1956

Evaluating the Mode of Inheritance in Tetraploid Alfalfa by Means of Genetic Intra-class Correlation

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EVALUATING THE MODE OF INHERITANCE IN TETRAPLOID ALFALFA BY MEANS OF GENETIC INTRA-CLASS CORRELATION

By

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A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science at South Dakota
State College of Agriculture
and Mechanic Arts

December 1956

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor

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ACKNOWLEDGEMENT

The author wishes to express his appreciation to Dr. M. W. Adams, Agronomist; Dr. W. W. Worzella, Head of the Agronomy Department; Dr. J. G. Ross, Agronomist; and other members of the Agronomy Department, South Dakota State College, for their encouragement and supervision in conducting this study. Thanks are due Mr. H. C. McKenzie, Professor of mathematics, for helpful suggestions during the course of the study.

Special thanks are due Mr. Dick Sowell and the photography laboratory staff for their advice and assistance in the preparation of the illustrations; to Mr. Everett Metcalf for helpful criticism during the preparation of the manuscript; and to Mrs. Virgil Riley for typing the thesis.

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INTRODUCTION

Observed segregation ratios for qualitative traits in tetraploid alfalfa have been interpreted on both disomic and tetrasomic modes of inheritance. In fact, some tetrasomic ratios cannot be distinguished from disomic ones (13) without intensive analysis. For quantitative traits, arbitrarily established segregation ratios in themselves are incapable of interpretation with respect to mode of inheritance.

Progressive change and fitness in species depend upon continuous variation; analogously, it may be reasonably inferred that artificial plant improvement too, rests upon the exploitation of continuous variation. The alfalfa breeder, in particular, to complement his art with science, must be able to select superior plants with a minimum of error and then to combine such plants in the most effective numbers, and in the most efficient manner. He must then be able to predict the behavior of given combinations in advanced generations. Application of the methods of biometrical genetics to the solution of these problems depends first upon a knowledge of the mode of inheritance. The main objective of this thesis was to determine the mode of inheritance of quantitative traits in tetraploid alfalfa.

Continuous variation, however, can only be studied through the statistical properties of the population. For purposes of this investigation, therefore, a statistic was required that would be amenable to both theoretical and experimental determination. It needed to be one to which some test of significance could be applied. A final requirement was that it be a statistic with theoretically different values dependent

upon mode of inheritance. These three requirements, rigorously adhered to, set the course of the investigation. The genetic intra-class correlation was found to fulfill these requirements satisfactorily in the analysis of first generation selfed (S_1) families. In this thesis, therefore, we have sought to evaluate the mode of inheritance, whether disomic or tetrasomic, by means of the genetic intra-class correlation.

REVIEW OF LITERATURE

Mode of Inheritance of Quantitative Traits

In an alfalfa inbreeding experiment of considerable proportions, Stewart (14) observed in S_1 families a high degree of uniformity. He stated that nearly 50 percent of 154 strains in the first generation of selfing were practically homozygous for one or more characters. The traits studied by Stewart were predominantly those showing continuous variation; namely, plant height, plant width, angle of erectness, stem diameter, and length and width of leaflet. The unusual degree of uniformity was attributed by Stewart to rather more self-fertilization than had been believed would normally occur under open-pollination situations. Stewart made no attempt to draw from his data any inferences with respect to mode of inheritance.

Because traits showing continuous variation are most important agronomically, they have been studied extensively in alfalfa. Among these have been percent leaves, number of stems, stem length, mature plant height, growth habit, root type, disease resistance, insect resistance, cold resistance, protein content, and ability to symbiose (2). Statisti-

cal properties related to the observed variation in these studies were in general not reported and no interpretations were offered on mode of inheritance.

Mode of Inheritance of Qualitative Traits

In contrast, where qualitative traits have been studied, a factorial analysis was generally attempted. Most of these were based on disomic inheritance, though in some cases a tetrasomic interpretation would have fit the data equally well, and in other cases the studies were not carried to the lengths necessary to distinguish critically the mode of inheritance. Stanford (13), however, studying one family of plants intensively, concluded that the family was segregating tetrasomically for flower color, and he observed quadrivalent formation in the mother plant, which enabled him to postulate the operation of double reduction at the flower color locus. No direct confirmation of double reduction was achieved in Stanford's paper, however, and indeed, his conclusions in that respect have been challenged (5).

More recently, evidence has been presented by Twamley (15) indicating that both disomic and tetrasomic segregation for flower color may be operative in tetraploid alfalfa. He suggested an autotetraploid origin for alfalfa, in which chromosome differentiation subsequently occurred to reduce the homology of certain pairs resulting in a shift to disomic or "semi-disomic" behavior.

Oldemeyer and Brink (11) found normal seed set and pollen in a hybrid alfalfa composed of 16 chromosomes from *M. media*, and 16 chromosomes from an autotetraploid *M. falcata* (doubled from the diploid state). They assumed that the *M. falcata* genomes substituted for and paired

readily with M. media genomes. If so, autotetraploidy would be indicated and a tetrasomic mode of inheritance should characterize normal alfalfa. No mention was made of the possibility, however, that in the meiotic cells of the hybrid, the 16 M. falcata chromosomes might pair inter se as 8 bivalents, and the 16 M. media chromosomes likewise, i. e. autosyndesis, the F_1 thus behaving strictly as an allotetraploid and giving disomic ratios.

More positive evidence that homology exists between the M. falcata and M. media genomes would be provided had normal pollen and seed set been observed in the F_1 hybrid of diploid M. falcata with diploid M. sativa.

In the diploid alfalfa nurseries at South Dakota State College, normal seed set has been noted in several inter-specific hybrids during the past two years, so the weight of the evidence, such as it is, tends to support an autoploid origin for common tetraploid alfalfa.

Nevertheless, studies of this kind, bearing on the origin and makeup of common tetraploid alfalfa, while suggestive and instructive, are not in themselves critical experiments so far as prevailing mode of inheritance is concerned.

Cytological Information

Grun (4) made an extensive study of the cytology in several clones of 4N alfalfa with particular emphasis on the frequency of multivalent pairing, the amount of variation in pairing from plant to plant, the degree and cause of lagging of chromosomes, and the effect on meiosis of different dates of fixation. His findings showed a limited number of quadrivalents, (as did Stanford's), and he stated that to a slight

extent autotetraploid origin can be assumed. Grun noted that there was a high degree of pairing irregularity in his material, and he suggested that the species is of recent origin as a tetraploid.

Mather's proposed value of alpha, depending upon quadrivalent formations, was noted by Little to be the most satisfactory explanation of observed tetraploid data (8). The index, alpha, was used by Mather to characterize observed ratios as a function of chromosomal behavior at meiosis. Alpha is a product of two cytological variables, a and g, where a equals the amount of genetic non-disjunction in a quadrivalent, and g equals the amount of equational separation of chromatids (8). These phenomena, jointly termed "double reduction", result in an increase in the proportion of homozygous gametes under inbreeding. Alpha is the symbol or measure of the degree of double reduction.

In order to have double reduction genes on sister chromatids must pass to the same pole at the first division. This phenomenon, known as genetic non-disjunction, a, is dependent upon the formation of quadrivalents or trivalents. Then, there must be at least one chiasma formed between the centromere and the gene in order to have equational separation, g. These cytological processes set the stage for double reduction and determine its frequency (alpha).

The value of alpha may vary from 0 to 1/3 depending upon the respective values of a and g. The range of a is 0 to 1/3 as quadrivalency varies from 0 to complete, and g changes from 0 to 1 as the cross-over percentage fluctuates between 0 and 50 percent.

Cytological phenomena which effect the ratios obtained in segregating families are mode of pairing, formation of quadrivalents, and

number of chiasmata (8). In cytological observations of M. sativa, Hanson found that his material had much lower quadrivalent frequency than that on which Grun reported in 1951. He suggested that the disparity in results was due to one or more factors, such as different environmental conditions, genotypes, or the limited sample examined (5). His conclusion that M. sativa is a segmental alloploid was based on, (a) the apparent prevalence of disomic inheritance, (b) the possibility that varying number of chracters may be inherited in a tetrasomic manner, and (c) the low frequency of multivalent associations at meiosis. Hanson also noted that if the original plant selected by Stanford had been duplex for a tetrasomic purple factor, P, and heterozygous for a disomic color factor, the presence of the non-segregating purple family in Stanford's nursery would not have to depend on the occurrence of double reduction. Atwood and Grun, in 1951, reported that no cases at that time indicated tetrasomic behavior only; however, some ratios have shown possibility of interpretation on either disomic or tetrasomic bases (2).

Bivalent pairing was noted by Julen in a report on hexaploid alfalfa (6). Twenty-four bivalents were found to pair regularly in a known allohexaploid, which was the product of tetraploid Ultuna alfalfa and octoploid M. sativa artificially produced with colchicine agar. Grun suggested that the genomic constitution of this hexaploid was AAAABB, with the necessity for at least one A set pairing freely with one B set in autoploid fashion (4). This case is more definitive than some others in favor of autoploidy.

Bolton and Greenshields reported a diploid form of Medicago sativa that was self sterile and cross sterile, except when crossed to diploid

M. falcata. The presence of two chromosomal satellites in this diploid, whereas four satellites had been observed in tetraploid M. sativa, was an indication that tetraploid forms may be autotetraploid (3).

MATERIALS AND METHODS

Parental Material

Parental material for this study was derived from a mixed hybrid population descended through several generations of open pollination from a cross of M. falcata by M. sativa made in 1914 at South Dakota State College. The population had been maintained in the same location at Brookings for about 40 years. During much of that time the field was part of a golf course, where vegetation was clipped regularly close to the ground. A group of 16 plants was chosen as a representative sample of the population.

Establishment of S_1 and Clonal Families

The 16 plants were transferred to the greenhouse in October 1953 in order to secure self-pollinated seed for establishment of an S_1 nursery the following season. Preparations were also made to establish an S_2 generation from one-half of the original 16 selections, for comparison and also for sterility studies. Self pollinations, using sterile technique, were carried on from November through April, with varying degrees of success. Seedlings were established in a field nursery, along with a set of clonal cuttings from each of the parent selections.

