CHIMERISM AND DIPLONTIC SELECTION

PROEFSCHRIFT TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS, MR. J. M. POLAK,
HOOGLERAAR IN DE RECHTS- EN STAATSWETENSCHAPPEN VAN DE
WESTERSE GEBIEDEN,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT VAN DE LANDBOUWHOGESCHOOL
TE WAGENINGEN
OP WOENSDAG 23 JUNI 1971 TE 16 UUR

DOOR

GIJSJE H. BALKEMA

A. A. BALKEMA
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Dit proefschrift met stellingen van Gijsberthe Henriëtte Balkema, landbouwkundig ingenieur, geboren te Amsterdam op 9 september 1942, is goedgekeurd door de promotor, Dr. Ir. J. H. van der Veen, hoogleraar in de erfelijkheidsleer.

De Rector Magnificus van de Landbouwhogeschool,
J. M. Polak

Wageningen, 17 mei 1971.

STELLINGEN

I

Chimaerie-onderzoek kan bijdragen tot een beter begrip van mutagenese.

Dit proefschrift

II

Diplontische selectie is een misleidende term want slechts in enkele gevallen, bijvoorbeeld tussen potentiële adventiefknoppen, treedt selectie op.

Dit proefschrift

III

Het model van een méristème d'attente is niet in strijd met het optreden van sectoriale chimaerie maar helpt om het te verklaren. Dit proefschrift

IV

Alleen de deelname van een besmettelijke DNA drager kan het ontbreken van chimaerie en de hoge frequentie van homozygotie bij de transformatie-proeven van Hess verklaren. D. Hess 1970. Z. Pflanzenphysiol. 63: 31–43

V

Als de commissie voor de samenstelling van de rassenlijst voor landbouwgewassen de belangrijkste toelatingscriteria vooruit bekend maakt kan dit de veredeling bevorderen.

VI

De rolverdeling man - vrouw is functioneel maar nu overbodig wanneer deze de voortplantingskansen bevordert.

VII

Zolang er geen algemene objectieve voorlichting is over ontwikkelingshulp kan deze term negatieve reacties oproepen.

VIII

Nederland profiteert van de gastarbeiders maar kan door een juist beleid tegelijk aan ontwikkelingshulp doen.

IX

De werkgelegenheid in Suriname dient door steun en hulp zodanig te worden bevorderd dat de vestiging van Surinamers in Nederland, waarbij Suriname meer verliest dan dat Nederland wint, wordt beperkt.

X

Niet alleen door gebrek aan de pil krijgen vrouwen kinderen.

K. Davis 1970 In: *The sociology of economic development.* Ed. G. D. Ness.

Gijsje H. Balkema,
Wageningen, 23 juni 1971.

aan mijn ouders
aan johan

VOORWOORD

Hooggeleerde Van der Veen, hooggeachte promotor, mag ik u hier bedanken voor uw discussies tijdens mijn onderzoek en voor het kritisch lezen van het manuscript.

Dit proefschrift is het resultaat van vier jaar onderzoek aan de afdeling Erfelijkheidsleer. Ik ben dankbaar voor de hulp en medewerking die ik heb gekregen van het tuin- en laboratoriumpersoneel, en vooral van de heer Knoop die de foto's en figuren heeft verzorgd.

De heer Labaar van de afdeling Wiskunde dank ik voor zijn hulp bij de computeranalyse.

De vriendelijke medewerking van het personeel van de Centrale Bibliotheek heb ik zeer gewaardeerd.

CHIMAERIE EN DIPLONTISCHE SELECTIE

Chimaerie is het voorkomen van genetisch verschillende weefsels binnen een individu, vaak als gevolg van een mutatie d.w.z. een plotselinge genetische verandering. Bij periclinale en sectoriale chimaerie komt het gemuteerde weefsel in een laag, respectievelijk sector (vaak binnen een laag) voor. De gemeenschappelijke celwanden leiden tot continue cellijnen of afstammelingen die, door de structuur van de plant, vaak beperkt blijven tot zekere cellagen of delen daarvan en die door de groei, hoofdzakelijk in de apicale meristemen, parallel lopen met de groeias van de plant. Lang voortgezette sectoren zijn meestal de afstammelingen van enkele centrale apicaalcellen van de groeitop.

Met behulp van chemicaliën en bestraling is het mogelijk mutaties te induceren meestal, uit practische overwegingen, in zaad, en daar het embryo meercellig is, treedt chimaerie op. De geïnduceerde mutaties en chimaerie van het zaad worden alleen ten dele later in de plant teruggevonden. Kennis van de anatomie en ontwikkeling van de plant bieden ook hier de verklaring. Slechts enkele cellen in het zaad zullen centrale apicaalcellen voor de hoofdas zijn (worden), andere in bladoksels (van de kiemlobben, van de eventueel aanwezige bladprimordia, of van de nog, uit het al aanwezige celmateriaal, te vormen bladprimordia) zullen centrale apicaalcellen worden als er in deze oksels zijknoppen ontstaan, maar het merendeel van de cellen komt in de wortel, kiemlobben en eerste bladeren terecht. De cellen kunnen buitendien nog verschillen in hun gevoeligheid voor de mutagentia.

Eigen onderzoek aan arabidopsis en de zonnebloem, na behandeling met de mutagentia EMS en/of colchicine, toonde gevoeligheidsverschillen aan tussen de centrale apicaalcellen van de hoofdas, en tussen behandelingsstadia (zaad en kiemplant). Duidelijke sectoren kwamen voor en hun verloop kon in verband gebracht worden met de bladstand en, eventueel, een iets gedraaide groei van de plant. De sectoren waren echter niet persistent en eindigden na enige tijd zodat de (sectoriale) chimaerie verdween.

Dit verdwijnen van (sectoriale) chimaerie wordt vaak toegeschreven aan diplontische selectie, d.w.z. selectie tussen twee weefsels binnen een plant, waarbij een selectief nadeel voor het gemuteerde weefsel wordt verondersteld. De, in de literatuur vermelde, diplontische selectie blijkt echter, bij nadere beschouwing grotendeels te verklaren uit de normale ontwikkeling van de plant. De meeste cellen zijn tijdens de (mutagene) behandeling al voorbestemd. Alleen in die gevallen waar keuze tussen cellen of knoppen mogelijk is kan diplontische selectie optreden en wordt diplontische selectie ook inderdaad waargenomen.

