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RADIATION-INDUCED POLYGENIC MUTATION IN *ARABIDOPSIS THALIANA*

I. SELECTION FOR FLOWERING TIME

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1. Introduction

Although it is well established that ionising radiations can induce mutations which affect quantitative characters (Scossiroli, 1954; Clayton and Robertson, 1955; Gregory, 1955), little is known about the nature, properties and rates of induction of such mutations. It is not known, for instance, whether it is possible to make generalisations concerning the direction of manifestation of these mutations (relative frequency of " plus " and " minus " mutations), whether they are usually recessive or not, and whether they exhibit non-allelic interactions either with normal alleles or among themselves. Similarly, the extent to which these properties vary with character studied, whether an important as opposed to less important component of fitness, and with the breeding system of the species used, is unknown. Information of this kind could be of value in two ways. First, it could throw light on the nature of the organisation and integration of genes within gene systems, the genome, and the gene-pool (Fisher, 1930; Mather, 1943; 1960; Dobzhansky, 1951; Lerner, 1954). Second, such data are necessary before a general assessment of the usefulness of radiation-induced quantitative variation in plant breeding can be made. Despite the work of Gregory (1955, 1961, 1965), Rawlings Hanway and Gardner (1958), Scossiroli (1959, 1965), Brock and Latter (1961), Brock (1965, 1966) and others, this evaluation is not yet possible.

The majority of existing information concerning the properties and rates of induction of polygene mutations has been obtained from experiments with a single species, *Drosophila melanogaster* (*e.g.* Wallace, 1957, 1963; Bateman, 1959; Muller and Falk, 1961; Yamada and Kitagawa, 1961; Stevens and Le Roux, 1963; Mukai, 1964; Mukai, Yoshikawa and Sano, 1966; Falk, 1967). The aim of the work reported in this and following papers has been to obtain further data concerning polygenic mutations using a different organism, and the crucifer *Arabidopsis thaliana* (Heyn.) was chosen for this purpose. Micro or polygene mutations affecting two characters have been examined, namely flowering time and number of seeds per pod. These two characters are probably exposed to different kinds of selection in wild populations and probably differ in their importance as components of fitness. For each character the work has proceeded in two phases. In the first phase selection has been carried out in populations obtained by irradiating homozygous individuals. In the second phase the selection lines have been analysed genetically by means of diallel crosses. This paper is concerned with selection for flowering time.

2. Materials and methods

Cultural method. An inbred line of *Arabidopsis thaliana* was maintained by self-pollination of a single plant for a further ten generations before its use in the first experiment. Plants were grown in beds, ten by six feet in size, on benches in the centre of a glasshouse. Each bed consisted of a six-inch deep tray lined with polythene sheet in which a few holes had been punched. The trays contained a two-inch layer of coarse gravel covered with four inches of John Innes potting compost (7 parts sterilised loam, 3 parts peat, 1 part sharp sand) and a surface of very finely sieved compost. Water was introduced directly into the layer of gravel, allowed to soak up into the compost and then drain away. The compost was very moist at the time of sowing.

Before sowing, seeds were placed on damp filter paper in petri dishes and kept in the dark at 1-2° C. for three to four days. Seeds were then transferred to shallow depressions on the soil surface (finger-tip size) using a fine paint-brush, one seed per depression. A spacing of two inches between and one and a half inches within rows was used. After sowing, a sheet of damp muslin was suspended a few inches above the soil surface to maintain a high humidity until germination was well advanced. Under these conditions germination was rapid and 90 per cent. or more control seeds gave rise to mature plants.

Irradiation. All irradiation treatments were given to seeds. The seeds were kept for at least four weeks after collection and then equilibrated over P_2O_2 to 6 per cent. moisture before irradiation, which was carried out in air. The seeds were hydrated in aerated conditions immediately following irradiation, allowed to imbibe for about an hour at room temperature and then stored at 1-2° C. as described above. Control seeds were handled in a similar manner.

In the first experiment a standard dose of 90 kr. of ^{60}Co gamma radiation was used. The first treatments were given at a dose rate of 120 kr./hr., and a dose rate varying between 300 and 240 kr./hr. was used in later treatments. In the second experiment doses of 30, 60, 90 and 120 kr. of ^{60}Co gamma radiation were given at a dose rate of 250 kr./hr. The LD_{50} of seeds handled in this manner is about 180 kr., and therefore all the doses used are relatively small.

Selection and testing. Selection for early and late flowering was carried out within lines maintained by self-pollination. Seed was collected from the earliest or latest flowering plant in families containing up to a hundred individuals. The actual number of individuals per family depended on the number of seeds surviving to the mature plant stage, the average number per group of lines being given in table 1. Each family was grown in one plot, randomised within a single block. This selection procedure was subject only to the requirements that the selected plants possessed sufficient fertility to give a hundred seeds, a very small proportion of the normal yield of control plants, and a normal phenotype. The most extreme early flowering plants almost always possessed the required fertility and phenotype, but failure to meet one or other of these criteria was more common among the most extreme late flowering plants, in which case the next most extreme plant was used. Families in which major gene mutations affecting flowering time were found segregating were also discarded. In the first experiment one early and

six late lines were removed in this way. In the second experiment one early and two late lines were discarded.

