

AN ANALYSIS OF HETEROSIS VS. INBREEDING EFFECTS
WITH AN AUTOTETRAPLOID CROSS-FERTILIZED PLANT:
MEDICAGO SATIVA L.

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ABSTRACT

Self-fertilization and crossing were combined to produce a large number of levels of inbreeding and of degrees of kinship. The inbreeding effect increases with the complexity of the character and with its supposed relationship with fitness. A certain amount of heterozygosity appears to be necessary for the expression of variability. With crossing of unrelated noninbred plants, genetic variance is mainly additive, but with inbreeding its major part is nonadditive. High additivity in crossing, therefore, coexists with strong inbreeding depression. However, even in inbreeding the genetic coefficient of covariation among relatives appears to be strongly and linearly related to the classical coefficient of kinship. This means that deviations from the additive model with inbreeding could be partly due to an effect of inbreeding on variances through an effect on means. An attempt to analyze genetic effects from a theoretical model, based upon the identity by descent relationship at the level of means and of covariances between relatives, tends to show that allelic interactions are more important and nonallelic interactions are less important for a character closely related to fitness. For a complex character, these results lead to the conception of a genome organized in polygenic complementary blocks integrating epistasis and dominance. Some consequences for plant breeding are also discussed.

HETEROSIS, or its corollary of inbreeding depression, plays as important a role in natural selection as it does in artificial selection. In spite of molecular genetic advances, however, the explanation of heterosis at the whole organism level has not progressed much since the first studies (SHULL 1914). The study of heterosis has relevance for population and quantitative genetics and for biochemical, physiological and ecological genetics. In this paper I use a biometrical approach for studying the phenomenon at the level of a single population, and I examine the influence of mating system (inbreeding followed by the crossing of unrelated individuals) on the means and variances of several quantitative characters. I hope that such studies will lead to testable hypotheses regarding the organization of the genome caused by natural selection, with concomitant implications for plant breeding.

Although the effects of heterosis or inbreeding are often studied for means,

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it is known that variances are also affected and must be considered in studies of natural or artificial selection. The partitioning of variances into components, for inbred populations, and the expression of covariances for inbred relatives, rests on the parameters developed by GILLOIS (1964) and HARRIS (1964). This theory has been extended to include autotetraploids by J. BOUFFETTE (1966), A. BOUFFETTE (1966) and GALLAIS (1967, 1974).

Although the autotetraploid plant, *Medicago sativa* L. is treated in this paper, the conclusions are general. Only mean results will be given, since details have appeared previously (GALLAIS 1976b, 1977b).

MATERIALS AND METHODS

Inbreeding phase material development: The aim was to develop many levels of inbreeding and kinship for the naturally cross-fertilized autotetraploid plant *M. sativa* L., variety Du Puits. Figure 1 shows how the inbreeding phase was developed. From the Du Puits population, 84 plants (S_0') were divided into four groups of 20–22 and were crossed, with emasculation, to give S_0 families with each plant being used once as a male and once as a female. From each S_0 family, one plant was self-fertilized to initiate an S pedigree by single seed descent to S_4 , and two plants were crossed, with emasculation, to initiate an S(FS) pedigree. This was also inbred by single-seed descent until S_3 (FS). Two half sib S_0 plants, one from each of two consecutive S_0 families, were crossed to give an HS family. Finally, S_0' plants were selfed to give the S_1' family from which S_2' families were derived.

This procedure led to ten inbreeding levels and 130 degrees of kinship. The S_3 (FS) level was lost because of high sterility levels, which also caused a reduction in size and number (31 from 84) of the S_4 families. The inbreeding coefficients varied from 0 to 0.52, and quadrigenic identity probabilities (GALLAIS 1967) varied from near 0 to 1. Kinship levels (MALECOT 1949) varied from 0.06 to 0.57.

Crossing phase material development: Unrelated plants at the same inbreeding levels (S_0, S_1, S_2 or S_3) were crossed in the following designs, expressed in the notation of COCKERHAM (1963):

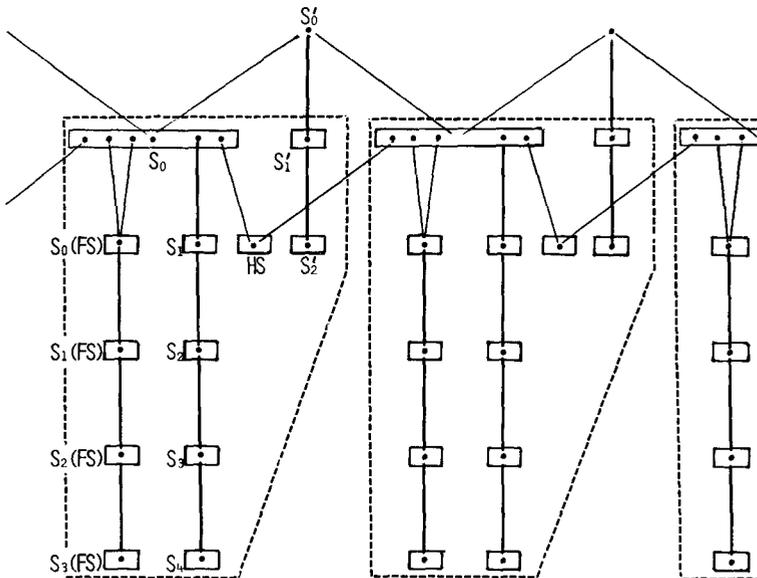


FIGURE 1.—Diagrammatic representation of the development of the material for the inbreeding phase (broken lines surround related families that were grouped in the split-plot design for field testing).

