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GENETIC ANALYSIS OF MUTAGEN-INDUCED FLOWERING TIME VARIATION IN *ARABIDOPSIS THALIANA* (L.) HEYNH.

H. A. S. HUSSEIN

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GENETIC ANALYSIS
OF MUTAGEN-INDUCED FLOWERING TIME VARIATION
IN *ARABIDOPSIS THALIANA* (L.) HEYNH.

Dit proefschrift met stellingen van

HASHEM AHMED EL-SAYED HUSSEIN,

B.Sc. (Agriculture) en M.Sc. (Horticulture), Cairo University, geboren te Akhmim, Egypte (V.A.R.), 11 oktober 1937, is goedgekeurd door de promotor, Dr. R. PRAKKEN, hoogleraar in de erfelijkheidsleer.

De Rector Magnificus der Landbouwhogeschool,
F. HELLINGA

Wageningen, 19 augustus 1968.

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GENETIC ANALYSIS OF MUTAGEN-INDUCED FLOWERING TIME VARIATION IN *ARABIDOPSIS THALIANA* (L.) HEYNH.

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS DR. IR. F. HELLINGA,
HOOGLERAAR IN DE CULTUURTECHNIEK,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG, 11 OKTOBER 1968, TE 16.00 UUR

DOOR

H. A. S. HUSSEIN

H. VEENMAN & ZONEN N.V. - WAGENINGEN

GENETIC ANALYSIS OF MUTAGEN-INDUCED FLOWERING TIME VARIATION IN *ARABIDOPSIS THALIANA* (L.) HEYNH.

THESIS

IN FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF AGRICULTURAL SCIENCES (GENETICS)
AT THE AGRICULTURAL UNIVERSITY,
WAGENINGEN, THE NETHERLANDS
ON FRIDAY, 11 OCTOBER 1968, AT 16 O'CLOCK

BY

H. A. S. HUSSEIN

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BIBLIOTHEEK
DER
LANDBOUWHOGESCHOOL
WAGENINGEN.

THEOREMS

I

In mutation breeding, more useful genetic variation can be obtained by chemical mutagens than by ionizing radiations.

This thesis

II

There is a better chance for the plant breeder to obtain improved varieties, if the technique of mutation breeding is combined with recombination breeding.

This thesis

III

Mutagen-induced male-sterility can provide the plant breeder with a significant tool for the use of heterosis in self-fertilizers.

IV

Methodological improvement of mutation induction may enable mutation breeding to compete with the conventional methods of plant breeding.

V

Response to vernalization is not an intrinsic property of late flowering plants of *Arabidopsis thaliana*. [This may hold true also for other plants with comparable responses to environmental conditions.]

This thesis

VI

Sex is a rather general phenomenon in bacteria.

VII

For a more efficient strategy in developing insecticides, the genetic mechanism of resistance and the mode of gene action should ultimately be known.

VIII

In developing countries, more emphasis should be placed on studying optimal methods of successful communication between research workers and farmers, in order to increase agricultural productivity.

H. A. S. Hussein
Wageningen, 11th October 1968

To my parents
To Laila, my wife
To Mona, my daughter
In much gratitude.

FOREWORD

This thesis is the outcome of my 4-year study at Wageningen. During its preparation, I feel that I have built up the basis on which my scientific career will stand. The theoretical knowledge and the practical experience I gained during this study, will enlighten my way for any future work I intend to do. Therefore, with warm feeling, I wish to express my deepest gratitude and sincere acknowledgements to all those who helped me to let this work take its present form.

In the first place, I should like to express my gratitude and sincere appreciation to my promotor, Professor Dr. R. PRAKKEN, the Director of the Department of Genetics at the Agricultural University, Wageningen, for his kindness, his interest in my study and for his valuable advice and fruitful criticism during preparation of the manuscript.

I owe a great debt of gratitude to Dr. Ir. J. H. VAN DER VEEN, for suggesting the problem of my study, for his guidance, his helpful advice, his constructive criticism and valuable help during the course of this study and during preparation of the manuscript, and for translating the summary in Dutch. Dr. VAN DER VEEN, I must admit that without your guidance and supervision, it would have been difficult to put this study in its present form. I deeply appreciate the friendly relationship grown between us and between our families during this period.

I thank Miss JOSJE BOSMA, for her kind assistance to me during the experimental work of this study. I very much acknowledge her carefulness in doing the crosses and for helping me during transplanting and other technical matters.

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I wish to express my thanks to Ir. C. BROERTJES at the Institute of Atomic Sciences in Agriculture, I.T.A.L. at Wageningen, for irradiating the X-ray material.

Further I should like to express my thanks to all the members of the Agricultural University at Wageningen.

My hearty thanks are due to my wife for helping me in my work, especially during the week-ends, and for making my life easy and quiet during my study.

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CONTENTS

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1. INTRODUCTION

Among the naturally occurring phenotypic contrasts between different varieties or ecotypes of a species, characters related to the moment of change from the vegetative to the generative phase form a conspicuous and prominent component. They can be expressed in terms of time between germination and the start of flowering, and (often closely related to it), the amount of vegetative production (e.g. number of rosette leaves).

These differences are often reflections of adaptation to the local environment. In an evolutionary sense, they are a critical moment in the sequence of events during subspeciation, because wide differences in flowering time form a barrier to intercrossing. In this way peripheral populations may acquire permanent genetical isolation from the population of origin.

However, natural seed- and seedling vernalization can make the flowering periods of 'early' and of 'late' varieties overlap, and isolation then depends on whether the breeding system has changed to self-fertilization, a change which is frequently observed in peripheral areas of the region of normal occurrence. Also, in the artificial environment of a greenhouse, large differences in flowering time can be greatly reduced by means of artificial vernalization.

From these considerations, it is clear that the architecture of the genetic system of flowering time deserves considerable attention.

In plant breeding, the components 'earliness' and 'vegetative production' are of primary economic interest. This provides a second motivation for the present study on the genetics of flowering time.

The choice of the experimental plant fell on *Arabidopsis thaliana* (L.) HEYNH. (Cruciferae). This plant is widely used in genetical studies, and it is an excellent organism for testing breeding procedures in self-fertilizers. This is due to its short generation interval, its little space requirements and several other features which are described in ch. 2. *Arabidopsis* shows a large spectrum of natural variation in flowering time and number of rosette leaves.

Flowering time is a character which often shows continuous variation. There are two ways to approach the genetic system of flowering time:
1. analysis of natural variation, and 2. analysis of artificial, that is mutagen-induced variation.

When crossing natural varieties of *Arabidopsis*, one generally finds in segregating generations typical quantitative variation (continuous variation) for flowering time and leaf number (See e.g. CETL, 1965). Identification of individual genotypes seems difficult. However, as argued by VAN DER VEEN (1967), it may often be possible, with some ingenuity and perseverance, to find major genic segregations (that is segregation of identifiable genes) in what at first sight appears as typical quantitative variation. For this, one or more of the following conditions have to be fulfilled: 1. uniform environmental conditions, 2. a differentiating environment (i.e. increasing the contrast in expression of the genotypes), 3. a uniform genotypic background (which can be obtained by repeated backcrosses or continued inbreeding), 4. a differentiating (i.e. a non-epistatic) genotypic background, and 5. a more precise assessment of the phenotypic expression of the genotypes. By fulfilling the conditions 1, 2 and 4, VAN DER VEEN (1965) identified in one of his crosses between natural varieties of *Arabidopsis* (viz. Di × Li_2, see also ch. 5) two genes with complementary-like interaction. The flowering time distributions found by BARTHLEMESS (1965) in an F_2 from two other lines can be interpreted in the same way, and probably the same two loci are involved (VAN DER VEEN, pers. comm.). However, attempts to identify more loci have not been very successful so far, although in many crosses a large amount of genotypic variation is present.

The other way to identify genes governing flowering time and leaf number is to induce variation by mutagenic treatment. Such variation is readily obtained by means of ionizing radiations and notably by the very effective chemical mutagens available nowadays.

Again, reports in literature speak of increased variation for these characters in M_2 and subsequent generations. In the absence of immediate indications for single gene differences, no attempts are made to try and analyse by means of classical Mendelian methods, but instead one resorts to the methods of biometrical genetics in order to describe the induced genotypic variation in statistical terms (estimates of components of variation), see e.g. BROCK (1965, 1967) and LAWRENCE (1965). One simply speaks of quantitative mutations or micromutations. In general, only rough generalizations could be reached.

Nevertheless, it would be very useful if single gene mutations could be identified, as in that case one can also study crosses between single gene mutant lines, with respect to linkage relationships, gene interactions and heterosis phenomena. In such crosses the distributions may approach continuity or even unimodality, but now one has the advantage that the positions are known of 3 homozygotes (parent line and the two mutant lines), of 2 monoheterozygotes (parent × mutant line), and of 1 diheterozygote (mutant line × mutant line). For reaching this purpose, it seems better not to score for single gene segregations in the often limited M_2-progenies from single M_1-sectors, but it seems preferable to select, from M_2 onwards, lines which have good vigour and good fertility. Thus one avoids large heterogeneity in the genotypic background and also disturbances in segregation ratios.

The working hypothesis, that in such lines single gene differences with the parent line would readily show up in segregating generations, will be confirmed by the present work. Indeed, mutant lines with more than one gene mutation appeared to be relatively rare. Also the further hypothesis, that the majority of such lines will contain mutations at different loci, proved to be true.

By virtue of this, the analysis of the genetic system of flowering time could be profitably tackled by means of induced mutations. In a sense, the present program is the reverse of what would be the case when using natural variation. In the latter case, single genes have to be isolated from crosses between varieties which differ in an unknown number of unknown genes, which involves a laborious test-cross program. In the former case one starts with identifiable single genes, obtained by mutations in a given parent-line, and builds up polygenic situations in which the between locus interactions and linkage relations can be readily assessed.

Of course, we do not know in how far natural variation existing at present is based on the same array of loci as mutagen-induced variation is. Nor do we know whether a possibly preponderant direction of dominance in nature will be the same for artificially induced mutations.

Finally, a program of mutation induction followed by a genetical analysis for a quantitative character like flowering time is of considerable interest from the point of view of practical mutation breeding, as it may give clues for increasing the efficiency of selection procedures.

Summarizing, the scope of the present investigation is as follows:

1. The analysis of a genetic system for a quantitative character by means of induced mutations and, as far as possible, by means of classical Mendelian methods.
2. Empirical evaluation of the possibilities of mutation breeding for an economically interesting quantitative character, especially with respect to the problem of macro- versus micromutation breeding. Also the problem of relative efficiency of different mutagens will be considered.

2. MATERIALS AND METHODS

2.1 STARTING MATERIAL: THE PARENTAL LINES C AND 51

The small cruciferous plant *Arabidopsis thaliana* (L.) HEYNH. ($2n = 10$) is almost ideal for genetical studies. With the earliest flowering varieties, the generation interval is only 5 to 6 weeks. It requires little space: if necessary, over 500 plants can be grown per m^2 greenhouse bench, so that a medium size experiment can be done in a homogeneous environment. Vigorous plants can yield over 100 siliquas, each with 40–60 seeds. For these reasons the plant has been nicknamed 'Botanical *Drosophila*'. Under greenhouse conditions, *Arabidopsis* is a strict self-fertilizer, so that bagging the inflorescences is not necessary.

As a starting material two lines were used: line C and line 51 (See plate 1).

Plate 1. The parental lines C (left) and 51 (right).

Line C has been obtained by REDEI after X-ray mutagenic treatment of race 'Landsberg'. This mutant line, Landsberg-'erecta', has a compact, erect growth habit, and is one of the earliest flowering types known in *Arabidopsis* (flowering 22–25 days after germination, 6–7 rosette leaves). It had been maintained in the Department of Genetics at Wageningen by selfing through one plant per generation during 10 generations, and can be considered to be completely homozygous.

Line 51 is one of the eleven late selections obtained by BHATIA and VAN DER VEEN (1965) after treatment of line C seeds with ethyl methanesulfonate (EMS).

Line 51 flowers about 5–6 days later than line C and has 6 to 7 more rosette leaves. On full development of its inflorescences, this line is taller than the rather short line C. It has a regular growth habit, is fully fertile, and had been maintained by selfing through one plant per generation during 8 generations. It is phenotypically very uniform. After seed vernalization, line 51 closely resembles line C in all respects, except for somewhat smaller rosette leaves (See further, ch. 5.1). It will be shown (ch. 5.1) that line 51 differs from line C mainly in one single partially recessive gene for late flowering and more rosette leaves.

For further details of the lines C and 51, the reader is referred to ch. 3.2 and ch. 5.

As BHATIA and VAN DER VEEN (1965) had not found mutant lines earlier than line C, it is conceivable that line C represents a 'physiological limit' to the expression of additional mutations towards 'early'. Line C does not respond to vernalization treatment either. As mentioned elsewhere (HUSSEIN and VAN DER VEEN, 1965), line 51, being derived from line C, does not represent such a 'physiological limit', and therefore, mutations towards 'early' to be induced in line 51 can be expected to phenotypically express themselves. This was the main argument for including line 51 in the present studies.

2.2 GROWING METHODS

The germination, transplantation and growing techniques used here, are essentially those developed by VAN DER VEEN (1965). These techniques aim at reducing environmental variation to a minimum.

Seeds harvested in the greenhouse are kept during about 2 weeks in the thermostat (dark, 32°C) to fulfill after-ripening requirements. The seeds are laid out in Petri-dishes on a 4 mm layer of agar (0.75% Oxoid no. 3 in tap-water) and transferred to a refrigerator (dark, 2–4°C) for 5 days in order to break dormancy. When dry seeds have to be subjected to mutagenic treatment, dormancy breaking can be done on water-saturated filter paper (in closed Petri-dishes) followed by drying on filter paper at 24°C. Sowing on agar is then after mutagenic treatment. Germination (on agar) is in the light (TL 55, 300 W / m^2, at 50 cm) at 24°C (within dishes). A powerful means to synchronize germination (apart from sufficient after-ripening and cold treatment) is the addition of a trace of KNO_3 (100 mg/liter) to the agar.

After 24 hours under light (or with certain mutagenic treatments, 32 hours) germination is about to become visible, and at this moment, the dishes are transferred to the dark (a thermostat at 24°C) during 2 days. Here visible germination follows, and the hypocotyles become $\frac{1}{2}$ to 1 cm long (etiolation). This is done in order to facilitate transplanting into soil. Then the dishes go back to light (as above) during 2 or 3 days. Here the cotyledons turn green and in general, the seedlings can recover from the stay in the dark.

Transplanting into soil (pots or pans in an airconditioned greenhouse) is with pre-incised (before etiolation) little blocks of agar (use spatula) into little holes, after which some soil is pressed over the agar. The agar serves as a water reservoir and decays after some time. It ensures uniformity of seedling growth after transplanting, as seedlings are very sensitive to drying out (a main cause for non-genetic disuniformity). Also soil splashing on the cotyledons can be detrimental to uniformity, but by using etiolated hypocotyledons, the cotyledons are well above the soil. Nevertheless, a fine spray is used in watering, and in the summer months the transplanted seedlings are shaded with a white wood lattice. Algal growth and hardening of soil surface are avoided by using a well balanced leaf-soil mixture.

The pots used have a diameter of 7 cm, and the pans are 25 × 25 cm. Per pan at the most 36 plants were grown, in order to avoid pronounced competition.

The greenhouse was airconditioned (22 °C throughout the year, and 70% rel. humidity). Additional light (in winter; continuous) was given by frames of Philips TL55 (180 W/m^2, at 50 cm), constructed so as to give a homogeneous field of light. In order to induce long day reactions in the winter, incandescent bulbs (4 × 25 W/m^2) were added to the TL 55 light.

2.3 EMASCULATION TECHNIQUE

The emasculation technique has been described by FEENSTRA (1965). The anthers are sucked out by means of a small pipette, drawn from a piece of thin walled glass tubing, or by means of a hypodermic needle from which the pointed end has been cut off. The tube is connected to a water-jet (or air-jet) pump with a vacuum tube. The air flow is controlled by a pedally operated valve. Flower buds higher than those to be crossed are removed, which is easily done with a forceps.

2.4 VERNALIZATION

For seed vernalization, sowing was as usual on agar in Petri-dishes. These were kept during 5 days at 2–4 °C (to break dormancy), and then transferred to light at 24 °C. After 24 hrs, the germination process is stimulated, but visible germination has just not taken place. Such a stimulation of germination is a prerequisite of successful and homogeneous vernalization. The dishes were then put back at 2–4 °C (dark) for about 4 weeks. At the end of this period, the hypocotyledons are about 1 cm long.

The unvernalized controls were also sown on agar, and treated as described in ch. 2.2. They were timed so that transfer to light (recovery period) was simultaneous for both vernalized and unvernalized sets. That is the vernalized set after 4 weeks cold, and the unvernalized set after 2 days at 24 °C (dark) for etiolation, were simultaneously transferred to light, and treated further as described in ch. 2.2.

2.5 MUTAGENIC TREATMENT

Mutation induction was done by treating seeds with the alkylating agent EMS (ethyl methanesulfonate) or with X-rays.

EMS was obtained from Eastman-Kodak Cy (New York).

X-ray treatments were given at the Institute for Atomic Sciences in Agriculture (ITAL) at Wageningen. The kind assistance of Ir. C. BROERTJES is gratefully acknowledged.

EMS-treatments were as follows: after dormancy breaking and redrying (cf. ch. 2.2), the seeds were submerged in EMS-solutions (0, 6, 9 and 12 mM, not buffered) in small flatbottom glass-tubes (diam. 19 mm, 0.7 ml solution) and left for 24 hrs in the dark at 24°C. After 5 min rinsing off with tapwater, the seeds were immediately laid out on agar, and treated further as described in ch. 2.2. For X-ray treatments, the redried seeds were allowed to imbibe water during 3 hours on water saturated filter-paper in small Petri-dishes prior to X-raying (doses 0, 8, 12, 16 and 24 Kr). The 250/25 X-ray apparatus was operated at 250 KV and 15 mA, without extra filter, at a focus distance of 28 cm, and at the rate of 700 r/min. In a later experiment (second-cycle treatment; ch. 8) a dose of 12 Kr was given at a rate of 290 r/min at a focus distance of 45 cm. Immediately after treatment, the seeds were laid out on agar, and treated as described in ch. 2.2.

A survey of the mutagenic treatments, the number of seeds treated, and the percentage of flowering plants obtained is given in table 1.

TABLE 1. Summary of the mutagenic treatments on 12-1-1965 (Experiment I) and 26-1-1965 (Experiment II). The numbers of M_1-plants obtained for line C and for line 51 are expressed as percentage of seeds treated (pooled over experiments I and II). X-raying was on presoaked seeds. EMS was applied at 24°C for 24 hrs.

Treatment	Number of seeds treated from each line		Mature M_1-plants as % of seeds treated	
X-rays (Kr)	Experiment I	Experiment II	Line C	Line 51
0	50	50	98	100
8	100	100	94	85
12	–	100	88	71
16	100	150	52	52
24	150	–	2	1
EMS (mM)				
0	50	50	98	96
6	50	50	48	64
9	50	50	53	79
12	50	50	43	62

It should be noted that the decrease in survival at increasing X-ray dosages (down to almost 0% for 24 Kr) is the normal picture obtained for *Arabidopsis*. For the EMS-treatments the % of flowering plants obtained could have been much higher, if all seeds germinated had been transplanted. However, only those with well elongated hypocotyledons (induced by the dark period, see ch. 2.2) were transplanted. After X-rays, all seedlings had well elongated hypocotyledons.

2.6 EXPERIMENTAL DESIGNS

The designs of the experiments carried out during this study were kept as simple as possible. The plants were either grown individually in small pots or groupwise in pans.

a. *Experiments in pots:* In a few cases, where extra precision was needed, plants of different families were individually randomized together per block. These are the leaf-size experiment of 4-3-1965 (ch. 5.1), containing the lines C, 51, their F_1 and F_2, and the vernalization experiments of 23-3-1966 (ch. 3.2.3), containing all mutant lines, and of 7-8-1966 (ch. 6.6), containing the large-effect late mutant lines.

In all other experiments grown in pots, plants of different families were not randomized together. E.g. with the diallel experiment of 10-2-1966 (See table 5, ch. 4), a plot containing all families of one cross in the order P_1, F_1, F_2, B.C. and P_2 was repeated a number of times in adjacent positions. The different crosses were grown in one block on the greenhouse bench. The replicated parent families enabled evaluation of and correction for any environmental gradient in the greenhouse. However, no gradients necessitating corrections for position on the greenhouse bench were met.

b. *Experiments in pans:* Per pan 5×5 or 6×6 plants were grown. The environment may sometimes change from pan to pan, not due to a gradient along the greenhouse bench, but simply as a 'pan-effect', sometimes resulting in differences between neighbouring pans. To be able to evaluate and correct for such pan-effects, the procedure adopted can be illustrated as follows: For example an F_2 grown in 4 pans each of 6×6 plants, did not consist of $4 \times 36 = 144$ F_2-plants but of $4 \times 30 = 120$ plants or $4 \times 24 = 96$ F_2-plants, as to each pan, 6 plants of one or of both parents were added. In other cases, not one of the corresponding parents, but a control variety (e.g. line 51 or line C) was added. Only in the experiments of ch. 7.4 (the small-effect-late-mutants from line C) it was not justified to pool the graphs of different pans, as the differences to be judged were so small, that the 'pan-effects' became a disturbing factor.

In all pan-experiments, the pans were individually randomized. E.g. in all F_2-diallel experiments (each F_2 represented by several replicated pans), all pans were individually randomized into one block.

For details of the different experiments, the reader is referred to the corresponding chapters. A summarizing table of experiments is given in ch. 4.

2.7 ANALYTICAL METHODS

2.7.1 *Scores*

'Flowering time' was measured as number of days from the end of cold treatment (for breaking dormancy) to the day of opening of the first flower on the main stem. For vernalized material, counting of days was from the moment of germination of the unvernalized control set, which was synchronized as described in ch. 2.4.

'Number of leaves' (or 'leaf number') is the number of rosette leaves including cotyledons. Both 'flowering time' and 'number of leaves' measure the extent of vegetative development.

The 'combined score' is the sum of 'flowering time' (in days) and 'leaf number'. This means that the two scores are combined with equal weight into one index for vegetative development. The rationale for using this score is based on the high positive correlation of the two characters between genotypes, and is best explained by describing the situation where its profit is maximal.
Suppose:

1. Genotypes which differ n days in flowering time, to have a leaf number difference n. This proportionality holds by approximation between lines C and 51 (ch. 5), and between the lines C and 51 and their different mutant lines, and the F_1's (Fig. 7, ch. 6.2). In other words, per day later flowering about one more leaf is produced (for exceptions, see ch. 6 and ch. 7).
2. Within each genotype, there is no (environmental) correlation between flowering time and leaf number. In fact, even with the late flowering mutant lines which have a larger variance for both characters, there is not much correlation.
3. Within each genotype, both characters have about equal variance. In reality, the variance in leaf number is often somewhat smaller than the variance in flowering time.

In this situation, the scatter diagrams for the different genotypes can then be represented by circles with centres on a line slope unity. If the genotypes differ little, i.e. the circles touch or overlap, then the overlap is minimal when projection is on the line slope unity (i.e. when the phenotypes are measured by the present equal weight index = 'combined score'), and the overlap is much larger when projection is on the Y-axis (flowering time) or on the X-axis (leaf number). Therefore, phenotypic classification of the genotypes in segregating generations is more precise when the combined score is used.

Of course when the genotypes differ much in expression, and the circles are far apart, absence of overlap on the Y-axis (or the X-axis) makes flowering time (or leaf number) sufficient for accurate classification. Therefore, sometimes only flowering time was used when very late lines are involved (also since counting the high number of leaves is time-consuming). Also, for the crosses with mutant lines which flower later, but fall short in leaf number increase (See ch. 6 and ch. 7), only 'flowering time' was used, since the leaf number distributions were almost completely overlapping.

2.7.2 *Graphs*

Frequency distributions of 'flowering time' or 'combined score' are given as simple frequency histograms with class intervals of 1, 2 or (sometimes) 4 units, depending a.o. on the range of phenotypes.

In some other graphs, family means for flowering time (ordinate) are plotted against means for leaf number (abscissa). For genotypic analysis in segregating generations, always the scatter diagrams of individual plant observations were used. However, these are not presented.

The non-segregating families were added to the frequency graphs of segregating families by means of a horizontal arrow, with a dot indicating the family mean. The arrow has a range including 95% of the plants, the 5% extremes being knocked off in the order of distance from the mean. If a choice had to be made between two plants at equal distance left and right from the mean, this was done at random. The absolute number of plants corresponding to 5% is always the nearest whole number, e.g. 2 plants in a family of 30. The construction of these ranges was always based on one-unit class intervals, also when two- or four-unit class interval graphs are presented.

Sometimes, the position of the mean of a non-segregating generation (in particular F_1's) was transferred from one experiment to another, provided no conspicuous genotype-season interaction was present as judged from a set of isogenic lines (cf. ch. 6.6). Transferred means are indicated by a vertical arrow under the abscissa. Transfer was done by linear interpolation relative to the parent lines (present in both experiments) .

Points of partitioning in the frequency distributions of segregating generations are also given by arrows. This partitioning was done in general by comparing with the frequency distributions of known genotypes. It gives estimates of the number of plants corresponding to the different phenotypic classes, and is basic to the genetical analysis.

3. THE MUTANT LINES

3.1 SELECTION PROCEDURES AND RESULTS

The survey of the number of families grown, and the number of mutants selected in successive generations, will be given in reference to table 2.

The M_1-plants were grown in pots. The M_2-families, each derived from a single M_1-fruit, were grown in pans. Each pan contained (if possible) 27 plants of a single M_2-family, and 9 plants from the parent of origin (line C or line 51), in order to avoid 'between pan' comparisons (pan-effects). Also in M_3 and M_4, the selections were grown in pans, but now with 16 plants per family, supplemented with 9 plants of the parent of origin. In M_5, plants of the selected lines to be used for crossing were grown in pots.

3.1.1 *Selection in M_1*

GAUL (1958, 1963) found for preformed barley tillers, that within a given mutagen treatment, the percentage of mutants in M_2 is equal for the different fertility classes of M_1-sectors. This has also been found in tomatoes by HILDERING and VAN DER VEEN (1966), and in *Arabidopsis* by VAN DER VEEN (1966) and by MÜLLER (1966). From M_1 to M_2, mean sterility decreases as a result of natural selection. Now, there is, also in *Arabidopsis* (MESKEN and VAN DER VEEN, 1968), a strong positive correlation between fertility in M_1 and M_2, both for EMS and X-rays. Therefore, the efficiency of a mutation selection program can be increased by harvesting from the more fertile M_1-sectors only, which then will result in improved M_2-fertility, without loss in the quantity of mutants in M_2.

For these reasons, the following selection procedure was adopted in M_1. From all M_1-plants on which sectors with good fertility were found, two fertile fruits among the first six fruits on the main stem were chosen to give *pairs* of M_2-families. This was done in the first place within the M_1's from 9 and 12 mM EMS and from 12 and 16 Kr X-rays. Already from the general appearance (especially the high level of fertility) of the M_1's from 6 mM EMS and 8 Kr X-rays, it was clear that these doses were too low to induce a reasonable amount of mutations. Nevertheless, a few M_2's were raised from the M_1 of line C after 6 mM EMS and from the M_1 of line 51 after 8 Kr X-rays (cf. table 2). As will be seen (Table 2), the latter treatment ultimately gave one mutant line, viz. $51XV_1$. As already mentioned (ch. 2.5), 24 Kr X-rays gave virtually complete M_1-lethality.

3.1.2 *Selection in M_2 and further generations*

A number of M_2-families which showed low germination percentage, segregated seedling chlorophyll mutants, or contained a majority of seedlings with abnormal growth, were discarded before transplanting. In total (pooled over lines and doses) 205 pairs of M_2-families were transplanted and grown to maturity (Table 2).

TABLE 2. Number of M$_1$-plants available, number of M$_1$-plants selected to give pairs of M$_2$-families, and number of selections (phenotypes) in M$_2$, M$_3$ and M$_4$.
Between brackets: selections discarded in M$_5$ (viz. 51XV$_2$ and 51C; see text).
Mutant lines ultimately continued:
From line C: 6 small-effect late mutant lines (CXl$_1$, CXl$_2$ and CXl$_3$ by X-rays; CEl$_1$, CEl$_2$ and CEl$_3$ by EMS), 1 large-effect late mutant line (CA by EMS).
From line 51: 6 small-effect early mutant lines (51XV$_1$ and 51XV$_3$ by X-rays; 51EV$_1$, 51EV$_2$, 51EV$_3$ and 51EV$_4$ by EMS), 5 small-effect late mutant lines (51Xl$_1$ and 51Xl$_2$ by X-rays; 51El$_1$, 51El$_2$ and 51El$_3$ by EMS), 4 large-effect late mutant lines (51A, 51B, 51D and 51E by EMS).
In the notation C and 51 stand for the parents of origin, X and E for X-rays and EMS. The symbol V derives from the Dutch word 'Vroeg' (= early), and the symbol 1 stands for (small-effect) late.

	X-rays (Kr)						EMS (mM)				
	8		12		16		6	9		12	
Line C											
M$_1$-plants available	188		88		130		48	53		43	
Pairs of M$_2$-families (= number of M$_1$-plants selected)	–		12		34		13	12		26	
Selections made			early	late	early	late		early	late	early	late
in M$_2$			4	–	5	9	–	3	7	1	1
in M$_3$			3	–	3	4	–	2	5	–	–
in M$_4$			–	–	–	3	–	–	4	–	–
Lines continued						CXl$_1$ CXl$_2$ CXl$_3$			CEl$_1$ CEl$_2$ CEl$_3$ CA		
Line 51											
M$_1$-plants available	170		71		129		64	79		62	
Pairs of M$_2$-families (= number of M$_1$-plants selected)	13		12		26		–	44		13	
Selections made	early	late	early	late	early	late		early	late	early	late
in M$_2$	2	1	–	–	3	7	–	7	19	4	4
in M$_3$	1	1	–	–	2	2	–	4	11	3	–
in M$_4$	1	–	–	–	2	2	–	3	8	1	–
Lines continued	51XV$_1$				(51XV$_2$) 51XV$_3$	51Xl$_1$ 51Xl$_2$		51EV$_1$ 51EV$_3$ 51EV$_4$	51El$_1$ 51El$_2$ 51El$_3$ 51A 51B (51C) 51D 51E	51EV$_2$	

On the basis of within pan comparison with the control variety (lines C or 51), M_2-plants which flowered earlier or later and/or had less or more leaves were selected. Only those plants which had a normal growth habit and good fertility were taken. This means that early or late types with reduced vigour or fertility were discarded. In general, 2 or 3 plants of a given type were selected per pan. In a number of cases, the same type could be selected from the sister-pan (from the sister-fruit on the same M_1-plant). However, in all cases only one line of a given flowering time type was ultimately (in M_4) selected per pair of M_2-families. In total, 31 and 46 selections in M_2 from X-rays respectively EMS were made (Table 2). Here the term 'selection' refers to a number of phenotypically similar M_2-plants, which, as said earlier, are derived from the same M_1-plant.