TABLE 1

The average number of mature plants from each hundred seeds sown. E = early selections, L = late selections. Plants were first irradiated in generation 1

		Generation			
		2	3	4	5
First experiment					
Control	E	100	96	89	91
	L		97	90	98
S group (90 kr.)	E	93	92	78	87
	L		97	65	91
A group (270 kr.)	E	93	87	82	81
	L		75	53	67
R group (450 kr.)	E	77	73	71	87
	L		67	51	40
Second experiment					
Control	E	66	100	96	92
	L		97	88	89
30 kr.	E	64	97	95	92
	L		96	93	85
60 kr.	E	62	99	95	89
	L		98	91	87
90 kr.	E	67	97	88	90
	L		93	82	76
120 kr.	E	75	97	88	94
	L		98	91	88

The first experiment consisted of three groups of lines, to which different irradiation treatments were given. The first group of lines (S) arose from three families, the selfed progeny of three plants, irradiated as seed. One early and two late selection lines were started from each family. The second group of lines (A) were derived from the first, separate lines being started following irradiation in the third generation (G_3). Irradiation treatment was also given in G_5 and, therefore the lines finally received an accumulated dose of 270 kr. The third group of lines (R) were initiated in a similar manner to the S group, but plants were irradiated as seeds each generation to G_5 so that the lines finally received an accumulated dose of 450 kr. Selection was also carried out in a control population. Selection continued from G_2 to G_6 inclusive. Samples of G_7 plants from each line were crossed to the control and, after selfing the resulting progeny, F_2 families were grown in a controlled environment room. The distribution of flowering data in two families suggested that the selection lines concerned differed from the control by a single gene only, and the lines were therefore excluded from the experiment. Together with those previously rejected eight out of eighteen late and one out of nine early lines were excluded.

The size of the selection response in the remaining lines was estimated in one trial using G_7, and two using G_8, plants. Only some of the lines were represented in the first G_8 trial, and they were accompanied by samples of a number of natural races from the collection of Professor Laibach. The

second G_8 trial included all lines and also the natural races. The three trials were grown in ten, twelve, and twelve randomised blocks respectively. Each line or variety was represented by one plot of ten plants per block.

The second experiment consisted of five groups of lines, each group arising from four plants which received either 0, 30, 60, 90 or 120 kr. One early and one late selection was initiated from each of the selfed progenies of those plants. Selection continued up to and including G_4. A trial containing twelve randomised blocks was grown in G_5.

3. Results

Selection response in the first experiment. A summary of the results from the first experiment is given in tables 2 and 3. Data from the early generations are not given since the irradiation treatments interfere with the estimation of selection responses. Table 2 shows the average number of days from sowing to flowering in G_7 and G_8 plants of the selection lines and in the varieties. Table 3 gives the results of an analysis of variance in flowering time of the selection lines, combining data from the first and third trials. Several lines were not grown in the second trial and therefore its data cannot be readily included in the analysis.

Table 2

The average number of days from sowing to flowering (opening of the first flower) in the three trials of the first experiment. Brackets indicate that data from some of the replicate lines are missing

Line or variety		Trial G_7 29/5/63	Trial G_8 15/6/64	Trial G_8 2/4/65	Average of Trials 1 and 3
Control	E	29·94	35·62	35·32	32·63
	L	30·09	—	36·07	33·08
S group (90 kr.)	E	29·31	(33·98)	35·16	32·24
	L	29·84	—	35·40	32·62
A group (270 kr.)	E	28·85	—	35·39	32·12
	L	31·75	(36·40)	35·38	33·57
R group (450 kr.)	E	28·41	34·80	35·01	31·71
	L	31·90	36·63	36·18	34·04
Enkheim		—	32·54	30·39	—
Estland 1		—	33·56	31·86	—
Estland 2		—	—	33·47	—
Maine		—	34·08	32·44	—
Eiffel		—	33·53	33·17	—
Limburg		—	36·03	36·15	—

The results in table 2 suggest that on average each group of lines had responded to selection and that the size of the response was greater than that in the control. This was confirmed by the analysis of variance. The difference between the control early and late lines, though in the expected direction, was not significant. The two trials were homogeneous in this respect. There was, however, a significant difference between early and late lines from irradiated families. Table 3 shows that the results from different early flowering lines within a given group were homogeneous and that data from the two trials were also homogeneous. The average size of the response to selection increased with increasing accumulated dose. The variation

TABLE 3

Analysis of variance in flowering time among selection lines of the first experiment