(1). $S_0 \times S_0$ in design A/B (two A plants for each B) and design (A/B) (C/D) (seven sets of two A plants for each two B plants, and two C plants for each two D plants). This gives five different levels of covariances between relatives.

(2). $S_1 \times S_1, S_2 \times S_2, S_3 \times S_3$ in design A/B (three A plants for each B). This gives 14 different covariances: half sibs and full sibs within each level S_1, S_2, S_3 , and eight types of relatives within and between levels S_1 and S_2 as shown in Figure 2.

This crossing phase provides four levels of inbreeding with inbreeding coefficients ranging from 0 to 0.14 (crossing unrelated plants does not completely remove inbreeding for autotetraploids). It also provides 19 types of relatives, with kinship coefficients ranging from 0.15 to 0.28.

Inbreeding phase field testing (1971-1972): For both inbreeding and crossing phases, seedlings were raised in a glass house before transplantation in the spring (mid-April). The two phases were tested separately.

Inbred families were studied in a split plot design with three replicates. The group of related families from S_0 and S'_0 formed the first level, and degree of inbreeding formed the second level. There was a systematic classification of families within plots according to inbreeding level in order to avoid interference among rows of different inbreeding levels (we verified later that there was no such interference). Each main plot was separated by one row of the reference population, whereas each subplot consisted of one row of ten plants with 50 cm between plants.

For dense sward studies, each of the ten inbreeding levels was studied with 10 cm between plants, 20 cm between rows in sets of three rows (two guard rows for the central row harvested). There were six replications. This was done for a mixture of all families with the same inbreeding level, whereas a separate dense sward study used 46 families at the ten inbreeding levels, with one row per plot and three replications.

During the development of the generations, the number of seeds per plot, the weight of seeds per pod and the weight of 100 seeds were observed. The heights of seedlings were observed for each family before transplantation. Since these characters were observed without a proper experimental design, environmental effects may be confounded with generation (inbreeding) effects.

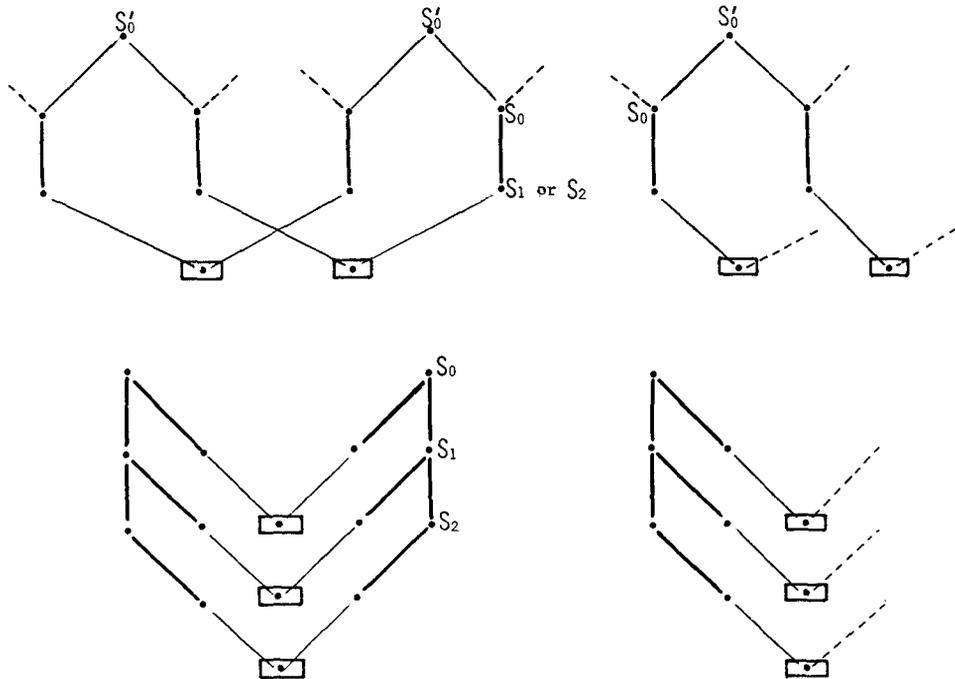


FIGURE 2.—Kinships in the crossing phase others besides half sibs and full sibs (related individuals are surrounded by a small rectangle).

For spaced plants in the field, green matter yield was observed for three cuts in the seedling year and for the first two cuts of the following year. Plant height was measured for the first two cuts of the seedling year. Only its log transformation, total green matter yield and average height will be considered here. Only green matter yield was observed in the dense sward conditions.

Crossing phase field testing (1973–1974): The aim in this phase was to estimate combining ability variances for each of the levels S_0 , S_1 , S_2 and S_3 . A trial was developed in four replications split for the level of inbreeding. Each plot had one row of ten plants with 50 cm between plants and rows. Plots of the reference Du Puits population were systematically replicated in each trial.

Green matter yield was observed in the three cuts of the seedling year and in the first two cuts of the following year. The total yield, its log transformation and plant height in the first cut of the seedling year will be considered.

In both inbreeding and crossing phases, covariances of relatives within an inbreeding level were estimated through analysis of variance, and covariances between inbreeding levels were estimated from the covariances between means of related families.

Genetic interpretation of sample moments: The general structure of expressions for means and covariances will be stated here—theoretical details were given by J. BOUFFETTE (1966) and by GALLAIS (1967, 1974, 1976b).

