RADIATION-INDUCED POLYGENIC MUTATION IN *ARABIDOPSIS THALIANA*

III. EFFECT OF PARENTAL GENOTYPE AND THE LIMITS TO CHANGE

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SUMMARY

Three experiments were carried out designed to examine further the properties of radiation-induced mutations affecting flowering time in *Arabidopsis thaliana* and some of the variables which influence these properties, such as the genotype of the variety that was irradiated (first experiment) and the factors which influence the accumulation of mutations within a single inbred line (second and third experiments). The average additive effect of the induced mutations was found to be independent of parental genotype, supporting the view that this property depends on the relation between the particular character studied and fitness rather than the frequency of "plus" and "minus" alleles in the parental genome as suggested by Brock (1965). Considerable response to selection was obtained in families derived by crossing inbred lines which had been previously irradiated and selected, indicating that few of the mutations fixed in these lines were allelic or linked. This result also suggests that each line contained few mutations, each of relatively large effect. Lines which were irradiated and selected every generation showed a linear relationship between response and accumulated dose in both the early and late flowering direction during generations one to five, but a negative response in the early direction and disproportionately large response in the late direction among generations six to nine inclusive. It is suggested that mutations induced in the later generations interact with those fixed in the early generations giving an adverse effect of multiple mutant combinations on fitness. Such interactions may impose limits on the usefulness of induced mutations in plant breeding.

1. INTRODUCTION

PREVIOUS papers in this series (Lawrence, 1968*a, b*) described experiments designed to examine the genetic properties of micro-mutations affecting flowering time, induced by ionising radiation in plants of a single standard genotype. They showed that equal amounts of variation were induced in the early and late flowering directions, that within the range studied the amount of variation induced was linearly related to accumulated dose when equal increments were given to seeds in successive generations, and that the mutations were sometimes dominant though usually recessive. The aim of the experiments reported in the present paper was to extend this analysis by comparing the variation in flowering time induced in two different inbred lines, one early and the other later flowering, and to examine the extent to which the flowering time of the standard inbred line could be

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changed by the accumulation of radiation-induced mutations, either by repeated irradiation and selection or by hybridisation between previously irradiated and selected individuals.

2. MATERIALS AND METHODS

(i) *Inbred lines*

The two inbred lines used in the first experiment reported here were Enkheim and the standard or control line used previously (Lawrence, 1968*a, b*). Enkheim is a very early flowering line and the standard a medium to late flowering variety, though earlier lines than Enkheim and much later ones than the standard have been described (Robbelen, 1965). Both were highly inbred when first received and were subsequently maintained by careful self-pollination of a single plant for at least 10 generations before use. The five lines used in the second experiment were all early flowering selections, one from the control or standard line, two from families which initially received 90 krad of ^{60}Co gamma radiation (S_2E ; S_3E), and two from families which received an accumulated dose of 450 krad (R_1E ; R_3E). The R lines were maintained by self-pollinating a single plant for three generations and the S lines for seven generations after the last irradiated generation before use in the second experiment. The seven lines used in the third experiment include an unselected standard line, three early selections from the R families (R_1E ; R_2E ; R_3E) and three late selections from the R families (R_1L_2 ; R_2L_2 ; R_3L_2). Further details of the derivation and performance of the lines used in all three experiments can be obtained from the previous reports (Lawrence 1968*a, b*).

