SUPERVITAL MUTANTS OF ARABIDOPSIS[1]

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THE occurrence of adaptively superior hereditary changes is a basic postulate of the microevolutionary theory. Evidence for new "supervitality" mutations is, however, rather scarce, and the superiority is generally slight and often dubious. Evolutionary changes, furthermore, have only rarely been witnessed.

Phage-resistant or antibiotic-resistant bacterial mutants have, however, definite selective advantage in a given environment. In higher organisms the melanotic forms of certain Lepidoptera provide examples of evolutionary change in natural populations. During the hundred years since the beginning of the industrial revolution, some melanotic mutants have increased from a rare aberrant to 95–98 percent of the populations in certain areas (KETTLEWELL 1955).

The possibility of inducing supervital mutants in higher organisms through various mutagens has also been claimed.

TIMOFÉEFF-RESSOVSKY (1939) in the 1930's studied "vitality mutations" in Drosophila which were classified on the basis of higher sex-ratio or better hatching rate. The former character can hardly be considered, however, as an adaptive trait, and the differences in hatching rate were small.

When BRÜCHER (1943) grew several induced Antirrhinum mutants in climate chambers, some genotypes poorly viable under natural condition proved to be superior to the wild type in certain quantitative characters. Even if we disregard the small number of plants per treatment, the experimental design could not give information about actual fitness.

GUSTAFSSON (1951) investigating the problem of induced mutations with eventual evolutionary significance, came to the conclusion that "one or two per thousand reach the viability level of the mother strain" and some "increased the possibilities of the population". The experiments carried out with induced mutants were not designed to test the survival value but rather the agronomic productivity, one possible component of supervitality.

STUBBE (1950, 1959) analyzed several induced mutants of snapdragon and various crop plants which exhibited higher fertility under certain conditions. These experiments provided valuable information about the viability of induced mutants. Evolutionary fitness is, however, a more complex phenomenon as both GUSTAFSSON and STUBBE pointed out.

DOBZHANSKY and SPASSKY (1947) irradiated *Drosophila pseudoobscura* strains

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carrying recessive genes or gene complexes deleterious to the flies. Homozygous and balanced heterozygous stocks were treated with 1,000r of X rays per generation. The homozygous lines were maintained in crowded populations, while the stocks with balanced chromosomes were kept under favorable conditions. Under the pressure of selection the irradiated population containing deleterious mutants improved.

ERNA REINHOLZ (1945) reported numerous X-ray-induced hereditary changes in *Arabidopsis thaliana* which included several vigorous types (kräftige Typen) and she remarks that besides the mainly deleterious types some must be of positive selective value. Unfortunately, no selection experiments were carried out, and the genetic analysis remained incomplete.

MATERIALS AND METHODS

Arabidopsis thaliana (L.) Heynh., an autogamous crucifer offers several useful features as an experimental tool for genetic studies. The complete life cycle of the early ecotypes is about five-six weeks under suitable conditions. The plants are very small and still produce large quantities of seed. It is easy to culture them in a well-controlled environment: in aseptic test tubes on artificial media and under artificial light. The chromosome number is low ($n = 5$). A great variety of useful genetic markers can be easily induced by X rays.

After genetic tests the homozygous progeny of a single individual of the early ecotype "Landsberg" was chosen for studies. In addition, some other ecotypes were used for special experiments: "Hauniensis" (obtained from the Hortus Botanicus Hauniensis, Copenhagen, Denmark); "Bergiana" (obtained from the Hortus Botanicus Bergianus, Stockholm, Sweden); "Coimbra" (obtained from the Botanical Garden of Coimbra, Portugal). All experiments, unless otherwise stated, were carried out under continuous light in a greenhouse with night-time illumination being provided by 200-watt incandescent bulbs (Long day). Short-day treatments were ones in which no supplementary illumination was given.

Soil and pots were steam-sterilized to prevent seed contamination. For aseptic cultures five ml medium per 150 × 16 mm test tubes were used. The basal medium (mg per liter bidistilled water: NH_4NO_3 200; $MgSO_4 \cdot 7\ H_2O$ 100; $CaH_4(PO_4)_2 \cdot H_2O$ 100; KH_2PO_4 100; K_2HPO_4 50; $FeC_6H_5O_7 \cdot 3H_2O$ 2.5) was solidified with 1.2% agar.

Floral induction was estimated by the appearance of flower buds visible with the aid of a watchmaker's magnifier.

Seeds presoaked 24 hours were given 8,000–12,000r of X rays at approximately 160 kv, 5 mA, 207r/min.

RESULTS

Description of the mutants: The seed of Arabidopsis is highly resistant to ionizing radiation. The germination of the seed was sometimes slightly delayed but the percentage of germination was almost the same as that of the untreated material under the conditions of radiation outlined. The survival of the treated

material was also very close to the untreated. In 1474 X_2 progenies four obviously highly vigorous new types were found in addition to a large number of different subvital and lethal types. It appears that the mutability at the loci controlling photoperiodic response is quite high in this material, but it still may be lower than that reported by REINHOLZ (1945). Calculation of the mutation rate after seed treatment is rather inaccurate because the X-radiated apical meristem becomes a genetic chimera, and the number of cells having the potential of giving rise to the sporogenous tissues may only be inferred from the segregation ratio of the F_2.