Item	d.f.	M.S.	V.R.
1. *Lines*	18	17·96	1·48
(i) Early lines	8	2·96	1·96*
between dose groups	3	5·06	3·35*
within dose groups	5	1·70	1·13
(ii) Late lines	9	11·77	< 1
between dose groups	3	19·33	1·32
within dose groups	6	8·00	< 1
(iii) Early *v.* late lines	1	193·73	2·63
2. *Lines* × *trials*	18	12·10	8·01***
(i) Early lines × trials	8	1·56	1·03
(ii) Late lines × trials	9	14·64	9·70***
(iii) Early *v.* late lines × trials	1	73·59	48·74***
3. *Trials*	1	1135·76	752·16***
4. *Blocks*	20	5·54	3·67***
5. *Error*	360	1·51	—
6. *Total*	417	—	—

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

between groups was significant ($P = 0{\cdot}05$-$0{\cdot}01$). Each group flowered significantly earlier than the control (SE $P = 0{\cdot}05$-$0{\cdot}01$, AE $P = 0{\cdot}01$-$0{\cdot}001$, RE $P < 0{\cdot}001$) and the R group flowered earlier than the S group ($P = 0{\cdot}05$-$0{\cdot}01$). The regression of response on dose was significant ($P = 0{\cdot}05$-$0{\cdot}01$) and showed that:

$$\text{Response} = 0{\cdot}18 \pm 0{\cdot}04 \text{ days/100 kr.} + 0{\cdot}31 \pm 0{\cdot}06 \text{ days.}$$

In this calculation the selection response in the control was assumed to be real, and equal in the early and late flowering directions. In contrast to these results, the response to selection in the late flowering lines was most erratic and irregular. The flowering times of lines within each group taking each trial separately, were markedly heterogeneous ($P < 0{\cdot}001$) and the results from the two trials were also very heterogeneous ($P < 0{\cdot}001$). Some individual lines within each group flowered significantly later than the control; others, however, did not. The two late lines from each original family did not differ in any consistent respect even in the S group, and are therefore treated as replicates. Although the variation between groups is not significant, the average size of the response is roughly proportional to dose.

Table 2 also shows the average flowering times of a number of natural races, grown in the same trials as the selection lines. These do not cover the whole range of flowering time in the species, and races flowering earlier than Enkheim and, to an even greater extent, later than Limburg 1, have been described (Robbelen, 1965). It therefore appears that the line used in the experiments is close to the average of the species with respect to its flowering time. It is also clear that the response in the selection lines represents only a small fraction of the potential response.

Fertility in the first experiment. G_8 plants from each selection line were grown in a controlled environment room (18 hr. illumination at 700 ft. C., 20° C.) and their fertility assessed by determining the number of good seeds

per pod and the proportion of pollen grains stained by aceto-carmine. Seeds were taken when fully ripe from the first twenty pods of two plants per line on each of two occasions. The number of pods within this twenty which had no good seeds was noted. Ripe pollen was taken from freshly opened flowers from at least two plants per line, and samples of two hundred pollen grains examined from each plant. The data, given in table 4, show that on average the irradiated lines produced fewer seeds than the control. In general, late selection lines were poorer than early lines. There was, however, no consistent decrease in the number of seeds formed with increasing accumulated dose. Although there was significant variation between lines, the variation between groups of lines receiving different treatments was not significant. The two sets of data, obtained on different occasions,

TABLE 4

Seeds per pod and seeds per fertile pod, expressed as per cent. control, and per cent. stained pollen grains in the selection lines of the first experiment

		% Control		% stained pollen grains
		Seeds/pod	Seeds/fertile pod	
Control	E	100	100	96
	L			97
S group (90 kr.)	E	80	83	99
	L	63	66	87
A group (270 kr.)	E	74	75	98
	L	40	51	93
R group (450 kr.)	E	78	82	98
	L	72	73	88

were homogeneous. The low production of the irradiated lines was due in part to an increase in the number of sterile pods, but even if these were excluded, the relative fertility of these lines was much the same. Pollen fertility seemed less affected by the irradiation than seed production, though this may merely reflect the inadequacies of the aceto-carmine staining method. The results from these two measures of fertility were, however, in broad agreement with one another.

Selection response in the second experiment. The average number of days from sowing to flowering in the different groups of lines in generations three, four and five is given in table 5. An analysis of variance in the G_5 trial is given in table 6. In the second experiment, unlike the first, there was a significant response to selection in the control populations. The overall response in the irradiated populations was also significant, and moreover significantly greater than the response in the control ($P < 0{\cdot}001$). There was, however, no evidence in the G_5 trial of an increasing response with increasing dose. This was, no doubt, due to the difficulty of accurately estimating small responses to selection. It was also due to heterogeneity between replicate selection lines, both early and late. Such heterogeneity was found only among late selection lines in the first experiment and, in keeping with this, the heterogeneity was greater ($P = 0{\cdot}05$-$0{\cdot}01$) among late than among early lines in the second experiment. Although it was not possible to demonstrate a linear dose response in the G_5 trial, a dose response of this kind seems very likely when the pooled results of G_3-G_5 are examined (table 5). Table 5 also

shows that the average response per unit dose in G_5 is closely similar to that in the first experiment. It appears likely therefore that the rate of induction and utilisation during selection of polygenic mutations is not materially affected by the manner in which the irradiation is carried out.