(ii) *Cultural methods*

In the first experiment, plants were grown in agar medium in 1.5×15 cm test-tubes closed with loosely fitting glass caps. The medium was prepared according to the method of Miksche and Brown (1965) with the exception that 0.75 per cent Difco Agar Noble was used, and 7.5 ml dispensed per tube. Seeds were sterilised by soaking in a 1 : 1 mixture of 3 per cent hydrogen peroxide and absolute alcohol for 10 min, followed by repeated washing with sterile demineralised water before sowing with the aid of a sterilised paint brush. The tubes were placed in white-painted wooden blocks, each containing a 10×10 array of holes with a 22 mm spacing between their centres and of sufficient depth such that the surface of the agar was level with the surface of the block. After sowing, the tubes were kept for 24 hours in the dark at 2°C and then transferred to a growth room, giving a temperature of $24 \pm 1^\circ\text{C}$ within the tubes and continuous illumination at an intensity of $10,800 \pm 1080$ lumens m^{-2} at the level of the agar. A considerable amount of trouble was experienced initially in this experiment which was eventually traced to a short length of PVC tubing through which hot agar medium was dispensed. Medium dispensed in this way proved to be completely lethal to the seedlings, but silicone rubber tubing was satisfactory and was used on all subsequent occasions. In the second and third experiments, plants were grown in soil in a glasshouse according to the method described previously (Lawrence, 1968*a*).

(iii) Irradiation

All irradiation treatments were given to seeds kept for at least 4 weeks after collection and equilibrated over P_2O_5 to 6 per cent moisture content. Seeds were irradiated in air and then soaked immediately in aerated water. Control seeds were handled in a similar manner. In the first experiment seeds were given 180 krad of ^{60}Co gamma irradiation at a dose-rate of 388 krad/hour, while in the third experiment the standard dose was 90 krad delivered at a dose-rate of 180 krad/hour.

(iv) Selection and testing

All lines were maintained by the self-pollination of a single plant and when grown in a glasshouse such plants were always transferred to a pot, their pods and open flowers removed, and self-pollination allowed to take place only under well-isolated conditions in a growth room. It is commonly believed that *Arabidopsis* is an obligate inbreeder but although self-pollination generally occurs, occasional cross-pollination can also take place when plants touch each other, particularly when they are semi-sterile. Insects have also been observed moving among the inflorescences and occasional outcrosses can only be avoided in a glasshouse by adopting stringent precautions.

In the first experiment, the earliest and the latest flowering plants were selected in families obtained by sowing 100 seeds taken from each of five plants irradiated as seed and five control plants of both the Enkheim and Standard line. Selection of either the extreme early or extreme late plant from similar sized families continued in the two subsequent generations and was followed by inbreeding without selection for two generations. The extreme plant was selected except if its phenotype was decidedly abnormal or if it failed to produce a hundred seeds. Early selections always produced a hundred seeds, but 10-20 per cent of the late selections failed to do so, particularly in the later generations, and the next latest plant was therefore chosen in those cases. One line (a late selection from an irradiated Standard line) was lost due to sterility in the X_4 generation. The performance of the remaining 39 lines was tested in two trials, each composed of 12 blocks. In the first, plants were grown in agar in a growth room and each line was represented by one plot of 10 plants per block. In the second, plants were grown in soil in a glasshouse and a similar experimental design was used except that there were only five plants per plot.

In the second experiment, selection for early flowering was practised in families obtained by selfing one plant from each of the 25 parents and hybrids in a 5×5 set of diallel crosses. The parents from which the set of diallel crosses were constructed were all early flowering selections taken from a previous experiment (see above). Two hundred seeds from each of these 25 plants were sown and the three earliest flowering plants selected from each of the resulting families. In the next two generations the 75 lines were maintained by selecting the earliest flowering plant in families containing up to 99 individuals, and this was followed by one generation of inbreeding without selection. The actual number of plants per family depended on the proportion of seeds germinating and surviving to maturity, which was usually better than 90 per cent. The performance of the 75

sowing to flowering (opening of the first flower) for each group of five replicate lines, and also the mean variance within the five families, in the X_2 , X_3 and X_4 generations. When grown in these conditions, Enkheim flowers about 4 days earlier than the Standard line, a substantial difference in this rapidly flowering species. A lower than usual light intensity and temperature probably accounts for the comparative lateness of all X_3 plants. The data from the X_2 generation appear to indicate that much more genetic variation was induced in Enkheim than in the Standard line, but subsequent selection responses show that this was not the case, supporting the previous contention (Lawrence, 1968a) that predictions based on X_2 distributions are usually extremely unreliable. Data from the

