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Cytoplasmic Effects on Peroxidase Activity in Crosses between two Genotypes of Flax (*Linum usitatissimum* L.)

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Summary. Crosses were made between two flax genotypes which differed markedly in dregee of basal branching and activity of peroxidase per gramme fresh weight. The two reciprocal F_1 hybrids and parental genotypes were crossed in all combinations to produce four F_2 progenies, four first backcross progenies to either parent, and the parental genotypes plus F_1's. Altogether, 16 progenies resulted; individual plants in each progeny were assayed for peroxidase activity. Plants were sampled at 35 and at 70 days after germination; activities were expressed on a log scale. A model was fitted by weighted least squares procedures to the mean log activities of the 16 progenies in each sample. The model contained parameters for additive and dominance genetic effects, as well as for cytoplasmic effects in terms of differences between all progenies of the one compared to the other reciprocal F_1. Such cytoplasmic effects could thus be evaluated in a maternal or a paternal direction, over F_2's and both backcrosses. The fitting technique involved the sequential elimination of redundant parameters in the model until the minimum group had been found adequately summarising the data. Additive and dominance parameters were significant in each sample; in the second sample (70 days) there were also significant cytoplasmic effects transmitted by male gametes of the reciprocal F_1's.

Introduction

Differences between reciprocal F_1 hybrids are routinely tested for significance in biometrical analyses of crosses between inbred lines. Their absence might be taken as evidence that maternal and/or cytoplasmic effects were of no importance. There is, however, little information available about the persistence of reciprocal differences into later generations derived from the two F_1's, apart from the study of Jinks, Perkins and Gregory (1972), or about the appearance of such differences in later generations when completely absent in the F_1. Techniques for analysis of reciprocal differences have been described by these authors and by Durrant (1965).

A straightforward extension of the basic cross between two inbred lines may be used to estimate maternal effects in F_1 reciprocal hybrids or in backcrosses with them, and, at the same time, estimate cytoplasmic effects in terms of differences in F_2 and backcross generations derived from the F_1 reciprocals. By crossing two inbred parental lines and their F_1 reciprocals in all combinations, in effect a four by four diallel, the parental lines, their F_1's, F_2's and backcrosses are produced. Various models may then be fitted to the 16 progenies. Estimation of additive and dominance genetic effects, as well as cytoplasmic effects in the F_2 and backcross progeny to the alternative F_1's may be made with one particular model. This approach has been used to examine a series of crosses between flax genotypes and genotrophs (Tyson, 1973), in which plant weight was measured. The same approach was employed here to see whether cytoplasmic effects on peroxidase activity could be detected, using two genotypes which differed markedly in activity per gramme fresh weight of main stem tissue. Peroxidase has been implicated in the control of indole acetic acid level (IAA) in plant tissues (Galston, Bonner and Baker, 1950). Differences displayed by various flax genotypes in the degree of branching from the axils of the cotyledons suggested that concomitant differences in peroxidase activity might be detectable. Techniques for measurement of peroxidase activity have been well worked out; in contrast, techniques for measurement of IAA level itself introduce severe practical limitations. The two flax genotypes used in this study differed markedly in their degree of basal branching, having been selected for high seed production on the one hand, and for long main stem length (for fibre production) on the other.

Materials and Methods

The two flax genotypes used were Royal (R) and Mandarin (M). R was much branched (oilseed type), whereas M was virtually unbranched (fibre type). A single plant progeny from each genotype provided the parental plants for the initial crosses. The 16 progenies resulting from the subsequent crosses in all combinations between the parental lines and their reciprocal F_1's were grown in a randomised complete block design with three replicates, the experiment being carried out in a growth chamber. Each replicate contained eight plants of each of the two parental lines, four of each of the reciprocal F_1's and nine plants each of all 12 F_2 and backcross progenies. Plants of each of the 16 progenies were completely randomised within each replicate. Replicates' positions were arranged to estimate the decreasing light intensity gradient which existed from the centre of the chamber to the corners.

With one replicate immediately under the central high intensity light zone, and the two others occupying the periphery of the chamber, light differences within any one replicate were minimised. Genetic and environmental variability among individuals within progenies was incorporated for weighting each progeny mean in the analytical procedures to be described. Two samples were grown; because of the size constraint of the 1.22 by 2.44 metre floor area, these samples followed one another consecutively in the chamber. Plants were sampled at 35 and at 70 days after germination. Peroxidase activity was measured on an individual plant basis; activity was assayed in dialysed extracts of main stem tissue from which leaves had been removed. Details of the preparation, dialysis, storage and assay of the individual plant extracts in this experiment have been described by Tyson (1970). Extracts from sample 2 displayed very high activities and all were accordingly diluted by a factor of 10 prior to assay. Activities were expressed in terms of the rate of increase in optical density per minute per gramme fresh weight, and were transformed to a log scale before analysis for the reasons detailed in the reference above. The transformed data are shown in table 1, where it may be noted also that each progeny is referenced through its row and column number in the four by four data matrix.