Het verdwijnen van een sector, d.w.z. een van de centrale apicaalcellen, uit de groeitop is een eenmalige en daardoor abrupte gebeurtenis. Noch uit de literatuur noch uit eigen waarnemingen blijkt dat dit verdwijnen het gevolg is van het, al dan niet, aanwezig zijn van een mutatie in de verdwenen sector.

Het voortbestaan van sectoren wijst op een heel regelmatige groei vooral van de centrale apicaalcellen want één onregelmatige deling is voldoende om een van de centrale apicaalcellen uit te stoten en dus tot verlies van een sector te leiden. Het is goed denkbaar dat factoren die de ontwikkeling van de groeitop anatomisch of fysiologisch beïnvloeden ook het evenwicht in de groeitop zelf, bij gevolg, de chimaerie zullen beïnvloeden. Dit kon uit eigen onderzoek worden bevestigd. Behandelingen of milieu omstandigheden die de ontwikkeling beïnvloedden van de (mutageen behandelde) planten veroorzaakten ook verschillen in chimaerie. Snelle ontwikkeling gaf zwakkere planten met minder chimaerie. Tijdelijke stagnatie van de ontwikkeling, vooral in een vroeg stadium, leidde ook tot verminderde chimaerie. Deze vorm van chimaerieverlies, volgens toeval m.b.t. de genetische samenstelling van de sectoren, zou men diplontische drift kunnen noemen.

Sectoriale chimaerie, vooral binnen de bloem, kan de splitsingsverhouding van de mutanten en daardoor detectie van mutaties verminderen. Dit werd ook in eigen proeven waargenomen en toont aan dat chimaerie de waargenomen mutatiefrequentie beïnvloedt, en dat verlies van chimaerie het vinden van mutaties bevordert. Daar tegenover staat het voordeel dat chimaerie biedt voor het vinden van meerdere verschillende mutaties binnen een individu wat vooral bij planten met een lange levenscyclus of planten die veel ruimte of aandacht vergen, nuttig kan zijn.

Het is nu mogelijk om, met kleine wijzigingen van de groeiomstandigheden de diplontische drift dusdanig te beïnvloeden dat de (sectoriale) chimaerie lang voortduurt of snel verloren gaat zonder differentieel mutatieverlies. Deze mogelijkheid kan én voor het mutatieonderzoek én voor de mutatieveredeling van practisch belang zijn.

CONTENTS

CHAPTER 1

INTRODUCTION

Chimerism is a common phenomenon after mutation induction, the mutation occurring only in part of the plant. Careful observation revealed that in arabidopsis (after seed treatment) the mutated tissue occurred in vertical sectors in the main inflorescence (van der Veen and Gerlach 1965). In the tomato however, the base and the top of the plant differed (Hildering and Verkerk 1964). This could be explained from the different growth habit, clearly illustrating the effect of the growth habit on chimerism.

Chimerism poses many questions: How many sectors are there in a plant? Why do they stay constant? Are the sectors related to the phyllotaxis, or to the vascular bundles? What happens when a leaf, or a bud, is formed on the border of a sector? Does this occur frequently? What happens when a flower is formed on the border? How is fertilization within a chimeric flower, random or per sector? Do different tissue layers interact? Do the sectors overlap? How can chimerism be prevented? How can it be lost? Is there selection against the mutation; within the plant, within a tissue? If not, why is chimerism lost?

To answer these questions requires an understanding of mutagenesis and plant morphogenesis. The literature reveals controversies even on the existence of the initial cells necessary for the occurrence of sectors. Often everything deviating from the expected results in mutation research is ascribed to chimerism and diplontic selection. Although much could be learned from the literature, it is so extensive that no attempt at a complete review will be made.

To study chimerism and its disappearance, arabidopsis and the sunflower were chosen. Arabidopsis is an easy to handle, small, fast-growing, self-fertilizing plant on which much mutation research has been done. The sunflower is larger and slower but has an inflorescence that might give a good impression of the pattern and stability of chimerism. Colchicine was used because it induces a specific mutation, polyploidy, which can be observed in the pollen. EMS (ethyl methane sulphonate)

was used to induce different mutations useful for establishing the maximum number of sectors and the relationship between sector size and number. A specific mutation, however, offers the advantage of interplant comparisons in the chimeric generation, theoretically it should even be possible to calculate a diplontic selection index for it.

The plants were subjected to different treatments in order to influence the time between mutation induction and scoring, and to influence the growth of the plants, as these two factors were expected to have the greatest influence on the loss of chimerism.

In the following the literature is briefly reviewed to give a general impression on which own observations and experiments expand. The first chapters describe the material i.e. the plant; methods i.e. mutations and mutagenesis; and results i.e. chimerism. In the next chapters loss of chimerism by diplontic selection is examined and a more plausible alternative viz. diplontic drift, is suggested.

A better understanding of chimerism and diplontic selection may help to explain what happens between mutation induction and detection, how this affects the scoring results and what can be done to influence it. This is of importance for both mutation research and mutation breeding.

CHAPTER 2

THE PLANT

The structure and developmental pattern of the plant determine the destiny of cells (or their derivatives) present at a certain stage. In the case of mutated cells (chimerism), e.g. after mutagenic treatment of the seed, it is important to understand this destiny. A short review is given of the literature on those aspects of plant anatomy, physiology and morphogenesis which may help to understand the destiny of the (mutated) cells, the origin of the organ(s) scored (for mutations) and factors which affect either.

2.1 MORPHOLOGY

2.1.1 *Phyllotaxis*

Phyllotaxis is the systematic arrangement of the leaves on the stem circumference. With almost mathematical precision (Richards 1951) the leaves follow each other in a spiral round the stem. After a certain number of circumventions (x) a leaf (no. y) will be exactly vertically above the leaf of departure. The fraction x/y gives the phyllotaxis, the most common being 1/2, 1/3, 2/5 and 3/8 though higher orders are found, e.g. in cones. Whorled and decussate phyllotaxes give some problems. In mosses the phyllotaxis (1/3) can be related to the three-sided initial cell. In higher plants no similar relationship exists and many speculations and theories have been made on the advantages and causes of phyllotaxis (Sinnott 1960).