The vigorous growth type is associated with a change in the flowering response. Planted in pots and under long-day treatment, the ecotype Landsberg develops visible flower primordia in 10–12 days. Some of the late mutants require a much longer period to reach flowering (Table 1). All the four mutant types can be distinguished easily from the wild type and from each other, by growing them in soil or in agar medium under various photoperiodic treatment (see Tables 1 and 2). The addition of sucrose or glucose promotes flower induction, especially under short-day treatment, but the different types have clearly different responses. Fructose, maltose, rhamnose, ribose, glycerol and malic acid are ineffective or toxic. Indole-3-acetic acid does not affect the flowering response, and

TABLE 1

Photoperiodic response of wild type and mutants cultured in winter on artificial medium in test tubes

	Number of days required from germination to visible flower primordia			
	Long day		Short day	
	No sugar	Sugar added	No sugar	Sugar added
	M ± S.E.	M ± S.E.	M ± S.E.	M ± S.E.
wild	13.2 ± 0.39	11.1 ± 0.48	49.3 ± 0.35	36.3 ± 1.47
ld	17.5 ± 0.11	17.0 ± 0.00	94.0 ± 4.00	. . . . .
co	17.1 ± 0.02	15.7 ± 0.03	40.0 ± 1.33	27.3 ± 0.61
gi^1	19.9 ± 0.36	19.4 ± 0.07	59.1 ± 3.08	41.4 ± 1.18
gi^2	38.9 ± 1.12	32.2 ± 0.88	71.4 ± 2.62	45.9 ± 1.65

TABLE 2

Seasonal variation of the photoperiodic response of wild type and mutants (Greenhouse, pots, long-day treatment)

	Number of days required from germination to visible flower primordia	
	December 3	April 7
	M ± S.E.	M ± S.E.
wild	11.1 ± 0.05	10.7 ± 0.19
ld	16.6 ± 0.50	19.9 ± 0.13
co	26.4 ± 0.54	19.3 ± 0.39
gi^1	24.8 ± 0.43	19.8 ± 0.53
gi^2	61.6 ± 0.88	37.1 ± 0.51

gibberellic acid promotes flower induction but is not specific for any genotype. Yeast extract, yeast hydrolysate, casein hydrolysate, coconut milk, extracts of wild-type Arabidopsis do not have any specific effect.

Each of the four mutants has a different photoperiodic response. Under long-day conditions *ld* (luminidependens) is not much later than the wild type, but under short-day treatment it needs the longest time for floral induction (Table 1). Mutant *co* (constans) is definitely later than the wild type in long days, but clearly earlier in short days. It may be noted, furthermore, that *co* is earlier than the wild type when the daily illumination is reduced to nine hours. Under long-day treatment *co* is recessive (see Table 6); under short days (nine hour intense illumination, sucrose-agar medium) the number of days (M ± SE) required for the appearance of visible flower primordia was the following for the three genotypes: wild 27.4 ± 0.72; *co* 20.0 ± 0.54; wild × *co* 21.0 ± 0.52. This indicates dominance reversal. Both gi^1 and gi^2 (gigantea), though much later than the wild, are affected similarly to the wild type by environmental factors. All vary in the time required for the appearance of visible flower primordia not only according to the factors demonstrated by Table 1, but also with the season. This seasonal change is very appreciable in the plants carrying the late gi^2 factor (Table 2), and is closely correlated to the natural day-length and light intensity. Cloudy days after germination retard all types somewhat.

Differences between mutants are most obvious in the first developmental stage, which ends in floral induction. Once flower primordia appear, the subsequent developmental phase, i.e., from induction to anthesis, and from anthesis to maturity show relatively little variation. For example, gi^2 in spring plantings requires a period approximately four times as long as the wild for flower initiation yet it requires about the same time as the wild from flower initiation to maturity. The delay in flower initiation results in mutant plants much larger than the wild type under long-day conditions. Under short-day conditions the differences are much smaller, but the overall growth is still positively correlated with the time of development (Figure 6). Data of Table 3 exemplify some quantitative characters of the wild type and mutants at different ages. At identical age, mutants and wild type are very similar in size. Any prolongation of the vegetative development is accompanied by an increase in size (Figure 1). Under the con-

TABLE 3

Some quantitative characters of wild type and mutants (First week of development under short day-length of winter, later constant illumination. Harvest at the earliest maturity)

Genotype	Days until visible floral induction	Diameter of the rosette (cm)	Number of rosette leaves	Plant height (cm)	Number of buds and flowers	Number of fruits	Fresh weight (mg)	Dry weight (mg)
wild	17.1 ± 0.14	3.3	6.9	16.7	10.0	25.4	210.0	35.6
ld	20.6 ± 0.50	4.9	8.4	18.4	9.4	28.1	262.0	51.2
co	34.4 ± 0.54	4.4	10.1	35.5	20.9	52.1	826.0	151.5
gi^1	30.8 ± 0.43	8.5	17.9	38.1	47.0	142.5	2290.0	324.5
gi^2	67.6 ± 0.88	11.7	34.9	42.9	101.0	250.0	3310.0	868.0

