the cultivated potato is an autotetraploid has created considerable interest in the genetics of this plant. Black (1935) reporting on the inheritance of resistance to wart disease in potatoes, concluded that inheritance was disomic, but conditioned by duplicate factors. Lunden (1937) reporting on numerous factors, concluded that many of them display autotetraploid inheritance, while some show disomic inheritance. This would seem to indicate that there is a difference among the chromosomes in regard to their degree of differentiation so that in some cases there are four homologous chromosomes, while other chromosomes are found behaving as two pairs differentiated from each other. Krantz, Becker and Fineman (1939), explained the inheritance of pollen sterility on an autotetraploid basis. Stevenson, Schultz and Clark (1939) explained the inheritance of immunity of Virus X on the same basis. Krantz and Kide (1941) in connection with studies on resistance to common scab, showed that the plants fell into five classes as regards breeding behavior, corresponding to the five possible genotypes in an autotetraploid. Cadman (1942) thoroughly reviewed the subject of autotetraploid inheritance in potatoes

and presented further evidence based on segregation of differences in reaction of virus inoculation.

The first artificially induced tetraploid to be studied genetically was the tomato. Sansome (1933) investigated the inheritance of seven characters and concluded that three of them displayed "chromatid segregation", one displayed "chromosome segregation", one was "intermediate", and in the other two the populations were not large enough to decide. As a matter of fact, the data indicate that probably with all the factors, segregation was "intermediate" as should be the case. At that time, Mather had not yet published his papers giving methods for calculating the amount of double reduction. When these papers were published, he drew on some unpublished data of Sansome's for use in illustrative examples.* (See Table V)

Upcott (1935) made a cytological study of tetraploid tomatoes and stated that the amount of quadrivalent formation was clearly compatible with the genetic results obtained by Sansome.

Lindstrom (1936) made a new approach to the study of quantitative genes by studying their inheritance in tetraploid as compared with diploid tomatoes.

Cytological and genetic studies on the garden Dahlia have been made by Lawrence (1929 and 1931) who arrived at the conclusion that Dahlia variabilis is an allo-octoploid resulting from doubling of the chromosome

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*Mather used the same unpublished data in two different papers (1935b) (1936) but obtained different indices of double reduction. On one case he solved for a parameter x and in another case α. The relation between these two is: $\alpha = 8x - 4$. But even when this substitution is made, the results do not agree. There are two reasons for this discrepancy. In the second paper, one of the classes was given as containing one more plant than in the first paper. This made a difference of .0032 in the value of α. Also, in the first paper two of the classes were lumped while they were kept separate in the second. This made a still further difference of .0304 in the value of α.

TABLE V

Ransome's data on the R factor in tetraploid tomatoes
(From Mather 1936)

	Ob.	Calculated on the basis of:					
		Chromosome Segregation		Chromatid Segregation		Mather's Parameters*	
		m	d^2/m	m	d^2/m	m	d^2/m
RRrr selfed	885	926.53	1.86	909.24	.66	889.90	.03
	68	26.47	65.16	43.76	13.43	63.10	.38
RRrr x rrrr	88	90.00	.04	84.86	.12	80.21	.76
	20	18.00	.22	23.14	.43	27.79	2.18
Rrrr selfed	606	619.50	.34	588.95	.44	599.46	.05
	221	206.50	1.02	237.05	1.09	226.54	.14
Rrrr x rrrr	48	58.00	1.72	53.86	.64	55.25	.95
	68	58.00	1.72	62.14	.55	60.75	.87
Chi square			72.08		17.35		5.36
df			4		4		2
P			<.01		<.01		.075

*Alpha equals .1896, beta equals .544

number in a hybrid between two tetraploid species. He found that some of the genetic factors display tetrasomic inheritance, while with others the inheritance is disomic. The Y factor for flavone formation was of the former type, while I, also a flavone factor, was of the latter type. This difference was explained by assuming that the Y factor was located on a chromosome that had not become differentiated, so that the four homologs could pair at random, while the I factor was located on a chromosome which had become differentiated into two dissimilar pairs. It was in Lawrence's first paper on this subject (1929) that double reduction was first suggested.

Tetraploid segregation in Rubus was studied by Crane and Darlington (1928 and 1932) in connection with the factor for presence or absence of prickles. They found an excess of recessives over those expected according to Muller's hypothesis, and they explained this in their first paper on the assumption that there was a tendency toward "allosyndesis". Later they explained their ratios on the basis of random chromatid segregation, but the populations were too small to permit any statistical conclusions other than that the deviation from Muller's ratios was significant.

Randolph (1935) published a paper on the cytogenetics of tetraploid Zea Mays. He states that segregation approximating 35:1 ratio were noted for several characters, but that the"data are inadequate to determine whether or not there are significant deviations from the expected ratios".

An interesting case of tetraploid segregation in Lotus corniculatus L. was reported by Dawson (1941). This species is tetraploid and two genetic types are found in natural populations, differing only in that one produces hydrogen cyanide and the other does not. Dawson found that this cyanogenetic character gave ratios which fit very closely those of Muller's "chromosome segregation" and concluded that the species is autotetraploid. The cytological observations

would give no evidence of its autotetraploid nature, since formation of quadrivalents is very rare, a condition which is usually assumed to indicate allotetraploidy. The failure of quadrivalent formation would account for the lack of double reduction found in the observed ratios.

MATERIALS AND METHODS

Plant Material. The present study was begun in the spring of 1941 on tetraploid lines of Antirrhinum kindly furnished by Mrs. Mabel Ruttle Nebel. These lines had been produced by her through the use of colchicine, and were first described by her (Nebel and Ruttle 1937). They consisted of tetraploids of commercial varieties, hybrids between tetraploids, and some segregating progenies. These plants were grown at the U. S. Horticultural Station, Beltsville, Md. in addition to plantings of some of best standard varieties. In 1942, a more complete collection of standard varieties was acquired in order to furnish as many genetic factors as possible for use in breeding and inheritance studies. By treatment with colchicine, it was possible to produce tetraploids of many more varieties. These were produced too recently to have yielded any data on segregation by this time, so that most of the data presented in this paper were obtained from the material furnished originally by Mrs. Nebel.

The first data on segregation in tetraploids were secured from F_2 populations in 1941. These populations were too small to be of much value for the study of segregation, but selected segregates were selfed and F_3 progenies of larger size were grown in the winter of 1941-42. The segregation of factors in the F_3 population was noted, and further selections were selfed from which large F_4 progenies were grown in the winter of 1942-43.

The F_1 hybrids grown in 1941 were noted for color and selfed, and

F_2 populations were grown in the winter of 1941-42. Plants were selected among these to furnish further data, and large F_3 populations grown in the winter of 1942-43.

Among the intervarietal tetraploids grown in 1941, four types were selected each possessing a different combination of three easily classified genetic factors, B, C and A (Baur's symbols). The colors of these types were White, Pink, Orange and Crimson. Four diploid lines were selected having the same colors except that purple was used instead of crimson. All of the possible crosses involving these eight lines were made, a total of 56 combinations, including reciprocals. The parent plants were also selfed in order to check their breeding behavior and to furnish material with which to compare the F_1's and F_2's . The F_1 populations were grown in the winter of 1941-42, their color was noted and selfed seed obtained from representative plants in each population except in sterile triploids. Large F_2 populations were then grown in the winter of 1942-43 when complete notes on seedling characters were obtained and as many plants as possible grown to maturity for notes on flower characters.

Methods. The seed planted during the first two seasons was sown by hand in 8-inch seed pans of sterilized soil. In planting the seed for the season of 1942-43, a method of sowing with a vacuum planter was used which possessed many advantages. This planter spaced 100 seeds 1/2 inch apart in a square pattern of ten rows and columns. The even spacing of the seeds is very effective in cutting down losses from damping off, and since there is less competition among seedlings, the effect of differential viability is reduced. Furthermore, this method of planting enables one to keep accurate records of the seedlings, designating their position in the pan by row and column for later reference. In taking notes of seedlings grown in this way, it was found convenient to use cards with lines marking off ten rows and ten columns. One of

these cards was used for noting each seed pan and the note for each seedling was written on the card in the position corresponding to that of the plant in the pan.

The germination of tetraploid seeds was considerably lower than that of diploids, 6600 seeds of tetraploids germinating 41.8% (in soil) and 6300 seeds of diploids, planted under the same conditions, germinating 73.4%.

During the first two seasons, all plants were grown in six inch pots, but in the winter of 1942-43, it was considered advisable to sacrifice optimum growing conditions in order to obtain the largest populations possible in the available space. Consequently, many of the notes on seedling characters were taken in the seed pans and the seedlings then discarded. For growing plants to maturity, some of the seedlings were transplanted into three inch pots while the majority were transplanted into flats, placing the plants about three inches apart. In this way, it was possible to grow about 20,000 plants in a relatively small space.

Selfing of flowers was accomplished merely by pinching each flower to bring the anthers in contact with the pistil and allowing the seed to set in the open without the protection of a bag. In the spring of 1941, several white and yellow varieties surrounded by plants with dominant color factors, were allowed to develop seed in the open, and this seed was planted the following fall to determine whether any crossing had taken place. Out of several hundred plants, no hybrids were found, so that this was considered a safe procedure. The greenhouses in which the plants were grown are well screened so that the entrance of large insects is prevented.

Genetic factors. The genetic factors which were studied were mostly ones which are easily classified and whose diploid inheritance is well known. Moreover, none of the factors are linked with each other.

The first observations on inheritance in Antirrhinum appeared in a series of reports by Mrs. Onslow (then Miss Wheldale) (1907, 1909 and 1910). Baur (1910)published the results of extensive studies and described many genetic factors in addition to those described by Onslow. Baur (1924) published a monograph on all the genetic studies in Antirrhinum made up to that time. Further reviews of the subject appeared in a series of papers by Kuckuck and Schick (1930), and Schick and Stubbe (1932 and 1934). In these reviews, all of the known genetic factors were described and a new set of symbols proposed to replace those given by Baur. The first chromosome maps appeared in a paper by Baur and others (1929), and these were amended and corrected by Renke (1930). Further linkage studies were reported in a series of eight papers by Schick (1930, 1933, and 1935), Hackbarth (1933), Kuckuck (1933, 1935 and 1938) and Kutscher (1935).