TABLE 5

Average number of days from sowing to flowering in generations 3, 4 and 5 of the second experiment

		Generation 3 25/5/64	Generation 4 14/5/65	Generation 5 4/5/66	Average of Generations 3, 4 and 5
Control	E	29·31	30·56	29·58	29·82
	L	29·04	30·47	30·08	29·86
30 kr.	E	29·08	30·70	29·31	29·70
	L	28·97	30·89	30·12	29·99
60 kr.	E	28·81	30·48	29·41	29·57
	L	29·06	31·04	30·03	30·04
90 kr.	E	28·93	30·66	29·57	29·72
	L	29·34	31·81	30·31	30·49
120 kr.	E	28·82	29·87	29·39	29·36
	L	29·11	31·00	30·07	30·06

TABLE 6

Analysis of variance in flowering time among selection lines in the second experiment

Item	d.f.	M.S.	V.R.
1. *Lines*	36	3·34	12·85***
(i) Early lines	18	1·10	4·23***
between dose groups	4	0·66	< 1
within dose groups	14	1·22	4·69***
(ii) Late lines	17	2·97	11·42***
between dose groups	4	0·57	< 1
within dose groups	13	3·71	14·27***
(iii) Early *v.* late lines	1	49·95	192·12***
2. *Blocks*	11	8·49	32·65***
3. *Error*	396	0·26	—
4. *Total*	443	—	—

4. DISCUSSION

The experiments described in this paper are concerned with micro or polygene mutations rather than all mutations which affect quantitative characters; that is mutations with large quantitative effects have not been studied. This is because micromutations are probably of greater importance than macromutations in the evolution, either natural or man-directed, of most organisms and because it is possible that they exhibit different properties. The screening procedure used was of course rather arbitrary since the phenotypic effects associated with different mutations are no doubt continuously distributed and in any case will depend on the particular genotype and environment used. The screening merely excluded mutations with gross effects whose presence might otherwise have tended to obscure the presence of the micromutations.

The nature of these micromutations, whether due to deletion, duplication,

base change, etc., is not known. Cytological examination of pollen mother cells in plants from the selection lines, and in the hybrids between these plants and the control, failed to reveal any chromosomal or meiotic abnormality. Variation in quantitative characters due to polyploidy, aneuploidy, and certain gross chromosome aberrations can therefore be ruled out, but more subtle changes in the chromosomes would escape detection. Spontaneous mutations will also fall into these various categories, even if with different relative frequencies, and so comparisons between the properties of spontaneous and induced mutations need not be invalid.

Since the aim of this investigation was to examine the properties of induced polygenic mutations, it was important to ensure that the original line used was homogeneous and as nearly homozygous as possible prior to irradiation. Since the inbred line chosen was maintained for a further ten generations by self-pollination of a single plant before use, it is likely that the level of heterozygosity was very low. This conclusion is supported by the small size of the response to selection in the control families of the first experiment which, although in the right direction, was statistically non-significant. In the second experiment, however, the response as estimated in the G_5 trial was both greater and significant. This result is puzzling since the same line was used and moreover it had been inbred for an additional five generations before the start of the second experiment. Examination of table 5 shows that a significant difference was not found in the two previous generations, and in fact in these cases was in the opposite direction to that expected. For this and other reasons, discussed in the following section, it seems likely that the response is of non-genetic origin and that the control plants in the second experiment, as in the first, were almost entirely homozygous.

Induced mutation rates. The size of the selection response among early flowering lines of the first experiment was linearly related to accumulated dose. The same appeared broadly true among the late flowering lines, though there was considerable heterogeneity betwen replicate lines in this case. A significant ($P = 0{\cdot}05\text{-}0{\cdot}01$) joint regression of response on dose can in fact be fitted to these two sets of data. It is likely, therefore, that successive dose increments induce approximately equal amounts of polygenic variation, at least in the range of one to five increments used in this experiment.

Although it was not possible to demonstrate a linear dose response unambiguously in the second experiment, the average size of the response per kilorad appeared to be approximately equal to that in the first. Crude estimates of the selection response, obtained by subtracting the mean flowering time of all the irradiated lines from that of the control and dividing by the average dose, are 2·3 and $1{\cdot}8 \times 10^{-3}$ days/kr. for early and late lines in the first experiment, and 2·1 and $1{\cdot}4 \times 10^{-3}$ days/kr. for similar lines in the second experiment. A greater response would probably have been obtained in the second experiment but for the uncharacteristically high mortality in the important second generation, due to a particularly adverse environment. Since the size of the response varies in different environments and in view of the relatively large standard errors involved, the agreement is good. It is likely, therefore, that if as in the present experiments the doses used kill no more than a small proportion of the X_1 plants, the rate of induction of polygenic mutations and their utilisation during selection is not materially affected by the manner in which the irradiation is carried out.