TABLE 2

The average number of days between sowing and flowering (opening of the first flower) of the 39 lines in the two trials of the first experiment. Trial 1 (top figure, agar) sown: 3/5/68, Trial 2 (bottom figure, soil,) sown: 9/7/68

Replicate line No.	Enkheim				Standard			
	0 krad		180 krad		0 krad		180 krad	
	Early	Late	Early	Late	Early	Late	Early	Late
1	17.33	18.35	17.45	18.35	20.33	21.10	20.54	21.70
	35.98	35.88	36.48	36.54	39.04	39.92	39.78	40.07
2	17.46	17.76	17.49	22.48	21.49	20.74	20.08	29.19
	37.02	35.45	36.49	42.38	38.93	39.67	39.08	49.72
3	18.43	18.32	18.13	20.98	20.95	20.90	21.01	21.42
	35.92	36.68	37.91	38.62	39.17	39.39	39.36	40.24
4	17.58	17.36	18.44	18.89	20.75	20.65	20.83	21.47
	36.63	36.01	35.82	39.01	39.07	39.13	39.63	40.73
5	17.42	16.98	17.77	18.50	20.44	20.73	21.53	—
	35.86	35.80	35.87	36.12	39.24	39.28	40.12	—
Trial 1	17.64	17.75	17.85	19.84	20.79	20.82	20.80	23.44
Trial 2	36.28	35.96	36.51	38.53	39.09	39.48	39.60	42.69
Average	26.96	26.86	27.18	29.19	29.94	30.15	30.20	33.07

X_3 and X_4 generations suggest that there was no response to selection in the control families but that such responses occurred in irradiated families, particularly in the late flowering direction. An asymmetrical response of this kind contrasts with earlier experience and may be due to the comparatively high acute dose used or to the environmental conditions. The high variation within X_4 families derived from the irradiated Standard line was due to a single early and two late selections, including the very late sub-vital family which was lost because of sterility, and probably arises from poor developmental stability rather than genetic segregation.

Analysis (table 3) of the results from the two trials (table 2) confirms the absence of selection responses in the control families and shows that the response in the irradiated families was similar for each of the two varieties. Since the line that was lost was very late, about 10 days later than its

TABLE 3

Analysis of variance in flowering time in the two trials of the first experiment

Item	d.f.	M.S.	V.R.
(1) <i>Lines</i>	38	138.66	42.40***
Varieties	1	2355.47	40.49***
Doses	1	374.10	1.28 n.s.
Selection	1	269.35	< 1 n.s.
V × D	1	52.68	< 1 n.s.
V × S	1	54.44	< 1 n.s.
D × S	1	291.82	5.02*
V × D × S	1	67.75	1.16 n.s.
Replicates	31	58.18	17.79***
(a) Control	16	2.28	< 1 n.s.
(b) Irradiated	15	117.81	36.03***
(2) <i>Lines</i> × trials	38	3.27	1.79*
(3) <i>Trials</i>	1	81243.20	44395.19***
(4) <i>Blocks</i> —trial 1	11	34.05	16.69***
trial 2	11	1.43	< 1 n.s.
(5) <i>Error</i> —trial 1	418	2.04	1.25*
trial 2	418	1.63	—
} 1.83			
(6) <i>Total</i>	935	—	—

* $P = 0.05-0.01$; *** $P < 0.001$.

control, response was in fact rather greater in the Standard families. There was no evidence of a response to selection in the early flowering direction in either variety. Heterogeneity among the replicate lines was almost entirely confined to the irradiated late selections, as found in previous experiments (Lawrence, 1968a).

(ii) *Second experiment*

This experiment was designed to estimate the extent to which further selection responses could be obtained after hybridisation between lines previously irradiated and selected for early flowering. Table 4 gives the average number of days from sowing to flowering in the F_2 to F_4 generations, during which selection was carried out, and in the F_6 trial. The relative performance of the families was estimated much less reliably in the F_2 to F_4 generations because the experimental design was suitable for selection rather than testing. The data from the F_6 trial were analysed by Hayman's method (Hayman, 1954) for diallel tables and the result is shown in table 5. This method of analysis is useful in the present case, even though it was devised for diallels composed of inbred parents and their F_1 hybrids rather than for sets of inbred selection lines; the various items in the analysis inevitably have a different meaning, however, and must be interpreted in a different manner.

