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

JEAN M. PERKINS, EVELYN G. EGLINGTON and J. L. JINKS
Department of Genetics, University of Birmingham, Birmingham B15 2TT, England

1. INTRODUCTION

IN several organisms specific heritable changes can be consistently induced by certain intra- or extracellular environments. The change may be chromosomal, extrachromosomal or a combination of the two. Frequently in the absence of the inducing environment the change can be reversed or it is gradually lost over succeeding generations (Lewis, 1950; Sonneborn, 1950; Nanney and Caughey, 1955; Highkin, 1958) but in other systems the change is more permanent (Brink 1958; Durrant, 1962a; Hill, 1965).

The eight combinations of the three fertiliser treatments nitrogen (N), phosphorus (P), and potassium (K), were applied by Hill (1965) to different individuals of a true breeding variety of *Nicotiana rustica* under glasshouse conditions in the winter 1962-63. This variety had been previously subjected to inbreeding by selfing for at least 15 generations. Three replicates, chosen at random from among the six grown for each fertiliser combination, were selfed and have been subsequently maintained as independent lines by selfing for seven successive generations. Differences in the two characters final height and flowering time obtained by the application of the different fertiliser combinations have been maintained undiminished in all succeeding generations when grown under uniform nutrient conditions (Hill and Perkins, 1969, and personal communication).

The fertiliser combinations NK and Nil induce the tallest persistent phenotype and P the shortest, while K induces the earliest flowering and NP the latest flowering. There is therefore no obvious correlation between the changes induced in the two characters.

The persistent changes as measured by fresh weight in two varieties of flax had previously been induced by Durrant (1962a, 1971) with the same three fertiliser treatments N, P and K in their eight possible combinations. The two extreme and stable phenotypes, large (L) and small (S) are induced by the fertiliser combinations NPK or N and by NK or P, respectively, depending upon the pH of the soil (Durrant, 1971). L and S also differ in DNA content, L having 12-16 per cent. more than S as measured by feulgen photometry. The DNA content was shown to increase and decrease respectively in the first 5 weeks of growth during induction of the L and S types (Evans, 1968). The L and S forms of the most thoroughly investigated flax variety, Stormont Cirrus, also differ by a single gene determining the presence or absence of hairs on septa in the capsules, S having hairs (HH) and L having none (hh) (Durrant and Nicholas, 1970).

The nature of the inheritance of the permanently induced changes in the flax variety Stormont Cirrus may be summarised as follows:

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(a) The heritable changes are chromosomal since they are not transmitted through reciprocal grafts and the two reciprocal crosses L × S and S × L show equilineal inheritance (Durrant, 1962a).

(b) The heritable changes within L and S display mainly additive genetic variation but some dominance variation (Durrant and Tyson, 1964).

(c) The L and S strains are genetically homogeneous since selection within these two types is ineffective (Durrant, 1971).

(d) The F_1 of the cross between L and S is genetically heterogeneous. The variation within the F_1 is greater than that of either parent and a significant parent/offspring correlation is obtained between F_1 individuals and the mean of their F_2 families (Durrant, 1962b). Furthermore, selection from different L × S individuals is successful in producing L and S types together with correlated changes in their DNA content in the expected direction (Evans, 1968). The single gene difference for presence or absence of hairy capsule septa also shows instability of expression in the F_1 (Durrant and Nicholas, 1970).

(e) The cytoplasm may be the site of a heritable change which determines the variability of expression of the heritable chromosomal change. In both the F_1 and F_2 generations the S × L reciprocal cross shows greater variation than the L × S cross (Durrant, 1962b). In the F_2 of crosses between L and S and other varieties of flax and linseed the larger variation which is found to be associated with the S genotype is paternally inherited and the smaller variation of the L genotype is maternally inherited (Durrant and Tyson, 1964).

The series of experiments described in this paper were designed to investigate the extent to which the inheritance of the permanently induced changes in *Nicotiana rustica* resembles that in flax.

2. EXPERIMENTAL MATERIAL

For each of the eight original inducing environments there are three replicate lines of *Nicotiana rustica*, making 24 in all. The extreme induced phenotypes for the two characters final height and flowering time were selected in the winter of 1966-67 on the basis of information from the original generation of induction and the three succeeding ones. The lines induced with P (*p*) were selected for their shortness and with NK (*nk*) and Nil (*nil*) for their tallness, while *nk* was also early flowering and *nil* late flowering. These selections differ from the extreme persistent phenotypes for flowering time mentioned in the introduction which were based upon information from the six generations after induction. It seems that the earlier selections for flowering time were biased by the effect of an additional induced change which was transient in nature, and possibly maternal as in flax (Durrant, 1971). Such a change would be present only in the original generation of induction and to a lesser extent in the first generation after induction.

One replicate line was chosen from each set of three for *p*, *nk* and *nil* and these were crossed in all possible combinations to give an F_1 diallel set of crosses (see table 1). Two independently derived such F_1 diallel sets were grown in 1967 (I and II) and one only in 1968 and 1969. Two replicate lines used to represent *p*, *nk* and *nil* in each of the crosses in each year are given in table 1. For the two F_1 diallel sets of crosses grown in 1967, from

crosses made by Hill in 1965, p, nk and nil were represented by choosing one line, from each of the three available, at random as a parent of each individual cross. Reciprocal crosses were however made between the same two individuals and therefore the same pair of replicate lines. Conversely, for the F_1 diallel set of crosses grown in 1968, from crosses made in 1967, just one replicate line was chosen at random from each set of three for p, nk or nil and used as a parent in all crosses involving that treatment. The F_1 diallel

