

THE NATURE OF THE INHERITANCE OF PERMANENTLY INDUCED CHANGES IN *NICOTIANA RUSTICA*

II. F_4 AND F_5 GENERATIONS OF SELECTED CROSSES

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SUMMARY

The F_4 and F_5 generations of the two selected crosses, $nk_2 \times nil_1$ and $p_3 \times nil_3$, from a diallel of all possible crosses between the three conditioned lines p , nil and nk were grown in successive years in the summers of 1970 and 1971 respectively in order to study (1) the persistence of the segregation between F_1 individuals seen in earlier generations and (2) the conventional segregation in the later generations. In the F_4 generation F_1 segregation in the cross $nk_2 \times nil_1$ approaches significance for the character final height. In the F_5 generation significant segregation between F_1 individuals was not found for the characters final height and flowering time for either cross. However, in the cross $nk_2 \times nil_1$ significant F_1 segregation was found for two other characters, height at flowering time and the diameter of the eighth leaf. The presence of segregation at the F_2 , F_3 and F_4 levels was confirmed for both characters in both crosses by the fitting of a standard additive genetical and additive environmental model. From the estimates of D obtained the numbers of effective factors by which these lines differ were found to range from three to five.

1. INTRODUCTION

PREVIOUS investigation (Perkins, Eglington and Jinks, 1971) of all possible crosses between three differently conditioned lines derived from pure-breeding variety 16 of *Nicotiana rustica* have established that F_1 individuals of the same cross differ for final height and flowering time and that these differences persist into the F_2 progenies of all crosses and the F_3 's of some of them. Furthermore, segregation in the F_2 and F_3 generations of these crosses appeared to be of the conventional kind that is observed in the same generations when derived from a cross between different inbred genotypes. In both respects the behaviour of the conditioned lines of *N. rustica* resembles that of similarly conditioned lines of flax (Durrant and Tyson, 1964). Two crosses, $p_3 \times nil_3$ and $nk_2 \times nil_1$, chosen because they provide the clearest evidence of differences among F_1 individuals which persisted to their corresponding F_2 's, have been chosen to provide F_4 and F_5 families. These were grown in the summers of 1970 and 1971 respectively, in order to study further the persistence of the F_1 segregation and the conventional segregations in the later generations.

2. EXPERIMENTAL MATERIALS

The F_4 and F_5 families were obtained by repeated selfing from the two crosses $p_3 \times nil_3$ and $nk_2 \times nil_1$ (Perkins, Eglington and Jinks, 1971).

Twenty F_4 families of each cross were grown in 1970, these families can be divided according to their ancestry into (1) five F_1 groups, (2) two F_2

TABLE 1

Analyses of variance of family means for the F_4 and F_5 generations of the $p_3 \times nil_3$ and $nk_2 \times nil_1$ crosses, for the character final height, measured in centimetres

Item	$p_3 \times nil_3$				$nk_2 \times nil_1$			
	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P
<i>(a) F₄ generation</i>								
1. F ₁ groups (F ₁)	4	71.04	V.R. (2)	n.s.	4	157.30	—	†
2. F ₂ groups in F ₁ (F ₂)	4	277.56	V.R. (7)	**	5	44.17	V.R. (3)	n.s.
3. F ₃ groups in F ₂ (F ₃)	9	36.60	χ^2 (8)	***	10	52.68	χ^2 (8)	***
4. Blocks (B)	1	0.34	χ^2 (8)	n.s.	1	43.89	V.R. (5)	n.s.
5. B \times F ₁	4	4.60	χ^2 (8)	n.s.	4	37.92	χ^2 (8)	*
6. B \times F ₂	4	2.80	χ^2 (8)	n.s.	5	12.91	χ^2 (8)	n.s.
7. B \times F ₃	9	21.40	χ^2 (8)	n.s.	10	15.28	χ^2 (8)	n.s.
8. Replicates	324	11.43			360	14.27		
<i>(b) F₅ generation</i>								
1. F ₁ groups (F ₁)	4	97.68	V.R. (2)	n.s.	4	311.82	V.R. (3)	n.s.
2. F ₂ groups in F ₁ (F ₂)	4	402.11	V.R. (4)	**	5	156.55	V.R. (3)	n.s.
3. F ₃ groups in F ₂ (F ₃)	9	83.53	V.R. (4)	n.s.	10	89.82	V.R. (4)	**
4. F ₄ groups in F ₃ (F ₄)	18	35.68	V.R. (9)	*	20	19.96	χ^2 (10)	*
5. Blocks (B)	1	539.81	V.R. (9)	***	1	928.32	χ^2 (10)	***
6. B \times F ₁	4	9.35	V.R. (9)	n.s.	4	11.61	χ^2 (10)	n.s.
7. B \times F ₂	4	7.08	V.R. (9)	n.s.	5	12.32	χ^2 (10)	n.s.
8. B \times F ₃	9	8.14	V.R. (9)	n.s.	10	12.20	χ^2 (10)	n.s.
9. B \times F ₄	18	12.13	χ^2 (10)	*	20	9.07	χ^2 (10)	n.s.
10. Replicates	592	7.49			698	11.66		