The 75 selection lines can be divided into three replicate sets of 25 lines, each set derived from either the first, second or third selections in the F_2 generation. Table 5 shows that there was significant variation between the 25 groups of lines, indicating the presence of genetic differences between the parents, and that the three replicate sets were homogeneous, which means that the response to selection was homogeneous in the three sets.

The average size of the selection response in the three sets varied significantly, however. Since the replicates/main effects interactions were not significant, they were pooled with the other interactions to provide a combined estimate

TABLE 4

Average number of days from sowing to flowering in the selection lines derived from the 5 × 5 diallel (second experiment) in the F₂-F₄ and F₆ generations. Each entry is the average of three replicate lines. F₆ trial sown: 19/8/68

Female parent		Male parent					Generation
		Control	S ₂ E	S ₃ E	R ₁ E	R ₃ E	
	Control	30.59	29.15	29.46	30.29	30.90	F ₂
	S ₂ E	29.31	29.14	29.46	29.47	29.74	
	S ₃ E	31.01	29.17	28.70	29.61	29.29	
	R ₁ E	29.28	30.16	29.31	29.51	29.89	
	R ₃ E	30.13	29.56	28.81	28.97	31.10	
	Control	29.70	29.33	28.53	29.03	28.60	F ₃
	S ₂ E	27.33	28.33	28.50	28.63	27.90	
	S ₃ E	29.63	28.27	27.87	29.03	27.60	
	R ₁ E	29.57	29.40	27.40	29.03	27.40	
	R ₃ E	28.70	28.43	28.97	27.27	29.17	
	Control	32.00	29.80	30.20	30.47	30.57	F ₄
	S ₂ E	30.37	31.67	30.17	30.40	30.83	
	S ₃ E	30.00	31.93	30.30	30.53	30.03	
	R ₁ E	30.30	30.50	30.27	30.97	29.03	
	R ₃ E	30.17	31.33	29.63	30.90	30.10	
	Control	32.66	31.78	31.50	30.75	31.44	F ₆ trial
	S ₂ E	31.70	31.68	30.80	30.73	30.23	
	S ₃ E	31.18	30.19	31.18	30.44	31.20	
	R ₁ E	30.89	30.74	30.41	30.72	30.15	
	R ₃ E	31.55	30.85	31.14	30.45	31.43	

TABLE 5

Analysis of variance in flowering time in the F₆ trial of the second experiment

Item	d.f.	M.S.	V.R.
(1) Lines	24	8.65	6.71***
a	4	32.16	24.93***
b	10	6.72	5.21***
b1	1	38.25	29.65***
b2	4	0.39	< 1 n.s.
b3	5	5.47	4.24***
Reciprocals	10	1.19	< 1 n.s.
(2) Replicates	2	21.59	16.74***
(3) Blocks	7	9.32	7.22***
(4) Error	566	1.29	—
(5) Total	599	—	—

*** P < 0.001.

of error variation. The *a* mean square measures variation between the five groups of lines, each derived from families possessing a parent in common, and therefore depends largely on the additive effects of the genes by which

the parents differ. Its significance confirms earlier results (Lawrence, 1968*b*) which demonstrated the existence of additive variation between the parental lines. The *b* mean square depends on the deviations of the "hybrid" line flowering times from the average performance of their parents and therefore in the present circumstance detects the response to selection in these families. Its significance shows that selection was successful and as might be expected the major portion of the *b* mean square depends on the average difference between the "hybrid" and "parent" lines (*b*1), though there is also evidence of some inconsistency in the selection response (*b*3). The "reciprocals" mean square, like the replicates/main effects interaction, measures the heterogeneity of selection response in replicate lines of common genetic origin and the lack of significance for this item also shows that the selection response was homogeneous in these groups.