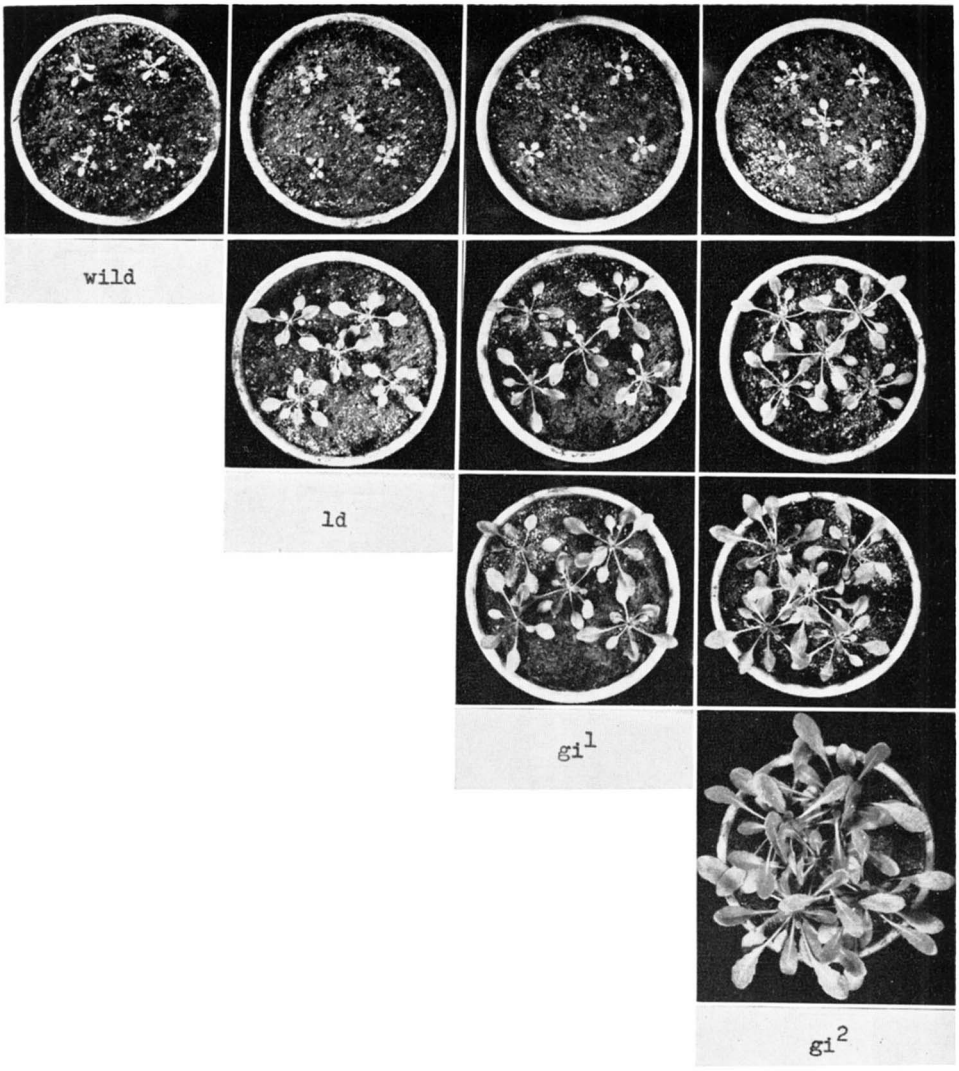

FIGURE 1.—Rosette development of wild type and three late mutants. Horizontal lines show the different types at the onset of flower development of the left most (age is identical). Vertical rows illustrate the rosette growth of the individual mutants. Lowest diagonal line compares the four types in identical developmental stage but at a different age.

ditions of the experiment summarized in Table 3, *gi*[2] needed 2.5–3 times as long a period as the wild for the completion of its life cycle, but it produced almost 25 times as much dry material and roughly ten times as much seed. Figure 2 shows the wild type and the *gi*[2] mutant at the beginning of fruit setting; the developmental stage is identical but the mutant is about twice as old.

Genetics of the mutants: Genetic studies demonstrated that all four late mutants are recessive (Figure 3; Table 6) and only a single factor is involved in each

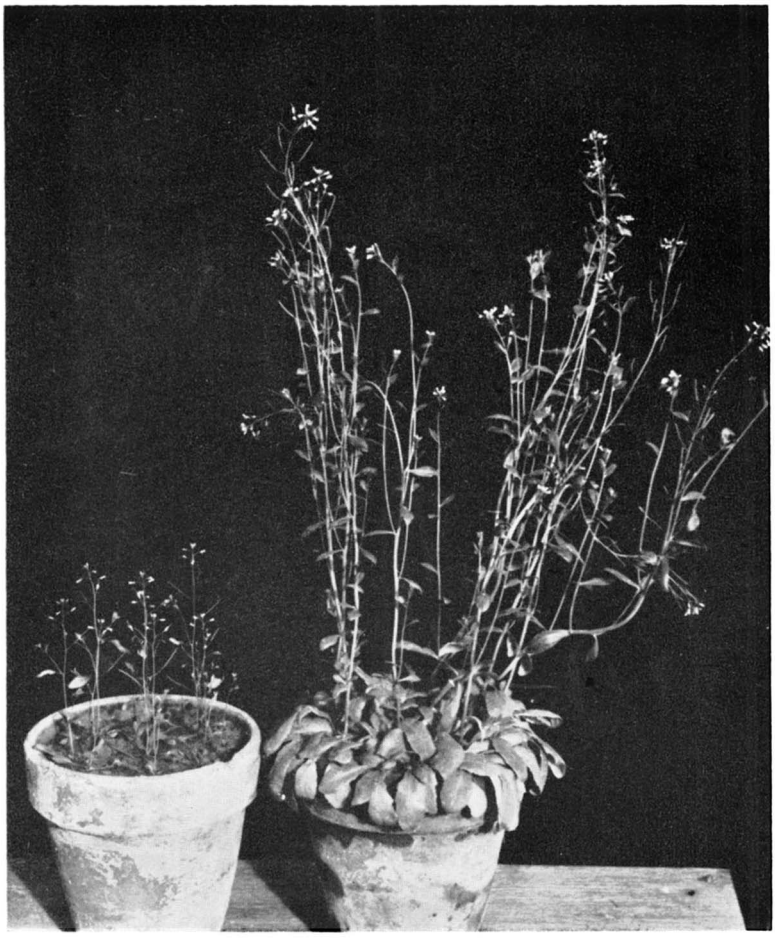

FIGURE 2.—Wild type (left) and mutant *gi*^{2} (right) at identical stage but different age.

case. Although the mutations affect the entire plant in a quantitative way the segregation is clearly qualitative (Figures 3 and 4). Under carefully controlled illumination even factor *ld*, and also *co* which differ the least from the wild under long-day conditions, can be readily recognized (Table 1).