† No real test of significance is available as both Items 3 and 5 are significant. n.s. Probability is non-significant. * Probability = 0.01-0.05; ** Probability = 0.001-0.01; *** Probability < 0.001.

TABLE 2

Analyses of variance of family means for the F_4 and F_5 generations of the $p_3 \times nil_3$ and $nk_2 \times nil_1$ crosses, for the character flowering time

Item	$p_3 \times nil_3$				$nk_2 \times nil_1$			
	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P
<i>(a) F₄ generation</i>								
1. F ₁ groups (F ₁)	4	16.70	V.R. (2)	n.s.	4	19.55	V.R. (2)	n.s.
2. F ₂ groups in F ₁ (F ₂)	4	46.10	V.R. (3)	**	5	17.51	V.R. (7)	***
3. F ₃ groups in F ₂ (F ₃)	9	2.20	χ^2 (8)	***	10	4.43	V.R. (7)	n.s.
4. Blocks (B)	1	0.15	χ^2 (8)	n.s.	1	0.55	V.R. (7)	n.s.
5. B \times F ₁	4	0.34	χ^2 (8)	n.s.	4	0.43	V.R. (7)	n.s.
6. B \times F ₂	4	0.31	χ^2 (8)	n.s.	5	1.46	V.R. (7)	n.s.
7. B \times F ₃	9	0.82	χ^2 (8)	n.s.	10	1.78	χ^2 (8)	*
8. Replicates	324	0.88			360	0.82		
<i>(b) F₅ generation</i>								
1. F ₁ groups (F ₁)	4	17.82	V.R. (2)	n.s.	4	14.66	V.R. (3)	n.s.
2. F ₂ groups in F ₁ (F ₂)	4	53.22	V.R. (3)	*	5	11.06	V.R. (3)	n.s.
3. F ₃ groups in F ₂ (F ₃)	9	11.64	V.R. (4)	**	10	7.67	V.R. (9)	***
4. F ₄ groups in F ₃ (F ₄)	18	2.63	χ^2 (10)	***	20	1.29	V.R. (9)	n.s.
5. Blocks (B)	1	3.32	χ^2 (10)	**	1	3.97	V.R. (9)	n.s.
6. B \times F ₁	4	0.32	χ^2 (10)	n.s.	4	0.18	V.R. (9)	n.s.
7. B \times F ₂	4	0.51	χ^2 (10)	n.s.	5	0.90	V.R. (9)	n.s.
8. B \times F ₃	9	0.30	χ^2 (10)	n.s.	10	0.59	V.R. (9)	n.s.
9. B \times F ₄	18	0.69	χ^2 (10)	n.s.	20	0.65	χ^2 (10)	***
10. Replicates	602	0.45			707	0.27		

Symbols as in table 1.

groups within each F_1 group, (3) two F_3 groups (= F_4 families) within each F_2 group. Similarly, 40 F_5 families were grown in 1971 which can be divided into the same groups with an additional division, (4) two F_4 groups (= F_5 families) within each F_3 group (tables 1 and 2), each group being descended from a single selfed plant in the generation specified. Each family consisted of 10 individually randomised plants in each of two replicate blocks.

In the cross $p_3 \times nil_3$ the entire data for one F_2 group, comprising two F_3 groups in the F_4 and four F_4 groups in the F_5 generation, has been removed from all analyses for reasons discussed by Perkins, Eglington and Jinks (1971).

3. RESULTS

(B) F_1 segregation

The analyses of variance are given for each cross and generation separately in table 1 for final height and in table 2 for flowering time. These analyses have the same structure as those presented on p. 450 of the previous paper (Perkins, Eglington and Jinks, 1971) but are extended to include the F_4 and F_5 generations.