Although the *b* items show that selection was successful, they do not show that mutations from each parent have been combined in the "hybrid" lines. To do this, it is necessary to show that the "hybrid" lines flower earlier than the earlier of their parents. If "reciprocal" lines are averaged, it can be seen that five out of six of the groups of "hybrid" lines flower earlier than the earlier parent, excluding the groups of "hybrids" in the Control array where such a response is neither expected nor observed. On average, the six groups of "hybrid" lines flower 0.38 day earlier than the earlier parent, a difference which is highly significant ($P < 0.01$). If it is assumed that the size of the maximum response can be estimated by adding the deviations of each parent from the control, an average of about 70 per cent of this maximum was achieved though there was a tendency for this proportion to be lower when the expected response was large and vice versa.

(iii) *Third experiment*

It has been shown that selection response is linearly related to accumulated dose when equal increments of 90 krad are given to seeds in up to five successive generations (Lawrence, 1968*a*). It is therefore of interest to determine up to what accumulated dose this linearity is maintained. It is clear from table 6 that such linearity breaks down between accumulated doses of 450 and 810 krad both in the early and late flowering directions, though in different ways. Comparing individual R and J lines which

TABLE 6

Mean number of days between sowing and flowering of the control and irradiated lines of the third experiment. Sown: 30/8/68

Replicate line No.	Control 0 krad unselected	R Lines		J Lines	
		Accumulated dose		Accumulated dose	
		450 krad		810 krad	
		Early	Late	Early	Late
1	38.24	37.80	39.87	37.15	42.92
2	37.84	37.09	37.97	37.46	47.99
3	—	37.74	38.72	40.74	43.02
Average	38.04	37.54	38.85	38.45	44.64

have common ancestry, it can be seen that the additional irradiation and selection gave an additional response in only one out of three early selections, the other two J lines being *later* than their R line counterparts, one substantially so. The J line late selections, however, showed disproportionately greater response and all flowered substantially later than their R line counterparts. Response in the R lines was rather smaller than observed previously, particularly among the early selections. An analysis of variance of the data (table 7) shows that variation between the lines was significant

TABLE 7

Analysis of variance in flowering time in the trial of the third experiment

Item	d.f.	M.S.	V.R.
(1) <i>Lines</i>	13	97.26	73.13***
<i>Treatments</i>	4	248.75	8.31**
<i>Replicates</i>	9	29.94	22.51
(2) <i>Blocks</i>	9	8.77	6.59
(3) <i>Lines</i> $\times$ <i>blocks</i>	117	1.33	—
(4) <i>Total</i>	139	—	—

** $P = 0.01-0.001$; *** $P < 0.001$.

and that there was significant variation between the five groups of lines receiving different radiation or selection treatments despite the existence of heterogeneity between the replicate lines.

4. DISCUSSION

These experiments were designed to examine the properties of radiation-induced mutations affecting flowering time in *Arabidopsis thaliana* and some of the variables which influence these properties, in particular the genotype of the variety that was irradiated (first experiment) and the factors which influence the accumulation of mutations within a single inbred line (second and third experiments).

With regard to the first of these points, contrasting varieties exposed to the same dose of radiation might possibly exhibit either different *amounts* or different *kinds* of induced variations, and might perhaps differ in both these ways. If it is assumed that the efficiency of selection was similar in the two varieties, results from the first experiment suggest that equal amounts of variation were induced in Enkheim and the Standard variety. The two varieties also had similar radiosensitivities with regard to the lethal effects of radiation, 78 per cent of the Standard and 83 per cent of Enkheim X_1 plants surviving to maturity after the dose of 180 krad. The LD_{50} of these seeds was about 200 krad, which is similar to the value of 180 krad found previously (Lawrence, 1968a).