Since the size of the response to selection is probably related directly to the amount of genetic variation induced, the data show that the mutation rate of polygenes influencing flowering is linearly related to dose, at least over the range of doses used. Previous dose response data generally depend on estimates of genetic variance in X_2 families, which as shown by the work of Kao *et al.* (1960) and as discussed below, are not usually reliable. Within this limitation, however, the work of Daly (1960) and Brock and Latter (1961) suggest that there is a linear relation between induced variance and dose when the doses used are no more than moderately lethal. At higher doses Kao *et al.* (1960), Brock and Latter (1961) and Scossiroli (1965) all find less variance than expected, which Scossiroli attributes to an association between lethal and mutational events. The present results demonstrate how such associated lethality can be avoided by dividing the dose into several increments. If given in one increment the highest dose used (450 kr.) would have killed all but a very few of the X_1 plants.

The expression of the mutation rate in terms of days response per unit dose is of limited value since the estimate obtained is unique to the particular character species, and treatment employed. It would be more useful if it could be expressed in such a form that it could be used for extrapolation to other treatments, characters or even species. In particular, it would be useful to compare the mutation rates of polygenes and major genes. Before this can be achieved, difficulties of two kinds must be overcome. The first, peculiar to quantitative characters, is that it is impossible to reliably estimate the number of loci concerned in any polygenic system. Mutation rates cannot therefore be expressed in terms of mutation per locus per unit dose. Second, common to all induced mutation rates, different species, varieties, or even tissues within individuals vary in radiosensitivity. The first difficulty can possibly be minimised by calculating doubling doses, which for polygenic systems are likely to be more or less independent of the number of loci involved. The ratio of induced to spontaneous mutation rate no doubt varies from locus to locus, but where many loci are concerned an average value will be obtained. The second difficulty is probably incapable of solution, although if mutations are induced in similar kinds of tissues, for example tissues in which the cells are capable of prolonged mitotic division, it might prove useful to scale the doubling doses by means of some survival parameter such as the LD_{50}. Different kinds of cells are known, however, to die in different ways (Bacq and Alexander, 1961) and a constant ratio between cell death and induced mutation rate cannot be assumed. Such scaling may therefore prove to be of limited value.

In the first experiment the response among the early flowering lines was linearly related to accumulated dose as follows:

$$\text{Response} = 0{\cdot}18 \pm 0{\cdot}04 \text{ days/100 kr.} + 0{\cdot}31 \pm 0{\cdot}06 \text{ days.}$$

In fitting this regression a symmetrical response to selection in the control population was assumed. According to this regression, response to selection ascribable to spontaneous mutation was $0{\cdot}31 \pm 0{\cdot}06$ days. Assuming that there is an equilibrium between spontaneous mutation induction and fixation due to inbreeding, this is approximately equal to 5×10^{-2} days per generation. More exact treatment is not justified in view of the errors involved. The doubling dose is therefore about 28 kr. A better estimate can probably be

obtained by combining data from the first and second experiments. The regression lines, all significant, are as follows:

Response (early lines) $= 0{\cdot}18 \pm 0{\cdot}03$ days/100 kr. $+0{\cdot}29 \pm 0{\cdot}04$ days
Response (late lines) $= 0{\cdot}21 \pm 0{\cdot}06$ days/100 kr. $+0{\cdot}13 \pm 0{\cdot}08$ days
Response (all lines) $= 0{\cdot}20 \pm 0{\cdot}03$ days/100 kr. $+0{\cdot}21 \pm 0{\cdot}05$ days

The slopes of the regressions are all closely similar, but the constants are rather variable. If it is assumed that the combined data for all lines is the most reliable, the best estimate of the doubling dose is 21 kr.

These calculations depend on the assumption that differences of flowering time between control early and late lines arise entirely from the selection of spontaneous mutations. Several lines of evidence suggest that in some generations this is not the case. In generation six of the first experiment, and generation five of the second, large and significant differences occurred, but they were not found either in previous or, in the first experiment, subsequent generations. The average flowering times of three early and three late control lines in generation seven of the first experiment are given in table 7.

TABLE 7

Average number of days from sowing to flowering and the average variance in flowering date for control selection lines in the first experiment and their F_2 hybrids

	Average days sowing to flowering		Average Variance	
Inbred families				
Early	35·32	35·35	4·581	4·410
Late	35·37		4·244	
F_2 families				
Early × late	35·49	35·40	4·675	4·367
Late × early	35·31		4·085	

Sowing date: 22/3/65.