Phyllotaxis fascinates because it shows that there is a very definite system to growth, a system which might in some way be related with sectorial growth (anisophylly) or with sectorial chimerism.

Theories on phyllotaxis

1. Phyllotaxis originates in the apical initial cells. This was refuted

when it was shown that no specialized apical initial cells exist (Hanstein 1868) but indirect evidence of apical initial cells has evoked new interest in a possible relationship (Frandsen 1968).

2. Folds in the dermatogen (outer layer of cells) caused by faster growth, initiate the leaves. Surgical experiments show that wounds tend to gape on the apex indicating a tension, whereas wounds in existing leaf primordia close (Snow and Snow 1948).

3. The vascular bundles, or procambium, determine the leaf position. They are closely connected (Nägeli 1858, Priestley and Scott 1936) but surgical experiments show that detaching the tissue in the position of the leaf-to-be from underlying tissue, in which the procambium develops, does not prevent initiation of the leaf (Snow and Snow 1948). The leaf is sometimes asymmetrically initiated, yet the vascular bundles develop symmetrically illustrating their independence (Catesson 1953). Sometimes empty sectors are found on the plant where no leaves are formed but which do have vascular bundles (Hadj-Moustapha 1958). Vascular bundles develop towards and from the leaf, and in that way are related to the phyllotaxis (Fig. 2-1).

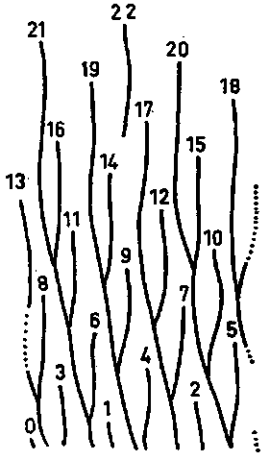


Fig. 2–1. Phyllotaxis and vascular bundles (from Nägeli 1858). Spiral: 3/8 phyllotaxis leaf 123····. Helix: 2 helices leaf 135····, 3 helices leaf 147····.

4. Repulsion or competition among leaves may cause the new primordium to develop as far away as possible from the previous ones. However, surgical experiments to isolate the older leaves or primordia did not affect the position of the next primordium (Snow and Snow

1948). For bud development, competition (Raciborski 1901) and repulsion (Snow 1965, Wardlaw 1968) may play a role.

5. The field theory states that chemical concentrations may vary in the apex producing fields in which the leaves develop (Richards 1948, Sussex 1964). Starting from a homogeneous apex small diffusion differences may eventually lead to a certain pattern (Turing 1952, Wardlaw 1957a,b).

6. The first available space theory (Hanstein 1868, Raciborski 1901, Priestley and Scott 1933), according to which the leaf develops where there is sufficient space, has been surgically demonstrated (Ball 1952, Snow and Snow 1933, 1935, 1947). Empty sectors (Hadj-Moustapha 1958) again cause problems. The phyllotaxis of axillary (Priestley and Scott 1933, Tucker 1962) and adventitious buds (Weisse 1894) seems to confirm this theory. Secondary extension of the leaf base may regulate the phyllotaxis (Catesson 1953, Snow and Snow 1962).

7. The anneau initial, a ring of actively dividing cells round the apex initiates leaves in a few slightly spiral helices (Fig. 2-1: leaves no. 1-3-5... or 1-4-7... giving two or three helices respectively). This theory solves the problem of decussate and whorled phyllotaxes, and leaf distances in the two helices compare better than within one spiral (Plantefol 1947a,b,c). Fused leaves in one (Dupuy 1968) or three spirals (Richards 1948) seem to illustrate the helices. This leads to the conclusion that the stem consists only of the fused leaf-bases (Buvat 1952) which is again refuted by the occurrence of empty sectors (Hadj-Moustapha 1958). One problem is; which parastiches are the helices (Fig. 2-1: 1-3-5... or 1-4-7...?) (Cutter 1959).

Anisophylly and anisoclady (differences in leaf, and bud, size) sometimes appear in helices, supporting the helix theory, but often occur in sectors (Champagnat 1948, Cutter 1966, Kumazawa and Kumazawa 1970, Raciborski 1901).

Surgical experiments have successfully split up the apex into two apices with one helix each (Plantefol 1958) or caused one helix to be lost (Loiseau 1965, 1969, 1970, Plantefol 1958, Tort 1969a), the latter usually being recovered again, sooner in vigorous plants (Loiseau 1965, 1970). Sometimes surgical experiments lead to anisophylly instead of complete loss of a helix (Tort 1969a). Fasciated stems have extra helices and often split up or fork to form apices with two or three helices (Plantefol 1947a).

In contrast to the former theories this theory not only explains but also describes phyllotaxis. As description the helix theory stresses the vertical connection between leaves originating from a few growth centres, while the spiral phyllotaxis has as many growth centres as leaves

(Wardlaw 1956) but all connected by a single spiral. Both components can be observed (Haccius 1950).

Chimeras show that there is a change in cells giving rise to the different leaves, also within a helix, for chimerism shifts independent of the phyllotaxis.

Changes in phyllotaxis. It has been mentioned that surgical experiments may influence phyllotaxis giving fewer helices (Loiseau 1965, 1969, 1970, Plantefol 1958, Tort 1969a), reversing the direction of the spiral (Ball 1952, Snow and Snow 1933) or changing the phyllotaxis from decussate to spiral (Snow and Snow 1935). Wounding may influence phyllotaxis by removing pressure (Weisse 1894, Snow 1951). Irradiation, e.g. X-rays (Gunckel 1957, Stein and Sparrow 1966, Zwintzcher 1955) and ^{32}P (Michaelis 1967); chemicals, e.g. giberellic acid and kinetine (Cutter 1963, 1964b, Tort 1969b); and changes in temperature (Tort 1967, 1969b) and light (Cutter 1966), may also influence phyllotaxis.