The mean μ_n of an inbred population is written as

$$\mu_n = \mu + \sum_i P_{i,n} E(X)_i + \epsilon_n$$

where μ is the mean of an equilibrium random mating population with the same gene frequencies, and i indexes possible interactions among the genes, with $E(X)_i$ denoting the appropriate expectation and $P_{i,n}$ the appropriate coefficient of identity by descent. If i represents a digenic interaction for example, $P_{i,n}$ will be the classical inbreeding coefficient F . The generation is denoted by n , and ϵ_n represents departures from the model.

Covariances between relatives Z_n and $Z_{n'}$, in generations n and n' , can be written as

$$\text{cov}(Z_n, Z_{n'}) = \sum_i \psi_i(n, n') E(X_1 X_2)_i + \epsilon_{nn'}$$

where $E(X_1 X_2)_i$ is now the expectation of the product of two types of genetic effects and $\psi_i(n, n')$ the corresponding identity coefficient. For example, if X_1 and X_2 represent additive effects, $E(X_1 X_2)$ will be an additive covariance and $\psi(n, n')$ the usual coefficient of kinship.

If the number of observations is sufficiently much greater than the number of parameters, then, it will be possible to estimate the parameters and test the goodness of fit of the models, including a comparison between restricted and more complete models. Unweighted least squares analyses have been used (results from weighted least squares, HAYMAN 1960, were very similar), and such estimates are not minimum variance since some covariances between mean products involved the same branch of a family (CHI, EBERHART and PENNY 1969). Least squares analyses also give high correlations between the estimates of some of the coefficients of identity and kinship (GALLAIS 1976a). For covariances, stepwise regression was also used to study the predictive value of some kinship coefficients.

In the absence of epistasis, the one-locus tetraploid model without restriction on allelic interactions will be denoted M4; without interactions among groups of four alleles, the model will be denoted M3; with interactions only between pairs of alleles, it will be denoted M2. When allelic interactions are only between pairs of alleles, and epistasis is only digenic by digenic, the model is denoted $M2 \times M2$.

RESULTS FOR INBREEDING PHASE

Means: Inbreeding has a strong effect on overall green matter yield in spaced plants: there is a 30% depression in the first generation of selfing and 70% in the fourth. There is less effect on plant height and green matter yield in competition: about 20% depression in the first generation for each character. Among the seed or seedling characters, the number of seeds per pod is strongly

affected with 55% depression in the first selfing generation; the weight of 100 seeds first increases and then decreases, whereas seedling height behaves similarly to adult height.

Although there is no information on experimental error, the 100-seed weight could be accounted for partly by maternal effects, with S_1 seed sizes being determined by S_0 rather than S_1 genetic parameters. This has been demonstrated by DESSUREAUX and GALLAIS (1971) and DATTÉE (1974) but cannot explain the increase from S_0 to S_1 (Table 1). Such a change could result from a negative relationship between seed size and numbers of seeds per pod. A favorable inbreeding effect due to tiller size and numbers of tillers being negatively related, with a strong inbreeding effect on tiller number, has been observed in cocksfoot (GALLAIS and GUY 1970).

Model M4 (no epistasis) explains 99.8% of the variation among inbreeding levels for yield and height, but deviations from this model are significant ($0.01 < P < 0.05$) for yield and for height ($P = 0.01$). These deviations could be due to epistasis which appears greater for height than for yield. This tends to be confirmed by a greater reduction of these deviations for height ($0.01 < P < 0.05$) than for yield ($0.05 < P < 0.10$), by "digenic heterozygous \times digenic heterozygous" epistasis.

The reduction in residual sum of squares for model M2 by allowing triallelic interactions (model M3) is greater for yield than for height (Table 2). Further significant reduction, for both characters, results from allowing quadriallelic interactions. This latter effect may be due to digenic by digenic epistasis because there is a high correlation between identity coefficients associated with quadrigenic effects and those associated with such epistatic effects.

Seedling height is entirely explained by the digenic model, and other seedling characters show significant maternal and embryonic effects (Table 3), with a greater maternal effect for seed size.

Variances and covariances: Table 4 shows that, for yield, the variance among

TABLE 1
Effect of inbreeding on means

Level of inbreeding		Characters in spaced plants ^a						
Generation	F	Yield	Height	Log yield	Yield in dense sward	Weight of 100 seeds	Seeds per pod	Seedling height
S_0	0	100 \pm 1.9	100 \pm 0.9	100 \pm 0.2	100 \pm 5.0	100	100	100
HS	0.04	92.2 \pm 3.0	94.3 \pm 1.6	98.7 \pm 0.8				
S_0 (FS)	0.08	87.0 \pm 1.4	92.2 \pm 0.6	97.5 \pm 0.3	87.1 \pm 4.5	105	87.3	95.4
S_1	0.17	68.4 \pm 1.2	81.2 \pm 0.6	95.3 \pm 0.1	81.8 \pm 6.2	103.5	45.2	91.0
S_1 (FS)	0.24	56.1 \pm 1.9	71.9 \pm 1.0	92.7 \pm 0.4	63.6 \pm 3.1	98.5	29.2	83.8
S_2	0.31	40.4 \pm 1.2	59.4 \pm 0.7	88.3 \pm 0.4	57.6 \pm 5.0	92.3	26.0	76.6
S_1 (FS)	0.36	36.5 \pm 2.0	56.1 \pm 0.9	85.1 \pm 0.9		88.6	20.0	68.5
S_3	0.42	24.7 \pm 1.9	48.3 \pm 1.0	82.3 \pm 1.0		84.4	19.7	67.6
S_4	0.52	24.3 \pm 2.6	47.7 \pm 1.6	82.3 \pm 1.3		76.8	15.0	58.5

^a With 95% confidence interval.