In M_3, both 'between line' and 'within line' selection for flowering time, and also for good growth habit and fertility, was practiced within each of the 31+46 M_2-selections. A considerable number of selections in M_2 showed in M_3 no flowering time difference with the parent type. This left 16 and 25 selections for X-rays respectively EMS (Table 2), each to be represented by one or two plants selected to give M_4-families. These two plants may or may not derive from the same M_2-plant.

In M_4, per M_2-selection, the most homogeneous line with good growth habit and fertility was chosen, and in that line, the 'best' plant was selected. In M_4 again several M_2-selections were dropped in view of reduced fertility, abnormal growth habit, and other defects. This left 8 X-ray and 16 EMS mutant lines in M_5, each derived from a different M_1-plant. (There was no objection, of course, to selection of an early and a late line, or of two late lines with a pronounced difference in flowering time, from the same M_1-plant, but this did not happen to be the case).

In M_5, two selections were discarded on the basis of insufficient fertility (line 51C from EMS-material) and of deviating growth habit (line $51XV_2$ from X-ray material).

With respect to the 24 M_4-selections, the following points have to be mentioned (cf. table 2):

1. No selections earlier than line C were obtained. In ch. 2.1, it was argued that the early line C probably represents a 'physiological limit' to the expression of mutations towards early.
2. The optimal doses with respect to the recovery of mutant lines are 16 Kr X-rays and 9 mM EMS.
3. In the case of line C, 3 lines were selected from 34 M_2-family pairs after 16 Kr X-rays, and 4 lines from 12 M_2-family pairs after 9 mM EMS. In the case of line 51, 4 lines were obtained from 26 M_2-family pairs after 16 Kr X-rays, and 11 lines from 44 M_2-family pairs after 9 mM EMS. That is, from in total $34+26=60$ M_2-family pairs after 16 Kr X-rays, and from $12+44=56$ M_2-family pairs after 9 mM EMS, respectively 7 and 15 lines were obtained. MESKEN and VAN DER VEEN (1968) found after 8.3 mM EMS 3 times as many chlorophyll mutants as after 12 Kr X-rays (these two doses gave the same level

of M_2-fertility). The present result for flowering time (and leaf number) mutants with good vigour and fertility is in accordance with these results, when allowing for the fact that here 16 Kr instead of 12 Kr was used.

4. No large-effect late mutant lines were obtained after X-rays, but 6 lines after EMS (See legends to table 2), which difference might indicate that EMS is more efficient to induce *large-effect* late mutants with good growth habit and fertility. See however ch. 9.3.

3.2 DESCRIPTION OF THE MUTANT LINES

3.2.1 *Flowering time and leaf number*

After discarding 2 selections in M_5 (51XV$_2$ and 51C), the remaining 22 selections were grown in further generations, during different seasons. Flowering time and (correlated to it) leaf number, varies with season. All lines flower earlier in spring and later in late autumn. Genotype-season interactions were in some cases rather pronounced. For the large-effect late mutant lines (CA, 51A, 51B, 51D and 51E), these are described in ch. 6. Also for the small-effect late mutant lines 51Xl$_1$ and 51El$_3$, genotype-season interaction was pronounced, and enabled to detect, in certain seasons, their single gene differences with line 51 (See ch. 7.2). Thus, these two mutant lines sometimes flower as late as, or even later than, the earliest line (51D) from the large-effect late mutant group (See below). This means that there is no systematic gap between small- and large-effect late mutant lines.

The following categories (already referred to above) were made in M_4, grown in winter, from 10-11-1965. (The mutant lines were genotypically compared with each other and with the parent line(s) by means of diallel crosses, ch. 6 and ch. 7, within but not between these categories).

1. Large-effect late mutants from lines C and 51, viz. CA, 51A, 51B, 51D and 51E, all by EMS. These mutants flower at least 10 days later and have at least 10 leaves more than the parent of origin.
2. Small-effect late mutants from line 51, viz. 51Xl$_1$ and 51Xl$_2$ by X-rays, and 51El$_1$, 51El$_2$ and 51El$_3$ by EMS. The mutants 51Xl$_1$ and 51El$_3$ (cf. above) can be taken as borderline cases with the large-effect late mutants, at least in some seasons.
3. Small-effect early mutants from line 51, viz. 51XV$_1$ and 51XV$_3$ by X-rays and 51EV$_1$, 51EV$_2$, 51EV$_3$ and 51EV$_4$ by EMS. None of these mutants flowers as early as line C.
4. Small-effect late mutants from line C, viz. CXl$_1$, CXl$_2$ and CXl$_3$ by X-rays and CEl$_1$, CEl$_2$ and CEl$_3$ by EMS. Only line CEl$_3$ flowers, at least in some seasons, as late as line 51, and therefore, it is considered as a borderline case with the large-effect late mutants.
5. In a second-cycle mutation-selection program, two revertants from line CA after EMS were studied, viz. CAE$_1$ and CAE$_2$, of which CAE$_2$ is about intermediate between the lines C and CA and CAE$_1$ is closer to CA (See further ch. 8).

3.2.2 *Fertility of the mutant lines*

In M_8, fertility and embryonic lethality were studied on 10 plants per line by means of MÜLLER's embryo-test (1963). When the fruits are opened just before ripening, one can score 1. ovules non-fertilized (dots of white tissue), 2. embryonic lethals at various stages of development (seed coat turns prematurely brown), 3. chlorophyll mutants and normal bright green embryos (visible through the transparent seed coat).

Fertility is expressed as percentage of ovules fertilized after pooling the fruits 5 and 6 over the 10 plants per line, and similarily, embryonic lethals are expressed as percentage of embryonic lethals among ovules-fertilized.

The lines C and 51 have fertility 90–95%, and about 1.0 to 1.5% embryonic lethals. Fertility of all mutant lines was over 85%, often over 90%, except for line CEl_2 (29%). This line was not discarded, while line 51C with similar sterility (but showing also chlorophyll deficiency) was. In general, fertility of the EMS-induced mutants shows the same range as that of the X-ray induced lines. In earlier generations, the percentages of selections discarded for sterility, were about equal for both mutagens.

A number of lines contained embryonic lethals, but it was not studied whether these are recessives, or are due to maternal physiological effects (cf. VAN DER VEEN, 1967). These lines are CEl_3 (25%), $51XV_2$ (9%), and $51EV_1$ (19%).

None of the mutant lines segregated chlorophyll mutants.

3.2.3 *Vernalization response*

The 22 mutant lines, in M_5, were grown from 23-3-1966, both as a vernalized and an unvernalized set, and in comparison with the lines C and 51. Each item consisted of 8 plants (in pots), and per item, 4 plants were grown in each of two adjacent blocks. In each block all $2 \times (22+2) \times 4 = 192$ plants were randomized individually. For vernalization treatment of the seeds and timing of the vernalized and the unvernalized sets, see ch. 2.4.

In the following 'good vernalization response' means that mutant lines, whether derived from line C or from line 51, are on vernalization close to the non-responding very early line C with respect to flowering time and leaf number. The results obtained can be summarized as follows:

1. Line C does not respond to vernalization. It can be taken as a 'physiological limit' towards early, flowering after 22 days and with 7 leaves (=22/7).
2. Line 51 (fl.t. 26 days/11 leaves=26/11) becomes after vernalization 23/8, which means a 'good vernalization response'.
3. Among the large-effect late mutant lines, the lines CA, 51D and 51E show a 'good response', whilst the lines 51A and 51B have a relatively small response. Several vernalization experiments were carried out with these lines, and further details are discussed in ch. 6.5 (51A and 51E) and ch. 6.6 (genotype-season interaction and vernalization response for all 5 lines).
4. The small-effect late mutant lines from line 51. In table 3, these lines together with C and 51 are arranged in the order of increasing flowering time. It is seen that vernalization response is good for leaf number, and ranges from good

to moderate for flowering time in going from the earliest to the latest mutant lines. None of the lines became as close to line C as line 51-vernalized.

5. The small-effect early mutant lines from line 51. These lines are about mid-way between the lines C and 51, i.e. rather close to C, and from this small scale experiment, response was difficult to evaluate. After vernalization, they all tended to become somewhat closer to line C, but none became as early as C. In fact, they closely resembled 51-vernalized.
6. The small-effect late mutant lines from line C. These (except for the mutant line CEI$_3$, see under 7) are about mid-way between line C and line 51, i.e. not much later than line C, and again, response to vernalization is difficult to evaluate. After vernalization they did not seem to become closer to line C, such in contrast with the early mutants from line 51 (See under 5).
7. The mutant line CEI$_3$ is the latest one of the small-effect late mutant lines from C, and is close to line 51. In contrast to line 51, it did not respond to vernalization in the present early spring experiment (Table 4). In a summer experiment (7-8-1966), it showed 'good response' (like 51), but again in a winter experiment (12-11-1966), it did not respond (unlike line 51). This is a clear case of interaction between vernalization response and season. Another case of interaction will be discussed in ch. 6.6 for the lines 51A and 51B.

TABLE 3. Vernalization response (Experiment of 23-3-1966) of the lines C and 51, and of the 5 small-effect late mutant lines from line 51, in the order of increasing flowering time. Flowering time (days) in bold face, leaf number in italics.

	C	51	51XI$_1$	51EI$_2$	51XI$_2$	51EI$_1$	51EI$_3$
Non-vern.	22	26	28	28	29	30	34
	6	*11*	*13*	*12*	*13*	*12*	*13*
Vern.	22	23	23	24	25	26	27
	7	*8*	*9*	*8*	*9*	*8*	*8*

TABLE 4. Interaction between season and vernalization response for the line CEI$_3$. Flowering time (days) in bold face, leaf number in italics.

	12-11-1966			23-3-1966			7-8-1966		
	C	51	CEI$_3$	C	51	CEI$_3$	C	51	CEI$_3$
Non-vern.	31	35	36	22	26	28	26	34	36
	7	*12*	*10*	*6*	*11*	*10*	*6*	*12*	*10*
Vern.	32	31	36	22	23	28	26	29	30
	7	*8*	*9*	*7*	*8*	*11*	*6*	*8*	*9*

4. TABLE OF EXPERIMENTS FOR GENETIC ANALYSES

The genetical analyses to be presented in the chapters 5 to 8, are based on experiments which have been conducted in different seasons, and which differ with respect to the generations entered. Although these points are explicitly stated in the text, it may be helpful to give a general survey by means of a comprehensive list (Table 5).

Throughout this thesis, the experiments are denoted by the date of germination, from which the days till the opening of the first flower on the main stem (flowering time) were counted.

TABLE 5. List of experiments for the genetic analyses.

Chapter	Date of experiment	Lines	Generations
ch. 5	20- 1-1965	C, 51	F_2
	4- 3-1965	C, 51	F_1, F_2
	24-11-1965	C_s, C, 51, 51_1	F_1-diallel
	10- 2-1966	C_s, C, 51, 51_1	F_1-diallel F_2-diallel B.C's to 51
	14- 7-1966	C_s, C, 51, 51_1, Li_2	F_1's to Li_2
	29- 9-1966	id.	F_1's to Li_2 F_2's of F_1's to Li_2 B.C.'s of F_1's to Li_2 with C_s, C, 51, 51_1
	24- 7-1967	51, (S96)	F_2
ch. 6	18- 5-1966	51, CA, 51A, 51B, 51D, 51E	F_1-diallel F_1's to C and Li_2
	11- 8-1966	51, CA, 51A, 51B, 51D, 51E	F_2-diallel
	9-10-1966	C, CA	F_1, F_2
		51, CA	F_1, F_2
	9-11-1966	51A, 51E	F_1's and F_2's to 51
	7-12-1966	51A, 51E	F_1's and F_2's to 51
	1- 2-1967	51A, 51B, 51D, 51E	F_1's and F_2's to C
	8- 3-1967	51, CA	F_1, B.C. to CA
	19- 4-1967	51A, 51B, 51D, 51E	F_1- and F_2-diallel
ch. 7	12- 5-1966	51 and its 5 small-effect late mutants	F_1-diallel
	20- 8-1966	id.	F_2-diallel
	5- 4-1967	id.	F_1's and F_2's to C
	5- 4-1967	C, CEl_3	F_1's and F_2's
	21- 7-1966	C and the 6 early mutants from 51	F_1- and F_2-diallel
	28- 7-1966	51 and the 6 small-effect late mutants from C	F_1- and F_2-diallel
ch. 8	18- 5-1967	C, CA, CAE_1, CAE_2	F_1- and F_2-diallel

5. GENETIC ANALYSIS OF THE LINES C AND 51

5.1 THE CROSS C/51

The parental lines C and 51, and their F_1 and F_2 were grown many times during the period November 1964 till August 1966 (in some of the experiments the backcrosses were added). This was done to compare the material in different seasons, under different growing conditions (e.g. in pots and in pans), and to study the effect of a leaf-size gene (See below). A representative sample of data from these experiments is given in table 6 (means and standard deviations of flowering time and leaf number of C and 51 and of two F_2-selections, in three different seasons), in fig. 1 (flowering time of these four lines plotted against leaf number), in fig. 2 (combined scores of the four lines and their six F_1's in these seasons), and in fig. 3 (the F_2 C/51 and the backcross (C/51) × 51).

The parental values and their differences vary with season. In the experiment of 10-2-1966 (ES-experiment), the parental differences (51–C) were 4 days and 5 leaves (Table 6) or 9 units combined score (Fig. 2); in the experiments of 24-11-1965 (W) and 18-5-1966 (S), these differences were 6 days and 5 resp. 8 leaves (Table 6) or 11 resp. 14 units c.s. (Fig. 2).

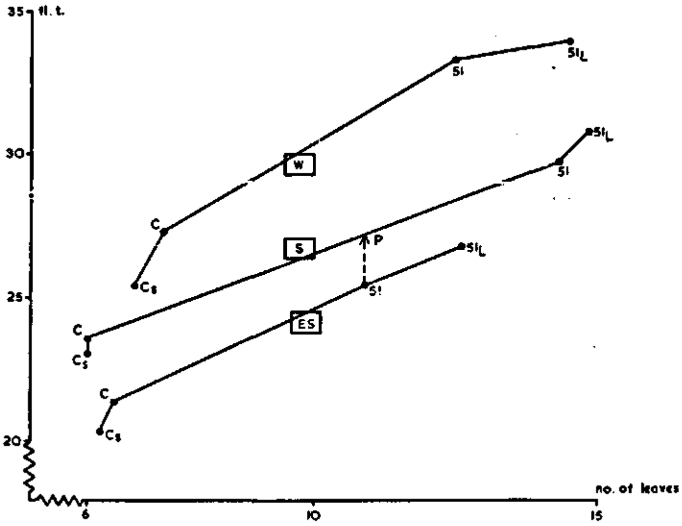
TABLE 6. Means and standard deviations for flowering time (bold face) and for number of leaves (italics) of the lines C and 51 and the two recombinant lines C_s and 51_l (selected from the F_2 C/51), grown in three seasons. Between brackets: number of plants. C_s and 51_l have small and large leaves respectively (See further text). The seasons are 'early spring' (ES; from 10-2-1966; in pots), 'summer' (S; from 18-5-1966; in pots), and 'winter' (W; from 24-11-1965; in pans).

Season	C_s	C	51	51_l
ES	20.6 ± 0.6	21.5 ± 0.5	25.6 ± 1.0	27.0 ± 1.0
	6.3 ± 0.4	*6.5 ± 0.5*	*11.4 ± 0.6*	*12.6 ± 0.9*
	(35)	(45)	(91)	(40)
S	23.2 ± 0.8	23.7 ± 0.5	30.2 ± 0.9	31.2 ± 0.9
	6.0 ± 0.3	*6.0 ± 0.3*	*14.3 ± 1.6*	*14.8 ± 1.6*
	(40)	(49)	(40)	(68)
W	25.7 ± 0.8	27.5 ± 0.9	33.5 ± 1.4	34.5 ± 1.0
	6.8 ± 0.4	*7.3 ± 0.5*	*12.5 ± 0.8*	*14.5 ± 1.2*
	(21)	(16)	(11)	(18)

On closer inspection of these three experiments (Fig. 1, constructed from table 6), and also of other experiments not presented here, two trends can be distinguished with respect to the seasonal differences per parent line and between parent lines. These trends will be discussed for the lines C and 51, whilst the selections C_s and 51_l (See later on) can be ignored for our present purpose since they closely follow C and 51 respectively. The trends are *defined* as follows:

Trend 1: 'Retarded growth'. A line flowers later in one season as compared with another season, but there is little or no increase in number of leaves.

FIG. 1. Flowering time (days) plotted against number of leaves for the lines C and 51, and for the two recombinants (C_s and 51_1) selected from F_2 C/51, grown in three seasons (ES, S and W). See also legend to table 6, from which this graph has been constructed.



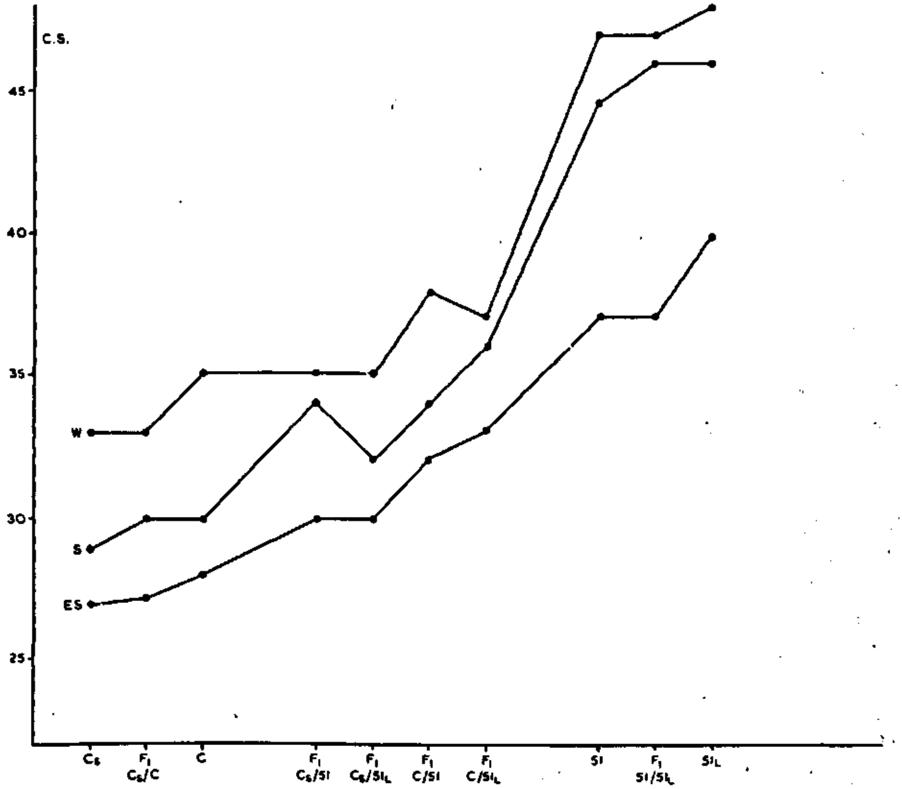


FIG. 2. Combined scores (c.s.) for the 2 parental lines (C and 51), the 2 selections (C_s and 51_1 from the F_2 C/51) and the 6 F_1's, grown in three different seasons, viz. 'early spring' (ES, from 10-2-1966; in pots), 'summer' (S, from 18-5-1966; in pots), and 'winter' (W, 24-11-1965; in pans). For number of plants of the 4 homozygotes, see table 6. The F_1's consisted of about 25 plants in the ES-experiment, of 15–25 plants in the S-experiment, and of 10–15 plants in the W-experiment.

Trend 2: 'Prolongation of vegetative development'. This involves both later flowering and increase in number of leaves.

Comparing the W-experiment with the ES-experiment (Fig. 1) one sees that in W, line C flowers 6.0 days and line 51 flowers 7.9 days later than in ES. However, the increase in leaf number of C and 51 is relatively small (one leaf only). Therefore, the predominant trend shown by W is 'retarded growth', affecting both lines.

Turning to the S-experiment in comparison with the ES-experiment, it is seen that in S, line C flowers 2.2 days later than in ES, without having more leaves. This again can be taken to reflect 'retarded growth'. Allowing for a similar amount of 'retarded growth' for line 51, i.e. transferring 51 in ES to point P in S (See fig. 1), it becomes clear that 51 also shows 'prolongation of vegetative development'. Thus, one finds that, relative to P, line 51 in S shows an extra delay in flowering time of 2.2 days, which now corresponds to 2.8 leaves. Therefore, the shift of 51 in S, is composed of both 'retarded growth' (also affecting C) and 'prolongation' (affecting 51 only).

No attempts were made to analyse these two types of response in terms of environmental components. However, as a general impression one may say that 'retarded growth' as measured by later flowering, refers back to retarded youth growth (from seedling stage onwards).

An important point which emerges from fig. 1 is that *within* each of the three experiments approximately the same proportionality exists between the *difference* in flowering time and in leaf number between C and 51; in other words the three connecting lines drawn in fig. 1 for W, S and ES do not diverge much in slope. Roughly speaking, in all three experiments one day difference in flowering time between C and 51 corresponds to a difference of one rosette leaf. The same holds when comparing C and 51 with the mutant lines derived from these, and with the different F_1's (ch. 6, where also some exceptions are mentioned). This justifies the use, in genetical analyses, of a 'combined score' in which flowering time and number of leaves are entered with equal weights, as is done by taking the sum of these two variables. See also ch. 2.7.1.

Turning to the cross C/51, it can be said that the F_1 C/51 is always closer to C, though to varying degree in different experiments (seasons; see fig. 2). No reciprocal differences were found.

The graphs of the F_2 C/51 and the backcross (C/51) × 51 (Fig. 3) were taken from the 10–2–1966 (ES-)experiment, grown in pots. The F_2 indicates a single gene segregation, since its three peaks correspond with C, F_1 and 51 respectively. This is more convincingly demonstrated by the backcross to 51. By interpolation one finds 42 plants like F_1 and 46 plants like 51 (expected 44 and 44; $\chi^2_1 = 0.18$; $P = 0.70-0.50$).

It is concluded that 51 differs from C in one major gene for flowering time and leaf number. Since 'early' is partially dominant, one denotes $C = E_1E_1$ and $51 = e_1e_1$.

From a preliminary F_2-experiment (20-1-1965) it was clear already that there was considerable segregation for leaf-size. This point deserves to be discussed in some detail.

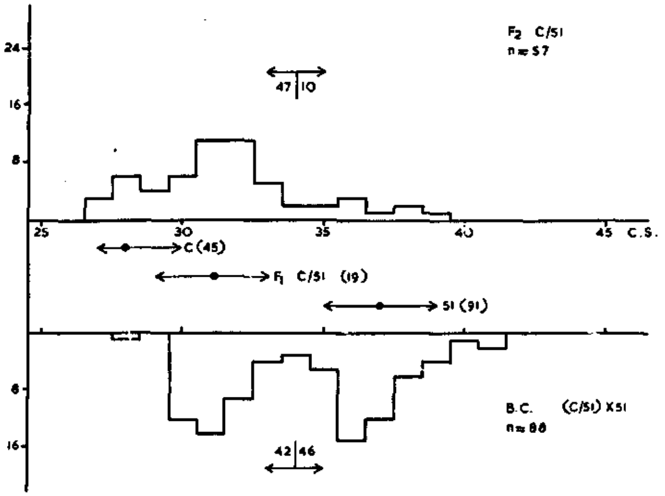


FIG. 3. Experiment of 10-2-1966. Frequency distributions (class interval 1 unit) of the combined scores (c.s.) for the F$_2$ C/51 (upper half) and the backcross (C/51) × 51 (lower half; in reverse). The parents and the F$_1$ (arrows) have been taken from the same experiment (Between brackets: number of plants).

In going from the first leaves upwards in the rosette, it is seen that leaf-size (both length and width) increases, except for the last few leaves, the size of which decreases again. When comparing leaves with the same number (counting from the cotyledons upwards), the leaves of C are considerably larger than the comparable ones of 51. Now, notably *within* line 51, a higher number of leaves implies smaller leaves at the lower nodes of the rosette. At first sight, this observation might also provide an explanation for the leaf-size differences between the lines C and 51.

However, in the F$_2$ it was apparent that among plants with a low number of leaves (C-type plants), there was considerable variation in leaf-size. The same holds for 51-like plants. Moreover, among the 51-like F$_2$-plants, the largest leaves tended to occur on plants with the highest number of leaves, which is opposite to the trend found *within* the parental line 51.

An F$_2$, size 216 plants, grown in pots from 4-3-1965, was grouped according to leaf number class. Within each class, leaf-size was classified visually by 3 observers independently from each other. The three classifications agreed almost completely. Among the 216 plants, 58 plants were having large leaves (expected 54; $\chi^2_1 = 0.39$; $P = 0.70-0.50$). Therefore, the contrast 'large leaves' (from C) versus 'small leaves' (from 51) is governed by a single gene difference. Furthermore, the range of variation among the plants with small leaves indicated incomplete dominance of small.

Attempts to replace this visual classification by measurements in such a way that a discrete classification on the basis of these measurements was possible, completely failed, although the F$_2$ and backcrosses were grown in several successive experiments. This is due to the fact that among plants with a given number of leaves and at a given node, variation in leaf-size of the two phenotypes was large and overlapping. Combinations of up to 4 measurements did not lead to a good criterion either, due to within plant correlations between different measurements. This is an instance where visual observation cannot easily be replaced by measurements.

To study whether the leaf-size gene can be considered as a modifier for flowering time and leaf number, the two extreme recombinant phenotypes were selected from the preliminary F_2 grown from 20-1-1965. The two selections bred true, viz. line C_s (C-type for flowering time and leaf number, but with small leaves), and line 51_1 (51-type, but with large leaves). After selfing for one more generation, a 4 × 4 F_1-diallel cross between C_s, C, 51 and 51_1 was made, and grown in different seasons to supplement the comparisons between C and 51 (Table 6 and fig. 2; W-, ES-, and S-experiments).

From table 6, it is seen that line C_s tends to flower somewhat earlier and to have slightly less leaves than line C, and that line 51_1 is somewhat later with slightly more leaves than line 51. This suggests a small effect of the leaf-size gene on flowering time and leaf number. It is not excluded yet that other modifiers giving this effect had accidently been fixed in the lines C_s and 51_1. However, in the segregating generations derived from the 4 × 4-diallel, viz. the F_2's and the backcrosses to 51, notably the F_2 C/C_s and the F_2 $51/51_1$ (not presented), showed that the large leaf segregants, indeed flowered somewhat later and had a somewhat higher number of leaves. Therefore, the leaf-size gene is a flowering time and leaf number modifier, but its effect is relatively small,

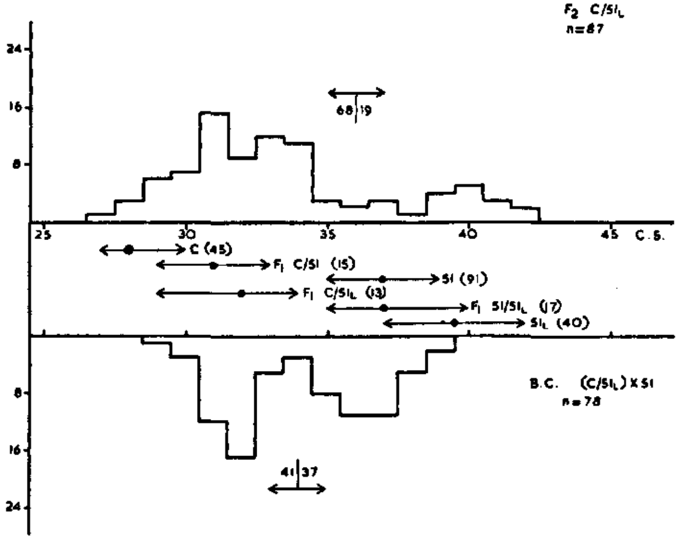


FIG. 4. Experiment of 10-2-1966. Frequency distributions (class interval 1 unit) of the combined scores (c.s.) for the F_2 $C/51_1$ (upper half) and the backcross $(C/51_1) \times 51$ (lower half; in reverse). The parents and the F_1's (arrows) have been taken from the same experiment (Between brackets: number of plants).

and can be neglected in crosses, where the segregation of $E_1 - e_1$, etc. is studied.

From 10-2-1966, all 4 parents, the 6 F_1's, the 6 F_2's and the 6 backcrosses to line 51 were grown in pots. All these families behaved as expected on the basis of the foregoing discussion. For illustration, the F_2 $C/51_1$ and the backcross $(C/51_1) \times 51$ are given in fig. 4. The range of this F_2 is well past line 51 and includes 51_1, as expected in a family homozygous for the large leaf allele.

Turning to the 4×4 F_1-diallel repeated over seasons (W-, ES- and S-experiments in fig. 2, combined score), the following points can be stated:

1. Shifts in flowering time (and leaf number) from one experiment to another follow the same direction for all families.
2. The scores of the summer (S)-experiment, are closer to those of the early spring (ES)-experiment for the E_1E_1-lines (C, C_s and their F_1), but closer to those of the winter (W)-experiment for the e_1e_1-lines (51, 51_1 and their F_1). This is a clear genotype-environment interaction.
3. The E_1e_1-heterozygotes ($C_s/51$, etc.) are relatively, but not absolutely, closer to the E_1E_1-types in winter (W) than in early spring (ES). This component of genotype-environment interaction refers to dominance relations.
4. In ES and W, the leaf-size heterozygotes tend, with respect to flowering time and leaf number, to be closer to the small leaf homozygote, (cf. F_1 C_s/C with C_s and C, and F_1 $51/51_1$ with 51 and 51_1; fig. 2). In summer (S), the reverse is true. It is interesting to note that this genotype-environment interaction (on dominance relations) does not only refer to flowering time effect of the leaf-size locus, but in a parallel way to leaf-size itself. Thus, in the 4-3-1965 experiment (See p. 21) only the large leaf homozygote could be classified as a separate group. However, in an experiment of 18-7-1965, large leaf was partially dominant, enabling e.g. in the F_2 C/C_s a classification of 17 small and 42 large-leaf plants (expected 15 and 45, $\chi^2_1 = 0.36$; $P = 0.70 - 0.50$).