In discussing genetic factors, it was decided to use Baur's symbols in this report, since they are much simpler than the revised symbols proposed by Kuckuck and Schick (1930), especially in writing genotypes of tetraploids.

The basic gene for flower color was designated by Baur (1910) as B, by Onslow (1907) as I, and by Kuckuck and Schick (1930) as Niv (an abbreviation of Nivea). A plant homozygous recessive for this gene can produce neither flavones nor anthocyanins in the flowers nor the plant, so the flowers are pure white and the plant green. None of the other color genes have any effect upon such a plant. The variety White Wonder had the constitution bb, while all other varieties were BB.

In the presence of B, flavones are always present, and the factor F determines the presence of anthocyanin, (Onslow's factor L and Kuckuck and Schick's Ino). Thus BF plants have both anthocyanin and flavone in the flowers, and the seedlings have anthocyanin, while Bf plants have only flavone in the flowers, and the seedlings are green and indistinguishable from bb plants.

The kind of flavone occurring in plants possessing the factor B is determined by a gene C. (Onslow's I and Kuckuck and Schick's Sulf.) Plants of the constitution BC have ivory colored flowers due to the presence of the flavone, apigenin, which is almost colorless. On the other hand Bc plants have yellow flowers due to the flavone luteolin. According to Onslow and Bassett (1913), the only difference between these two pigments is that apigenin has only one OH group on the side benzene ring of the flavone molecule, while luteolin has two OH groups on this ring. Thus, as Scott-Montrieff (1930) has pointed out, the mutation from dominant to recessive has involved oxidation resulting in the addition of an OH group. Most ivory colored flowers have a patch of yellow in the mouth and at the base of the tube. The size and intensity of this yellow patch is generally greater in Cc plants than in CC plants, and it is further altered by the presence of numerous modifying factors. Some varieties, such as Pierre Schneider, have almost no yellow in the mouth and can scarcely be distinguished from bb plants by ordinary observation. Since the ammonium salts of apigenin are bright yellow (Onslow, 1913) the genetic constitution of white flowered plants can be determined by placing the flowers in a bottle with a few drops of ammonium hydroxide. Flowers with the constitution bb will remain white, while BC flowers will turn a brilliant yellow. This method has proven very helpful in distinguishing pure white from ivory-white flowers.

The type of anthocyanin in BF plants is determined by a gene A. (Onslow's B, Kuckuck and Schick's Eos). Plants with the constitution BFA contain the anthocyanin, antirrhinin, which gives the flowers a magenta color in the presence of ivory flavone or a crimson color in the presence of yellow flavone. Plants with the constitution BFa contain a different anthocyanin, pelargonin, and flowers with ivory flavone are then pink, while with yellow

flavone they are bronze. Onslow and Bassett (1914) and Scott-Monteriefi (1930) have shown that antirrhinin contains two OH groups on the side benzene ring of the anthocyanin molecule while pelargonin contains only one OH group on this ring. Therefore, as Scott-Monteriefi (1938) has pointed out, the change from the dominant to the recessive form involves a reduction resulting in the loss of an OH group, a situation just the opposite from that found in the case of the flavone pigments. Seedlings with the constitution HfA contain purple anthocyanin, especially noticeable on the under side of the leaves and the region around the growing point, while in Hfa plants the color is light red. The type of flavone has no effect on the color of the seedlings.

These four genes H, C, F and A are the ones used most extensively in the present study. Their interaction may be summarized briefly as follows: h gives white flowers and green seedlings regardless of the other genes present, hcfa or hcfA plants have yellow flowers and green seedlings, hCfa or hCfA plants have ivory flowers and green seedlings, HCfa plants have bronze flowers and seedlings tinged with light red, HCfA plants have pink flowers and tinged seedlings, hCFA plants have crimson flowers and purple seedlings, HCFA plants have magenta flowers and purple seedlings. Table VI shows in more detail the interaction and breeding behavior of various genotypes involving these four genes. It will be noticed that there are seven possible phenotypes which can segregate in 43 different ways. Since, in a tetraploid there are three different kinds of heterozygotes for any gene, there are 421 ways in which tetraploids may segregate for these four genes.

It is important to know whether differential viability has any effect in altering the diploid ratios obtained with any of these factors, since such a condition would have a pronounced effect on the tetraploid

TABLE VI

Ratio expected upon selfing a plant heterozygous for the factors B, C, F and A, in Antirrhinum, and the breeding behaviour of the progeny.

Abbreviations of phenotypes: M - magenta; C - Crimson; P - Pink; B - Bronze; I - Ivory; Y - Yellow; W - White.

Phenotypes		Genotypes	Breeding behavior upon selfing
81 BCFA (Magenta)	1	BBCCFFAA	All magenta
	2	BBCCFFAa	3 M : 1 P
	2	BBCCFfAA	3 M : 1 I
	2	BBCcFFAA	3 M : 1 C
	2	BbCCFFAA	3 M : 1 W
	4	BBCCFfAa	9 M : 3 P : 4 I
	4	BBCcFFAa	9 M : 3 P : 3 C : 1 B
	4	BbCCFFAa	9 M : 3 P : 4 W
	4	BBCcFfAA	9 M : 3 I : 3 C : 1 Y
	4	BbCCFfAA	9 M : 3 I : 4 W
	4	BbCcFFAA	9 M : 3 C : 4 W
	8	BbCcFfAA	27 M : 9 I : 9 C : 3 Y : 16 W
	8	BbCcFFAa	27 M : 9 I : 9 C : 3 Y : 16 W
	8	BbCCFfAa	27 M : 9 P :12 I :16 W
	8	BBCcFfAa	27 M : 9 P : 9 C : 3 B : 12 I : 4 Y
	16	BbCcFfAa	Same as parent.
27 BCFa (Pink)	1	BBCCFFaa	All Pink
	2	BBCCFfaa	3 P : 1 I
	2	BBCcFFaa	3 P : 1 B
	2	BbCCFFaa	3 P : 1 W
	4	BbCcFFaa	9 P : 3 B : 4 W
	4	BbCCFfaa	9 P : 3 I : 4 W
	4	BBCcFfaa	9 P : 3 I : 3 B : 1 Y
	8	BbCcFfaa	27 P : 9 I : 9 B : 3 Y : 16 W
27 BcFA (Crimson)	1	BBccFFAA	All Crimson
	2	BBccFFAa	3 C : 1 B
	2	BBccFfAA	3 C : 1 Y
	2	BbccFFAA	3 C : 1 W
	4	BBccFfAa	9 C : 3 B : 4 Y
	4	BbccFFAa	9 C : 3 B : 4 W
	4	BbccFfAA	9 C : 3 Y : 4 W
	8	BbccFfAa	27 C : 9 B :12 Y : 16 W
9 BcFa (Bronze)	1	BBccFFaa	All Bronze
	2	BBccFfaa	3 B : 1 Y
	2	BbccFFaa	3 B : 1 W
	4	BbccFfaa	9 B : 3 Y : 4 W
36 BCf - (Ivory)	4	BBCCff--	All Ivory
	8	BBCcff--	3 I : 1 Y
	8	BbCCff--	3 I : 1 W
	16	BbCcff--	9 I : 3 Y : 4 W

TABLE IV (continued)

Phenotypes	Genotypes	Breeding behavior upon selfing
12 B-ff - (Yellow)	4 BBccff--	All Yellow
	8 Bbccff--	3 Y : 1 W
64 b--- (White)	64 bb------	All White

ratios. Table VII shows the ratios of colored to green seedlings in all families segregating for the factor pair B-b. The value of X^2 based on the expectancy of 3:1 is 0.195. The probability that deviations greater than this would occur by chance is 67%, so that the observed deviation can not be considered significant.

Table VIII gives the ratios of purple to tinged seedlings for all diploid populations segregating for the A-a factor pair. Here the X^2 value of 5.361 would be exceeded by chance only about 3% of the time, so if we adopt the 5% level of significance, these data must be considered to deviate significantly from a 3:1. Since some of these data are from progenies which were also segregating for green, giving expected ratios of nine purple: three tinged: four green, the question arises whether the deficiency of tinged plants is due to a weak linkage between B and A. Such a linkage, in the coupling phase*, would tend to increase the proportion of purples and decrease the tinged. For this reason, the families in Table VIII are divided into two groups, the first of which segregated only for purple and tinged, while the second group segregated for purple, tinged and green. The deficiency of tinged plants is somewhat greater in the first group than in the second. Therefore, we must conclude that linkage is not the factor responsible, but that a slight amount of differential viability (about 8%) reduces the proportion of tinged plants.

Table IX gives data on the ratios of ivory (or ivory ground) to yellow (or yellow ground) flowers in diploid progenies segregating for the C-c factor pair. Since such data can be obtained only from flowering plants

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*These data would be coupling data, since the white parents had the constitution bbcc.

TABLE VII

Segregation of colored and green seedlings in progenies from diploid plants heterozygous for the B factor.

Cross	Pedigree	Observed		Expected		
		B	b	B	b	Chi^2
Rose Queen x White Wonder	43-297	227	85	234.00	78.00	.838
	43-356	113	46	119.25	39.75	1.310
White Wonder x Christmas Cheer	43-300	561	160	540.75	180.25	3.033
	43-359	445	132	432.75	144.25	1.387
White Wonder X Velvet Beauty	43-301	190	61	188.25	62.75	.065
	43-360	273	92	273.75	91.25	.008
	43-361	222	82	228.00	76.00	.631
	43-302	144	43	140.25	46.75	.401
	43-362	265	93	268.50	89.50	.186
	43-363	361	134	371.25	123.75	1.132
Velvet Beauty x Pink Tetraploid*	43-303	56	18	55.50	18.50	.018
	43-364	242	74	237.00	79.00	.422
Afterglow x White Wonder	43-305	191	65	192.00	64.00	.021
Velvet Beauty x White Wonder	43-308	185	56	180.75	60.25	.400
Total		3475:1141				9.852
Expected 3:1		3462:1154				P .77
Chi square		.195				
P		.67				

*The pink tetraploid male parent was heterozygous for B. Since the F_1 was diploid pink, it perhaps arose by androgensis from a hybrid diploid male gamete.