The parent clones were studied for cytological behavior during the time plants were in the greenhouse and also later when plants were transferred to the field nursery.

Notes Taken on Morphological Traits

During August and September, when the plants had sufficient time to exhibit their characteristic growth behavior, notes were taken in the field on the following traits: growth habit (erectness or prostrateness), foliage color, stem length, leaf width, leaf length, and internode length. Four traits were chosen for their descriptive qualities as to growth type and overall growth form of the plant. These traits—growth habit, stem length, leaf score (leaf width x leaf length), and internode length—had been studied before by other investigators (see lit. rev.).

Analysis of 1954 Data

After calculation of genetic variances and covariances, it was then possible to calculate intra-class correlations for each trait in all families using the first generation selfed families as class.

To illustrate calculation of r_I, the intra-class correlation, from an analysis of variance assume there are n families of k plants each, then the form used was as follows:

Source of Variation	DF	SS	MS	MS EXP
Between Families.....	n - 1		MS I	$V_e+V_w+kV_b$
Between Plants Within Families.....	n(k - 1)		MS II	V_e+V_w
Within Plants From Clonal Analysis.....	(estimated from clonal analysis)		MS III	V_e
Total.....	nk - 1			

Significance of MS I provides evidence that the families are genetically different; the variance component measuring the magnitude of the family difference between family means. Similarly, among S_1 families, there are expected to be genetic differences between plants within families. This can be found as MS II - MS III = V_w.

There has been isolated, thus, the genetic variances between and within families. These estimates are the bases for calculation of the intra-class correlation. If the within family genetic variance is zero, then all plants of a family will resemble each other very closely, and the r_I will be one. On the other hand, when V_w is very large, relative to the family variance V_b, r_I approaches zero.

The statistical concept of the intra-class correlation, r_I, can be visualized more clearly in the following manner: Let X and Y be members of the same family, but let Z be a member of another family, then $r_{XY} = \text{Cov } XY/\sqrt{V_x \cdot V_y}$; $V_{(X-Y)} = V_X + V_Y - 2 \text{ Cov } XY = B$, (the within family variance); $V_{(X-Z)} = V_X + V_Z - 2 \text{ Cov } XZ = A + B$. (Cov XZ = 0 because of no relationship). Since $V_X = V_Y = V_Z$, the extra variance between members of different families as a fraction of total variance between them, i.e. A/A + B, is: $V_{(X-Z)} - V_{(Y-Y)}/V_{(X-Z)} = 2 \text{ Cov } XY/ 2 V_Y = r_{XY}$. An unbiased estimate of A is critical to the observed r_I, for if by chance, A = 0; $r_I = 0$, irregardless of the size of B. Reliance must be placed on the parents as being truly a random sample of the population. if the r_I derived from A and B is to represent accurately the genetic intra-class correlation of that population.

It is evident that r_{II}, the correlation between members of the same family, here is actually intra-class correlation (9).

Theoretical Determination of Intra-class Correlation

Comparisons were made with theoretical values of the intra-class correlation derived by various means. A method for determining the r_I made use of path coefficients (17). Also, theoretical arithmetic models were set up and variances calculated for determination of intra-class correlations based on various assumptions, such as disomy, tetrasomy, dominance, and additive gene action. A third method was the use of an inbreeding coefficient ratio for determining the intra-class correlation. The use of path coefficients gave results most appropriate for purposes of this study, hence greatest reliance was placed on this method.

1954 Discriminant Function

After analysis of the traits individually over all families and for each family separately, a method of combining characters was used, that involved the calculation of a discriminant function (10). The discriminant function as used in this study was intended to serve a two-fold purpose. In the first place, the various families appeared to be visibly distinct as to type, and yet it was obvious that this overall growth form could not be expressed by one score except in a most subjective way. A subjective scoring system would not be particularly useful genetically however; a metric score was required. The discriminant based on objective measurements, insofar as possible, of the basic variable traits that seemed to constitute growth form, more accurately describes the observed variation in form, and does it metrically so that it is useful in a genetic analysis. Secondly, since the discriminant is

a linear combination of certain underlying variables which are uncorrelated among themselves, the function itself behaves as a single variable in analysis, i.e. it possesses a mean and variance. Being based on the variation of several quantitative traits, its genetic variance probably results from gene differences at many loci, randomly distributed over several chromosomes. Linked gene effects thus cannot be a serious source of bias; the possible effect of complicated gene inter-actions can not be evaluated.

The intra-class correlation based on a discriminant function would seem to reflect, unavoidably, an average behavior of a large of the germ plasm in inheritance so far as mode of inheritance is concerned.

Analysis of 1955 Data

The estimate of error by clonal replication was not satisfactory in all respects; therefore, it was determined that another set of data should be taken in such manner as to estimate error more satisfactorily. Multiple measurements on the same plant was adopted as the best method of estimating the error. This method of taking notes yielded two sets of data for each trait, on which a complete analysis was made as before. The form used in 1955 was as follows, where n = number of families, k = average number of plants per family, and r = determinations per plant:

Source of Variation	DF	SS	MS	MS EXP
Between Families	n-1		MS I	$V_e + rV_w + rkV_b$
Between Plants Within Families	n(k-1)		MS II	$V_e + rV_w$
Within Plants	nk(r-1)		MS III	V_e
Total	nkr-1			

1955 Discriminant Function

Only three traits were used in the discriminant function the second time; the trait which was most affected by environmental variables being eliminated in 1955. The new method of error estimation resulted in statistics on which valid conclusions could be based.

EXPERIMENTAL RESULTS

Self-Fertility

Parental plants used varied considerably in self-fertility; first generation selfed families ranged in size from 26 to 98 plants. In the S_2 generation, on account of increased self-incompatibility of S_1 parents, the families were in general quite small in size, ranging from four to forty-three plants per family. No relationship was evident between uniformity of progenies and readiness with which parents would set seed by selfing.

Cytological Observations

In no instance were numerous quadrivalent formations found in meiotic cells of the parent clones (Figure 1 and Figure 2). A number of the plants were examined at meiotic pachytene and very few multivalent pairings were noted, which indicated that the frequency of double reduction was also low. Incomplete union of the chromosomes throughout their entire length in the pachytene stage was typical of the cells examined (Figure 2). Some of the chromosomes appeared to be pairing doubly throughout their length in twos and fours. Limited number of quadrivalents were also observed at diakinesis (Figure 1), to substantiate the findings at pachytene. If there had been many multivalent formations during prophase,

they apparently had terminalised before reaching diakinesis. Differences in pairing relationships may be observed in the same plant on different dates of collection, and between plants at the same or different dates (4); therefore, observations over a number of days, and involving a number of plants, would be expected to be significantly different. As Grun noted, alfalfa chromosomes are very small, a fact which may have been instrumental in partially inhibiting the formation of quadrivalents.

The principal effect of quadrivalency is on the rate of approach to homozygosity. Double reduction, dependent on quadrivalency, increases slightly the proportion of homozygous gametes; and in selfing speeds up the rate at which homozygosity is approached. Since quadrivalent frequency, though variable among plants and in time, appears on the average to be low, no great influence can be attributed to the phenomenon of double reduction in alfalfa; particularly is this likely to be the case with quantitative characters.

1954 Field Data

The observations of uniformity obtained in this study might very well parallel the uniformity which Stewart noted in 1931. Uniformity was noted in some S_1 families upon visual inspection; however, with continuously varying traits it is necessary to measure and analyse impartially the variability in each family to determine quantitatively how much uniformity they actually exhibit.

This measurement was accomplished by calculating from the data the genetic intra-class correlation, r_I. Since r_I was based on variance components, it was first necessary to find the appropriate mean squares from analysis of variance of S_1 families and replicated clonal parents.

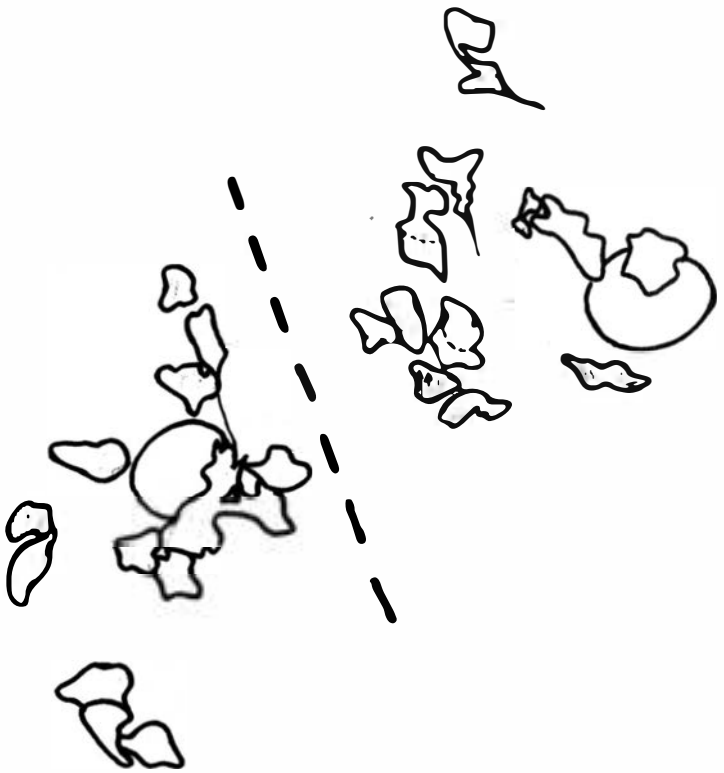


Figure 1. Camera lucida drawing of two M. sativa cells at diakinesis showing the pairing relationships.