In no case is there significant evidence of segregation between F_1 groups although for both generations and characters of the $nk_2 \times nil_1$ cross the relevant mean square is the largest main effect apart from that between blocks. In the F_4 generation of the cross $nk_2 \times nil_1$ for the character final height (table 1) there is no test for Item 1 owing to the significance of both its interaction with blocks (Item 5) and Item 3 (between F_3 groups within F_2 groups). However, by calculating either $4\sigma_{b_1}^2$ or $2\sigma_3^2$ and subtracting from the empirical mean square for Item 1, it is possible to obtain an estimate of the "mean square" for differences between F_1 groups in order to test its significance. Either adjustment provides a derived mean square which is, however, still non-significant against the original error items.

(B) F_2, F_3, F_4 segregation

It can be seen from tables 1 and 2 that there is evidence that segregation is occurring at the F_2, F_3 and F_4 levels for both final height and flowering time in both crosses although all the relevant mean squares (Items 2 and 3 in the F_4 and 2, 3 and 4 in the F_5 generations) are not necessarily significant in each case.

To investigate the nature of the variation in the F_5 generation that occurred from the F_2 generation onwards, the four rank variances, $V2F_c5$, $V3F_c5$, $V4F_c5$ and $V5F_c5$, which measure the mean variation ascribable to differences between F_2 great grandparents, F_3 grandparents, F_4 parents and F_5 progenies within F_4 groups, respectively, have been calculated from the analysis of variance for each of the eight combinations of characters, crosses and blocks (table 3). These have been equated to the standard biometrical genetical expectations in terms of a model with an additive (D), dominance (H) and additive environmental (E_1) component of variation which assumes Mendelian autosomal inheritance, independence of the genes in action and in distribution and no genotype-environmental interactions. This model was found to be satisfactory in all cases when fitted by least squares procedures (Mather and Jinks, 1971) but in no case was the estimate of H

TABLE 3

Rank variances for F_6 generation of crosses $p_3 \times nil_3$ and $nk_2 \times nil_1$ grown in 1971

Cross $p_3 \times nil_3$		$V2F_65$	$V3F_65$	$V4F_65$	$V5F_65$
Final height					
Block 1		42.5713	15.4820	21.9608	69.9173
Block 2		59.6687	30.4096	25.8501	79.0203
Blocks 1+2		102.2400	45.8916	47.8109	148.9376
Flowering time					
Block 1		6.7549	2.5760	1.5708	4.0244
Block 2		6.6792	3.3957	1.7468	4.9928
Blocks 1+2		13.4341	5.9717	3.3176	9.0172
Cross $nk_2 \times nil_1$		$V2F_65$	$V3F_65$	$V4F_65$	$V5F_65$
Final height					
Block 1		28.3941	30.5101	13.8785	105.1270
Block 2		13.8219	20.4993	15.1550	128.4373
Blocks 1+2		42.2160	51.0094	29.0335	233.5503
Flowering time					
Block 1		0.8153	2.0534	0.7672	2.3928
Block 2		2.1744	2.0757	1.1741	3.0457
Blocks 1+2		2.9897	4.1291	1.9413	5.4385

significantly different from zero. A model was therefore refitted which omitted H . The analyses of goodness of fit of this model are presented in table 4. The significance of the following items are given, the joint regression over blocks, the residual variation (not accounted for by the model), the heterogeneity of the regression over the two blocks and the remainder. For flowering time the model still fits, the only significant item being the joint regression. The least-squares estimates of the parameters for flowering

TABLE 4

Analysis of variance to test the fit of the D and E_1 model to data from the F_6 generation of the crosses $p_3 \times nil_3$ and $nk_2 \times nil_1$ grown in 1971

Item	$p_3 \times nil_3$				$nk_2 \times nil_1$			
	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P
1. Joint regression	2	9137.6443	V.R. (AMS)	***	2	14876.5100	V.R. (AMS)	***
2. Residual	2	119.1875	V.R. (3)	n.s.	2	66.6722	V.R. (3)	n.s.
3. Heterogeneity of regression	2	148.7945	V.R. (4)	*	2	212.3597	V.R. (4)	**
4. Remainder	2	4.4970			2	2.2009		
Flowering time								
1. Joint regression	2	76.3384	V.R. (AMS)	***	2	13.7938	V.R. (AMS)	***
2. Residual	2	0.7748	V.R. (4)	n.s.	2	1.0403	V.R. (4)	n.s.
3. Heterogeneity of regression	2	0.2823	V.R. (4)	n.s.	2	0.5582	V.R. (4)	n.s.
4. Remainder	2	0.1294			2	1.1194		

AMS = Average mean square. Symbols as in table 1.

time have been tested (table 5) against errors determined in the usual way from the pooled mean squares of the error items (Items 2, 3 and 4 of table 4). Both D and E_1 are significant in both crosses.