TABLE VIII

Segregation of purple and tinged seedlings in progenies from diploid plants heterozygous for the A factor.

Cross	Pedigree	Observed		Expected*		
		B	b	B	b	Chi^2
Rose Queen x Crimson Diploid	43-298	133	56	130.8	58.2	.109
	43-357	244	80	248.2	75.8	.304
Crimson Diploid x Christmas Cheer	43-304	378	120	367.7	112.3	1.833
Velvet Beauty x Afterglow	43-306	392	92	370.8	113.2	5.183
	43-367	238	78	242.1	73.9	.297
	43-309	134	53	143.3	43.7	2.583
Afterglow x Tetraploid Crimson**	43-311	117	35	116.4	36.6	.013
	43-368	38	16	41.4	12.6	1.197
Sub-total		1734	513			
White Wonder x Velvet Beauty	43-301	132	58	145.6	44.4	5.436
	43-360	212	61	209.1	63.9	.172
	43-361	175	47	170.1	51.9	.604
	43-302	105	38	110.3	33.7	.716
	43-362	198	67	203.0	62.0	.526
	43-363	278	83	276.6	84.4	.030
Velvet Beauty x White Wonder	43-308	142	43	141.7	43.3	.003
Sub-total		1243	397			
Grand total		2977	909	Total Chi^2		18.325
Expected	3:1	2914.5	971.5	P		.20
Chi square		5.403				
P		.02				

*Based on the proportions in the total.

**Since the F_1 was diploid crimson, it presumably arose through the functioning of a haploid gamete from the tetraploid male parent.

TABLE IX

Segregation of flower color with ivory and yellow flavone in progenies from diploid plants heterozygous for the C factor.

Cross	Pedigree	C	c
Rose Queen x White Wonder	43-297	17	10
Rose Queen x Crimson Diploid	43-298	20	6
Pinkie x Afterglow	43-299	11	4
White Wonder x Christmas Cheer	43-300	44	5
White Wonder x Velvet Beauty	43-301	10	3
	43-302	25	2
Velvet Beauty x Pink Tetraploid*	43-303	22	9
Crimson Diploid x Christmas Cheer	43-304	5	1
Velvet Beauty x Afterglow	43-306	20	3
	43-309	11	6
Velvet Beauty x White Wonder	43-307	2	1
	43-308	2	1
Afterglow x Christmas Cheer	43-312	27	5
	Total	216	56
	Exp.	204	68
	Chi^2.	2.824	
	P	.11	

*See note in Table VII.

the populations are much smaller than in the two preceding tables where the data were based on seedlings. The probability that the X^2 value of 2.824 would be exceeded by chance is about 11%, so the deviation can not be considered significant, although the population is too small to enable one to draw any very reliable conclusions as to the existence of differential viability.

No diploid data on the F factor are available from cultures grown, in the present study, but the data presented by Baur (1910) on three progenies give a total of 592 F to 192 f plants. The X^2 of 0.109 would be exceeded by chance 75% of the time, so that differential viability is evidently not functioning to alter the segregation of this factor.

It was mentioned earlier that four types of tetraploids and four similar types of diploids were selected in 1941 for the purpose of making crosses to study the inheritance of easily classified characters. The genes involved in these selected parents were B, C and A. The results of the crosses have revealed the genotypic constitution of these parents. The white tetraploids had the constitution b_4a_4, some of them being homozygous C_4 and others being heterozygous for this factor, though it could not be determined whether they were C_3c_1 C_2c_2, or C_1c_3. The pink tetraploids had the constitution $B_4C_4a_4$, the bronze tetraploids $B_4c_4a_4$, and the crimson tetraploids $B_4c_4A_4$. Among the diploids, the whites were bbccaa, the pinks BBCCaa, the bronzes BBccaa and the purples BBCCAA.

In the case of the F_1 and F_2 populations grown in 1941, it was impossible to deduce the exact genotypes of the original parents, since the populations during the first two seasons were too small. It is known, however, that the genes C, F and A but not B were involved. From the crosses made in 1941 and the hybrids grown at that time, it was therefore possible to study the inheritance of four genes. Another gene, which caused the pig-

ment in the flowers to occur in flecks instead of evenly distributed, appeared in the segregating tetraploids. This gene proved to be very unstable, mutating frequently to the self color condition. Its expression also appeared to vary considerably according to the dosage of each allele in the genotype. Baur (1924) and Buckuck and Schick (1930) described several such genes, but it is impossible to say whether the one in the present cultures corresponds to any of theirs. Due to its irregular behavior, it was not possible to make any accurate analysis of its inheritance.

RESULTS

The basic color factor. Table X. shows the ratios of colored to green seedlings obtained in all tetraploid progenies segregating for the B-b factor pair. Since this factor was involved only in the 1941 crosses, all of the data are from progenies of duplex hybrids. The deviation of the observed ratio from a 35:1 is highly significant as shown by the X^2 value of 50.05. This is also bought out by the value of β ,which deviates from zero by more than six times its standard error. The value of β , .3824, is greater than the theoretical maximum of .3333, but the standard error of .0675 indicated that the true value may lie in the vicinity of this latter figure. The homogeneity of the data is tested by adding the value of X^2 for each individual progeny, calculated from the expectancies based on the percentages of the two classes in the total. The total X^2 thus obtained clearly indicates that the data are homogeneous. In fact, its value is so low that it might cast some suspicion on the validity of the data. It can only be said in this regard, that in noting these, as well as all other progenies, an attempt was made to avoid any subconscious bias in the classification of the plants. To accomplish this, each plant was noted on a card in a space indicating its position in th planting, and no totals were computed until the notes were completed.

TABLE X

Segregation of colored and green seedlings in progenies from tetraploid plants with the constitution BBbb.

Cross	Pedigree	Observed		Expected		
		B	b	B	b	Chi^2
Tet. Pink x Dip. White*	43-285	145	6	143.0	8.0	.528
	43-345	78	5	78.6	4.4	.086
	43-286	103	5	102.3	5.7	.091
Tet. White x Tet. Crimson	43-287	22	1	21.8	1.2	.035
Tet. Crimson x Tet. White	43-290	147	8	146.8	8.2	.005
	43-348	192	11	192.2	10.8	.004
	43-349	519	27	517.0	29.0	.146
	43-350	480	29	482.0	27.0	.156
Tet. Bronze x Tet. White	43-292	52	2	51.1	2.9	.295
	43-351	18	1	18.0	1.0	.000
	43-352	141	12	144.9	8.1	1.983
	43-293	74	3	72.9	4.1	.312
	43-353	27	2	27.5	1.5	.176
	Total	1998	112			3.817
	Expected 35:1	2051.4	58.6		df	12
	Chi^2	50.05			P	.985
	P	<.01				

Beta: .3824 ± .0636

*The tetraploid pink F_1 evidently resulted from the functioning of an unreduced gamete in the male parent.

The conclusion that can be drawn from the data on the B-b factor pair is that its locus is probably at a considerable distance from the centromere, so that the amount of crossing-over between the locus and centromere is close to the upper limit of 50%.

The anthocyanin modifying factor. Data on the ratios of purple to tinged seedlings in all progenies from duplex hybrids with the constitution AAaa are shown in Table XI. Comparison with the 35:1 and 21:1 ratios shows that the observed ratio differs significantly from both of these. The total χ^2 indicates that the data are homogeneous. In considering the value of β, it must be remembered that in diploids, there was evidence that a small proportion of aa plants were eliminated due to differential viability. If this likewise holds true in the tetraploids, it is highly probable that the true value of β is somewhat higher than .14724 as calculated here. It can therefore be concluded that the A factor is located somewhat more than 20 crossover units from the centromere, but that an accurate estimation is impossible, due to the existance of differential viability.

Data on progenies from simplex hybrids with the constitution Aaaa appear in Table XII. The ratio of the total does not differ significantly from a 3:1 ratio, but upon applying the χ^2 test to each progeny and obtaining the total χ^2, it is found that the data are decidedly heterogeneous. Upon examining the deviations from expected with respect to sign, it is seen that the first eight progenies, which are descended from Afterglow, all contain an excess of recessives. Of the remaining eighteen progenies, descended from the varieties Orange Shades, Pinkie, and Golden Rod, fourteen show a deficiency of recessives and only four show an excess. Therefore if these two groups are analysed separately, it is found that each one is homogeneous and that the values of χ^2 differ widely. There are two possible explanations

TABLE XI

Segregation of purple and tinged seedlings in progenies from tetraploid plants with the constitution Aaaa.