TABLE 1

Experimental material

	Experiment			
	1967		1968	1969
No. of blocks	2		2	3
	I	II		
	$p\ 2^* \times p\ 2$	$p\ 3\ \times p\ 3$	$p\ 1\ \times p\ 1$	
	$p\ 1\ \times nk\ 3$	$p\ 1\ \times nk\ 3$	$p\ 1\ \times nk\ 3$	
	$p\ 3\ \times nil\ 3$	$p\ 2\ \times nil\ 2$	$p\ 1\ \times nil\ 2$	
	$nk\ 3 \times p\ 1$	$nk\ 3 \times p\ 1$	$nk\ 3 \times p\ 1$	
	$nk\ 3 \times nk\ 3$	$nk\ 3 \times nk\ 3$	$nk\ 3 \times nk\ 3$	
	$nk\ 2 \times nil\ 1$	$nk\ 2 \times nil\ 1$	$nk\ 3 \times nil\ 2$	
	$nil\ 3 \times p\ 3$	$nil\ 2 \times p\ 2$	$nil\ 2 \times p\ 1$	
	$nil\ 1 \times nk\ 2$	$nil\ 1 \times nk\ 2$	$nil\ 2 \times nk\ 3$	
	$nil\ 3 \times nil\ 3$	$nil\ 2 \times nil\ 2$	$nil\ 2 \times nil\ 2$	
No. of sibs/cross	25	25	10	5
Total over crosses and blocks	450	450	180	135
F_2 crosses			from 1967 I F_1s	from 1968 F_1s
	—	—	$p\ 1\ \times nk\ 3$	$p\ 1\ \times nk\ 3$
	—	—	$p\ 3\ \times nil\ 3$	$p\ 1\ \times nil\ 2$
	—	—	$nk\ 3 \times p\ 1$	$nk\ 3 \times p\ 1$
	—	—	$nk\ 2 \times nil\ 1$	$nk\ 3 \times nil\ 2$
	—	—	$nil\ 3 \times p\ 3$	$nil\ 2 \times p\ 1$
	—	—	$nil\ 1 \times nk\ 2$	$nil\ 2 \times nk\ 3$
No. of F_2 families/cross	—	—	10	5
No. of sibs/F_2 family	—	—	20	10
Total over crosses and blocks	—	—	2400	900
F_3 crosses				from 1968 F_2s
	—	—	—	$p\ 3\ \times nil\ 3$
	—	—	—	$nk\ 2 \times nil\ 1$
No. of F_3 family groups/cross	—	—	—	7
No. of F_3 families/group	—	—	—	7
No. of sibs/F_3 family	—	—	—	10
Total over crosses and blocks	—	—	—	2940

* The fertiliser combination which provided the original environment of induction is given in small letters followed by the replicate number (1-3) of the particular conditioned line used.

set grown in 1969 was an independent repeat of that grown in 1968. The number of replicate blocks and the number of sibs per cross in each block for the three years 1967, 1968 and 1969 are given in table 1.

A random sample of F_1 individuals from each cross, which were grown in the first diallel set of 1967 and that in 1968, were selfed to give a corresponding number of F_2 families. A number of sibs from each F_2 family were grown in the following year as part of the 1968 and 1969 experiments. The number of F_2 families per cross and the number of sibs per F_2 family are given in table 1.

For reasons that will be discussed later in the paper, F_2 individuals belonging to two particular crosses, *p 3 × nil 3* and *nk 2 × nil 1*, were selfed in 1968 to give a corresponding number of F_3 families which were grown in the following year as part of the 1969 experiment. For each of these crosses in 1968 there were ten F_2 families derived by selfing ten F_1 individuals in 1967. Of these ten F_2 families seven were chosen at random and seven individuals from each family were selfed to give seven F_3 families. This meant that for each cross in 1969 there were seven F_3 families in each of seven groups the seven families within each group being traceable to seven F_2 individuals belonging to the same family in 1968. The seven groups can likewise be traced back to seven different F_2 families in 1968 and ultimately to seven different F_1 individuals of the first diallel set in 1967. Ten sibs were grown for each F_3 family in each of the three blocks of the experiment (table 1).

In any one year all the generations were grown together in the same experiment. All the plants in each block of each experiment were individually randomised. The two characters final height (in centimetres) and the days to flowering (after sowing) were scored in each of the three years 1967, 1968 and 1969.

3. RESULTS

(a) *Analysis of family means*

The means and variances of the families of the four F_1 diallel sets of crosses are given for final height in table 2 and for flowering time in table 3. Examination of the diagonal terms in table 2 shows that *p* is short in stature

TABLE 2

The family means and variances within families for the character final height of the two F_1 diallel sets of crosses grown in 1967 and of the single sets grown in 1968 and 1969

		p		nk		nil	
		$\bar{x}$	$Vx*$	$\bar{x}$	$Vx*$	$\bar{x}$	$Vx*$
p	1967 I	81·08	43·49	101·91	52·76	81·74	43·49
	1967 II	90·48	52·06	112·08	68·37 (46)	97·93	73·19 (47)
	1968	97·60	50·20	110·40	80·67	112·50	61·73
	1969	85·67	31·03	96·27	79·83	91·27	13·07
nk	1967 I	109·75	72·30 (46)	109·37	94·35	101·60	99·73
	1967 II	100·33	58·77	111·56	61·79	98·15	49·39
	1968	117·90	55·06	123·35	70·45	132·55	75·14
	1969	98·60	57·20	102·33	44·20	105·60	64·27
nil	1967 I	80·82	43·88	101·30	39·89	93·17	44·75
	1967 II	94·09	56·65	100·23	61·86	111·71	99·25
	1968	110·00	70·61	126·45	56·76	122·00	42·96
	1969	98·73	20·03	107·33	42·87	103·33	41·70

* The degrees of freedom attributable to each variance are 48, 48, 18 and 12 for 1967 I, 1967 II, 1968 and 1969 respectively. The degrees of freedom are given in brackets when they have been reduced by the loss of plants.

and *nk* and *nil* are tall, as expected (see section 2). The expected differences also hold for flowering time in table 3 between *p*, *nk* and *nil*, although they are smaller than originally anticipated (see section 2).

The corresponding analyses of variance (Hayman, 1954) are given for the two characters in tables 4 and 5, respectively. Despite the difference in derivation of the 1967 I and II and the 1968 and 1969 diallel sets of

crosses (see section 2) the results of the analyses are fairly consistent for final height (table 4). Both additive and non-additive components of variation are present (items a and b respectively). The significance of the b_1 item for each diallel set indicates the presence of a directional non-additive effect which does, however, vary from the direction of tallness in 1967 I, 1968 and 1969 to shortness in 1967 II. The significance of the b_2 item means that there are significant non-additive effects which are not accounted for by b_1. General differences between reciprocal crosses are usually present (item c) and for the 1967 II diallel set specific differences (item d) also.