Genes *gi*^{1} and *gi*^{2} proved to be allelic with dominance of the earlier allele, *gi*^{1}. In an F_2 population of 447 individuals no earlier phenotypes than *gi*^{1} could be found. A testcross demonstrated a 1:1 ratio. Mutants *ld* and *co* occupy different loci. A preliminary study indicates that the *gi* locus belongs to the fourth linkage group about two units distal to the pigmentation gene *xv* (xanthaviridis). Since no markers are available distal to *gi*, it is assumed to occupy a fairly terminal position. Factor *co* belongs to the fifth linkage group about 27 units from *ti* (tigrina) and about half that distance from *lu* (lutescens), both involved in the control of leaf pigmentation. Attempts to locate *ld* have not been successful so far.

The transmission of all four factors is perfect. In both homozygotes and hetero-

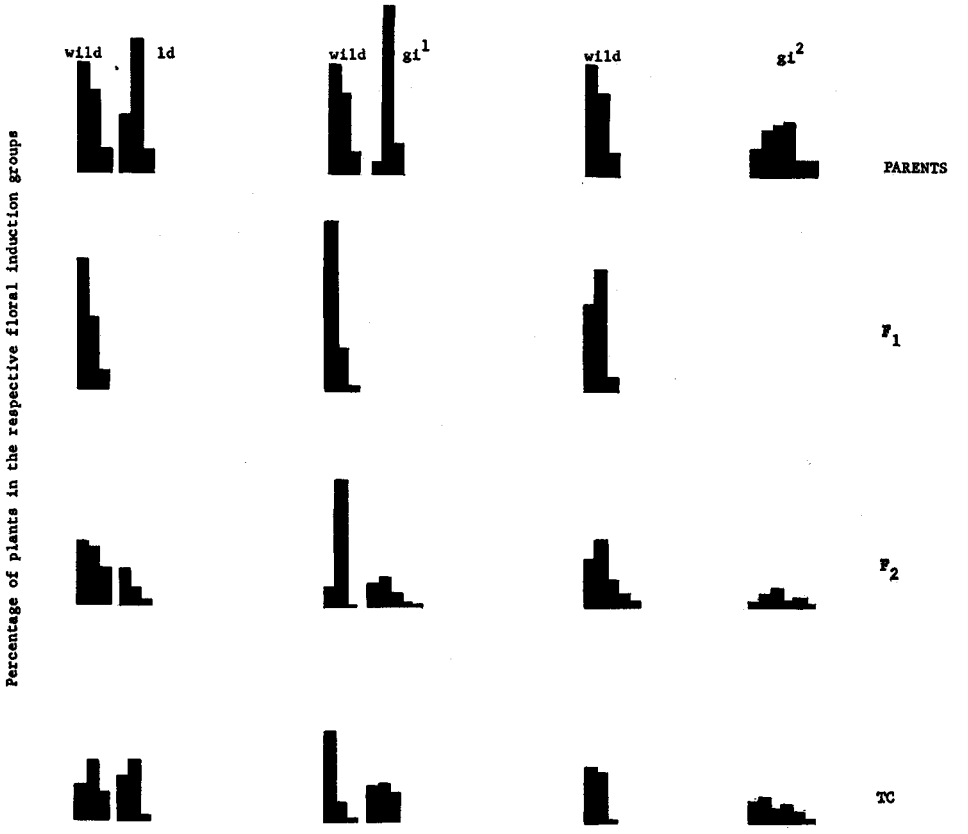

FIGURE 3.—Segregation for photoperiodic response. Upper row wild type and mutant parents, second row F_1, third F_2, fourth testcross.

zygotes male and female fertility is normal, or even improved. Linkage studies with the *gi* locus do not indicate any irregularity of the genetic recombination. This chromosome is relatively well-marked and crossing-over values are additive and essentially the same in both repulsion and coupling with different marker combinations.

Cytological studies by DR. LOTTI M. STEINITZ-SEARS did not reveal any chromosomal irregularity in the mutants (L. M. STEINITZ-SEARS, in preparation).

Population studies: The increased vigor and the excellent reproductive ability of the mutants suggest that they may have an advantage in certain environments. The high reproductive value must be associated with superior competitive ability in order to leave more offspring and have a selective advantage. Sometimes highly fertile genotypes seem to be inferior in a population while others of lower reproductive ability get the upper hand in competition (Montgomery effect, see GUSTAFSSON 1951).

In order to test the real survival value of the mutants, population studies were started in the greenhouse during the summer of 1958. Five F_1 seeds of each of

