A HYPOTHESIS OF DEVELOPMENTAL SELECTION EXEMPLIFIED BY LETHAL AND SEMI-LETHAL MUTANTS OF *ARABIDOPSIS*

By J. LANGRIDGE*

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Summary

A hypothesis of developmental selection is presented in which the internal factors of selection, barriers imposed by ontogenetic development, are considered in relation to the degree of survival of newly arisen deleterious mutations. This hypothesis states that the survival of such mutant genes to the stage of germination depends on the diffusibility of the metabolite required by the mutants and the ontogenetic stage at which it is required. As a corollary of the hypothesis, those lethal mutants of flowering plants that appear after germination should, with certain predictable exceptions, have requirements for low molecular weight substances which may be supplied from external sources.

As an experimental test of the hypothesis, the requirements for normal growth of 11 lethal or semi-lethal mutants of *Arabidopsis thaliana* (L.) Heynh. have been examined. The six "reparable" mutants require the following: (1) thiamin; (2) choline; (3) coconut milk; (4) sucrose or glucose; (5) a high osmotic pressure; (6) an alternation of temperatures for flowering. Of the five "irreparable" mutants, (1) has decreased embryo growth; (2) lacks cotyledons; (3) lacks chlorophyll; (4) and (5) lack chloroplasts. It is considered that the behaviour of these mutants is generally in accord with the hypothesis.

I. INTRODUCTION

Lethal mutants, although frequently appearing in flowering plants, have been regarded with little interest, and with the exception of chlorophyll-deficient mutants, no systematic examination of their frequency of occurrence or physiology has been made. The reason for this is that precise work on the organic nutrition of intact flowering plants is technically difficult. In contrast with the majority of micro-organisms, higher plants are diploid and thus the isolation of mutants is laborious, their large-scale aseptic culture is seldom practicable, and their average life cycle is generally a matter of months. However, it has recently been shown (Langridge 1955) that biochemically deficient mutants of the *Neurospora* type may be isolated and characterized in the crucifer, *Arabidopsis thaliana* (L.) Heynh. The examination of the first 11 lethals or semi-lethals isolated in this plant has now been completed, and a summary of their behaviour is presented below as an experimental test of a hypothesis of developmental selection.

This hypothesis states that the selection against deleterious mutations during the ontogenetic development of a flowering plant is determined (i) by the time of action of the gene concerned, and (ii) by the diffusibility of its metabolically active product.

*Division of Plant Industry, C.S.I.R.O., Canberra.

It is expected that selection of this nature will be most pronounced in flowering plants. Animals have only limited capacity for synthesis of essential substances in the low molecular weight range, so that there is little possibility of deleterious mutations being maintained by synergism. Such mutations will normally cause the early death of the embryo. In most microorganisms, on the other hand, a relative lack of pre-germinal differentiation excludes rigorous selection against newly arisen mutations. When the mature conidia of a fungus such as *Neurospora* are irradiated and immediately placed on a nutrient medium, the first possibly gene-controlled developmental process to occur is that of germination. No developmental selection is involved, and therefore, only about 7 per cent. of non- or slow-growing mutants have synthesis deficiencies which can be made good with *Neurospora* autolysate (Lein, Mitchell, and Houlahan 1948).

However, when the seeds or pollen grains of angiosperms are irradiated, any induced mutation may be subject to elimination by any one of a series of developmental barriers before it can be expressed as a homozygous sporophytic character. This selection against certain mutations by the sieves of gametophyte, endosperm, and embryo development is important when considering the types of biochemical mutants to be expected in flowering plants.

It will be shown below that X-irradiation of the seeds of *A. thaliana* may cause a mutation in one cell of a usually two-celled apical meristem. This mutation, except when it is a dominant inhibitor of an essential cellular function, will be maintained as a chimera in the X_1 plant both by its wild-type allele in the same cell and by the normal tissue of the remainder of the plant. The mutant allele will thus remain shielded from selection until it becomes separated from its wild-type allele at meiosis. Without considering the details of selection during the critical part of the plant's life cycle between meiosis and germination, one may predict that there will be two categories of mutants with respect to this selection.

