RADIATION-INDUCED POLYGENIC MUTATION IN *ARABIDOPSIS THALIANA*

II. ANALYSIS OF LINES SELECTED FOR FLOWERING TIME

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1. Introduction

The aim of the work reported in this paper has been to examine the genetic properties of mutations induced in polygenic systems by ionising radiation. The general background to these and related experiments has been described in an earlier publication (Lawrence, 1968). The present results are concerned with the analysis, by means of diallel crosses, of inbred lines containing mutations affecting flowering time.

2. Material and method

All lines used in the experiments originated from a single long inbred line of *Arabidopsis thaliana* Heyn., and details of their derivation and performance can be obtained from the earlier report (Lawrence, *loc. cit.*). Briefly, selection in both the early and late flowering direction was initiated in families obtained by self-pollinating single plants irradiated as seed with 90 kr. of ^{60}Co gamma rays. Selection continued from the second to the sixth generation inclusive after treatment. In the R group of lines, seed from selected plants were reirradiated with a dose of 90 kr. in each generation up to and including the fifth. Plants in these lines, therefore, received an accumulated dose of 450 kr. In the A group of lines, seed were reirradiated only in the third and fifth generations, and plants therefore received an accumulated dose of 270 kr. Finally, seed from the S group of lines received no further treatment. Selection was also carried out in control families. All lines were maintained by self-pollinating single plants and at least three generations intervened between the last irradiation treatment and use of the plants as parents for constructing sets of diallel crosses. All lines were test crossed to the control and with one exception those which were found to contain only a single major gene mutation were not used as diallel parents.

Five sets of full diallel crosses, including reciprocal hybrids and selfs, were constructed. The parents for three of these sets were chosen randomly from the available selection lines, while a fourth set was concerned only with early lines from the S group. The fifth set of diallel crosses involved inbred lines from the Laibach standard collection of natural races (see Robblelen, 1965). In most instances each parental genotype was represented by a single plant, used both as male and female, and seed taken from at least three well-filled pods in each cross or self. Two parent plants, giving two completely independent sets of crosses, were used in the first 7×7 diallel and one of the 4×4 diallels, while in the 14×14 diallel one plant was used as female and one as male parent. All crossing was carried out in a growth

room and great care taken to avoid unintentional cross or self-pollination. To ensure that the crossing technique was adequate, tests were carried out using suitably marked stocks. Since no offspring resulting from contaminant pollen were found in this test, which involved nearly a thousand progeny, such precautions were evidently successful. Each set of diallel crosses was grown in a glasshouse using a randomised block design in which each family was represented by one plot per block. Ten plants per plot were grown in each experiment except the 14×14 and third 7×7 diallel in which five plants were sown. Details of the cultural method are given in the previous paper (Lawrence, *loc. cit.*).

3. Results

(a) *Mean flowering time*

The mean flowering times of each family, expressed as days from sowing to the opening of the first flower in the five experiments involving the selection lines are given in tables 1 to 4. The parents for the 14×14 diallel include a control plant and plants taken almost equally from early and late selections in each of the three groups of lines (S, A and R). It was the most comprehensive set of crosses attempted. Parents for the first 7×7 diallel were drawn from one early and one late line in each of the three groups, while for the second 7×7 diallel the three early and three late lines from the S group were used. In both cases a parent from the control was included. The first three experiments, therefore, include approximately equal numbers of parents from early and late selection lines, and presumably a random sample of the induced mutations which survived the selection process. Since the aim of these experiments is to make inferences concerning the general population of induced mutations of this kind, an Eisenhart (1947) model II situation is envisaged in the analyses of variance. Individually, the two 7×7 diallels are a little small for this purpose though the 14×14 is probably of an adequate size. Parents for the 4×4 diallel were taken from the three early lines of the S group and a control line, and the progeny were grown on two occasions.

The performance of the inbred lines in the five experiments is in broad agreement with previous results (Lawrence, 1968), but there are several exceptions. The R early lines in the 14×14 diallel flower later than the control, and lines S_1L_2 and A_1L_1 flower earlier than the control in the first 7×7 experiment. Since other evidence (Lawrence, *loc. cit.*) suggests that plants derived from irradiated families possess poorer developmental stability than control plants, these exceptions are to be expected. The mean flowering time of the plants varies considerably from experiment to experiment, owing partly to the time of year at which they were sown, and hence to the length of the photoperiod. Since plants in the second 7×7 diallel were sown very late in the season, even the early lines took over seven weeks to come into flower, and supplementary illumination had to be used to do this.