Two distinct patterns of significant items are found among the analyses for flowering time in table 5 which coincide with the manner in which the crosses of the diallel sets were derived (see section 2). For the analyses of the 1967 I and II diallel sets no additive component of variation has been detected (item a). This presumably arises from using derivatives of three independent

TABLE 3

The family means and variances within families for the character flowering time of the two F_1 diallel sets of crosses grown in 1967 and of the single sets grown in 1968 and 1969

	p		nk		nil	
	$\bar{x}$	Vx^*	$\bar{x}$	Vx^*	$\bar{x}$	Vx^*
1967 I	78·24	1·92	75·52	0·90	75·18	1·21
1967 II	77·00	1·67	79·17	3·87 (46)	78·76	3·16 (47)
1968	78·30	3·02	76·25	4·85	77·90	8·60
1969	70·00	0·73	69·93	3·03	70·67	3·63
1967 I	78·69	1·88 (46)	77·06	1·38	74·90	1·72
1967 II	76·58	3·28	78·28	2·74	75·50	1·33
1968	76·70	3·74	74·90	9·78	76·65	3·03
1969	69·67	0·63	68·60	1·27	69·53	1·17
1967 I	75·64	1·02	75·76	4·15	78·14	2·01
1967 II	78·28	2·31	76·12	3·15	76·96	1·43
1968	75·95	4·36	77·05	7·05	79·35	10·36
1969	70·27	0·83	69·13	0·77	71·67	4·77

* The degrees of freedom are as given in table 2.

replicate lines from each conditioning treatment as parents of the crosses (table 1) and the failure to correctly detect the extreme permanent changes for flowering time at the time the selections were made. There are, however, non-additive effects (item b_2) which are not directional in nature and specific differences between reciprocal crosses for both F_1 diallel sets (item d). For the 1967 I set there is also a directional non-additive effect (item b_1), which is in the direction of early flowering (table 3) and there are general differences between reciprocal crosses (item c). In contrast, an additive component of variation was detected in the analyses of the 1968 and 1969 diallel sets (item a). For both of these sets there are no reciprocal differences (items c and d). In the case of the 1968 diallel set there is however the suggestion of a directional non-additive component (item b_1) which is again in the direction of early flowering, and of a non-additive component not accounted for by b_1 (item b_2).

The use of independently replicated conditioned lines, which are known to differ in their properties (Hill and Perkins, 1969) as parents in the 1967 I and II experiments will have spuriously inflated the specific effects such as

TABLE 4

Hayman analysis of variance for the character final height of the two F_1 diallel sets of crosses grown in 1967 and of the single sets of crosses grown in 1968 and 1969

		1967 I			1967 II				1968				1969		
	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P
1. a	2	904·28	V.R. (8+9)	**	2	198·20	V.R. (3)	n.s.	2	785·36	V.R. (3)	†	2	445·39	V.R. (3)	†
2. b	3	126·70	χ^2 (10)	***	3	162·05	V.R. (7)	*	3	34·12	χ^2 (10)	***	3	28·13	V.R. (10)	**
2a. b_1	1	10·79	χ^2 (10)	*	1	67·73	χ^2 (10)	***	1	63·47	χ^2 (10)	**	1	42·31	V.R. (10)	**
2b. b_2	2	184·65	χ^2 (10)	***	2	209·21	V.R. (7b)	†	2	19·45	χ^2 (10)	*	2	13·54	V.R. (10)	*
3. c	2	19·32	V.R. (8+9)	n.s.	2	72·95	χ^2 (10)	***	2	47·32	χ^2 (10)	***	2	48·69	V.R. (10)	***
4. d	1	28·83	V.R. (8+9)	n.s.	1	11·29	χ^2 (10)	*	1	5·07	χ^2 (10)	n.s.	1	3·38	V.R. (10)	n.s.
5. blocks, bl	1	32·32	V.R. (8+9)	n.s.	1	4·14	V.R. (7)	n.s.	1	19·43	χ^2 (10)	n.s.	2	74·05	V.R. (10)	***
6. $a \times bl$	2	2·89	V.R. (10)	n.s.	2	1·12	χ^2 (10)	n.s.	2	3·88	χ^2 (10)	n.s.	4	5·06	V.R. (10)	n.s.
7. $b \times bl$	3	0·47	χ^2 (10)	n.s.	3	12·83	χ^2 (10)	**	3	2·62	χ^2 (10)	n.s.	6	8·98	V.R. (10)	n.s.
7a. $b_1 \times bl$	1	0·68	χ^2 (10)	n.s.	1	7·99	χ^2 (10)	n.s.	1	0·02	χ^2 (10)	n.s.	2	7·68	V.R. (10)	n.s.
7b. $b_2 \times bl$	2	0·36	χ^2 (10)	n.s.	2	15·25	χ^2 (10)	**	2	3·92	χ^2 (10)	n.s.	4	9·63	V.R. (10)	n.s.
8. $c \times bl$	3	8·41	χ^2 (10)	*	2	3·93	χ^2 (10)	n.s.	2	0·84	χ^2 (10)	n.s.	4	7·08	V.R. (10)	n.s.
9. $d \times bl$					1	1·22	χ^2 (10)	n.s.	1	0·12	χ^2 (10)	n.s.	2	8·43	V.R. (10)	n.s.
10. Error	430	2·39			429	2·60			162	6·06			108	4·30		

n.s. Probability is non-significant. † Probability = 0·05-0·10. * Probability = 0·01-0·05. ** Probability = 0·001-0·01. *** Probability = <0·001.