Cross or Hybrid ancestor	Pedigree	Observed		Expected		
		A	a	A	a	Chi^2
Tet. Pink x Tet. Crimson	43-282	270	12	271.7	10.3	.291
	43-342	190	9	191.7	7.3	.411
Tet. Crimson x Tet. Pink	43-288	157	4	155.1	5.9	.635
	43-346	42	2	42.4	1.6	.104
	43-347	137	5	136.8	5.2	.008
Tet. Crimson x Tet. White	43-290	141	6	141.6	5.4	.069
	43-348	185	7	185.0	7.0	.000
	43-349	506	13	500.0	19.0	1.967
	43-350	461	19	462.6	17.7	.133
	43-291	153	5	152.2	5.8	.115
Tet. Bronze x Tet. Crimson	43-294	100	3	99.2	3.8	.175
	43-354	55	3	55.9	2.1	.400
	43-355	67	4	68.4	2.6	.783
Dip.Pink* x Tet. Crimson	43-296	108	3	106.9	4.1	.306
Light purple tet. from Afterglow (F_4)	43- 96	84	3	83.8	3.2	.013
	43- 98	214	8	213.9	8.1	.001
	43-105	105	3	104.1	3.9	.208
	43-106	65	4	66.5	2.5	.934
	43-109	64	2	63.6	2.4	.069
	43-118	18	1	18.3	.7	.133
Unknown (F_4)	43-129	145	7	146.4	5.6	.363
	43-314	472	15	469.2	17.8	.437
	43-122	107	4	106.9	4.1	.003
	43-127	40	4	42.4	1.6	3.736
	43-139	51	2	51.1	1.9	.005
	43-143	49	4	51.1	1.9	2.407
Tet. Crimson x Tet. Bronze (F_3)	43-149	152	8	154.8	5.8	.866
	43-369	68	4	69.4	2.6	.782
	43-371	428	18	429.7	16.3	.184
Tet. Pink x Tet. Crimson (F_3)	43-155	33	1	33.8	1.2	.036
	43-157	175	3	171.5	6.5	1.956
	43-158	95	4	95.4	3.6	.046
	43-160	79	3	79.0	3.0	.000

TABLE XI (continued)

Cross or Hybrid ancestor	Pedigree	Observed		Expected		
		A	a	A	a	Chi^2
Tet. Yellow x Tet. Crimson (F_3)	43-161	154	7	155.1	5.9	.213
	43-321	109	5	109.8	4.2	.158
	43-322	56	1	54.9	2.1	.598
	43-324	108	5	108.9	4.1	.205
	43-165	55	1	54.0	2.0	.519
	43-167	100	2	98.3	3.7	.810
	43-170	75	2	74.2	2.8	.237
	43-171	45	1	44.3	1.7	.299
Total		5718	217			20.633
Expected 35:1		5770.14	164.86		P	.99
Chi square		16.9613				
P		<.01				
Expected 21:1		5665.23	269.77			
Chi square		10.813				
P		<.01				
Beta		.14724 ± .03822				

*The tetraploid purple F_1 evidently arose through the fertilization of an unreduced gamete in the diploid female parent.

TABLE XII

Segregation of purple and tinged seedlings in progenies from tetraploid plants with the constitution Aaaa.

Cross or Hybrid Ancestor	Pedigree	Observed		Calculated from grand total			Calculated from sub-totals		
		A	a	A	a	Chi^2	A	a	Chi^2
Lt. purple tet. from Afterglow	43- 99	28	17	33.9	11.1	4.163	31.4	13.6	1.318
	43-100	19	8	20.3	6.7	.335	18.9	8.1	.062
	43-107	140	56	147.7	48.3	1.629	136.8	59.2	.248
	43-108	28	16	33.2	10.8	3.318	30.7	13.3	.786
	43-117	39	33	41.4	13.6	8.631	38.4	16.6	3.534
	43-110	77	31	81.4	26.6	.966	75.4	32.6	.112
	43-111	130	46	136.4	44.6	.088	126.4	54.6	1.940
	43-313	73	33	78.9	26.1	2.420	74.0	32.0	.045
Sub-total		542	230				Chi square		7.885
Alpha:		.39619	± .19108				P		.40
T. Velvet Beauty x T. Orange Shades	43-146	23	4	20.3	6.7	1.447	21.0	6.0	.857
	43-315	67	11	61.2	16.8	2.659	62.9	15.1	1.431
	43-147	33	9	31.7	10.3	.217	32.7	9.3	.012
	43-316	28	5	24.9	8.1	1.573	25.7	7.3	.930
	43-150	61	15	49.7	16.3	.158	51.3	14.7	.008
	43-317	113	25	108.3	33.8	3.042	106.5	30.5	1.376
	43-151	16	7	17.3	5.7	.394	17.9	5.1	.910
	43- 17	39	13	39.1	12.9	.004	40.4	11.6	.211
T. Pinkie x Tet. Torchlight	43-154	161	46	156.0	51.0	.650	161.0	46.0	.000
	43-318	413	132	410.7	134.3	.032	422.9	121.1	1.661
	43-160	45	14	44.5	14.5	.022	45.9	13.1	.079
T. Yellow x Tet. Crimson	43-331	94	48	107.0	35.0	6.408	110.4	31.6	10.948
	43-166	27	7	25.6	8.4	.313	26.4	7.6	.081
	43-169	91	13	83.9	27.1	5.312	86.5	24.5	1.589
	43-333	68	9	58.8	19.2	7.188	60.7	17.3	0.117
	43-335	57	14	53.6	17.5	.059	55.2	15.8	.264
	43-336	30	5	26.4	8.6	1.938	27.2	7.8	1.292
	43-341	16	6	16.8	5.2	.164	16.3	4.7	.463
Sub-total:		1361	389				Chi square =		26.709
Alpha:		-.23243	± .09432				P		.07

Grand Total: 1903 619 Chi square: 51.927
Alpha: -.03877 ± .06338 P: <.01

for this behavior. One is the possibility that there are two different genes involved for the modification of anthocyanin. This is largely ruled out by the fact that crosses in the diploids, involving the ancestors of both these groups, failed to show any evidence of duplicate or complementary factors for anthocyanin modification. The other possibility is that the differential viability, noted in the diploids, functions more effectively in one group than in the other. It appears plausible that the detrimental effect of a recessive gene should vary according to its genetic as well as its physical environment. The negative value of α obtained in the second group indicated that some factor, other than the failure of chiasma formation between the gene and centromere, was functioning to reduce the proportion of recessives.

The basic anthocyanin factor. Data on progenies from duplex hybrids with the constitution FFff are presented in Table XIII. The total ratio obtained corresponds almost exactly to the 21:1 ratio calculated by Haldane (1930) on the basis of "random chromatid segregation". However, as Mather (1935a) has pointed out, such a ratio should be interpreted as resulting from an amount of crossing-over between the centromere and the gene such that 6/7 of the first meiotic divisions will be equational and 1/7 will be reductional at the locus in question. The total X^2 calculated from the last column, indicates that the data are homogeneous.

Table XIV shows data from progenies of simplex hybrids with the constitution Ffff. The total ratio obtained contains only a slight excess of recessives, and does not deviate significantly from a 3:1 ratio. The value of α is also considerably lower than the value of β derived from duplex data.

When the plants from these progenies came into bloom, an attempt

TABLE XIII

Segregation of colored and green seedlings in progenies from tetraploid plants with the constitution *FFff*.

Cross or Hybrid Ancestor	Pedigree	Observed		Expected		
		F	f	F	f	Chi^2
Light Purple Tet. from Afterglow	43- 98	222	9	220.5	10.5	.224
	43- 99	45	1	43.9	2.1	.604
	43-105	108	7	109.8	5.2	.653
	43-107	196	12	198.6	9.4	.758
	43-108	44	1	43.0	2.0	.533
	43-111	287	13	286.4	13.6	.028
Unknown	43-122	111	4	109.8	5.2	.290
	43-123	42	2	42.0	2.0	.000
Tet. Yellow x Tet. Crimson	43-165	56	4	57.3	2.7	.655
	43-166	34	2	34.4	1.6	.105
	43-167	102	6	103.1	4.9	.258
	43-168	83	2	81.1	3.9	.970
	43-331	142	7	142.2	6.8	.006
	Total	1472	70			5.069
	Beta:	.27837	±.07464		P	.95

Segregation of flower colors and colored and green seedlings in progenies from tetraploid plants with the constitution Ffff.

Cross or Hybrid Ancestor	Pedigree	Flowers					Seedlings				
							Observed		Expected		
		FFFF + FFFf	FFff	Ffff	ffff	Unclassified dominants	F	f	F	f	Chi^2
Light Purple Tet. from Afterglow	43-109		7	28	13	2	66	28	69.3	24.7	.598
	43-117		3	19	16	20	55	19	54.6	19.4	.011
	43-110		16	42	32	25	108	40	109.1	38.9	.053
Tet. Yellow x Tet. Crimson	43-169	2	11	30	42	9	188	87	202.8	72.2	4.118
	43-170		13	24	12	2	77	16	68.6	24.4	3.020
	43-171	2	12	14	8	1	74	27	74.5	26.5	.013
Unknown	43-127		6	15	11	3	44	21	47.9	17.1	1.325
	43-139		2	15	7	1	53	12	47.9	17.1	.004
	43-145		9	29	15	3	53	17	51.6	18.4	.145
	43-120	1	35	100	40		181	53	172.6	61.4	1.556
	Total	5	126	332	194	66	859	320			13.800
	Expected:	7.22	148.25	298.52		Alpha:	.093	± .008		P:	.15

Chi square 4.0570
df: 2
P: .14

was made to classify the dominant F plants into quadriplex or triplex, duplex, and simplex. Only plants also carrying the dominant A could be classified, so that the pink and bronze flowered plants which are phenotypically Fa appear in the table as unclassified dominants. The observed proportions of these three classes of dominant F plants agrees fairly well with the values calculated. The classification was based upon the intensity of purple or crimson color. A similar classification was attempted in 1942, and the genotypes of various plants tested by progeny tests. It was found that most of the classifications were correctly made, but even a small amount of error in data of this type, interferes seriously with any statistical analysis.

A few progenies appeared which apparently came from triplex hybrids with the constitution FFFf. While only one family segregated a nulliplex plant, the assumption that these progenies sprung from triplex hybrids was based upon the fact that some of the plants had flowers whose color was less intense than normal quadriplex plants and judged to be duplex or simplex. The population is too small to allow for a very accurate estimate of α, but its value agrees rather well with the value of β obtained from duplex data. A summary of these data appears in Table XV.

The flavone modifying factor. The C-c factor pair differs from the others studied in that plants cannot be classified in the seedling stage, but must be grown to flowering. Consequently, the population size is somewhat more limited. The data for all progenies from duplex hybrids with the constitution CCcc is presented in table XVI. The deviation from a 35:1 ratio is significant and the data are shown to be homogeneous. The value of β, .16474 indicates that this gene is located between twenty and thirty cross-over units from the centromere.