The data were first analysed by the method of Yates (1947) and Hayman (1954). In this analysis variation in the full diallel table is partitioned into genetic (a and b) and non-genetic (c and d) items. The a mean square depends substantially on the additive effects of the genes by which the lines differ, while the b mean square detects non-additive effects; that is, they detect general and special combining ability respectively. The non-additive

TABLE 1

Average number of days between sowing and flowering in the 14 × 14 *diallel. Sown:* 20/7/64

Female parent \ Male parent	S_2E	S_3E	S_3L_1	A_1E	A_3E	A_1L_2	A_3L_2	R_1E	R_2E	R_3E	R_1L_2	R_2L_2	R_3L_2	Control
S_2E	**33·41**	33·64	32·82	33·37	33·32	34·06	34·09	34·84	34·81	34·12	33·45	35·47	31·92	34·70
S_3E	32·37	**33·62**	32·96	35·23	35·68	33·21	32·21	33·56	32·64	32·58	32·30	32·80	32·44	33·18
S_3L_1	33·07	33·28	**34·76**	33·22	33·82	33·08	35·59	33·82	33·92	32·56	33·90	33·19	33·72	33·88
A_1E	36·19	35·35	34·89	**34·61**	34·91	35·67	35·16	35·98	36·51	35·90	34·33	36·33	35·34	34·48
A_3E	33·93	33·14	35·49	34·28	**33·52**	33·04	32·72	33·36	34·37	33·70	32·29	34·68	35·78	33·48
A_1L_2	32·72	34·22	33·55	33·48	34·87	**36·30**	33·58	34·28	36·00	33·64	33·59	33·55	33·93	34·54
A_3L_2	34·07	35·85	37·44	37·26	34·40	35·66	**37·28**	36·50	36·09	35·85	35·24	35·97	35·98	34·60
R_1E	34·36	33·72	34·53	34·70	33·38	34·19	34·84	**34·54**	34·12	34·00	35·37	33·20	34·02	35·15
R_2E	32·94	31·22	33·96	35·28	34·48	33·99	34·64	33·52	**35·62**	34·37	32·92	33·52	34·65	33·95
R_3E	34·18	33·40	33·88	33·79	34·49	34·00	33·00	33·04	34·29	**34·76**	32·86	35·32	35·85	34·38
R_1L_2	34·29	33·98	34·22	33·76	34·08	35·11	33·27	34·96	35·24	34·97	**35·17**	33·79	34·44	33·98
R_2L_2	33·96	34·10	34·53	35·27	32·54	34·06	32·58	34·46	35·46	32·72	33·67	**34·23**	33·96	34·87
R_3L_2	32·11	34·32	35·04	32·61	33·88	33·52	32·26	33·37	33·94	34·44	33·40	32·63	**35·37**	34·08
Control	33·34	33·31	33·48	33·30	35·86	33·03	34·06	33·72	35·42	34·36	34·66	35·32	34·75	**33·85**

E = early and L = late selection lines.
S, A and R = 90, 270 and 450 kr. accumulated dose respectively. See test for further details concerning the lines.

TABLE 2

Average number of days from sowing to flowering in the first 7 × 7 diallel.
Sown: 4/7/67

Female parent \ Male parent	S_3E	S_2L_1	A_3E	A_1L_1	R_1E	R_3L_2	Control
S_3E	**23·90**	23·70	23·96	24·32	25·16	24·89	24·74
S_2L_1	23·74	**23·54**	24·15	24·12	24·29	24·54	24·22
A_3E	25·16	24·64	**23·92**	24·17	24·31	24·51	24·55
A_1L_1	24·24	24·64	24·71	**23·96**	24·97	25·54	24·49
R_1E	24·39	23·89	24·09	24·07	**23·84**	24·84	24·37
R_3L_2	24·37	24·75	24·77	24·99	25·04	**26·04**	24·56
Control	24·65	25·17	23·92	24·25	24·09	24·71	**24·36**

TABLE 3

Average number of days between sowing and flowering in the second 7 × 7 diallel.
Sown: 29/8/63

Female parent \ Male parent	SE_1	SE_2	SE_3	Control	SL_1	SL_2	SL_3
SE_1	**52·63**	49·48	52·03	53·28	65·83	53·30	52·10
SE_2	50·58	**51·28**	50·35	50·90	71·43	52·90	52·30
SE_3	53·10	51·63	**48·68**	50·70	62·15	48·03	49·85
Control	52·10	49·45	51·78	**52·60**	68·33	52·20	52·33
SL_1	57·93	71·43	64·88	73·43	**82·88**	69·23	69·80
SL_2	51·65	52·53	47·83	51·23	71·18	**52·65**	48·73
SL_3	52·93	51·70	55·53	53·15	56·70	57·30	**54·20**

TABLE 4

Average number of days from sowing to flowering in the two 4 × 4 diallels.
Expt. 1 *sown:* 12/8/63. *Expt.* 2 *sown:* 1/5/64.

Female parent		\ Male parent	S_1E	S_2E	S_3E	Control
Expt. 1		S_1E	**44·52**	44·18	44·94	46·10
		S_2E	44·59	**45·21**	44·03	44·53
		S_3E	44·60	44·96	**43·51**	45·12
		Control	45·84	45·47	45·62	**47·75**
Expt. 2		S_1E	**30·90**	30·57	30·17	30·27
		S_2E	30·28	**30·91**	29·86	30·03
		S_3E	30·24	29·53	**30·92**	30·29
		Control	30·16	30·70	30·03	**32·33**

variation is divided into three parts. In the absence of non-allelic interaction b_1 depends on the net effect of all dominant alleles, b_2 on variation in dominance attributable to the different parental lines, and b_3 on dominance not detected by the other two items. The c mean square depends on the average difference between the reciprocal progeny of each parent, and d on residual variation between reciprocal hybrids. The a mean square provides an unambiguous test for additive variation only in the absence of significant maternal/paternal influences, or at least only when these are small relative

to *a*. Similarly, *d* interferes with the detection of non-additive variation. Results of the analyses of variance which were carried out with the aid of an Atlas computer (see Cooper, 1965) are given in tables 5 and 6.