As well as exhibiting similar amounts of induced variation, the two varieties were also similar with regard to the kind of variation induced, at least as far as the additive effects of the induced mutations are concerned. There was little evidence for any variation in the early flowering direction in either variety, and similar amounts were induced in the late flowering direction. If anything, the response was greater in the Standard variety

since the line that was lost was unusually late, but replicate lines were very heterogeneous in both varieties and it is unlikely that the difference would have been significant. Hypotheses of two kinds, leading to different predictions, have been proposed concerning the relation between the parental genotype and the additive effects of the induced mutations. Brock (1965) has suggested that "plus" alleles mutate to a "minus" condition and vice versa, though with the proviso that "the degree to which the genes of the parental genome operate as a balanced set" is also significant. According to this scheme, therefore, the net additive effect of the induced mutations relative to the performance of the parental variety depends substantially on the proportions of "plus" and "minus" alleles in the parental genome. That is, emphasis is laid on the kind of selection which the particular variety has experienced in the immediate past, which changes these proportions. Lawrence (1965, 1968*a*), on the other hand, has related the average additive effects of the induced mutations to the genetic architecture of the character studied and has laid emphasis on the relation of the character to fitness, that is on the kind and intensity of selection to which the characters have been exposed in the species as a whole over long periods.

These two schemes predict similar results when the characters studied are those which are exposed to natural selection largely of the directional variety, that is characters which are directly or linearly related to fitness, but differ with regard to the expected properties of mutations affecting characters previously selected for an intermediate or optimum phenotype whose relation with fitness is non-linear. Flowering time is probably a character of this second type. According to Brock's scheme, the average additive effect of the induced mutations relative to the parental phenotype will differ in very early and very late flowering varieties, since more average phenotypes are expected to predominate among the mutants in both cases. According to the second scheme, however, the average additive effects of the mutations are expected to be relatively independent of parental genotype at least within broad limits imposed by the developmental physiology of flowering. Most mutations reduce fitness, which can be manifest both as earlier and later flowering, and moreover since flowering time in a largely self-pollinating species is probably a relatively minor component of fitness, its control is likely to be less stringent. There is, however, another important factor. Many "flowering time" mutations seem to exhibit pleiotropic effects on other characters which are more major components of fitness (Sakai and Suzuki, 1964; Scossiroli *et al.*, 1966; Brock, 1967), such as the number of seeds per pod (Lawrence, 1968*a* and unpublished), and these are likely to act predominantly in the late flowering direction. Sub-vital mutants of many kinds, for example, might be expected to grow slowly and therefore flower late. From the fitness point of view, therefore, the average additive effect of the induced mutations is expected to be small or zero when pleiotropic effects are absent, or act predominantly in the late flowering direction when they are present. In either case, however, the relative proportion of mutations acting in the "plus" and "minus" direction is expected to be largely independent of parental genotype.

The results from the first experiment therefore tend to support the fitness and not Brock's hypothesis. A similar conclusion has also been reached from experiments with wheat (Gaul and Aastveit, 1966). The absence of any response in the early flowering direction in the first experiment contrasts

with previous results using the Standard variety. An explanation based on physiological limits can hardly be maintained, particularly with the Standard variety, since early flowering varieties are known. Similarly, although selection was somewhat more intense in the late flowering direction, some response to selection in the early flowering direction was expected. It is more likely that lack of response in the early flowering direction is related to the exacting environment that these miniaturised plants experienced, in which most mutants could be sub-vital, though it might also arise from the use of a relatively high acute dose; two-hit deletions with sub-vital effects might be expected to predominate at higher doses for example.

The object of the second and third experiments was to examine the extent to which more extreme phenotypes can be produced by accumulating mutations within a single genome either by hybridisation between previously irradiated and selected lines or by repeated irradiation and selection. Such experiments provide information about the properties and behaviour of the mutations and are also relevant to the question of potential use of induced quantitative variation for crop plant improvement. When hybridisation is used, success in producing more extreme lines depends not only on the efficiency of selection but also on the gene action of the mutations, whether they exhibit non-allelic interactions, and the extent to which the mutations are allelic or linked. Results from the second experiment suggest that few mutations are allelic or linked and therefore indicate that the number of loci which influence flowering time must be high. Nevertheless, the high efficiency of selection, in which an average of 70 per cent of the potential response was realised, implies that the parental lines contained only a few mutations each with relatively large effect. These are, of course, precisely the kind of mutations that are most likely to be selected. Selection appeared to be rather less efficient in families derived from hybrids between the more extreme parents, and although this might depend on non-allelic interaction it is more probably due to the extreme parents possessing more mutations than the less extreme ones, and the consequent smaller probability of simultaneously fixing all of the required alleles at each of the segregating loci during inbreeding.