TABLE XV

Segregation of colored and green seedlings in progenies from tetraploid plants with the constitution FFFf.

Pedigree	F	f
43-106	69	0
43-162	64	0
43-163	12	0
43-164	119	0
43-119	146	1
Total:	410	1

Alpha: .39461 ± .19707

TABLE XVI

Segregation of flower color with ivory and yellow flavones in progenies from tetraploid plants with the constitution CCcc.

Cross or Hybrid Ancestor	Pedigree	Observed		Expected		
		C	c	C	c	Chi^2
Light purple tet. from Afterglow	42-131	19	1	19.2	.8	.007
	42-130	11	1	11.5	.5	.522
	43- 98	170	5	168.4	6.6	.403
	42-133	18	2	19.2	.8	1.875
	42-134	18	1	18.3	.7	.133
	43-105	74	4	75.1	2.9	.567
	43-107	131	6	131.8	5.2	.128
	43-109	48	2	48.1	1.9	.005
	43-117	63	1	61.6	2.4	.848
	43-110	113	2	110.7	4.3	1.398
Unknown	43-138	32	2	32.7	1.3	.392
	43-143	52	2	52.0	2.0	.000
	43-133	20	1	20.2	.8	.070
	43-131	42	1	41.4	1.6	.234
	43-174	89	2	87.6	3.4	.599
Tet. Pink x Tet. Crimson	42-162	42	3	43.3	1.7	1.033
	43-282	105	5	105.9	4.1	.205
	Total	1047	41			8.319
						df:16
	Beta: .16474 ± .08922					P: .93

DISCUSSION

Conditions necessary for the genetic analysis of tetraploids. In spite of the fact that a large number of tetraploids have been discovered or produced artificially, many of these are not amenable to genetic analysis, due chiefly to three difficulties commonly encountered. The first and commonest of these difficulties is the high degree of sterility often found in autotetraploids. The very nature of tetraploid segregation requires that very large populations must be grown and analyzed in order to obtain data that will have any statistical significance. In general, larger populations are required in order to draw valid conclusions in tetraploid material than in diploids. The high sterility of many tetraploids makes it impossible or at least extremely difficult to secure such large populations.

The second difficulty, curiously enough, is most often found when the first difficulty is absent. It consists of the amphidiploid-like behavior found in some tetraploids. It is now generally recognized that autotetraploidy and amphidiploidy are rather arbitrary terms and that many tetraploids are intermediate in their behavior so that the distinction is not always clear-cut. Amphidiploidy is not confined to species hybrids, but may arise from autotetraploids through structural changes in the chromosomes or some other form of chromosome differentiation. Increased fertility and decreased segregation are simultaneous effects of a change from autotetraploidy to amphidiploidy, so that many of the most fertile diploids display little or no genetic segregation.

The third difficulty is the lack, in many species, of clear-cut genetic characters which lend themselves readily to classification and analysis. A prominent example of this is found in the species of Lilium. Nearly all of the genetic characters which distinguish varieties within a species

such as L. longiflorum, are quantitative in nature, and a study of their inheritance would be extremely difficult even in diploids. Another example is that of scab resistance in the potato, reported on by Krantz and Kide (1941), a genetic character very difficult to classify with any high degree of precision.

Tetraploids of Antirrhinum majus provide exceptionally favorable material for the study of segregation, due to the fact that none of these common difficulties present themselves to any serious extent. While some of the plants display considerable sterility, there is a great deal of variation in this regard, and fertile lines can be selected which will set seed nearly as abundantly as the diploids. A single capsule will often contain two or three hundred seeds, and a single flower spike may produce from ten to thirty capsules.

It has been suggested by Ruttle (Emsweller and Ruttle 1941) that these tetraploids display a certain amount of amphidiploid-like behavior, and she states that varietal hybrids are more fertile than non-hybrid tetraploids. While Sparrow, Ruttle and Nebel (1942) make the suggestion that this phenomenon may be due to heterozygosity per se, there is another explanation which seems to be more in accord with the following observations. In the present material (much of which was kindly furnished by the second author of the above cited paper, and closely related to the material discussed therein) the majority of hybrid tetraploids were more fertile than the homozygous tetraploids. However, there were exceptional cases of hybrid tetraploids being almost completely sterile and also of intravarietal tetraploids being highly fertile. Furthermore, it was found that selection for several generations was effective in producing lines of either high or low fertility, independent of the homozygosity of the lines. For

example, lines of tetraploid "White Wonder" resulting from two years' of inbreeding and divergent selection for high and low fertility show marked differences in fertility, although all lines are extremely homozygous and indistinguishable from each other in morphological characters. The lines selected for high fertility set an average of 3.76 capsules per plant and 1.92 per spike, while those selected for low fertility set an average of .44 capsules per plant, and .24 per spike.

A further observation which conflicts with the idea that heterozygosity per se is responsible for high fertility, has been made in connection with progenies segregating for genetic characters. In one such progeny segregating for color, where the heterozygous individuals could be distinguished from those which were homozygous, there were also clear cut differences in fertility. The plants were classified according to both color and fertility, and the results are shown in Table XVII. It is clear that in this case at least, there is no relation between fertility and heterozygosity of the character involved. By growing progenies from many of these plants, their heterozygosity was checked and in all cases the classification was confirmed. Many other segregating progenies have been observed in which the average fertility of the homozygous recessives was as high as that of the dominant plants which, of course, included a large proportion of heterozygotes. It might be argued with justification that heterozygosity for only one or two genetic factors may not be sufficient to affect fertility so that these last stated observations would not constitute a valid argument. However, the argument that most strongly refutes the idea of amphidiploid-like behavior lies in the very fact that they segregate according to the expectations for a true autotetraploid. Any amount of selective pairing between genetically similar chromosomes in a hybrid should have the effect of reducing the proportion of recessives below the expected, and such a reduction has never been observed.

TABLE XVII

Comparison between heterozygosity and fertility in segregating line 42-334

Genetic Constitution	Fertile Plants	Sterile Plants	Total
FFFF	2	0	2
FFFf	6	3	9
FFff	13	5	18
Mff	7	2	9
ffff	5	0	5
Total	33	10	43

TABLE XVIII

Types of dihybrid ratios found in diploids and the corresponding ratios expected in tetraploids, disregarding double reduction.

Diploid	Examples in Antirrhinum	Tetraploid	
		Both factors duplex	One factor duplex the other simplex
9 : 3: 3 : 1	BBFfCc	1225 : 35 : 35 : 1*	105 : 35 : 3 : 1*
9 : 3 : 4	BbFFCc	1225 : 35 : 36*	105 : 35 : 4* or 35 : 1 : 12*
9 : 6 : 1		1225 : 70 : 1	105 : 38 : 1
9 : 7	BbffCC	1225 : 71	35 : 13
10 : 6		613 : 35	53 : 19
13 : 3	BbffCc	1261 : 35	141 : 3 or 109 : 35
15 : 1		1295 : 1	143 : 1

*Types of ratios that have been observed in Antirrhinum.

In view of these observations, it seems logical to suppose that the increased fertility frequently observed in the tetraploid hybrids may simply be due to the covering up of complementary recessive factors for sterility. This is, of course, merely a special case of the general explanation of hybrid vigor in plants, and seems to be compatible with the observed facts.

The abundance of clear-cut genetic factors is very helpful in carrying out an analysis of inheritance in tetraploid Antirrhinum. Many of these characters have been the subject of extended studies by Baur (1910) and others, and their inheritance is well understood. The most convenient factors are those involving the formation of anthocyanin pigments, since these factors are not only expressed in the flowers, but are also expressed in the colors of the young seedlings. This makes it possible to grow very large populations in a limited space and secure data on segregation without growing the plants to flowering.

Another useful type of factor is that in which the three types of heterozygotes can be distinguished from each other and from the homozygotes. This is true of the anthocyanin factor found in our cultures which produces a different phenotypic effect for each of five different genotypes.

Cytological behavior and tetraploid segregation. As stated previously, cytological behavior has a more pronounced effect on genetic segregation in tetraploids than in diploids. There are three main variables in cytological behavior which may affect the proportion of recessives found in a segregating progeny. These will be discussed in the following order: first, mode of pairing; second, quadrivalent formation; and third, chiasma frequency.

Pairing in a tetraploid may be classified as random or selective with reference to any given group of four homologous chromosomes. Even within the same tetraploid, some members of a genom may undergo random pair-

ing while others may display verying degrees of selective pairing. In random pairing, any one of the four homologous chromosomes may pair with any one of the other three with equal probability. The hypotheses of Muller, Haldane and Mather all assume random pairing, but Gregory's hypothesis assumes a special type of selective pairing.