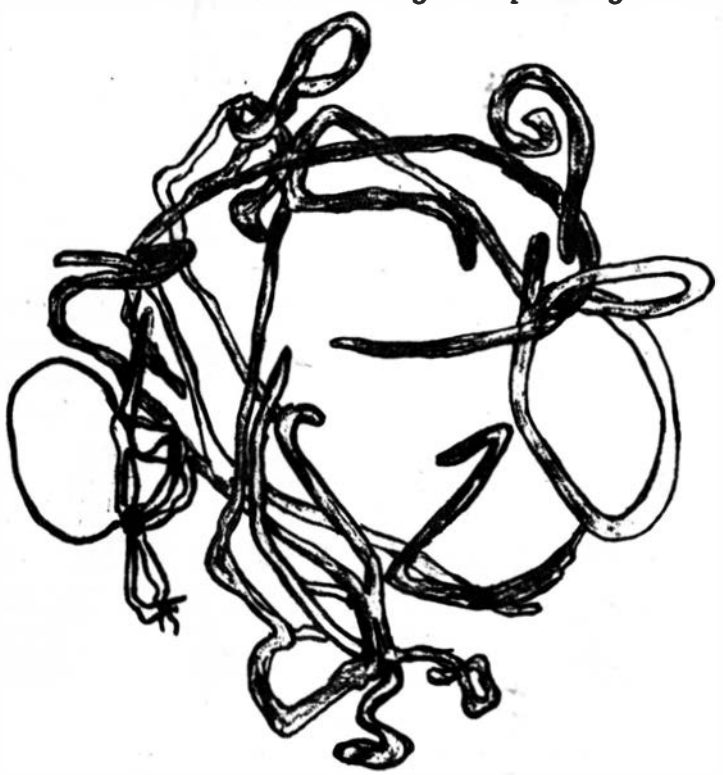


Figure 2. Camera lucida drawing of one M. sativa cell at pachytene showing the pairing relationships.

1954 Analysis of variance and covariance

Variance components, necessary for the intra-class correlations, are to be found in Table 1.

1954 Discriminant function

For reasons set forth earlier, it was decided to calculate a discriminant function. Such a function, based on variation of several component characters, becomes, in effect, a "super-character", encompassing the independent variation of its components. Statistically, it tends to maximise the genetic variation as contrasted to the non-genetic variation, and should normally result in a higher variance ratio than any one of the component factors. It is important to point out that, as calculated here, it does not differentially effect the variances that enter into the r_I.

The discriminant function for 1954, the calculation of which is given in Appendix A, takes the following form:

$$D = 37.56\ (X_1) - 0.29\ (X_2) + 1\ (X_3) - 1.88(X_4).$$

This linear equation was then expanded by squaring both sides to give a new equation of symbolic variances and covariances in terms of the component variables X_1 to X_4,

$$D^2 = [37.56\ (X_1) - 0.29\ (X_2) + 1\ (X_3) - 1.88\ (X_4)]^2.$$

When the actual values of these variance and covariances were inserted and algebraically summated, there resulted the analysis of variance of the discriminant itself (Table 2).

Table 1. Summary of 1954 analysis of variance and covariance

Source of variation	DF	SS	MS	F
Clones:				
X_1 (Growth Habit)				
Between Families	15	28.37	1.891	**
Within Families	368	16.12	0.044	
X_2 (Stem Length)				
Between Families	15	30.02	2.001	**
Within Families	368	48.68	0.132	
X_3 (Leaf Score)				
Between Families	15	4,728.03	315.202	**
Within Families	368	5,345.92	14.500	
X_4 (Internode Length)				
Between Families	15	183.66	12.244	**
Within Families	368	255.21	0.694	
S_1 Families:				
X_1 (Growth Habit)				
Between Families	15	183.96	12.264	**
Within Families	990	237.00	0.239	
Clonal Error			0.044	
X_2 (Stem Length)				
Between Families	15	45.06	3.004	**
Within Families	990	84.98	0.086	
Clonal Error			0.132	
X_3 (Leaf Length)				
Between Families	15	8,432.22	562.281	**
Within Families	990	21,008.18	21.220	
Clonal Error			14.500	
X_4 (Internode Length)				
Between Families	15	416.47	27.765	**
Within Families	990	719.12	0.786	
Clonal Error			0.694	

** Significant at 1% level
(k_0 for 1954 data = 62.19)

Source of covariation	DF	SS	MS	F
Clones:				
X_1X_2				
Between Families	15	1.67	0.111	**
Within Families	368	7.39	0.020	
X_1X_3				
Between Families	15	-8.02	-0.535	**
Within Families	368	48.09	0.131	
X_1X_4				
Between Families	15	40.60	2.707	**
Within Families	368	11.24	0.031	
X_2X_3				
Between Families	15	29.19	1.946	**
Within Families	368	108.53	0.295	
X_2X_4				
Between Families	15	18.45	1.230	**
Within Families	368	39.89	0.108	
X_3X_4				
Between Families	15	-32.75	-2.183	**
Within Families	368	241.13	0.655	
S_1 Families:				
X_1X_2				
Between Families	15	35.78	2.385	**
Within Families	990	23.07	0.023	
Clonal Error			0.020	
X_1X_3				
Between Families	15	-175.77	-11.718	**
Within Families	990	-6.16	-0.006	
Clonal Error			0.131	
X_1X_4				
Between Families	15	171.46	11.431	**
Within Families	990	24.58	0.025	
Clonal Error			0.031	
X_2X_3				
Between Families	15	43.75	2.917	**
Within Families	990	141.41	0.143	
Clonal Error			0.295	
X_2X_4				
Between Families	15	85.16	5.677	**
Within Families	990	98.96	0.100	
Clonal Error			0.108	
X_3X_4				
Between Families	15	-395.88	-26.392	**
Within Families	990	793.67	0.800	
Clonal Error			0.655	

Table 2. Analysis of variance of 1954 discriminant function

Source of Variation	DF	SS	MS
Between Families.....	15	770,894.04	51,392.94
Within Families.....	990	2,211,238.73	2,233.57
Within Plants.....Estimated from clonal analysis		562,450.27	1,528.40

1954 Intra-class correlations

The genetic intra-class correlations were calculated on each individual trait over all families, then for each family separately. The intra-class correlations for 1954 are summarized in Table 3.

Table 3. Intra-class correlations for 1954

Source (S_1 families)	rI assuming error	rI assuming no error
X_1 (Growth Habit).....	0.4965	0.44
X_2 (Stem Length).....	6.8910	0.95
X_3 (Leaf Score).....	0.5642	0.28
X_4 (Internode Length).....	0.9157	0.37
D (Discriminant Function).....	0.5285	

The intra-class correlations for two of the characters; X_2 (stem length), and X_4 (internode length) were much too high, and this logically resulted from insufficient error control. A measure of the lack of error estimation can be gained by comparison with intra-class correlations on the same material assuming no error whatsoever. An intra-class correlation calculated with the assumption of no error would be a minimum value. As was explained in the methods, measurement error and variation due to differences in cultural factors, soil heterogeneity and other uncontrollable factors should have been accounted for by an analysis of replicated

clonal families. This unexpected amount of variation was believed due to differential crown development and initiation of new shoots on vegetatively propagated cuttings within a clonal genotype. The differential growth factor was not noticeable in the S_1 families, which were started as seedlings.

It appears that the r_I for traits X_1 and X_3 (growth habit and leaf score, respectively), are more in accord with expected values, i.e. somewhat higher than the corresponding values wherein no error was assumed.

The r_I of 0.5283 over all families for the discriminant function of the four factors—growth habit, stem length, leaf score, and internode length—cannot be considered completely reliable in view of the lack of error control for certain of the components. Calculation of the r_I for the stem length trait alone gave an unreasonably high value. The error represented in this calculation was given very little weight in the discriminant function, -0.29 (X_2), in relation to the other traits, especially X_1, which was 37.56 (X_1).

It is possible too that the errors were compensating to a degree that would result in a meaningful estimate, but until additional estimates become available, there is no way of evaluating this initial figure.

1955 Field Data

The provision for new error control, made in 1955, was quite effective as may be seen from results of analysis of 1955 data.

1955 Analysis of variance and covariance

A summary of the analysis of variance and covariance for data on the three morphological traits—X_1 (growth habit), X_3 (leaf score), and X_4 (internode length)—in addition to flower color notes, are found in

Table 4. Summary of 1955 analysis of variance and covariance

Source of Variation	DF	SS	MS
X_2 (Growth Habit)			
Between Families	15	49,864.88	3,324.33
Within Families	946	38,036.42	40.21
Within Plant Error	962	22,480.56	23.39
X_3 (Leaf Score)			
Between Families	15	694,715.09	46,314.34
Within Families	946	848,322.57	896.75
Within Plant Error	962	40,346.42	41.98
X_4 (Internode Length)			
Between Families	15	104,764.24	6,984.28
Within Families	946	180,557.02	190.86
Within Plant Error	962	13,341.85	13.88
P (Purple-Qualitative)			
Between Families	15	49.049	3.270
Within Families	974	62.402	0.064
Pq (Purple-Quantitative)			
Between Families	15	458.286	30.552
Within Families	974	741.456	0.761
Yq (Yellow-Quantitative)			
Between Families	15	180.40	12.027
Within Families	974	551.22	0.566
Source of Covariation:			
X_1X_3			
Between Families	15	39,926.45	2,661.76
Within Families	946	9,219.30	9.75
Within Plant Error	962	408.69	0.43
X_1X_4			
Between Families	15	57,626.75	3,841.78
Within Families	946	15,392.47	16.27
Within Plant Error	962	28.31	0.03
X_3X_4			
Between Families	15	22,380.26	1,492.02
Within Families	946	64,260.10	67.93
Within Plant Error	962	434.62	0.45

(k_o for 1955 data= 59.5)

Table 4. The flower color notes, taken in 1955, were also analyzed in order to obtain the respective intra-class correlations. The components of flower color measured were P (purple-qualitative), Pq (purple-quantitative), and Yq (yellow-quantitative).

1955 Discriminant function

A discriminant function was calculated from the 1955 data, but only on three traits instead of the four used in 1954. This analysis as presented in appendix A yields the discriminant function, $DF = 1(X_1) - 2.72(X_3) + 3.39(X_4)$.

The procedure of applying the discriminant to the data followed in 1954 was also followed in 1955. A summary of the analysis of these data is presented in Table 5.

Table 5. Analysis of variance of 1955 discriminant function

Source of Variation	DF	SS	MS
Between Families.....	15	6,515.67	434.38
Between Plants Within Families.....	946	7,197.47	7.61
Within Plants Error.....	962		0.51

1955 Intra-class correlations

Intra-class correlations were calculated as in 1954. A summary of the intra-class correlations for 1955 may be found in Table 6.