TABLE 2

F values of the test of reduction of deviations from digenic model (M2) by M3 and M4

Test	d.f.	Yield	Height	Weight of 100 seeds	Seeds/pod	Seedling height
Dev 2-M3 ^a	1	11.3*	3.6(*)	21.5**	31.9**	1.5
Dev 2-M3 ^b	1	38**	40**	430**	71.2**	1.9
Dev 2-M4	3	18**	35**	185**	26.9**	1.9
Dev 3-M4	3	8*	33**	60**	4.8(*)	2.1

Dev 2, Dev 3 are deviations from M2 or M3 models. (1), Test on Dev 3 with 6 d.f.; (2), test on Dev 4 with 4 d.f.

^a Test on Dev 3 with 6 d.f.

^b Test on Dev 4 with 4 d.f.

**, *, (*) significant at 0.01, 0.05, 0.10, probability level, respectively.

TABLE 3

Test of influence of mother plant (MP)

Test	Yield	Height	Weight of 100 seeds	Seeds/pod	Seedling height
MP after embryo (E) ^a	NS	NS	**	**	NS
<i>F</i> (<i>F</i> _{MP} after <i>F</i> _E) ^b	NS	NS	**	**	NS
<i>r</i> with <i>F</i> _E	0.982	0.983	0.931	0.914	0.993
<i>r</i> with <i>F</i> _{MP}	0.913	0.930	0.992	0.782	0.975
<i>r</i> with (<i>F</i> _E , <i>F</i> _{MP})	0.998	0.985	0.995	0.980	0.996

*F*_E, *F*_{MP}: coefficient of inbreeding of the embryo and of the mother plant, respectively. *r* = coefficient of simple or multiple correlation.

^a Test *F* with model M4 for the embryo and first parameter of mother plant introduced by stepwise regression.

^b Test with model M2 for mother plant and embryo.

** Significant at the 0.01 probability level. NS = not significant.

inbred families increases from level *S*₀ to level *S*₁(FS) and then decreases significantly. There appears to be an optimum level of inbreeding for the expression of variance. For height the same tendency is observed; however, the optimum appears to be at a higher level of inbreeding. The log transformation of yield tends to suppress the observed optimum and reveals a high increase in variance from *S*₀(FS) to *S*₂(FS), followed by a plateau. Such linearization is also observed if we consider the genetic coefficient of variation: it increases from level *S*₀ to level *S*₄. The behavior of yield with competition, as for means, is more similar to that of height than to that of yield: it increases from levels *S*₀ to *S*₂.

When we consider all covariances between relatives for yield and height, only 41% of their variation is explained by the classical coefficient of kinship (ψ). The log transformation for yield increases this degree of determination to 69%. Furthermore, if, instead of the covariances, we consider the genetic coefficient of covariation (GCC) among relatives, *i.e.*, the square root of the covariances, assumed positive, divided by the product of the generation means of the two related zygotes, the classical coefficient of kinship ψ explains 81% of the variation. Because the intercept of the regression of GCC on ψ is not

different from zero, it appears possible to approximate the covariance between two inbred relatives Z_I, Z_J with generation means μ_I and μ_J by

$$\text{cov}(Z_I, Z_J) = C\psi_{IJ}^2 \mu_I \mu_J$$

(C being a constant). This is a very simple expression of covariances between inbred relatives, with a low number of parameters, in contrast to the number necessary with the classical formulation (GALLAIS 1976b). For example, five components of variance or covariance are necessary with the digenic model (M2).

Without transformation for yield and height, there is also a high correlation between the covariances and the coefficient of kinship $\psi(ijk|ijk)$, i.e., the probability of three nonidentical alleles in one zygote being identical with three alleles drawn in the other zygote. The correlation is higher for yield than for height, 0.90 and 0.77, respectively (Table 5).

TABLE 4

Effect of inbreeding on genetic variances among independent inbred families, giving the value 1 to S_0 variances

Level of inbreeding	ψ	Yield ^a			Height ^a			Log yield variance ^a	Yield GCV ^a	Yield in sward		
		Ll	Variance	Lu	Ll	Variance	Lu			Ll	Variance	Lu
S ₀	0.12	0.7	1	1.3	0.7	1	1.4	1.0	1.0	0.6	1	2.2
S ₀ (FS)	0.19	1.1	1.4	1.7	0.9	1.1	1.5	1.8	1.3	0.7	1.2	3.0
S ₁	0.25	1.2	1.4	1.8	1.3	1.7	2.1	3.8	1.7	0.8	2.1	4.0
S ₁ (FS)	0.31	1.3	1.7	2.4	1.8	2.4	3.4	8.8	2.3	2.9	4.9	8.7
S ₂	0.37	1.0	1.3	1.7	1.7	2.3	2.8	11.5	2.8	3.8	5.9	9.4
S ₂ (FS)	0.43	0.8	1.1	1.6	2.0	2.7	3.8	32.0	2.9			
S ₃	0.48	0.7	1.0	1.5	1.3	1.8	2.5	22.0	4.1			
S ₄	0.57	0.5	0.8	1.3	0.5	1.0	1.8	23.0	3.6			

For yield in spaced plants, height and yield in sward, 90% confidence interval Ll-Lu is given. ψ is the classical coefficient of kinship.

^a Characters in spaced plants.