Table 1. *Peroxidase activity, expressed as log ΔO.D. 470 nm. min^{-1} g. fresh weight^{-1}. Mean log activities for the 16 progenies at 35 and at 70 days. Genotypes: R = Royal, M = Mandarin*

Sample 1 (35 days)

♀ \ ♂		R .1	F_1 ($R\times M$) .2	F_1 ($M\times R$) .3	M .4	Mean
R	1.	11 1.2988	12 1.1387	13 1.1272	14 0.8528	1.1044
F_1 ($F\times M$)	2.	21 1.1307	22 1.0117	23 1.0872	24 0.9914	1.0553
F_1 ($M\times R$)	3.	31 1.1359	32 1.0508	33 1.0654	34 0.9896	1.0604
M	4.	41 0.9554	42 1.0141	43 0.9282	44 1.0407	0.9846
Mean		1.1302	1.0538	1.0520	0.9686	

Sample 2 (70 days)

♀ \ ♂		R .1	F_1 ($R\times M$) .2	F_1 ($M\times R$) .3	M .4	Mean
R	1.	11 0.8094	12 0.7090	13 0.7994	14 0.6218	0.7349
F_1 ($R\times M$)	2.	21 0.7417	22 0.6660	23 0.7603	24 0.6334	0.7004
F_1 ($M\times R$)	3.	31 0.7970	32 0.6632	333 0.7545	34 0.7398	0.7386
M	4.	41 0.5416	42 0.6432	43 0.5926	44 0.5754	0.5882
Mean		0.7224	0.6704	0.7267	0.6426	

Results

For each of the 16 progenies in each replicate at each sampling, a mean activity and its variance were calculated from the individual plant log peroxidase activities. An analysis of variance (Tyson, 1970) of these mean activities showed that there were no significant differences among replicates, and no interaction between progenies and replicates. The activity data were, therefore, averaged over replicates within each sample, and the variances for these 16 means within each sample were obtained from the pooled within-replicate, within-sample sums of squares for the corresponding progenies.

The analysis of variance mentioned above was centred on an orthogonal breakdown of the progenies' sum of squares, a breakdown which examined cytoplasmic and maternal effects within segregating generations. The comparisons made within the F_2 and backcross generations are summarised in table 2 (part of table 8, Tyson, 1970). Their application (table 2) to the peroxidase activities (table 1) revealed, for example, signification differences in the F_2 between the progeny of reciprocal F_1's when these F_1's were used as males in generating the four F_2 progenies.

It is also possible to examine cytoplasmic effects across segregating generations with orthogonal comparisons; this was described by Tyson (1973). A set of orthogonal comparisons which estimates additive [d] and dominance [h] genetic effects, as well as estimating cytoplasmic effects across segregating generations, is shown in table 3. With the inclusion of a dummy variable to estimate the mean of all 16 progenies, this coefficient matrix (X) was fitted by weighted least squares procedures to the vector (y) of observed progeny means in order to estimate a total of 15 parameters. The weights used were the reciprocals of the variances of the progeny means, supplied as a diagonal matrix (V^{-1}) in:

$$b = (X\,V^{-1}\,X')^{-1}\,X\,V^{-1}\,y\,.$$

After fitting the model implicit in table 3 by this method, the residual sum of squares was distributed as chi-square; this allowed a test to be made of the model's adequacy in summarising the data. Estimation of [d] and [h] as in table 3 was simply an extension of the coefficients employed by Mather and Jinks (1971) for [d], [h] estimation, given means for P_1, P_2, F_1, F_2, B_1 and B_2.