These results have a number of features in common. All show the presence of significant non-additive variation in some form or other and all but the 14 × 14 diallel significant additive variation. The 14 × 14 data provide no evidence for the presence of additive variation because *c* is not only significant but also greater than *a*. As there clearly is genetic variation between the lines in this experiment it seems likely that the additive variation is merely confounded with the maternal/paternal effects rather than absent. The large size of the *c* mean square might at first sight suggest that much of

TABLE 5

Analyses of variance in flowering time using the method of Hayman (1954)

	14 × 14			1st 7 × 7			2nd 7 × 7			2nd 4 × 4		
Item	d.f.	M.S.		d.f.	M.S.		d.f.	M.S.		d.f.	M.S.	
a	13	37·67		6	6·61***		6	1,885·5**		3	4·93**	
b	91	9·05*		21	1·49*		21	43·3		6	9·00***	
b_1	1	55·46***		1	3·50*		1	3·6		1	42·63***	
b_2	13	8·63		6	1·52		6	5·5		3	2·60	
b_3	77	8·52**		14	1·33		14	62·4*		2	1·78	
c	13	42·15***		6	1·74		6	18·1		3	0·31	
d	78	6·32		15	0·92		15	44·9		3	1·15	
a × blocks	117	6·25		42	0·76		18	30·1		33	1·50	
b × blocks	819	4·94		147	0·82		63	23·8		66	1·27	
b_1 × blocks	9	4·34		7	1·97		3	44·9		11	1·29	
b_2 × blocks	117	5·15	5·46	42	0·47	0·91	18	23·8	27·6	33	1·91	1·02
b_3 × blocks	693	4·91		98	0·89		42	22·3		22	0·30	
c × blocks	117	6·61		42	1·23		18	25·1		33	0·55	
d × blocks	702	5·74		105	0·96		45	32·9		33	0·51	
Blocks	9	83·44***		7	42·87***		3	4,096·9***		11	6·86***	
Total	1959	—		391	—		195	—		191	—	

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

the response to selection was due to maternal effect or cytoplasmic rather than nuclear mutations. Significant variation between reciprocal hybrids was not found in the other experiments, however. Further, if this was so the progeny of early selections in the female arrays of the 14 × 14 diallel should flower consistently earlier than their reciprocals in the male arrays and there should be a converse situation for progeny of late selections. However, the difference between male and female arrays is in the expected direction in only six out of thirteen cases, though the difference is on average 50 per cent. greater in these six cases. The reason for these abnormal maternal/paternal effects is unknown.

Two independent sets of families were grown in the first 4 × 4 diallel. Since the two sets of results are entirely homogeneous there is no evidence for residual heterozygosity in the lines or of non-genetic parental effects.

Although non-additive variation was found in each experiment, it appeared in different forms. In some diallels b_1 was significant, indicating

that the non-additive effects acted predominantly in one direction, while in others b_2 or b_3 was significant. Such items give an indication of the overall expression of dominance, and epistasis if present, in the experiment. They do not, however, provide detailed information concerning individual lines. This is best obtained by examining the different variances and covariances derived from the diallel tables.

(b) *Variances and covariances*

The variances and covariances derived from the diallel tables can be examined in two ways. First, the mean variance and mean covariance of arrays together with other variances, can be used to estimate the components of variation in the manner described by Jinks (1954). Since five equations

TABLE 6

Analysis of variance in flowering time in the first 4 × 4 diallel

Item	d.f.	M.S.
a	3	83·80***
b	6	12·35
b_1	1	4·54
b_2	3	20·55*
b_3	2	3·96
c	3	3·52
d	3	5·84
$a \times$ sets	3	5·75
$b \times$ sets	6	8·11
$b_1 \times$ sets	1	22·81
$b_2 \times$ sets	3	7·49
$b_3 \times$ sets	2	1·69
$c \times$ sets	3	10·48
$d \times$ sets	3	17·53
Sets	1	0·08
Blocks	11	57·54***
Sets × blocks	11	5·03
Error	329	7·08
Total	383	—

* $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

are available to estimate five components, a perfect fit is obtained and standard errors cannot be estimated. The estimates of the components, given in table 12, are therefore used only to summarise the data. In view of the results of the analyses of variance, however, it is likely that D and H are significantly different from zero. Some idea of the reliability of the estimates can also be obtained by comparing results from different experiments. Second, the relation between individual variances and covariances of arrays can be examined to give more detailed information about the contribution of each parental line to the non-additive variation. Plots of *Wr* against *Vr* are given in fig. 1. The environmental component has been subtracted from each of these variances and covariances. If significantly heterogeneous, estimates of environmental variance were calculated for each individual array; otherwise a pooled estimate was used.