TABLE 5

Correlations between covariances and some coefficients of kinship

Coefficient of kinship ^a	Yield	Height	Log yield	Yield in dense sward
$\psi(i i)$	0.64	0.64	0.83	0.89
$\psi(ii ii)$	0.33	0.25	0.79	0.79
$\psi(ij ij)$	0.74	0.70	0.85	0.89
$\psi(ii iii)$	0.35	0.28	0.78	0.73
$\psi(ii ii)$	0.55	0.53	0.90	0.83
$\psi(ijk ijk)$	0.90	0.77	0.62	0.78
$\psi(iii iii)$	0.32	0.20	0.73	0.69
$\psi(ijkl ijkl)$	0.66	0.40	0.12	0.30

^a A letter represents a class of identity by descent, and vertical trait separates genes of one individual from those of its relative.

Covariances in dense sward are highly related to the simple coefficient of kinship ($r = 0.89$); however, we must note that there are only 30 covariances without level S_4 .

Model M2 explains 86, 84 and 76% of the variation for yield in spaced plants, log yield and height of spaced plants, respectively. Reductions of the deviations from the additive model by M2 have not been tested, because they are obvious for yield and height. Model M3 explains 90, 89 and 78% of the variation for yield, log yield and the height, respectively. Reduction of deviations from M2 is highly significant and at about the same level of significance in spaced plants for yield and height. It is not significant for yield in dense sward. Model M4 was not tested due to its great number of parameters.

For model $M2 \times M2$, the reduction of deviation from M2 is more significant for height ($F = 6.5$) than for yield ($F = 3.8$). This tends to confirm a greater effect of digenic by digenic epistasis for height than for yield. The reduction is also significant for yield in dense sward ($P < 0.01$). If we consider this character to be clearly related to height in spaced plant (GUY 1970), this also supports the presence of epistasis for height.

Due to the great number of parameters in M3, M4 and $M2 \times M2$ models, it is difficult to estimate the variance and covariance components. Another difficulty is due to the interdependence among coefficients of kinship (GALLAIS, 1976b) which induces a high variance of the estimators. Hence, the parameters were not estimated, and they are less important than the model testing.

The effect of the log transformation on yield is to decrease inbreeding depression and to increase the dependence between second degree statistics (variances and covariances) and the classical coefficient of kinship. As expected, it increases mainly the weight of interactions between identical alleles (Table 5). Its main effect can be interpreted as a correction for scaling. Indeed, in spite of the fact that residual errors do not increase with level of inbreeding, and of the near-normal distribution of phenotype values of families with the same level of inbreeding, it is clear that, in absolute value, weak (highly inbred) plants cannot vary as much as vigorous (noninbred) plants. It is remarkable that the genetic coefficient of covariation (*i.e.*, the correction of covariance for differences in means) greatly increases, more than the log transformation, the dependence between covariance and coefficient of kinship. The effects of such transformations can also reflect the presence of multiplicative effects for yield.

RESULTS FOR CROSSING PHASE

When expressed in relative values, the means of the crossing phase (Table 6) appear on the same curve relating means to the coefficient of inbreeding F as those of the inbreeding phase (Table 1). This shows that if there is some genotype \times year interaction—as crossing phase was disconnected in the time of inbreeding phase—it does not affect the relative means. It is verified (GALLAIS 1967) that the more inbred the parents the weaker the single crosses. The difference between $S_0 \times S_0$ and $S_3 \times S_3$ is about 25%.

For variances, the main result is the absence of significance for the specific combining ability variance. On the average (Table 7), the covariance between

TABLE 6

Effect of inbreeding on the performances of crosses in relative value and with 95% confidence intervals

Level of inbreeding			
Generation	F	Yield	Height
$S_0 \times S_0$	0	100 ± 2.0	100 ± 1.1
$S_1 \times S_1$	0.06	93 ± 2.2	94 ± 1.1
$S_2 \times S_2$	0.10	85 ± 1.8	91 ± 1.0
$S_3 \times S_3$	0.14	73 ± 2.7	85 ± 1.7

TABLE 7

Effect of inbreeding on covariances between half sibs (HS) and full sibs (FS), giving the value 1 to covariance between $S_0 \times S_0$ half sibs

Level of inbreeding	Yield		Height		Log yield		
	cov HS	cov FS	cov HS	cov FS	cov HS	cov FS	
$S_0 \times S_0$	0.06	1.0 ± (0.3)	1.5 ± (0.3)	1.0 ± (0.5)	2.6 ± (0.5)	1.0 ± (0.4)	1.4 ± (0.4)
$S_1 \times S_1$	0.09	1.2 ± (0.6)	1.9 ± (0.7)	2.1 ± (8.9)	3.0 ± (1.0)	1.7 ± (0.8)	2.7 ± (0.9)
$S_2 \times S_2$	0.12	0.8 ± (0.5)	2.8 ± (0.6)	0.4 ± (0.4)	1.8 ± (0.5)	2.1 ± (1.1)	4.8 ± (1.2)
$S_3 \times S_3$	0.14	0.9 ± (0.7)	1.3 ± (0.8)	1.3 ± (0.9)	2.6 ± (1.5)	2.7 ± (1.5)	4.9 ± (1.8)
Mean of covariances		1.0	1.9	1.2	2.5	1.9	3.5

The standard deviation of the estimate is given in parentheses.

full sibs is twice the covariance between half sibs, as expected if genetic variance is mainly additive. Variation in this relation according to the level of inbreeding appears due to a lack of accuracy. Such a lack of accuracy could explain why, for yield and height, an increase in the covariances with increasing level of inbreeding of the parents is not observed.