Fitting of parameters by weighted least squares was employed by Barnes (1968) for his data from crosses in all combinations between two inbred lines of Drosophila and their reciprocal F_1 hybrids. He began with a basic model to estimate additive and dominance genetic effects, and inserted additional parameters for maternal and other effects until an adequate fit to his data had been obtained. In contrast, the fitting of the model in table 3 to the per-

Table 2. *Orthogonal comparisons among F_2 and first backcross progenies: cytoplasmic and maternal effects within segregating generations. F ratios shown for comparisons over samples 1 and 2, with tests against progeny by sample by replicate interaction as error*

Comparison		Progeny 11	12	13	14	21	22	23	24	31	32	33	34	41	42	43	44	F[1]
F_2	4						1	1			−1	−1						—
	5						1	−1			1	−1						6.47*
	6						1	−1			−1	1						—
B_1[2]	7		1	1		−1				−1								—
	8		1	−1		1				−1								1.65
	9		1	−1		−1				1								—
B_2[2]	10								1				1		−1	−1		2.65
	11								1				−1		1	−1		—
	12								1				−1		−1	1		4.95*

[1] F ratios for these comparisons over both samples; no significant interaction with samples for any of these 9 comparisons.
[2] B_1 = backcrosses to parent 1(R); B_2 = backcrosses to parent 2(M)
* Significant at probability 0.05.

Table 3. *Orthogonal comparisons among progenies; estimation of d, h, and cytoplasmic effects across segregating generation*

Parameter:		Progeny 11	12	13	14	21	22	23	24	31	32	33	34	41	42	43	44
Additive gene effect, [d]		1 1	1/2	1/2		1/2			−1/2	1/2			−1/2		−1/2	−1/2	−1
Dominance gene effects, [h]		2	1/2	1/2	1	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1	1/2	1/2	
Comparisons: Cytoplasmic effects	female	3				1	1	1	1	−1	−1	−1	−1				
	male	4	1	−1			1	−1			1	−1			1	−1	
	interaction	5					1	−1			−1	1					
F_1 effects	female	6 1	1	1	1	−1	−1	−1	−1	−1	−1	−1	−1	1	1	1	1
	male	7 1	−1	−1	1	1	−1	−1	1	1	−1	−1	1	1	−1	−1	1
	interaction	8 1	−1	−1	1	−1	1	1	−1	−1	1	1	−1	1	−1	−1	1
Additive-cytoplasmic interactions		9				1/2			−1/2	−1/2			1/2				
		10	1/2	−1/2											−1/2	1/2	
Parental-F_1 interactions		11 1	−1	−1	1									−1	1	1	−1
		12 1			−1	−1			1	−1			1	1			−1
Cytoplasmic-F_1 interactions		13				1	−1	−1	1	−1	1	1	−1				
		14	1	−1			−1	1			−1	1			1	−1	

oxidase activity data here was carried out in the reverse direction. The orthogonality of the comparisons in table 3 meant that comparisons, or parameters, which were redundant in the sense of contributing little to the variability among progeny means, could be removed sequentially one at a time from the matrix of coefficients (X). Re-fitting of the reduced model and re-calculation of the chi-square at each stage in the re-fitting allowed the minimum group of parameters adequately summarising the data to be determined. Comparisons were removed from the matrix X sequentially until the point was reached at which further deletion resulted in a significant chi-square value. Degrees of freedom for this chi-square (number of progeny means minus number of parameters estimated) increased, of course, as the elimination of redundant parameters proceeded.

The lengthy calculations inherent in the construction of adequate models by the reduction procedure used here were handled by writing an appropriate programme in IBM Matlan (matrix language). Any set of orthogonal parameters could thus be reduced, in the course of successive weighted least squares solutions, to the minimum group for adequate fit to the data. Orthogonality introduced limitations in the parameters which could be estimated; nevertheless, parts of the analysis of four by four tables for row and column effects have biological implications in the context of this type of experiment. Here, differences between the means of rows 2 and 3 in the data matrix (table 1), and between columns 2 and 3, may be recognised as stemming from female or male gametes of the alternative F_1's. In addition to the principal genetic and cytoplasmic effects, F_1 effects

in a male or female direction were estimable, with interactions between these three sets of effects also included in the comparisons of table 3.

The models fitted to each sample are shown in table 4. In both samples, additive and dominance effects were significant. In sample 1 there was a significant interaction between F_1 effects in a maternal direction and male cytoplasmic effects. Reference to table 3 shows that the effects of the male gametes from the two F_1's were not the same within the backcrosses to either parent as they were within the F_2's. In sample 2 there was a significant effect of the reciprocal F_1 male gametes across the backcross and F_2 generations, and thus evidence of transmission of cytoplasmic effects by male gametes. There was also a significant F_1 maternal effect, in that the eight progenies derived from F_1 female gametes, that is, from (R×M) and (M×R) as females, were superior to the eight from R and M as female parents. The interaction between F_1 maternal and paternal effects stemmed from the much larger superiority in the maternal direction, although the trend of superiority for F_1 derivatives was also apparent in the paternal direction. The two final interactions both involved cytoplasmic effects.