The discriminant function calculated on 1955 data involved only three traits, X_1 (growth habit), X_3 (leaf score), and X_4 (internode length), eliminating the one variable which contributed the most environmental

Table 6. Intra-class correlations for 1955

Intra-class Correlations Over All Families	
X_1 (Growth Habit)	0.767
X_3 (Leaf Score)	0.472
X_4 (Internode Length)	0.379
D (Discriminant Function)	0.503
P (Purple-Qualitative)	0.450
P_q (Purple-Quantitative)	0.390
Y_q (Yellow-Quantitative)	0.250
X_1 (Rep 1-Growth Habit)	0.444*
X_3 (Rep 1-Leaf Score)	0.589*
X_4 (Rep 1-Internode Length)	0.451*
X_1 (Rep 2-Growth Habit)	0.446*
X_3 (Rep 2-Leaf Score)	0.361*
X_4 (Rep 2-Internode Length)	0.356*

* Calculated assuming no error

Intra-class Correlations for Individual Families

Family	X_1	X_3	X_4
1	0.9603	0.3978	0.5512
2	0.7340	0.5506	0.2672
3	0.8609	0.6259	0.4139
4	0.7783	0.5495	0.4265
5	0.6998	0.5125	0.3433
6	0.6043	0.5602	0.5849
7	0.7667	0.6830	0.5755
8	0.7210	0.4618	0.2752
9	0.6029	0.5843	0.3716
10	0.7766	0.6028	0.4681
11	0.6662	0.2919	0.4025
12	0.8030	0.5854	0.4130
13	0.4250	0.4813	0.3725
14	0.0287	0.4738	0.5016
15	0.7626	0.5934	0.3573
16	0.9420	0.2069	0.3382

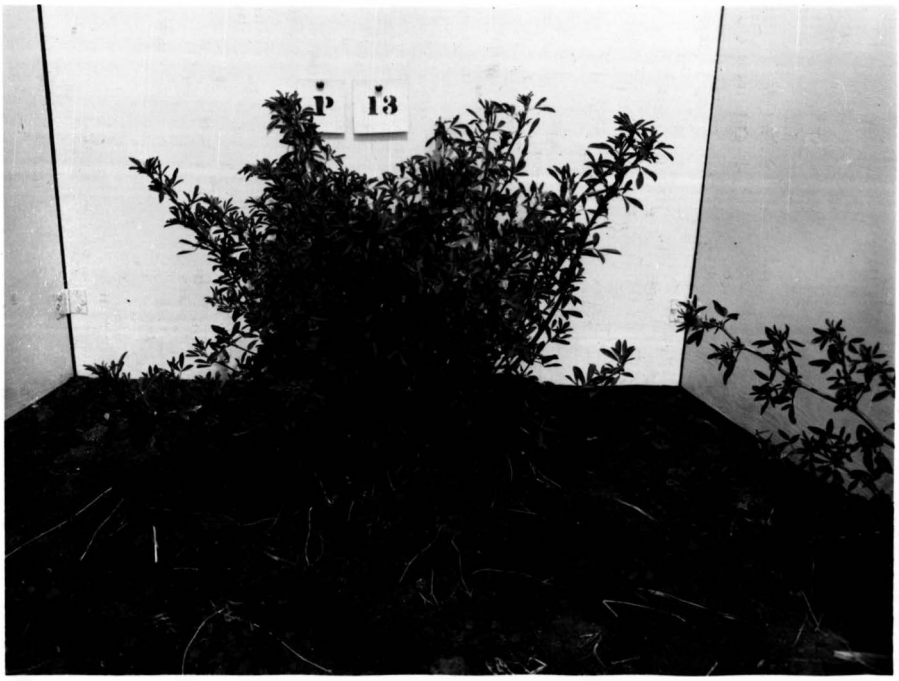
variance in 1954. To determine within plant variation, duplicate measurements were made on each plant; therefore, two sets of data for each trait were used in the 1955 analysis.

Pictorial Comparisons with Intra-class Correlations

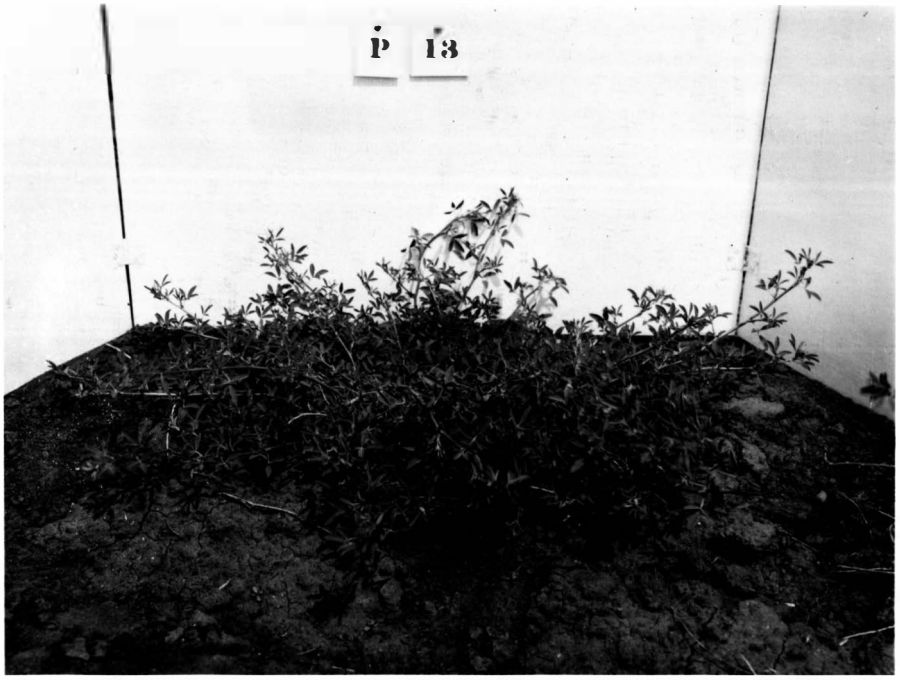
Family 13 gave the lowest growth habit intra-class correlation on the individual family basis, 0.4250 for 1955. This particular family was noticed to be especially variable in the field. Others, such as families 1, 14, 15, and 16 were remarkably uniform for growth habit in the field.

Pictures were taken to contrast the differences in appearance of families with low intra-class correlations with those yielding high intra-class correlations. Family 15, whose intra-class correlation for growth habit was 0.7626 in 1955 contrasts with family 13 as may be seen in pictures 1 through 8. Picture 2 and 3 show close-up evidence of the wide range in growth type exhibited in family 13. An overall picture of the entire family can be seen in picture 4. Other families exhibited varying degrees of uniformity with corresponding values of intra-class correlation for the growth habit trait. Pictures, 5, 6, and 7 show uniformity of growth type in family 15 and 1, respectively.

Picture 1. Clonal parent (family 13), intermediate growth habit.



Picture 2. S_1 plant (family 13), upright growth habit.

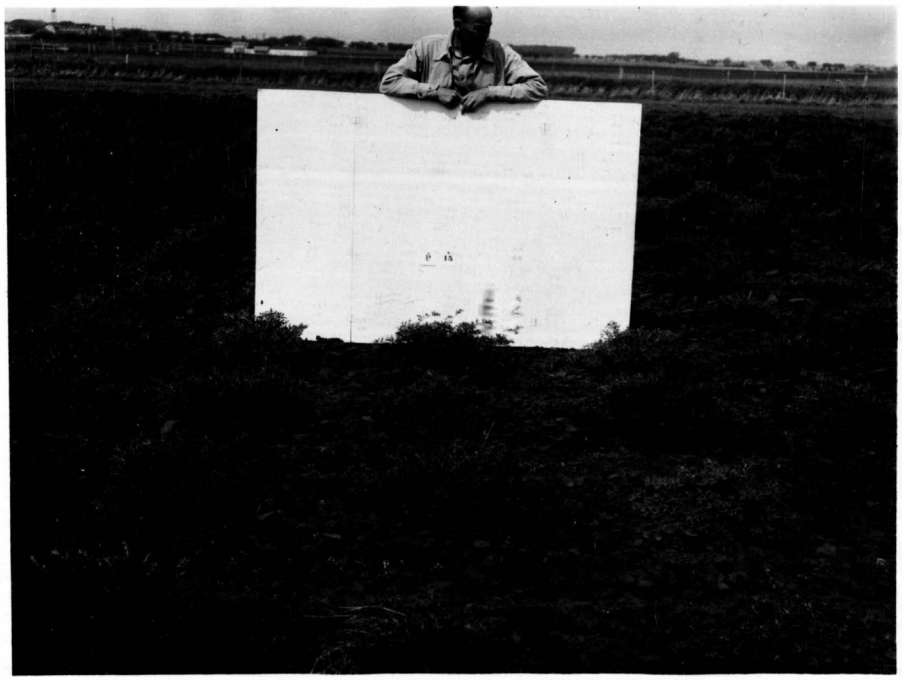


Picture 3. S_1 plant (family 13), decumbent growth habit.

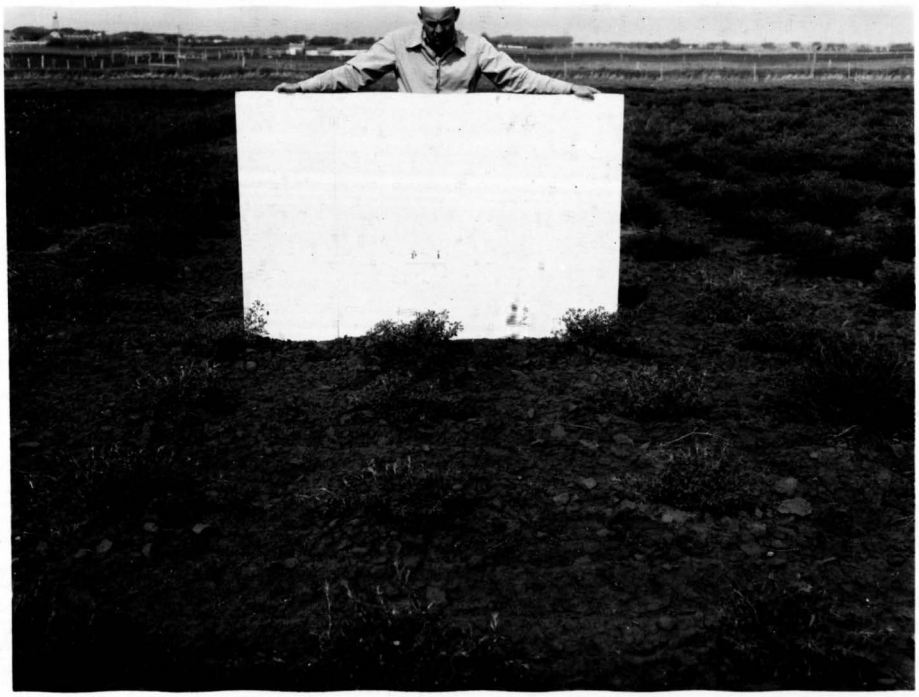
Picture 4. 9 S_1 plants (family 13), segregating growth habit.