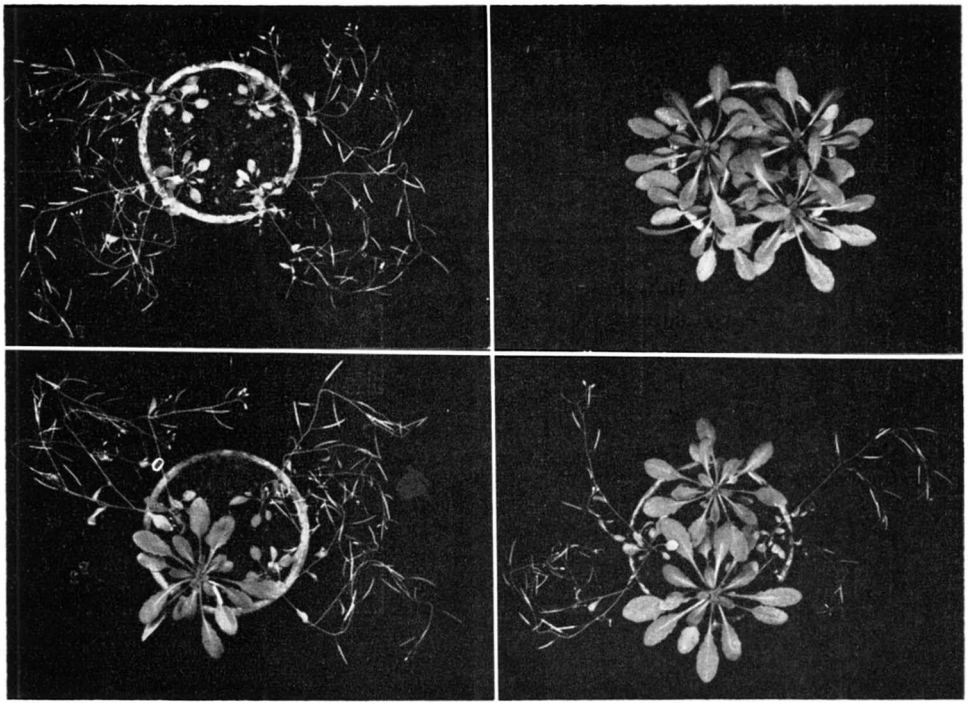

FIGURE 4.—Monogenic segregation of *gi*^{2} × wild cross. Upper line parents, left bottom F_2, right bottom testcross.

three different mutant × wild crosses were planted in ten-inch pots with four replications. Each "population" started thus with an equal initial gene frequency. The cultures were given long-day treatment. The pots were watered according to need. During the summer, although the greenhouse roof was painted with whitewash, the temperature rose to 40–45°C during the day for several hours. During the winter when the heating was thermostatically controlled the temperature rose above 20°C only on sunny days. Insects were regularly controlled by spraying and fumigation. No artificial selection was practiced, the plants being left alone to reproduce themselves according to their abilities. From the second generation on, the cultures were crowded. Only *gi*^{2} × wild populations could be classified by direct observation. By the time the wild phenotypes were in approximately the fifth generation, the advantage of the mutant was apparent, and the recessive mutant outnumbered the wild type by a factor of 7.8. After 60 weeks the wild and *gi*^{2} phenotypes were classified in the four pots. During this time the wild type presumably completed about ten generations. The crowded condition and the smaller differences in size did not permit a direct classification in the other two populations. Seed samples were therefore taken for spaced planting out of three pots each. The sampling was repeated twice at two-week intervals to avoid the possibility of sampling error due to the different cyclic maturity of the different genotypes. Any error in sampling probably favored the

FIGURE 5.—The selection experiment in F_{10}. The few smallest plants in the left pot may be the wild type.

wild type. Figure 5 shows *ld* × wild and gi^2 × wild populations at about the time of F_{10}.

The seed samples from each pot were mixed and planted and the resulting plants were classified (Table 4). By the tenth generation the heterozygotes had presumably decreased, assuming no heterozygote advantage to $(1/2)^{10}$, a negligible frequency. Thus, the phenotypic classes may be taken to represent also the genotypes. During this relatively short time, in a crowded, closed population, the mutant alleles exhibited a very significant selective advantage. In the two populations in which mutants at the *gi* locus were involved, the wild allele was practically eliminated. Mutant *ld* also performed well; from the initial frequency of 0.50 it increased to a level of 0.87. Due to the special physiological differences between wild type and mutants the amount of selective advantage cannot be calculated as usual. In the case of *ld* and gi^1 which take about 10–25 percent longer to mature the differences in the life cycle as such were not considered in the estimate of the selective value. A direct calculation indicated that with 0.3

TABLE 4

Effect of ten generations of natural selection on populations with an initial equal gene ratio

Population	Number of phenotypes		Allelic frequency		Estimated selective value of the mutant
	Wild	Mutant	Wild	Mutant	
ld × wild	68	452	13.08	86.92	1.3
gi^1 × wild	3	614	0.49	99.51	2.0
gi^2 × wild	2	661	0.30	99.70	>2.0

or 1.0 selective advantage, respectively, in a small, limited population the gene frequency may be subject to shifts of the extent actually observed (Table 4). Similar direct calculations were used to estimate the selective value of the *gi*^{2} populations, but with the additional assumption that while the wild reproduces twice, *gi*^{2} completes only one life cycle. Since the populations are limited in area, only that space is available to the intermediate wild phenotype generation which is not occupied by the mutant genotype. Competition for space is restricted to every second wild generation, when both types mature simultaneously. Such a calculation is not quite justified because the difference of time required for the completion of the life cycle is subject to considerable variation (Tables 1 and 2). In our roughly estimated selection coefficient the differences in maturity, reproductive and competitive abilities are all expressed in a common approximate figure.

Random change versus *selective advantage:* From WRIGHT's classical studies (1921, etc.) on the evolutionary significance of random genetic drift it is known that in small populations chance alone may change the gene frequency even against selective pressure. In a self-fertilizing community random events have a greater chance than in a freely interbreeding population. The effective size of an autogamous population is only half the actual number of individuals. Periodic fluctuation of the number of plants also affects the effective size and it tends toward the lowest number by following the harmonic mean of the consecutive population sizes. The actual number of plants per experimental series varied between 500–1,000. If we take 250 as the effective size, some correction for random individual differences in reproduction is also included. The variance of the gene frequency may be readily calculated by using the available formula (LI 1955) and the probability of random sampling of the gametes may be easily calculated. According to such a calculation there is only a very small chance that random events alone would bring about a shift in the gene frequency of the extent actually observed.