(*a*) *Mutations Escaping Pre-germinal Developmental Selection*

There is a class of genes which, although normally active throughout the whole of the plant's life cycle, show a mutant phenotype during only part of this cycle. This device lowers the deleterious effect of a particular mutation. In plants carrying the mutant allele *wx*, for example, only amylopectin is formed in the pollen grain, female gametophyte, and endosperm instead of both amylose and amylopectin (Sprague, Brimhall, and Hixon 1943). However, both types of starch are found in the post-germination sporophyte. Another example is the gene *Y* (yellow endosperm) in maize. The mutant lacks carotenoids in the endosperm (Mangelsdorf and Fraps 1931), yet, since recessive plants grow normally, carotenoids must be present in large amounts in the leaves, where they are probably essential for the maintenance of chlorophyll (Griffith *et al.* 1955). It is an open question as to whether there are separate genes acting sequentially to perform two apparently identical functions, or whether a genetic block in one tissue is not necessarily a block in another tissue differently differentiated.

Certain evidence suggests that some mutant genes may escape elimination because they are mutations of genes which are inactive in the seed. The embryo of

the pea, for example, has a complete set of genes for the formation of nicotinic acid, yet it is unable to carry out this synthesis (Bonner 1938), although it is improbable that a light-catalyzed reaction is involved. The young pea plant may acquire the ability to form nicotinic acid adaptively, for one would expect an extensive synthesis of adaptive enzymes at or soon after germination, when the organism is exposed to a new set of environmental stimuli. *Arabidopsis* embryos are unable to utilize nitrate (Rijven, unpublished data), yet the seedling can do so, presumably because nitrate reductase is then formed. The requirements of embryos for other organic compounds may also be a reflection of failure of the genes to act at this stage.

A third class of gene effect also is not subject to selection during seed growth because the product of the biosynthetic chain in which the gene acts is not required before germination. Mutational blocks in photosynthesis are of this type, the most common being those affecting the formation of chlorophyll and plastids.

A final group of genes whose functions may be lost by mutation, although the mutated gene does not cause the death of the seed, consists of those genes active in the synthesis of diffusible substances. In these cases it is expected that the deficiency will be made good by diffusion of the required substance from the maternal plant.

(*b*) *Mutations Eliminated by Pre-germinal Development*

It seems probable that those mutations which lead to inviable gametophytes or seeds and thus are not expressed in the growing plant, may be of three types with respect to function.

The largest class will consist of mutations affecting the synthesis of high molecular weight materials which cannot diffuse from one genetic type of tissue to another. Included here are mutations blocking the formation of polypeptides and proteins, many phosphorylated compounds, conjugated vitamins, and nucleic acids.

Another type of mutation that will be eliminated is concerned with the metabolism of the resting seed or catabolic reactions of the germination process. Mutations of the genes active in the Krebs cycle, for example, may survive until the seed is mature, but thereafter they will be lethal and germination will fail.

Finally, it is doubtful if gene mutations which cause a multiple loss of essential metabolites will survive to germination, even if the metabolites are diffusible. This class includes genes acting early in biosynthetic pathways such as amino acid synthesis (Davis 1951), and genes controlling relatively non-specific enzymes (e.g. Rudman and Meister 1953).

The conclusion to be drawn from this discussion is that, subject to the restrictions outlined above, most of the non-growing mutants of a flowering plant should be lacking in the ability to form diffusible substances and, therefore, they should be capable of responding to supplements.

II. Methods

A description of the experimental plant, *A. thaliana*, and of the method used for its aseptic culture has been published previously (Langridge 1957).

(*a*) *Production of Mutations*

Mutations were induced by X-irradiation of seeds of the race Estland at the dosage recommended by Reinholz (1947). Seeds soaked for 40 hr received 6000 r at 316 r/min, 130 kV, 20 mA, and with a half-value layer of 2.9 mm of aluminium. The seeds were germinated on filter paper, the seedlings transferred to pots of steam-sterilized soil, and the X_1 plants grown in the glass-house.