Picture 5. S_1 plants (family 13, bounded by the stakes), segregating growth habit.

Picture 6. S_1 plants (family 15, bounded by the stakes), uniform growth habit.



Picture 7. 9 S_1 plants (family 15), uniform growth habit.



Picture 8. 9 S_1 plants (family 1), uniform growth habit.

Theoretical Values of Intra-class Correlation

To evaluate a population with respect to mode of inheritance by means of genetic intra-class correlation comparisons must be made with theoretical values of intra-class correlation.

Wright's path coefficients

Such theoretical values have been determined for the disomic and the tetrasomic cases, employing Wright's method of path coefficients (17). Only the situation wherein gene effects are additive is considered (Appendix B).

By this method r_I for the disomic organism is 2/3 or 0.67; whereas, the r_I for the tetrasomic organism is 2/7 or 0.29.

Inbreeding coefficient ratio

A second method that was used depends on the fact that as inbreeding progresses more and more of the genetic variance comes to be between families and less and less within families. The relative proportion between and within families at any given level of inbreeding is a function of F, the inbreeding coefficient. This relationship can be stated in the form 2F/1-F; at fixation F equals 1, and the total variance is twice that under random mating and it all lies between families. The within family variance goes to zero as it should when homozygosity has been attained (17).

The intra-class correlation would thus be given by 2F/2F + (1-F) which will equal 2F/1+F. This relationship is valid only for additive genetic variance. Application to disomic plants, wherein F equals .5, (r_I = 1/1+.5 or 0.67), gave the same value obtained from path coefficients.

For tetrasomic plants, F equals 1/6 after one generation of

selfing (Appendix B). Therefore, as before, $2F/1+F$ equals 0.333/1.167 or 0.29, the same value obtained for tetrasomic behavior by means of path coefficients for the tetrasomic case.

Arithmetic models

In addition to other methods, attempts were made to find theoretical values of r_I using arithmetic models. These models are identical to those developed by Mather (1949) for variances between and within F_3 families. Using such models, there was definite indication that dominance would result in lowering the r_I; in the diploic case with complete dominance r_I would fall to 0.60.

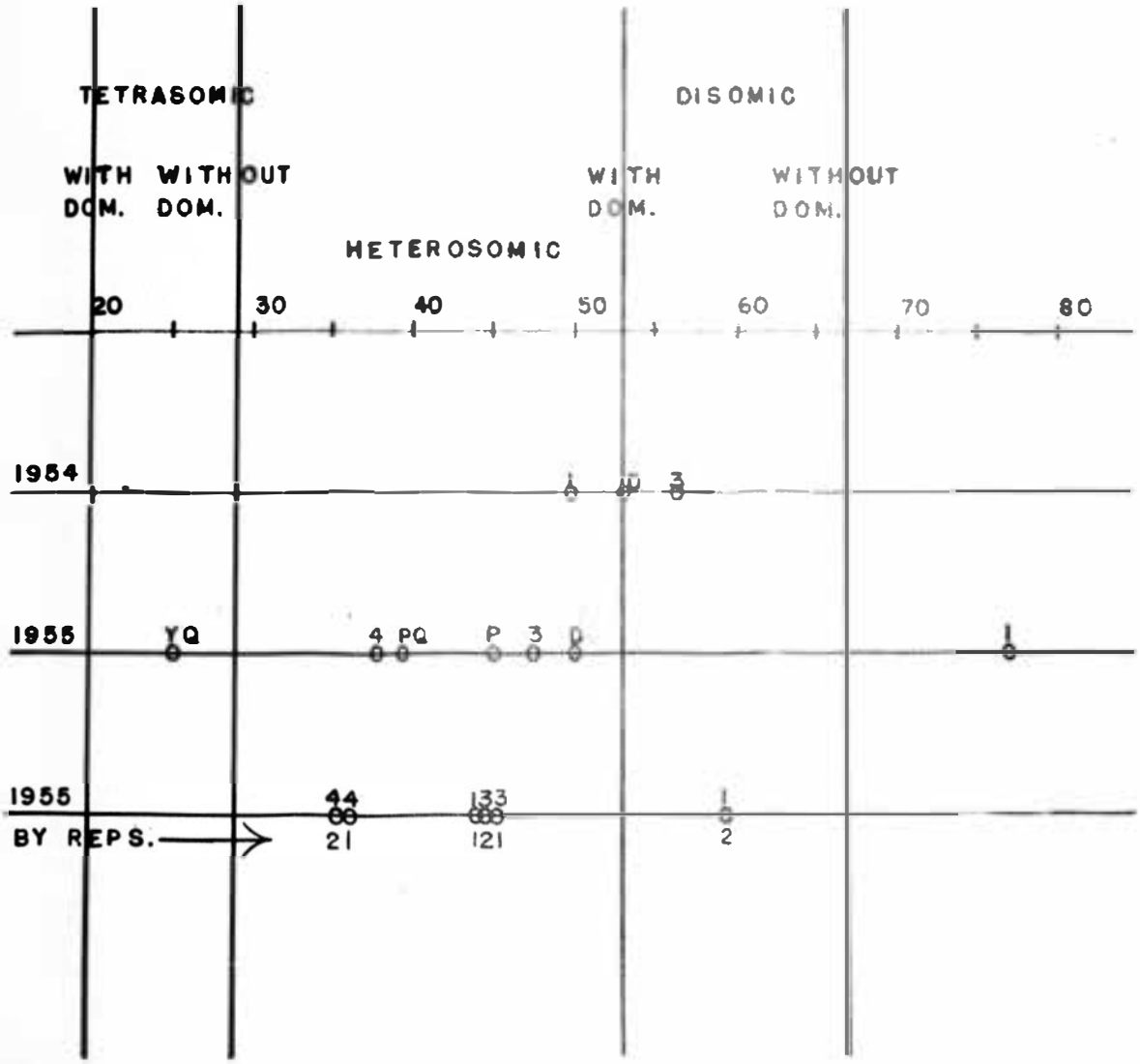
Wright (17) has shown with path coefficients that in the case of dominance, r_I in diploic organisms is equal to 0.53; extension of his formula to the tetrasomic case gives an r_I of 0.20.

It is evident that the general effect of gene interactions is to lower the correlation between members of the same family. In tetraploids dominance may occur as in diploids, but there are several additional possibilities for genic interactions not accounted for by Wright's formula, $r_{oo} = 2a^2b^2 (1+m)/1+F$ (17).

A comparison between theoretical values of intra-class correlation and experimentally determined values may be portrayed graphically as in Figure 3.

Tests of Significance

To test the validity of the observations, it was necessary to make comparisons of expected and observed r_I's. This was attained by transformation of the r_I values to z and applying a t-test(12). The calculated value of z always yields a variance equivalent to $1/n-3$; therefore, it is



LEGEND OF OBSERVED INTRA-CLASS CORRELATIONS:

1- Trait 1 (Growth Habit)
3- Trait 3 (Leaf Score)
4- Trait 4 (Internode Length)
D- Discriminant Function

P- Purple-Qualitative
PQ- Purple-Quantitative
YQ- Yellow-Quantitative
1955 replications 1 and 2 are identified below the line.

Figure 3. Graphical comparison of theoretical intra-class correlations with observed intra-class correlations

possible to find a probability level between theoretical and observed intra-class correlations by using the average number of plants per family as n. Probability levels from the t-tests are found in Table 7.

Table 7. Probability levels from t-test for significance between theoretical and observed intra-class correlations

		Theoretical r_I values:	
		Disomic No Dominance 0.667	Tetrasomic No Dominance 0.286
Source	Observed r_I	Probability level	
1954:			
X_1 (Growth Habit)	0.4965	0.05	0.10
X_2 (Stem Length)	6.8910	0.01	0.01
X_3 (Leaf Score)	0.5642	0.20	0.01
X_4 (Internode Length)	0.9157	0.01	0.01
D (Discriminant Function)	0.5285	0.10	0.05
1955:			
X_1 (Growth Habit)	0.767	0.20	0.01
X_3 (Leaf Score)	0.472	0.05	0.10
X_4 (Internode Length)	0.379	0.01	0.50
D (Discriminant Function)	0.500	0.10	0.10
P (Purple-Qualitative)	0.450	0.02	0.20
P_Q (Purple-Quantitative)	0.390	0.01	0.40
Y_Q (Yellow-Quantitative)	0.250	0.01	0.50
1955:			
X_1 Growth Habit-Rep 1)	0.444*	0.02	0.20
X_3 Leaf Score-Rep 1)	0.451*	0.02	0.20
X_4 Internode Length-Rep 1)	0.361*	0.01	0.50
X_1 Growth Habit-Rep 2)	0.589*	0.40	0.01
X_3 Leaf Score-Rep 2)	0.446*	0.02	0.20
X_4 Internode Length-Rep 2)	0.356*	0.01	0.50

*Calculated on each replication separately assuming no error

The levels of probability show a higher degree of coincidence between the theoretical and the observed for the tetrasomic case than for the disomic case. In only two cases, in 1955, does the probability get very high for the disomic case. The first is replication 2 of the growth habit trait in which no error was used for calculation, and the second is the combined replication 1 and 2 for growth habit. This difference between replications 1 and 2 for the growth habit trait is undoubtedly due to the fact that replication 1 was data which was taken in 1954; whereas, replication 2 was taken in 1955. Upon examination of the scores for individual plants it was apparent that a marked (genotype x year) interaction effect was being manifested. This effect showed up in the error term for I_1 and finally biased the combined years r_I upward considerably. The fact that most cases are neither strictly disomic nor tetrasomic, but lie generally somewhere between the two, may indicate that both disomic and tetrasomic behavior are operative in determining the genetic variance of some traits. This condition of joint influence by both disomy and tetrasomy may be termed "heterosomy."