In conclusion of this chapter, it can be said that line 51 differs from line C in a major gene for flowering time ($C = E_1E_1$ and $51 = e_1e_1$), and in a major gene for leaf-size, which has a small effect on flowering time.

5.2 CROSSES WITH ECOTYPE Li$_2$

5.2.1 *Introduction*

The very early lines C and C_s, and the early lines 51 and 51_1, were also studied in crosses with the medium late race Limburg-2 (Li_2). This race originates from Laibach's collection of natural races, which at present is kept in the Institute of Agronomy and Plant Breeding at Göttingen (cf. Röbbelen, 1965). From 1960 onwards, Li_2 was kept at the Department of Genetics at Wageningen by harvesting from one plant per generation, during about 10 generations.

According to van der Veen (pers. comm.), Li_2 gives an F_1 which is heterotic towards late when crossed with early types (e.g. line 51), but not when crossed with some very early types (e.g. line C). This author studied in particular the cross Li_2/Dijon (van der Veen, 1965), and found Li_2 to contain an almost completely dominant gene B for late flowering (and high number of leaves), whilst the medium early race Di contains a gene A for late. Gene A has only a small effect in the presence of bb, but a large effect, with partial dominance, in the presence of Bb or BB. Conversely, the effect of B (already large in the presence of aa) is inflated in the presence of Aa or AA. Here, heterosis towards late results from the combination of two dominant major genes, of which notably gene A is dependent for its expression on gene B: a complementary-like interaction. For the analysis, the very late homozygote S189 (AABB) and the 'early to very early' homozygote S96 (aabb) were selected from the F_2.

Now, due to the dominance of gene B, the F_1 S96/Li_2 (aaBb) coincides with Li_2 in flowering time. In later experiments (VAN DER VEEN, pers. comm.) also the lines C (very early) and 51 (early) were crossed with Li_2. The F_1 C/Li_2 was found to be only slightly earlier than Li_2. When crossing the 'early to very early' selection S96 with the very early line C, the F_1 is intermediate, the F_2 shows little genotypic variation, and the testcross $Li_2 \times (F_1$ S96/C) resembles Li_2: it is obvious that line C contains an allele similar or equal to b (write line C = bb). Finally, the F_1 51/Li_2 is heterotic, being somewhat later than Li_2.

As discussed in ch. 5.1, line 51 differs from line C at mainly one locus: e_1e_1 vs. E_1E_1, where E_1 is partially dominant. As seen above, substitution of E_1E_1 by e_1e_1 in the crossing partner of Li_2 leads to a shift from a non-heterotic F_1 C/Li_2 to a heterotic F_1 51/Li_2. Thus heterosis for flowering time results here from the joint action of a completely dominant gene for late (B) and a *partially* recessive gene for late (e_1). Of course gene B additively acting with a *completely* recessive gene for late would not give heterosis, but an F_1 which is equal to Li_2.

The situation described above led the present author to include the crosses of the lines C_s, C, 51 and 51_1 with Li_2 in his studies. Moreover, Li_2 offers an opportunity to compare an identified *dominant* major gene for late occurring in a natural race (gene B) with mutagen-induced *recessive* genes for late (such as e_1).

5.2.2 *Additional crosses*

From 14-7-1966, the 'very early' lines C_s and C, and the 'early' lines 51 and 51_1 were grown along with Li_2 and with their reciprocal F_1's to Li_2. The F_1 C_s/Li_2 and the F_1 C/Li_2 proved to be non-heterotic, the F_1 51/Li_2 and F_1 51_1/Li_2 heterotic, which is in accordance with the observations by VAN DER VEEN (See earlier). All F_1's were selfed to give F_2's, as well as backcrossed to the corresponding early parent.

These segregating generations (and again the F_1's and the parents) were grown in pots from 29-9-1966. The material was not randomized but grown in four family groups corresponding to the four early parents. Hence the slightly different ranges of Li_2 given in fig. 5. The positions of the combined scores of the F_1's relative to Li_2, in terms of heterotic and non-heterotic, are strictly comparable to those in the F_1-experiment of 14-7-1966. There was no objection to pool the data of families derived from reciprocal F_1's. Due to lack of seeds, the F_1 C/Li_2 was not grown simultaneously, but had to be transferred from the 14-7-1966 experiment by linear interpolation between C and Li_2 (See vertical

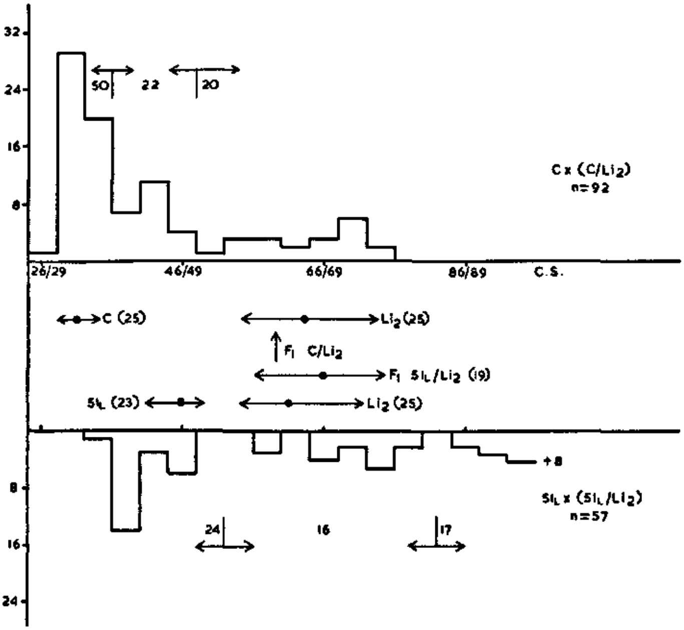


FIG. 5. Experiment of 29-9-1966. Frequency distributions (class interval 4 units) of the combined scores (c.s.) for the backcross C×(C/Li_2), and the backcross 51_1 × (51_1/Li_2). Interpolation was done in graphs with one unit class interval. In the backcross 51_1 × (51_1/Li_2), no c.s. could be given for 8 late plants (See text). Ranges of parents and F_1 are indicated by horizontal arrows (Between brackets: number of plants). The F_1 C/Li_2 (vertical arrow) had been transferred from the F_1 experiment of 14-7-1966 (See text).

arrow in fig. 5). The combined score distributions for the backcrosses C × (C/Li_2) and 51_1 × (51_1/Li_2) are given as an illustration in fig. 5, where the class interval is 4 units. Interpolation to obtain phenotypic frequencies was of course done in graphs with a class interval of one unit, where in fact, the points of interpolation could be established with little ambiguity. After day 58, leaf counting was suspended, so that e.g. for 8 plants in the backcross 51_1 × (51_1/Li_2) only flowering time is known, and consequently no combined score could be calculated (cf. fig. 5). This, however, did not affect the analysis. (Where a high number of leaves is difficult to count, due to decay of the bottom leaves, the obvious solution is to mark, during the course of growth, leaves with a specified number).

5.2.3 *Hypothesis*

For ease of presentation of the analysis, the genetical hypothesis to be constructed from the data will be presented first. For this hypothesis the reader is referred to table 7, where the effects and interactions of the genes are described in the legend. The presentation of the experimental data will follow in ch. 5.2.4.

TABLE 7. Hypothetical relationships between race Li_2, selection S96, and lines C and 51. The bb genotypes gradually range from very early (line C) to early (line 51). In the presence of the dominant gene B for late, E_1E_1 can still be called early (though it is clearly later than C), $E'_1.$ is medium late and e_1e_1 is late. In the $E'_1.$-group variation ranges from somewhat earlier than Li_2 (cf. the non-heterotic F_1 C/Li_2) to somewhat later than Li_2 (cf. the heterotic F_1 $51/Li_2$). For ease of comparison the combined scores as found in the 29-9-1966 experiment are given between brackets. Those of S96 and F_1 $S96/Li_2$ have been interpolated from other experiments.

	E_1E_1	$E_1E'_1$	$E'_1E'_1$	E'_1e_1	e_1e_1
BB			$Li_2(62)$		
Bb	early (42)		medium late $C/Li_2(59)$ $S96/Li_2(62)$ $51/Li_2(68)$		late (96)
bb	very early C(32)		very early to early S96(37)		early 51(44)

The hypothesis is based on the following considerations:

1. Given C = bb and Li_2 = BB (as stated earlier), C and Li_2 must differ at a 'second locus', as the F_2 ratio corresponds to 7 very early and early: 9 medium late (like F_1 and Li_2). See experiment 1 in ch. 5.2.4.
2. As S96 and Li_2 differ at only one locus (as discussed earlier), which was denoted by b–B, it follows that S96 and C, being both bb, must differ at the 'second locus'.
3. Line 51 differs from line C at locus E_1–e_1. *If* this is a third locus, then 51 and Li_2 should differ at three loci. However, 51 and Li_2 differ at two loci, giving an F_2 ratio corresponding to 4 early: (9 medium late + 3 late). See experiment 2 in ch. 5.2.4. Therefore, e_1 is a mutation either at the B–b locus or at the 'second locus'.

3a. Now, if e_1 is a mutant at the B–b locus, it follows from 2) that line 51 and S96 differ at two loci, viz. the 'second locus' and the B–b locus. The F_2 should then segregate types later than line 51.

3b. However, if e_1 is a mutation at the 'second locus', it follows from 2) that line 51 and S96 differ at only one locus, viz. the 'second locus'. The F_2 then should segregate early types only, ranging from S96-like to 51-like types.

The F_2 51/S96 actually behaves conform alternative 3b); see experiment 3 in ch. 5.2.4. Therefore, e_1 is a mutant at the 'second locus'. This second locus can be called E_1–E'_1–e_1, and one can write C = bbE_1E_1, S96 = $bbE_1'E'_1$, 51 = $bb\,e_1e_1$ and Li_2 = $BB\,E'_1E'_1$.

It will now be shown, what experimental data support this hypothesis (cf. tables 8 and 9).

5.2.4 *Experimental results*

Experiment 1

In both the C/Li_2 and the C_s/Li_2 material, a digenic segregation with complementary-like interaction was found, giving in the backcrosses to C and C_s the ratio 3 very early and early: 1 medium late (like F_1) and in the F_2's the ratio

7 very early and early: 9 medium late (like F_1 and Li_2) (See table 8). Points of interpolation in backcrosses and F_2's corresponded well. The backcross C × (C/Li_2) is presented in fig. 5. Here a further point of interpolation was indicated by a one unit class interval graph, giving 50 very early (like C), 22 early and 20 medium late plants (like F_1) which correspond to the ratio 2 (bb):1 (E_1E_1 Bb): 1 ($E_1E'_1$ Bb). This means that B has a relatively small effect in the presence of E_1E_1 and a relatively large effect in the presence of $E_1E'_1$ (cf. F_1 C/Li_2). In the F_2, however, no further interpolation (into 4:3:9 ratio) was possible, probably due to the presence of bb $E'_1E'_1$. Corresponding to the fact that the F_1 C/Li_2 is somewhat earlier than Li_2, the F_2 graph (not presented) extends somewhat past the backcross graph in the direction of late.

TABLE 8. Experiment 1. Numerical results of interpolation in backcross- and F_2-generations of the crosses C/Li_2 and C_1/Li_2; $C = E_1E_1$ bb, $Li_2 = E'_1E'_1$ BB.

Generation		Very early (bb) + early (E_1E_1 B.)	Medium late (E'_1. B.)	χ^2_1	P
C × (C/Li_2)	obs.	72	20		
	exp. (3:1)	69	23	0.52	0.50–0.30
C_1 × (C_1/Li_2)	obs.	36	8		
	exp. (3:1)	33	11	1.09	0.30–0.20
F_2 C/Li_2	obs.	39	55		
	exp. (7:9)	41	53	0.18	0.70–0.50
F_2 C_1/Li_2	obs.	44	53		
	exp. (7:9)	42	55	0.17	0.70–0.50

Experiment 2

Also in the $51/Li_2$ and $51_1/Li_2$ material, a digenic segregation is indicated, giving in the backcrosses a ratio of 2 early (like 51 and earlier): 1 medium late (like F_1): 1 late, and expected to give in the F_2's a ratio of 4 early: 9 medium late (like F_1 and Li_2): 3 late (See table 9). However, in the F_2's no unambiguous point of interpolation could be found between the medium late and late group, although there is little doubt about the presence of these two groups in the F_2-graph when comparing it with the backcross-graph. In this cross the interaction between the genes is not of a complementary nature, as lateness is here conditioned by a dominant gene (B) and a recessive gene (e_1). Among the graphs, the backcross 51_1 × ($51_1/Li_2$) has been chosen to illustrate some significant points (Fig. 5; cf. also the diagram in table 7). In the early group many plants are earlier than 51_1, though none is as early as C. This indicates at least partial dominance of E'_1 (from Li_2) over e_1 (from 51). The medium late group in the backcross 51_1 × ($51_1/Li_2$) is somewhat later than the medium late group in C × (C/Li_2), which corresponds to the fact that the somewhat heterotic F_1 $51/Li_2$ is later than the non-heterotic F_1 C/Li_2.

TABLE 9. Experiment 2. Numerical results of interpolation in backcross- and F_2-generations of the crosses $51/Li_2$ and $51_1/Li_2$; $51 = e_1e_1$ bb, $Li_2 = E'_1E'_1$ BB.

Generation		Early (bb)	Medium late (E'_1. B.)	Late (e_1e_1 B.)	χ^2	P
51 × ($51/Li_2$)	obs.	32	22	16		
	exp. (2:1:1)	35	18	18	1.37	0.50
51_1 × ($51_1/Li_2$)	obs.	24	16	17		
	exp. (2:1:1)	28	14	14	1.50	0.50–0.30
F_2 $51/Li_2$	obs.	25	71			
	exp. (4:(9 + 3))	24	72		0.06	0.80
F_2 $51_1/Li_2$	obs.	14	59			
	exp. (4:(9 + 3))	18	54		1.35	0.30–0.20

Experiment 3

The F_2 S96/51 along with line 51 was grown from 24-7-1967 in pans. The F_1 S96/51 and line S96 were grown in an earlier experiment by VAN DER VEEN (pers. comm.). He found that the F_1 was somewhat closer to the early parent (line S96) than to the less early parent (line 51). In the present experiment, the F_1 and the parent S96 were not grown due to lack of seeds. Since the main purpose of this experiment was to test whether the F_2 S96/51 will segregate types later than line 51 or not, it was sufficient to grow the F_2 and line 51 only.

The frequency distribution of flowering time for the F_2 S96/51 is given in fig. 6. The graph strongly suggests that types later than line 51 did not occur. Moreover, the shape of the F_2 graph suggests the segregation of 1 (like S96): 2 (like F_1) : 1 (like 51). As argued when presenting the hypothesis (ch. 5.2.3), this means that e_1 is a mutation at the 'second locus' which locus we call $E_1-E'_1-e_1$.

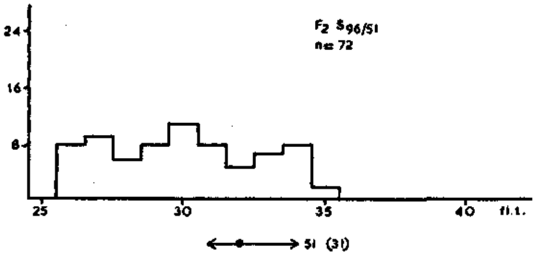


FIG. 6. Experiment of 24-7-1967. Frequency distribution (class interval 1 unit) of flowering time for the F_2 S96/51. The range of line 51 is indicated by a horizontal arrow (Between brackets: number of plants).

Finally it must be repeated that the positions of the F_1's of Li_2 (BB $E'_1E'_1$) with respectively C (bb E_1E_1), S96 (bb $E'_1E'_1$) and 51 (bb e_1e_1), do not in the first place depend on the dominance relations at the $E_1-E'_1-e_1$ locus, but rather are proportional to the effect of these alleles in the very early and early parents. This results in the non-heterotic F_1 C/Li_2 and F_1 $S96/Li_2$, and in the heterotic F_1 $51/Li_2$.

6. GENETIC ANALYSIS OF THE LARGE-EFFECT LATE MUTANTS

6.1 INTRODUCTION

In this chapter, it will be shown that the large-effect late mutant lines CA, 51A, 51B and 51E differ from their parent of origin (C or 51) each in one single gene. As here identification of individual genes is possible by means of phenotypic classification in segregating generations, one may speak of major gene mutations or macromutations. For the line 51D, no identification of one or more individual genes was possible. In this case, the phenotypic difference with the parent of origin can be considered to result from one or more minor gene mutations or micromutations. It will be remembered (See also ch. 6.4.4) that 51D is the earliest of the large-effect late mutants, and it can be taken as a borderline case with the small-effect late mutant lines.

The starting material for the genetical analyses is a 7 × 7 diallel cross between C, 51, CA, 51A, 51B, 51D and 51E. The order of presentation is as follows.

a. A discussion of the F_1's between the 7 lines (ch. 6.2).
b. The segregating generation from the cross C/CA (ch. 6.3).
c. The segregating generations from the crosses between line 51 and respectively its mutants 51A, 51B, 51E and 51D (ch. 6.4).
d. The segregating generation from the cross 51/CA, to test for allelism between the mutations in 51 and CA (ch. 6.7).
e. The segregating generations from the crosses between on the one hand 51A, 51B and 51E and on the other hand C and CA, to test whether the former three lines contain mutations allelic to the mutations in 51 or CA (ch. 6.8).
f. The segregating generations from the crosses between 51A, 51B and 51E to test for mutual allelism (ch. 6.9).

6.2 THE 7 × 7 F_1-DIALLEL FOR THE LATE MUTANTS

6.2.1 *Presentation of the F_1-data*

A 7 × 7 F_1-diallel cross was made between line C, its first-cycle mutant lines 51 and CA, and its second-cycle mutant lines 51A, 51B, 51D and 51E. The material was grown from 18-5-1966 (in pots), in a randomized block with plots consisting of 2 lines and their reciprocal F_1's, each entered with 5 plants. The cross C/51 was missing due to lack of seeds, but its position between C and 51 could be obtained by linear interpolation from several other experiments involving these two lines and their F_1's.

The line means for flowering time and number of leaves were then obtained from 30 plants (or in the case of C and 51, from 25 plants), and the F_1 means from 10 plants. The reciprocals were pooled as there were no reciprocal differences. The data are given both in table 10 and in fig. 7. At the same time (18-5-1966), but in adjacent position in the greenhouse, the ecotype Limburg-2 (Li_2) and its F_1's with CA, 51A, 51B, 51D and 51E were grown in a similar design.

The F_1's of Li_2 with C and 51, however, were grown from 14-7-1966. Their data were added to the 18-5-1966 experiment by linear interpolation with the help of the lines C, 51 and Li_2. In this way the 7 × 7 diallel was extended by means of a Li_2-array to an 8 × 8 diallel. This Li_2-array has been added in table 10 and fig. 7.

TABLE 10. Means of flowering time (normal figures) and number of rosette leaves (italics) for the 7 × 7 F_1-diallel (18-5-1966), extended by the Li_2-array (18-5-1966) to an 8 × 8 diallel. The F_1-data for C/51, C/Li_2 and 51/Li_2 were transferred from other experiments by linear interpolation (See text). Parental means from 25 or 30 plants, all F_1-means from 10 plants (reciprocals pooled). For ease of inspection, the parental means are given at the entries instead of in the diagonal, and the F_1-means are entered twice (in reciprocal positions). For subdivision of the diallel table see text.

		C	CA	51	51D	51E	51B	51A	Li_2
	self.	25	38	30	34	38	43	42	36
		6	*22*	*12*	*16*	*17*	*29*	*28*	*27*
C	25	–	27	27	27	28	27	31	34
	6	–	*8*	*8*	*9*	*8*	*8*	*13*	*23*
CA	38	27	–	30	30	30	32	36	41
	22	*8*	–	*12*	*12*	*12*	*13*	*21*	*31*
51	30	27	30	–	30	32	30	36	39
	12	*8*	*12*	–	*12*	*13*	*12*	*21*	*31*
51D	34	27	30	30	–	33	30	36	35
	16	*9*	*12*	*12*	–	*13*	*14*	*22*	*28*
51E	38	28	30	32	33	–	31	36	38
	17	*8*	*12*	*13*	*13*	–	*13*	*20*	*27*
51B	43	27	32	30	30	31	–	35	42
	29	*8*	*13*	*12*	*14*	*13*	–	*23*	*32*
51A	42	31	36	36	36	36	35	–	45
	28	*13*	*21*	*21*	*22*	*20*	*23*	–	*36*
Li_2	36	34	41	39	35	38	42	45	–
	27	*23*	*31*	*31*	*28*	*27*	*32*	*36*	–

6.2.2 *Interpretation of the F_1-data*

Table 10 and fig. 7 are two ways of presenting the same data for flowering time and number of leaves.

Leaving apart Li_2 for the moment, i.e. confining ourselves to the 7 × 7 diallel, it appears from table 10 and fig. 7 that the F_1-means can be grouped in a systematic way, as will be described in detail. At the same time, one can attempt to give one or more hypotheses about the actions and interactions of the mutant genes.

For ease of presentation, and also in order to avoid alternative hypotheses which have to be rejected later on, genotypes will be assigned to the mutant

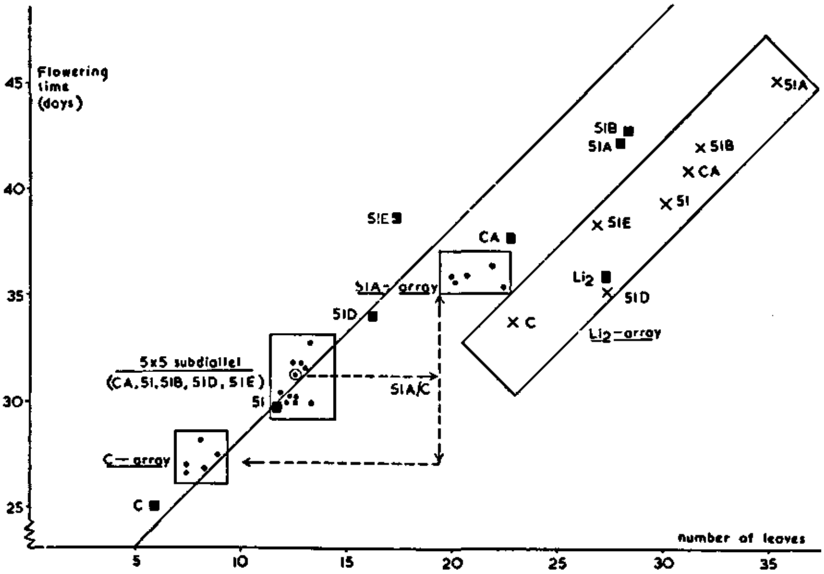


FIG. 7. Mean flowering time and number of leaves plotted for the 7 × 7 F_1-diallel and the Li_2-array (18-5-1966). The F_1-data for C/51, C/Li_2 and 51/Li_2 were transferred from other experiments by linear interpolation (See text). Parental means (■) from 25 or 30 plants, all F_1-means (● for the 7 × 7 diallel, × for the Li_2-array) from 10 plants. Note the separate F_1-positions of the 51A- and C-arrays, and in particular the position of F_1 51A/C (arrows, see further text). A line slope unity has been added to compare the mutant lines derived from C and 51. Note the relative position of the Li_2-array.

lines in agreement with the results of an extensive analysis of segregating generations to be presented later (ch. 6.3 and onwards). This means that the one hypothesis is given, which will be confirmed by further analysis.

These genotypes are:

C = E_1E_1 E_2E_2 E_3E_3 E_4E_4 E_5E_5 E_6E_6
CA = e_2e_2
51 = e_1e_1
51A = e_1e_1 e_3e_3
51B = e_1e_1 e_4e_4
51D = e_1e_1 e_5e_5?
51E = e_1e_1 e_6e_6

Thus, C differs from CA in one locus (E_2—e_2), and from 51 in a different locus (E_1—e_1). Line 51 differs monogenically from the mutant lines derived from it, viz. 51A, 51B and 51E. (For 51D no major gene could be identified, but for the moment a single gene difference will be assumed). This implies that C and CA differ from these three mutant lines in 2, respectively 3 loci.

Secondly, the dominance relations inferred from the monoheterozygote F_1's are as follows:

a. E_1 is incompletely dominant over e_1; cf. the position of the F_1 C/51 between C and 51 (See also ch. 5.1).

b. E_2 is almost completely dominant over e_2; cf. the position of the F_1 C/CA between C and CA (See also ch. 6.3).

c. E_3 is *not* dominant over e_3; the F_1 51/51A is *intermediate* between 51 and 51A (See also ch. 6.4.1).

d. E_4, E_5 and E_6 are virtually completely dominant over respectively e_4, e_5 and e_6; the F_1 51/51B, the F_1 51/51D and the F_1 51/51E are very near to line 51 (See also ch's 6.4.2, 6.4.4 and 6.4.3 resp.).

The positions of all other F_1's, i.e. the diheterozygotes and triheterozygotes, are conform (on an additive between locus base) to those of the monoheterozygotes, as will now be shown with the help of table 10 and fig. 7, where it is seen that the F_1's fall in a number of distinct groups.

The C-array. Not only the monoheterozygote F_1's between C and the mutant lines derived from it, viz. F_1 C/51 and F_1 C/CA, are near line C, but also the diheterozygote F_1's between C and three mutant lines derived from line 51, viz. F_1 C/51B, F_1 C/51D and F_1 C/51E. E.g. F_1 C/51B $= E_1e_1\ E_4e_4$, where e_1 expresses itself only slightly (See a) and e_4 does not express itself (See d). For the F_1 C/51A see later.

The 4 × 4 subdiallel of F_1's. Not only the monoheterozygote F_1's between 51 and three mutant lines derived from 51, viz. F_1 51/51B, F_1 51/51D and F_1 51/51E, are near 51, but also the 3 diheterozygote F_1's between 51B, 51D and 51E. E.g. F_1 51B/51E $= e_1e_1\ E_4e_4\ E_6e_6$, where e_4 and e_6 do not express themselves (See *d*).

The CA-array. The monoheterozygote F_1 C/CA is excepted from this group, as it belongs to the C-array (See above). Now the diheterozygote F_1 51/CA $= E_1e_1\ E_2e_2$ is *not* near C, as E_1 and E_2 are not completely dominant (See a and b). The result is that this F_1 happens to be near 51. The triheterozygote F_1's between CA and three mutant lines derived from 51, viz. F_1 CA/51B, F_1 CA/51D and F_1 CA/51E, are also near 51, since they are all $E_1e_1\ E_2e_2$ (51-level) and since in heterozygotes e_4, e_5 and e_6 do not express themselves (See d).

The 5 × 5 subdiallel of F_1's. From the discussion of the 4 × 4 diallel and of the CA-array it follows, that all 10 F_1's between CA, 51, 51B, 51D and 51E cluster near line 51. Therefore, this F_1 cluster will be denoted as the 5 × 5 subdiallel of F_1's.

The 51A-array. The monoheterozygote F_1 51/51A is intermediate between 51 and 51A, as stated under c. Now the diheterozygotes F_1 51A/51B, F_1 51A/51D and F_1 51A/51E are also near the F_1 51/51A. E.g. F_1 51A/51B $= e_1e_1\ E_3e_3\ E_4e_4$, where e_4 does not express itself (See d), and E_3e_3 is intermediate between E_3E_3 and e_3e_3 (See c). This leads to equality with the F_1 51/51A $= e_1e_1\ E_3e_3\ E_4E_4$. Similarly, the triheterozygote F_1 51A/CA $= E_1e_1\ E_2e_2\ E_3e_3$ equals F_1 51/51A $= e_1e_1\ E_2E_2\ E_3e_3$, as $E_1e_1\ E_2e_2 = F_1$ 51/CA is at the same level as $e_1e_1\ E_2E_2 = 51$, see also ch. 6.8.2. All F_1's of 51A except F_1 C/51A are in one cluster.

The F_1 C/51A. The diheterozygote F_1 C/51A $= E_1e_1\ E_3e_3$ is not equal to but much earlier than F_1 51/51A $= e_1e_1\ E_3e_3$, since E_1 is partially dominant over e_1 (See a). The result is that the F_1 C/51A, in fig. 7 indicated by ⊙, happens to coincide with the cluster of the 5 × 5 subdiallel of F_1's.

In conclusion, given the genotypes of the 7 lines, and after inferring the dominance relations at each of the 6 loci from the position of the monoheterozygote F_1's relative to the parents, one can understand the relative position of all other F_1's in a simple way: in di- and triheterozygotes the effects at the individual loci are additive. As said above, the justification of the parental genotypes assigned, will be given in ch. 6.3 and onwards, where the data of the segregating generations are presented.

The Li_2-array. Among the 6 induced mutations for later flowering and higher number of leaves, 5 are partially or wholly recessive, and one, at locus E_3-e_3 (line 51A), showed an intermediate effect. Li_2 contains a *dominant* gene (B) for late flowering, as discussed in ch. 5.2, where the crosses C/Li_2 and 51/Li_2 are presented. As it seemed interesting to study the effects of the mutations in the presence of the gene B, the Li_2-array was added to the 7 × 7 diallel. In ch. 5.2.2, it was concluded that Li_2 also differs from C in a gene at the E_1-e_1 locus, viz. gene E'_1, in the order of increasing lateness, $E_1-E'_1-e_1$.

Thus $Li_2 = BB\ E'_1E'_1$, $C = bb\ E_1E_1$, and $51 = bb\ e_1e_1$, where E'_1 is (almost) completely dominant over both E_1 and e_1, so that F_1 51/Li_2 is somewhat later than F_1 C/Li_2, and the first F_1 is slightly heterotic, whilst the second F_1 is not (cf. fig. 7). Now among the loci E_2-e_2 till E_6-e_6 only one may coincide with the B-b locus. This point has not been studied, as no F_2's between Li_2 and CA, 51A, 51B, 51D and 51E were studied. In the present context it will, however, not make much difference when one assumes none of the mutations e_2 till e_6 to be at the B-b locus.

It is seen from fig. 7 that relative to line C and its first- and second-cycle mutants, the Li_2-array is shifted to the right. As a line slope unity can be fitted to Li_2 and its F_1's, one day difference in flowering time still corresponds with one more leaf in this array. This means that at the early stages of development, leaf production of Li_2 and its F_1's must proceed at a higher rate. In this respect either gene B, or other genes in the background of Li_2, are dominant over respectively gene b or other genes in the background of line C and its first- and second-cycle mutants. Comparing the positions of F_1 Li_2/51D, F_1 Li_2/51E and Li_2/51B, it is seen that e_5, e_6 and e_4 are not completely recessive in Li_2 background. The F_1 Li_2/51A is the latest flowering, which of course reflects the intermediate inheritance at the E_3-e_3 locus.