If random factors had been a major role in shaping the composition of the populations, considerable differences among the subdivisions (replications) of the genotypically different populations would be expected. A homogeneity test indicates that the subdivisions are generally close to each other thus random drift does not seem to have taken place (Table 5). For *ld* and *gi*^{1} populations only three parallels were compared. (The fourth was omitted because in each case the pots were heavily infested with algae.) The average of the replications was considered as standard for the chi-square technique. The numbers representing the two phenotypes are the total actual numbers in the population pots in the case of *gi*^{2}. In the cases of *ld* and *gi*^{1} they were only a sample drawn as described above, and the actual total number of plants in F_{10} was higher.

Analogy between wild late races and late mutants: HÄRER (1950) and NAPP-ZINN (1957) studied the inheritance of flowering response in various Arabidopsis ecotypes and reported incomplete dominance of the late types. Similar observations were reported by LAIBACH (1943), who in one cross, however, observed almost complete dominance of the early race in the F_1 but an apparently multi-

TABLE 5

Test of homogeneity of the subdivisions of the three F_{10} populations. The average is chosen as standard arbitrarily

Populations		Phenotypes wild	*ld*	Total	χ^2	Probability
A-1	obs.:	21.0	159.0	180	0.316	>0.50
	exp.:	23.5	156.5	180		
A-2	obs.:	18.0	129.0	147	0.081	<0.80
	exp.:	19.2	127.8	147		
A-3	obs.:	29.0	164.0	193	0.656	<0.50
	exp.:	25.2	167.8	193		
		wild	*gi*[1]			
B-1	obs.:	1.0	161.0	162	0.050	>0.80
	exp.:	0.8	160.2	162		
B-2	obs.:	1.0	225.0	226	0.010	>0.90
	exp.:	1.1	224.9	226		
B-3	obs.:	1.0	228.0	229	0.010	>0.90
	exp.:	1.1	227.9	229		
		wild	*gi*[2]			
C-1	obs.:	2.0	183.0	185	1.841	<0.20
	exp.:	0.6	182.4	185		
C-2	obs.:	0.0	194.0	194	0.602	<0.50
	exp.:	0.6	193.4	194		
C-3	obs.:	0.0	183.0	183	0.602	<0.50
	exp.:	0.6	182.4	183		
C-4	obs.:	0.0	101.0	101	0.031	>0.80
	exp.:	0.3	100.7	101		

TABLE 6

Number of days required for the appearance of visible flower primordia in the F_1 of the induced late mutants and some late races of natural origin

Genotype	Days	Genotype	Days
	M ± S.E.		M ± S.E.
wild	9.6 ± 0.13	Hauniensis	32.3 ± 0.14
ld × wild F_1	10.4 ± 0.35*	wild × Hauniensis F_1	19.0 ± 0.01
co × wild F_1	9.8 ± 0.15	*gi*[2] × Hauniensis F_1	31.2 ± 0.66
gi[1] × wild F_1	9.5 ± 0.27	Coimbra	19.4 ± 0.50
gi[2] × wild F_1	9.8 ± 0.18	wild × Coimbra F_1	13.9 ± 0.19
ld × *co* F_1	11.0 ± 0.18*	*gi*[2] × Coimbra F_1	15.6 ± 0.21
ld × *gi*[2] F_1	11.7 ± 0.33*	Bergiana	27.5 ± 0.23
co × *gi*[2] F_1	12.6 ± 0.36	wild × Bergiana F_1	19.8 ± 0.31
gi[1] × *gi*[2] F_1	21.9 ± 0.41	*gi*[2] × Bergiana F_1	26.2 ± 0.29

* Slight delay in germination.

genic segregation in the F_2. All three authors claim that one major and a few minor factors control the flowering response in Arabidopsis.

Since the phenotype of our late mutants is very similar to the various late ecotypes and two (out of four) of our mutants are allelic, tests for allelism were

performed with five different late ecotypes. Data from three such crosses are shown in Table 6. Similar results were obtained with two other additional crosses. The late wild races are semidominant over our standard wild type and almost completely dominant over our *gi*^{2} mutant. Differences were also observed in the F_1 of other late mutant × late wild crosses, only the differences were smaller. In both standard wild × late wild and late mutant × late wild F_2 progenies, early-flowering plants segregated which seemed identical with our standard wild type. None of our late mutants is, therefore, allelic to any of the major factors controlling flowering in the following ecotypes: Hauniensis, Bergiana, Coimbra, $Graz_1$, $Graz_3$. (It may be noted that most of these wild ecotypes are mixtures containing several more or less different genotypes.) All these wild races, however, carry modifier genes which are active on our late mutants. Figure 7 shows the clear difference between the F_1's of standard wild × Hauniensis and *gi*^{2} × Hauniensis, respectively.

DISCUSSION

In the greenhouse under favorable photoperiodic treatment our late mutants greatly surpass the wild type in reproductive ability. Occasionally gigas forms