DISCUSSION

At the inception of this study the major problem to be solved was whether mode of inheritance in tetraploid alfalfa was disomic or tetrasomic. During the course of the study it became apparent that there were six (6) possibilities to be considered; disomy with or without dominance, tetrasomy with or without dominance, tetrasomy with double reduction, and heterosomy.

In order to evaluate these hypotheses it was necessary to consider double reduction, dominance, natural selection, gene frequency, and the statistical concept of sampling from a normal distribution.

Disomy with no dominance

In the case of disomy with no dominance, the theoretical r_I value of .67 is far above the range of any of the observed r_I's. Only the r_I for one trait in 1955, (X_1) growth habit, had very high probability level of fitness to the theoretical, and this was explained to be due to a large clone x years interaction, which influenced the intra-class correlation upward. There is little likelihood, in sampling from a population wherein disomy is the mode of inheritance, that all observed values will fall below the parameter 0.67. The hypothesis of disomy cannot, on this account, be accepted for the quantitative traits studied. There is, of course, the possibility that strict disomy might result for a trait conditioned by only one or two loci. In the present study no such effects were observed.

Disomy with dominance

Additive gene action is the simplest mechanism that can be assumed although possibly the most meaningful for continuously varying traits; nevertheless dominance must be considered as a possibility. Complete dominance decreases the theoretical intra-class correlation to 0.53 for the disomic case, as determined by path coefficients. This value of r_I lies at the upper end of the range of observed intra-class correlations. Integration of theoretical and observed values of the intra-class correlation by the statistical concept of the normal curve is dependent upon a distribution of observed values in a normal fashion

about the theoretical intra-class correlation as the mean. Significantly, if the observed values represented the disomic case with dominance there would be expected a sampling distribution of these values around 0.53 as the mean of that distribution. This is not the case.

Also, it is quite unlikely that complete dominance would be operating to any great degree with traits which depend upon numerous genes distributed over several chromosomes. Although dominant disomic loci may well be operative, in general, they would not be detected unless a trait conditioned by only one or two loci were being studied. The r_I's obtained in the present study are based on multigenic variance, and it is illogical to assume that complete dominance operates for every such locus.

Tetrasomy with no dominance

Non-selective chromosomal pairing where no dominance is involved in the tetraploid gives an r_I of 0.29. A normal distribution of sample r_I's for the tetrasomic case with no dominance then would be centered on 0.29 as the mean; however 0.29 is lower than any of the observed values, excepting one r_I for flower color. An autoploid origin (6, 4, 3, 11) is evidence for a tetrasomic mode of inheritance in tetraploid alfalfa; however, the experimental values of r_I obtained in this study do not confirm the hypothesis of strict tetrasomy.

As was indicated above for disomic loci, it appears that a simple trait conditioned by one gene might readily be inherited in a tetrasomic fashion; but the probability that the several loci conditioning a quantitative character are all tetrasomic is necessarily low.

Tetrasomy with dominance

With complete dominance (digenic interaction) in the tetrasomic

case the intra-class correlation falls to 0.20. Dominance effects, if present, may appear largely as additive effects in the analysis between families, especially when gene frequency is low for the dominant allele. This could have occurred either at the disomic or tetrasomic level in the present study in the case of growth habit. It has been noted that the original field from which the parents were taken had been closely mowed for several years as a golf course. Such selection is believed to have significantly reduced the gene frequency for the upright habit (1). The upright habit of growth has been noted to be effected by dominance.

Thus, though dominance may not be effective in lowering the intra-class correlation appreciably, it would nevertheless fall in the range 0.20 to 0.29. Only one experimental value was observed in this range (yellow flower color; r_I equals 0.25). This may well be a trait that is conditioned by only one or two loci, and it is recognized that where this simple genetic basis prevails tetrasomy may be more readily detected by the intra-class correlation. The r_I for the more multigenic traits however are not found in this range, therefore the tetrasomic hypothesis alone, with or without dominance, does not satisfactorily account for the observed behavior in the material studied.

Tetrasomy with double reduction

An intra-class correlation of 0.29 for tetrasomy could be influenced upward by double reduction, however the conditions necessary for its common occurrence, namely a high level of quadrivalency, have not been observed in this study in any great degree.

Cytological examination at pachytene showed pairing to be predominately as bivalents. In most instances, only one quadrivalent was

evident, and critical examination showed that chromosomes in the quadrivalent were not fully paired throughout their length. At diakinesis generally only one quadrivalent was observed per cell, and in some none was observed.

Obviously if double reduction has an effect it is very slight, and may influence only a few loci. This would possibly be evident if those few loci happened to control a qualitative trait such as flower color, but it is unreasonable to postulate any major effect upon quantitative traits which are controlled by numerous genes over several chromosomes.

In the opinion of some writers, double reduction has a major role in determining the inheritance pattern of the tetraploid (8), however more recent workers reported only a slight and inconsistent degree of quadrivalency in alfalfa (4, 13).

Grun's work was especially definitive in describing the unpredictable occurrence of quadrivalents. Hanson's (5) findings of very low quadrivalent frequency lead him to postulate disomy, in contrast to Stanford's (13) statement suggesting tetrasomy.

The weight of the cytological evidence does not support the theory of tetrasomy with double reduction, therefore it must be rejected, at least in the major part, as an important explanation of the intra-class correlations found in the present study.

Heterosomy

A theoretical value representing the heterosomic situation cannot be found except in a most arbitrary fashion. This is true because heterosomy can result in r_I's at any point between the values for strict

tetrasomy and strict disomy, dependent upon the relative contributions of tetrasomic and disomic loci to the character being measured.

None of the hypotheses discussed above can be accepted unreservedly for quantitative characters for the reasons previously given, but heterosomy, defined as it is above, is a flexible enough category that nearly all the observed r_I's can be accounted for by postulating joint contributions to the trait from genes at both disomic and tetrasomic loci; that is, heterosomy.

Recent findings of Twamley (15), Wilsie and Dudley (16), in addition to the conclusion of Hanson that alfalfa is a segmental allopolyploid, point to this interpretation.

It is remarkable that different workers, employing different methods of attack on this problem, rather unanimously have arrived at a common interpretation of the mode of inheritance in tetraploid alfalfa. This fact, in itself, tends to make the hypothesis of heterosomy more acceptable.

SUMMARY

1. A study was made of 16 S_1 families from a mixed hybrid population to determine whether tetraploid alfalfa behaves disomically or tetrasomically in inheritance.

2. Morphological traits, dependent upon quantitative inheritance, were studied in the field nursery in 1954 and in 1955. Four traits—growth habit, stem length, leaf score, and internode length—were studied in 1954. Because of unreliable estimate of error in 1954 only three traits —growth habit, leaf score, and internode length—were used for 1955

calculations. The stem length trait was subject to excessive non-genetic variation.

3. Traits dependent upon continuous variation were used because artificial plant improvement depends upon characters exhibiting this type of inheritance.

4. A combination of these morphological traits was achieved by calculation of a discriminant function in 1954 and in 1955 to determine an overall growth type score, and to provide a more suitable genetic basis for inference with respect to the meaning of the intra-class correlation.

5. A method of evaluating mode of inheritance by biometrical genetic means was sought and was found to be the genetic intra-class correlation. This statistic was calculated for all morphological growth form traits in both years and for three flower color traits in 1955.

6. Theoretical values of intra-class correlation were found by Wright's method of path coefficients, by a ratio of variances that was really a function of the inbreeding coefficient, and by arithmetic models.

7. Observed intra-class correlations were transformed to Fisher's Z, then tested for significance with theoretical values by the t-test.

8. Six hypotheses were considered; disomy with or without dominance, tetrasomy with or without dominance, tetrasomy with double reduction, and heterosomy.

9. The first four hypotheses appear to be of limited importance, applying perhaps in special cases of traits influenced by one or a very few genes, but not in the more generally encountered cases of multigenic

traits.

10. The fifth hypothesis cannot apply generally because cytological observations have given no evidence of the conditions necessary for a significant amount of double reduction.

11. The majority of observed intra-class correlations for both years were found in the range between 0.29 and 0.67 where values may best be accounted for by postulating joint contributions from disomic and tetrasomic loci. Thus, with the exceptions previously noted, it may be concluded that heterosis is the prevailing mode of inheritance for the great majority of quantitative characters in tetraploid alfalfa.