6.3 THE CROSS C/CA

From 9-10-1966, the F_2 C/CA, size 178 plants, was grown in rows of 10 pots as follows: 1 row C, 1 row CA, 1 row F_1, 5 rows F_2, which sequence was replicated 4 times.

Like in the F_1-experiment of 18-5-1966, the F_1 C/CA flowered slightly later than line C. The F_2 (Fig. 8) clearly indicates segregation for one major gene, as interpolation gives 142 plants resembling C and F_1, and 36 plants resembling CA (expected 134 and 44 plants; $\chi^2_1 = 1.84$; $P = 0.20-0.10$).

It should be noted however, that in the early group plants occur which are

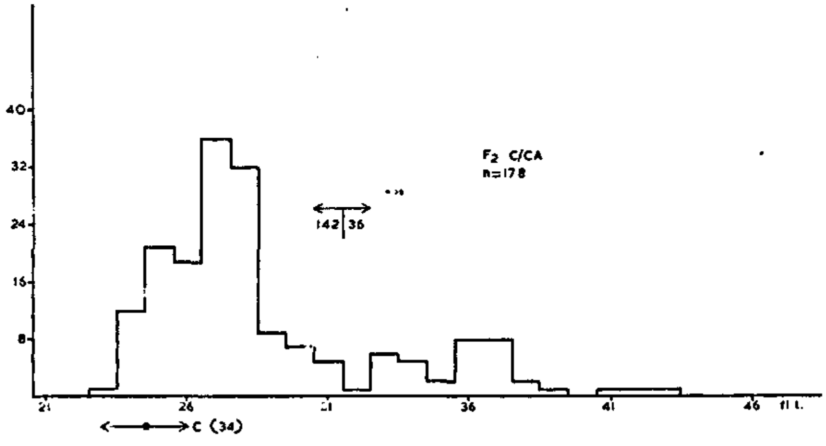


FIG. 8. Experiment of 9-10-1966. Frequency distribution (class interval 1 unit) for flowering time (fl.t.) of the F_2 C/CA. Ranges of parents and F_1 C/CA are indicated by horizontal arrows (Between brackets: number of plants).

clearly later than the F_1, and that in the later group, plants occur which are clearly earlier than CA. This indicates segregation at one or more modifier loci, which also complicates the analysis of crosses between CA and other mutant lines.

The same F_2, along with C, CA and F_1, were grown from 18-5-1967 in an experiment with reversed mutants from CA (See ch. 8.2.1). An F_2-graph for combined score (not presented) showed precisely the same features as the present F_2-graph for flowering time. The 1967 F_2 gave by interpolation 90 early and 26 late plants (expected 87 and 29; $\chi^2_1 = 0.41$; $P = 0.70-0.50$).

In conclusion, one can say that the mutant line CA differs from line C in one almost completely recessive gene, in addition to which modifier segregation is apparent.

6.4 THE CROSSES OF LINE 51 WITH ITS MUTANT LINES 51A, 51B, 51E AND 51D

In the following it will be shown that the late mutant lines 51A, 51B and 51E each differs in one major gene from the parental line 51, and that 51D may differ from line 51 in two recessive genes, rather than one major gene.

The F_2's between line 51 and its late mutants were grown from 11-8-1966 (Table 5), as part of a 6 × 6 diallel (without reciprocals) between CA, 51 and the 4 mutants from 51. Each F_2 was grown in 5 pans of 30 plants each to which 6 plants of line 51 were added. Similarly, 2 pans of each parent were grown. All pans were randomized into one block. In the F_2 flowering time graphs, the positions of the F_1's were added from the 18-5-1966 experiment by means of linear interpolation between 51 and the corresponding parent. The justification of this is given in ch. 6.6.

6.4.1 *The cross 51/51A*

The F_1 51/51A is about intermediate between the two parents (See table 10 and fig. 7). The shape of the F_2-graph for flowering time, size 143 plants (Fig. 9a), clearly indicates segregation of one major gene with intermediate heterozygote. Numerical interpolation is only feasible between the early group (like 51) and the medium late group (like F_1). The result is 32 early plants and 111 medium late and later plants (expected 36 and 107; $\chi^2_1 = 0.90$; $P = 0.50-0.30$). The transgression past 51A is due to a soil-born infection which especially delayed some of the late flowering plants. The parent 51A was not infected.

The F_2 51/51A, and also the parents and the F_1, were repeated from 9-11-1966 and also from 7-12-1966 (See fig. 11). In these F_2's, interpolation between early (like 51) and medium late (like F_1) was easy again. Moreover, the F_2 distribution now showed a gap of several days between the medium late (like F_1) and the late group (like 51A). For further discussion of this winter gap see ch. 6.5. The results for the 9-11-1966 experiment (192 F_2-plants) were 45, 105 and 42 plants, and for the 7-12-1966 experiment (191 F_2-plants; see fig. 11) 45, 100 and 46 plants. Jointly, 90, 205 and 88 plants like resp. parent 51, F_1 and 51A (expected 96, 192 and 96; $\chi^2_2 = 1.76$; $P = 0.50-0.30$). The two F_2's did not show any transgression past 51A, confirming that the slight transgression in the 11-8-1966 experiment has no genetic basis.

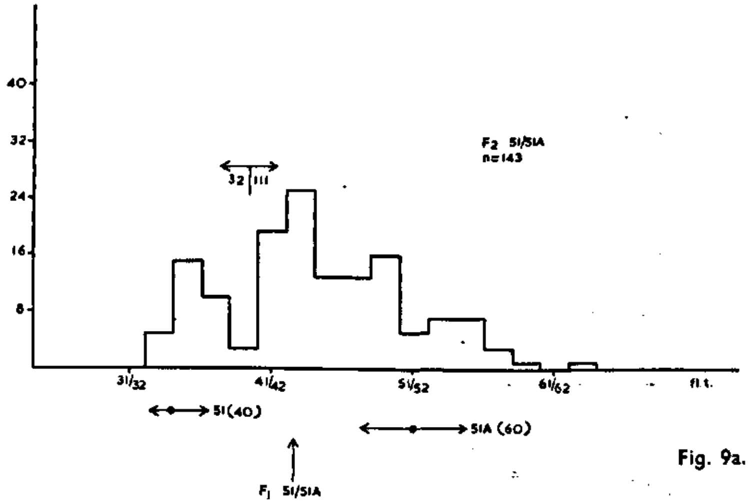


Fig. 9a.

FIG. 9. a-d. Experiment of 11-8-1966. Frequency distributions for flowering time (fl.t.) of the F_2 51/51A (a), the F_2 51/51B (b), the F_2 51/51E (c) and the F_2 51/51D (d). Class interval 2 units, except for F_2 51/51D (1 unit). Horizontal arrows indicate the ranges of the parents (Between brackets: number of plants). The positions of the F_1's (vertical arrows) have been transferred by means of linear interpolation from the F_1-experiment of 18-5-1966 (Table 10 and fig. 7, ch.6.2).

In conclusion: it can be said that the late mutant line 51A differs from the parental line 51 in one major gene (e_3e_3) with an intermediate expression of the heterozygote.

6.4.2 *The cross 51/51B*

The F_1 51/51B coincides with the position of line 51 (See table 10 and fig. 7). The F_2-graph for flowering time (146 plants; fig. 9b) clearly indicates segregation for one major gene, viz. 113 plants like 51 and F_1, and 33 plants like 51B (expected 109.5 and 36.5; $\chi^2_1 = 0.45$; $P = 0.50$).

Like in the F_2 51/51A, also in the present F_2 51/51B, the transgression past the late parent (51B) is due to a slight soil-borne infection, while the late parent itself was not infected.

In conclusion: one can say that the late mutant line 51B differs from line 51 in one completely recessive major gene (e_4e_4).

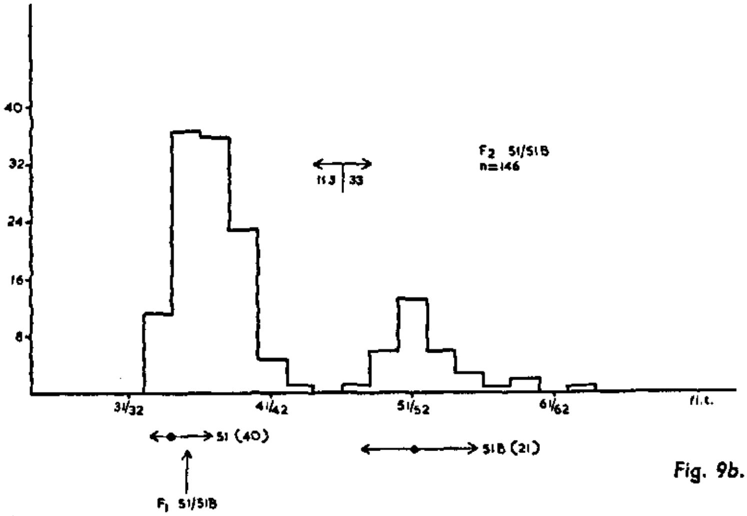


Fig. 9b.

6.4.3 *The cross 51/51E*

The F_1 51/51E is close to line 51 (See table 10 and fig. 7). Although the mutant line 51E is medium late, and in fact only a few days later than line 51D, the F_2-graph (146 plants; fig. 9c) shows clear bimodality, and by interpolation one obtains 115 plants like line 51 and F_1, and 31 plants like parent 51E (expected 109.5 and 36.5 plants; $\chi^2_1 = 1.11$; $P = 0.30-0.20$).

The F_2 51/51E, and also the parents and the F_1, were repeated from 9-11-1966, and also from 7-12-1966 (See fig. 10). The graphs show clear bimodality and by interpolation one obtains for the 9-11-1966 experiment (189 F_2-plants), 151 plants like line 51 and F_1, and 38 plants like 51E, and for the 7-12-1966 experiment (191 F_2-plants) respectively 143 and 48 plants. Jointly, 294 and 86 plants (expected 285 and 95; $\chi^2_1 = 1.14$; $P = 0.30-0.20$).

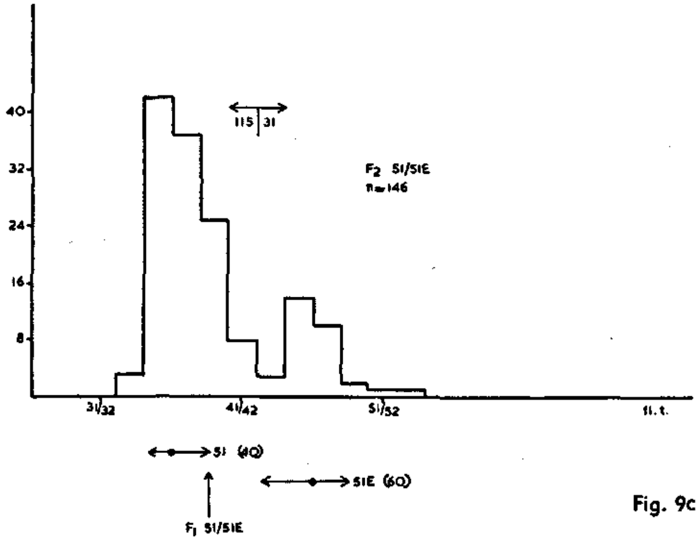


Fig. 9c.

It can be concluded that the late mutant line 51E differs from line 51 in one completely recessive major gene (e_6e_6).

6.4.4 *The cross 51/51D*

The F_2-graph (Fig. 9d; 141 plants) is based on a single unit scale for flowering time, as line 51D is the earliest of the 4 mutant lines from 51, and consequently the F_2 has a smaller range.

The F_1 51/51D is close to 51. The F_2-graph shows little or no indication of bimodality and numerical interpolation is hazardous. The graph indicates a

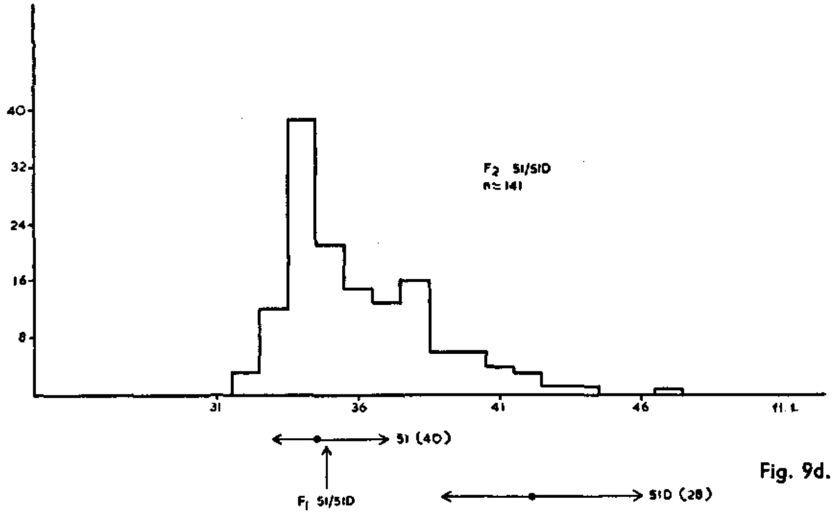


Fig. 9d.

shortage of plants flowering simultaneously with the parent 51D, at least when one expects a 3:1 ratio. There are two possibilities:

1. Line 51D contains one major gene plus one or more modifiers towards late.

When tentatively shifting 51D 2 days in the early direction, one can partition 13 plants as later than the new parental mean, and thus estimate $2 \times 13 = 26$ plants as comparable with 51D. This still gives a considerable shortage compared with the expected $1/4 \times 141 = 35$ plants.

2. Line 51D differs from 51 in two or more minor genes. In order to argue from a contrasting hypothesis, assume 2 genes with equal effect, and which are completely recessive (since the F_1 coincides with 51). If not linked, one expects a 9:6:1 ratio. Relative to the mid-point of 51D, 4 to 5 plants are later, that is 9 plants coincide with 51D. This is the closest possible fit to the expected $1/16 \times 144 = 9$ plants.

The F_2-graph may also be partitioned into 80 plants equal to, and 61 plants later than F_1, which corresponds to the ratio $9:(6+1)$.

In ch. 6.8.7 it will be shown, that also in the cross C/51D, the hypothesis of two recessive genes with equal effect, is at least as acceptable as the hypothesis of one major gene with some recessive modifier(s) towards late.

6.5 VERNALIZATION OF THE CROSSES 51/51A AND 51/51E

From 7-12-1966, the following F_2's were grown in pans, each with the corresponding parents and F_1:

F_2 51/51A non-vernalized, 191 plants (Fig. 11 top)
F_2 51/51A vernalized, 192 plants (Fig. 11 bottom)
F_2 51/51E non-vernalized, 191 plants (Fig. 10 top)
F_2 51/51E vernalized, 184 plants (Fig. 10 bottom)

The non-vernalized and vernalized sets were timed to simultaneous post-germination development (See ch. 2.4).

The experiment was carried out by the student J. J. C. JANSSEN, whose kind cooperation is acknowledged here. The two non-vernalized F_2's have already been mentioned in ch. 6.4.1 (the cross 51/51A) and in ch. 6.4.3 (the cross 51/51E).

Turning to the cross 51/51E first, one can see from fig. 10 that line 51, the F_1 51/51E, and line 51E show a pronounced response to vernalization: all three families flower clearly earlier than line 51 non-vernalized. The narrow distribution of the vernalized F_2 fits well with the almost coinciding ranges of the vernalized parents and F_1.

The cross 51/51A, however, behaves in a different way (Fig. 11). There is a clear response of 51, F_1 and 51A, and the vernalized F_1 is earlier than 51 non-vernalized. The late parent (51A) however, clearly lags behind in response. The distribution of the vernalized F_2 fits well with the ranges of the vernalized parents and F_1. Vernalization changes the 1:2:1 segregation into a 3 early: 1 late segregation (obs. 136 early and 56 late; exp. 144 and 48; $\chi^2{}_1 = 1.78$, P =

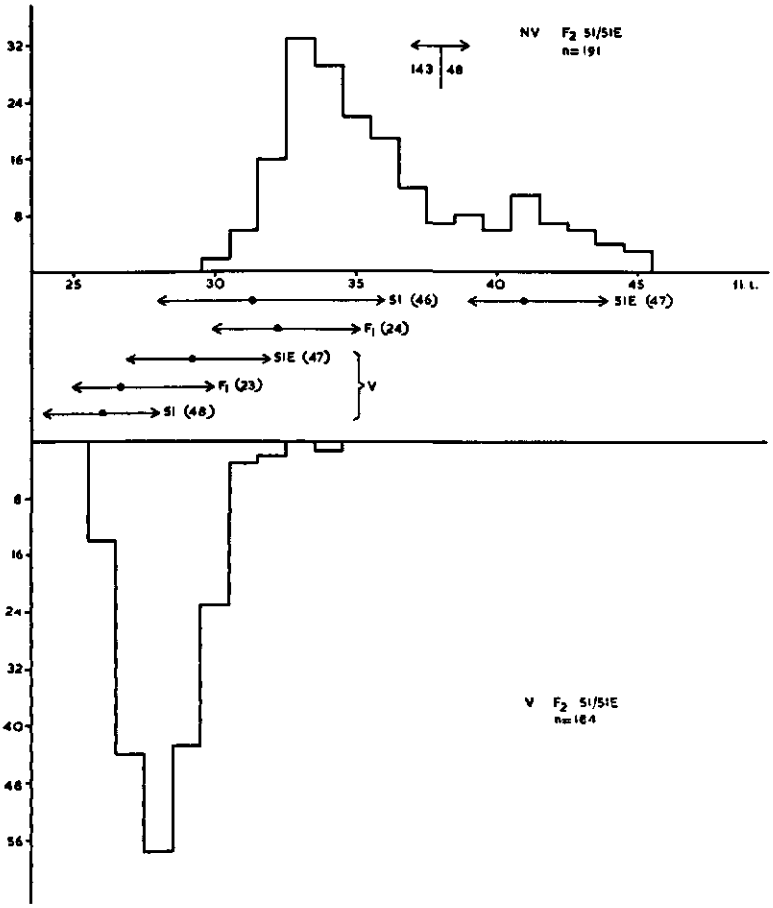


FIG. 10. Experiment of 7-12-1966. Frequency distribution for flowering time (fl.t.) of the F_2 51/51E (NV = non-vernalized; V = vernalized). The ranges of parents and F_1's (non-vernalized and vernalized) are indicated by horizontal arrows (Between brackets: number of plants).

0.30–0.20). In other words, after vernalization e_3 becomes almost completely recessive.

It must be added that the response to vernalization of line 51A is larger than that of the F_1 and of line 51, when measured in absolute number of days. However, it is clear from fig. 11 bottom, that the *relative* response of line 51A is much smaller.

The relative lack of response of line 51A can be quantitatively stated as follows:

In several vernalization experiments, line C did not show any response to vernalization. In the vernalization experiment of 7-8-1966 (ch. 6.6; fig. 12) the non-responding line C flowered 2.8 days earlier than line 51 vernalized. In that material:

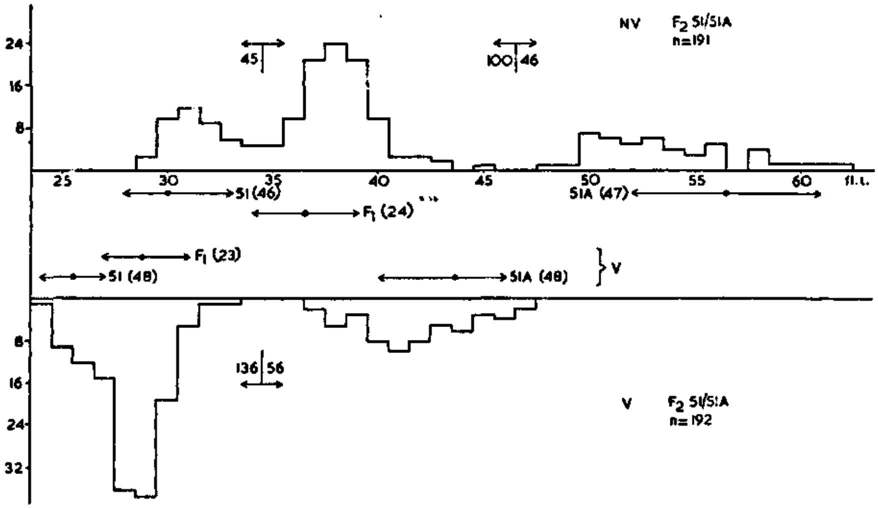


FIG. 11. Experiment of 7-12-1966. Frequency distributions for flowering time (fl.t.) of the F_2 51/51A (NV = non-vernalized; V = vernalized). The ranges of parents and F_1's (non-vernalized and vernalized) are indicated by horizontal arrows (Between brackets: number of plants).

(51–C) non-vern./(51–C) vern. = 8.7/2.8 = 3.1 and
(51A–C) non-vern./51A–C) vern. = 29.0/18.4 = 1.6
This indicates a *relatively* smaller response of line 51A.

In the present experiment (7-12-1966), line C was not grown, but assuming it to flower 2.8 days earlier than 51-vern. (as in the experiment of 7-8-1966), i.e. at day 25.5 – 2.8 = 22.7, a similar comparison can be made. One obtains:
(51–C) non-vern./(51–C) vern. = 7.3/2.8 = 2.6,
(F_1 51/51A–C) non-vern./(F_1 51/51A–C) vern. = 13.8/6.0 = 2.3 and
(51A–C) non-vern./(51A–C) vern. = 33.7/20.9 = 1.6

The parental values of both experiments (3.1 and 2.6 for line 51 and 1.6 and 1.6 for line 51A) correspond reasonably well, and the F_1 of the present experiment responds as well as line 51 (values 2.3 and 2.6 respectively). The relative response of line 51A (value 1.6) is much smaller than that of line 51 and F_1.

Since line 51A = e_1e_1 e_3e_3 (See ch's 6.2.2, 6.8.1 and 6.8.2), one may wonder whether the response of line 51A perhaps solely depends on e_1e_1 (line 51 = e_1e_1 E_3E_3 gives a clear response). In other words, one may wonder whether the genotype E_1E_1 e_3e_3 responds at all. However, this genotype is not available as a selection.

6.6 GENOTYPE-SEASON INTERACTION AND VERNALIZATION RESPONSE

Table 11 gives the mean flowering times of lines C and 51, of their late mutants, and of two F_1's, grown in 8 experiments in different seasons. Within each of the following three groups of experiments, the flowering times showed close similarity:

TABLE 11. Mean flowering time of lines C and 51, of their late mutants and of two F_1's, in three seasons. Note the differences in size of the experiments. In the experiments of 23-3-1966, 7-8-1966 and 7-12-1966, also a vernalized set was grown (See fig. 12).

Date of experiment	C	51	F_1 51/51E	F_1 51/51A	CA	51D	51E	51A	51B	Number of plants
23- 3-1966	22	26	–	–	36	33	38	41	44	8
19- 4-1967	–	30	–	–	–	34	38	41	39	50 or more
18- 5-1966	25	30	32	36	38	34	38	42	43	25 to 30 F_1's 10
7- 8-1966	26	34	–	–	43	43	43	55	55	10–15
11- 8-1966	–	34	–	–	44	42	44	52	52	30 or more
9-11-1966	–	31	32	40	–	–	40	54	–	50
7-12-1966	–	31	32	36	–	–	41	56	–	F_1's 25
1- 2-1967	27	32	–	–	–	37	39	54	54	50 or more

a. Spring experiments (23-3-1966, 19-4-1967 and 18-5-1966). Only lines C and 51 from 23-3-1966 deviate towards earlier.

b. Summer experiments (7-8-1966 and 11-8-1966). All lines show a delay compared with the spring experiments. This delay increases more or less proportionally to the degree of lateness.

c. Winter experiments (9-11-1966, 7-12-1966 and 1-2-1967). The flowering times are almost equal to those of the spring experiments, except for a pronounced delay with the latest flowering lines (51A and 51B).

Comparing the 18-5-1966 and the 7-12-1966 data for the cross 51/51A one sees that line 51 and F_1 have the same flowering time (as stated under c), but that line 51A flowers after 42 and 56 days respectively. Thus the F_1 is intermediate in the spring experiment, but relatively closer to line 51 in the winter experiment, which implies a conspicuous change in the degree of dominance. This was also reflected by a clear gap between the heterozygote (E_3e_3) and the late homozygote (e_3e_3) in the winter F_2-graph (Fig. 11; ch. 6.5).[1]

In each of the three seasons (Table 11), one experiment contained both non-vernalized and vernalized lines (though not always a complete set of vernalized lines), viz. the experiments of 23-3-1966, 7-8-1966 and 7-12-1966. Fig. 12 gives the vernalization responses (vertical bars) in these three experiments.

The lines 51, 51D, CA, 51E and the F_1's 51/51E and 51/51A, show a large response, i.e. their flowering time drops below the horizontal broken line in

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[1] In the presence of such genotype-season interaction, transfer by linear interpolation of F_1 positions from one experiment to another can lead to erroneous comparisons. Throughout this publication, F_1-positions have only been transferred between the following experiments:

a. Within the spring group from 18-5-1966 to 19-4-1967, viz. the F_1's between line 51 and its late mutants. In general, transfer between experiments within the same season can be safely done (cf. table 11).

b. From the spring group to the summer group, i.e. from 18-5-1966 to 11-8-1966. This is justifiable as all lines of the summer experiment show a relative delay which increases about proportionally in going from early to late flowering.

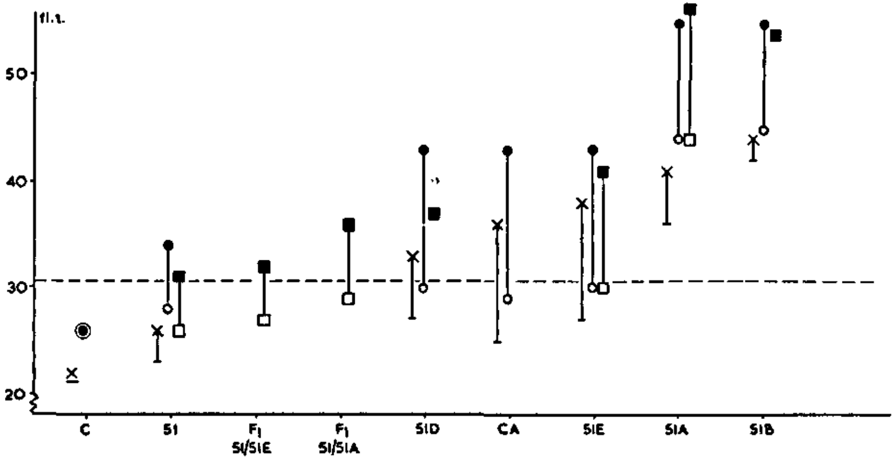

FIG. 12. Vernalization response of the lines C and 51, of their late mutants, and of the F_1 51/51A and the F_1 51/51E, in three seasons.
×–| = spring experiment (23-3-1966)
●–○ = summer experiment (7-8-1966)
■–□ = winter experiment (7-12-1966)
The broken horizontal line has been added for ease of comparison.

fig. 12. However, 51A and 51B show a relatively much smaller response (See ch. 6.5 for line 51A). It is conspicuous that the response of these two lines is very small in the spring experiment where they flower relatively early (without vernalization).

Summarizing, it can be said that the lines 51A and 51B are distinct from the other lines in three respects:

1. They are the latest flowering lines in this material.
2. In the winter experiments, they show a pronounced relative delay in flowering time.
3. They show a relative lack of response to vernalization. This response itself is again subject to interactions with season and with lateness.

The genotypes e_3e_3 and e_4e_4 behave strictly similar in these three aspects. As will be remembered the dominance relations at these two loci are strikingly different from each other. In ch. 6.9.3, it will be shown that E_3–e_3 and E_4–e_4 are closely linked.

It would be interesting to know what causal relations exist between the different aspects of relative developmental pattern. No experiments have been done to throw further light on this complex of response.

6.7 THE CROSS 51/CA

The lines 51 and CA both derive from line C and each differs from C in one major gene (ch. 5.1 and ch. 6.3, respectively). In ch. 5.1, the following notation was introduced: $C = E_1E_1$ and $51 = e_1e_1$. It now has to be tested whether or not the gene for late flowering of CA is allelic to E_1–e_1. If allelic, the F_2 51/CA should segregate 1 like 51:2 like F_1:1 like CA, and the backcross (51/CA)/CA should segregate 1 like F_1:1 like CA. Now the F_1 51/CA coincides with 51 in flowering time and in number of leaves (See table 10 and fig. 7; ch. 6.2). This means that under the one-locus hypothesis no types earlier than 51 should segregate in F_2 and backcross. Also no types are expected to be later than CA.

An F_2, size $n = 157$, grown from 9-10-1966 (simultaneously and in the same way as the F_2 C/CA; ch. 6.3), contained many plants clearly earlier than line 51, and also showed transgression towards late past line CA. This indicated digenic segregation. However, this F_2 was not very favourable for a more detailed examination, partly due to the fact that the F_1 coincides with line 51, but mainly as a result of modifier segregation. In ch. 5.1, it was shown that line 51 contains a modifier for flowering time (connected with the leaf-size), and in ch. 6.3, it was shown that CA also contains modifiers for flowering time.

For these reasons, the *backcross* (51/CA)/CA, size 235 plants, was grown in pots from 8-3-1967 along with the lines C, 51, CA and the F_1 51/CA. Fig. 13 presents the combined score of this backcross. Under the digenic hypothesis ($C = E_1E_1\ E_2E_2$, $51 = e_1e_1\ E_2E_2$, and $CA = E_1E_1\ e_2e_2$) the following types are expected to segregate:

$E_1E_1\ E_2e_2$ (like F_1 C/CA; somewhat later than C; cf. ch. 6.3)
$E_1e_1\ E_2e_2$ (like F_1 51/CA; about equal to line 51; cf. ch. 6.2, F_1's).
$E_1E_1\ e_2e_2$ (like CA)
$E_1e_1\ e_2e_2$ (later than line CA, since E_1e_1 is somewhat later than C in the cross C/51).