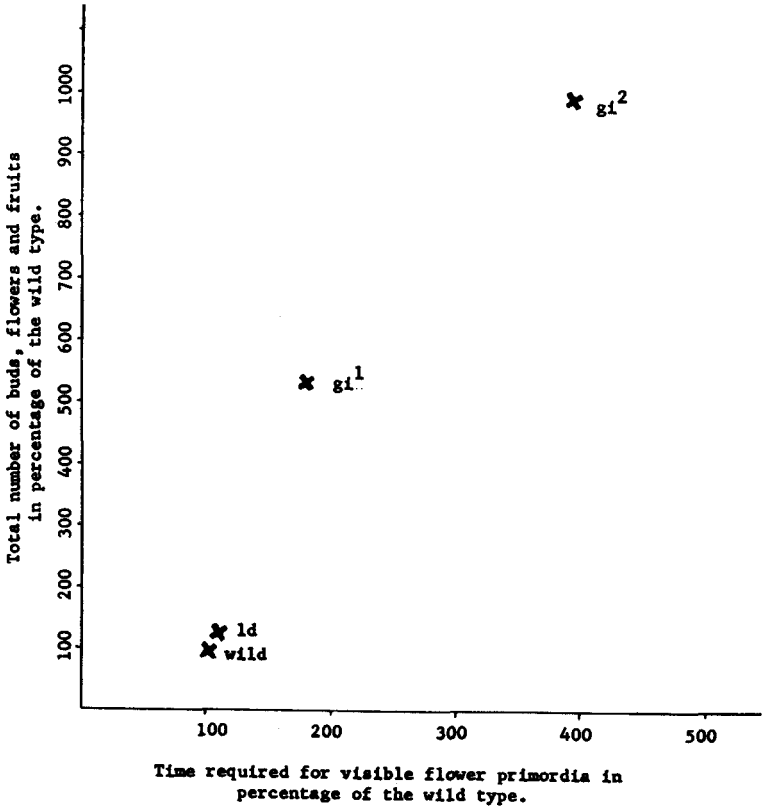

FIGURE 6.—Correlation diagram of flowering response and reproductive ability.

FIGURE 7.—The effect of modifiers of the Hauniensis ecotype on the gi^2 mutant. Standard wild × Hauniensis F_1 (left) and gi^2 mutant × Hauniensis F_1 (right).

arise due to X-ray treatments in other species, *e.g.* Pisum, Vicia (GELIN 1954), Hordeum (GUSTAFSSON 1954), Soya and Lycopersicum (STUBBE 1959). Quantitative characters may also be improved in Drosophila (SCOSSIROLI 1953; CLAYTON and ROBERTSON 1955 and others) or in microorganisms (RAMAKRISHNAN, RAINA and GADGIL, 1958). Large changes in all quantitative characters may be produced, however, only when the flowering response is affected in a photoperiodically, very sensitive organism.

This supervitality is based mainly not on adaptation to a wider range of environmental factors but rather on the specific adaptation to longer daily illumination. The mutations actually weaken the ecological homoeostasis of the mutants. Supervitality should result in reproductive success under special conditions, but the latter may not be equivalent to increased fitness (MAYR 1959). Adaptation is an extremely complex phenomenon. An organism highly specialized to a certain environmental peak may find itself at a disadvantage with any change of the milieu and may also be unable to conquer new habitats.

The higher selective advantage of our mutants in the crowded, small populations may be due to the fact that the late genotypes develop larger rosettes which occupy the available space during the time of seed shedding of the wild type and thus frustrate the proper multiplication of the latter. This could take place even if the successful genotype was in the long run less well fitted to a natural environment. If this was the basis of the selective advantage, we should rather call these late mutants "criminal genotypes" (DOBZHANSKY 1955), because their spread over the biotope would jeopardize the future of the species.

All these possible objections against the probable fitness of the mutants seem to be invalidated by the fact that in nature late ecotypes are prevalent which appear physiologically similar to the X-ray mutants. However, until the adaptive value is tested in nature, any estimate of the microevolutionary value of the mutants remains tentative.

FISHER (1958) pointed out that in an infinite population the newly arisen

mutants have a very dim prospect. Mischance and blind accident eliminate approximately one third of the neutral dominants during the first reproduction, and there is no hope at all for survival after numerous generations. If the mutation is slightly advantageous, the outlook is better. For the recessives, even if they are advantageous, the situation is similar to the neutral dominants because selection acts only on homozygotes. A neutral mutant has a probability of 0.63 for survival after one generation. In the case of selective values of 1.05 and 1.10 the chances are 0.65 and 0.67, respectively, only a little higher. Advantageous dominant mutants ultimately must survive.

A systematic survey of the geographical and ecological distribution of genes affecting photoperiodic response in the species has not been made. In a paper by LAIBACH (1951) the data seem to indicate, however, that out of 70 Arabidopsis races (ecotypes) 68 are later than Landsberg (our standard wild type) and only one Li_5 (Limburg) is earlier. This sample of 70 ecotypes may not be truly representative of the total gene pool of the species, but gross misrepresentation does not seem likely.

Mutants *ld*, gi^1 and gi^2 are later than the wild type even under short-day conditions (Table 1). Late maturity and reproductive ability seem to be closely correlated (Figure 6).

Most Arabidopsis ecotypes are spring annuals and under natural day-length, especially in Northern habitats, similar mutants may enjoy a reproductive advantage. It may not be an unreasonable assumption that such genes have a good chance to be spread by natural selection.

If we disregard the complications of the different life cycle and try to express all the differences only with the selection coefficient, we may readily calculate the number of generations needed to bring about certain changes of the gene frequency in a mixture of two noninterbreeding infinite populations by using the formula given by HOGBEN (1946).

According to such a calculation—with self-fertilization—33, 126, 472, 2314 generations are required to change an initial 1/99.999 phenotypic ratio to 99.999/1 in case of 1.0, 0.2, 0.05 and 0.01 selective advantage, respectively. The selection is more efficient in an autogamous population than in a random mating one; in case of 0.01 selective advantage the progress is almost fifteen times faster.

Since phenotypes similar to the mutants are abundant in nature the question can be asked whether these mutants are really the products of the radiation. There is enough evidence to exclude contamination: (a) Our standard stock has been checked for homozygosity before irradiation started, (b) Arabidopsis is strictly autogamous (cross-fertilization from closely neighboring plants is in about the 10^{-4} range), (c) In the vicinity of the laboratory no Arabidopsis have ever been found in nature, (d) During the time the first late mutants were obtained only ecotype Landsberg was in our possession, (e) The late flowering of the wild ecotypes is controlled by several factors and it is semidominant.