The estimates of genetic and cytoplasmic effects made in these crosses could not be extrapolated to other environments or genotypes. Nevertheless, male gamete transmission of cytoplasmic effects appearing in later, rather than earlier, developmental stages, and absence of immediate F_1 reciprocal differences, contrasted with the not uncommon detection of transient maternal effects in F_1's of crosses between inbred lines.

Table 4. *Estimates of parameters detailed in table 3. Standard errors shown with each estimate*

Parameter:	Sample 1 (35 days)	
	Mean =	1.1817 ± 0.0122
1	Additive effects, [d] =	0.1467 ± 0.0101
2	Dominance effects, [h] =	-0.2541 ± 0.0222
14	F_1 maternal by cytoplasmic male interaction =	0.0229 ± 0.0070
	$\chi^2_{(12)}$ =	20.15, $0.05 < P < 0.10$
Parameter:	Sample 2 (70 days)	
	Mean =	0.7427 ± 0.0179
1	Additive effects, [d] =	0.1108 ± 0.0138
2	Dominance effects, [h] =	-0.0946 ± 0.0361
4	Male cytoplasmic effect =	-0.0311 ± 0.0087
6	F_1 maternal effect =	-0.0268 ± 0.0073
8	F_1 maternal by paternal interaction =	-0.0133 ± 0.0072
10	Additive by male cytoplasmic interaction =	-0.0663 ± 0.0252
13	F_1 paternal by cytoplasmic female interaction =	-0.0175 ± 0.0089
	$\chi^2_{(8)}$ =	14.64, $0.05 < P < 0.10$

Discussion

Compared to control of inheritance through nuclear genes, there is insufficient information about the mechanism of cytoplasmic inheritance in higher plants for the construction and testing of models in an analogous way. In spite of this, cytoplasmic effects across segregating F_2 and first backcross generations were demonstrable with the type of model and weighted least squares procedure used here; they were also found in the F_2 with the unweighted analysis within segregating generations previously used.

Barnes (1968) fitted an additive, dominance and maternal effect model to eight generation means from his Drosophila data, means generated in a similar arrangement of crosses to those here, and derived from all 16 possible progenies. Additional genetic parameters in Barnes' models, such as those for additive and dominance components of a maternal genotype, required additional generations for fitting; Barnes showed also how such extended models could be developed for data from a correspondingly enlarged experiment. However, amalgamation of progenies, so as to supply the generation means to which his models were fitted, inevitably discarded information among the 16 progenies. This information was not retrievable in terms of any parameters derived from diploid genetic mechanisms, or as simple maternal or paternal effects.

It was this source of information upon which attention was focussed here, to reveal differences between the progeny of the one compared to the other F_1 reciprocal hybrid. The model used here, and its fitting by reduction to the simplest form with chi-square tests in successive weighted least squares solutions, were immediately applicable to Barnes' data, except that appropriate weights for the means of the 16 progenies in his data were not available. An arbitrary elimination of all but the first nine parameters (including the mean) was accordingly made in the model used here, so as to allow these remaining parameters to be fitted by an unweighted least squares procedure, with standard errors obtained from the least squares residue. It could then be shown that there were significant ($P < 0.05$) male transmitted cytoplasmic effects in Barnes' data of exactly the kind detected here for peroxidase activity in flax. This at least suggested the possibility of checking more often for such effects; were they to be reliably demonstrable in various situations, their explanation might allow more realistic models to be constructed.

Peroxidase is present in variant forms, or isozymes, in this plant. These isozymes

may be readily separated by standard electrophoretic techniques, with subsequent measurement of their relative mobility and individual activity. Separation and measurement in the progenies here, for example, would allow the cytoplasmic effects described for 'total' activity to be examined in respect of each isozyme. It might then be possible to examine the ways in which such cytoplasmic effects on, say, activities of individual isozymes were reflected in terms of enzyme composition or structure. This, in turn, might provide an insight into the presumably differential cytoplasmic contributions to gametes during their formation on the reciprocal F_1's.

Several possibilities exist in the area of differential cytoplasmic contributions. Among these, for example, would be long-lived messenger RNA, mitochondrial or chloroplast DNA, level of IAA, or different thresholds of response among genes to switching on for RNA synthesis by IAA. Available techniques make an attack on some of these areas feasible.

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