This table also shows the average flowering time of the three pairs of F_2 families arising from reciprocal crosses between these early and late lines. These results show that the large difference of flowering time between control early and late lines neither persisted nor gave rise to segregation. Such an irregular and transitory response seems more likely to be due to maternal influences than spontaneous mutation. Significant maternal influences on flowering time have been found on several occasions in sets of dialled crosses (Lawrence unpublished) and seem to be common in *Arabidopsis*.

Since maternal influences inflate estimates of the selection response in control families in some but not all generations, the best estimate of the response due to spontaneous mutation will be obtained by pooling all the data from the controls in tables 2, 5 and 7. These data were weighted according to the number of generations of selection involved and gave an estimate of $2{\cdot}4 \times 10^{-2}$ days/generation selection response due to spontaneous mutation, compared to the previous estimate of $4{\cdot}2 \times 10^{-2}$ days/generation obtained from the regression analysis of the combined data of experiments one and two. Taking the response in the irradiated populations as $0{\cdot}2 \times 10^{-2}$ days/kr., the best estimate of the doubling dose is 12 kr. The estimates

therefore range between 12 and 28 kr. with the most probable value lying towards the lower end of this range. These doubling doses can be compared with those derived from the data of Robbelen (1964) and Veleminsky *et al.* (1964). In both cases the effect of X-rays on dry seeds of *Arabidopsis* was studied. The first set of data was concerned with the induction of chlorophyll mutations, scored in X_2 and the second set was concerned with chlorophyll and embryonic lethal mutations, scored in immature pods of X_1 plants. In both cases the doubling dose was about 4 kr. It seems unlikely therefore that induced mutation rates per locus are higher for polygenes than major genes (*cf.* Burdick and Mukai, 1958; Mukai, 1964), a conclusion also supported by the work of Kitagawa (1967). It must be admitted that maternal influences may have led to an overestimate of the doubling dose in the present experiments and it is possible that the seeds used had greater inherent radioresistance than those used by Robbelen and Veleminsky *et al.* by virtue of different genotype or conditions of treatment. Nevertheless, it is unlikely that polygenic mutation rates are, for instance ten times greater than major gene mutation rates. It is likely, therefore, that the apparently high polygenic mutation rates observed by Bateman (1959), Mukai (1964) and Gregory (1965) arise from the large number of loci controlling quantitative characters rather than a high rate/locus.

Mutation induction with repeated irradiation. The results from the first experiment show that successive dose increments induced approximately equal amounts of polygenic variation, and moreover equal amounts in both the early and late direction. In the work of Jalil and Yamaguchi (1964), Khadr and Frey (1965) and Brock (1966), however, smaller amounts of variation were induced with a second dose increment. In Brock's experiment the response in the first cycle of irradiation and selection was large when compared with the variation between normal varieties of the plant used. It is possible, therefore, that less response was obtained in the second cycle of irradiation and selection because of an approach to some kind of limit. In the present results, the response to selection even after five dose increments was small in comparison with the potential response indicated by natural varieties (table 2) and therefore no limitation would be apparent. Comparable data are not given by the other authors, but in neither case is the response to the first dose increment particularly large and it is unlikely that limitations can be invoked in these experiments. An alternative explanation of the reduced response to a second dose increment in all three experiments is suggested by data from the control families. Brock found a large response in unirradiated families in the first but not the second cycle of selection. Although this may in part arise from a genotype × years interaction (Brock, *loc. cit.*), such a result suggests that in irradiated families a considerable proportion of the response in the first but not second cycle of selection arose from pre-existing and not induced variation. Approximately equal responses in the two cycles are obtained if they are expressed as a proportion of the response in unirradiated families. Since genetic heterogeneity also occurs in the unirradiated material used by Khadr and Frey (*loc. cit.*) a similar explanation may also apply in their experiment. The data of Jalil and Yamaguchi (*loc. cit.*) are presented in such a way that it is impossible to determine whether the same is true in this case.

Fertility in the selection lines. The number of seeds per pod is almost invariably lower in plants selected from irradiated populations than in

control plants, even when several generations of inbreeding have intervened between treatment and testing. This is not very surprising since there can have been very little selection for high fertility during the course of the experiment. The reduction in fertility could arise either from pleiotropic effects of mutations influencing flowering time or from mutations quite independent of these which were fixed randomly during inbreeding. If pleiotropy occurs, the size of the response to selection and the number of seeds per pod should be negatively correlated. Apart from the uniformly lower fertility of the late flowering lines, there is no evidence of this correlation in the variation between different dose groups (tables 2 and 4); the R group of lines show both greater average response and higher average fertility than the A group. Further, the average correlation between replicate early lines within each dose group is only $-0{\cdot}09$. It is therefore most unlikely that mutations both advance flowering and reduce fertility. The average correlation between replicate late lines within dose groups is however $-0{\cdot}35$. This is not significant, but high correlations may not occur even when there is a complete identity between the sets of mutations affecting flowering and fertility. Different mutations can be expected to show different relative effects on these two characters. Pleiotropy cannot therefore be ruled out in the late flowering lines. Such pleiotropy would explain why the late selection lines have lower average fertility than early lines. It may also be significant that viability is lower in late than early flowering plants. Mutations which impair vigour and viability could well reduce growth rate, hence delaying flowering, and also reduce fertility. Brock (1967) has evidence for a correlation between flowering time and growth rate in *Arabidopsis* plants selected from irradiated families. Extensive pleiotropy has also been found in rice (Sakai and Suzuki, 1964), wheat (Scossiroli *et al.*, 1966), but not oats (Khadr and Frey, 1965). In the latter case correlations may have been revealed if the irradiated populations had been subdivided.