Changes during development are common, many plants starting with decussate phyllotaxis and changing from 1/2 to 1/3, 2/5, 3/8 and higher phyllotaxis. This can be related to apex size and shape (Koch 1893, Priestley and Scott 1933). Detailed study showed that the apex size increases while the leaf primordium size stays constant so that more primordia per apex circumference become possible (Soma and Kuriyama 1970). This may also be the reason for the induced changes in phyllotaxis through injury, chemicals, light and temperature. The growth habit of the plant, e.g. sympodial growth, may complicate matters (Brunaud 1968a,b). When the plant passes from the vegetative to the generative stage the phyllotaxis often changes (Cutter 1964b, Schwabe 1959) but may remain unchanged even when the plant apex returns to the vegetative stage (Diomainto-Bonnand 1970). Usually the apex has to exceed a minimum size before passing into the generative stage, this may concur with a certain phyllotaxis. As a whole the development of phyllotaxis is very regular.

Differences in phyllotaxis have been correlated with economic production in some plants (Thomas et al. 1970) though correlation with the direction of the spiral could not be proved significant (Satyabalan et al. 1964).

Torsion. Normally the leaves develop in a spiral round a vertical stem, but some types of teratological growth give plants where the leaves are almost vertically above each other and the stem is twisted into a spiral, often because the leaf bases are fused (de Vries 1892, Dupuy 1968). Mutated sectors in chimeric plants often reveal a gradual twist in the apparently vertical stem (Balkema 1970, Kaukis and Reitz 1955,

Michaelis 1967, Steffensen 1968). This may be related to a slight twist in the vascular bundles (Fig. 2-1). Studies of the apex revealed a rotating movement probably due to differential growth at leaf initiation (Catesson 1953, Schüepp 1966). Still another form of twisted growth (Plate IC) has been observed (Balkema 1971, Reinholz 1947) in which the plants all seem to be slightly twisted in a clockwise direction, probably related to the sun.

Anisophylly and anisoclady has already been mentioned; large and small leaves, or buds, occur according to a certain pattern along the stem (Champagnat 1948, Cutter 1966, Goebel 1928, Sell 1969, Hadj-Moustapha 1958). Leaves may even be asymmetrical or completely absent, or the prophylls of the side shoots may show a certain periodicity (Kumazawa and Kumazawa 1970). External factors probably influence the periodicity (Cutter 1966, Kumazawa and Kumazawa 1970) or induce anisophylly (Tort 1969a).

2.1.2 *The leaf*

The position of the leaf primordium can be forecast from the phyllotaxis of the stem. The first clear signs are periclinal cell divisions in the subepidermis (von Guttenberg 1960). Actually the plane of cell division in the next (deeper) cell layer changes even earlier (Lyndon 1970b). The first primordia are formed very near to, or on the apex (Catesson 1953, von Guttenberg et al. 1955) including the greater part of the apex (Cornu 1970, von Guttenberg et al. 1955, Hagemann 1963, Senghas 1957). Later leaves originate further from the summit of the (now larger) apex so that the ratio primordium size to apex size changes although the primordium size stays constant (Soma and Kuriyama 1970). As the apex is the area between the youngest leaf primordia it must increase in size when the leaves are formed further from the summit.

Leaf initiation time, or the plastochron, may vary during the development of the plant (Abbe and Stein 1954) or stay constant (Berg and Cutter 1969) but becomes shorter nearer to the inflorescence (Schüepp 1966, Senghas 1957). The number of primordia simultaneously found in the apex is related to the phyllotaxis (Priestley and Scott 1936). Development starts in the subepidermal layer but other layers participate in forming the leaf (Avery et al. 1959, Dermen 1960, von Guttenberg 1955, 1960, Schmidt 1924, Thielke 1951). The development of the primordium influences the apex which rotates (Catesson 1953, Kaplan 1970, Loiseau 1962, Schüepp 1966) or is tilted (Rougier 1955) away from the developing leaf. In ferns a leaf primordium may still be surgically induced to develop into a bud (Steeves 1959, Wardlaw 1968) but

in angiosperms radially symmetrical leaves are only occasionally formed (Snow and Snow 1959).

The primordium develops into a leaf with a characteristic shape which may even be obtained without cell division (Haber 1962). The relative importance of cell division and expansion varies according to the developmental stage of the plant (Sunderland 1960). The primordium is not necessarily in the centre of the future leaf which may extend asymmetrically at first (Catesson 1953, Majumdar 1942, Snow and Snow 1962), after which the veins develop symmetrically (Catesson 1953).

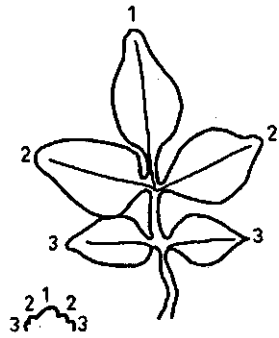


Fig. 2–2. Origin of a leaf. The leaflets develop from adjacent parts in the primordium.

The shape of the leaf was originally ascribed to division in lateral initials or meristems (Clowes 1963, von Guttenberg 1960), but studies with chimeras show that the whole leaf develops from a single base, the middle forming the top of the leaf (Bartels 1960, Dulieu 1965, 1968). This means that in compound leaves the leaflets originate next to each other on one leaf base and not above each other. This could be observed (Weberling 1956, Williams 1970) and deduced from chimeras (Balkema 1965 unpublished). If the veins are formed early in the developing leaf they follow the cell lineages and chimeric pattern (Bugnon et al. 1969, Guédès 1969).

2.1.3 *The bud*

The axillary bud is usually formed near to the apex in the axil of the fourth or even younger (nearer to the apex) leaf. The first cell divisions are usually below the subepidermis (Hanstein 1868, Schmidt 1924, Cutter 1964b, Gifford 1951, Hsü 1944, Klopfer 1965a,c, Lawalrée 1948) but may also be in the subepidermis only (Howard 1970, Howard

et al. 1963). Primary axillary buds may have a deeper origin than secondary i.e. axillary axillary buds (Champagnat 1954b). A "shell zone" separates the bud meristem from the apical meristem, especially when the bud develops near to the apex (Champagnat 1954b, Lamine-Gueye 1967).