For the isolation of mutants, all seeds from an X_1 plant were harvested, mixed thoroughly by shaking, and 16 seeds taken at random were sown separately in test tubes containing sterile mineral agar. When the X_2 segregation ratio is 7 : 1 (see below), a family size of 16 is only about 90 per cent. efficient in detecting mutants. However, by this isolation method each seed may be accounted for, so that all lethal and semi-lethal segregants have been detected, although many of the minor morphological mutants may have been missed. When an X_1 plant was found to be heterozygous for a mutation, further seeds were usually sown to determine the X_2 segregation ratios shown in Tables 4, 5, and 6.

(*b*) *Supplements*

The following supplements are the standard ones used to determine the requirements of growth mutants. None of them increases the dry weight of wild-type plants growing at normal temperatures. All extracts, hydrolyzates, or solutions are sterilized by filtration through sintered-glass filters (Gallenkamp, porosity 5), and the aseptic liquids are transferred to heat-sterilized vaccine bottles for storage. When required, aliquots are taken from the bottles using an alcohol-sterilized hypodermic syringe and added to autoclaved agar–mineral medium before gelling has occurred. If the mutant shows a response to amino acid or vitamin solutions, absence of interaction is initially assumed and the specific compound required is determined by means of the screening procedure recommended by Lindegren and Lindegren (1951).

(i) *Coconut Milk.*—Milk from green coconuts was brought to pH 3.0, extracted four times with freshly distilled ether, and adjusted to pH 6.0. Residual ether was removed by heating the milk on a water-bath at 35°C. It is used at the rate of 0.4 ml per plant—i.e. per 5 ml medium.

(ii) *Pea Seed Diffusate.*—This was prepared according to the method of Bonner, Haagen-Smit, and Went (1939). Five diffusate fractions were obtained by soaking sterilized seeds for 24 hr in successive volumes of sterile distilled water. The first fraction was discarded, and the remaining ones were combined and used without concentration. Only 0.25 ml could be supplied to each plant, as higher concentrations delayed germination by about 6 days and strongly inhibited root growth.

(iii) *Nucleic Acid Hydrolysate.*—Sodium nucleate (from yeast) was dissolved in 0.1N NaOH, hydrolysed by heating for 30 min at 50°C, neutralized, and made up to the required volume. It was used at the rate of 0.4 mg nucleic acid derivatives per plant.

(iv) *Vitamins.*—The relative amounts of the different water-soluble vitamins were based on published figures for their occurrence in plant tissues (Cheldelin and

Williams 1942; Schopfer 1949). The concentrations in the stock solution were adjusted so that an aliquot of 0.1 ml would contain the amounts of vitamins normally present in a plant of 20 mg dry weight (Table 1).

TABLE 1
VITAMIN SOLUTION

Vitamin	Amount Supplied per Plant (μg)	Vitamin	Amount Supplied per Plant (μg)
Thiamin hydrochloride	0·2	*i*-Inositol	40·0
Riboflavin	0·2	Folic acid	0·2
Nicotinic acid	1·0	Ascorbic acid	4·0
D-Pantothenate, calcium salt	0·2	Choline chloride	0·2
Pyridoxine hydrochloride	0·2	*p*-Aminobenzoic acid	0·2
D-Biotin	0·01		

(v) *Amino Acids.*—The amino acid solution contained individual amino acids in the proportions found in the plant protein edestin (Tristram 1949). However, the plants are very sensitive to amino acids, tolerating only about 1/20 of the calculated optimal supplement when a complete mixture is used. Therefore, the amino acids were grouped according to structural relationships, the tolerance level determined for each group, and solutions of these were usually used in preference to the complete mixture (Table 2).