APPENDIX A: DISCRIMINANT FUNCTION

Discriminant function for 1954

The function, DF, was found as a linear combination of the X-variables, X_1 (Growth Habit), X_2 (Stem Length), X_3 (Leaf Score), and X_4 (Internode Length), each weighted by an appropriate coefficient, which was to be estimated:

$$DF = b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4.$$

Following Mather (10), from the variance and covariance analyses a set of simultaneous equations was written:

$$bX_1(AX_1X_1 - \phi aX_1X_1)\ bX_2(AX_2X_1 - \phi aX_2X_1)\ bX_3(AX_3X_1 - \phi aX_3X_1)\ bX_4(AX_4X_1 - \phi aX_4X_1) = 0$$

$$bX_1(AX_1X_2 - \phi aX_1X_2)\ bX_2(AX_2X_2 - \phi aX_2X_2)\ bX_3(AX_3X_2 - \phi aX_3X_2)\ bX_4(AX_4X_2 - \phi aX_4X_2) = 0$$

$$bX_1(AX_1X_3 - \phi aX_1X_3)\ bX_2(AX_2X_3 - \phi aX_2X_3)\ bX_3(AX_3X_3 - \phi aX_3X_3)\ bX_4(AX_4X_3 - \phi aX_4X_3) = 0$$

$$bX_1(AX_1X_4 - \phi aX_1X_4)\ bX_2(AX_2X_4 - \phi aX_2X_4)\ bX_3(AX_3X_4 - \phi aX_3X_4)\ bX_4(AX_4X_4 - \phi aX_4X_4) = 0$$

where AX_1X_1, AX_1X_2 etc. are total sums of squares and cross products; aX_1X_1, aX_1X_2 etc. are the corresponding sums of squares and cross products between families; and ϕ (phi) is an adjustable quantity, whose value is to be estimated. (10) After substitution of 1954 data into the above equations, the result may be written as a determinant:

$$\begin{vmatrix} 461.2 - 421.0\,\phi & 79.0 - 58.8\,\phi & -51.2 + 181.9\,\phi & 226.2 - 196.0\,\phi \\ 79.0 - 58.8\,\phi & 260.8 - 130.0\,\phi & 466.8 - 185.2\,\phi & 284.7 - 184.1\,\phi \\ -51.2 + 181.9\,\phi & 466.8 - 185.2\,\phi & 44059.6 - 29442.4\,\phi & 1031.6 - 397.8\,\phi \\ 226.2 - 196.0\,\phi & 294.7 - 184.1\,\phi & 1031.6 - 397.8\,\phi & 1829.7 - 1135.6\,\phi \end{vmatrix} = 0$$

The quantity ϕ was inserted in the matrix and the resulting determinant equated to zero in order to force a solution. The determinant in ϕ was expanded by matrix algebra to yield the following quartic equation:

$$33377.80 - 89665.60\,\phi + 88282.70\,\phi^2 - 37392.73\,\phi^3 + 5749.74\,\phi^4 = 0.$$

This quartic equation was solved for ϕ by Horner's method of synthetic division in order to determine the lowest positive root. This root was calculated to be 2.026167. Substitution of the root for ϕ into the original equation then gives four simultaneous equations.

$$\begin{aligned} -3.92\,bX_1 - 0.41\,bX_2 + 3.18\,bX_3 - 1.71\,bX_4 &= 0 \\ -0.41\,bX_1 - 0.02\,bX_2 + 0.92\,bX_3 - 0.88\,bX_4 &= 0 \\ -3.18\,bX_1 + 0.92\,bX_2 - 155.94\,bX_3 + 2.26\,bX_4 &= 0 \\ -1.71\,bX_1 - 0.88\,bX_2 + 2.26\,bX_3 - 4.72\,bX_4 &= 0 \end{aligned}$$

These equations were solved by addition and subtraction to give

$$\frac{bX_1}{bX_3} = 37.56, \quad \frac{bX_2}{bX_3} = -0.29, \quad \text{and} \quad \frac{bX_4}{bX_3} = 1.88$$

where bX_3 is set equal to 1. The discriminant function is

$$37.56\,(X_1) - 0.29\,(X_2) + 1\,(X_3) - 1.88\,(X_4).$$

This discriminant was then applied to the original data which yielded a new set of data for analysis.

Discriminant function for 1955

The same procedure in calculating the discriminant was followed as for the previous year; however, only three variables enter into the 1955 discriminant function. These three variables were X_1 (growth habit), X_3 (leaf score), and X_4 (internode length). After substitution of sums of squares and cross products into the original equations, the resultant determinant,

$$\begin{vmatrix} (119.1 - 87.9\phi) & (-27.5 + 27.7\phi) & (72.9 - 73.0\phi) \\ (-27.5 + 27.7\phi) & (1583.4 - 1543.0\phi) & (86.2 - 86.6\phi) \\ (72.9 - 73.0\phi) & (86.2 - 86.6\phi) & (298.7 - 285.3\phi) \end{vmatrix} = 0$$

was expanded to give the following cubic equation:

$$4,645.8 - 12,107.2\phi + 10,397.5\phi^2 - 2,924.4\phi^3 = 0,$$

in which ϕ is found by Horner's method to have a value of 1.51343. Substitution of the value for ϕ into the original equations gives three new equations,

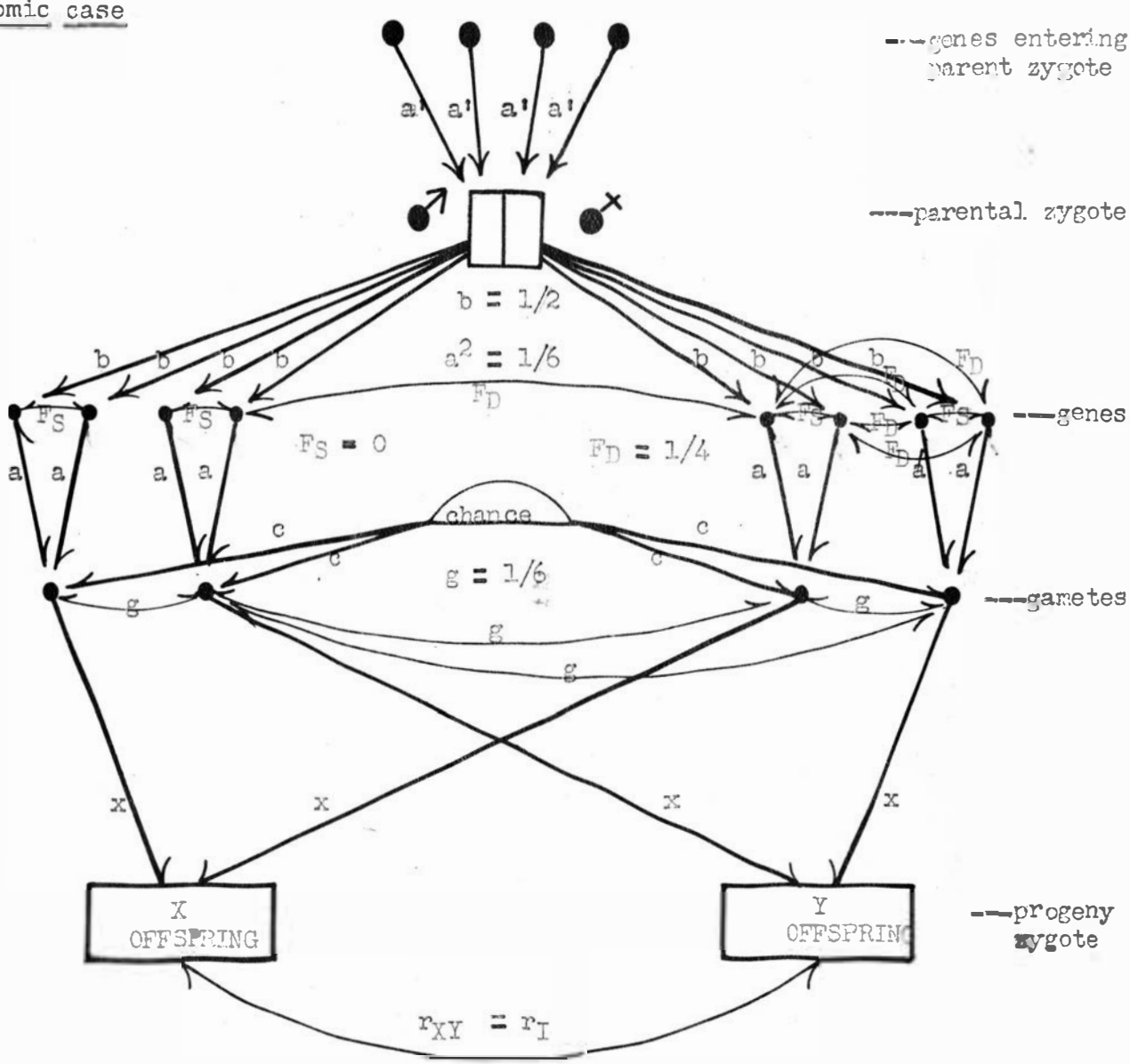
$$-13.93\ bX_1 + 14.42\ bX_3 - 37.58\ bX_4 = 0$$
$$14.42\ bX_1 - 751.82\ bX_3 - 44.86\ bX_4 = 0$$
$$-37.58\ bX_1 - 44.86\ bX_3 - 133.08\ bX_4 = 0$$

which yield a discriminant function as follows:

$$D = 1\ (X_1) - 2.72\ (X_3) + 3.39\ (X_4),$$

(when the X_1 variable is set to equal to 1).

Tetrasomic case



Path coefficient diagram illustrating the calculation of intra-class correlation in the case of self fertilization in a tetrasomic organism.

Symbols used in the path coefficient diagram are explained as follows:

a' - path coefficient to parental zygote from genes in preceding generation. a' equals 1/2 when f' equals 0.

b - path coefficient from parental zygote to its component genes.

a - path coefficient from component genes to gametes.

x - path coefficient from gametes to progeny zygote.

F' - inbreeding coefficient in preceding generation.

F_D - correlation between genes of different gametes.

F_g - correlation between genes of the same gamete.

g - correlation between gametes.

r_{XY} - correlation between progeny.

k - level or degree of ploidy; "k" in a tetraploid = 2.

In the overall diagram of the path coefficient determination, it was not feasable to enter all the correlation paths. In order to preserve the overall picture of the entire diagram with sufficient clarity, it was necessary to separate the diagram into several sections for more critical explanation.

Genes in any two gametes from a single diploid parent are correlated to the extent of b^2, as shown in sub-diagram 1.