The axillary buds develop from the apical meristem when they originate in the apex (Cutter 1964a, Loiseau and Nougarède 1963, Thomson and Miller 1962) or from a detached meristem which originated in the apex and may have to dedifferentiate again when the axillary buds develop later or further from the apex (Koch 1893, Buvat 1952, Champagnat 1954a, Cutter 1965, Garrison 1949a,b, Gifford 1951, Lamine-Gueye 1967, Lance 1957, Lawalrée 1948, Philipson 1948, 1949). The bud forms nearer to the apex just before the generative stage (Gifford and Tepper 1961, Hagemann 1963, Schüepp 1966). Some authors find the bud originating on the leaf base (Majumdar 1942, Majumdar and Datta 1946, Philipson 1949), others find a cauline origin (Koch 1893, Snow and Snow 1959). It has been shown that the first activity occurs in the stem just above the leaf base (van Fleet 1959) and chimeras show that a mutation is sometimes found in the axillary bud and the main stem above the leaf but absent from the leaf itself (Michaelis 1967). Fusion of the stem with the leaf base, and physiological influences from the leaf may cause an apparently foliar origin (Snow and Snow 1942, 1959). It is even suggested that the buds in monocotyledons may be formed under the next leaf (von Guttenberg 1960).

Axillary buds are associated with leaves. When two form in one axil, one is usually a secondary axillary bud of the other (Chassat 1962) but without a visible leaf (Cutter 1964a). Leaves may be so reduced that they can only be indicated anatomically (Hagemann 1963). The bud primordium covers less of the stem-circumference than the leaf (Clowes 1957) yet correlation for chimerism between the leaf and its axillary bud is high (Bartels 1961) though not 100% (Michaelis 1967). An interesting case of bud chimerism was found in a gladiolus flower having the top and base tepal similarly mutated (Buiatti et al. 1969). This may be a case where the mutated sector passed through the centre of the bud meristem leaving both sides normal.

The position of the bud in the leaf axil is influenced by older leaves and buds and is often acentric, usually anodic (Snow 1965, Wardlaw 1968) while the prophylls of the bud may be preferably cathodic (Kumazawa and Kumazawa 1970). This may be related to the asymmetrical extension of the leaf base (Catesson 1953). Bud development may show some periodicity.

The bud develops to some extent and then becomes dormant. This

development is correlated with that of the main apex so that there is a gradient in the plant and younger buds are nearer to the generative stage (Fardy et al. 1953, Heslop-Harrison and Heslop-Harrison 1969). This gradient also appears in tissue cultures from nodes at different positions (Chouard and Aghion 1961).

During dormancy the DNA in the bud is in the 2C stage (Naylor 1958). Dormancy can end naturally or be artificially broken. The older buds are first out of dormancy but the younger buds develop faster (Philipson 1948). However, many buds stay dormant, probably as a result of competition between buds for available nutrients (Raciborski 1901, Gillet et al. 1969). External conditions, e.g. light, can cause excessive bud development (Arnal et al. 1969).

Adventitious buds are formed from differentiated tissue which dedifferentiates, when other buds and the apex have been killed or removed. They may develop on roots, in which case they develop from deeper (L_3,C) tissue (Bateson 1921, Robinson and Darrow 1929), or even from roottips (Ballade 1970). On tubers they also have a deeper origin (Dermen 1960, Howard 1969). When they develop on the hypocotyl they may originate from epidermal cells (Crooks 1933) or subepidermal cells (Champagnat et al. 1962). On leaves they may develop from deeper cells (Marchal 1968) but are usually formed in epidermal cells (Broertjes et al. 1968, Naylor and Johnson 1937), sometimes in special positions, e.g. near haircells (Bigot 1970), or from detached meristematic cell groups on the leaf-edge (Naylor and Johnson 1937). The first chimeras were produced from adventitious buds (see Neilson-Jones 1934, 1969; see 4.1.1) which usually develop from two or more cells (Stewart and Dermen 1970b) except in the leaf where they develop from one (Arisumi and Frazier 1968, Broertjes 1969a) or occasionally from two cells (Clowes and Juniper 1968).

The phyllotaxis of the bud is influenced by the main stem and the adjacent leaf which is the first leaf of the new phyllotactic spiral (Priestley and Scott 1933). Other developing buds may influence the phyllotaxis (Fosket 1968). In adventitious buds the first leaf may even be dorsal or ventral to the bud (Weisse 1894).

2.1.4 *Vascular bundles*

These form a continuous network in the plant from the roots to the leaves and flowers. They develop at an early stage thus giving an impression of the development of the plant. Though they are related to the phyllotaxis they do not cause it, differentiating after the leaves (Nägeli 1858) (Fig. 2-1). They also develop towards organs which degen-

erate (Hagemann 1963, van Heel 1966). This may explain the vascular bundles in an empty sector (Hadj-Moustapha 1958). The vascular tissue is first seen as procambium which differentiates very near to the apex so that it is difficult to observe the direction of differentiation (Bersillon 1955). Grégoire (1938) found the direction to change when the plant enters the generative stage but this was later refuted (Lawalrée 1948). The observation that the vascular tissue develops in both directions (Nägeli 1858) still holds for xylem (Esau 1945, Priestley and Scott 1936). The phloem differentiates earlier and from the leaf towards the stem (Esau 1945). Actually the procambium is meristematic, becoming visible through the differentiation of the surrounding cells (Newman 1961).

Buds and apices can induce vascular tissue in callus tissue (Camus 1945, Wetmore 1953, 1956), but this effect is even obtained with blocks of agar with auxin and sugar, the concentrations influencing the type (phloem, xylem or both) and position of the vascular tissue (Wetmore 1956, Wetmore and Rier 1963).

The vascular bundles form a pattern in the stem, spaced equally, almost vertical except at the nodes where they fork or bend in one direction, so that they stay parallel and slightly spiral (Nägeli 1858, Priestley and Scott 1936). After some nodes the bundles fuse with those from a lower leaf (Fig. 2-1). The number of bundles is related to the leaf traces and the phyllotaxis (Clowes 1961, Priestley and Scott 1936). Secondary growth is proportionate to primary growth so that the relative positions stay constant (Thoday 1922). Vascular bundles from the bud join the leaf traces (Hagemann 1963, Majumdar 1942) or go directly to the stem (Sachs 1970).