With the assumption of the absence of specific combining ability, estimates of the covariance between half sibs (general combining ability variance) are more accurate (Table 8). For yield, there is an increase from $S_0 \times S_0$ to $S_2 \times S_2$, followed by a decrease at the $S_3 \times S_3$ level. A change in variance for height also appears if we exclude anomalous values of the S_2 level. The log transformation for yield clearly shows, as for direct estimates, an increase in general combining ability variance from $S_0 \times S_0$ to $S_3 \times S_3$. Such an increase is also observed for yield and height if we consider the genetic coefficient of variation of the general combining ability.

The relationship between all of the 19 covariances estimated in the crossing phase and the simple coefficient of kinship ψ is generally very high ($r = 0.81, 0.90, 0.78$ for yield, log yield and height, respectively). This confirms the nonsignificant specific combining ability variances, *i.e.*, a great importance of additivity. This is quite different from the result of the inbreeding phase.

TABLE 8

Estimation of the general combining ability variance

	$S_0 \times S_0$	$S_1 \times S_1$	$S_2 \times S_2$	$S_3 \times S_3$
Yield	1.0 ± (0.3)	1.3 ± (0.4)	1.6 ± (0.5)	1.0 ± (0.4)
Log yield	1.0 ± (0.3)	1.9 ± (0.4)	3.1 ± (0.6)	5.2 ± (1.0)
Height	1.0 ± (0.2)	1.1 ± (0.2)	0.8 ± (0.2)	1.2 ± (0.2)
GCV yield	1.0	1.2	1.5	1.4
GCV height	1.0	1.1	1.0	1.3

The assumption is made that specific combining ability variance is zero giving the value 1 to $S_0 \times S_0$ level. General ability variance is the relative genetic coefficient of variation of GCA. Values in parentheses are the standard deviations of the estimates.

As expected from the effect of log transformation and from the study of the genetic coefficient of variation of general combining ability, the GCC among relatives is, as in the inbreeding phase, strongly related to the classical coefficient of kinship ψ ($r = 0.85$). However, it appears that the relationship is not of the same form. The square of the GCC, *i.e.*, the covariance divided by the product of the two generations' means, is more related to the coefficient of kinship ψ ($r = 0.90$), and the intercept of the regression on ψ is not different from zero, which was not the case with regression of GCC on ψ . Therefore, under crossing, it appears possible to write the covariance between two inbred relatives Z_i, Z_j with generation means μ_i and μ_j as

$$\text{cov } Z_i Z_j = C\psi_{ij}\mu_i\mu_j.$$

Model M2 explains 81, 88 and 81% of the variance among covariances for yield, log yield and height, respectively. Reductions of deviations from the additive model are significant for the three characters.

The reduction of deviations of M2 by some parameters of the model M3, introduced by stepwise regression, is greater for yield ($F = 4.7, 0.01 < P < 0.05$) than for height ($F = 2.5, P \approx 0.10$), whereas some parameters of the $M2 \times M2$ model reduce these deviations more for height ($F = 4.7, 0.01 < P < 0.05$) than for yield ($F = 3.6, 0.05 < P < 0.10$). Then, as in the inbreeding phase, epistasis appears more important for height than for yield.

DISCUSSION

The effect of inbreeding on means and variances: In studies of inbreeding effects in cross-fertilized organisms (LERNER 1954; FALCONER 1961), it is generally observed that characters highly correlated with fitness are more affected by inbreeding depression. In lucerne, if we accept that yield and seeds per pod are more related to fitness than height, this is also verified. The difference between yield and height in their relationship with natural selection could also explain why, for means, interactions between more than two alleles appear more important for yield than for height. Indeed, natural selection can favor interactions only between alleles of degree greater than the level of ploidy of the gametes.

Also, for variances, height and yield have a different behavior. For yield, it

appears that there is an optimum level of inbreeding for the expression of the variability, but such an optimum is not so clear for height. The presence of an optimum level of inbreeding could be interpreted as the result of two phenomena with increasing levels of inbreeding: a direct favorable effect due to segregation and an unfavorable effect due to a relationship between means and variances. As the inbreeding effect is greater for yield, this could explain why an optimum is observed for a lower level of inbreeding for yield than for height.

It is remarkable that the "correction" of covariances by the differences in means of the two relatives, through the genetic coefficient of covariation, allows an explanation of the covariances among inbred relatives with only the classical coefficient of kinship. So, after elimination of inbreeding effect on means, results of inbreeding, and crossing phases show a high additive variance. Without such a transformation the nature of the genetic variance under inbreeding and crossing appears quite different, and many parameters are necessary to describe it under inbreeding (GALLAIS 1976b).

The statistical relationship between the square of the genetic coefficient of covariation and the classical coefficient of kinship does not seem the same in the two phases: it appears curvilinear for inbreeding and linear for crossing. As the two phases were disconnected, it is not possible to have a precise test of this difference, and genotype \times environment interactions could explain it. Furthermore, we have to consider a direct effect of inbreeding on variances and covariances, unexplained by the change in means and the classical coefficient of kinship. The curvilinearity under inbreeding could be due to a greater effect of dominance or epistasis than in crossing.

To generalize our results to face the difficulty of estimating variance components under inbreeding, due to a great number of parameters [see GILLOIS (1964) and HARRIS (1964) for diploids], we propose to apply the classical decomposition of covariances between noninbred relatives to the square of the genetic coefficient of covariation among inbred relatives. We do not know of experiments with inbred and noninbred relatives to compare with our results and to see the general value of our proposal. However, it is based on a relationship between means and variances under inbreeding that can be considered as general for species with marked inbreeding effects. Hence, it could be efficient for prediction purposes in plant or animal breeding schemes that use inbreeding.