TABLE 2
AMINO ACID SOLUTIONS

Solution No.	Amino Acid	Amount Supplied per Plant (μg)	Solution No.	Amino Acid	Amount Supplied per Plant (μg)
I	Glycine	7	IV	L-Cystine	12
	β-Alanine	82		L-Cysteine	12
	DL-Serine	25		L-Methionine	26
	L-Threonine	52		L-Histidine	63
				L-Proline	54
II	L-Valine	52			
	L-Leucine	50	V	L-Aspartic acid	24
	L-Isoleucine	61		L-Glutamic acid	34
III	L-Phenylalanine	10	VI	L-Lysine	25
	L-Tyrosine	10		L-Arginine	54
	L-Tryptophan	7			

III. RESULTS

(*a*) *The X_1 Generation*

About 50 per cent. of the X-irradiated seeds germinated at the same time as unirradiated ones, the remainder having delayed germination or none at all (Table 3).

Most of the seedlings from seeds with definitely delayed germination had poorly developed cotyledons and failed to grow on planting into soil. Many others, whose seeds were only about 1 day later than the controls in germinating, produced only a few leaves which were pale green and of a smooth, round, drooping appearance. They became yellow and died within 3 weeks of planting out.

During growth, many of the plants showed the non-inherited morphological abnormalities characteristic of high-dosage X-ray treatment. These abnormalities included split leaves, multiple and fused flower stalks, fused leaves, single flowers at the leaf axils, and death of the main floral primordium. The first three leaves of one plant contained a chlorophyll-deficient sector, but its progeny were of a normal green colour. No dominant mutations were seen.

TABLE 3

GERMINATION AND SURVIVAL AFTER IRRADIATION

Race	No. of Seeds Irradiated	Germination (%)	Plants Surviving and Producing Seed (%)
Estland (control)	100	87	72
Estland (irradiated)	600	80	21
Graz (irradiated)	750	69	8
Enkheim (irradiated)	600	73	16

(*b*) *The X_2 Generation*

The irradiation of seeds causes mutations as a result of ionization in a multicellular apical meristem. Consequently, the X_1 plants will be chimerical for any mutations so induced.

From segregation data, allowance may be made for the chimerical state and the mutation rate per primordial cell calculated as follows. The mean segregation ratio of normal to mutant for 15 mutations, obtained from sowing mixed seeds from X_1 plants, was 8 : 1. This segregation ratio approximates to the 7 : 1 expected if the mean number of primordial cells, which eventually form flowering shoots bearing seed, is two at the time of irradiation. Then, assuming two primordial cells per plant, both of which are sampled yielding a 7 : 1 ratio in families of 16 plants, the apparent mutation rate will be $2\chi\ (1-(0.875)^{16})$, i.e. 1.76χ, where χ is the mutation rate per primordial cell.

With a dose of 6000 r, the apparent mutation rate, based on the examination of 112 X_2 families, was 21 per cent. Thus the probability of any given X_1 plant having a detectable mutation will be 0·21 and the probability per primordial cell 0·12.

These are rather inaccurate calculations, for only 60 per cent. of the mutant families give the expected 7 : 1 ratio, the remainder having X_2 segregations suggestive of origin in a one-, four-, or five-celled meristem. In addition, the diagrams given

by Kaukis and Reitz (1955) indicate that, between normal and mutant tissue in a chimera, there may exist competition leading to a gradual suppression of the mutant sector.

Four sorts of abnormality occur in X_2 families:

(i) Single gene mutations.

(ii) Variations produced by the contemporary environment, accounting for 60–70 per cent. of the total apparent variation. Included here are fasciations of the type found in the X_1 generation.

(iii) Variations environmentally induced at the time of seed development, e.g. mono- and tricotyledonous seedlings.

(iv) Abnormal plants, usually with reduced fertility, which continue to segregate in successive selfed generations. When examined cytologically, these are found to be due to chromosomal deletions or rearrangements.

TABLE 4

LIST OF MORPHOLOGICAL MUTANTS

Mutant No.	Phenotype	X_2 Segregation	
		Normal	Mutant
1014/12	Pointed leaves	14	1
2075/4	Rounded leaf tips and short petioles	38	3
1072/9	Leaves two-thirds normal size	15	1
1064/1	Angular, toothed leaves	43	5
1010/15	Early flowering	15	1
1036/16	Late flowering	15	1
1090/1	White seeds	*	*

*Segregated in X_3 generation.