This is convincingly confirmed by fig. 13. The large contrast E_2e_2–e_2e_2 leads to pronounced bimodality, giving on interpolation 121 'early' and 114 'late' (expected 117.5 and 117.5; $\chi^2_1 = 0.21$; $P = 0.70$–0.50). The contrast E_1E_1–

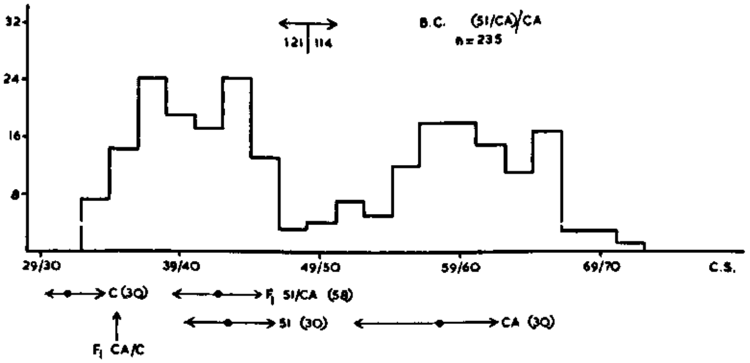

FIG. 13. Experiment of 8-3-1967. Frequency distribution (class interval 2 units) for combined score (c.s.) of the backcross (51/CA)/CA. Ranges of C, 51, F_1 51/CA, and CA, are indicated by horizontal arrows (Between brackets: number of plants). The position of F_1 C/CA (vertical arrow) has been transferred by means of linear interpolation from the 18-5-1966 experiment (Table 10 and fig. 7, ch. 6.2).

E_1e_1 is small in the cross C/51, ch. 5.1, (both E_2E_2), but in the presence of E_2e_2 and of e_2e_2 this contrast is obviously somewhat larger (cf. F_1 C/CA and F_1 51/CA; fig. 13). In the E_2e_2 group, about equal amounts of plants coincide with the range of F_1 51/CA and are earlier than F_1 51/CA, i.e. coincide with F_1 C/CA. Similarly, in the e_2e_2 group, about equal amounts of plants coincide with the range of CA and are later than CA.

In conclusion: it can be said that the lines 51 and CA differ at two loci which are not linked, or at least are not closely linked.

6.8 THE CROSSES OF THE LATE MUTANTS FROM 51 WITH THE LINES C AND CA

It will now be tested whether any of the mutations of 51A, 51B, 51E or 51D (ch. 6.4) is allelic to E_1-e_1 (contrast C–51) or allelic to E_2-e_2 (contrast C–CA).

The F_2's between line C and the four late mutants from line 51 were grown from 1-2-1967 in 5 pans of 24 plants each, while to each pan 6 plants from line 51 and 6 plants from the late mutant parent were added. Also 2 pans of each parent and 1 pan of each F_1 were grown. All pans were randomized into one block. Line 51 was added to each pan because all F_1's are near C (except the F_1 C/51A which is near 51), so that segregation of types like 51 in the F_2 is a main criterion to distinguish between allelism and digenic segregation.

The F_2's between CA and the four mutant lines were grown from 11-8-1966 as part of a 6 × 6 diallel (without reciprocals) between CA, 51 and the 4 late mutants from 51. Each F_2 was grown in 5 pans of 30 plants, and to each pan 6 plants of line 51 were added. Similarly, 2 pans of each parent were grown. All pans were randomized into one block. This experiment was affected by a soil-borne infection (See ch. 6.9), so that the cross CA/51 was repeated from 9-10-1966 (ch. 6.7) and the 4 × 4 diallel between the late mutants was repeated from 19-4-1967 (ch. 6.9). However, the F_2's between line 51 and its late mutants did not suffer much from this handicap and were used for analysis (ch. 6.4), nor did the F_2's between CA and the late mutants from 51. The F_1's of CA with 51A, etc., as well as other F_1's were added to the F_2 flowering time graphs by linear interpolation from the 18-5-1966 experiment. The justification of this was given in ch. 6.6. Finally, the position of line C was taken at day 26.5, from the almost simultaneous experiment of 7-8-1966.

6.8.1 *The cross C/51A*

It will be shown that the mutant line 51A did not arise from line 51 by a mutation at the E_1-e_1 locus. Denote $C = E_1E_1\ E_3E_3$, $51 = e_1e_1\ E_3E_3$ and $51A = e_1e_1\ e_3e_3$.

The F_1 C/51A (Fig. 14) is close to line 51, as was found earlier (ch. 6.2; F_1's).

The F_2 (Fig. 14) segregates 3 $E_3.$: 1 e_3e_3 (obs. 91 and 29 plants; exp. 90 and 30; $\chi^2_1 = 0.04$; $P = 0.90-0.80$). The large contrast between E_3e_3 and e_3e_3 is typical for the winter experiments (cf. ch. 6.6). Allelism is rejected by the following points:

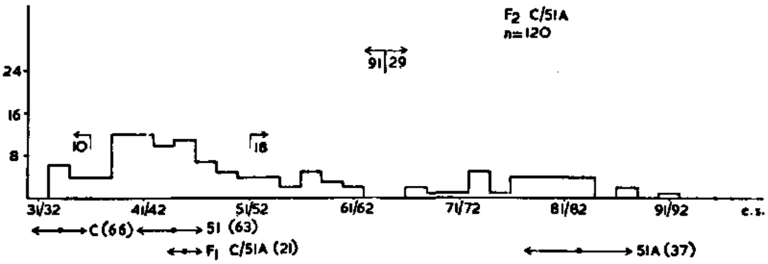

FIG. 14. Experiments of 1-2-1967. Frequency distribution (class interval 2 units) for the combined score (c.s.) of the F_2 C/51A. Ranges of C, 51, F_1 C/51A and 51A are indicated by horizontal arrows (Between brackets: number of plants).

1. Interpolation (Fig. 14) gives at the most 10 C-type plants, which corresponds to $1/16 \times 120 = 7$ to 8 plants, and not to $1/4 \times 120 = 30$ plants as expected in case of allelism.
2. Tentative interpolation (Fig. 14) among the 91 $E_3.$ plants gives 18 plants later than line 51 and the F_1 C/51A. These are not expected with allelism, and must be the recombinant e_1e_1 E_3e_3. This is the genotype of the F_1 51/51A, which is considerably later than line 51 (See ch. 6.4.1, and ch. 6.5 for a winter-experiment). With independent segregation one expects $2/16 \times 120 = 15$ plants.
3. Among the e_3e_3 plants, a good many are earlier than 51A, and thus represent the phenotype $E_1.$ e_3e_3. Interpolation is not feasible here.

In conclusion: 51A differs from line C at two loci which are not closely linked.

6.8.2 *The cross CA/51A*

It will be shown that line 51A did not arise from line 51 at the same locus as CA did from C, viz. $51A = e_1e_1$ E_2E_2 e_3e_3, and not $51A = e_1e_1$ $e'_2e'_2$. Further denote $C = E_1E_1$ E_2E_2 E_3E_3, $51 = e_1e_1$ E_2E_2 E_3E_3 and $CA = E_1E_1$ e_2e_2 E_3E_3.

The F_1 CA/51A is slightly earlier than CA (Fig. 15), and could still be accommodated by E_1e_1 $e_2e'_2$ versus E_1E_1 e_2e_2, implying intragenic complementation at an e_2–e'_2 locus. However, considerable transgression towards early (past F_1 CA/51A) and towards late (past 51A) rejects the hypothesis of allelism. The position of the F_1 can be understood as follows. The F_1 $51/CA = E_1e_1$ E_2e_2 E_3E_3 happens to coincide with line 51 (ch. 6.2). From this level, substitution of E_3E_3 by E_3e_3 raises the flowering time to the level of F_1 51/51A, which is somewhat earlier than line CA (Fig. 15). Thus the position of F_1 $CA/51A = E_1e_1$ E_2e_2 E_3e_3 corresponds to expectation based on additivity between the three loci.

The following points support the trigenic interpretation:

1. E_1e_1 E_2e_2 E_3E_3 equals line 51, as stated above, so that the other $E_1.$ $E_2.$ E_3E_3 types are expected to be earlier than line 51, i.e. $(9/16 - 4/16) \times 1/4 \times 85 =$ about 7 F_2-plants. Interpolation (Fig. 15) gives 6 plants. A combined

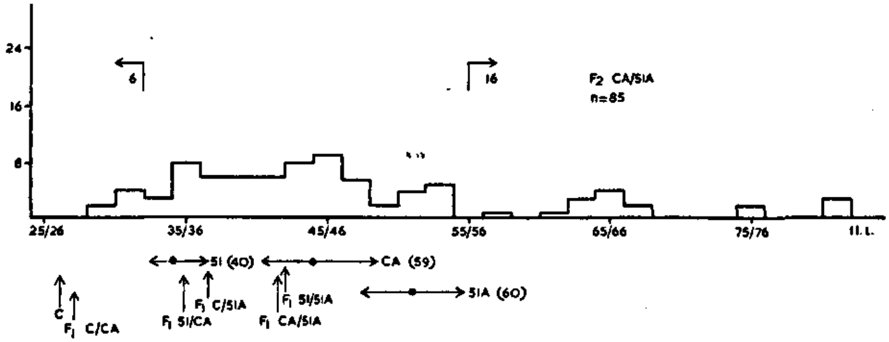

FIG. 15. Experiment of 11-8-1966. Frequency distribution (class interval 2 units) for the flowering time (fl.t.) of the F_2 CA/51A. Ranges of 51, CA and 51A are indicated by horizontal arrows (Between brackets: number of plants). The positions of the F_1 CA/51A and the other F_1's (vertical arrows) have been transferred by means of linear interpolation from the F_1-experiment of 18-5-1966 (Table 10 and fig. 7, ch. 6.2). The position of line C is put at day 26.5 from the experiment of 7-8-1966 (See text).

score graph (not presented) for the early flowering range showed 2 C-type plants (exp. $1/64 \times 85 = 1$ to 2 plants).

2. Genotypes expected to be later than 51A ($= e_1e_1\ E_2E_2\ e_3e_3$) are $e_1e_1\ e_2e_2\ e_3e_3$ (but not $e_1e_1\ E_2e_2\ e_3e_3$: dominance of E_2), $E_1.\ e_2e_2\ e_3e_3$ (as the contrast $E_1E_1 - e_1e_1$ is smaller than $E_2E_2 - e_2e_2$), and $e_1e_1\ e_2e_2\ E_3e_3$ (as the contrast $E_2E_2 - e_2e_2$ is larger than $E_3e_3 - e_3e_3$; cf CA–C with 51A–F_1 51/51A in fig. 15). This gives $(1+3+2)/64 \times 85 = 8$ plants. By interpolation (Fig. 15) one obtains 16 plants. So that, probably also $E_1e_1\ e_2e_2\ E_3e_3$ is later than 51A.

Since all genotypes expected to be earlier than line 51 arise from one or two E_2E_3 gametes, and those expected to be later than 51A arise from one or two e_2e_3 gametes, there is no indication for linkage between $E_2 - e_2$ and $E_3 - e_3$ (with close linkage a shortage of transgressive types is expected).

In conclusion: line 51A differs from line CA at three loci, which are not closely linked.

6.8.3 *The cross C/51B*

It will be shown that line 51B did not arise from line 51 by a mutation at the $E_1 - e_1$ locus. Denote $C = E_1E_1\ E_4E_4$, $51 = e_1e_1\ E_4E_4$ and $51B = e_1e_1\ e_4e_4$.

The F_1 C/51B is clearly earlier than line 51 (Fig. 16); in the F_1-experiment of 18-5-1966 (ch. 6.2) it was even closer to line C than to 51.

Due to the large contrast $E_4e_4 - e_4e_4$ (cf. ch. 6.4.2; F_2 51/51B), the graph of the F_2 C/51B shows two distinct groups, viz. 84 'early' and 33 'late' plants (exp. 88 and 29; $\chi^2{}_1 = 0.73$; $P = 0.50 - 0.30$).

Allelism is rejected by the following points:

1. Interpolation (Fig. 16) gives 4 C-type plants corresponding to $1/16 \times 117 =$ about 7 plants. With allelism, one expects $\frac{1}{4} \times 117 =$ about 29 C-type plants. With close linkage between $E_1 - e_1$ and $E_4 - e_4$ one also expects many more than 7 C-type plants.

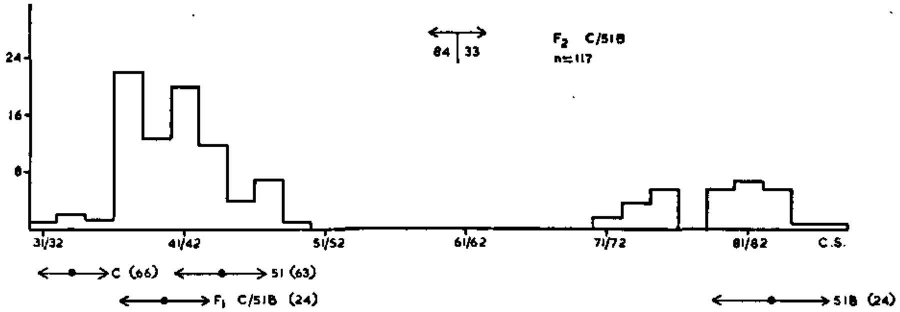

FIG. 16. Experiment of 1-2-1967. Frequency distribution (class interval 2 units) for the combined score (c.s.) of the F_2 C/51B. Ranges of C, 51, F_1 C/51B and 51B are indicated by horizontal arrows (Between brackets: number of plants).

2. Fig. 16 shows a considerable number of plants like 51, viz. the $e_1e_1E_4$. recombinants.
3. Among the e_4e_4-group, several plants are earlier than line 51B and represent the recombinants $E_1.\ e_4e_4$. Interpolation within this group is not feasible.

In conclusion: it can be said that line 51B differs from line C at two loci (E_1-e_1 and E_4-e_4), which are not closely linked.

6.8.4 *The cross CA/51B*

In ch. 6.8.3, it was tacitly assumed that 51B did not arise from line 51 at the same locus as CA did from C. Denote $C=E_1E_1\ E_2E_2\ E_4E_4$, $CA=E_1E_1\ e_2e_2\ E_4E_4$, $51=e_1e_1\ E_2E_2\ E_4E_4$ and $51B=e_1e_1\ E_2E_2\ e_4e_4$. This point will now be tested.

The F_1 $CA/51B=E_1e_1\ E_2e_2\ E_4e_4$ is not much later than line 51, i.e. much earlier than the earlier parent CA (cf. fig. 17). Its position can be understood

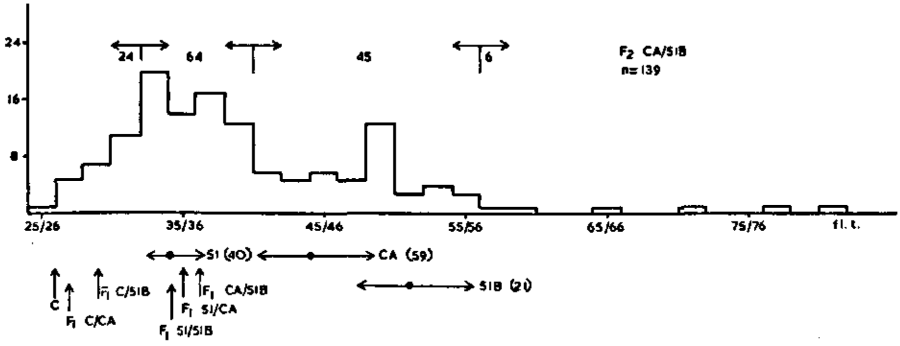

FIG. 17. Experiment of 11-8-1966. Frequency distribution (class interval 2 units) for the flowering time (fl.t.) of the F_2 CA/51B. Ranges of 51, CA and 51B are indicated by horizontal arrows (Between brackets: number of plants). The positions of the F_1 CA/51B and the other F_1's (vertical arrows) have been transferred by means of linear interpolation from the F_1-experiment of 18-5-1966 (Table 10 and fig. 7, ch. 6.2). The position of line C is put at day 26.5 from the experiment of 7-8-1966 (See text).

from the fact that the F_1 51/CA ($=E_1e_1\ E_2e_2\ E_4E_4$) happens to be equal to line 51 (ch. 6.2), and from the almost complete dominance of E_4 over e_4 (cf. F_1 51/51B, ch. 6.4.2). The effects at the individual loci appear to be of additive nature. However, the F_1-position may still be explained on the basis of allelism, with intragenic complementation towards early between e_2 and e'_2 (F_1 CA/51B $= E_1e_1\ e_2e'_2$).

Allelism, however, is rejected by the following points obtained from the F_2 (Fig. 17):

1. Types expected to be earlier than line 51 ($=e_1e_1\ E_2E_2\ E_4E_4$) and thus definitely earlier than the F_1 ($=E_1e_1\ E_2e_2\ E_4e_4$) are: $E_1.\ E_2.\ E_4E_4$, except $E_1e_1\ E_2e_2\ E_4E_4$ ($=F_1$ 51/CA, which equals line 51, see above), and $E_1.\ E_2.\ E_4e_4$, except $E_1e_1\ E_2e_2\ E_4e_4$ ($=F_1$ CA/51B, which is slightly later than line 51). This amounts to $[(9-4)+(18-8)]/64 \times 139 = 32$ plants, all from one or two E_2E_4 gametes. Tentative interpolation (Fig. 17) gives 24 plants earlier than line 51. A combined score graph (not presented) was made for the early flowering range and indicated 28 such plants, whilst 1 plant was typical C-type (expected $1/64 \times 139 = 2$ plants). With close linkage or allelism, one expects few resp. no plants earlier than line 51.
2. Types expected to be as late as or later than CA are: $E_1E_1\ e_2e_2\ E_4E_4$ (= CA) and all other e_2e_2-types, $E_1E_1\ E_2E_2\ e_4e_4$ (not much earlier than 51B; see F_2 C/51B in fig. 16) and all other e_4e_4-types. The genotype $e_1e_1\ E_2e_2\ E_4e_4$ (not identified) is probably not as late as line CA, as it must resemble $e_1e_1\ E_2E_2\ E_4e_4$ ($=F_1$ 51/51B, fig. 9b) or even be slightly later in view of the almost complete dominance of E_2 over e_2 (cf. F_1 C/CA, ch. 6.3). In total, one expects $(1/4+1/4-1/16) \times 139 = 61$ plants to be as late as or later than line CA. Interpolation in fig. 17 gives $45+6=51$ plants ($\chi^2_1 = 2.90$; $P = 0.10-0.05$).
3. Types expected to be later than line 51B ($=e_1e_1\ E_2E_2\ e_4e_4$) are: all $e_2e_2\ e_4e_4$-types (as the contrast $E_2E_2-e_2e_2$ is larger than $E_1E_1-e_1e_1$). In total, $1/16 \times 139 = 8$ to 9 $e_2e_2\ e_4e_4$ recombinants. Interpolation gives 6 plants. It is interesting to note that the range of transgression past 51B (Fig. 17) coincides with the range of transgression past 51A in the F_2 CA/51A (Fig. 15). In the latter F_2 the number of transgressants is higher due to the intermediacy of E_3e_3.

In conclusion: The lines CA and 51B arose from C resp. 51, at different, not closely linked loci, viz. E_2-e_2 and E_4-e_4. It should be noted that E_2-e_2 and E_3-e_3 are not closely linked either (ch. 6.8.2), nor is E_1-e_1 closely linked to any of these loci. This leaves open the possibility of close linkage between E_3-e_3 and E_4-e_4. This will be shown to be the case in ch. 6.9.3.

6.8.5 *The cross C/51E*

It will be shown that line 51E did not arise from line 51 by a mutation at the E_1-e_1 locus. Denote $C=E_1E_1\ E_6E_6$, $51=e_1e_1\ E_6E_6$ and $51E=e_1e_1\ e_6e_6$.

The F_1 C/51E is closer to line C than to line 51 (Fig. 18). In the F_2 C/51E (Fig. 18), all e_1e_1- and e_6e_6-types are expected to be as late as or later than line 51. In total, $(1/4+1/4-1/16) \times 120 = 52$ to 53 plants. Partitioning at the midpoint between the means of F_1 C/51E ($=E_1e_1\ E_6e_6$) and line 51 ($=e_1e_1\ E_6E_6$)

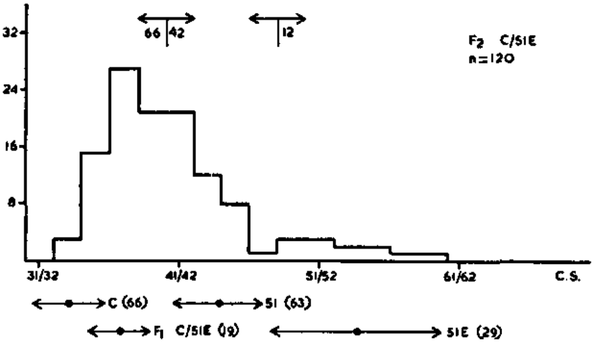

FIG. 18. Experiment of 1-2-1967. Frequency distribution (class interval 2 units) for the combined score (c.s.) of the F_2 C/51E. Ranges of C, 51, F_1 C/51E and 51E are indicated by horizontal arrows (Between brackets: number of plants).

gives $42+12=54$ plants. Types like 51 are not expected in the case of allelism·

Allelism is further rejected by the following points:

1. With independent segregation one expects $1/16 \times 120 = 7$ to 8 C-type plants. Interpolation in the F_2-graph is not feasible, though it is not likely that more than 10 plants resemble C. With allelism, one expects $1/4 \times 120 = 30$ C-type plants. With close linkage between E_1-e_1 and E_6-e_6 one expects many more than 7 to 8 plants.
2. Interpolation gives 12 plants resembling line 51E. With independent segregation, one expects $1/16 \times 120 = 7$ to 8 plants.

In conclusion: it can be said that line 51E differs from line C at two loci (E_1-e_1 and E_6-e_6), which are not closely linked.

6.8.6 *The cross CA/51E*

In ch. 6.8.5 it was assumed that line 51E did not arise from 51 at the same locus as CA did from C. Denote $C = E_1E_1\ E_2E_2\ E_6E_6$, $CA = E_1E_1\ e_2e_2\ E_6E_6$, $51 = e_1e_1\ E_2E_2\ E_6E_6$ and $51E = e_1e_1\ E_2E_2\ e_6e_6$. This point will now be tested.

The F_1 CA/51E $= E_1e_1\ E_2e_2\ E_6e_6$ is only 2 days later than line 51 (cf. fig. 19). This position can be understood on the basis of between locus additivity, as the F_1 51/CA $= E_1e_1\ E_2e_2\ E_6E_6$ happens to be equal to line 51 (ch. 6.2), and as E_6 is almost completely dominant over e_6 (cf. F_1 51/51E, ch. 6.2). However, the F_1-position can also be explained on the basis of allelism, with dominance of e'_2 over e_2 (F_1 CA/51E $= E_1e_1\ e_2e'_2$).

The F_2 CA/51E (Fig. 19) shows the following features:

1. Types expected to be earlier than line 51 ($= e_1e_1\ E_2E_2\ E_6E_6$), and thus definitely earlier than the F_1 ($= E_1e_1\ E_2e_2\ E_6e_6$) are $E_1.\ E_2.\ E_6E_6$, except $E_1e_1\ E_2e_2\ E_6E_6$ ($= F_1$ 51/CA, which equals 51), and $E_1.\ E_2.\ E_6e_6$, except $E_1e_1\ E_2e_2\ E_6e_6$ ($= F_1$ CA/51E, which is somewhat later than 51). This amounts to $[(9-4)+(18-8)]/64 \times 111 = 26$ plants, all from one or two E_2E_6 gametes. Interpolation in the F_2-graph (Fig. 19) is not feasible. However, a combined score graph (not presented) was made for the early flowering range and clearly indicated that not more than 32 plants were earlier than line 51, whilst 3 plants were typical C-type (expected $1/64 \times 111 = 2$ plants). With close linkage or allelism, one expects few resp. no plants much earlier than line 51.

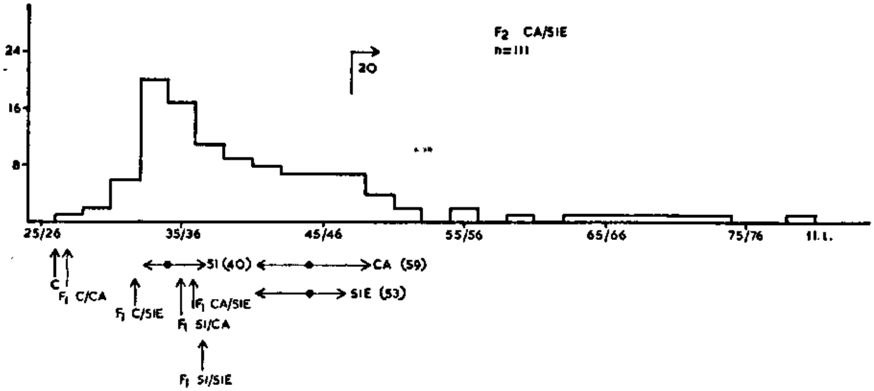

FIG. 19. Experiment of 11-8-1966. Frequency distribution (class interval 2 units) for the flowering time (fl.t.) of the F_2 CA/51E. Ranges of 51, CA and 51E are indicated by horizontal arrows (Between brackets: number of plants). The positions of the F_1 CA/51E and the other F_1's (vertical arrows) have been transferred by means of linear interpolation from the F_1-experiment of 18-5-1966 (Table 10 and fig. 7, ch. 6.2). The position of line C is put at day 26.5 from the experiment of 7-8-1966 (See text).

2. Types expected to be later than line CA ($=E_1E_1$ e_2e_2 E_6E_6) and line 51E ($=e_1e_1$ E_2E_2 e_6e_6) are all e_2e_2-types, except E_1E_1 e_2e_2 E_6.-types (as E_6 is almost completely dominant over e_6, see above). In total 13/64 × 111 = about 23 plants. In fig. 19, one finds at the most 20 plants. With allelism or absolute linkage, one expects among the e_2E_6 e_2E_6 group, the E_1e_1- and e_1e_1-types to be later than CA. In total 3/16 × 111 = about 21 plants. Therefore, transgression past CA gives no information in this respect.

It is seen that in the F_2 CA/51E, the range of the transgressants past CA extends as far as the ranges of the F_2 CA/51A and the F_2 CA/51B (See figs. 15 and 17). This is surprising as the effect of e_6e_6 is much smaller than that of e_3e_3 and e_4e_4. The explanation could be that the triple recessives (Expected 1 in 64) happened to be absent in the latter two F_2's, but that e_1e_1 e_2e_2 e_6e_6 is present in the F_2 CA/51E.

In conclusion: lines CA and 51E arose from C resp. 51 at different, not closely linked loci, viz. E_2-e_2 and E_6-e_6.

6.8.7 *The cross C/51D*

The F_1 C/51D is clearly earlier than line 51 (Fig. 20). Therefore, the presence of many 51-like plants in the F_2 is already sufficient evidence for rejecting the hypothesis that line 51D arose from line 51 by a single gene mutation at the E_1-e_1 locus. Another hypothesis (cf. ch. 6.4.4) is that 51D arose from 51 by 2 (recessive) mutations. If one of these is at the E_1-e_1 locus, one expects 1/4 × 3/4 × 115 = 21 to 22 plants to resemble line C and 1/16 × 115 = 7 to 8 plants to resemble line 51D. The F_2-graph (Fig. 20) shows a clear deficit when compared with these expectations.

These deficits are also an argument against the hypothesis that $51D = e_1e_1$

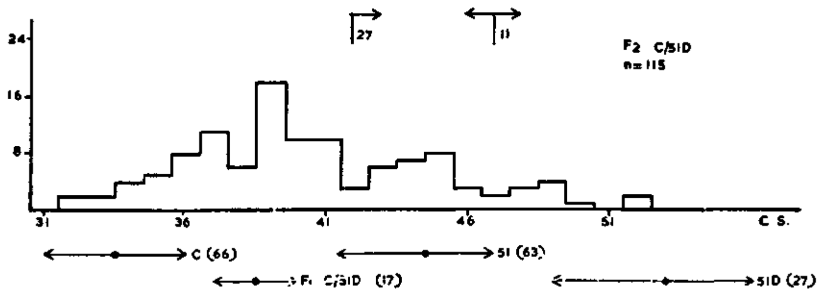

FIG. 20. Experiment of 1-2-1967. Frequency distribution (class interval 1 unit) for the combined score (c.s.) of the F_2 C/51D. Ranges of C, 51, F_1 C/51D and 51D are indicated by horizontal arrows (Between brackets: number of plants).

e_5e_5, i.e. that 51D arose from 51 as a single gene mutation at a locus *different* from E_1-e_1. On the other hand, interpolation in the F_2-graph (Fig. 20) gives 27 plants like line 51 and 11 plants later than line 51, which might indicate a single gene difference between the lines 51 and 51D. Additional modifiers could then account for the shift of e_1e_1 e_5e_5-types towards earlier as compared with line 51D.

In conclusion: no definite decision can be reached whether line 51D arose from line 51 by mutations at one or two loci.

6.8.8 *The cross CA/51D*

The F_2 CA/51D gave no further possibilities to distinguish between the two hypotheses about the differences between line 51 and its mutant 51D. Therefore, no details will be presented.

6.9 THE CROSSES BETWEEN 51A, 51B AND 51E

In ch. 6.4 it has been shown that 51A, 51B and 51E each differs from 51 in one major gene. No definite conclusions could be reached for line 51D.

It remains to be tested whether the lines 51A, 51B and 51E arose from line 51 at different loci. Anticipating the results of the diallel cross, the genotypes were assigned as follows (ch. 6.8 and elsewhere): $51A = e_3e_3$, $51B = e_4e_4$ and $51E = e_6e_6$. Crosses of 51D with these three lines, did not allow any definite conclusions, so that no further statements about line 51D can be made. Therefore, these crosses will not be presented.

From 11-8-1966, a 6 × 6 F_2-diallel was grown (without reciprocals) including the parents CA, 51, 51A, 51B, 51D and 51E. As discussed in ch. 6.6, it is justified to transfer the positions of the F_1's by linear interpolation from the F_1-diallel experiment, grown from 18-5-1966 (Table 10 and fig. 7). However, the data of the 6 × 6 F_2-diallel were partly useless, as several pans were affected by a soil-borne infection. In fact, only the arrays of line 51 (ch. 6.4) and CA (ch. 6.8) could be used with sufficient confidence. From the 4 × 4 F_2-subdiallel of 51A, 51B, 51D and 51E, only tentative conclusions could be drawn. This 4 × 4 F_2-diallel was therefore repeated from 19-4-1967. Parents, F_1's and also line 51, were added. No reciprocals were grown.

It was originally intended to use a combined score, but this led to difficulties in interpretation as a result of the genotypic constitution of line 51E. Compared with the lines 51A and 51B, the leaf number of line 51E falls short of what would be expected from its flowering time (cf. fig. 7). In the present experiment (19-4-1967) this feature is even more pronounced than in fig. 7. The cross 51B/51E gives information about the probable cause of this phenomenon. Therefore, this cross will be presented first, followed by the crosses 51A/51E and 51A/51B.