TABLE 5

Hayman analysis of variance for the character flowering time of the two F_1 diallel sets of crosses grown in 1967 and of the single sets grown in 1968 and 1969

	1967 I				1967 II				1968				1969			
	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P
1. a	2	1·22	V.R. (3)	n.s.	2	1·54	V.R. (8)	n.s.	2	8·47	χ^2 (10)	***	2	7·76	V.R. (6)	*
2. b	3	7·07	χ^2 (10)	***	3	5·07	χ^2 (10)	***	3	2·51	χ^2 (10)	**	3	1·74	V.R. (7)	n.s.
2a. b_1	1	13·93	χ^2 (10)	***	1	0·01	χ^2 (10)	n.s.	1	2·35	χ^2 (10)	†	1	0·71	V.R. (10)	n.s.
2b. b_3	2	3·65	χ^2 (10)	***	2	7·60	χ^2 (10)	***	2	2·59	χ^2 (10)	*	2	2·25	V.R. (7b)	n.s.
3. c	2	3·37	χ^2 (10)	***	2	3·29	V.R. (8)	n.s.	2	0·78	χ^2 (10)	n.s.	2	0·15	V.R. (10)	n.s.
4. d	1	4·24	χ^2 (10)	***	1	0·74	χ^2 (10)	**	1	2·61	V.R. (9)	n.s.	1	0·11	V.R. (10)	n.s.
5. blocks, bl	1	1·04	χ^2 (10)	***	1	0·50	V.R. (8)	n.s.	1	4·80	V.R. (9)	n.s.	2	2·51	V.R. (7)	n.s.
6. $a \times bl$	2	0·19	χ^2 (10)	n.s.	2	0·03	V.R. (8)	n.s.	2	0·51	χ^2 (10)	n.s.	4	0·74	V.R. (10)	*
7. $b \times bl$	3	0·20	χ^2 (10)	n.s.	3	0·11	χ^2 (10)	n.s.	3	0·17	χ^2 (10)	n.s.	6	1·35	V.R. (10)	***
7a. $b_1 \times bl$	1	0·01	χ^2 (10)	n.s.	1	0·04	χ^2 (10)	n.s.	1	0·36	χ^2 (10)	n.s.	2	0·62	V.R. (10)	n.s.
7b. $b_2 \times bl$	2	0·29	χ^2 (10)	n.s.	2	0·14	χ^2 (10)	n.s.	2	0·08	χ^2 (10)	n.s.	4	1·94	V.R. (10)	***
8. $c \times bl$	2	0·31	χ^2 (10)	n.s.	2	1·23	χ^2 (10)	***	2	0·55	χ^2 (10)	n.s.	4	0·20	V.R. (10)	n.s.
9. $d \times bl$	1	0·20	χ^2 (10)	n.s.	1	0·11	χ^2 (10)	n.s.	1	3·01	χ^2 (10)	*	2	0·23	V.R. (10)	n.s.
10. Error	430	0·07			429	0·10			162	0·65			108	0·25		

n.s. Probability is non-significant. † Probability = 0·05–0·10. * Probability = 0·01–0·01. ** Probability = 0·001–0·01. *** Probability = <0·001.

items b_2 and d at the expense of the general effects such as items a, b_1 and c. Comparisons of the corresponding items in the analyses of variance (tables 4 and 5) of the four experiments give clear indications that this has happened and throw considerable doubt, therefore, on the significant and large mean squares for the specific non-additive and specific differences between reciprocal crosses which are found for both characters in the 1967 experiments only. It seems probable that both these effects are of the general kind as detected in the later experiments.

TABLE 6

The generation means, average variances within families, V2F$_c$2, and variances of family means, V1F$_c$2, for the final height of the two sets of F$_2$ crosses grown in 1968 and 1969 respectively

		Generation	V2F$_c$2		V1F$_c$2		
Experiment	Cross	mean	Variance	d.f.	Variance‡	d.f.	r_{F_1/F_2}
1968	*p 1 × nk 3*	123·22	108·05	376	4·86 n.s.	9	n.s.
(from	*nk 3 × p 1*	122·72	99·69	377	7·70 n.s.	9	n.s.
1967 I F$_1$)	*p 3 × nil 3*	99·22	98·63	376	11·74 *	9	0·92 ***
	nil 3 × p 3	99·67	94·32	377	24·44 ***	9	n.s.
	nk 2 × nil 1	125·67	115·86	376	21·64 ***	9	0·74 *
	nil 1 × nk 2	125·45	108·72	374	9·27§ n.s.	9	n.s.
1969	*p 1 × nk 3*	93·83	72·98	133	13·65 n.s.	4	n.s.
(from	*nk 3 × p 1*	97·24	78·43	134	20·03 *	4	n.s.
1968 F$_1$)	*p 1 × nil 2*	91·77	65·18	134	36·29 ***	4	n.s.
	nil 2 × p 1	92·25	79·33	134	113·34 ***	4	n.s.
	nk 3 × nil 2	106·02	78·46	133	6·39§ n.s.	4	1·00 ***
	nil 2 × nk 3	106·11	86·93	133	29·39 *	4	n.s.

‡ The corresponding mean square for the differences between F$_2$ family means has been tested against the variance of these means within families or the block interaction, if significant (marked §).

n.s. Probability is non-significant.   * Probability = 0·01-0·05.   ** Probability = 0·001-0·01
*** Probability = <0·001.

TABLE 7

The generation means, average variances within families, V2F$_c$2, and variances of family means, V1F$_c$2, for the flowering time of the two sets of F$_2$ crosses grown in 1968 and 1969 respectively

		Generation	V2F$_c$2		V1F$_c$2		
Experiment	Cross	mean	Variance	d.f.	Variance‡	d.f.	r_{F_1/F_2}
1968	*p 1 × nk 3*	78·21	13·20	378	1·72 **	9	n.s.
(from	*nk 3 × p 1*	77·73	9·52	377	2·71 ***	9	n.s.
1967 I F$_1$)	*p 3 × nil 3*	78·36	13·76	376	4·85§ n.s.	9	n.s.
	nil 3 × p 3	79·15	16·84	377	9·58 **	9	n.s.
	nk 2 × nil 1	76·87	9·80	375	2·76 ***	9	0·70 *
	nil 1 × nk 2	77·08	8·94	374	4·04 ***	9	n.s.
1969	*p 1 × nk 3*	71·01	6·82	133	4·74 ***	4	n.s.
(from	*nk 3 × p 1*	70·67	4·56	134	3·00 ***	4	n.s.
1968 F$_1$)	*p 1 × nil 2*	71·38	11·06	134	3·29 *	4	n.s.
	nil 2 × p 1	71·53	5·37	134	1·03§ n.s.	4	n.s.
	nk 3 × nil 2	71·26	7·92	133	3·63 **	4	n.s.
	nil 2 × nk 3	70·71	3·46	133	1·18 *	4	n.s.