In view of the highly significant maternal/paternal effects in the 14×14

diallel, covariances and variances were first calculated separately from male and female arrays, and an analysis of the kind suggested by Allard (1956) carried out. The result of this analysis, given in table 7, shows that the maternal/paternal effect greatly reduced the size of *Vr* and *Wr* within female arrays but did not change either the array order, that is, the relative dominance of the parents, or the proportionality between $\bar{V}r$ and $\bar{W}r$. Overall there is a significant variation between the lines, and $\bar{W}r$ is significantly smaller than $\bar{V}r$. Since this analysis shows that the data from male and female arrays give essentially the same information, *Vr* and *Wr* were calculated using the pooled results from reciprocal hybrids, and the plot of *Wr* against *Vr* is shown in fig. 1. Although the points are widely scattered, there is a significant regression of *Wr* on *Vr* ($P = 0{\cdot}01 - 0{\cdot}001$) with a slope of $+0{\cdot}74 \pm 0{\cdot}19$. This slope is appreciably smaller than the expected slope of $+1{\cdot}00$, but because of the scatter the deviation is not significant. It is not clear, therefore, whether some of the induced mutations exhibit non-allelic interactions or whether the scatter is entirely due to maternal/paternal

TABLE 7

Analysis of variance in Vr *and* Wr *calculated separately from male and female arrays in the 14 × 14 diallel*

Item	d.f.	M.S.	
1. Lines	13	0·196***	
2. $\bar{W} - \bar{V}$	1	0·853**	
3. ♂ versus ♀ arrays	1	1·818***	
1 × 2	13	0·085	
1 × 3	13	0·073	
2 × 3	1	0·002	0·073
1 × 2 × 3	13	0·068	
Total	55	—	

** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

influence and error. In the absence of data suitable for scaling tests (Mather, 1949) the question must remain open. The position of the control near the origin indicates that most of the induced mutations are recessive compared with their wild type alleles. In general, there are fewer recessive mutations in the early than late lines, hence the significance of b_1 found previously (table 5). Alternatively, individual mutations in the late lines produce a greater effect than those in early lines.

A significant regression of *Wr* on *Vr* is also found in the two 7×7 diallels ($P = 0{\cdot}01 - 0{\cdot}001$ and $P < 0{\cdot}001$ respectively) with slopes ($+1{\cdot}29 \pm 0{\cdot}24$ and $+1{\cdot}00 \pm 0{\cdot}08$) which do not differ significantly from expectation (fig. 1). Unlike the 14×14 data, however, the *Wr*/*Vr* regression does not pass through or near the origin. In each case the intercept on the *Wr* axis is significantly positive ($+0{\cdot}07 \pm 0{\cdot}02$, $P = 0{\cdot}02 - 0{\cdot}01$ and $+31{\cdot}09 \pm 1{\cdot}37$, $P < 0{\cdot}001$). In diallel crosses between homozygous parents this intercept equals $\frac{1}{4}(D - H_1)$ (Jinks, 1954) and a positive value therefore indicates that dominance is incomplete. This is also shown by the ratio H_1/D which is less than unity (table 12). In the first 7×7 diallel the induced mutations are again recessive to their wild type alleles and late selection lines contain more

recessive alleles, or those with greater effect, than early lines. Surprisingly this is so even though two of the " late " lines flowered earlier than the control. In complete contrast, wild type alleles are almost completely recessive in the second 7×7 diallel, and there is little distinction between early and late lines. This result may be due partly to the unusual environment in which the plants were grown, but it mostly arises from the inclusion of S_1L_1 and its progeny in the diallel. Differences between these and other families