Epistasis and the importance of additivity in crossing: For means or covariances in the inbreeding phase, as in the crossing phase, there is a tendency to have more epistasis for height than for yield. This is the opposite of what was expected on the basis of the complexity of each character. However, such a tendency appears quite consistent with what we know now on the theoretical effect of natural selection.

Since the first comments of MATHER (1941) on internal and relational balances, several theoretical studies (LEWONTIN 1964, 1974; HEDRICK 1976; MAYNARD-SMITH 1980), have shown that natural selection for an intermediate phenotype, or selection in fluctuating environments, could act to favor the devel-

opment of complementation (associations of genes in repulsion) between gametes. Epistasis would affect the development of such complementary structures. Combined with selection for the rate of recombination, it could be integrated with dominance in balanced units of segregation of greater size than a single locus. Such an organization of the genome in large and not strictly defined units, called "linkats" by DEMARLY (1972), could give a compromise between the preservation of variability for long-term adaptation and short-term adaptation. A consequence would be that natural selection increases "apparent" additivity. With a "wild" cross-fertilized species, it could be possible that revealed epistasis and dominance are lower for a character clearly related to fitness. This could be the case for yield in our experiment. Such organization would also explain the following point.

Coexistence of additivity under crossing with inbreeding depression and nonadditivity under inbreeding: A main conclusion from this study is that, for yield, a high additive variance in the noninbred population coexists with a strong inbreeding depression and with a nonadditive variance under inbreeding. Coexistence of additivity and inbreeding depression is theoretically possible with partial or complete dominance, with low frequencies of favorable genes (FALCONER 1961). In such situations, a lack of accuracy in the estimation of the components of the variance under crossing could explain why dominance variance is not significant. This lack of accuracy is illustrated in our experiment by the fact that specific combining ability variance does not appear significant but that the M2 model significantly reduces deviations from an additive model when all covariances under crossing are considered. The situation is then not as paradoxical as it appears at first sight. However, the major part of the variance under crossing appears additive, and such coexistence of additivity and strong inbreeding depression can also be deduced from reviews on corn by MOLL and STUBER (1974) and in perennial forage grasses by BREESE and HAYWARD (1970). Therefore, for a cross-fertilized unselected species, we may wonder if the assumption of gene frequencies is always sufficient to explain such a phenomenon.

The previous comment on the effect of natural selection could explain the importance of additive variance in the noninbred population. In crossing, there would be a systematic complementation between unrelated homologous units of segregation. Furthermore, it is quite possible that such complementation is developed by some gametic selection favoring the fusion of most unrelated gametes (a generalization to the whole genome of the incompatibility systems). Unfavorable (deleterious) genes integrated in the unit of segregation will be masked. If we assume that such unfavorable genes are present at low frequencies at a great number of loci, the probability of homozygosity of such genes in all individuals will be high. On one hand, a general effect of inbreeding on the mean with some "masking" effect explaining a decrease in variance with increasing levels of inbreeding could result, whereas, on the other hand, a change in the nature of the variability due to a change in the set of the expressed genes or in their regulation could be the case. Such an effect of the genetic structure (homozygosity, heterozygosity) on the expression of variability has been observed by MUKAI, YOWHIKAWA and SANO (1966) for *Drosophila*.

Another effect of inbreeding could be to deeply disturb the genetic organization developed by a great number of cycles of cross-fertilization and natural selection. The relative stability of the assumed units of segregation could disappear. So, again, new variation could be revealed, decreasing the apparent weight of additivity. From a more general point of view, inbreeding in normally cross-fertilized species can be considered as a system of mating changing the environment of genes. With gene \times environment interactions (dominance, epistasis, nucleocytoplasmic interactions, etc.), when the effect of natural selection is taken into account, it is quite conceivable that expression and organization of the variability is affected by inbreeding.

Consequences for the plant breeder: From the strict point of view of autotetraploid breeding, this experimental study allowed the verification of several theoretical predictions. The most specific for autotetraploids is the decrease in expected value of crosses with increasing level of inbreeding of their parents. A consequence of this, combined with the change in variance, in the population studied is that the best "single crosses" will be at the S_0 level. So, in such a situation, to exploit hybrid vigor in hybrid varieties if inbreeding is used, it will be necessary to develop double-cross hybrids (GALLAIS 1969; GALLAIS and GUY 1970). More generally, an optimum level of inbreeding can exist for the use of the variance in a given type of variety.

The other consequences from the experimental results are not strictly related to the autotetraploid state. The main result is that the variance among inbred families, with increasing levels of inbreeding, does not necessarily increase. Such situations can also be observed in diploids.

Therefore, in the presence of a masking effect due to inbreeding, selection on phenotypic values for performance of crosses during inbreeding, mainly with high levels of inbreeding, will be inefficient. Indeed, the genetic organization discussed previously induces a poor relationship between the value of an inbred plant and its value in crossing (*i.e.*, its general combining ability). So, if phenotypic selection is envisaged for inbred material, it must be restricted to a low level of inbreeding.

With such a situation, it will be difficult to develop lines, because there will be a strong elimination of material. It will be better to develop several cycles of recurrent selection without inbreeding first, and then to develop lines. Recurrent selection, even for combining ability, will also increase the value *per se* of the material and then of the lines that could be derived from (GALLAIS 1977a).

If the organization in linkats is confirmed by some other studies, it remains to determine how to induce recombination within them. Can inbreeding destroy the stability of such units? Clearly, if it could, self-fertilization might be excluded because it will "fix" the genome too quickly. Milder inbreeding could be more efficient.