Selective pairing occurs when the four homologs are not equally homologous but tend to fall into two groups such that the two chromosomes within a group display more affinity than two chromosomes from different groups. In other words, they have differential affinity. Homology is a relative term and can vary from "absolute identity", such as is found in doubled haploids, to the very weak homology found in secondary pairing. As a result, the pairing in tetraploids can vary from completely random where the four chromosomes are equally homologous, to completely selective, where the plant behaves functionally as a diploid. All degrees of selective pairing between these two extremes may exist. To illustrate how selective pairing may effect segregation in autotetraploids, let us assume that we have a duplex hybrid with the constitution $\underline{AAaa}$. Furthermore, let us designate the two similar pairs of chromosomes carrying their genes with subscripts 1 and 2. Then in a plant with the constitution $\underline{A_1A_1a_2a_2}$, if selective pairing is complete there will be no segregation, since only gametes with the constitution $\underline{A_1a_2}$ can be formed and these will unite to form progeny with the constitution $\underline{A_1A_1a_2a_2}$. If selective pairing is not complete so that $\underline{A_1}$ occasionally pairs with $\underline{a_2}$, then gametes can be produced with the constitution $\underline{A_1A_1}$ or $\underline{a_2a_2}$, the latter type may unite to give a homozygous recessive segregate. This is known to occur in Primula kewensis where a limited amount of segregation takes place. Chromosome doubling in a diploid hybrid $\underline{A_1a_2}$ or crossing of two autotetraploids $\underline{A_1A_1A_1A_1}$ and $\underline{a_2a_2a_2a_2}$ will give rise to amphidiploids with the constitution

$A_1A_1A_2a_2$ just discussed. However, under some conditions, we may have tetraploids with the constitution $A_1a_1A_2a_2$. In this case, complete selective pairing will produce gametes with the constitutions A_1A_2, A_1a_2, a_1A_2, and a_1a_2 in equal proportions, and the phenotypic ratios on selfing will be 15 A:1a. Moreover, under these conditions, two types of duplex hybrids will be produced, those like the parent $A_1a_1A_2a_2$ and non-segregating types $A_1A_1a_2a_2$ or $a_1a_1A_2A_2$. This type of segregation is found in diploids when duplicate factors are involved, and its occurrence is sometimes considered an indication of amphidiploid ancestry. We have already pointed out that newly arisen tetraploid hybrids are almost certain to have the constitution $A_1A_1a_2a_2$. The origin of the second type, $A_1a_1A_2a_2$ is probably secondary, resulting from the occasional pairing and consequent crossing-over between A_1 and a_2 chromosomes in such a way that there is an exchange of alleles A and a without conspicuously altering the original differentiation of the chromosomes. This is borne out by the fact that practically all cases of duplicate factor ratios are found in plants which, on other grounds, show evidence of being old polyploids. Gregory's hypothesis was based on the assumption that there was complete selective pairing between chromosomes from opposite parents. This type of pairing was later referred to as "allosyndesis", and Crane and Darlington (1928) spoke of deviations from the 35:1 ratio in Rubus as due to "a tendency toward allosyndesis", an explanation which they later retracted (1931). The terms autosyndesis and allosyndesis have been purposely avoided in the foregoing discussion to avoid the confusion that is common in their usages. Autosyndesis is generally defined as pairing between chromosomes derived from the same parent, and allosyndesis as pairing between chromosomes from opposite parents. However, it makes a difference whether we refer to the original parents or to the immediate parents. Darlington (1937 p.200)

states "The distinction between the two is only relative to the immediate parents ....", He further restricts the use of the terms to "pairing of dissimilar chromosomes". Hence he says, "Autosyndesis occurs in allopolyploids under the following conditions:- 1. In the normal polyploid species as an exceptional occurrence". Cadman, (1942) on the contrary, speaks of "duplex allotetraploids ....where strict autosyndesis prevails". (Italics mine) Unlike Darlington, he is referring to autosyndesis as pairing between chromosomes from the original parents of the allotetraploid. Sharp also uses the terms in this latter sense, although he refers to them as auto- and allosynapsis. The terms are used in still another sense in the literature, when they refer to genetic differences. Thus in a duplex hybrid AAaa, pairing between A and A or a and a is sometimes called autosyndesis, while pairing between A and a is called allosyndesis. Whenever the terms are applied to autotetraploids, this is the meaning usually inferred, since the differences that normally exist among the four homologous chromosomes are genetic.

Arguments were presented earlier in this discussion to support the conclusion that selective pairing is rare or entirely absent in tetraploids of Antirrhinum majus, and that the genetic ratios obtained are therefore not affected by this cytological variable.

The second cytological variable that affects segregation is the formation of quadrivalents. Quadrivalent formation involves pairing among all four homologous chromosomes. Hence, Gregory's ratio, based on pairing only between chromosomes from opposite parents, cannot be obtained in plants where quadrivalent formation is common. Muller's ratios, on the other hand, are not affected by the presence or absence of quadrivalents as long as crossing over does not occur between the gene involved and the centromere.

In fact, his ratios can be obtained in the complete absence of quadrivalent formation. This appears to be the case in Lotus as reported by Dawson (1941). Haldane's ratios can only be obtained if the chromosomes involved form quadrivalents regularly. In Mather's formulae, the quantity g, representing the amount of genetical nondisjunction, is dependent upon the amount of quadrivalent formation, attaining its random value of 1/3 only if quadrivalents are regularly formed.

Sparrow, Buttle and Nebel (1942) have presented data on the frequency of quadrivalent formation in Antirrhinum. They observed frequencies ranging from two to seven with an average of five per cell. There is no way of knowing whether these quadrivalents tend to involve the same chromosomes each time or if all of the chromosomes form quadrivalents at about the same frequency.

The third cytological variable is the frequency and position of chiasmata formation. Since separation at first division is always reductional at the centromere, equational separation at a given locus depends upon the formation a chiasma between that locus and centromere. In Mather's formulae, the amount of equational separation is designated as e. If one chiasma is always formed between the centromere and the locus in question, separation at that locus is always equational and e has the value of 1. Stated in terms of cross-over percentage, this would be equivalent to 50% crossing over, and in general terms, e is twice the cross-over percentage.

Oehlkers (1940) has reviewed the subject of environmental effects on chiasma formation, and Ernst (1938) has shown specifically that sudden changes in temperature reduce the chiasma frequency and hence the amount of crossing-over in Antirrhinum. Therefore, since the segregation in a tetraploid varies with the amount of chiasma formation, and this is in turn influenced by environmental factors, tetraploid ratios should be expected to

vary according to the environmental conditions which prevailed when the parent plant was undergoing meiosis.

In a tetraploid, when the two chromosomes in a gamete are derived from sister chromatids, double reduction is said to have occurred. Since double reduction tends to increase the proportion of homozygous gametes (both recessive and dominant), it affects the genetic ratios by increasing the proportion of recessive phenotypes above that expected on the assumption that the chromosomes behave as units and do not undergo double reduction. Hence, any cytological behavior that favors double reduction tends to increase the proportion of recessives in a ratio. In order for double reduction to take place, a special set of conditions must be satisfied. First, paired chromosomes bearing opposite alleles must pass to the same pole at the first division. This is termed genetic non-disjunction and its frequency was designated by Mather with the symbol "s". Genetic non-disjunction is in turn dependent upon the formation of trivalents or quadrivalents, since with bivalents, the two members of the pair almost invariably pass to opposite poles. The second condition which must be fulfilled is that at least one chiasma must be formed between the centromere and the locus of the gene in question, resulting in equational separation at the first division. When both of these conditions are fulfilled, two chromosomes pass to the same pole at the first division, each consisting of two chromatids bearing opposite alleles. At the second division, when the centromeres divide and pass to opposite poles, the two chromatids bearing the same allele may pass to the same pole, thus resulting in double reduction. Inasmuch as double reduction depends upon these two conditions, its frequency may be expressed as the product of the frequency of genetic non-disjunction (s) and the fre-

quency equational separation (e). This product, a^e, is designated by Mather as α in simplex and triplex, β in duplex hybrids.

The parameters α and β, give us the products of the amount of genetical non-disjuction, a and the amount of equational separation, e, but tell us nothing about the relative part that each of these variables plays in affecting segregation. The value of a will vary from zero, when no quadrivalents are formed, to 1/3, the random value obtained when the chromosomes always associate in quadrivalents. The value e, on the other hand, can vary from zero to one as the cross-over percentage between the gene and the centromere varies from zero to 50%. Thus the value of α and β should vary from zero to .33. If we observe cytologically, that the frequency of quadrivalents is very high, we can assume that the value of a approaches it upper limit of 1/3 and as a result, gain an approximation of e from the value 3%.

We have seen what relationship exists between tetraploid segregation and several specific aspects of cytological behavior. It should also be pointed out that segregation in tetraploids has an important bearing on several general cytological concepts. One of these is in regard to the time of crossing-over. Deviations from the Muller ratios furnish us with genetic proof that crossing-over takes place in the four strand stage between two of the strands. The first demonstration of this fact was furnished by Anderson (1925) in connection with attached X-chromosomes in Drosophila. It was further demonstrated by Bridges and Anderson (1925) in the X-chromosomes of triploid females of Drosophila. It is an interesting fact that the data on tetraploid segregation published previously by Blakeslee, Belling and Farnham (1927), can now be interpreted to prove the same fact. The appearance of recessive progeny from triplex hybrids could not have taken place

without double reduction and hence crossing-over in the four strand stage. The first genetic demonstration of double strand crossing-over in Zea was made by Rhoades (1932) and later discussed more fully (1933). His work was based on the genotypic constitution of trisomic types. Emerson and Rhoades, (1933) pointed out the significance of chromatid double-strand crossing-over in relation to the upper limit of recombination. Finally, the ingenious work of Lindegren on Neurospora (1933) proved the occurrence of double-strand crossing by an entirely different method. His recent report (1941) on more extensive experiments with this plant have shed light on further details in the behavior of chromatids and chromosomes during meiosis.

Another general problem, upon which tetraploid segregation can throw light, is the old problem of reductional and equational separation of chromosomes during meiosis. It was long thought that the first division of meiosis was "reductional" or "heterotypic" while the second division was "equational" or "homotypic". This distinction is now known to apply only to the centromere and closely adjacent regions of the chromosomes, for the other portions of the chromosomes may divide equationally at the first division as the result of crossing over. As a result of these "equational exceptions", double reduction can occur, a fact first suggested by Lawrence (1929) and further discussed by Darlington (1929). This problem has been discussed in detail by Mather (1935 and 1936).

Genetical behavior and tetraploid segregation. Not only does tetraploidy have a close relation to various cytological problems, but also it is of interest in connection with various genetical problems. The foremost of these is the problem of locating the centromere on the linkage map of a chromosome. It is impossible to solve this problem in the the study of diploid ratios, but a solution can be approximated if we have autotetraploids from which to secure data on segregation. This method was used by DeWinton and Haldane

(1938) to determine the approximate position of the centromere on the linkage map of one of the chromosomes in Primula sinensis. As already pointed out, we have no method of determining the amount of crossing-over between a gene and the centromere with any high degree of accuracy. However, we can obtain relative values, so that with chromosomes on which we have a large number of markers, we should be able to locate the centromere within rather close limits. There is one difficulty which is apt to be encountered rather commonly in connection with interpretation of ratios. This is the problem of differential viability of various phenotypes. A great many recessive genes tend to reduce viability, and when a tetraploid is segregating for such a character, the introduction of another variable renders the interpretation of results almost impossible. It is true that we can sometimes calculate the extent of differential viability in a diploid, and make corresponding allowances in calculating tetraploid expectancies. However, it is scarcely safe to assume that the extent of viability will be the same in the tetraploid as in the diploid. Therefore, any results obtained with characters that show differential viability should be regarded as only very rough approximations.