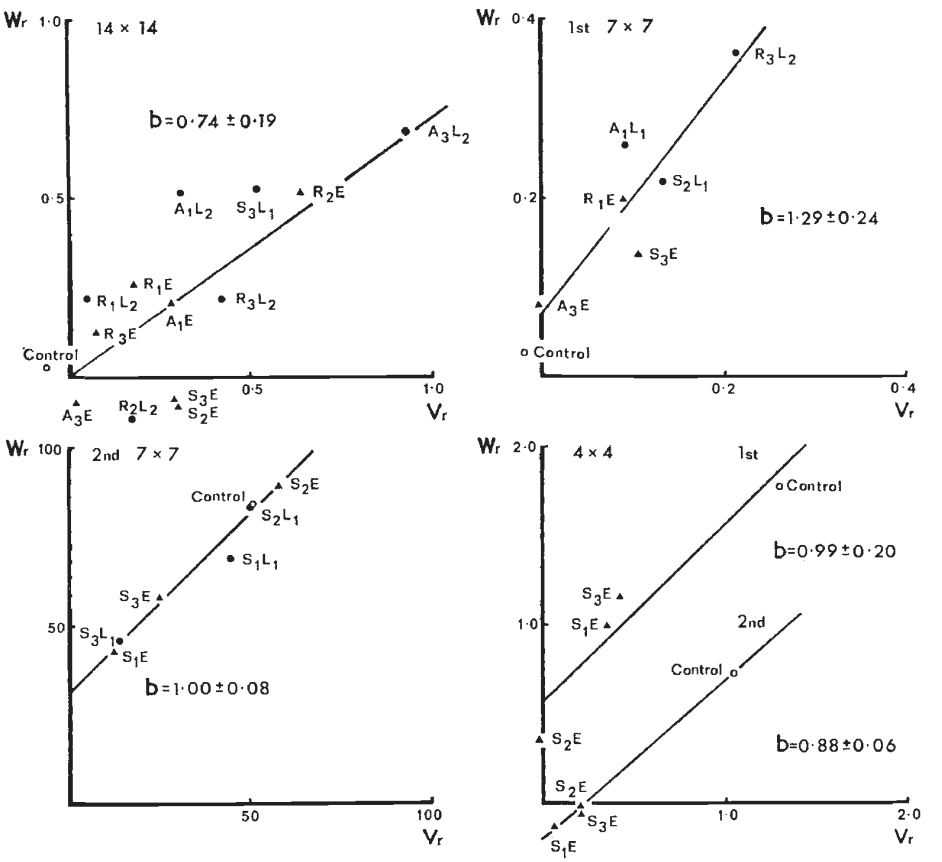

FIG. 1.—*Wr*/*Vr* graphs for the four sets of diallel crosses concerned with the selection lines.

account for most of the variation in the experiment. After it had been grown it was found that the greater part of the difference between S_1L_1 and the control could be ascribed to the action of a single gene. If this line and its hybrids were removed from the set of crosses, the mutations in the remaining lines were found to be mostly recessive to the alleles in the control.

Although the results from the second 7×7 diallel suggest that the dominance of the induced mutations depended on an unusual genetic background and environment, the data from the two 4×4 diallels show that at least some mutations possess this property in more normal genotypes and environments. The regression of *Wr* on *Vr* is significant in each case ($P = 0{\cdot}05 - 0{\cdot}01$,

P = 0·01 − 0·001) with slopes of +0·99 ± 0·20 and +0·88 ± 0·06 respectively. In each case the induced mutations were dominant over their alleles in the control, even though the two environments were quite different, as shown by the time taken for the plants to flower. The two experiments differed, however, in the sign of $\frac{1}{4}(D-H_1)$, which was significantly positive in the first (+0·57 ± 0·10) and significantly negative in the second (−0·20 ± 0·02). Similarly the ratio H_1/D (table 12) is less than one in the first experiment, indicating incomplete dominance, and greater than one in the second experiment, apparently indicating overdominance. An analysis of variance of *Vr* and *Wr* (Allard, 1956) showed that the differences between the two experiments were significant, though there was an overall correspondence in the order of arrays (table 8).

TABLE 8

Analysis of variance in Vr *and* Wr *in the two 4 × 4 diallels*

Item	d.f.	M.S.
1. Lines	3	0·898*
2. $\bar{W}-\bar{V}$	1	0·100
3. Experiments	1	1·165
1 × 3	3	0·089**
2 × 3	1	0·643***
1 × 2	3	0·008 } 0·009
1 × 2 × 3	3	0·009 }
Total	15	—

* P < 0·05; ** P < 0·01; *** P < 0·001.

(c) *Naturally occurring variation*

Since it was of interest to compare the properties of induced mutations with those of naturally occurring or wild type mutations, a set of diallel crosses was constructed using parents from six inbred lines from the Laibach standard collection and a control line. The data are summarised in tables 9 and 10. As found previously (Lawrence, *loc. cit.*), the variation between these lines was considerably greater than the variation between the selection

TABLE 9

Average number of days from sowing to flowering in the 7 × 7 diallel (natural races). Sown: 9/8/65

		Male parent						
		Enk.	Est. 1	Est. 2	Maine	Eiffel	Limb. 1	Cont.
Female parent	Enk.	**22·96**	24·70	26·44	26·47	25·90	27·30	26·61
	Est. 1	23·46	**25·06**	25·18	25·00	25·30	26·60	25·20
	Est. 2	24·10	25·20	**26·79**	26·58	26·42	26·66	27·41
	Maine	23·84	25·60	25·85	**26·71**	26·52	27·19	27·98
	Eiffel	25·12	25·50	26·67	26·49	**26·87**	27·64	27·58
	Limb. 1	25·81	25·75	25·88	26·82	26·62	**28·54**	28·57
	Control	26·19	26·48	27·23	27·14	27·22	29·30	**28·45**

Enk. = Enkheim; Est. 1 and 2 = Estland 1 and 2; Limb. 1 = Limberg 1; Cont. = Control line.