For final height the refitted model shows a highly significant joint regression but also a significant heterogeneity between blocks. The latter item was also large for the three parameter model but non-significant due to the small number of degrees of freedom available for the test. The residual is also large, indicating the presence of some other unfitted parameter, possibly H , but it is not significant against the block heterogeneity. The parameters have been tested against an error derived from the pooled mean square of Items 2 and 3 in table 4. D was found to be significant for the $p_3 \times nil_3$ cross, but not for $nk_2 \times nil_1$. E_1 was again significant in both crosses.

TABLE 5
Estimates of the parameters D and E_1 in the F_5 generation

$p_3 \times nil_3$		Block		
		1	2	1 and 2 combined
Final Height	D	56.4584*	85.1967*	70.8275*
	E_1	66.8093	74.1889*	70.4992*
Flowering time	D	9.2095***	9.6053***	9.4075***
	E_1	3.4123**	4.3807***	3.8966***
$nk_2 \times nil_1$				
	Final height	D 45.6582 n.s.	21.3365 n.s.	33.4974 n.s.
	E_1	102.3259**	127.2848**	114.8054**
Flowering time	D	2.1876*	3.7557**	2.9717**
	E_1	2.3194**	2.8670**	2.5932**

(iii) *The number of effective factors*

The values of D from the two parameter model for the two crosses and characters were used to obtain estimates of the numbers of effective factors by which the parental lines used in the crosses differed. The formula used to estimate the numbers of effective factors was

$$k = \frac{(\frac{1}{2} \text{ range})^2}{D},$$

where k is the number of effective factors and "range" is the difference between the greatest and smallest family mean among the families within each cross of the F_5 generation (averaging over blocks for flowering time, but treating blocks separately for final height in the $p_3 \times nil_3$ cross where there was heterogeneity between blocks). There is of course no estimate of the number of effective factors by which nk and nil differ for final height as D was non-significant. For the purpose of this estimation the F_5 families are being treated as pure-breeding lines.

The estimated number of genes, to the nearest whole number, for the cases where D was significant, are $p_3 \times nil_3$, final height, 3, flowering time, 3, and $nk_2 \times nil_1$, flowering time, 5. These are of course minimal estimates for the usual reasons (Mather and Jinks, 1971). The distribution of the family means, averaged over blocks, in the F_5 generation is given in histogram form in figs. 1a and 1b for final height and in figs. 2a and 2b for flowering time