In the inflorescence the vascular system may be reduced (Bersillon 1955). In the flower they usually take the shortest routes, still growing slightly oblique (van Heel 1966). The vascular bundles form a semicircle in the petiole but can be induced to form a circle (Warren-Wilson and Warren-Wilson 1963). If they form early they run parallel to the tissue type in chimeric leaves (Bugnon et al. 1969, Guédès 1969).

2.1.5 *The flower*

Wolff, and later Goethe, considered the flower to be a modified branch (Nozeran 1955). This idea is still found (McCoy 1940) but often adapted to differentiate between the fertile parts, stamens and carpels, and the petals and sepals. The latter are regarded as modified leaves (Melville 1962, 1963) as they have similar positions (Bersillon 1955, Grégoire 1938, Plantefol 1948) and origin (Avery et al. 1959). The stamens

and carpels have the same origin as branches (Avery et al. 1959) and are classified as a combination of leaf and fertile stem (van Heel 1966, Melville 1962, 1963) so that the flower is actually a condensed inflorescence (Melville 1962, 1963). The generative apex for a flower resembles that for an inflorescence (Grégoire 1938). Simple flowers may indeed be the result of continuous reduction and simplification from complex inflorescences, while complex flowers are still in the prefloral stage (Emberger 1951, Nozeran 1955). This may explain a.o. the structure of the crucifer flower (Motte 1946, Nozeran 1955). Aberrant "flowers" with three flowers on one stalk are still found (Arnal et al. 1969, Buggert and Röbbelen 1970).

The normal pattern of floral parts within a flower is physiologically determined as was shown by surgical experiments (Cusick 1956, 1959), and the number (of parts) may be influenced by environmental conditions, e.g. reduced by cold (Cutter 1965). Their initiation affects the apex less than leaf initiation (McCoy 1940). The early generative apex differs from the vegetative apex, in that active cell division occurs in the whole apex instead of only at the sides as in the vegetative apex (Buvat 1952, Fardy et al. 1950, Grégoire 1935, 1938, Lawalrée 1948). The first flowers may include most of the apex (Cornu 1970). When more flowers are formed they are arranged as the leaves, in a certain phyllotaxis, probably because they occur in the axils of leaves which, however, may be very rudimentary (Hagemann 1963). The apex then usually shows zonation with an active flank meristem (Nougarède 1967). When only one flower is formed it is not necessarily terminal (Grégoire 1938).

Changes back to the vegetative state (Arnal 1966, Codaccioni 1958, Hagemann 1963) show that the generative apex is not fundamentally different from the vegetative apex. However, the morphologic difference is often so clear that it can be used when studying the physiology of flowering (Besnard-Wibaut 1967). The generative apex resembles (Newman 1965) or is a modification of (Monet and Bastard 1969) the vegetative apex at the end of the season.

2.2 APICAL STRUCTURE

Cell division, growth and organ formation is oriented mainly in the apical meristems of the plant. The shoot apex will be considered first.

When specialized apical initial cells had been found in the fern they were also sought in the angiosperm apex. In 1868 Hanstein showed that the apex consists of different cell layers. Since then initial cells

have been found or postulated in these layers. In 1935 Grégoire stressed the anatomical differences between vegetative and generative apices. This led to more careful observations of the apex and its zonation, ending in the theory of the méristème d'attente, a resting meristem which is inactive in the vegetative stage and takes over in the generative stage. These three main ideas on apex organization (layers, initials and zones) are not necessarily incompatible, since they concern three different aspects of the apex: zonation, stratification within this zonation and cells or cell lineages within this stratification (Fig. 2-3). This is clearly

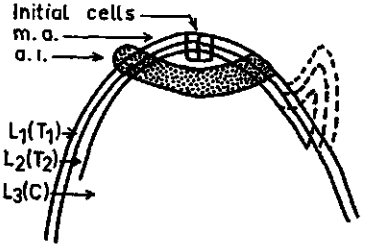


Fig .2-3. The zones, layers and initial cells of the apex. L_1, L_2, L_3 (T_1, T_2, C) = layers, m.a. and a.i. = zones viz. méristème d'attente and anneau initial.

demonstrated by the occurrence of zonation in apices of mosses or ferns which do have specialized initial cells (Hallet 1969a,b, Michaux 1969).

Maybe the controversy arises from the different interests of people studying the different aspects: cytologists and physiologists the zones, geneticists and histologists the layers and geneticists the initial cells. The use of the word initial for the ultimate cell source (initial cell) and for initiating or forming (anneau initial) may have added to the controversy.

2.2.1 *Layers*

Hanstein (1868) studying apices of different plant species (angiosperms) could find no specialized initial cells, as had been found in ferns, but noticed a certain stratification. Usually the plants had an outer layer, one cell deep, which only divided anticlinally and covered the whole plant. The cells in the core of the plant divided in all directions. Between this core and the outer layer there was another layer, usually one cell deep and only dividing anticlinally. This layer however also showed periclinal cell division at leaf and bud initiation and, further from the apex, to form the cortex.

Later a sharp distinction was made between the anticlinally dividing outer layers and the core, the tunica and the corpus respectively, the

tunica usually consisting of two independent layers (Schmidt 1924). Periclinal chimeras have given evidence that the plant can usually be seen as three almost independent layers, the L_1, L_2 and L_3 or T_1, T_2 and C (Satina et al. 1940). The anticlinal division in the outer cell layers has been explained as the result of pressure on the cells, and of the cell shape (Dermen 1969a,b). Sometimes an extra tunica layer is found (Dermen 1951) or only one tunica layer as in many monocotyledons (Gifford 1954, Popham 1951, Thielke 1951) or no tunica at all (Thielke 1959). These discrepancies may be the result of an occasional periclinal division in the L_2, absence of clear layering in the L_2, or the presence of some layering in the L_3, but can often be solved by studying the situation in the bud (von Guttenberg 1960). The embryo is usually not yet differentiated into L_1, L_2 and L_3 but the L_1 is generally present and the L_2 develops from the internal tissue (von Guttenberg 1960). The L_1 seems to be mainly a covering layer but after damage to the apex the L_1 is important for regenerating a new apex (Iqbal 1970, Linsbauer 1917, Pilkington 1929).