TABLE 10

Analysis of variance in flowering time in the 7 × 7 (natural races) diallel

Item	7 × 7 d.f.	7 × 7 M.S.		6 × 6 (excluding Enkheim) d.f.	6 × 6 (excluding Enkheim) M.S.		6 × 6 (excluding Maine) d.f.	6 × 6 (excluding Maine) M.S.	
a	6	105·61*		5	66·37**		5	109·42*	
b	21	3·04		15	3·05***		15	3·99***	
b_1	1	2·61		1	11·71***		1	1·57	
b_2	6	4·88		5	1·34		5	6·01***	
b_3	14	2·29		9	3·04**		9	3·14***	
c	6	13·93***		5	3·77**		5	11·90***	
d	15	2·25**		10	1·39		10	1·46	
a × blocks	54	0·86		45	1·02		45	0·85	
b × blocks	189	0·79		135	0·95		135	0·81	
b_1 × blocks	9	0·47		9	1·35		9	1·11	
b_2 × blocks	54	1·10	0·80	45	1·31	0·94	45	0·82	0·84
b_3 × blocks	126	0·67		81	0·78		81	0·76	
c × blocks	54	0·35		45	0·46		45	0·65	
d × blocks	135	0·98		90	1·12		90	0·98	
Blocks	9	1·68		9	1·73		9	1·36	
Total	489	—		359	—		359	—	

* P < 0·05; ** P < 0·01; *** P < 0·001.

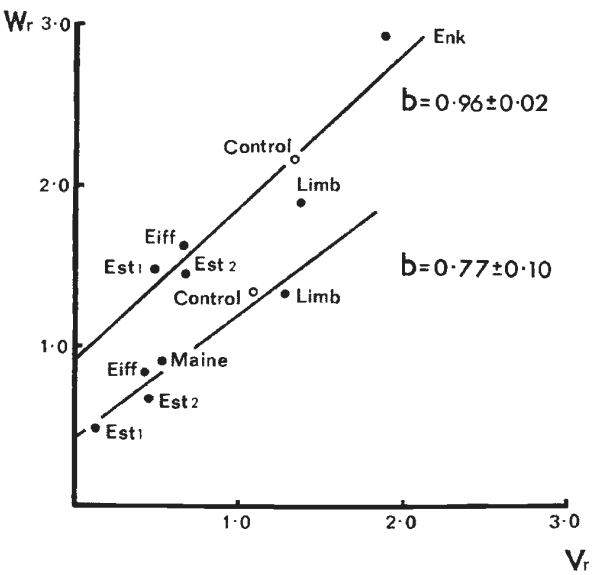

FIG. 2.—*Wr*/*Vr* graphs for the set of diallel crosses concerned with natural races from the Laibach standard collection.

lines. The majority of this was genetic, and due to additive effects. The nature of the genetic component of variation was obscured, however, by significant differences between reciprocal hybrids, both consistent (*c*) and residual (*d*). Examination of table 9 shows that the significance of *c* was due to the progeny of Enkheim and of Limberg 1, while the significance

of *d* arose entirely from two crosses, Enkheim × Maine and Enkheim × Estland 2, particularly the former. Unambiguous evidence for the presence of both additive and non-additive variation can be found if the data from Either enkheim and its progeny or Maine and its progeny are excluded (table 10). The plot of *Wr* against *Vr* (fig. 2) suggests that the non-additive variation was due only to dominance; neither of the two regression slopes deviates significantly from expectation ($+0{\cdot}77 \pm 0{\cdot}10$, $+0{\cdot}96 \pm 0{\cdot}02$). Dominance is incomplete since $\frac{1}{4}(D - H_1)$ is significantly positive in each case ($+0{\cdot}43 \pm 0{\cdot}04$ and $+0{\cdot}91 \pm 0{\cdot}09$, $P < 0{\cdot}001$ in both cases). If Enkheim and its hybrids were removed from the diallel table, dominance appeared to act exclusively in the early flowering direction. If, however, Maine and and its hybrids were excluded, Enkheim, which is the earliest of all the lines in the sample, was also found to contain the most recessive alleles. This was so in both male and female arrays. Although maternal/paternal influence modified the expression of dominance in some lines (table 11),

TABLE 11

Analyses of variance in Vr *and* Wr *in the two 6 × 6 diallels derived from the 7 × 7 (natural races) diallel*

Item	d.f.	Excluding Enkheim M.S.		Excluding Maine M.S.	
1. Lines	5	0·611**		1·167*	
2. $\bar{W} - \bar{V}$	1	0·354***		3·368*	
3. ♂ versus ♀ arrays	1	0·049		3·313*	
1 × 3	5	0·054*		0·227**	
2 × 3	1	0·002		0·267*	
1 × 2	5	0·016	0·012	0·037	0·028
1 × 2 × 3	5	0·009		0·018	
Total	23	—		—	

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

there was an overall agreement between the results from male and female arrays. It would appear, therefore, that dominance can be associated both with alleles which delay as well as those which advance flowering. The sample of lines is too small, however, to provide reliable information about the relative frequencies within the species of these dominant alleles.