When repeated irradiation and selection are used, success in producing more extreme lines depends on factors that are similar to those in the case of hybridisation, with the exception that linkage is not important and there is less opportunity for the elimination of deleterious and sub-vital mutations. Results from the third experiment suggests that this reduced opportunity for elimination of deleterious mutations may be of considerable importance. Over the first five generations, response to selection in either the early or late flowering direction was directly proportioned to accumulated dose (Lawrence, 1968a), and there is no reason to believe that the same dose increments were not equally mutagenic, or produced different kinds of mutations, in later generations. There was, however, on average a negative response to selection for early flowering during generations six to nine. The additional mutations induced in these generations which individually would promote earliness presumably fail to be selected because their action is masked by other mutations, or if selected have little effect because of similar non-allelic interactions. In the late flowering direction, on the other hand, there was a disproportionately large response to selection. It would therefore appear as if the mutations induced in the later generations

act non-additively in combination with those fixed during the early generations to produce late flowering phenotypes and this may arise because of pleiotropic effects on some other characters, such as growth-rate perhaps, which are more directly related to fitness. The results from the second experiment suggest that not all mutations exhibit these interactions, and since they were also not detected in the first five generations of the third experiment they may arise as a consequence of the accumulation of numerous mutations which individually have very little or no effect on flowering time but which are randomly fixed during inbreeding. If so, they may be avoided by interposing generations of inbreeding, or better intercrossing, with selection between the generations which are irradiated, but in any case their existence has important implications for the use of induced mutations in plant breeding and may impose limits to the extent of the change in phenotype achieved in this way. Nevertheless, substantial change can be achieved by a combination of irradiation, selection and hybridisation as shown by the second experiment and also by the work of Brock and Shaw (1969).

The existence of such interactions also has important implications for plant breeding with regard to the choice of selecting macro- or micro-mutations. It has been suggested (Gregory, 1965; Gaul *et al.*, 1969) that micro-mutations are more favourable because they have a high mutation rate and because they have a better chance of improving fitness. The basis for the second contention is a model proposed by Fisher (1930), for which Gregory (1965) has experimental support, which shows that for species which are well but not perfectly adapted, a small change in phenotype is more likely to improve adaptation than a large one, with a limiting probability of 50 per cent for very small changes. While this model may well be correct, the deductions made from it with regard to mutation breeding are not. The point at issue in this case is whether it is better to produce a given change in phenotype by *one* mutational step or by *many*, and on Fisher's hypothesis the chances of improving adaptation will be the same either way. Similarly, although the frequency of induced micro-mutations may well be high, this may be entirely compensated for by the very small effect of individual mutations. The choice between micro- and macro-mutation breeding will therefore depend on other factors, such as the relative ease with which the required change can be achieved by the two methods, and the extent of undesirable correlated changes. It is still not clear what the result of such an assessment would be. Brock and Shaw (1969) have observed that the largest response to selection is usually dependent on macro-mutations, and similar observations have been made in the present series of experiments. Although such mutations often have undesirable side-effects, the extent to which these can be minimised by alteration of the genetic background is unknown, and it would appear that strains containing many micro-mutations may also have such side-effects. In general, Fisher's model, which is essentially static and non-genetic, does not provide a suitable theoretical basis for mutation breeding. It assumes a relatively constant environment though it could equally be turned upside down and assume a constant genotype and varying environment, while in plant breeding adaptation is sometimes sought to a new or different environment, as in the case of short-strawed cereal mutants and high soil-nitrogen levels.

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