The role of the layers in organ formation still gives some controversy. Usually all three participate in forming the leaf and bud (Schmidt 1924, Dermen 1951, Satina et al. 1940) but the leaf may consist of L_1 only (Hsü 1944, Rösler 1928) or of L_1 and L_2 (Dermen 1951). The participation of the layers in the tuber (Klopfer 1965a) and fruit (Dermen 1960) may also vary. The sporogenic tissue usually derives from the L_2 (Dermen 1947, Popham 1964). Periclinal divisions in L_1 or L_2 can disturb the genetic independence of the layers so that L_1 or L_3 may also contribute to the spores (Dermen 1947, Stewart and Burk 1970). This may occur spontaneously (Dermen 1947, Popham 1964, Stewart and Burk 1970) or be caused by damage to the apex (Crockett 1957, 1968) or by genetic differences between the layers (Thielke 1954).

The plant apex can be placed in one of 7 classes according to the layers and specialized initial cells (Popham 1964). This has again been reduced to 3 classes; monoplex with a specialized initial cell, simplex with no specialized initial cell and no layers, and duplex without a specialized initial cell but with one or more cell layers dividing only anticlinally, covering a core with cells dividing in all directions (Newman 1961). Angiosperms belong to the last class.

2.2.2 *Initial cells*

When specialized initial cells were found in ferns an attempt was made to find these in angiosperms too. It became apparent that if initial cells existed in angiosperms, they were morphologically hardly identifiable

(Hanstein 1868). The number of initial cells seen or calculated by different authors varies but, as pointed out by Schwendener in 1879, maximally 3 or 4 apical initial cells can exist (in the apex centre) and divide, without being lost (Schwendener 1879).

Initial cells here are the beginning of cell lineages. They must retain their position and divide to form new cells (Tepper 1964). The number of initial cells is dependent on how far back into the ontogeny of the plant we go as the zygote, from which the whole plant derives, is unicellular. It also depends on the part of the plant studied and the layering. Usually the apical initial cells of one layer in the apex are meant when an estimate of the number of initial cells is given. Douliot (1890) mentions columns of initial cells (a group per layer).

Vandendries (1909) clearly shows 6 subepidermal initial cells in the ovular bud of some crucifers. Other authors, studying the different layers of the apex anatomically find one (von Guttenberg 1955, Kliem 1937), one or a few (Bartels 1960, Cross 1937, von Guttenberg et al. 1955, Klopfer 1965a, Rauh and Rappert 1954, Rösler 1928, Stant 1952), or a group (Hamilton 1948, Hsü 1944) of initial cells. Studying the apical epidermis maximally 3 adjacent cells can be seen (Dermen 1945, Stewart and Dermen 1970a). Evidence from chimeras shows that sectors of mutated tissue in one layer usually cover 1/4, 1/2, 2/3, or 3/4 of the circumference (Bartels 1960, Stewart and Dermen 1970a; see also 4.4.2) when they are continuous and, therefore, derived from the apical initials, but may be much smaller when they are localized (Dermen 1945). Chimeric potato tubers may have 6 initial cells (Howard 1961b). Others find no anatomic evidence for initial cells (Favard 1954, Foster 1941) or dispute their existence (Bergann 1965). Evidence for only one initial cell from "chimeras" (Moh 1961) may be caused by lack of apical organization in the seed of this plant (coffee) (Varossieau 1940) at the time of treatment. If the apical cells are not stable eventually only cells from one cell lineage will be found.

Photos of the apex show that the epidermal cells constantly shift, i.e. no cells remain continuously in the centre (Ball 1960). This effect may however have been produced by the movement of the apex during leaf formation (Catesson 1953). Marking or wounding shows that the cells do move to the sides of the apex (Loiseau 1962, Soma and Ball 1964). Removal of the top cells, however, shows that these are not necessary for further growth (Ball 1948, 1950).

Other observations on the apex demonstrate the existence of cell groups (Gavandan and Gastelier 1970, Tolberg and Johnson 1966), oriented cell division in the apex (Stebbins 1965, Thielke 1954, 1955) and interaction between layers (Thielke 1955).

2.2.3 *Zones*

The idea of fundamentally different zones in the apex was probably started by Grégoire who pointed out anatomic differences between the vegetative and generative apex (Grégoire 1935, 1938). Plantefol developed a theory on phyllotaxis which differentiates between the summit of the apex and the sides, or anneau initial, where the leaves are initiated (Plantefol 1947b). Buvat found cytological differences between these zones. The cells in the summit do not have the expected meristematic appearance but, rather, appear to be differentiated. These he therefore called the méristème d'attente, which becomes active and dedifferentiates when the plant passes into the generative phase (Buvat 1952). Although the idea of zonation was not new (Fardy et al. 1950, Foster 1941) the extreme idea of the méristème d'attente without cell division stimulated much research on the apex. Buvat (1955) himself admits that the cells in the summit do divide, but they have no specific organogenic properties. They provide the cells for an increase in size of the apex and also, by dedifferentiation, some cells for the actively dividing anneau initial, and eventually they form the floral apex.

Anatomically cell divisions were found but fewer than at the sides of the apex (Brown et al. 1964, Buvat 1955, Catesson 1953, Favard 1954, Gifford and Tepper 1962, Lance 1952, Loiseau 1962, van Parijs 1969, Popham 1958, Savelkoul 1957, Staff 1968, Stant 1952). However, the time of day (Edgar 1961, Lance 1952) and the duration of mitosis (Jacqmard 1970, Lyndon 1970a) may influence these results. Even when differences in cell size are taken into account the cells at the sides divide about four times more often (Lance 1959). Direction of cell division and cell displacement vary during the plastochron (Lyndon 1970a), especially during and before leaf initiation (Lyndon 1970b). Observations on living apices (Newman 1956), photos of the apex (Ball 1960) and marking or killing apical cells (Loiseau 1962, Soma and Ball 1964), also show that the cells in the summit do divide. Cell impressions (Frank 1965, Frank and Pohl 1965) or the use of the scanning electron microscope (Falk et al. 1970) may make more observations possible.