4. DISCUSSION

Since the phenotypic variation between the selection lines was small, the data from the experiments described above are potentially vulnerable to distortion from factors which would not be of importance when the variation is greater. This is of course a different matter from the precision of the experiments which by use of suitable replication was generally adequate to detect the small differences involved. One source of ambiguity found in some experiments arose from differences between reciprocal hybrids. These could be minimised or eliminated by taking seed in each cross from at least three or four well-filled pods, and by replicating the set of diallel crosses

with independent sets of parents. This procedure may be too laborious in large sets of crosses. Another possible source of distortion which could give rise to variation between inbreds and hybrids, was an effect of emasculation on seed development. It was shown not to be important, however, since offspring from naturally self-pollinated flowers and from flowers which were emasculated and artificially self-pollinated were identical.

Although some of the experiments may be ambiguous in detail, together they point clearly to certain general conclusions. These can be inferred from a summary of all of the data given in table 12. First, the great majority of the variation between the selection lines was due to the additive effects of the induced mutations. Only in the 14×14 diallel was the evidence for additive variation ambiguous, owing to pronounced maternal/paternal influence. The reason for this unusual result is not known, but may arise from either genetic or non-genetic heterogeneity within the lines since separate plants were used as male and female parent. Neither kind of heterogeneity could be detected in the control or S lines, but their existence in the A and R lines cannot be ruled out. Second, although the greater part of the variation between lines was additive, significant non-additive variation was found in all experiments. Covariance (Wr)/variance (Vr) analysis suggested that most of this non-additive variation was due to dominance, though it is possible that non-allelic interaction between the induced mutations occurred in the 14×14 diallel experiment. The evidence is far from conclusive on this point, however. The majority of the induced mutations in two of the larger diallels were either completely or incompletely recessive to their wild type alleles. Others, however, were neither recessive nor dominant, and in the other 7×7 diallel the majority of the mutations were incompletely or completely dominant. In general, late lines contained more recessive mutations, or recessive mutations with greater effect, than early lines. The average level of dominance, whether complete or incomplete, no doubt depended partly on which sample of lines, and hence which sample of mutations, were examined. It also varied, as shown by the two 4×4 diallels, with the environment in which the plants were grown. It is hardly surprising that the properties of genes influencing flowering time should vary in experiments grown at different times of the year and hence in different temperatures and photoperiods. Wild type alleles also exhibited incomplete dominance (fig. 2, table 12) and this appears to be common in the gene system controlling flowering in other species (Jinks, 1954, 1955).

The nature of the induced mutations and the relation between their properties and the genotype of the control.

Following the original work of Stadler (1932) it has been widely assumed that the great majority of radiation-induced mutations in plants arise from deletion and duplication rather than " point effects ". A review of recent evidence (Nilan, 1966) confirms the importance of deletions though not of duplications, at least in maize. It is likely that most spontaneous mutations in maize are also due to structural changes such as deletion (Nilan, 1966). Inevitably, such evidence refers to major gene mutations. Whether induced or spontaneous mutations in polygene systems are also associated with structural changes is not known. Since at least some of the induced mutations studied in the present experiments were dominant over their wild-type alleles, deletion cannot explain all of the induced variation. Indeed, if the

TABLE 12

Summary of the data. *The components of variation and their derivatives, given in the top half of the table, are as defined by Jinks* (1954). *Items in the lower part of the table refer to the slope and constant of the* Wr/Vr *regression, and the average of* Wr *and* Vr *for the control, early lines, and late lines respectively.*

	Selection lines					Natural races	
	14×14	1st 7×7	2nd 7×7	1st 4×4	2nd 4×4	6×6 excluding Enkheim	6×6 excluding Maine
D	0·672	0·586	132·182	2·981	0·423	1·577	4·470
H_1	0·990	0·200	30·488	0·721	1·396	0·453	0·991
H_2	0·718	0·145	7·942	0·445	1·330	0·426	0·647
F	+0·484	+0·439	+22·108	+1·673	+0·326	−0·578	+1·200
E_2	0·546	0·113	6·895	0·295	0·085	0·094	0·084
H_1/D	1·474	0·341	0·231	0·242	3·303	0·287	0·222
$H_2/4H_1$	0·181	0·181	0·065	0·154	0·238	0·235	0·163
b Wr/Vr	0·74±0·19	1·29±0·24	1·00±0·08	0·99±0·20	0·88±0·06	0·77±0·10	0·96±0·02
$\frac{1}{4}(D-H_1)$	0·00±0·05	+0·07±0·02	+31·09±1·37	+0·57±0·10	−0·20±0·02	+0·43±0·04	+0·91±0·09
$(\overline{W+V})C$	−0·016	0·003	67·5	1·534	0·884	—	—
$(\overline{W+V})E$	0·193	0·101	47·4	0·539	0·041	—	—
$(\overline{W+V})L$	0·373	0·214	51·1	—	—	—	—

results from the second 7 × 7 diallel are reliable, rather little can arise from this cause. Although an unusual genetic background and environment were used in this experiment, it is difficult to visualise how a deletion can be dominant over a normal allele in any circumstances. Such dominant mutations may, of course, arise from duplications, even though no evidence for this kind of mutational event was found in maize (Nilan, 1966).