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LITERATURE CITED

- BOUFFETTE, A. R., 1966 Contribution mathématique à la génétique des tétraploïdes. Thèse Doctorat 3ème cycle, Fac. Sci., Lyon, France.

- BOUFFETTE, J., 1966 Expression de la covariance chez les tétraploïdes. Thèse Doctorat 3ème cycle, Fac. Sci., Lyon, France.
- BREESE, E. L. and M. D. HAYWARD, 1972 The genetic basis of present breeding methods in forage crops. *Euphytica* **21**: 324-336.
- CHI, R. K., S. A. EBERHART and L. H. PENNY, 1969 Covariance among relatives in a maize variety. *Genetics* **63**: 511-520.
- COCKERHAM, C. C., 1963 Estimation of genetic variances. pp. 53-94. In: *Statistical Genetics and Plant Breeding*, Edited by W. D. HANSON and H. F. ROBINSON. Publication 982 from the National Academy of Science, National Research Council, Washington D.C.
- DATTÉE, Y., 1974 Influence des interactions entre génotypes parentaux sur la production de graines chez la luzerne. *Ann. Amélior. Plant.* **24**: 37-43.
- DEMARLY, Y. 1972 Commentaires sur les aptitudes à la combinaison. *Ann. Amélior. Plant.* **22**: 187-200.
- DESSUREAUX, L. and A. GALLAIS, 1971 Evolution of fertility in advanced generation of an alfalfa single cross hybrid. *Can. J. Genet. Cytol.* **13**: 834-841.
- FALCONER, D. S., 1961 *Introduction to Quantitative Genetics*. Oliver and Boyd, London.
- GALLAIS, A., 1967 Moyenne des populations tétraploïdes. *Ann. Amélior. Plant.* **18**: 5-15.
- GALLAIS, A., 1969 Interactions between alleles and their variability in autotetraploid cross-fertilized plants. *Genet. Agrar.* **23**: 312-323.
- GALLAIS, A., 1974 Covariances between arbitrary relatives in autotetraploids with panmictic disequilibrium. *Genetics* **76**: 587-600.
- GALLAIS, A., 1975 The use of heterosis in autotetraploid cross-fertilized plants with some applications to luzerne and cocksfoot. pp 50-56. In: *Proceedings of the Meeting of Eucarpia Fodder Crops Section*, Edited by B. NUESCH. Swiss Federal Station for Agronomy, Zurich.
- GALLAIS, A., 1976a Sur la signification de l'aptitude générale à la combinaison. *Ann. Amélior. Plant.* **26**: 1-13.
- GALLAIS, A., 1976b An experimental check of quantitative genetics on a autotetraploid plant, *Medicago sativa* with a special reference to identity by descent relationship. pp. 519-540. In: *Proceedings of the International Conference on Quantitative Genetics*, Edited by O. KEMPTHORNE, E. POLLAK, and T. B. Bailey Jr., Iowa State University Press, Ames.
- GALLAIS, A., 1977a Amélioration des populations, méthodes de sélection et création de variétés. I. *Ann. Amélior. Plant.* **27**: 281-329.
- GALLAIS, A., 1977b Contribution à l'étude théorique et expérimentale de l'hétérosis chez une plante allogame autotétraploïde. Thèse Doctorat d'Etat, Fac. Sci., Orsay, France.
- GALLAIS, A. and P. GUY, 1970 Breeding for heterosis in autotetraploids. pp. 105-118. In: *Section Eucarpia, Lusignan. Rapport S.E.I., I.N.R.A., Fodder Crops*, Versailles, France.
- GILLOIS, M., 1964 La relation d'identité en génétique. Thèse Fac. Sci., Paris, France.
- GUY, P., 1970 Quelques aspects de la morphologie d'une luzerne en couvert végétal dense. 5ème Colloque d'Ecologie E.N.S. March 12-14, 1970, E.N.S., Paris, France.
- HARRIS, D. L., 1964 Genotypic covariance among inbred relatives. *Genetics* **50**: 1319-1348.
- HAYMAN, B. I., 1960 Maximum likelihood estimation of genetic components of variation. *Biometrics* **16**: 369-381.
- HEDRICK, P. W., 1976 Genetic variation in heterogeneous environment. II. Temporal heterogeneity and directional selection. *Genetics* **84**: 145-147.
- LERNER, I. M., 1954 *Genetic Homeostasis*. Oliver and Boyd, Edinburgh.
- LEWONTIN, R. C., 1964 The interaction of selection and linkage. II. Optimum models. *Genetics* **50**: 757-782.

- LEWONTIN, R. C., 1974 The genetic basis of evolutionary change. Ph.D. Thesis. Columbia University, New York.
- MALÉCOT, G., 1949 Les mathématiques de l'hérédité. Masson et Cie, Paris.
- MATHER, K., 1941 Variation and selection of polygenic characters. *J Genet.* **41**: 159–193.
- MAYNARD-SMITH, J., 1980 Selection for recombination in a polygenic model. *Genet. Res.* **35**: 269–277.
- MOLL, R. H. and C. W. STUBER, 1974 Quantitative genetics: empirical results relevant to plant breeding. *Adv. Agron.* **26**: 277–313.
- MUKAI, T., I. YOWHIKAWA and K. SANO, 1966 The genetic structure of natural populations of *Drosophila melanogaster*. IV. Heterozygous effects of radiation induced mutations on viability in various genetic backgrounds. *Genetics* **53**: 513–527.
- SHULL, G. H., 1914 Duplicate genes for capsule form in *Bursa Bursa pastoris*. *Z. Indukt. Abstammungs. Vererbungsl.* **12**: 97–179.

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