The relative frequency of " plus " and " minus " mutations. Information concerning the relative frequency of mutations increasing (" plus ") and decreasing (" minus ") the manifestation of a character is usually obtained by comparing results from X_2 and control families, by similar comparisons in later generations, or from asymmetry of response to selection (*e.g.* Bateman, 1959; Scossiroli, 1965; Brock, 1965). Different generations give different kinds of information since genetic variance in X_2 contains both non-additive and additive components whereas in later generations the non-additive component will be greatly diminished. It is generally assumed that the average effect, either additive or non-additive, of " plus " and " minus " mutations is of the same size but this may be incorrect. In the present experiments replicate selection lines gave similar responses in the early flowering direction, but were very heterogeneous in the late flowering direction. This suggests that mutations delaying flowering are relatively less frequent but produce a larger average effect. It is also significant that more major gene mutations were found among the late than early lines. It is therefore safer to refer to relative amounts of variation in the " plus " and " minus " direction rather than to mutation frequencies.

The similarity in dose-response relations in early and late flowering lines, assessed in G_7 and G_8, indicates that equal amounts of additive variation were induced in each direction at any one dose level. This refers, of course, only to polygenic mutations which were recoverable, that is to those which

did not decrease viability or fertility to such an extent that they were eliminated in earlier generations of the experiment. Although at first sight the X_2 data (table 8) suggests a preponderance of mutations in the " plus " direction, the difference in average flowering time between X_2 and control families arises solely from increased skewness in the X_2 distribution; the modal flowering date is the same in each kind of family. The marked deviation from normality in the distribution of flowering time in X_2 and control families is typical of this character and line, and is also common in other species (Scossiroli *et al.*, 1966). Part of the increased skewness in X_2 families is due to major gene mutations which are more common in late than in early lines. Since lines showing these mutations were removed before the G_7 or G_8 trials were grown, they did not influence the estimates of selection response. The increased skewness is also due to greater genotype × environment interactions among late flowering plants. This can be seen

TABLE 8

Distribution of flowering time in the control, X_2 and irradiated X_2 families of the first experiment. g_1 *which estimates skewness and* g_2 *which estimates kurtosis are as defined by Fisher* (1958)

Family	Average days sowing to flowering	Average variance	Average g_1	Average g_2
Control	40·57	2·167	$+1·579 \pm 0·240$	$+4·661 \pm 0·476$
X_2 (90 kr.)	41·19	9·049	$+1·838 \pm 0·250$	$+5·522 \pm 0·495$

from table 3 and is also shown by the lower X_2 parent/X_3 offspring correlation in late compared with early lines. Chromosome aberrations which impair growth, hence delay flowering, and which are elimintaed by natural selection may also lower the heritability. Finally, X_1 plants probably provide a poorer maternal environment for seed formation than control plants, leading to delayed flowering in their X_2 progeny (cf. Scossiroli *et al.*, 1966). In general, reliable genetical information cannot be derived from X_2 data, and it is safer to conclude from the present results that approximately equal amounts of additive variation were induced in the " plus " and " minus " directions.

The relation between the properties of induced mutations and the control genotype. This relation could be of two main types. First, the properties, either additive or non-additive, of each induced mutation could be related directly and in some simple manner to the properties of the alleles before the mutational event took place. Assuming that most loci have similar mutation rates, it follows that a simple relationship should be found between the average phenotype of plants derived from irradiated families and that of the control. A model of this kind has been proposed by Brock (1965). Referring presumably to additive effects, he envisages that " plus " alleles mutate to a " minus " condition and vice versa. Lines with extreme phenotypes, containing a high proportion of either " plus " or " minus " alleles, will therefore give rise after irradiation to families with more average phenotypes. Second, the properties of the induced mutations could, as suggested previously (Lawrence, 1965), depend on the genetic architecture (Mather, 1960) of the particular character studied; that is, their properties would be determined by interactions between the mutations and other genes, including those responsible for modifying the properties of the " wild type " alleles. Accord-