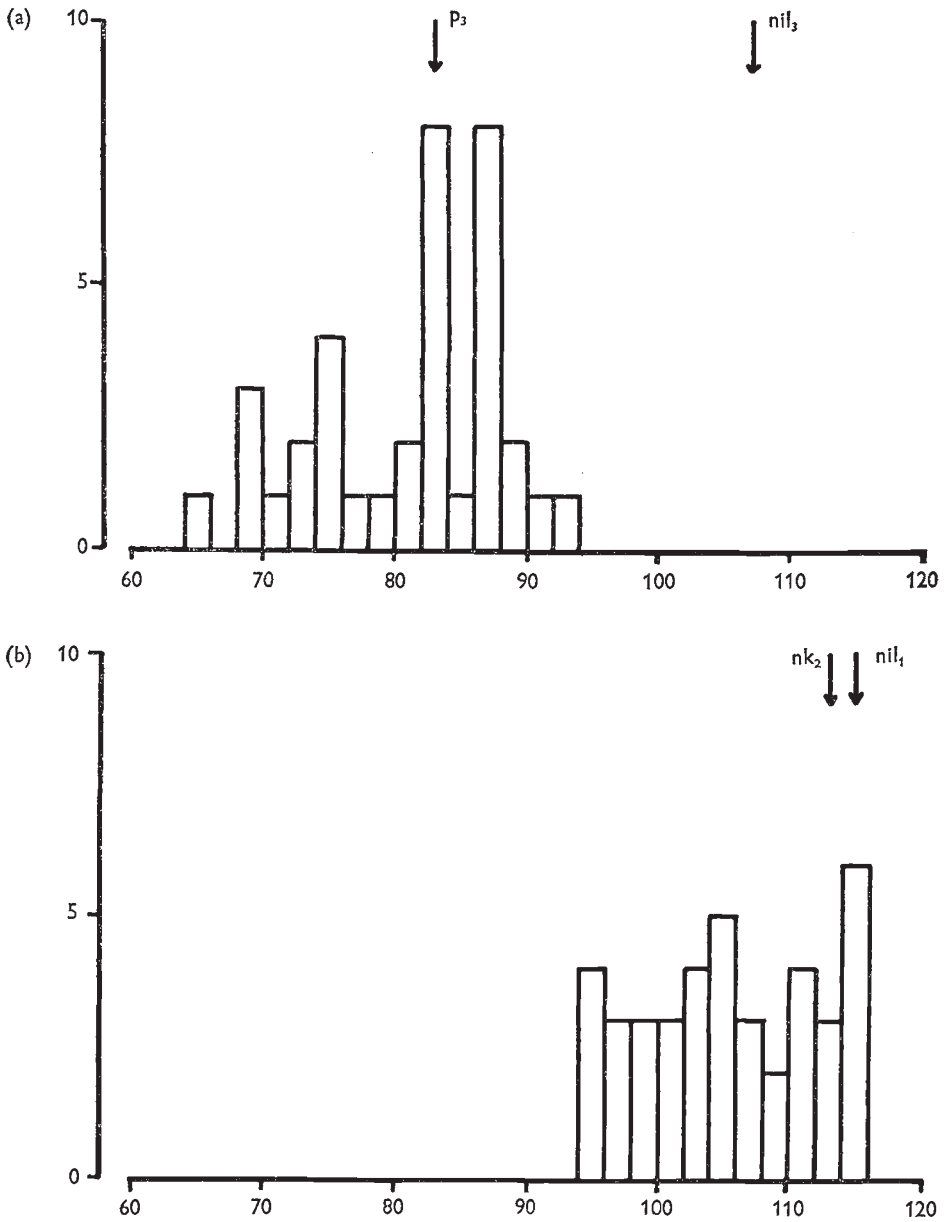


FIG. 1.—Histograms to show the distribution of the F_2 family means for the character final height for (a) the 36 families of the cross $p_3 \times nil_3$ and (b) the 40 families of the cross $nk_2 \times nil_1$.

in the $p_3 \times nil_3$ and $nk_2 \times nil_1$ crosses respectively. The mean parental line values are indicated in each diagram (these parental values were obtained from an experiment adjacent to the experiment under consideration and sown on the same day, ensuring maximum similarity between the conditions experienced by the two experiments).