Differential viability has an interesting effect upon the relative values of β and α derived from duplex and simplex data respectively. Since differential viability generally decreases the proportion of recessives, it tends to decrease the values of α and β below what their value would be if the dominants and recessives were equally viable. However, this decrease is not the same for both parameters. For example, where double reduction is at a maximum, the value of α is .333, but if differential viability eliminates 18.7% of the recessives, the value of α in progeny from a simplex plant will be reduced to zero. On the other hand, in progeny from a duplex plant, the value of β will only be reduced to .208. In general, the same amount of

differential viability will reduce the value of α to a greater extent than the value of β. Mather (1936) has noted just such differences in the values of α and β, but has explained them on cytological grounds. The cytological conditions which he postulates for causing the excess of β over α are extremely restricted. A change of pairing partner must occur which involves a change in pairing relationship with respect to the heteroxygous factor. Furthermore, two chiasmata must be formed between the centromere and the locus of the gene involved, and one of these must occur on either side of the partner exchange. It hardly seems possible that such a restricted set of conditions could account for all the differences that have been found between the two parameters. On the other hand, if we explain such differences on the basis of differential viability, we are dealing with a very common phenomenon. It is well known that a great many, if not most recessive genes have a detrimental effect upon the well-being of the plant.

In regard to the phenotypic results produced by genes, several unique phenomena are found in tetraploids. Whereas in diploids there are only three possible genotypes involving a pair of alleles, in tetraploids there are five. In two of these genotypes, the simplex and triplex, the proportion of dominant to recessive genes differs from the equal proportions always found in diploid hybrids. Often when two alleles are present in equal proportions, one is completely dominant over the other, but it may not be capable of completely masking the effect of those recessive genes in a simplex hybrid. For this reason, incomplete dominance is more common in tetraploids than in diploids. In Primula, for example, Somme (1930) has shown that in diploids the factor G for green stigma is completely dominant over the factor g for red stigma, effecting not only stigma color but also supressing color in the flowers. In tetraploids, on the other hand, plants with the constitution Gggg have green stigmas, but "the flowers have a darker shade, in some cases

nearly as dark as that of the pure recessive form". In addition the factor B for magenta flower color is completely dominant over red in the diploid, but in the tetraploid the class Bbbb "exhibits colours varying from magenta to almost pure red". An even greater increase of incomplete dominance was found in connection with interaction involving the dominant white gene, I and the green stigma factor G. In tetraploids, the number of combinations of genotypes is so increased that they "may, according to their coloring, be arranged in an almost continuous series, ranging from pure white through lighter and darker shades to pure magenta or red", Sansome (1933) has reported similar cases in tomato, where one dominant gene is not completely dominant over three recessives. In Antirrhinum, the basic color factor B, and the magenta anthocyanin factor A appear to be completely dominant even in the simplex condition. However, plants with the constitution Cccc contain considerable more yellow than those containing two or more genes C for ivory color. It was also found that the F factor produced an almost continuous gradation of colors from full to very dilute as the number of recessive genes increased from zero to three. The great variation in the flecking character is probably due, in part, to the phenomenon of incomplete dominance. It can be seen that with quantitative characters, the occurrence of the "blending type" of inheritance will be even more prounced in tetraploids than in diploids.

Another subject of genetic interest in tetraploids is the interaction of factors producing modified dihybrid ratios. Table XVIII shows the various dihybrid ratios found in diploids with corresponding ratios that would be expected in tetraploids on the basis of chromosome segregation only.

In the second column are shown examples of genotypes in Antirrhinum which can give some of these ratios. The starred ratios in the third and fourth columns indicate those which have actually been obtained, further modified of course by chromatid crossing-over.

Statistical analysis of tetraploid segregation. The unfixed nature of tetraploid ratios introduces problems in statistical analysis not encountered in dealing with the fixed diploid ratios. It is rather common in the literature for geneticists to refer to "chromosome" and "chromatid segregation" as though these were two fixed ratios between which one must choose in fitting a set of data. As a matter of fact, these two types of segregation are in reality, only ideal limiting ratios which are seldom attained, and probability favors the occurrence of ratios which are intermediate between these two limits.* The important statistical problem is therefore, not to determine which ratio the data fit more closely, but to find out to what extent the two opposing forces of equational and reductional separation have affected the data. Mather's parameters α and β conveniently characterize a set of data in this regard. It has been shown that the data published by Blakeslee, Belling and Farnham (1923) differ significantly from both a 35:1 and 2l:1 ratio.

On the other hand, it has been shown that Gregory's data do not differ significantly from either ratio. These two examples bring out the point that large populations are necessary in order to draw valid conclusions in regard to tetraploid ratios. For example, it is necessary to have a population of at least 1707 plants in order to be certain that any ratio obtained can be proven to deviate significantly from either a 35:1 or a 2l:1 ratio.

The method of calculating expected ratios when the values of α and β are given is merely one of substituting these values in the formulae in Table I, column 5. The converse problem of calculating the values of α

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*As Mather (1935a) has pointed out, random chromatid segregation is really not the upper limit as far as proportion of recessives is concerned. Data may simulate the random chromatid ratio due to the balance between 6/7 equational and 1/7 reductional separation. Complete equational separation at a locus will produce and even higher proportion of recessives.

and β from observed data is discussed fully by Mather (1935a, 1935b, and 1936). Briefly the method consists of setting up a log likelihood equation in which the likelihood is expressed as the sum of the products of the observed number in each class and the log of the expectancy in terms of α or β. This expression is then differentiated with respect to α or β and the derivative equated to zero. The solution of this equation is then the value of α or β giving the "maximum likelihood". The variance is then obtained by taking the second derivative of the original equation, substituting the value of α or β, and taking the negative reciprocal of the result. When data are available on several types of matings, the maximum likelihood equations will be rather complex and must be solved by successive approximations. When we have only data on progeny from selfed plants, as is often the case, the equations reduce to quite simple forms. Thus to calculate α from a progeny of a selfed simplex plant, we can use the equation: $\alpha = 8\sqrt{z} - 4$, where z represents the percentage of recessives found in the population. The standard error will be: $4\sqrt{(1-z)/n}$. For data from selfed triplex data the equation is: $\alpha = 8\sqrt{z}$, and the standard error is the same as in the last case. The parameter β derived from a progeny of a selfed duplex plant can be calculated from the formula: $6\sqrt{z} - 1$, and the standard error will be: $3\sqrt{(1-z)/n}$.

Practical significance of tetraploid segregation. A question that naturally arises in the discussion of any scientific problem is whether that problem has any practical significance. Inasmuch as there are several horticultural crops which are autotetraploid or behave partly as autotetraploids, the problem of autotetraploid genetics is of considerable importance to the plant breeder. As an example, let us consider the common problem in plant breeding of obtaining a true-breeding line. In diploids this is relatively simple, for when a selection is made in an F_2 or later generation which pro-

duces a uniform progeny, all of the plants in such a progeny are concluded to be homozygous and we feel safe in bulking the seed and using it to obtain and increase. Such a procedure would be unsafe in dealing with autotetraploids, for the plant selected may be triplex. In this case, its progeny would be phenotypically uniform, but genotypically it would still be segregating, producing some duplex and even simplex plants which would segregate in the following generation. Just such a problem has been pointed out by Cadman (1943) in connection with obtaining a strain of potatoes homozygous for resistance to virus. Another problem which arises is the difficulty in obtaining multiple recessive types from a cross. For example, let us suppose a cross is made between two parents which differ by three genetic factors in an endeavor to obtain a plant recessive for all three. In a diploid, the probability of obtaining such a triple recessive is 1 in 64. In an autotetraploid, on the other hand, the probability ranges from 1 in 8,000 to 1 in 46,656, depending upon the amount of double reduction operating to increase the proportion of recessives. Obviously, one must either grow very large populations or devise some method of obtaining the desired type in several steps.

Besides being of practical significance to the plant breeder, the study of autotetraploid genetics has another more indirect value which consists of furnishing an approach to the study of quantitative inheritance. This problem has been one of the most baffling and complex encountered in the field of genetics, and it is still far from being solved. Yet it is of the utmost economic importance, since a great majority of the genes which contribute to the value of cultivated plants are quantitative in character, and a thorough understanding of their hereditary behavior is greatly to be desired. Lindstrom (1935) states that "Comparison of quantitative inheritance in 2n and 4n affords another approach to the problem of quantitative inheritance", and he has taken the first step in this direction through a study of quant-

itative genes in tetraploid tomatoes.

SUMMARY

The segregation of four genetic characters was studied in colchicine induced tetraploids of Antirrhinum majus. It was found that these plants behave as true autotetraploids. Segregation for each factor was different, depending on the amount of double reduction due to crossing-over between the gene and the centromere. The amount of double reduction was greatest in the basic color factor, B, least in the A factor, and between these two extremes in the F and G factors. In all cases, large enough populations were grown to demonstrate a significant difference between the observed ratio and 35:1 ratio. In the case of the A factor, it was possible also to show a significant deviation from a 21:1 ratio. Differential viability was discussed and it was pointed out that this phenomenon might account for the frequently observed apparent differences in double reduction between duplex and simplex plants. Tetraploid segregation is dependent upon such variables as type of pairing, quadrivalent formation, number and position of chiasmata, and differential viability. Since the last three of these variables are in turn influenced by environment, it was pointed out that segregation is indirectly dependent upon the external conditions existing at the time of meiosis and during the early stages of seedling development.