6.9.1 *The cross 51B/51E*

The F_1 51B/51E is not much later than line 51 (Fig. 21 a). This position can be understood on the basis of two loci. Denote 51 = E_4E_4 E_6E_6, 51B = e_4e_4 E_6E_6 and 51E = E_4E_4 e_6e_6. Since E_4 and E_6 are almost completely dominant over resp. e_4 and e_6 (See fig. 7), additivity of the effects at the two loci leads to almost complete complementation in the F_1 51B/51E. This case of heterosis can be considered as a good example of the dominance theory of heterosis.

Interpolation in the flowering time graph of the F_2 51B/51E (Fig. 21 a), gives 55 plants earlier than the parent 51E and 59 plants as late as or later than 51E. On the basis of independent segregation, one expects 9/16 × 114 = 64 plants earlier and 7/16 × 114 = 50 plants as late as or later than 51E ($\chi^2_1 =$ 2.89; P = 0.10–0.05). The cause for this shortage of the early group lies in line 51E as will be explained when presenting the cross 51A/51E (ch. 6.9.2).

Allelism, already improbable in view of the F_1-position, is rejected by the transgression towards late past the parent 51B. In the present F_2, 5 to 6 plants out of 114 are later, and in the F_2 from 11-8-1966 experiment, 5 among 81 plants were later. In total, 10 to 11 out of 195 plants (expected 1/16 × 195 = 12 plants representing the genotype e_4e_4 e_6e_6).

As said before, line 51E falls short in the number of leaves. In a scatter diagram (not presented) for flowering time and leaf number of the F_2 51B/51E, one would expect among the 59 late plants, or rather (when excluding the 5 e_4e_4 e_6e_6-plants) among the 54 late plants, equal numbers to more or less resemble 51E and 51B, viz. the F_2-phenotypes $E_4.$ e_6e_6 and e_4e_4 $E_6.$ respectively. That is, when assuming e_6e_6 to have a much smaller effect on leaf number than on flowering time (as compared with e_4e_4). However, at the most 11 plants fall in the 51E area (in the scatter diagram). It is therefore more likely that 51E is homozygous for a separate recessive gene, d, which decreases the number of leaves, but has no effect on flowering time. In this case the types e_4e_4 $E_6.$ dd and $E_4.$ e_6e_6 dd are expected to be in the area of 51E, that is 1/4 × 54 = 13 to 14 plants, which agrees well with the 11 plants observed.

It is clear from the foregoing that in the present cross 51B/51E, the flowering time distribution is easier to interpret than the leaf number distribution, as three genes affect leaf number, viz. E_4-e_4, E_6-e_6 and D–d.

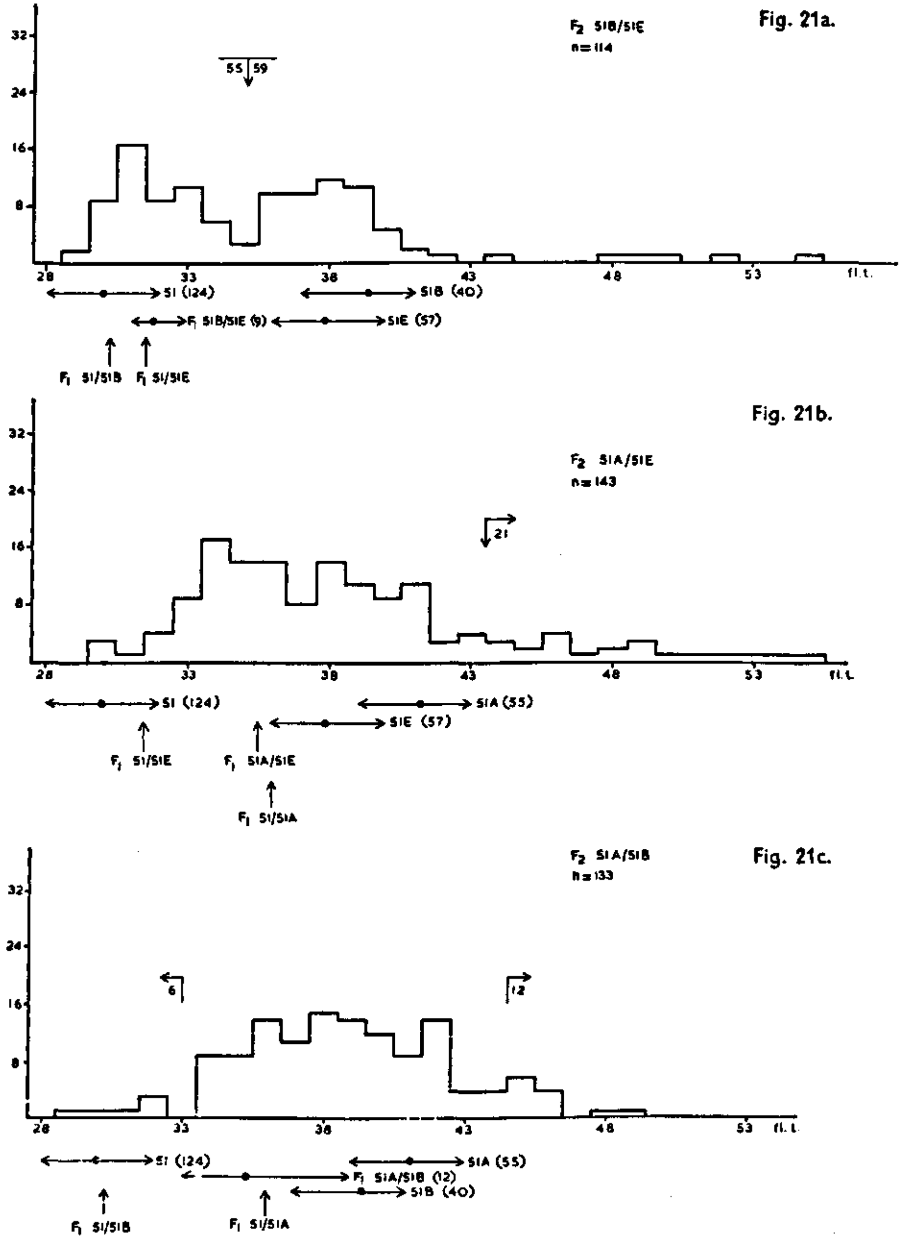

FIG. 21. a–c.

Experiment of 19-4-1967. Frequency distributions for flowering time (fl.t.) of the F_2 51B/51E (a), the F_2 51A/51E (b) and the F_2 51A/51B (c). Class interval 1 unit. Horizontal arrows indicate the ranges of line 51, F_1's and parents (Between brackets: number of plants). The positions of the F_1's (vertical arrows) have been transferred by means of linear interpolation from the F_1-experiment of 18-5-1966 (Table 10 and fig. 7, ch. 6.2).

6.9.2 *The cross 51A/51E*

The F_1 51A/51E was not grown due to lack of seeds in this experiment. However, in another experiment (18-5-1966, ch. 6.2), this F_1 was found to be close to the F_1 51/51A, which in its turn is intermediate between the parents 51 and 51A. The position of the F_1 51A/51E (Fig. 21b, flowering time of F_2) can be understood on the basis of two loci. Denote $51 = E_3E_3\ E_6E_6$, $51A = e_3e_3\ E_6E_6$ and $51E = E_3E_3\ e_6e_6$. As E_6 is almost completely dominant over e_6 (Fig. 7), the substitution $E_6E_6 \rightarrow E_6e_6$ has very little or no effect. On the other hand, the substitution $E_3E_3 \rightarrow E_3e_3$ corresponds to the shift from line 51 to the F_1 51/51A. Thus additivity of the effects at the two loci leads to a position of the F_1 51A/51E equal to that of the F_1 51/51A.

Allelism is rejected by the F_2-transgression towards late past the parent 51A (Fig. 21b). Not only genotype $e_3e_3\ e_6e_6$, but also genotype $E_3e_3\ e_6e_6$ is expected to be later than 51A, as can be seen from fig. 21b, when one adds the difference between 51 ($=E_3E_3\ E_6E_6$) and F_1 51/51A ($=E_3e_3\ E_6E_6$) to the level of 51E ($=E_3E_3\ e_6e_6$). In total, one expects $3/16 \times 143 = 27$ plants to be later than 51A (observed 21 plants). Among the 21 late plants, at least 8 plants are in the range of the very late plants found in the cross 51B/51E (Fig. 21a), and by analogy they can be taken to represent the genotype $e_3e_3\ e_6e_6$ (expected $1/16 \times 143 =$ about 9 plants).

One can conclude that the lines 51A and 51E differ at two loci (E_3-e_3 and E_6-e_6), which are not closely linked.

So far we have ignored the shortage of plants like 51 (Fig. 21b). One expects 1/16 ($E_3E_3\ E_6E_6$, like 51) + 2/16 ($E_3E_3\ E_6e_6$, like F_1 51/51E), out of 143 plants, which is about 27 plants. The shortage is considerable, and would clearly indicate linkage. However, in another F_2 51A/51E grown from the same seedlot, JANSSEN (experiment of 7-12-1966, pers. comm.) found on progeny testing by means of F_3's, that not only 51-like types ($E_3E_3\ E_6E_6$) were rare or absent, but also the 51E-genotype ($E_3E_3\ e_6e_6$), which means that all E_3E_3-types fall numerically very short of expectation. Now, 51E has rather small seeds, and since also in the F_2-seedlot a considerable quantity of small seeds occurred, unconscious selection against these small seeds during sowing could have taken place. This can result in a shortage of E_3E_3-genotypes, if seed size is determined by the embryonic genotype, and if 51E happens to contain such a recessive seed-size mutation linked to E_3(!).

The shortage in JANSSEN's F_2 was even more pronounced than in our own F_2. Since the seeds used by him were poured from the original envelope into another envelope, it can well be that by preference the larger, more round-shaped and heavier seeds were transferred.

To test this point, the few F_2-seeds which remained after sowing the F_2 of 19-4-1967, were divided into a lot of smaller seeds and a lot of larger seeds. These two lots were sown on 31-8-1967. At day 39, i.e. at the beginning of flowering of 51E, from the 'small seed'-F_2, 16 out of 31 plants had started flowering, and from the 'large seed'-F_2, 9 out of 32 plants were flowering. This confirms our hypothesis.

It is clear that in e.g. the cross 51/51E, where F_2 seeds also show variation in size, no connection with flowering time differences occurs, since the seed-size locus is not linked to E_6-e_6 and since both parents are E_3E_3. However, in the cross 51B/51E (ch. 6.9.1) also a shortage of early flowering types was found. This can be readily explained if the hypothetical seed-size mutation is also linked to E_4, in other words if E_3 and E_4 are linked. This is precisely what is found in the cross 51A/51B.

6.9.3 *The cross 51A/51B*

The F_1 51A/51B is about intermediate between the lines 51 and 51A (Fig. 21c), and about coincides with the F_1 51/51A. This position can be understood on the basis of two loci. Denote $51 = E_3E_3\ E_4E_4$, $51A = e_3e_3\ E_4E_4$ and $51B = E_3E_3\ e_4e_4$. As E_4 is almost completely dominant over e_4 (Fig. 7), the substitution $E_4E_4 \rightarrow E_4e_4$ has little or no effect. On the other hand, the substitution $E_3E_3 \rightarrow E_3e_3$ corresponds to the shift from 51 to the intermediate F_1 51/51A. Thus additivity of the effects at the two loci leads to a position of the F_1 51A/51B equal to that of the F_1 51/51A.

With these two loci, one expects in the F_2 51A/51B, two genotypes to be earlier than the F_1 51A/51B, viz. $E_3E_3\ E_4E_4$ (like 51) and $E_3E_3\ E_4e_4$ (like F_1 51/51B, which is practically equal to 51), and two genotypes to be later than 51A ($= e_3e_3\ E_4E_4$), viz. $E_3e_3\ e_4e_4$ (due to intermediate inheritance at the E_3-e_3 locus) and $e_3e_3\ e_4e_4$.

In the flowering time graph of the F_2 51A/51B, size 133 plants, given in fig. 21c, one sees a considerable shortage of 51-like types (only 6 plants), and at the other side of the range absence of genotypes as late as the latest found in the F_2 51A/51E (Fig. 21b), and the F_2 51B/51E (Fig. 21a), which indicates absence of $e_3e_3\ e_4e_4$-plants. The number of plants which are later than 51A is tentatively estimated as 12.

With a recombination value p, one expects the fractions $p^2/4\ E_3E_3\ E_4E_4 + 2p(1-p)/4\ E_3E_3\ E_4e_4$ and $2p(1-p)/4\ E_3e_3\ e_4e_4 + p^2/4\ e_3e_3\ e_4e_4$ to be resp. like 51 and later than 51A. Jointly $2p(2-p)/4$, which after equating to $(6+12)/133$, leads to $p = 0.15$. Then, one expects $133.(0.15^2)/4 = 0.75$ plants representing the $e_3e_3\ e_4e_4$-genotype, so that it is not surprising that no such individuals of this genotype have been found.

In conclusion: it can be said that 51A and 51B differ in two rather closely linked loci (E_3-e_3 and E_4-e_4), and that the heterosis towards early can be understood by the effects at the individual loci.

7. ANALYSIS OF THE SMALL-EFFECT MUTANTS

7.1 INTRODUCTION

The mutant lines analysed in ch. 6, viz. CA from C and 51A, 51B, 51D and 51E from 51, all showed a large phenotypic difference (all towards late) with the line of origin. Therefore, the prospect of phenotypic identification of genotypes was good. Once identified, a mutation is called a major gene mutation, and the line a major gene mutant (cf. ch. 6). The above mentioned lines were treated as a group, a.o. by entering them into one diallel cross. Only line 51D did not lead to definite classification, which means that it still has to be called a minor gene mutant.

The present chapter discusses mutant lines which show only a small phenotypic difference with the line of origin. There was little prospect for identification of genotypes, i.e. the mutants were expected to remain in the category of minor gene mutants. Nevertheless, a few lines proved to contain a directly identifiable mutation, i.e. a major gene mutation.

Depending on the line of origin and direction of change, three groups are distinguished:

1. Small-effect late mutants from line 51: $51Xl_1$, $51Xl_2$, $51El_1$, $51El_2$ and $51El_3$; ch. 7.2.
2. Small-effect early mutants from line 51: $51EV_1$, $51EV_2$, $51EV_3$, $51EV_4$; $51XV_1$ and $51XV_3$; ch. 7.3.
3. Small-effect late mutants from line C: CEl_1, CEl_2, CEl_3; CXl_1, CXl_2 and CXl_3; ch. 7.4.

It should be remembered that no (small-effect) early mutants from line C could be found.

The symbols E and X refer to EMS and X-rays; the symbols l and V to late and early respectively.

The three groups were analysed seperately, i.e. no crosses *between* these groups were made.

7.2 THE SMALL-EFFECT LATE MUTANTS FROM LINE 51

A 6 × 6 F_1-diallel (without reciprocals) between the lines 51, $51Xl_1$, $51Xl_2$, $51El_1$, $51El_2$ and $51El_3$, was grown from 12-5-1966. The corresponding 6 × 6 F_2-diallel (including parents) was grown in pans from 20-8-1966 as follows. Each F_2 was represented by 4 pans (30 F_2-plants each) and each mutant line by 2 pans (30 plants each). To each pan 6 plants from line 51 were added. All pans were randomized into one block.

The 5 mutant lines were also crossed with line C, and the F_1's were selfed. The F_1's and the F_2's were grown from 5-4-1967. Each F_2 was represented by 4 pans (24 F_2-plants + 6 51-plants + 6 mutant line-plants) and each F_1 was represented by 1 pan (24 F_1-plants + 6 C-plants + 6 mutant line-plants). All pans were randomized into one block.

TABLE 12. Differences in combined scores (Experiment of 12-5-1966 and 5-4-1967) and in flowering time (Experiment of 20-8-1966) between the 5 small-effect late mutant lines and the line of origin 51.

Experiment	51Xl$_1$	51Xl$_2$	51El$_1$	51El$_2$	51El$_3$
12-5-1966 (comb. score)	4.5	6.6	3.1	3.4	13.2
5-4-1967 (comb. score)	6.0	7.0	6.6	3.9	10.7
20-8-1966 (fl. time)	13.5	4.7	4.3	4.9	13.2

Table 12 gives the differences between the 5 mutant lines in each of the three experiments (combined scores; but flowering time for the 20-8-1966 experiment). It is seen that line 51El$_3$ is clearly later than the other four lines (except line 51Xl$_1$ in the experiment of 20-8-1966). Notably in the experiment of 20-8-1966, the flowering time difference of 51El$_3$ with 51 turns out to be of an order of magnitude which one might call 'large'. More surprisingly even, line 51Xl$_1$ in the experiment of 20-8-1966, also shows a large difference with 51, such in contrast to its behaviour in the two other experiments. This is a particularly striking case of genotype-environment interaction. As will be shown in the following, the lines 51Xl$_1$ and 51El$_3$ each differ from line 51 (the parent of origin) in one major gene.

Turning to the crosses of the 5 mutant lines with line 51 first, one finds (Experiment of 12-5-1966; table 13), that all 5 F_1's are close to 51. Each F_2 (Experiment of 20-8-1966; graphs not presented) included in its range line 51 and the mutant line. Clear-cut bimodality was found in the graph of the F_2 51/51Xl$_1$: out of 109 plants, 76 resembled line 51 and the F_1, and 33 resembled 51Xl$_1$ (expected 82 and 27; $\chi^2_1 = 1.76$; $P = 0.20 - 0.10$). Line 51Xl$_1$ will now be denoted by e_7e_7.

TABLE 13. Experiment of 12-5-1966. Combined scores for line 51 and the 5 small-effect late mutant lines from 51 (in italics) and their 5 + 10 F_1's. The arrows indicate F_1's which are not heterotic towards early (3 cases). Underlined are those crosses of which the F_2's show clear-cut transgression towards late.

	51	51Xl$_1$	51Xl$_2$	51El$_1$	51El$_2$	51El$_3$
51	*43.0*					
51Xl$_1$	44.0	*47.5*				
51Xl$_2$	45.6	→ 48.9	*49.6*			
51El$_1$	44.7	43.7	44.6	*46.1*		
51El$_2$	44.2	43.0	→ 48.4	41.7	*46.4*	
51El$_3$	43.5	45.7	47.0	→ 48.0	45.2	*56.2*

The graph of the F_2 51/51El$_3$ gave a slight suggestion of trimodality corresponding to a 1:2:1 segregation (partial dominance of the heterozygote towards early). This is in contrast with the complete dominance of early as found in the 12-5-1966 experiment. The other three F_2's were unimodal.

Discussing next the crosses of the 5 mutant lines with line C, it was found (Experiment of 5-4-1967) that none of the F_1's is much later than C. The largest difference (3.3 units c.s.) is found for the F_1 C/51El$_3$. These positions can be understood from the fact that the F_1 C/51 = E_1e_1 is close to C and that the F_1's of the 5 mutant lines with 51 are close to 51, except for the F_1 51/51El$_3$. For all 5 F_2's (only the graph of F_2 C/51El$_3$ is presented; fig. 22) it can be said that the distributions ranged from C-like types to types like the mutant line, and that in all cases at least 15% of the F_2-plants resembled line 51. The latter point proves that *if* the mutant lines arose from 51 by a single gene mutation each, this mutation did not occur at the E_1-e_1 locus, nor at a locus closely linked to E_1-e_1.

So far, such a single gene mutation could only be demonstrated for 51Xl$_1$, but now the F_2 C/51El$_3$ (Fig. 22) gives evidence that 51El$_3$ too arose from 51 as a single gene mutation. Therefore, 51El$_3$ will now be denoted as e_8e_8. Suppose that the substitution $E_1E_1E_8E_8$ (= C) to $E_1E_1e_8e_8$ has the same effect as the substitution $E_1E_1E_8E_8$ to $e_1e_1E_8E_8$ (= 51), which is not unlikely, and suppose that in the presence of e_1e_1 the heterozygote E_8e_8 approaches intermediacy between E_8E_8 and e_8e_8, as indicated by the F_2 51/51El$_3$ (Experiment of 20-8-1966; see above), then one expects the following F_2 ratio:

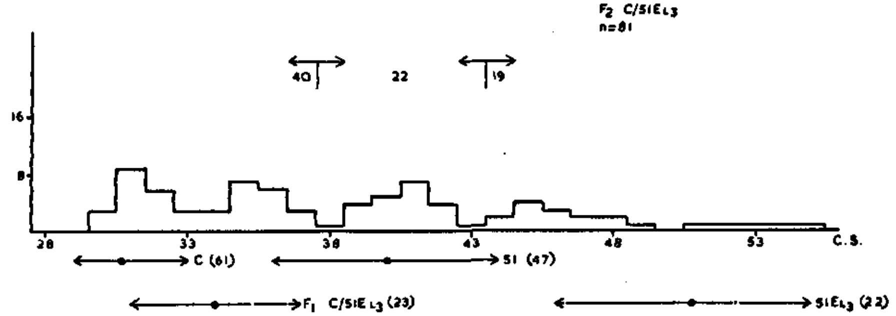

FIG. 22. Experiment of 5-4-1967. Frequency distribution (class interval 1 unit) for the combined score (c.s.) of the F_2 C/51El$_3$. Ranges of C, 51, F_1 C/51El$_3$ and 51El$_3$ are indicated by horizontal arrows (Between brackets: number of plants).

9 $E_1.E_8.$ (earlier than 51):3 $E_1.e_8e_8$ (like 51) + 1 $e_1e_1E_8E_8$ (like 51):2 $e_1e_1E_8e_8$ (like F_1 51/51El$_3$) + 1 $e_1e_1e_8e_8$ (like 51El$_3$). For 81 F_2-plants, this means resp. 45, 20 and 15 plants. By interpolation in fig. 22, one finds 40, 22 and 19 plants ($\chi^2_2 = 1.82$; P = 0.50–0.30).

Turning finally to the 5 × 5 diallel between the 5 mutant lines, one finds that among the 10 F_1's (Table 13; experiment of 12-5-1966), 7 F_1's are heterotic towards early, and that of the 3 remaining F_1's (indicated by →), the F_1 51X$_1$/51Xl$_2$ and the F_1 51Xl$_2$/51El$_2$ are intermediate, and the F_1 51El$_1$/51El$_3$ is close to the earlier parent (51El$_1$). The F_2's (Experiment of 20-8-1966; no graphs presented) show the following:

a. All three F_2's between 51Xl$_2$, 51El$_1$ and 51El$_2$ (F_1's underlined in table 13) show clear transgression past the later parent, whilst it is significant that in the F_2 $51Xl_2/51El_2$ (F_1 not heterotic towards early) types like 51 are recovered. Therefore, *if* these three lines arose as single gene mutations (which has not been demonstrated!), then none of these three mutations can have occurred at the same locus or at closely linked loci.

b. The F_2 of $51Xl_1$ and $51El_3$, which two lines were identified earlier as major gene mutants, contains types like 51, but shows no transgression towards late. This means that $51Xl_1$ and $51El_3$ probably arose as single gene mutations at closely linked loci.

c. The three remaining F_2's involving $51Xl_1$ show only slight transgression towards late past $51Xl_1$. However, the F_2 $51Xl_1/51Xl_2$, the F_1 of which was not heterotic towards early, included types like 51, and therefore allelism or close linkage can be ruled out. It then remains that either $51El_1$ or $51El_2$ (but not both, see under a) can have arisen as a single gene mutation at a locus closely linked to that of $51Xl_1$ (viz. locus E_7-e_7).

d. The three remaining F_2's involving $51El_3$ show only slight transgression towards late past $51El_3$. However, the F_2 $51El_1/51El_3$, the F_1 of which was not heterotic towards early, includes types like 51, and therefore allelism or close linkage can be ruled out. It then remains to prove that either $51Xl_2$ or $51El_2$ (but not both, see under a) can have arisen as a single gene mutation at a locus closely linked to that of $51El_3$ (viz. locus E_8-e_8).

e. Now, *$51Xl_1$ and $51El_3$ probably contain closely linked mutations* (See b). Any locus linked to E_7-e_7 should also be linked to E_8-e_8. From c) and d) it follows that only line $51El_2$ may contain a mutation at a locus linked to E_7-e_7 and E_8-e_8. Allelism to E_7-e_7 is not likely as the F_1 $51Xl_1/51El_2$ is heterotic towards early (coincides with 51), and allelism to E_8-e_8 is not likely either as on the one hand the F_1 $51El_2/51El_3$ is only one unit combined score earlier than $51El_2$, and on the other hand 4 plants occurred which are unmistakenly coinciding with 51.

In conclusion: it can be said that $51Xl_1$ contains a completely recessive mutation e_7 and $51El_3$ an incompletely recessive mutation e_8. The loci E_7-e_7 and E_8-e_8 are probably closely linked, but not closely linked to E_1-e_1. It should be noted that no tests for allelism or close linkage have been done between E_7-e_7 and E_8-e_8 on the one hand, and E_2-e_2, E_3-e_3, E_4-e_4 and E_6-e_6 (ch. 6) on the other hand.

No single genes could be identified from $51Xl_2$, $51El_1$ and $51El_2$, but if these lines arose as single gene mutants from 51, then the three loci are not closely linked to each other nor to E_1-e_1. Finally, the hypothetical single gene mutation of $51El_2$ *might* be linked to E_7-e_7 and E_8-e_8.

7.3 THE SMALL-EFFECT EARLY MUTANTS FROM LINE 51

The 6 early mutants from line 51 are $51EV_1$, $51EV_2$, $51EV_3$ and $51EV_4$ (by EMS), and $51XV_1$ and $51XV_3$ (by X-rays). None of the 6 mutant lines flowers as early as line C (cf. table 14). In fact, all 6 mutants, in terms of combined score, are about mid-way between lines C and 51. Only $51XV_1$ is somewhat closer to line C. Considering the fact that identification of the single gene difference between C and line 51 had not been too easy (ch. 5.1), it was not expected that, in the 6 early lines, single gene differences with line 51 might be detected. Therefore, their crosses with line 51 (the parent of origin) were not studied, but instead a 7 × 7 F_2-diallel (between the 6 lines and line C) was thought sufficient to draw some rough conclusions about the lines, that is conclusions from presence or absence of transgressive segregations, and in particular from presence or absence of 51-like plants in the different F_2-families.

TABLE 14. Experiment of 21-7-1966. Flowering time, leaf number and combined score of the 6 small-effect early mutants from line 51, in comparison with the lines C and 51. All means derived from 60–70 plants.

	C	$51EV_1$	$51EV_2$	$51EV_3$	$51EV_4$	$51XV_1$	$51XV_3$	51
Flowering time	25.9	28.0	29.1	28.4	29.4	27.6	27.8	30.6
No. of leaves	6.9	10.2	10.2	10.0	8.8	8.8	9.9	13.1
Combined score	32.8	38.2	39.3	38.4	38.2	36.4	37.7	43.7

It will be shown that the analysis of the 7 × 7 F_2-diallel strongly suggests that the 6 lines arose from line 51 as single gene mutations at 6 different loci, one of which (that of $51XV_3$) is probably allelic or very closely linked to E_1-e_1. In retrospect, it can be said that the lack of direct information about the position of the F_1's with line 51 (the parent of origin) proved to be a considerable handicap.

The 7 × 7 diallel between the 6 lines (selfed for 5 generations) and line C was grown from *21-7-1966*. Each F_2-family was represented by 4 pans, each with 24 F_2-plants, 6 C-plants and 6 51-plants. Each parent was represented by 2 pans, each with 36 plants. F_2- and parent-pans were randomized into one block. In an adjacent randomized block, each F_1 was represented by one pan containing 10 F_1-plants and 5 plants of each of the two corresponding parents, so that the relative position of the F_1's can be ascertained.

The data of flowering time and leaf number of the 6 lines, and of C and 51, are given in table 14. Roughly speaking, the lines are for both characters about half-way between the lines C and 51. Only line $51XV_1$ is somewhat closer to C. Line $51EV_4$ compared with line 51 shows a relatively large decrease in number of leaves, but only a relatively small decrease in flowering time.

Turning to the 6 crosses with line C first, it is found that all F_1's are much closer to the mutant lines than to line C. In fact, their positions are near the point where the F_1 C/51 ($=E_1e_1$) is expected. The simplest hypothesis to account for these positions is that the 6 lines arose from line 51 as single gene mutations which are partially or wholly recessive, so that the diheterozygote F_1's with C ($E_1e_1V_iv_i$) are close to the monoheterozygotes $E_1e_1V_iV_i$. In the case of independent segregation, one then expects in the F_2-families 3 in 16 plants to be close to line 51, viz. the $e_1e_1V_i.$-plants. In the case of allelism or very close linkage, no 51-like plants are expected to be recovered in F_2. Interpolation in the scatter diagrams was not difficult and gave the following results presented in table 15: (Fig. 23 gives by way of illustration the frequency distribution of the F_2 C/51EV$_2$). It is seen that in all F_2-families, except in the F_2 C/51XV$_3$, the data fit to the hypothesis of two independently segregating genes. The F_2 C/51XV$_3$ (Fig. 24) shows no transgression at all towards late past the parent 51XV$_3$, and at the most 25% of the plants are within the range of line C. Therefore, line 51XV$_3$ either arose as a mutation at the E_1-e_1 locus or at a locus very closely linked to it. In the former case, line 51XV$_3$ may be denoted by $e'_1e'_1$. However, in contrast to e_1, the allele e'_1 must be dominant over E_1, considering the fact that the F_1 C/51XV$_3$ is close to line 51XV$_3$ (See fig. 24). This means that by X-rays the recessive allele e_1 has been mutated to a dominant allele e'_1. This would be a very interesting situation. On the other hand, the mutation of 51XV$_3$ can also have arisen at a locus very closely linked to E_1-e_1, e.g. at locus V_6-v_6, in which case the mutation must be a recessive (v_6), as the F_1 C/51XV$_3$ ($=E_1e_1V_6v_6$) about coincides with the F_1 C/51. The present data do not allow a definite decision between the two alternatives.

TABLE 15. Experiment of 21-7-1966. Numerical results of interpolation in the F_2-generation of the crosses between line C and the 6 early mutants from line 51; n = number of plants per each F_2-family.