(c) *Mutants*

Twenty-four mutants were obtained from testing 112 families. Eleven of these were lethals or semi-lethals, six were chromosomal mutants, and seven were morphological mutants whose viability was not noticeably decreased. Of the mutants designated as chromosomal, only two have been examined cytologically, but mutants of this class have a characteristic appearance and a much reduced fertility. Typically, they have irregular asymmetric leaves, smaller and narrower than the wild type, often with the leaf edges curled downwards. Most of the pollen grains are aborted and the anthers indehiscent. Plants homozygous for the deficiency appear rarely or not at all.

A list of the morphological and chromosomal mutants is presented in Tables 4 and 5. The lethal and semi-lethal mutants and their X_2 and F_2 segregations are shown in Table 6. As this study is primarily concerned with the growth requirements of mutants of low viability, these mutants are described more fully. Each of these

mutants was tested for its response to the following treatments: sucrose (2 per cent.), coconut milk, vitamins, nucleic acids, amino acids, low osmotic pressure (0·5 atm) and high osmotic pressure (2·0 atm as given by 0·025M K_2SO_4), low temperature (20°C) and high temperature (28°C).

TABLE 5

LIST OF CHROMOSOMAL MUTANTS

Mutant No.	Phenotype	X_2 Segregation	
		Normal	Mutant
1024/14	Variable expression. Leaves coarsely serrate, with leaf edges folded inwards, or of irregular sizes. Pollen shrivelled and empty, anthers indehiscent	40	6
1102/11	Sharply pointed leaves, one-half normal size. 80 per cent. aborted pollen	14	1
1007/10	Leaves curled over at ends, smaller than normal. Heterozygous for a reciprocal translocation	14	2
1084/16	Mutant with only three rosette leaves each about one-third the normal size and having a chlorotic strip down the centre. Sterile	13	2
1097/3	Light-green leaves, very narrow and pointed. Flower stalk short and weak. Sterile	14	1
1046/12	Cotyledons spatulate; leaves small, dark green and curled; late flowering. About 60 per cent. aborted pollen. Heterozygous for a chromosome deletion	65	7

IV. DISCUSSION

The practical implication of the hypothesis under consideration is that, with certain predictable exceptions, all non-growing mutants of flowering plants should have requirements for diffusible substances. A test of this conclusion is provided by the behaviour of the mutants described above. Of these 11 mutants, five respond to chemicals and one responds to an altered physical environment, but it has not been possible to increase the growth of the remaining five mutants. It remains to be seen if these "irreparable mutants" invalidate the hypothesis.

Mutants 1005/7 and 2079/8 appear to belong to the same class, for they both lack chloroplasts. However, they cannot utilize sugars as would be expected if only photosynthesis were inactivated by the mutations. It seems that their lethality could be due to the loss of some component such as a protein, sterol, or lipid which is necessary both for chloroplast formation and general growth. Alternatively, the chloroplasts may be required to carry out essential functions other than photosynthesis. The latter alternative seems the more probable, although no synthetic properties besides photosynthesis have yet been shown to be unique to the chloroplasts. However, Sisakyan (1955) reports that the formation and oxidation of fatty acids and