The question of the nature of the induced mutations, whether point effects or structural changes in chromosomes, clearly has a direct bearing on the relation between their properties, particularly non-additive ones, and the genotype of the control. As discussed previously (Lawrence, 1968), two main kinds of relationship can be envisaged. First, the properties of an induced mutation could be determined by the properties of the particular allele before the mutational event took place, as for instance might be the case if all mutations arose from duplication. If it is assumed that all genes are equally susceptible to mutation, a marked deviation in the average properties of induced mutations influencing any given character is expected when different genotypes are irradiated. In addition, a relatively simple dependence of these properties on those of the wild type genes is expected. Second, the properties of the induced mutations could be determined by the factors responsible for the genetic architecture (Mather, 1960) of the particular character studied; that is, they could depend on a complex network of interactions between the mutations and the wild-type alleles, including alleles responsible for dominance modification. Since it is likely that the effect of this network of interactions is relatively independent of small changes in genotype, the properties of induced mutations might be expected similarly to be relatively constant with regard to any one particular character. Such properties would vary with regard to different characters, however, according to the kind of selective forces to which the character had over long periods been previously exposed.

With respect to additive effects, it was concluded (Lawrence, 1968) that although in principle it should not be difficult to distinguish between these alternatives, existing data were not adequate to do so unambiguously. Nevertheless it was felt that the evidence favoured the second hypothesis. The present information concerning the non-additive effects of induced mutations can also be interpreted according to either scheme. Unless the majority of the mutations are deletions and duplications, however, the second hypothesis seems the more reasonable. One feature of the results which requires explanation by either model is the varying frequency of dominant and recessive mutations occurring in early as opposed to late flowering times. On average, late lines contained more recessive mutations in two out of the three larger diallel experiments, including the 14 × 14. Unless deletions occur only, or more frequently, in late flowering lines, this implies directional dominance; that is, early alleles are more frequently dominant in the particular genotype irradiated, according to the first hypothesis or in the species as a whole, according to the second. Although designed to do so, the results from the set of diallel crosses between natural races could not be used to examine this problem bacause of complications arising from maternal effects. In the absence of suitable data from *Arabidopsis*, results from other species were examined, making the assumption that the genetic architecture of flowering is similar in most plants. The data, given in table 13, were selected using only two criteria; first, that each experiment involved several

lines or races, and second, that each exhibited significant non-additive variation. It would appear from this presumably random sample of experiments that non-additive effects act predominantly in the direction of early flowering. If this is true for *Arabidopsis*, the present results are compatible with the second hypothesis.

In conclusion, it appears that relatively unselected samples of radiation-induced mutations in the polygenic system influencing flowering time are quite similar to their spontaneous counterparts. They probably contrast in this respect with radiation-induced major gene mutations (see Gaul, 1964, for references concerning the properties of major gene mutations).

TABLE 13

Predominant direction of non-additive effects found in experiments concerned with flowering time in different plant species. Significant non-additive variation occurred in each of the listed examples

Species	Predominant direction of non-additive effects	Reference
Cultivated plants		
Maize	Early	Moll *et al.* (1965)
Tobacco	Early	Jinks (1954)
Tobacco	Early	Matzinger *et al.* (1962)
Tobacco	Late	Marani and Sachs (1966)
Oats	Early	Petr and Frey (1967)
Cotton	Early	Marani (1964)
Sorghum	—	Niehaus and Pickett (1966)
Rye grass	—	Hayward (1967)
Subterranean clover	—	Davern *et al.* (1957)
Egg plant	Early	Gotoh (1953)
Wild plants		
Galeopsis	Late	Hagberg (1952)
Melandrium	Early	Lawrence (1963)
Melandrium	—	Lawrence (1964)
Papaver	Early	Lawrence (1965)

Whether the similarity arises because such mutations result from addition or loss of pre-existing genetic material, or its rearrangement, or because genotypes have become adapted to recurrent mutation, remains unclear. Genetic analysis of mutations induced in a number of contrasting genotypes by chemical mutagens less prone to break chromosomes than radiation, would help to solve this problem.

5. SUMMARY

1. Genetic analysis of a number of inbred lines of *Arabidopsis thaliana* containing radiation-induced mutations in the polygenic system influencing flowering time has been carried out by means of sets of diallel crosses.

2. These lines were obtained by selecting for both early and late flowering in families resulting from the self-pollination of single, previously homozygous plants irradiated as seed.

3. The majority of the variation between the lines arose from the additive effects of the mutations (general combining ability) but significant non-additive variation (special combining ability) was also found.

4. In most experiments the majority of the mutations were recessive to their wild type alleles, but dominant mutations were also found. Deletions

cannot therefore give rise to all of the variation and it is possible that they are of relatively minor importance in this context.

5. Mutations in the late flowering selection lines are generally recessive to those in the early flowering lines, a situation which probably reflects the average dominance relationship among wild type alleles.

6. In general, these relatively unselected samples of radiation-induced mutations possess properties quite similar to those of their spontaneous counterparts. Whether this is because such mutations merely result from addition or loss of pre-existing genetic material, or its rearrangement, or because genotypes have become adapted to recurrent mutation, is not known.

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