ing to this scheme, the relationship between the average phenotypes of mutant and control families would vary little for any one character, though different characters would exhibit different relationships. Since most mutations decrease fitness, the distribution of mutant phenotypes about the control mean at any given level of induced variance would show greater asymmetry when the character in question was an important component of fitness and when it has been exposed over long periods to strong directional rather than stabilising selection. Thus mutant families show great changes relative to the control with regard to yield, fertility and viability characters, which are almost certainly exposed to directional selection (see Mukai (1964) and Brock (1965) for references). Flowering time, on the other hand, has probably been exposed to stabilising selection since it is reasonable to assume that both very early and very late flowering individuals are less fit than those with intermediate phenotypes. This does not preclude the possibility that selection is more intense in one direction. In keeping with this, the mean flowering time of mutant plants is generally close to that of the control (Brock, 1965, and present results). Small differences between control and mutant families probably arise from pleiotropic effects of mutations, which may influence other characters which are more important components of fitness. Mutations which delay flowering, for instance, may do so by means of a reduction in growth rate (*cf.* Brock, 1965).

In principle it should not be difficult to distinguish between these two types of hypothesis. This could be achieved by examining the properties of mutations induced in at least two genotypes well contrasted with regard to a character which is exposed to stabilising selection. Satisfactory data of this kind does not, however, exist. The present data, obtained using a single line of average performance, is compatible with either hypothesis. To support his model, Brock (1965) has examined published results obtained from different species and characters. Apart from the objections that no distinction is drawn between additive and non-additive effects and that comparisons involving different characters can be interpreted in several ways, a number of the examples cited are unsatisfactory owing to the presence of genetic heterogeneity in control families. Such data were generally obtained from crop plants in which it is extremely difficult to ensure homozygosity and homogeneity in control families. If the data are re-examined, and also the results cited by Mukai (1964), they provide better support for the second rather than the first hypothesis. The matter cannot be regarded as settled, however.

Although the great majority of mutations decrease fitness, it is unlikely that all do so. The hypotheses outlined above are concerned with all mutations affecting quantitative characters, no distinction being made between macro- and micromutations. Gregory (1965) has emphasised, however, the importance of magnitude of mutational change in this problem, and has drawn attention to a model proposed by Fisher (1930). This model relates magnitude of mutational change to probability of increase in fitness, the probability being nearly zero for large changes but approaching 0·5 when the changes become vanishingly small. It follows that the distribution of mutant phenotypes about the control mean becomes more symmetrical when large mutational changes are excluded from the distribution and completely symmetrical when all but mutations with vanishingly small effect are rejected. Gregory's data (1965) concerning yield of fruit from the peanut

Arachis hypogea support this model and a reduction in asymmetry is commonly found after screening irradiated material for macromutations (*e.g.* Scossiroli, 1965; Brock, 1965). From the practical point of view the model is valuable in suggesting that micromutations have relatively greater merits than macromutations in plant breeding programmes. In view of uncertainty concerning the random nature of mutations and their associated phenotypes it is less clear whether this essentially static and non-genetic model will prove quantitatively adequate.

The importance of the breeding system in determing the genetic structure of populations has long been recognised (Mather, 1943) and it is reasonable to inquire whether the properties of induced mutations are different in inbreeders and outbreeders. Much of the previous evidence concerning the properties of mutations has been obtained from experiments with *Drosophila* (see Introduction for references), an outbreeding species. *Arabidopsis* is commonly regarded as a complete inbreeder, though recent evidence questions this view (Deneen, 1966). In outbreeding species the deleterious effects of most mutations can be minimised by the action of dominance, which in Fisher's (1930) view has evolved for this purpose. Such a strategy is of course much less effective in predominantly inbreeding species, but Gregory (1961) has argued that in these organisms non-allelic interactions may play a role analogous to that of dominance in outbreeders. Deleterious mutations may therefore be " absorbed " rather than eliminated. There is, however, insufficient evidence at present to examine this idea critically.

5. Summary

1. Selection for early and late flowering was practised in families derived by selfing single irradiated plants from a homozygous line of *Arabidopsis thaliana* (Heyn.). Selection was also carried out in the control.

2. In the first experiment, seeds from selected plants in one group of lines were re-irradiated with the same dose (90 kr.) of ^{60}Co gamma rays in the second, third, fourth and fifth generations; those in a second group of lines were re-irradiated in the third and fifth generations only, while those in the last group of lines were not re-irradiated. In the second experiment single doses of either 30, 60, 90 or 120 kr. were given to seeds in the first generation only.

3. Selection response in the first, and probably also the second, experiment was symmetrical and linearly related to dose, and the two sets of data gave similar estimates of the response per unit dose. The combined data gave estimates of the doubling dose lying between 12 and 28 kr., the lower value being the most likely.

4. The average number of seeds per pod in plants derived from irradiated families was almost invariably lower than that in the control. In the early flowering lines the reduction in fertility is due to mutations independent of those influencing flowering, but pleiotropy cannot be ruled out in the late flowering lines.

5. The results are compatible with the hypothesis that the properties of induced mutations depend on the genetic architecture of the character studied.

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