F_2	n	Plants like or near 51 exp.	obs.	χ^2_1	P
C/51EV$_1$	87	16	17	0.08	0.80–0.70
C/51EV$_2$	90	18	20	0.28	0.70–0.50
C/51EV$_3$	94	18	20	0.27	0.70–0.50
C/51EV$_4$	96	18	13	1.71	0.20–0.10
C/51XV$_1$	87	16	12	1.23	0.30–0.20
C/51XV$_3$	66	12	0	14.67	< 0.01

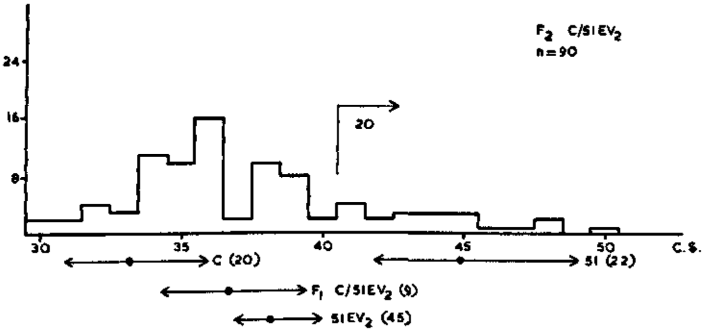

FIG. 23. Experiment of 21-7-1966. Frequency distribution (class interval 1 unit) for the combined score (c.s.) of the F_2 C/51EV$_2$. Ranges of C, F_1 C/51EV$_2$, 51EV$_2$, and 51 are indicated by horizontal arrows (Between brackets: number of plants).

Two further points must be mentioned about the F_2's with line C (except the F_2 C/51XV$_3$):

1. There is some clustering of plants at the early side of the C-range, but no

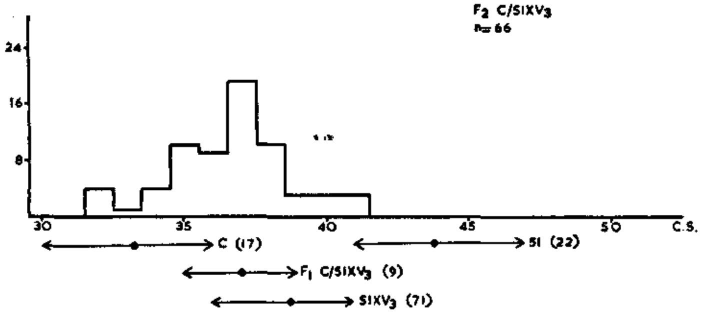

FIG. 24. Experiment of 21-7-1966. Frequency distribution (class interval 1 unit) for the combined score (c.s.) of the F_2 C/51XV$_3$. Ranges of C, F_1 C/51XV$_3$, 51XV$_3$ and 51 are indicated by horizontal arrows (Between brackets: number of plants). Note the absence of 51-like plants in this graph.

clear-cut transgression, as would be expected for the types $E_1E_1v_iv_i$. However, line C can be considered as a (physiological) limit of expression towards early. In this connection, it will be remembered that no mutant lines earlier than C could be derived from C, and that it is not possible to make line C flower earlier by vernalization treatment (cf. ch. 3.2.3).

2. *In the F_2 C/51EV$_2$ and F_2 C/51EV$_3$ interpolation gave 3 in 16 plants* practically coinciding with line 51. In the F_2 C/51EV$_1$, in the F_2 C/51EV$_4$, and to some extent in the F_2 C/51XV$_1$, this point of interpolation includes also types somewhat earlier than line 51 in the 51-like class. This could mean that the mutations in 51EV$_2$ and 51EV$_3$ are not far from completely recessive, and those in 51EV$_1$ and 51EV$_4$ are only partially recessive.

Turning now to the 6 × 6 diallel between the 6 early mutants from line 51, it is found that:

1. The F_1's are closer to the mutant lines than to line 51, except for F_1 51EV$_2$/51EV$_3$ which is about intermediate between the two mutant lines and line 51. By contrast, the F_1 51EV$_1$/51EV$_4$ practically coincides with the two parents. This fits with the supposition made above that 51EV$_2$ and 51EV$_3$ contain about completely recessive mutations, and that 51EV$_1$ and 51EV$_4$ contain mutations which are only partially recessive. Judging from these F_1 positions, also 51XV$_1$ and 51XV$_3$ contain practically recessive mutations. Especially in this connection, it is a handicap that the F_1's between the 6 mutant lines and line 51 were not included in the experiment.
2. The F_2's show little transgression past the mutant lines towards early. One would expect on the basis of independent segregation 1 in 16 plants ($v_iv_iv_jv_j$) to be definitely earlier. As linkage is excluded (See below), one must assume an interaction of the 'double recessive epistasis' type (7 'early' : 9 'late').
3. The F_2's all show many plants coinciding for flowering time and leaf number with line 51. It was not possible to find clear-cut points for interpolation. Nevertheless, this is sufficient evidence to conclude that all 6 lines arose from line 51 as single gene mutations at different loci which are not closely linked to each other.

4. The highest proportion of 51-like plants is found in the F_2 51EV$_2$/51EV$_3$, the lowest in the F_2 51EV$_1$/51EV$_4$. This is in accordance with the statement made earlier that the former two lines contain almost completely recessive mutations, the latter two lines only partially recessive mutations.
5. The F_2's of 51XV$_3$ with the other 5 mutants in general show a low proportion of 51-like plants. This indicates partial recessivity of the mutation in 51XV$_3$.

Summarizing, one can now denote:

C $= E_1E_1\ V_1V_1\ V_2V_2\ V_3V_3\ V_4V_4\ V_5V_5\ (V_6V_6)$
51 $= e_1e_1$
51EV$_1 = e_1e_1\ v_1v_1$
51EV$_2 = e_1e_1\ v_2v_2$
51EV$_3 = e_1e_1\ v_3v_3$
51EV$_4 = e_1e_1\ v_4v_4$
51XV$_1 = e_1e_1\ v_5v_5$
51XV$_3 = e_1e_1\ (v_6v_6)$

The only linked loci among these six are E_1-e_1 and V_6-v_6, which are very closely linked. However, v_6 can also be at the E_1-e_1 locus in which case, this locus can be denoted as $E_1-e'_1-e_1$. All mutations are recessive to their alleles in line 51: nearly completely recessive (v_2,v_3) or partially recessive (v_1, v_4, v_5, v_6).

7.4 THE SMALL-EFFECT LATE MUTANTS FROM LINE C

Among the late mutant lines derived from line C, only line CA was later (in fact much later) than line 51, and its single gene difference with C could be easily identified (ch. 6). The 6 other late mutant lines from C, viz. CEl$_1$, CEl$_2$, CEl$_3$ (by EMS), and CXl$_1$, CXl$_2$, CXl$_3$ (by X-rays) were not later than line 51. Considering the fact that identification of the single gene difference between C and line 51 had not been too easy (ch. 5.1), it was not expected that, in the 6 lines, single gene differences with C might be detected. Therefore, their crosses with line C were not studied, but instead, a 7 × 7 F_2-diallel (between the 6 lines and line 51) was thought sufficient to draw some rough conclusions about the lines, that is conclusions from presence or absence of transgressive segregations, and in particular for presence or absence of C-like plants in the different F_2-families. Such a diallel cross is of course a powerful means of analysis, as it provides many cross comparisons. It will be shown that the analysis of the 7 × 7 F_2-diallel strongly suggests that the 6 lines arose as single gene mutations at 6 different loci (also different from E_1-e_1, the C–51 locus). In retrospect, it can be said that the lack of direct information about the position of the F_1's with C proved to be a considerable handicap. Only the cross of CEl$_3$ (the latest of the 6 lines) with C was studied in a later experiment, and this revealed a single gene difference (See below).

The 7 × 7 diallel between the 6 lines (selfed for 5 generations) and line 51 was grown from 28-7-1966. Each F_2-family was represented by 4 pans, each with 24 F_2-plants, 6 C-plants and 6 51-plants. Each parent was represented by 2 pans, each with 36 plants. F$_2$- and parent-pans were randomized into one block. In an adjacent randomized block, each F$_1$ was represented by one pan containing 10 F$_1$ plants and 5 plants of each of the two corresponding parents, so that the relative position of the F$_1$'s can be ascertained. A drawback in this experiment was the rather large environmental variance between pans.

The data of flowering time and leaf number of the 6 lines, and of C and 51, are given in table 16. It is seen that all 6 lines, and notably the lines CEl$_1$ and CEl$_2$, show a smaller increase in leaf number than would be expected on the basis of their increase in flowering time. That is, if one takes the contrast between C and 51 (about one more leaf per day later flowering) as a basis of comparison. This means that one cannot use one single criterion for a combined score (cf. ch. 2.7.1). It proved necessary for the analysis to compare in all combinations the scatter diagrams (flowering time plotted against leaf number) of the different crosses (per cross: parents, F$_1$, F$_2$, and C). It is not feasible to present the complete sequence of analysis by means of scatter diagrams or graphs. Therefore, the presentation must be limited to the conclusions supported by the main arguments.

TABLE 16. Experiment of 28-7-1966. Flowering time and leaf number of the 6 small-effect late mutants from C in comparison with C and 51. All means derived from 60–70 plants.

	C	CEl$_1$	CEl$_2$	CEl$_3$	51	CXl$_1$	CXl$_2$	CXl$_3$
Flowering time	25.4	29.4	28.5	33.4	32.1	30.8	31.6	29.8
Leaf number	6.2	7.4	7.5	10.3	12.7	9.0	10.8	8.9

Turning first to the EMS-induced mutant lines from C, viz. CEl$_1$, CEl$_2$, CEl$_3$ (and also line 51!), two points must be stated:

1. From table 16, it is seen that CEl$_1$ and CEl$_2$ flower about half-way between C and 51 (but show only a small response in leaf number), CEl$_3$ flowers slightly later than 51, but has somewhat less leaves (in other experiments CEl$_3$ did not flower later than 51).
2. The F$_1$ CEl$_1$/CEl$_2$ is heterotic towards early, and flowers about half-way between these two mutants and C. The F$_1$ 51/CEl$_3$ is also heterotic towards early, and flowers somewhat later than the mid-way between C and 51 (i.e. somewhat later than lines CEl$_1$ and CEl$_2$). The F$_1$ CEl$_1$/51 and F$_1$ CEl$_1$/CEl$_3$ flower hardly later, if later at all, than CEl$_1$, and similarly the F$_1$ CEl$_2$/51 and F$_1$ CEl$_2$/CEl$_3$ flower about simultaneously with CEl$_2$.

The simplest hypothesis to account for the positions of the F$_1$'s between CEl$_1$, CEl$_2$, CEl$_3$ and 51 is as follows: Suppose the lines CEl$_1$, CEl$_2$ and CEl$_3$ arose from C by single recessive mutations (like 51 did; and as will be shown below for CEl$_3$ in a direct way). Denote CEl$_1$ = l_1l_1, CEl$_2$ = l_2l_2, CEl$_3$ = l_3l_3 (51 = e_1e_1). Furthermore, suppose L_1 and L_2 to be partially dominant, L_3 to be also partially dominant but with a tendency towards intermediacy (such in contrast with the situation in the C/CEl$_3$ experiment of 5-4-1967 to be discussed later), and E_1 to be partially dominant, but again with a tendency towards intermediacy (ch. 5.1).

On the basis of this hypothesis, the positions of the diheterozygotes listed above (under 2) can be readily understood. Put for ease of comparison and by way of simplification $C=0$, $CEl_1=4$, $CEl_2=4$, $CEl_3=8$ and $51=8$. Then from F_1 $C/CEl_1=1$ and F_1 $C/CEl_2=1$ (partial dominance), the F_1 CEl_1/CEl_2 could be $=2$, which indeed is intermediate between C and the two parents. Furthermore, from F_1 $C/51=3$ and F_1 $C/CEl_3=3$ (partial dominance with a tendency towards intermediacy), the F_1 $51/CEl_3$ could be $=6$, which indeed is somewhat later than mid-way between C and 51. Finally, from F_1 $C/CEl_1=1$ and F_1 $C/51=3$, the F_1 $CEl_1/51$ could be $=4$, which indeed equals CEl_1 (The same argument goes for the F_1 CEl_1/CEl_3, the F_1 $CEl_2/51$ and the F_1 CEl_2/CEl_3).

This hypothesis contains two elements: 1. the lines as single gene mutations from C, and 2. the positions of the monoheterozygotes. So far the hypothesis accounts for the positions of the diheterozygotes, but it should also accommodate the different aspects of all corresponding F_2-distributions.

It will now be shown that this is the case. These F_2-distributions (scatter diagrams, not presented) are mainly continuous, and therefore, interpolations are tentative, unless stated otherwise.

If E_1-e_1 and L_3-l_3 segregate independently, then in the F_2 $51/CEl_3$ (size 90 plants) one expects $1/16 \times 90=5$ to 6 C-like plants (standing apart from the monoheterozygotes) and $5/16 \times 90=28$ to 29 plants later than 51 and CEl_3, among which $1/16 \times 90=5$ to 6 plants much later than 51 or CEl_3. One finds 2 distinct C-like plants, and 12–15 plants later than 51, but none very much later. This suggests that 51 and CEl_3 differ at two loci which are probably linked (but not closely). In a later experiment (5-4-1967) the F_2 C/CEl_3, showed monogenic segregation, i.e. segregated 72 plants like C and F_1, and 20 plants like CEl_3 (expected 69 and 23; $\chi^2{}_1=0.52$; $P=0.50-0.30$). It should be noted that in the 5-4-1967 experiment the F_1 is close to C, in contrast to what has to be assumed for the heterozygote L_3l_3 in the present 28-7-1966 experiment.

If L_1-l_1 and L_2-l_2 segregate independently, then in the F_2 CEl_1/CEl_2 (size 85 plants), one expects with dominance of L_1 and L_2, $9/16 \times 85=47$ to 48 plants ranging from C to F_1 CEl_1/CEl_2. One finds about 51 plants. Furthermore, $1/16 \times 85=5$ to 6 plants should be clearly later than the two parents. One finds about 4 plants as late as line 51. This supports the hypothesis that CEl_1 and CEl_2 arose as single gene mutations at different loci. These loci are not closely linked.

If L_1-l_1 and L_2-l_2 on the one hand, segregate independently from L_3-l_3 and E_1-e_1 on the other hand, then one expects, in the 4 corresponding F_2's, 3 in 16 plants to range from C-type to close to C. This follows from the assumption that L_1l_1 and L_2l_2 are near to C, and L_3l_3 and E_1e_1 are not so near to C. In view of the dominance of L_1 and L_2, 1 in 16 plants are expected to be later than 51 or CEl_3. The results are given in table 17 as follows (all 4 F_2's contain C-like plants, but the interpolations are rather tentative):

TABLE 17. Experiment of 28-7-1966. Numerical results of interpolation in the F$_2$'s from the crosses between the lines CEl$_1$ and CEl$_2$ on the one hand, and the lines 51 and CEl$_3$ on the other hand; n = number of plants per each F$_2$-family.

		Plants like or near C				Plants later than 51 or CEl$_3$			
F$_2$	n	exp.	obs.	χ^2_1	P	exp.	obs.	χ^2_1	P
CEl$_1$/51	84	16	13	0.70	0.30–0.50	5.0	6	0.22	0.70–0.50
CEl$_1$/CEl$_3$	87	16	19	0.67	0.30–0.50	5.5	4	0.44	0.50
CEl$_2$/51	93	17	13	1.16	0.30	6.0	8	0.74	0.50–0.30
CEl$_2$/CEl$_3$	91	17	22	1.85	0.20	6.0	5	0.18	0.70

The numbers 'observed' agree well with those 'expected'. The fact that both crosses with 51 show a shortage of 'types like or near C' and both crosses with CEl$_3$ show an excess of these types, may throw some doubt on the reliability of interpolation in these F$_2$-distributions. Nevertheless, taking into account all facts given so far, there seems no objection to the conclusion that CEl$_1$, CEl$_2$, CEl$_3$ (and 51) arose as single gene mutations at different loci, viz. L_1-l_1, L_2-l_2, L_3-l_3 (and E_1-e_1), of which L_3-l_3 and E_1-e_1 are probably linked (but not closely).

Turning now to the second group of the small-effect late mutants from line C, viz. the X-ray induced mutants CXl$_1$, CXl$_2$ and CXl$_3$, one sees from table 16 that CXl$_2$ is nearest to line 51, but otherwise the three mutant lines do not diverge very much in flowering time. The three F$_1$'s flower between the two corresponding parents. The three F$_2$'s show transgression, which is pronounced towards early (including C-like plants), but not very pronounced towards late. As a preliminary hypothesis, one may propose that CXl$_1$, CXl$_2$ and CXl$_3$ arose from C as single gene mutations (X_1, X_2, X_3), and that these three mutations are not recessive, but somewhere between intermediate and completely dominant. As e.g. $X_1X_1X_2X_2$ is not very much later than $X_1x_1X_2x_2$, the partitioning of phenotypes may in terms of classical F$_2$-ratios be anywhere between $1{:}4{:}(6+4+1)$ and $1{:}(4+6+4+1)$. Though the F$_2$-distributions tend to correspond somewhat more to the first alternative, no definite conclusion can be drawn, as the F$_1$'s with C (the monoheterozygotes) were not grown. As all three F$_2$'s contained 85 to 90 plants, one then expects 5 to 6 C-like plants in each F$_2$-family. In the F$_2$ CXl$_1$/CXl$_2$ one finds 3 distinct C-type plants, and 12 such plants in the F$_2$ CXl$_2$/CXl$_3$. So far the data do not contradict the hypothesis of three loci x_1-X_1, x_2-X_2, and x_3-X_3, which are not closely linked.

More supporting information comes from the crosses between CXl$_1$, CXl$_2$ and CXl$_3$ on the one hand, and CEl$_1$ and CEl$_2$ on the other hand. It will be remembered that the mutations l_1 and l_2 act very similar to each other, and that also the mutations X_1, X_2 and X_3 are very similar in action to each other. All 6 F$_1$'s are in flowering time rather close to the CX-parent. As the mutations l_1 and l_2 are almost completely recessive, this diheterozygote position strongly indicates that the mutations X_1, X_2 and X_3 are almost completely dominant. All 6 F_2's show transgression, which is pronounced towards early (including C-types), and reasonably pronounced towards late.

On the basis of two independent genes (X_1 or X_2 or X_3 and l_1 or l_2), one would, with the given dominance relationships, expect 3 in 16 plants to be like C or close to C, and 3 in 16 plants to be later than the later parent. However, the transgression towards late is not pronounced enough to allow partitioning of a late phenotype from the rest. One may speak of partial epistasis of X_1, X_2 and X_3 over L_1–l_1 and L_2–l_2. Interpolation at the early side of the distributions gives the following results presented in table 18:

TABLE 18. Experiment of 28 7-1966. Numerical results of interpolation in the F_2's from the crosses between the lines CXl_1, CXl_2, CXl_3 on the one hand, and the lines CEl_1 and CEl_2 on the other hand; n = number of plants per each F_2-family.

F_2	n	Plants like or near C exp.	Plants like or near C obs.	χ^2_1	P
CXl_1/CEl_1	93	17	22	1.80	0.20–0.10
CXl_1/CEl_2	92	17	18	0.07	0.80–0.70
CXl_2/CEl_1	94	17	17	0.03	0.90–0.80
CXl_2/CEl_2	89	17	20	0.66	0.50–0.30
CXl_3/CEl_1	85	16	18	0.32	0.70–0.50
CXl_3/CEl_2	94	17	20	0.64	0.50–0.30

The final group of crosses to be considered are those between CXl_1, CXl_2 and CXl_3 on the one hand, and CEl_3 and 51 on the other hand. The latter two lines are later than the former three (cf. table 16). All 6 F_1's are later than their CX-parents (sometimes approaching intermediacy). This is precisely the expected position of the diheterozygotes, since the mutant alleles X_1, X_2 and X_3 are between intermediate and almost completely dominant and the mutant alleles l_3 and e_1 are not completely recessive. On the basis of two independent genes (X_1 or X_2 or X_3 and l_3 or e_1), one would expect 3 in 16 plants to be later than the later parent (51 or CEl_3). In some of the 6 F_2's, the transgression is sufficiently pronounced to allow partitioning of a late phenotype from the rest. One may again speak of partial epistasis of X_1, X_2 and X_3 over l_1 and e_1. Furthermore, one expects 1 in 16 plants to flower as early as C-type (L_3 and E_1 are far from completely dominant). Interpolation at this side of the distributions gives the following results presented in table 19.

Only the F_2 $CXl_2/51$ does not segregate types like C, and in fact can be taken to show no transgression at all past the two parents. It seems likely that x_2–X_2 and E_1–e_1 are closely linked. The F_2 CXl_2/CEl_3 gives only 2 plants which are close to C but not typical C-type. On the other hand a number of plants are clearly later than CEl_3. This suggests that x_2–X_2 and L_3–l_3 are probably loosely linked, which conclusion is completely in accordance with the earlier conclusion that E_1–e_1 and L_3–l_3 are probably loosely linked.

TABLE 19. Experiment of 28-7-1966. Numerical results of interpolation in the F_2's from the crosses between the lines CXl_1, CXl_2 and CXl_3 on the one hand, and 51 and CEl_3 on the other hand; n = number of plants per each F_2-family.

F_2	n	Plants like C		χ^2_1	P
		exp.	obs.		
CXl_1/51	93	6	4	0.72	0.50–0.30
CXl_1/CEl_3	90	6	8	0.71	0.50–0.30
CXl_2/51	86	5	0	5.25	0.05–0.02
CXl_2/CEl_3	94	6	2?	2.87	0.10–0.05
CXl_3/51	93	6	8	0.71	0.50–0.30
CXl_3/CEl_3	92	6	9	1.51	0.30–0.20

Summarizing it can be said:

1. That the mutant lines CEl_1, CEl_2, CEl_3, (51), CXl_1, CXl_2 and CXl_3 arose from line C as single gene mutations at 7 different loci: L_1–l_1, L_2–l_2, L_3–l_3, (E_1–e_1), x_1–X_1, x_2–X_2 and x_3–X_3.
2. Among these loci, it is probable that x_2–X_2 and E_1–e_1 are closely linked, and that L_3–l_3 is loosely linked to these two loci.
3. It is remarkable that all 4 EMS-induced mutations with small effect towards late are recessive (l_1, l_2, l_3 and e_1), and that all 3 X-ray induced ones are dominant (X_1, X_2 and X_3).

8. REVERSE MUTATION

8.1 SECOND-CYCLE MUTANTS

This chapter describes the author's attempts to obtain mutant lines reverted towards early from the 6 large-effect late mutant lines CA and CEI_3 (derived from line C) and 51A, 51B, 51D and 51E (from line 51). These lines were, in M_6 or M_7, subjected to a second-cycle treatment with X-rays (12 Kr) or with EMS (10 mM, 24 hrs, 24 °C) on 21-3-1966. Germination of the treated items (size 200 seeds) was over 90% (like the controls), except for CEI_3 after EMS which gave only 32% germination. Per line per treatment, 10 pans with 20 M_1 plants each were grown, and almost all seedlings transplanted survived till harvest. From each pan one fully fertile siliqua was harvested on the main stem of the 5 most fertile plants. Thus each of the $6 \times 2 = 12$ M_1-items (except CEI_3 after EMS) gave $10 \times 5 = 50$ M_2-families. All M_2-families which did not segregate seedling mutants (chlorophylls, etc.), were transplanted along with C, 51 and the 6 parental lines as controls.

Each of the 6 treated parent lines showed in M_2 a conspicuous increase in variation towards later flowering and more leaves, whilst only the lines CA, CEI_3 and 51D gave also conspicuous segregants towards earlier flowering and less leaves. Nevertheless, for *all* 6 lines, the 2 or 3 earliest plants were selected in each M_2-family which released variation towards early. The M_3 confirmed the impression obtained from M_2: only the treated lines CA, CEI_3 and 51D gave M_3-families which deviated sufficiently towards early to be interesting for our present purpose. In total 5 non-segregating early mutant M_3-families of independent origin were obtained, viz. CAE_1 and CAE_2 (from CA by EMS), $51DE_1$ and $51DE_2$ (from 51D by EMS), and CEI_3X_1 (from CEI_3 by X-rays). These 5 second-cycle mutant lines were selfed for one more generation to enable further within line selection for uniformity and vigour. For details of these lines, see point 5 in ch. 3.2.1.

As in the meantime attempts to identify major genes for the contrast between 51 and 51D had failed (See ch. 6.4.4 and ch. 6.8.7), the two second-cycle mutants from 51D were not subjected to further analysis. The mutant line CEI_3X_1 gave in the F_2 with its parent line CEI_3, no indication for single gene segregation, and consequently was also dropped from further studies. This leaves the revertants CAE_1 and CAE_2 from CA.

8.2 THE 4 × 4 DIALLEL CROSS BETWEEN C, CA, CAE_1 AND CAE_2

The 6 F_2's between C, CA, CAE_1 and CAE_2 (no reciprocals included) along with the F_1's and the 4 parents, were grown in pans from 18-5-1967.

8.2.1 *The cross C/CA*

The F_2 C/CA segregated 90 plants early like C and F_1, and 26 plants late like CA (expected 87 and 29; $\chi^2_1 = 0.41$; $P = 0.70-0.50$), and also gave evidence for additional modifier segregation. This confirms the findings in earlier experiments (See ch. 6.3). C = E_2E_2 and CA = e_2e_2.

8.2.2 *The crosses CA/CAE$_1$ and CA/CAE$_2$*

The line CAE$_2$ is about intermediate between C and CA, and the line CAE$_1$ is about intermediate between CAE$_2$ and CA. The F_1 CA/CAE$_2$ is about intermediate between its two parents, the F_1 CA/CAE$_1$ is close to CAE$_1$, so that these two F_1's roughly coincide. See fig. 25 for position of parents and F_1's. The frequency distributions (not presented) of the combined scores of the F_2 CA/CAE$_1$, size 115 plants, and the F_2 CA/CAE$_2$, size 103 plants, did not show any bi- or trimodality, but their ranges include the parents in such a way, that about 25% of the F_2 plants can be considered to represent the CA genotype. This suggests a single gene segregation in both F_2's.

8.2.3 *The crosses C/CAE$_1$ and C/CAE$_2$*

The F_1 C/CAE$_1$ and the F_1 C/CAE$_2$ are very close to line C, like the F_1 C/CA itself. See fig. 25, where the frequency distributions of the combined scores are given for the F_2 C/CAE$_1$, size 119 plants, and for the F_2 C/CAE$_2$, size 114 plants. Both F_2's clearly show the E_2-e_2 segregation, though there is a shortage of e_2e_2 types, viz. only 19 or at the most 23 plants (expected 30 plants) and 21

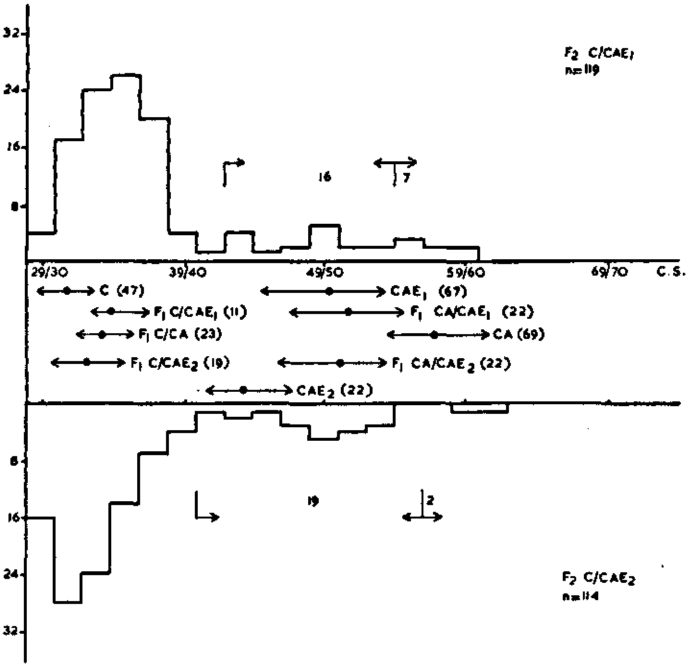

FIG. 25. Experiment of 18-5-1967. Frequency distributions (class interval 2 units) for the combined score (c.s.) of the F_2 C/CAE$_1$ (upper) and of the F_2 C/CAE$_2$ (bottom). Ranges of parents, F_1's and CA are indicated by horizontal arrows (Between brackets: number of plants).

plants (expected 28 to 29 plants), respectively. The cause of this shortage is unknown.

Now, the range of the e_2e_2 group includes CAE_1 and CA in case of the F_2 C/CAE_1, and includes CAE_2 and CA in case of the F_2 C/CAE_2. (These e_2e_2-ranges coincide with the ranges of the F_2 CA/CAE_1 and the F_2 CA/CAE_2 respectively). The range and also the shape of the e_2e_2-part of the two F_2-graphs (Fig. 25) give support to the hypothesis that both CAE_1 and CAE_2 differ from CA in one major gene. At the same time, recovery of CA types in the two F_2's shows that the two mutants are not at the E_2-e_2 locus. By interpolation one finds for the F_2 C/CAE_1, 7 in 19 or 23 e_2e_2-plants like CA (expected 5, $\chi^2{}_1 = 1.06$; $P = 0.30$) and for the F_2 C/CAE_2, 2 in 21 plants (expected 5; $\chi^2{}_1 = 2.87$; $P = 0.10-0.05$).

It now remains to be tested whether the mutations in CAE_1 and CAE_2 arose at different loci.

8.2.4 *The cross CAE_1/CAE_2*

The parents, the F_1 and the F_2 (size 114 plants) of this cross are given in fig. 26 (combined score, single unit).

The F_1 is intermediate between the parents CAE_1 and CAE_2. The F_2 CAE_1/CAE_2 shows transgression towards late past CAE_1 and towards early past CAE_2. This suggests digenic segregation.

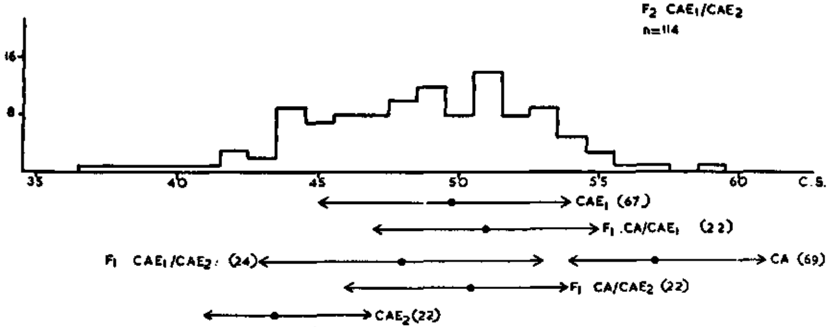

FIG. 26. Experiment of 18-5-1967. Frequency distribution (class interval 1 unit) for the combined score (c.s.) of the F_2 CAE_1/CAE_2. Ranges of CAE_1, CAE_2, F_1 CAE_1/CAE_2, F_1 CA/CAE_1, F_1 CA/CAE_2 and CA are indicated by horizontal arrows (Between brackets: number of plants).