‡ and § as in table 6.
n.s. Probability is non-significant.   * Probability = 0·01-0·05.   ** Probability = 0·001-0·01.
*** Probability = <0·001.

The generation means, average variances within families ($V2F_c2$) and the variances of family means ($V1F_c2$) for the F_2s grown in 1968 and 1969 are given in successive columns of table 6 for final height and of table 7 for flowering time. The corresponding analyses of variance are given in tables 8 (*a*) and 8 (*b*). These analyses show no evidence of differences between reciprocal crosses (item 2). Differences between blocks (item 4) are highly significant in all cases except for flowering time in 1968 (table 8 (*b*)). For final height there are highly significant differences between the F_2s from different initial crosses in both years (item 1) and in one of these years,

TABLE 8

The analyses of variance for the two characters' final height and flowering time of the F_2 crosses grown in 1968 (from 1967 I F_1 crosses) and 1969 (from 1968 F_1 crosses)

(*a*) *Final height*

Item	1968				1969			
	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P
1. Crosses	2	8282·19	V.R. (3)	***	2	1604·62	V.R. (7)	***
2. Reciprocal crosses	3	1·65	V.R. (3)	n.s.	3	29·72	V.R. (7)	n.s.
3. F_2 families within crosses	54	13·28	χ^2 (8)	***	24	36·52	V.R. (7)	n.s.
4. Blocks	1	68·21	χ^2 (8)	***	2	465·81	V.R. (7)	***
5. Crosses × blocks	2	5·08	χ^2 (8)	n.s.	4	13·74	V.R. (7)	n.s.
6. Reciprocal crosses × blocks	3	4·24	χ^2 (8)	n.s.	6	20·29	V.R. (7)	n.s
7. F_2 families × blocks within crosses	54	6·20	χ^2 (8)	n.s.	48	28·04	χ^2 (8)	***
8. Error	2256	5·27			807	7·69		

(*b*) *Flowering time*

Item	1968				1969			
	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P
1. Crosses	2	31·85	V.R. (3)	**	2	3·14	V.R. (3)	n.s.
2. Reciprocal crosses	3	3·01	V.R. (3)	n.s.	3	1·12	V.R. (3)	n.s.
3. F_2 families within crosses	54	4·27	V.R. (7)	***	24	2·81	V.R. (7)	**
4. Blocks	1	4·13	V.R. (7)	†	2	13·45	V.R. (7)	***
5. Crosses × blocks	2	0·80	V.R. (7)	n.s.	4	1·00	V.R. (7)	n.s.
6. Reciprocal crosses × blocks	3	0·10	V.R. (7)	n.s.	6	0·33	V.R. (7)	n.s.
7. F_2 families × blocks within crosses	54	1·03	χ^2 (8)	**	48	1·09	χ^2 (8)	*
8. Error	2256	0·61			807	0·71		

n.s. Probability is non-significant. † Probability = 0·05-0·10. * Probability = 0·01-0·05. ** Probability = 0·001-0·01. *** Probability = <0·001.

1968, there are also significant differences between F_2 families from the same initial cross (item 3). The same item fails to reach significance in 1969 because of its large interaction with blocks (item 7). For flowering time, on the other hand, there are significant differences between F_2 families from the same initial cross and significant interactions with blocks in both years (items 3 and 7 respectively). Only for 1968 are there differences between the F_2s from different initial crosses (item 1) which significantly exceed those between F_2 families from the same initial cross.

The significance of item 3 in these analyses means that there are differences among individuals of the same F_1 cross which have been transmitted to their F_2 progenies. That is, there appears to be segregation within the

2 F

F_1s of the kind reported for flax (Durrant and Tyson, 1964; and see section 1*d*). These analyses, however, give an assessment of the situation over all the initial F_1 crosses. For each cross the significance of the variance between F_2 families (V1F$_c$2) is given in tables 6 and 7. A further, albeit less sensitive test for segregation is provided by the parent-offspring correlation between F_1 individuals of the same cross and their corresponding F_2 family means. This test, of course, involves comparisons over seasons and for any one initial cross few degrees of freedom. The correlations and their significances are given in the last column of tables 6 and 7.

The individual analyses of variance of final height in 1968 (table 6) show evidence of segregation in the three F_1 crosses, *p 3 × nil 3*, *nk 2 × nil 1* and *nil 3 × p 3*. In 1969 two of these crosses, although involving independent

TABLE 9

The analyses of variance for the two characters' final height and flowering time of the F_3 generation of the
p 3 × nil 3 and nk 2 × nil 1 crosses, grown in 1969, from the 1967 I F_1 generation

(a) *Final height*

| | | *p 3 × nil 3* cross | | | *nk 2 × nil 1* cross | | |
Item	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P
1. F_1 groups (F_1)	6	86·02	V.R. (2)	n.s.	6	164·20	V.R. (2)	*
2. F_2 groups in F_1(F_2)	41	88·78	χ^2 (6)	***	42	35·69	V.R. (5)	***
3. Blocks (B)	2	342·17	χ^2 (6)	***	2	375·46	V.R. (5)	***
4. B × F_1	12	7·59	χ^2 (6)	n.s.	12	11·30	V.R. (5)	n.s.
5. B × F_2	82	8·27	χ^2 (6)	n.s.	82	12·92	χ^2 (6)	*
6. Replicates	1277	7·45			1319	8·72		