Since X-ray mutants are generally more deleterious than spontaneous ones is there is a possibility that these mutants arose spontaneously in the irradiated material? The probability of this is small since Arabidopsis is very stable geneti-

cally, the overall mutation rate being extremely low. So far no spontaneous mutation has been observed in our quite extensive material. On the other hand, many mutants with normal viability have been induced by X rays in our laboratory. REINHOLZ (1945) also reported a large number of vital and vigorous X-ray mutants in the same species.

For the most intriguing problem, e.g., what kind of change occurred in the genetic material, unfortunately only circumstantial evidence is available. These mutations cannot be distinguished from the wild type by such criteria as genetic transmission, visible chromosomal alterations, modified crossover value, etc. It has to be noted, however, that in our material the resolving power of the last two mentioned techniques is not high.

In the light of the few available clues as to the evolution of the photoperiodic response in Arabidopsis, we may suppose that the ancestral type was some kind of a late form similar to the semidominant common late ecotypes. From these through successive mutational steps early flowering forms similar to Landsberg arose. The recessive mutants, enjoying some advantage in certain niches, were maintained. The recessive gene responsible for earliness would be an active one, manufacturing a substance which directly promotes development or renders the carrier more photoreceptive. The early mutation may be based on two different genetic mechanisms: genic interaction or changes in an independent locus. If the ionization inactivates or deletes the gene suppressing lateness, the tardiness may again manifest itself. Various early $\times$ early crosses, however, do not yield late types nor do they exhibit any tendency toward lateness.

Saving the assumption that the early phenotype is the mutant we may conceive that lateness is produced either through the removal or through the mutation of the "early gene" both resulting in a return to the original physiological base line (lateness). If floral induction is controlled by several genes we may expect recessive X-ray mutants differing in lateness depending on deficiencies or inactivations at different loci.

The occurrence of multiple allelism at the *gi* locus could be attributed to false allelism (MCCLINTOCK 1944) or pseudoallelism. There seems to be another difficulty in supposing that the presence of the dominant allele or its absence would result in a similar phenotype. It is not easy to conceive of a dominant allele which would not carry other functions than to mutation to an active recessive allele.

Both of these interpretations would be compatible with the most generally accepted view that X rays do not induce "progressive" changes in the hereditary material and that the majority of the relatively normally transmitted mutations represent submicroscopic deficiencies.

If we may concede that X rays also induce "intragenic changes" (WOODWARD, PARTRIDGE and GILES 1960; GREEN 1961) similar to those that occur in spontaneous mutation, we could consider Landsberg as a multistep early mutant and the recessive late X-ray mutants as reversions to or toward an original late allele deprived from the dominance modifiers.

Though several other interpretations are possible, the following seems to be favored by the information available. The genuine predecessor of the present

days late ecotypes may have been an early one, similar to our standard stock. Late recessive mutants may have occurred spontaneously in the natural habitats where the mutants did not carry a handicap in spite of the later maturity; on the contrary they enjoyed an advantage due to the better reproductive ability. The recessive mutation became semidominant through the acquisition of modifiers. Such modifiers, specific also for the late X-ray mutants, are as a matter of fact present in all the late ecotypes tested (Table 6). These modifiers may be additional late factors and the dominance modification is achieved through some kind of dosage effect. Dominance *versus* recessiveness is not only an attribute of the gene itself but also of its background, genetic and environmental, and thus subject to natural selection. FISHER (1958) actually suggests that the successful new gene should in some way become dominant to its competitors. It is not quite obvious what the advantage is in a self-fertilizing organism to convert the prevalent alleles to dominant, but as a matter of fact the wild type is dominant also in autogamous plants. The different degrees of dominance in the late ecotypes may be an indication of an emerging new wild type. KETTLEWELL has actually demonstrated that the melanism of the moth *Biston betularia* has become "more dominant" since it began to spread over the industrial areas (FORD 1960).

Apart from the microevolutionary potentials of these induced mutants, they may be of some interest to the plant breeder. It may be expected that in photoperiodically sensitive agricultural and horticultural crops, mutants with altered photoperiodic response of practical value may be produced by X rays.

SUMMARY

At three loci four phenotypically different mutations have been induced by X rays which affect the photoperiodic response of the carrier. The standard wild type develops visible flower primordia in 10–12 days after germination. The mutants have various flowering response and may need a period up to 4–6 times as long for the completion of the same developmental stage. In spite of the large differences in the time required for the onset of flowering, the life cycle of the latest mutant is only 2–3 times that of the wild type. The slower development is accompanied by very vigorous growth; dry matter production may increase over 20 times and seed production may rise tenfold. These hereditary changes do not involve impaired genetic transmission, altered crossing-over value or visible chromosomal alterations. In three different competitive populations (mutant × wild) under long-day conditions, in the greenhouse, during a 60-week period (about ten generations of the wild type), the mutant allele increased from the initial 0.500 frequency to 0.869, 0.995, and 0.997, respectively. The roughly estimated selective advantage of the mutants under the experimental conditions varies from 0.3 to over 1.0. These are extremely high values. Since the progress of selection is rapid in a self-fertilizing plant community, if vigorous late mutants occur at a fair rate, their rapid increase may be expected. In nature late ecotypes are prevalent. The possible evolution of the photoperiodic response in the species

is discussed. The suggestion is made that the different degree of dominance of the late ecotypes, based on modifier genes, indicates the evolution of dominance of the character concerned and the late flowering forms in nature represent the emergence of the new wild type of the species.

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