TABLE 6

LIST OF LETHAL AND SEMI-LETHAL MUTANTS

Mutant No.	Phenotype	Nutritional Requirement	Segregation Ratio	
			X_2	F_2
1005/7	Light yellow cotyledons and hypocotyl; no leaves or secondary roots. Plastids undeveloped ($1{\cdot}32 \pm 0{\cdot}36\ \mu$ in diameter; wild-type, $3{\cdot}36 \pm 0{\cdot}41\ \mu$)	No response to supplements	133:22	44:13
2079/8	Similar to 1005/7. Lacks chloroplasts	No response to supplements	45:3	86:23
2071/13	Normal-sized chloroplasts, but no chlorophyll. One pair of small, light yellow leaves; root system normal	With glucose or sucrose in the medium the mutant produces 4–6 rosette leaves and sometimes flowers	44:1	42:22*
1090/10	Cotyledons reduced to membranous chlorophyll-less structures which usually remain within the seed coat	No response to supplements	26:9	27:6
1018/6	Rosette leaves entirely chlorotic or chlorotic towards the tips; cotyledons mottled	Thiamin (1 μg/plant) completely restores growth. The pyrimidine and thiazole portions are ineffective	27:3	18:7
1025/3	Small leaves, light green with dark green veins; very slow growing. Chlorophyll content reduced to two-thirds that of wild type	Growth accelerated by sucrose or glucose, and to a slight extent by fructose	39:2	33:9
1138/1	At 28°C, plants have thin, small, light-green, curled leaves. At 23°C, only one pair of minute leaves are formed and the plant dies early	When coconut milk is supplied, leaves are much larger, wider, and not so closely curled	58:9	41:10
1031/13	Dwarf, attaining only one-third the wild type dry weight. Relative growth rates: wild type 0·26, dwarf 0·29; net assimilation rates: wild type 0·43, dwarf 0·43; mean seed weights: wild type 33 μg, dwarf 12 μg. Dwarfness, therefore, results from poor embryo growth	No response to supplements	58:3	20:4
1053/11	Mutant with temperature requirements for flowering that are not found in the wild type. Requires 28°C for flower stalk formation, followed by low temperature (20°C) for flower production	No response to supplements	120:16	30:9

*35 seeds failed to germinate.

TABLE 6 (*Continued*)

Mutant No.	Phenotype	Nutritional Requirement	Segregation Ratio	
			X_2	F_2
1023/13	At 23°C differentiation is upset and multiple apices producing small asymmetrical leaves result. At 28°C plants are small but with normal differentiation	Requires glucose, sucrose, or K_2SO_4 to increase the osmotic pressure of the medium to at least 1·5 atm	43:6	57:14
EST_9	Poor growth above 27°C. Leaf expansion and secondary root growth retarded	Normal appearance and growth with 20 μg of choline per plant	Spontaneous	35:10

the incorporation of phosphorus in phospholipids takes place in the chloroplasts, and it is known that even non-photosynthesizing flowering plants (parasites and saprophytes) still retain their plastids (Schürhoff 1924). If the additional activities of the chloroplasts include the formation of a non-diffusible substance that is not required before germination, the hypothesis holds. There is evidently insufficient information to make a definite decision from a consideration of these mutants.

The mutant 2071/13 lacks only chlorophyll. The chlorophyll molecule cannot be supplied to the plant, and, although sucrose does not fully restore normal growth, this is to be expected from the work of Spoehr (1942) and Rischkow and Bulanowa (1931) with similar mutants. The roots of flowering plants do not appear to be able to take up sugar at a rate sufficient to compensate for a lack of photosynthesis.

In the two remaining mutants, 1090/10 and 1031/13, it is obvious that the genetic defect expresses itself in the embryo. The first mutation affects a process dispensable in the embryo but essential to the growing plant, while the second lowers the efficiency of a process essential to the embryo but not required by the plant. Both these mutations affect genes that are time-limited in action, and thus they have escaped pre-germination elimination.

The behaviour of the limited number of mutants that has been studied conforms, in general, with what would be expected if the hypothesis were correct. Some of the mutants do not respond to supplements, and although their behaviour does not contribute to the proof of the hypothesis, their presence was expected and is explained by it. The fact is established that, in contrast with the majority of mutants of *Neurospora* (Atwood and Mukai 1953), *Aspergillus* (Pontecorvo 1953), and *Glomerella* (Markert 1952), most of the non-growing mutants of *Arabidopsis* require diffusible substances for the restoration of their growth. The flowering plants, then, by removing through developmental selection, mutants that are unsuitable for experimental work, compensate to some extent for their diploid condition and difficulties of culture. Thus the use of biochemical mutants in small, rapidly growing plants for chemical and genetic studies should be entirely practicable.

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