(b) *Flowering time*

| | | *p 3 × nil 3* cross | | | *nk 2 × nil 1* cross | | |
Item	d.f.	M.S.	Test	P	d.f.	M.S.	Test	P
1. F_1 groups (F_1)	6	17·33	V.R. (2)	n.s.	6	9·48	V.R. (2)	*
2. F_2 groups in F_1(F_2)	41	10·39	V.R. (5)	***	42	3·40	V.R. (5)	***
3. Blocks (B)	2	22·84	V.R. (5)	***	2	15·81	V.R. (5)	***
4. B × F_1	12	1·77	V.R. (5)	n.s.	12	1·52	V.R. (5)	n.s.
5. B × F_2	82	2·34	χ^2 (6)	***	84	1·12	χ^2 (6)	*
6. Replicates	1277	1·66			1319	0·70		

n.s. Probability is non-significant.   * Probability = 0·01-0·05. ** Probability = 0·001-0·01.
*** Probability = < 0·001.

replicate lines (*i.e. p 1 × nil 2* and *nil 2 × p 1*), also show evidence of segregation. In addition, two further crosses, *nk 3 × p 1* and *nil 2 × nk 3*, show evidence of segregation at a lower level of significance (P = 1 − 5 per cent.). Two of the crosses showing significant segregation in 1968, *p 3 × nil 3* and *nk 2 × nil 1*, have significant parent-offspring correlations in 1968, while a further cross, *nk 3 × nil 2*, that showed segregation in 1968 but not in 1969, has a significant correlation in 1969.

The individual analyses of variance of flowering time (table 7) show evidence of segregation in all the F_1 crosses except for one cross in each year. For one of the crosses showing segregation in 1968, *nk 2 × nil 1*, there is a corresponding significant parent-offspring correlation. Indeed, this particular cross showed evidence of segregation for both characters on both tests.

Although we have so far attributed the significance of these tests to segregation in the F_1 of the kind reported by Durrant (1962*b*) for the heritable-

induced changes in flax, there is one further possibility. It could be argued that the differences that inevitably arise among genetically homogeneous individuals due to environmental variation, *e.g.* among members of a typical F_1, could be transmitted to their progenies, *e.g.* their F_2s. This would be an extreme view to take for genetical differences of the normal kind but not for differences which have arisen in lines due to persistent heritable responses to inducing environments. An F_3 generation was, therefore, derived from the F_2 of 1968 for two of the original 1967 I, F_1 crosses in a way which allows this possibility to be examined. The two crosses chosen, *p 3 × nil 3* and *nk 2 × nil 1*, were those which from all previous analyses (see tables 6 and 7) gave the best evidence for such segregation.

The results of the analyses of variance of the F_3 generation of the two crosses for final height and flowering time are given in table 9. One family in the *p 3 × nil 3* cross showed extremes of expression for both characters, being more than 50 per cent. taller and flowering about 10 days later, on average, than other families of the same cross. Because of this clear discontinuity the data for this family has been omitted and an analysis for unequal groups (Snedecor and Cochran, 1967, pp. 289-290) was used on the remaining data.

The two crosses differ significantly from each other $(P < 0.001)$ for both characters, as might be anticipated as a consequence of the criteria on which the parent lines were originally selected. The Nil treatment, giving plants which are all tall and late flowering, was common to both crosses as male parent (though two different *nil* lines were used), but the female parent differed, the *p 3* line being short and of intermediate flowering time and the *nk 2* line being tall and early flowering (tables 2 and 3).

The interaction between blocks and F_2 groups within F_1 groups (table 9, item 5) is significant for both characters in the *nk 2 × nil 1* cross and for flowering time in the *p 3 × nil 3* cross, and is used as the error term against which its two random main effects, blocks and F_2 groups within F_1 groups, are tested in these three analyses. F_2 groups within F_1 groups (item 2) show highly significant differences for both characters in both crosses. This would strongly indicate the presence of the conventional chromosomal gene segregation of an F_2. In this hierarchical design the differences between F_1 groups (items 1) are tested against differences between F_2 groups within F_1 groups. Though the F_2 generation from which the parents of the F_3 were taken had given evidence for segregation in the F_1, in the F_3 generation only the *nk 2 × nil 1* cross shows significant differences between F_1 groups for both characters. For final height in this cross this significance has a probability of nearly 0.01.

The generation means and variances within and between F_2 groups are given sequentially, along with their degrees of freedom for each of the seven F_1 groups in the *p 3 × nil 3* cross (section *a*) and *nk 2 × nil 1* cross (section *b*) for final height (table 10) and flowering time (table 11). The two sets of variances summarise the breakdown of items 2, 5 and 6 in both sections of table 9. From tables 10 and 11 it can be seen that differences between F_2 group means are significant for final height and flowering time in the *p 3 × nil 3* cross for all F_1 groups except for the flowering time of group 2. In the *nk 2 × nil 1* cross final height shows significant differences between F_2 groups in four F_1 groups and flowering time in three F_1 groups, though only two groups show significance for both characters. Where significance is not attained,

TABLE 10

Analyses of individual F_1 groups of the F_3 generation of the p $3 \times$ nil 3 and nk $2 \times$ nil 1 crosses grown in 1969 f
the 1967 I F_1 generation, for the character, final height

Cross	F_c1 group	Generation mean	V3F$_c$3 Variance	V3F$_c$3 d.f.	V2F$_c$3 Variance‡	V2F$_c$3 P	V2F$_c$3 d.f.	V1F$_c$3 Variance	V1F$_c$3 P	d
p 3 × nil 3	1	80·66	169·57	177	60·02	***	5			
	2	83·40	60·06	189	24·08	***	6			
	3	79·57	74·79	188	31·03§	*	6			
	4	80·14	64·05	189	22·10	***	6			
	5	78·71	49·91	187	15·81	***	6			
	6	82·21	70·34	188	36·97	***	6			
	7	81·26	75·25	189	22·21	***	6			
Average		81·26	74·46	1293	29·59	***	41	3·91	n.s.	
nk 2× nil 1	1	100·65	115·91	189	44·08	***	6			
	2	101·39	75·24	188	17·09§	†	6			
	3	105·73	63·93	189	10·68	***	6			
	4	100·02	74·49	188	7·79	**	6			
	5	100·30	118·05	188	6·80§	n.s.	6			
	6	103·06	68·75	188	21·90§	n.s.	6			
	7	96·97	74·80	189	21·59	***	6			
Average		101·29	87·17	1320	11·90	***	42	7·82	*	

‡ and § as in table 6.
n.s. Probability is non-significant.   † Probability = 0·05-0·10.   * Probability = 0·01-0·05.
** Probability = 0·001-0·01.   *** Probability = <0·001.