LITERATURE CITED

Anderson, E. G. 1925. Crossing-over in a case of attached X chromosomes in Drosophila melanogaster. Genetics 10:403-417.

Baur, E. 1910. Vererbungs- und Bastardierungsversuche mit Antirrhinum. Zeits. Induktive Abstam. u. Vererbungslehre 3:34-98.

_____ 1924. Untersuchungen uber das Wesen, die Enstehung und die Vererbung von Rassenunterscheiden bei Antirrhinum majus. Leipzig. Biblioth. Genetica, Bd. 4.

_____, O. H. Frankel, B. Husfeld, N. Saulescu, and E. Schiemann. 1929. Koppelungserscheinungen bei Antirrhinum majus. Zeits. Induktive Abstam. u. vererbungslehre. 50:314-347.

Black, W. 1935. Studies on the inheritance of resistance to wart disease in potatoes. Jour. Genetics 30:137-146.

Blakeslee, A.F., J. Belling, and A. Farnham. 1923. Inheritance in tetraploid Daturas. Bot. Gaz. 76:329-373.

Bridges, C. B., and E. G. Anderson. 1925. Crossing-over in the X chromosomes of triploid females of Drosophila melanogaster. Genetics 10:418-441.

Cadman, C. H. 1942. Autotetraploid inheritance in the potato: some new evidence. Jour. Genetics 44:53-61.

Gra , E. B., and C. D. Darlington. 1928. The origin of new forms in Rubus. I. Genetica 9:241-278.

_____. 1932. Chromatid segregation in tetraploid Rubus. Nature 129:869.

_____and W.J.C. Lawrence, 1928. The genetics of garden plants. 2nd ed., London, Macmillan Co.

Darlington, C.D., 1929. Chromosome behaviour and structural hybridity in Tradescantiae. Jour. Genetics 21:207-286.

_____. 1937. Recent advances in cytology. 2nd ed., Philadelphia, P. Blakiston's Son and Co.

Dawson, C.D.F., 1941. Tetrasomic inheritance in Lotus corniculatus L. Jour. Genetics 42:49-72.

Darlington, C., and J.B.S. Haldane. 1931. Linkage in the tetraploid Primula sinensis. Jour. Genetics. 24:131-144.

_____. 1935. The Genetics of Primula sinensis III. Linkage in the diploid. Jour. Genetics 31:67-100.

Emerson, R. A., and M. M. Rhoades. 1933. Relation of chromatid crossing-over to the upper limit of recombination. Amer. Nat. 67:374-377.

Emsweller, S. L., and M. L. Ruttle. 1941. Induced polyploidy in floriculture. Amer. Nat. 75:310-328.

Ernst, H. 1938. Meiosis und Crossing-over, Zytogenetische Untersuchungen an Antirrhinum majus L. Zeits. f. Bot. 33:241-294.

_____. 1939. Zytogenetische Untersuchungen an Antirrhinum majus L. Zeits. f. Bot. 34:81-111.

Fisher, R. A. 1934. Statistical methods for research workers. 5th. ed. London, Oliver and Boyd.

Gregory, R. P. 1914. On the genetics of tetraploid plants in Primula sinensis. Proc. Roy. Soc. London Ser. B. 87:484-492.

Hackbarth, J. 1933. Untersuchungen uber Koppelung bei Antirrhinum majus II. Zeits. Induktive Abstam. u. Vererbungslehre 64:15-53.

Haldane, J. B. S. 1930. The theoretical genetics of autopolyploids. Jour. Genetics 22:359-372.

Krantz, F. A., and C. L. Becker, and R. M. Fineman. 1939. Incidence and inheritance of pollen sterility in the potato. J. Agric. Res. 58:593-609.

_____and Carl J. Eide. 1941. Inheritance of reaction to common common scab in the potato. J. Agric. Res. 63:219-231.

Kuckuck, H., and R. Schick. 1930. Die Erbfaktoren bei Antirrhinum majus und ihre Bezeichnung. Zeits. Induktive Abstam. u. Vererbungslehre 56:51-83.

_____. 1933. Untersuchungen uber Koppelung bei Antirrhinum majus. III. Zeits. Induktive Abstam. u. Vererbungslehre 65:243-252.

_____. 1935. Untersuchungen uber Koppelung bei Antirrhinum majus. VI. Zeits. Induktive Abstam. u. Vererbungslehre 69:335-344.

_____. 1938. Untersuchungen uber Koppelung bei Antirrhinum majus. VIII. Die Genkarte des Uni-chromosomes. Zeits. Induktive Abstam. u. Vererbungslehre 75:24-54.

Kutscher, Lotte. 1938. Untersuchungen uber Koppelung bei Antirrhinum majus. V. Zeits. Induktive Abstam. u. Vererbungslehre. 68:464-485.

Lawrence, W. J. C. 1929. The genetics and cytology of Dahlia species. Jour. Genetics 21:135-159.

_____. 1931. The genetics and cytology of Dahlia variabilis. Jour. Genetics 24:257-324.

Lindegren, Carl C. 1933. The genetics of Neurospora III. Pure bred stocks and crossing-over in N. crassa. Bull Torrey Bot. Club. 60:133-154.

_____, and Gertrude Lindegren. 1942. Locally specific patterns of chromatid and chromosome interference in Neurospora. Genetics 27:1-24.

Lindstrom, E. W. 1935. Segregation of quantitative genes in tetraploid tomato hybrids as evidence for dominance relations of size characters. Genetics 20:1-11.

_____. 1936. Genetics of polyploidy. Bot. Rev. 2:193-215.

Lunden, A. P. 1937. Inheritance studies in the potato. Meld. Norg. Landbr.Høisk. 17:1-156.

Mather, K. 1935a. Reductional and equational separation of the chromosomes in bivalents and multivalents. Jour. Genetics 30:53-78.

_____. 1935b. The combination of data. Ann. Eugenics 16:399-410.

_____. 1936. Segregation and linkage in autotetraploids. Jour. Genetics 32:287-314.

Muller, H. J. 1914. A new mode of segregation in Gregory's tetraploid Primulas. Amer. Nat. 48:508-512.

Oehlkers, Friedrich, 1940. Meiosis and crossing-over. Biol. Zentralbl. 60:337-348.

Onslow, M. Wheldale. 1907. The inheritance of flower color in Antirrhinum majus. Proc. Roy. Soc. London, Ser. B. 79:288-305.

_____. 1909. Further observations upon the inheritance of flower color in Antirrhinum majus. Roy. Soc. London, Evolution Com. Rpts. 5:1-26.

_____. 1910. Die Vererbung die Blutenfarbe bei Antirrhinum majus. Zeits. Induktive Abstam. u. Vererbungslehre 3:321-333.

_____. 1913. The flower pigments of Antirrhinum majus. I. Method of preparation. Biochem. Jour. 7:87-91.

_____, and H. L. Bassett. 1913. The flower pigments of Antirrhinum majus. II. The pale yellow or ivory pigments. Biochem. Jour. 7:441-444.

_____. 1914. The flower pigments of Antirrhinum majus. III. The red and magenta pigments. Biochem. Jour. 8:204-208.

Propach, H. 1934. Cytologische Untersuchungen an Antirrhinum. I. Planta 22:368-374.

Randolph, L. F. 1935. Cytogenetics of tetraploid maize. Jour. Agric. Res. 50:591-605.

Hanke, M. 1930. Faktorenkoppelung und Faktorenanalyse bei Antirrhinum majus. Zeits. Induktive Abstam. u. Vererbungslehre 53:235-378.

Rhoades, M. M. 1931. The frequencies of homozygosis of factors in attached X females of Drosophila melanogaster. Genetics 16:375-385.

_____. 1933. An experimental and theoretical study of chromatid crossing-over. Genetics 18:535-560.

Ruttle, M. L., and B. R. Nebel. 1939. Cytogenetic results with colchicine. Biol. Zentralblatt. 59:79-87.

Sansome, F. W. 1933. Chromatid segregation in Solanum lycopersicum. Jour. Genetics 27:105-132.

Schick, R. 1930. Untersuchungen uber Koppelung bei Antirrhinum majus. I. Koppelung bei Blattfaktoren. Zeits. Induktive Abstam. u. Vererbungslehre. 56:84-106.

_____ 1933. Untersuchungen uber Koppelung bei Antirrhinum majus. IV. Die Lokalisation von funf weiteren Genen im Auren-Chromosome. Zeits. Induktive Abstam. u. Vererbungslehre. 65:293-313.

_____. 1935. Untersuchungen uber Koppelung bei Antirrhinum majus. VII. Zeits. Induktive Abstam. u. Vererbungslehre. 69:345-373.

_____, and H. Stubbe. 1933. Die Gene von Antirrhinum majus. II. Zeits. Induktive Abstam. u. Vererbungslehre. 63:249-290.

_____. 1934. Die Gene von Antirrhinum majus. III. Zeits. Induktive Abstam. u. Vererbungslehre. 66:425-462.

Scott-Moncrieff, R. 1930. Natural anthocyanin pigments. I. The magenta flower pigment of Antirrhinum majus. Biochem. Jour. 24:752-766.

_____. 1936. A biochemical survey of some Mendelian factors for flower colour. Jour. Genetics 32:117-170.

Somme, A. Sverdrup. 1930. Genetics and cytology of the tetraploid form of Primula sinensis. Jour. Genetics. 23:447-509.

Sparrow, A. H., M. L. Ruttle, and B. R. Nebel. 1942. Comparative cytology of sterile intra- and fertile intervarietal tetraploids of Antirrhinum majus L. Am. Jour. Bot. 29:711-716.

Stevenson, F. J., E. S. Schultz, and C. F. Clark. 1939. Inheritance of immunity from Virus X (latent mosaic) in the potato. Phytopathology 29:363-368.

Upcott, M. 1935. The cytology of triploid and tetraploid Lycopersicum esculentum. Jour. Genetics 31:1-20.