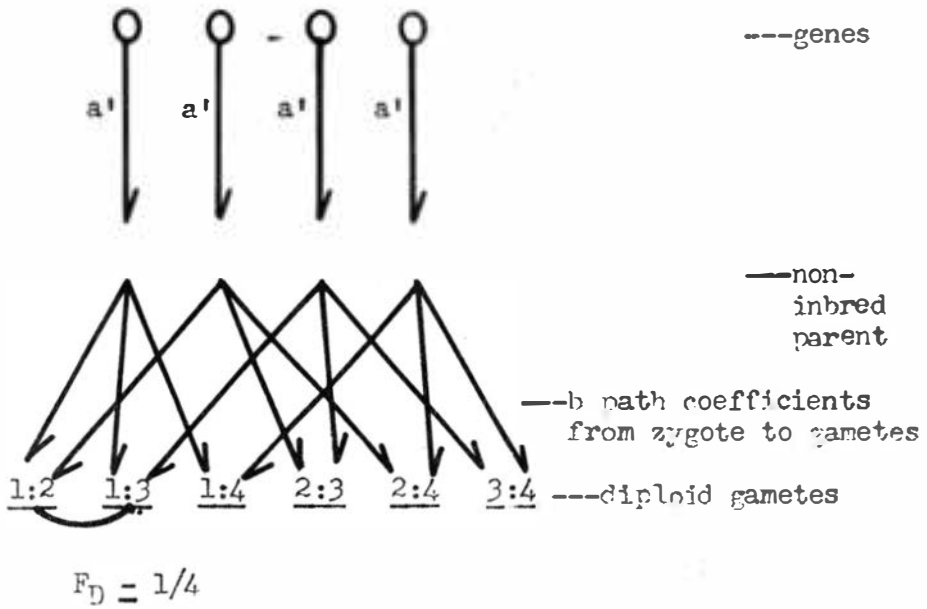
From sub-diagram 1, the complete determination, genetically, of the initial non-inbred parent may be expressed as $4a'^2 = 1$, from which

$$a' = \sqrt{1/4},$$

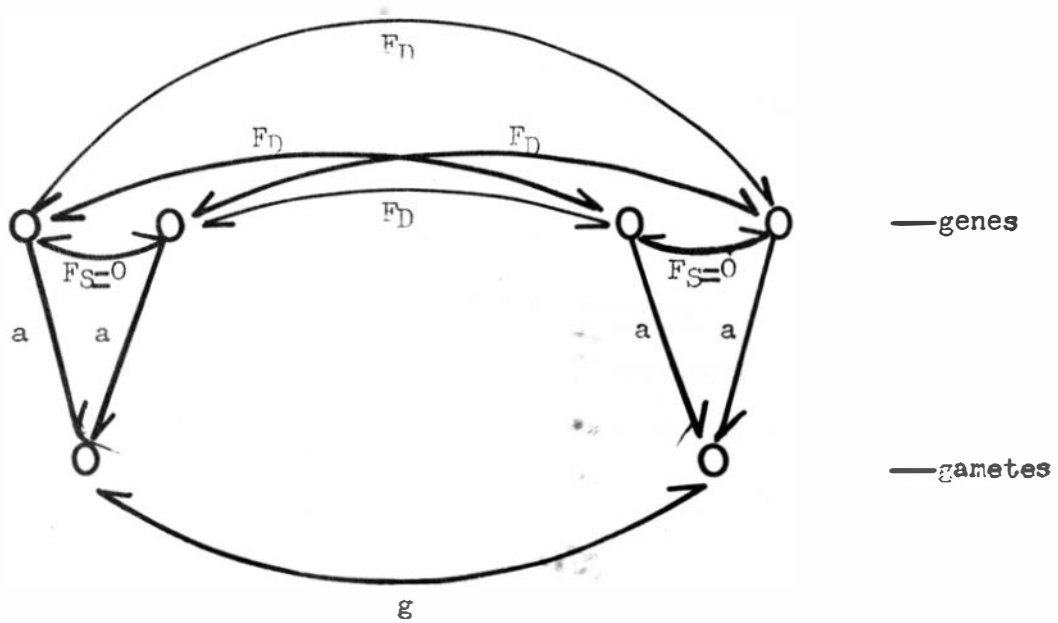
$$a'^2 = 1/4 - b^2 = 1/2k\left[1 + (2k-1)F'\right] \quad (18),$$

$$a' = b, \text{ also } b = a' = 1/4,$$

$$F_D = b^2 = 1/4.$$



Sub-diagram 1. Illustration of pathways from genes entering parent zygote to the diploid gametes of the parent zygote.



Sub-diagram 2. Illustration of pathways from genes to gametes for complete determination of the gametic correlation (g).

That this value is correct may be shown in a different manner. Consider gametes 1:2 and 1:3 together; it is possible to find the genic correlation of all combinations of two genes at a time, thus:

gene 1 with gene 1 gives an r which equals unity,
gene 1 with gene 3 gives an r which equals zero,
gene 2 with gene 1 gives an r which equals zero,
gene 2 with gene 3 gives an r which equals zero,

hence, once in four times the correlation will equal one (1), and three out of four times the correlation will equal zero (0), therefore

$$F_D = (1/4 \cdot 1) + (3/4 \cdot 0) = 1/4,$$

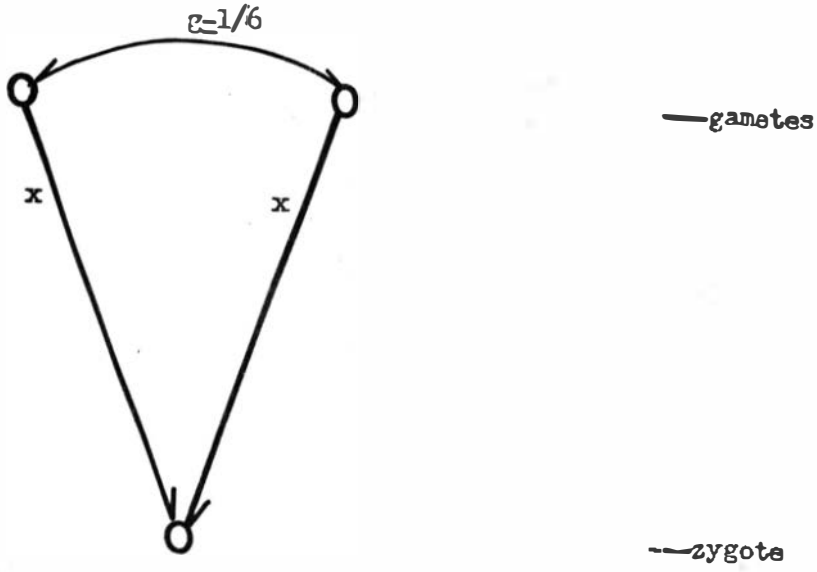
as already obtained above; or we may use Wright's formula for the determination of F_D, where F_A equals 0 and k equals 2: (F_A = F of common ancestor)

$$F_D = 1/2k \left[1 - (2k-1)F_A\right]$$

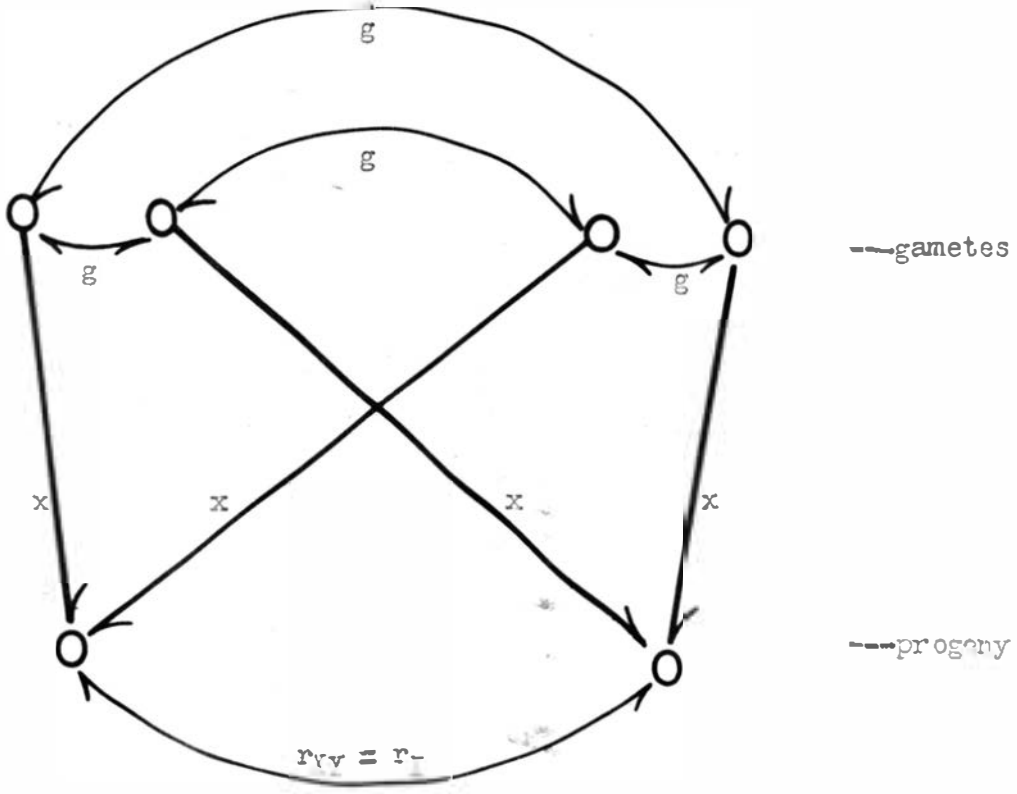
$$F_D = 1/4.$$

With a correlation, F_D, between genes of different gametes and with the value of "g" at $\sqrt{1/6}$ (18), the correlation, g, between gametes can be calculated, as shown in sub-diagram 2 in which $g = 4a^2 F_D = 4 \cdot 1/6 \cdot 1/4 = 1/6$; therefore "g", the correlation between gametes, is the same as the average F, inbreeding coefficient. After "g" was determined as the correlation between gametes under self-fertilisation, the correlation between any two sibs may be found. First the value of "x", the path from gamete to offspring, was found as in sub-diagram 3.

Expressing the complete determination of the zygote from sub-diagram 3 as follows:



Sub-diagram 1. Illustration of pathways for the additive genetic determination of the zygote.



Sub-diagram 2. Illustration of pathways in the determination of sib correlation (r_I).

$$x^2 + x^2 + 2x^2g = 1$$

$$2x^2(1+g) = 1$$

$$x^2 = 1/2(1+g)$$

$x^2 = 1/2(7/6) = 3/7$, which is the value of the "x" path; then the sib correlation (r_I) can be finally determined as found in sub-diagram 4.

$$r_{XX} = xgx + xgx + xgx + xgx = 4x^2g$$

$$= 4 \cdot 3/7 \cdot 1/6 = 12/42 = 2/7$$

$r_I = 2/7$ or 0.2857

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