TABLE 11

Analysis of individual F_1 groups of the F_3 generation grown in 1969 from the 1967 I F_1 generation, for the chara
flowering time

Cross	F_c1 group	Generation mean	V3F$_c$3 Variance	V3F$_c$3 d.f.	V2F$_c$3 Variance‡	V2F$_c$3 P	V2F$_c$3 d.f.	V1F$_c$3 Variance	V1F$_c$3 P	d
p 3 × nil 3	1	70·62	13·37	177	7·99§	***	5			
	2	72·60	11·30	189	2·79§	n.s.	6			
	3	71·90	7·99	188	4·66§	**	6			
	4	72·30	12·68	189	3·25§	*	6			
	5	71·68	19·97	187	2·83§	**	6			
	6	74·23	17·87	188	2·29	**	6			
	7	72·60	13·50	188	1·21	*	6			
Average		72·31	11·61	1293	3·46	***	41	0·85	n.s.	
nk 2 × nil 1	1	69·31	5·41	188	1·89	***	6			
	2	69·72	8·76	189	1·35§	n.s.	6			
	3	70·51	5·12	189	0·79§	n.s.	6			
	4	69·78	4·32	188	0·15	n.s.	6			
	5	71·30	11·70	188	1·70	*	6			
	6	69·72	4·29	188	0·25§	n.s.	6			
	7	70·13	6·61	189	1·82	***	6			
Average		70·07	6·97	1320	1·13	***	42	0·45	*	

‡ and § as in table 6.
n.s. Probability is non-significant.   * Probability = 0·01-0·05.   ** Probability = 0·001-0·01.
*** Probability = <0·001.

the mean sums of squares for differences between families are being tested against significant block interactions, apart from flowering time of group 4 in the *nk 2 × nil 1* cross.

(b) *Analysis of family variances*

The variance within families for each F_1 cross (given in table 2 for final height and table 3 for flowering time) have been tested for homogeneity over the 1967 I, 1967 II, 1968 and 1969 experiments. Using the Bartlett χ^2 test, the variances for final height were homogeneous over the four experiments for all crosses except $p \times nil$ (P = 0·01 − 0·05) and were, in contrast, heterogeneous for flowering time for all crosses except $nk \times nil$.

In table 12 the pooled variance over experiments within F_1 crosses are given for the final height since, with one exception, the variances within

TABLE 12

The pooled variance for final height and the average variance for flowering time over experiments (1967 I, 1967 II, 1968 and 1969) within F_1 crosses

	Final height			Flowering time		
	Variance	d.f.	P	Variance	d.f.	P
p × p	46·53	126		1·84	126	
nk × nk	73·76	126		3·79	126	
nil × nil	64·97	126		4·64	126	
p × nk†	65·22	124	n.s.	3·16	124	n.s.
nk × p	63·10	124		2·38	124	
p × nil†	54·36	125	n.s.	4·15	125	n.s.
nil × p	50·29	126		2·13	126	
nk × nil†	73·66	126	***	1·81	126	
nil × nk	50·95	126		3·78	126	= 0·05

† The larger reciprocal variance has been tested against the smaller as given in the text.
n.s. Probability is non-significant. *** Probability = < 0·001.

crosses are homogeneous. For flowering time the average variance over experiments has been given for each cross since, with one exception, the variances are heterogeneous within crosses. In the case of final height the pooled variances of reciprocal crosses have been compared. Because of the large number of degrees of freedom in the numerator and denominator a c test was used. The variance of the cross $nk \times nil$ is significantly larger than that of its reciprocal cross (table 12). A variance ratio was computed for flowering time by dividing the χ^2 for the heterogeneity of variances between reciprocal crosses (1 d.f.) by the pooled heterogeneity χ^2 within crosses having first divided the latter by its six degrees of freedom. The variance of the cross $nil \times nk$ is just significantly greater than that of its reciprocal cross (table 12).

There were no significant correlations over the six crosses for either character between the magnitude of the variance between the F_1 individuals of 1967 I and 1968 (tables 2 and 3) and the magnitude of the corresponding variance between their F_2 family means of 1968 and 1969 (tables 6 and 7). Neither were any reciprocal differences detected in the magnitude of the variance between F_2 family means for either character in either year (tables

6 and 7). However, the variance within F_1 families of the cross $nk \times nil$ differs consistently from that of its reciprocal cross over four different experiments for both final height and flowering time (table 12). It is also in this cross that differences between F_1 individuals have been shown (see section 3a) to be transmitted through the F_2 to the F_3 generation.

In the F_3 generation further investigation of the variances resulting from segregation in the F_1 can be made for the two crosses, $p \ 3 \times nil \ 3$ and $nk \ 2 \times nil \ 1$, and the variation due to segregation in the F_2 can also be studied on a family rather than on an individual plant basis.

Firstly it can be seen from tables 10 and 11 that the variance arising from segregation in the F_1 (V1F_c3) is significant in the cross $nk \ 2 \times nil \ 1$ at the 5 per cent. probability level for both characters. Confirmation is thus given of the genetic nature of the variance between F_1 groups which was seen in the 1968 F_2 generation (tables 6 and 7). It is this cross that shows consistent significant differences with its reciprocal cross, $nil \times nk$, for the variance between F_1 individuals in the four F_1 experiments, though the variances of $nk \times nil$ are larger for final height only. Estimates of the variance due to differences between F_1 groups in the F_3 for the $nk \ 2 \times nil \ 1$ cross are $\sigma^2 1F_c3 = 6.120$ for final height and 0.289 for flowering time and for the $p \ 3 \times nil \ 3$ cross, $\sigma^2 1F_c3 = 0.338$ for flowering time and 0 for final height, though for this cross the variances are not significant.