Let CAE_1 be denoted by $e_2e_2\ R_1R_1\ r_2r_2$, CAE_2 by $e_2e_2\ r_1r_1\ R_2R_2$ and CA by $e_2e_2\ r_1r_1\ r_2r_2$. With two independently segregating genes, one expects $3/16 \times 114 =$ about 21 plants to be earlier than CAE_2. However, at the most 7 plants are found. Similarly, at the most 4 plants resemble CA (expected $1/16 \times 114 = 7$ plants). This indicates linkage between the loci R_1-r_1 and R_2-r_2. With recombination fraction p, the frequency of $R_1.\ R_2R_2$ is $p^2/4 + 2p(1-p)/4$ and that of $r_1r_1\ r_2r_2$ is $p^2/4$. Jointly, $p/2$. Equating $p/2 = 11/114$, one obtains $p = 0.19$, as a maximum estimate.

8.2.5 *Summary of the 4 × 4 diallel*

Line CAE_1 arose from CA by a dominant mutation (R_1), and line CAE_2 arose from CA by a mutation (R_2) with intermediate heterozygote. R_1-r_1 and R_2-r_2 are rather closely linked ($p=0.19$), but not closely linked to E_2-e_2. It should be noted that the present study does not contain tests to establish whether R_1 or R_2 arose at one of the loci E_1-e_1, E_3-e_3, E_4-e_4 and E_6-e_6.

In both the F_2's C/CAE_1 and C/CAE_2, no plants earlier than line C were found, which means that the genotypes $E_2E_2\ R_1R_1\ r_2r_2$ and $E_2E_2\ r_1r_1\ R_2R_2$ are phenotypically similar to $E_2E_2\ r_1r_1\ r_2r_2$ (= line C). Thus in the presence of E_2., the contrasts $R_1R_1-r_1r_1$ and $R_2R_2-r_2r_2$ do not come to expression. In terms of these loci, E_2 can be called epistatic. On the other hand, line C may be taken to represent a 'physiological limit' towards early, as no mutants earlier than C could be found (ch. 3.1.2), and as C did not appreciably respond to vernalization (ch. 3.2.3 and ch. 6.6). In this sense, the *total* genotype of line C can be considered as epistatic over any mutations towards early.

9. GENERAL DISCUSSION

The chapters 5, 6, 7 and 8 present the genetical analyses of flowering time and (correlated to it) leaf number for line 51 (originated from line C), the 22 mutant selections from the lines C and 51, and the 2 mutant selections from line CA. The results are summarized in table 20, which lists the mutations identified, the direction and magnitude of the phenotypic effects (towards early or late; small-,medium-or large-effect), the direction and degree of dominance, and (in the legend) the linkage relationships *within* certain groups of mutant lines. In the following discussion of some points which deserve special attention references are made to this table, unless stated otherwise.

9.1 MACRO- VERSUS MICROMUTATIONS. PRACTICAL IMPLICATIONS

In all 8 early mutant lines, and in 12 out of 16 late mutant lines (line 51 = e_1e_1, not included) single gene differences with the parent of origin (C or 51 or CA) could be identified. This has a number of implications. In the first place, mutant lines with more than one gene mutation for flowering time in a given direction appear to be relatively rare, although such lines, if present, would have an increased likelihood of being selected in M_2 and later generations. LAWRENCE (1965) analysed flowering time mutants of *Arabidopsis* by means of biometrical methods, and arrived at the conclusion, predicted by him, that 'selection will give rise to association', which conclusion is now being contradicted by the present direct genetical analysis. Secondly, the concept of micromutations needs to be considered. As GAUL (1965) has implicitly stated, classifications into macro- and micromutations (or major genes and polygenes), are meaningless and arbitrary: 'there seems to be only one sort of variability and only one sort of gene'. Indeed, the classification in table 20 into small-, medium- and large-effect mutations is arbitrary. As implied in the 'Introduction' (ch. 1) and in ch. 6.1, a mutation once identified, can be called a major gene mutation, and as long as not identified, it can be called a minor gene mutation. In the present study it was possible to individually identify single gene mutations also in most of the small-effect mutants. This was possible by providing a homogeneous environment, by comparing the F_2's from mutant × parent of origin with isogenic lines (parents and F_1's) and by mutual comparison of F_2-ranges (especially transgression) within sets of diallel crosses.

BROCK and LATTER (1961) ascribed only 10% of flowering time variation induced by X-rays in subterranean clover, to major gene mutations. Similarly, all but one of the X-ray mutations in *Arabidopsis* have a small effect (Table 20, see also below). The genes responsible for the remaining 90% of the induced variation in clover were not identified. But from our *Arabidopsis*-results, it now becomes clear that this not necessarily means that these small-effect genes are unidentifiable. Nor does it mean, as discussed earlier, that individual late flowering plants in M_2-lines with continuous phenotypic distributions must

TABLE 20. List of mutant genes, 17 towards late (including e_1 in line 51!) and 6 towards early. In 4 mutant lines (indicated by '?') no single gene differences with the parent of origin could be identified (viz. the small-effect late mutants $51El_1$, $51El_2$, $51Xl_2$ and the medium-effect late mutant 51D). It should be noted that no attempts were made to obtain late mutants from CA.

Dominance relationships (between brackets):

r = complete or almost complete recessiveness of the mutant gene.
±r = partial recessivity.
i = heterozygote about intermediate.
±r/i = changing from season to season.
d = completely or almost completely dominant.

The linkage relationships were only established *within* each of the 5 diallel crosses (See below). It will be noted that e_1 (in line 51) was entered into all diallel crosses, except in that between e_2 and the second-cycle mutants R_1 and R_2.

Linkage relationships as inferred from this study:

e_3–e_4 (ch. 6; diallel cross e_1, e_2, e_3, e_4, e_6)
e_7–e_8 (ch. 7.2; diallel cross e_1, e_7, e_8)
e_1–v_6 (ch. 7.3; diallel cross e_1, v_1, v_2, v_3, v_4, v_5, v_6)
$\left.\begin{array}{l} e_1\text{–}l_3 \\ e_1\text{–}X_2 \end{array}\right\}$ (ch. 7.4; diallel cross e_1, l_1, l_2, l_3, X_1, X_2, X_3)
R_1–R_2 (ch. 8; diallel cross e_2, R_1, R_2)

	Towards late				Towards early			
	from line C		from line 51		from line 51		from line CA	
	EMS	X-rays	EMS	X-rays	EMS	X-rays	EMS	X-rays
Small (up to 5 days)	l_1 (±r) l_2 (±r)	X_1 (d) X_2 (d) X_3 (d)	? (r) ? (r)	? (r)	v_1 (±r) v_2 (r) v_3 (r) v_4 (±r)	v_5 (±r) v_6 (±r)	R_1 (d)	
medium (6–10 days)	l_3 (±r/i) e_1 (±r)		e_8 (±r) ? (r) e_6 (r)	e_7 (r)			R_2 (i)	
large (> 10 days)	e_2 (r)		e_3 (i) e_4 (r)					

'automatically' contain *several* small-effect mutant genes (polygenes), a number of which is expected to be in the heterozygous state. On the contrary, our conclusion is that in general flowering time mutants selected in M_2 are single gene homozygotes at least when the mutations are recessives (See below), and that no further segregation will occur in M_3. This means that once individual plant selection has been applied in M_2, no within line selection for flowering time is necessary in further generations. For small-effect mutants, individual plant selection in M_2 must be followed by between line selection in M_3 and further generations, as genotypic and environmental effects cannot be distinguished in M_2 on an individual plant basis. However, when individual M_1-sectors can give rise to reasonably large M_2-lines, the same mutant genotype will be present in a number of M_2-individuals, so that, in a sense, 'line' selection can already be practiced in the M_2-generation.

Finally, when no large-effect mutants of the desired phenotype appear in M_2, but only small-effect mutants in the desired direction, crosses can be made already between individuals of different M_2-lines, in order to obtain recombinants with phenotypic effect of sufficient magnitude. This procedure finds its justification in the fact that the M_2-mutants selected are most often single gene recessives, and in the fact that the majority of the mutations are at different loci, as will be discussed later.

9.2 LATE VERSUS EARLY DIRECTION

It is significant that no early mutants could be selected following mutagenic treatment of the very early flowering line C. In this connection, two more facts are important. As pointed out in ch. 3.2.3 (See also ch. 6.6), vernalization did not have any detectable effect on line C. In ch. 8.2.5, it is seen that when substituting r_2r_2 in line C by R_2R_2 (R_2 is a medium-effect mutation towards early induced in line CA), no phenotypic shift towards early occurred. The same holds for substitution of the small-effect early mutants from line 51 ($v_1 \ldots v_6$; ch. 7.3) and from line CA (R_1, ch. 8.2.5). Jointly, these three points strongly support that line C represents a 'physiological limit' to the expression of effects towards early.

In literature, it is frequently stated for various organisms that after mutagenic treatment variation is mainly released away from the direction of previous selection. It seems logical that this is the result of previous selection having approached a 'physiological limit' to further expression, rather than the result of reduced possibilities of further mutations in that direction.

This was precisely the reason why line 51 and on a smaller scale line CA were included in the present experiments. As both lines 51 (BHATIA and VAN DER VEEN, 1965) and CA (this study) were obtained as late mutants from line C, they do not represent a physiological limit. From table 20, it is seen, that the number of mutants from line 51 towards early is not conspicuously less than the number of mutants from line 51 towards late. Moreover, as far as these numerically restricted data go, EMS and X-rays do not seem to differ much in the ratio of the late versus early mutants derived from line 51. As said in ch. 8.1, no attempts were made to select mutants towards late from line CA.

9.3 RELATIVE EFFICIENCY OF EMS AND X-RAYS

The majority of the mutations, both towards early and towards late were obtained after EMS treatment. As discussed in ch. 3.1.2, this is the outcome of the higher efficiency of EMS. For the doses 9 mM and 16 Kr, which doses gave rise to the vast majority of the present mutant lines, about 2 times as many mutant lines were obtained after EMS. Judging from the ratios of chlorophyll mutants in *Arabidopsis* given by MESKEN and VAN DER VEEN (1968), this ratio would be about 3 for doses which give equal M_2-fertility (viz. 8.3 mM and 12 Kr). Thus, taking equal M_2-fertility as a basis of comparison, EMS is about 3 times as efficient as X-rays in inducing flowering time mutants, with good fertility and vigour. It will be remembered that an important step in the selection procedure is to harvest and sow from the most fertile M_1-sectors only. This however, does not affect the above comparison, as within a given mutagen treatment in *Arabidopsis*, M_2-mutants are independently distributed of M_1-fertility (See also ch. 3.1.1).

A second point is whether EMS and X-rays differ with regard to the frequencies of mutations in the three magnitude of effect classes (Table 20). For this comparison, one has to exclude e_1 (which was induced in an EMS-experiment by BHATIA and VAN DER VEEN, 1965), and also the medium-effect EMS mutant 51D for which it could not be decided whether the difference with line 51 depends on one medium-effect or two or more small-effect mutations (ch. 6.4.4 and ch. 6.8.7). Furthermore, the early mutants from line 51 simply cannot have a large effect as they are near the 'physiological limit' represented by line C (ch. 9.2). When assuming the 3 small-effect late mutants from line 51 to be due to single gene mutations, one obtains for EMS 5, 4 and 3, and for X-rays 4,1 and 0, small-, medium- and large-effect mutations respectively. Or, combining the medium- and large-effect classes, 5 and 7 for EMS and 4 and 1 for X-rays. Applying the exact test for a 2 × 2 contingency table (FISHER, 1954) to judge whether this represents a significant deviation from equal proportions, one finds $P = 0.16$. This is not significant at the 10% level of probability. Moreover, X-ray induced medium- and large-effect late flowering mutants have been reported by several authors, viz. REINHOLZ (1947), REDEI and STEINITZ-SEARS (1961), and MCKELVIE (1962). LAWRENCE (1965), using gamma-rays found 8 out of 18 late flowering mutants to differ in a single major gene from the parent of origin.

A set of MCKELVIE'S mutants were grown in this Department, and the X-ray mutants showed a higher reduction in fertility than the EMS-mutants (VAN DER VEEN, pers. comm.). Unfortunately, no data on the corresponding mutagen doses are available, so that for the mutagens, as such, a differential association between large-effect flowering time mutations and sterility cannot be assessed. In our own material, examination of the selection data shows that for EMS and

X-rays, resp. 18 and 6 medium- and large-effect mutants of independent origin were selected in M_2. This does not deviate significantly from the 2:1 ratio for EMS versus X-rays mentioned earlier. In M_3 and further generations, 11 resp. 5 of these mutant lines were discarded, almost all on the basis of sterility which was strongly selected against (cf. ch. 3). This leaves the 7 EMS and 1 X-ray mutant lines mentioned earlier (cf. also table 20). Applying the exact test on the 2×2 table for discarded and not discarded with EMS and X-rays, one obtains $P = 0.25$. Moreover, most of the mutants in M_2 were derived from 9 mM and 16 Kr. The latter dose gives a higher overall M_2-sterility in *Arabidopsis* (MESKEN and VAN DER VEEN, 1968). Therefore, one already expects more mutants to be discarded after 16 Kr.

In conclusion: no preferential effects could be detected for EMS with respect to the induction of medium- and large-effect flowering time mutations, nor with respect to better fertility of such mutants.

9.4 DIRECTION AND DEGREE OF DOMINANCE. HETEROSIS.

At the loci identified so far after crossing natural varieties of *Arabidopsis thaliana*, the alleles towards late flowering are partially or completely dominant. Single gene differences were identified by HÄRER (1951), DIERKS (1958) and NAPP-ZINN (1963), whilst two dominants with complementary-like interaction were studied by VAN DER VEEN (1965). It seems that in all these studies on genes with large effect, at the most 3 loci are involved (VAN DER VEEN, pers. comm.).

In contrast, and not unexpectedly, the majority of the induced mutations towards late are partially or almost completely recessive. The same holds for the majority of mutations towards early. The exceptions are (cf. table 20) the large-effect EMS-mutation towards late, e_3 (which has intermediate heterozygote), the EMS-mutations towards early (R_1, completely dominant, and R_2, intermediate), and most conspicuously, no less than 3 small-effect X-ray mutants towards late from line C (X_1, X_2, X_3 are almost completely dominant; ch. 7.4).

Restricting ourselves to the small-effect late mutations and assuming the 3 small-effect late mutants from line 51 (not clearly identified) to be monogenic, it is seen that all 4 EMS-mutations are recessive, whilst 3 out of 4 X-ray mutations are dominant. Applying the exact test for a 2×2-table, this difference is significant at the level $P = 0.07$. Therefore, with some reserve, one can say that X-rays induce relatively more (small-effect late) dominant mutations than EMS does. In passing through literature, it may be noted that MCKELVIE (1962) lists a dominant X-ray induced mutation (F) with a very large-effect towards late. The 4 large-effect X-ray induced late flowering mutations (at 3 loci) mentioned by REDEI and STEINITZ-SEARS (1961) are all recessive, and so are the other late flowering mutations listed by MCKELVIE (l.c.).

With respect to between locus interactions, one may speak of epistasis in those cases where a mutation towards early is transferred to the genotypic background of line C, and consequently does not express itself, since line C represents a 'physiological limit' towards early (cf. ch. 9.2). An example is presented by the cross C/CAE_2 (ch. 8.2.3, see also ch. 8.2.5). Away from this limit, it was seen in ch. 7.4, that X_1, X_2 and X_3 are partially epistatic over L_1–l_1 and L_2–l_2. In all other comparisons made, the effects (including dominance effects) at the individual loci are approximately additive. This is clear in particular for the loci with medium- and large-effect (ch. 6.2). LAWRENCE (1965), using a statistical method, concluded for flowering time mutants in *Arabidopsis*, that there is no evidence of non-allelic interactions.

The crosses between the large-effect late mutants (ch. 6) provide good examples of heterosis in the absence of non-allelic interactions. Heterosis here means that the F_1-mean is outside the range of the parental means. In the dominance theory, heterosis depends on direction and magnitude of the dominance deviations at the individual loci, and also on the relative magnitude of the homozygote differences at the individual loci. The following crosses are good illustrations of different numerical aspects of the dominance theory of heterosis. For this purpose, the combined score of line 51 is put at 0, and the c.s. of the mutant lines are obtained by difference (from table 10; see also fig. 7):

a. The F_1 of the almost completely recessive mutants 51B (e_4e_4 E_6E_6) and 51E (E_4E_4 e_6e_6). The c.s. values are: F_1 51/51B = 0, F_1 51/51E = 3 and F_1 51B/51E = 2 (exp. 0 + 3 = 3). Cf. 51E = (38 + 17)–(30 + 12) = 13 and 51B = 30.

b. The F_1 of the completely recessive mutant 51B (E_3E_3 e_4e_4) and the mutant with intermediate heterozygote 51A (e_3e_3 E_4E_4). Here, F_1 51/51B = 0, F_1 51/51A = 15, and F_1 51A/51B = 16 (exp. 0 + 15 = 15). Cf. 51A = 28 and 51B = 30.

c. The F_1 of the completely recessive mutant 51D (assume E_3E_3 e_5e_5; however, see ch. 6.4.4) and the mutant with intermediate heterozygote 51A (e_3e_3 E_5E_5). Here, F_1 51/51D = 0, F_1 51/51A = 15, and F_1 51A/51D = 16 (exp. 0 + 15 = 15). Cf. 51D = 8 and 51A = 28. Here, the F_1 51A/51D is not heterotic as the sum of the dominance contributions is less than half the parental difference.

Similar comparisons can be made for the triheterozygote F_1's CA/51B, CA/51E, etc.

Heterosis towards late was encountered in the F_1 51/Li_2, where Li_2 contains a completely dominant gene B towards late (ch. 5.2). Equating the combined score of S96 to 0 (See table 7), one finds F_1 S96/Li_2 = 25, Li_2 = 25, F_1 S96/51 = 3 (See ch. 5.2.4), and F_1 51/Li_2 = 31 (exp. 25 + 3 = 28). The F_1 51/Li_2 is later than the later parent, viz. Li_2.

In short, all these crosses are good examples of heterosis based on intralocus interactions in the absence of inter-locus interactions (or in the case of Li_2 in the presence of small inter-locus interactions).

9.5 LINKAGE RELATIONSHIPS

The linkage relationships had been tested only *within* certain groups of mutations identified. These groups are given in the *legend* of table 20, and are arranged on the basis of parent of origin and direction and magnitude of phenotypic effect as assessed in the M_4-experiment described in ch. 3.7.1. On the basis of later observations (a.o. in different seasons) the magnitudes of effect were reclassified as given in the body of table 20. Line 51 (e_1e_1) was entered in all but one of the 5 diallel crosses. The analysis of the diallel crosses (ch.'s 6, 7 and 8) enabled only to detect relatively close linkage, as 1. flowering time is subject to considerable environmental variation, and 2. the crosses between the mutant lines were preponderantly in repulsion phase (recessive mutations!) in F_2's of limited size.

It is noteworthy that in each of the diallel crosses, even in the two 3 × 3-diallels, close linkage could be detected (See legend to table 20). Of particular interest is the large linkage group $e_1/v_6/l_3/X_2$ (no inferences were made about the linear order!). Moreover, the ecotype Li_2 contains an allele E'_1 at the E_1-e_1 locus (ch. 5.2.3). It remained undecided whether v_6 (ch. 7.3) is also an allele at the E_1-e_1 locus.

On the one hand, if the loci governing flowering time in *Arabidopsis* are evenly distributed all over the genome ($2n = 10$), one does not expect to find close linkages in each of the relatively small sets of mutants tested. If tests had also been done between mutants of different diallel sets, no doubt more cases of linkage would have been detected. On the other hand, it can still be said that the majority in each of the 3 larger sets are not closely linked, and therefore, the loci for flowering time in *Arabidopsis* genome are not restricted to a few clusters only.

9.6 GENOTYPE-ENVIRONMENT INTERACTIONS

Interactions between flowering time genotype and environment (season in the greenhouse) are often very pronounced in *Arabidopsis*. These interactions are studied in detail by BARTHELMESS (1967), who stated: 'Thus selection under different environments out of the same set of F_2-populations led to different results in respect to flowering time ...'. Similarly, our initial classification into small- and large-effect late mutant lines made in M_4 grown in the winter (ch. 3.2.1) had to be revised on the basis of later experiments throughout the year (cf. table 20).

Comparing early lines (e.g. C and 51) in winter and spring, their flowering time differences are about equal (ch. 5.1; fig. 1), but the late lines (e.g. 51A and 51B) show a conspicuous delay in the winter (ch. 6.6; table 11). Similar results for early and late genotypes from natural varieties were found by VAN DER VEEN (1965), who gave as extreme contrasts for flowering time:

	S96	Li_2	S189
Early-mid-summer	22	30	47
Winter	27	35	77

On the other hand, in (late) summer the combined score difference between the lines C and 51 was *larger* than in winter and spring, due to a relative delay of line 51 (ch. 5.1; fig. 1 and 2). The late mutant lines also flowered proportionally later (ch. 6.6; table 11). Very conspicuous is the large delay of line $51Xl_1$ in summer, which delay is not shown in winter and spring, nor in summer by the lines $51Xl_2$, $51El_1$, etc., which in winter and spring flower about simultaneously with line $51Xl_1$ (ch. 7.7; table 12).

In two crosses, where the wild allele is far from completely dominant, dominance × season interactions were met, i.e. the relative position of the monoheterozygote F_1 between the mutant line and the parent of origin fluctuated with season. Flowering time of the F_1 51/51A is about intermediate in the spring (and summer), but closer to line 51 in the winter (ch. 6.6; table 11), which reflects the differential delay of line 51A in the winter (See above), as the difference between line 51 and F_1 51/51A is the same in spring and winter. The F_1 C/CEl_3 behaves in a contrasting way: it is closer to line C in the spring (parental difference 6 days), but approaches intermediacy in the summer (parental difference 10 days).

When one ranges vernalization treatment under the environmental components, a conspicuous vernalization × genotype interaction is found for the lines 51A, 51B and CEl_3 which show a relatively small response to vernalization, such in contrast to the nearly complete response of all other lines (except of course line C, which represents a 'physiological limit'; see ch. 9.2). Of these three lines, the only monoheterozygote studied for vernalization response was the F_1 51/51A, and its response was almost complete (ch. 6.5). It is interesting to note that for all three lines, vernalization response varied with season, i.e. the three lines showed genotype × vernalization × season interaction.

The lines 51A and 51B behave very similarly to each other (ch. 6.6; fig. 12): In summer and winter, where they flower relatively late, they show a moderate response to vernalization, but in spring, where they flower relatively early, the response is almost absent. Line CEl_3 flowers relatively late only in the summer (not in winter, where it flowers as early as in spring), and correspondingly vernalization response is good (even very good) in summer, but almost absent in winter and in spring (ch. 3.2.3; table 4). Therefore, some causal relationship may exist between flowering time delay due to season and response to vernalization.

9.7 SIMILARITIES IN EFFECT

The large-effect late mutations e_3 (in 51A) and e_4 (in 51B) are similar in effect, in showing a relative lack of vernalization response, which lack of response is almost complete in spring, where these two lines suffer the smallest delay in flowering time (ch. 9.6). The only difference is that e_4 is completely recessive, whilst e_3 has intermediate inheritance.

Lack of vernalization response is not an intrinsic property of late mutations as such, as the large-effect mutation e_2 (in CA) shows complete response in all seasons. McKELVIE (1962) emphasises the exceptional behaviour of his large-effect late mutant line florens-1 (f_1f_1), which lacks response to vernalization, and also resembles our e_3 in showing intermediate inheritance.

If the mutations e_3 and e_4 are considered as changes which impair the function of the normal alleles E_3 and E_4, the above similarities in effect of the two mutant alleles may imply similarity in function of the normal alleles. It is now tempting to speculate, whether the E_3–e_3 and E_4–e_4 loci are in duplicate chromosome segments, the more as these two loci are closely linked (ch. 6.9.3).

SUMMARY

Arabidopsis thaliana (L.) HEYNH. was chosen as a model plant to study the genetic system of flowering time (start of flowering) and, often closely correlated to it, number of rosette leaves (vegetative production). The duration of the vegetative phase is of interest from the point of view of both natural selection (local adaptation) and artificial selection (plant breeding).

Genotypic variation was induced by seed treatment with EMS (ethyl methanesulfonate) and with X-rays. Two pure lines were used as a starting material, viz. the very early flowering Landsberg- 'erecta' (line C) and a later flowering mutant line derived from it (line 51). In total 24 flowering time mutants with good fertility and vigour, and of independent origin, were obtained through sector selection in M_1 (for fertility), and individual selection in M_2, followed by line selection in M_3 and further generations (for flowering time and fertility).

After grouping the 24 lines according to parent of origin, and direction and magnitude of effect, diallel crosses (F_1 and F_2) were made within the groups, in order to identify the individual mutations by means of classical Mendelian methods, rather than to describe and analyse the induced quantitative variation by the statistical methods of quantitative inheritance. In nearly all cases, including the small-effect lines, single gene differences with the parent of origin, could be successfully identified with the experimental and analytical procedures used for this purpose.

The analysis of the flowering time mutants led to the following conclusions:

1. No significant differences could be detected between EMS and X-rays, with respect to magnitude and direction of effect. There is some indication that X-rays induce more (small-effect late) dominant mutations. With respect to mutant frequency at equal levels of M_2-fertility, EMS is 2 to 3 times as efficient as X-rays.
2. The majority of the mutant lines selected differ in one single recessive gene from the parent of origin. Selections containing two or more unidentified mutations (micromutations) are relatively rare.
3. Mutagenic treatment of the medium early line 51, releases variability in both directions: towards early and towards late. However, no earlier mutant lines were obtained from the very early line C. Moreover, line C did not respond to vernalization treatment, and early mutant alleles when transferred to line C did not come to expression. Therefore, it is concluded that line C represents a 'physiological limit' towards early.
4. There was no evidence for allelism of mutations of independent origin. Several linkage groups were established. On the one hand, the loci for flowering time in *Arabidopsis* are not distributed at random over the genome, on the other hand they are not restricted to a few chromosome segments either.
5. Except for occasional evidence for non-allelic interaction (epistasis), the effects at the individual loci are additive over loci. Crosses between recessives and intermediates give, in connection with the relative magnitudes of the gene

contrasts, interesting numerical examples for the dominance theory of heterosis.

6. Pronounced genotype × environment interactions were frequently met: homozygotes effect × season, dominance effect × season, and also genotype × vernalization × season interactions were described.

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SAMENVATTING

Dit onderzoek betreft het genetisch systeem van de 'bloeitijd' (begin van de bloei) en van het 'aantal rozetbladeren' (vegetatieve productie): twee veelal sterk gecorreleerde kenmerken. De duur van de vegetatieve phase is een belangrijk aspect zowel bij de natuurlijke selectie (locale adaptatie) als bij de kunstmatige selectie (plantenveredeling).

Als object werd gekozen de modelplant *Arabidopsis thaliana* (L.) HEYNH., en als uitgangsmateriaal dienden twee zuivere lijnen, te weten het zeer vroeg bloeiende type Landsberg-'erecta' (lijn C) en een hieruit ontstane minder vroeg bloeiende mutant (lijn 51).

Ter inductie van genotypische variatie werden zaden behandeld met EMS (ethyl methaan sulfonaat) en met Röntgenstralen. In totaal werden verkregen 24 bloeitijdsmutanten, onafhankelijk van elkaar ontstaan, en met goede fertiliteit en groeikracht. Dit als resultaat van sector selectie op fertiliteit in M_1, gevolgd door selectie op bloeitijd en fertiliteit in M_2 (individuele selectie) en in M_3 en volgende generaties (lijn selectie).

Na groepering der 24 lijnen volgens ouder van herkomst, en volgens richting en grootte van het phenotypisch effect, werden binnen de groepen diallele kruisingsschema's (F_1 en F_2) uitgevoerd teneinde de individuele mutaties te identificeren met behulp van een klassieke Mendel-analyse. Hieraan werd de voorkeur gegeven boven de bij dergelijke geinduceerde kwantitatieve variatie vaak gevolgde weg van beschrijving en analyse met behulp van de statistische methoden der kwantitatieve genetica. Voor bijna alle lijnen, ook die met gering phenotypisch verschil, konden monogene contrasten met de ouder van herkomst worden geïdentificeerd, dankzij de voor dit doel gebezigde experimentele en analytische methoden.

De analyse van de bloeitijd leidde tot de volgende conclusies:

1. Tussen EMS en Röntgenstralen konden geen significante verschillen worden aangetoond wat betreft grootte en richting van het effect der bloeitijdsmutaties. Er is een zwakke aanwijzing dat Röntgenstralen meer dominante mutaties (met gering effect naar laat) induceren. Bij gelijk niveau van M_2-fertiliteit geeft EMS 2 à 3 × zoveel mutaties als Röntgen.
2. De meeste der geselecteerde mutante lijnen berusten op één recessieve mutatie. Selecties met 2 of meer niet te identificeren mutaties (micromutaties) waren weinig frekwent.
3. Mutagene behandeling van de niet extreem vroege lijn 51, geeft genetische variabiliteit zowel in de richting van vroeg als van laat. Uit lijn C werden geen vroegere mutanten verkregen. Voorts kon lijn C niet door koudebehandeling van het kiemend zaad worden vervroegd. Tenslotte kwamen in lijn 51 en in lijn CA verkregen mutaties naar vroeg niet meer tot expressie na inkruising in lijn C. Daarom wordt lijn C beschouwd als een 'physiologische limiet' voor vroegheidsexpressie.
4. Voor zover onderzocht, kon geen allelie tussen de verkregen mutaties wor-

den aangetoond, doch wel werd een viertal nauwe koppelingsgroepen gevonden (Tabel 20). Aan de ene kant zijn de bloeitijdsloci niet 'aselekt' over het *Arabidopsis*-genoom verdeeld, anderzijds is hun aanwezigheid ook weer niet beperkt tot slechts enkele chromosoomsegmenten.

5. Afgezien van enkele gevallen van (geringe) niet-allele interactie, zijn de effecten op de individuele loci additief over de loci. Kruisingen tussen recessief en intermediair vererverende mutanten, in combinatie met verschillen in grootte der geneffecten, leverden illustratieve kwantitatieve voorbeelden van de dominantietheorie voor het heterosisverschijnsel.
6. Er worden meerdere gevallen beschreven van uitgesproken genotype × milieu interactie: homozygotenverschil × seizoen, dominantie × seizoen, en genotypen × vernalisatie × seizoen interactie.

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