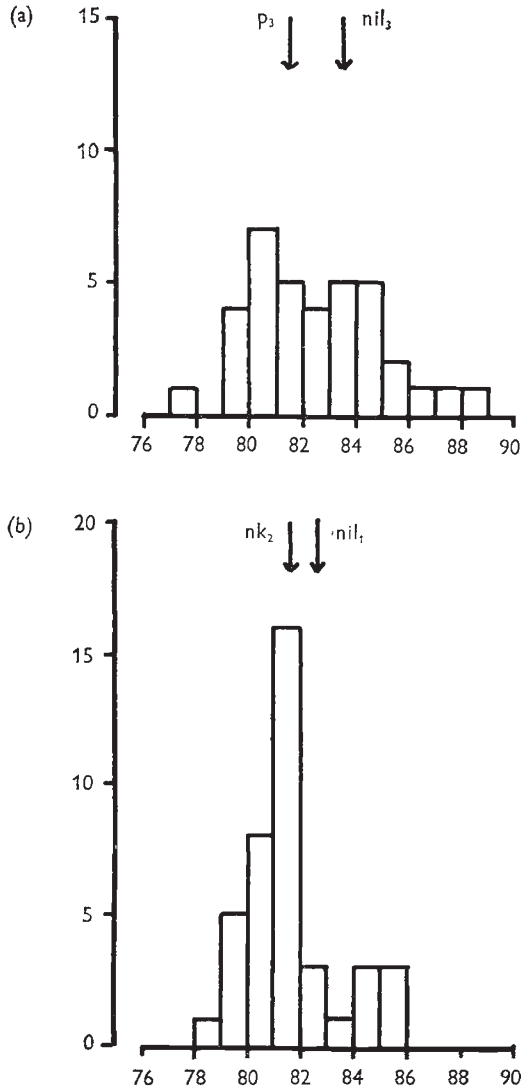


FIG. 2.—Histograms to show the distribution of the F_5 family means for the character flowering time for (a) the 36 families of the cross $p_3 \times nil_3$ and (b) the 40 families of the cross $nk_2 \times nil_1$.

The range of family means for final height is similar between parents and offspring in the $p_3 \times nil_3$ cross and much larger for the offspring than the parents in the $nk_2 \times nil_1$ cross, suggesting association of genes controlling height in the former and dispersion in the latter. For flowering time both crosses have a larger range of F_5 family means than of parental values, from which it is concluded that the genes controlling flowering time are in dispersion in both crosses. However, the nk_2 and nil_1 parents show very similar flowering times in the 1971 data which may reflect the change in flowering time of some of the original standard conditioned lines in the first few generations after conditioning (Perkins, Eglington and Jinks, 1971) and changes in the environmental conditions.

4. CONCLUSIONS

For neither character could the segregation which occurred in the F_1 , and which could still be detected between F_1 groups in the F_2 and F_3 generations, be detected in the F_4 and F_5 generations of the crosses $p_3 \times nil_3$ and $nk_2 \times nil_1$ although in the latter cross the mean square between F_1 groups was the largest item, apart from blocks. However, part of the failure to find significant F_1 segregation must be attributable to the reduction in the number of degrees of freedom available for this item in the F_4 and F_5 data compared with earlier generations. This resulted from a reduction in the number of F_1 groups represented in the later generations because of the rapid expansion in the size of the experiment that follows from a hierarchical breeding design. The same argument applies to the alternative method of detecting the persistence of the F_1 segregation by comparing the means of F_1 groups over successive generations. Using correlations to make these comparisons, the coefficients were 0.87 and 0.79 for final height and 0.73 and 0.87 for flowering time in the two crosses between the F_3 and F_4 generations and 0.52, 0.86, 0.75 and 0.79 for the corresponding comparisons between the F_4 and F_5 generations. These are all relatively high values considering that they are comparisons over both generations and seasons, but because of the small number of F_1 groups represented in these generations, none is significant, only three degrees of freedom being available for each coefficient. There are, therefore, some indications that the segregation observed in the F_1 of these crosses is still leading to differences between F_1 groups in these later generations and this is strongly supported by significant differences ($P = 0.01 - 0.05$) between F_1 groups in the F_5 generation for two other characters, height at flowering time and width of the eighth leaf (Eglington and Moore, unpublished).

That a biometrical genetical model consisting of additive genetical and additive environmental effects only, adequately describes the variation from the F_2 generation onwards, leaves no reason to doubt that this segregation is of the conventional kind. This confirms the earlier analysis of 3×3 diallel sets of crosses in respect of the additive genetical component but not for the smaller non-additive component. This failure to detect a non-additive component is not surprising since the selfing series is relatively insensitive to the effects of non-additive variation and hence is unlikely to detect a small component of this kind.

The minimal number of effective factors detected in the crosses between the conditioned lines are of the same order of magnitude as those detected by comparable methods in crosses between conventionally derived inbred lines for the same characters (Eaves and Brumpton, 1972). That more than one and possibly many effective factors are involved in the differences between lines which have received different conditioning treatments is further supported by:

- (i) the distributions of the F_5 family means for the two characters which are given in figs. 1 and 2;
- (ii) the occurrence of families in the F_5 whose means fall well outside of the parental range;
- (iii) the independence or low correlations between the differences in final height and flowering time among the F_5 families ($r = 0.40$ and 0.16 for the $p_3 \times nil_3$ and $nk_2 \times nil_1$ crosses, respectively); and

- (iv) the many other characters for which there is significant variation among the F_5 families which is partly or wholly independent of final height and flowering time (Moore and Eglinton, 1973). For the present, therefore, we must proceed on the assumption that sites on may chromosomes have undergone heritable changes as a result of the conditioning treatments.

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