Secondly, it may be noted that the variance due to differences between F_2 groups within F_1 groups is very highly significant for both crosses (table 9, items 2). This is a total variance within each cross due to the normal segregation processes which occur in the F_2 after recombination and reassortment of chromosomal genetic differences between the two parental lines. From tables 10 and 11 it can be seen that the variance due to segregation between F_2 individuals (V2F_c3) is significant for all F_1 groups of the $p \ 3 \times nil \ 3$ cross for both characters, except for the flowering time of one group, showing there to be significant variation around the F_1 group mean of the F_2 families within the group. In the $nk \ 2 \times nil \ 1$ cross the variance due to segregation in the F_2 (V2F_c3) is significant in about half the F_1 groups for each character though there is no obvious relationship between the statistics of the two characters.

4. DISCUSSION

We can now compare the conclusions that can be drawn from the analyses of the $N.$ $rustica$ experiments presented in the last section with those derived from comparable investigations in flax which were summarised as items a to e in section 1. The conclusions from the $N.$ $rustica$ experiments are itemised below under corresponding headings.

(a) There are differences between the means of reciprocal F_1 families for both characters in the diallel sets of crosses but there is no evidence that these differences persist into their reciprocal F_2 progenies. Examination of the individual reciprocal crosses shows that differences vary in direction over the four F_1 diallel experiments and that the majority of the large, significant differences are confined to the $p \times nk$ and $nk \times p$ crosses.

(b) Evidence for the nature of the heritable differences among the lines which have received different conditioning treatments comes from the diallel

among three contrasting lines and from F_2 and F_3 families raised from two of the crosses. After allowing for the different designs used in the four F_1 diallel sets of crosses it is clear that the differences are mainly additive with some non-additive effects for both characters. The general non-additive component for final height is with one exception (1967 II experiment) in the direction of tallness, while for flowering time it is for earliness in the two experiments in which it has a significant directional element. Not only is this pattern characteristic of the inheritance of these two characters in crosses between conventionally derived inbred lines of N. *rustica* (Mather and Jinks, 1971) but there is segregation both in the F_2 and F_3 generations of the conventional kind. This is similar to the situation in flax (Durrant and Tyson, 1964).

(c) Although the differences between the lines induced by different treatments have remained stable throughout these experiments it has been noted earlier (Hill and Perkins, 1969) and again in this paper that there are significant differences between replicate lines derived from different plants which received the same inducing treatment. But, while these differences are significant they are smaller than those between lines which were induced by different treatments (Hill and Perkins, 1968). On the other hand, in the generation immediately following the conditioning treatment Hill (1967) found it possible to select for early and late flowering within families derived from a single plant grown in the inducing environment. The relative mean flowering times and the variances of the conditioned lines changed from the generation of induction to the second generation after induction. There were changes in the ranking of the lines for mean flowering time (as referred to in section 2) and the variances within lines declined in magnitude (Hill, 1967). From this evidence an unstable transitory induced effect can be inferred as found in flax (Durrant, 1971) upon which the selection may have been operative.

(d) The heterogeneity of the F_1s of crosses between pairs of differently conditioned lines has been investigated at three levels, namely, comparisons of F_2 families derived from different individuals of the same F_1 cross; comparisons between such F_1 parents and their F_2 progeny means and comparisons of groups of F_3 families derived from different F_2 parents which are themselves progenies of different F_1 individuals from the same cross. At all three levels there is evidence of segregation in some of the original F_1 crosses for both characters, and for one cross *nk 2 × nil 1* there is evidence of such segregation for both characters at every level through to the F_3.

(e) The cytoplasm may control the variability of expression of the induced heritable changes as in flax. In N. *rustica* the F_1 of the cross *nk 2 × nil 1* shows significantly greater variation than its reciprocal for final height and significantly smaller variation for flowering time. Unlike flax these differences could not be detected in their reciprocal F_2s. This, of course, is the cross for which there is unambiguous evidence of segregation in the F_1 in all tests for both characters. For each character the direction of the difference between the variances of the reciprocal F_1s can be related to the difference in the variances of their respective mothers (see tables 2 and 3 1967 I experiment and table 12).

Overall, therefore, the N. *rustica* experiments confirm the most important properties of the inheritance of differences between conditioned lines previously reported in flax (Durrant, 1971, as summarised in section 1).

It is quite clear that the phenomena as found in N. *rustica* and flax are essentially the same. At the same time there are a number of properties which appear to be peculiar to the three conditioned lines used in the N. *rustica* experiments. One of these is the transitory nature of the reciprocal differences in the F_1 already referred to. Another is the dominance properties of the induced differences for final height. Both the *nk* and the *nil* lines were chosen for their tallness, *nk* being also early flowering and *nil* late flowering. However, in both reciprocal crosses to p, which is short, the F_1 and F_2 of $nk \times p$ are tall and the F_1 and F_2 of $nil \times p$ are short. Hence, there are two kinds of induced tallness. That of the *nk* line is dominant and that of the *nil* line is recessive.

5. SUMMARY

1. The nature of the heritable differences for final height and flowering time induced in an inbred variety of *Nicotiana rustica* by environmental treatments consisting of all eight possible combinations of the presence and absence of N, P and K fertilisers have been investigated by raising the F_1 and F_2 generations of a complete diallel set of crosses between three differently conditioned lines and the F_3 of two of these crosses. Four independent diallel sets of F_1 crosses were raised in three successive seasons and the F_2 generations were raised in two of these.

2. The experiments were designed to compare the pattern of inheritance of the conditioned differences in N. *rustica* with that previously described in flax.

3. The results obtained agree with those from flax in all essential details the more important common properties being:

(i) the heritable variation is mainly additive with some non-additive variation;

(ii) there is segregation in the F_1 the consequences of which can be detected in the F_2 and F_3 progenies of some of the crosses, and there is also additional segregation in the F_2 and F_3 families of a more conventional kind;

(iii) a possible cytoplasmic effect which appears to be maternally transmitted and leads to a difference in the variation within reciprocal F_1 families.

4. In addition there were differences between the means of reciprocal F_1 crosses which were transitory in that no differences were detectable between the F_2 progenies of the reciprocal crosses.

5. There is also evidence that different inducing treatments have produced two distinct types of tall lines, one being dominant and the other recessive in crosses to short lines.

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