Surgical experiments (removing or damaging the summit) have proved that the plant can continue growth without these cells (Ball 1948, 1950, Plantefol 1958). Tissue cultures show that these cells themselves cannot continue on their own (Wetmore 1956). They may be the ultimate source of cell material but they are dependent on the rest of the apex where organ formation occurs (Sussex 1964).

Indirect evidence also comes from damage caused by chronic irradiation. The sides are more sensitive than the summit, as is expected if

they are more active (Gunckel and Sparrow 1953). Labelling with DNA precursors shows a difference between the summit and the sides (Gifford and Tepper 1962, Lance-Nougarède 1961, Miksche and Brown 1963, van Parijs 1969) but the, possibly disturbing, influence of labelling must be taken into account (Wimber 1959) and DNA synthesis does not necessarily indicate cell division (Partanen and Gifford 1958, van der Walle and Bernier 1967). Chemical and physiological differences have also been found (Gifford and Tepper 1962, Nougarède 1967, West and Gunckel 1968).

Some experiments show no difference in labelling (Clowes 1959a) or rate of cell division, or even show more active division in the summit (Cross 1937). There may be several explanations for this. Young seedlings or embryos are often not yet differentiated into zones (Buvat 1955, Catesson 1953, Nougarède 1967) and some plants never develop zonation (Lance-Nougarède 1961, Loiseau 1962). The inactive zone is very small in small apices (Catesson 1953, Loiseau 1962) and may be difficult to discern when the apex oscillates (Catesson 1953). During the transition from the vegetative to the generative stage the zonation is lost and the summit becomes as active as the sides (Bernier et al. 1967, Besnard-Wibaut 1966, Buvat 1952, 1955, Corson 1969, Fardy et al. 1953, Lance 1952, 1959, Saint-Côme 1965). This transition may take long under unfavorable circumstances giving however, a less clear zonation.

The relationship between zonation and transition to the generative stage is so clear that it is now used as a first indication of floral induction (Bernier et al. 1967). It also explains the properties of certain chemicals to induce flowering. GA (giberellic acid) stimulates cell division and, given at the right time, it will stimulate division in the inactive summit cells and cause earlier flowering (Besnard-Wibaut 1967, Jacqmard 1967). BUdR (Bromodeoxyuridine), a thymidine analogue, temporarily inhibits the cells at the sides from dividing, thereby releasing the cells at the summit and causing earlier flowering (Brown 1962, 1967). In the generative apex new zonation may develop (Besnard-Wibaut 1966, 1967).

Evidence from chimeras shows that less division occurs in the summit (Gymnosperms: Hejnowicz 1959). No extended chimerism is found in plants with no méristème d'attente at and shortly after the treatment (Dulieu 1969, Fardy et al. 1953). Chimeric sectors do not follow the helices produced by the anneau initial, which must be seen as superimposed on the cell lineages (Michaelis 1967).

It has been said to be necessary for geometric reasons that the cells at the summit divide less frequently (von Guttenberg 1960, Wardlaw 1970). For stability it seems better that they divide less, while physiolog-

ically this can be explained (Newman 1965, Wardlaw 1956). Logically it is expected as the cells at the sides are actually the first cells of the fast-growing meristematic leaves.

These three theories (2.2.1, 2.2.2, 2.2.3), each emphasizing a single aspect of the apex structure, do not exclude each other as an apex can have three layers each with a few initial cells, a less active summit and a very active anneau initial forming the leaves. If one of the initial cells mutates it will eventually form a sector of cells with this mutation which may, however, only be seen when it has reached the anneau initial and is incorporated into the leaves and/or axillary buds. One initial cell or the absence of stable initial cells will lead to the absence of sectors. In this case either periclinal chimeras (if the layers are stable), or totally mutated (and normal) plants are eventually formed.

2.2.4 *The root apex*

The root apex is more symmetric as it is not disturbed by the origin of leaves, lateral roots originating further from the apex, but the root cap poses new problems. Here too controversy is found between "initial cells" and "zonation".

Initial cells. Some authors observed one initial cell (von Guttenberg 1948, von Guttenberg et al. 1955) or a few dividing in two directions to form the root cap and the root (Schwendener 1879). Usually groups of initial cells are observed (Clowes 1950). After wounding one side of the root, repair is found only at that side, and not the whole apex is reorganized, as would be expected if there were only a few initial cells (Clowes 1953). Root chimeras have been used to estimate the number of initial cells. As no stable cell layers are found the mutations usually occur as solid sectors (Brumfield 1943a). The size of the sector found varies from 1/2 (Brumfield 1943a, Němec 1962b, 1963) to 1/3 or 1/4 (Brumfield 1943a, Juhl 1953, Rickard 1958 see Clowes 1961) to 1/6, 1/8 and 1/16 (Brumfield 1943a, Davidson 1959, Nawaschin 1926, O'Dell and Foard 1968). This may depend on whether an apical initial cell was mutated (Brumfield 1943a). Lateral roots which derive from many cells give small sectors (Davidson 1959, Němec 1961, O'Dell and Foard 1968). Other reference to chimerism in the root is made by Kachidze (1932) and van der Mey (1970). Using chimeras Němec could demonstrate the different origin of root cap and root (Němec 1962b).

It must be taken into account that using X-rays to induce chimerism may damage the root and give a distorted impression of the normal situation (Clowes 1959d). Chimeric roots differ from normal roots in

the origin of lateral roots (Němec 1962b) or by various other abnormalities. Among the latter are forking of the root tip (Němec 1965) which is also found after wounding (Clowes 1963), the formation of a new root tip at one side (Němec 1963), or even the casting off or cell death of mutated tissue (Němec 1961, 1962b, 1965). Yet after decapitation a new chimeric apex is formed (Němec 1962a).

Zonation. As in the shoot a part of the apical meristem of the root is found to be less active. This was already mentioned for a root initial cell (von Guttenberg 1948). Later a group of cells was found differing in rate of cell division (Clowes 1956a, 1961, Jensen 1959, Thompson and Clowes 1968), in cell growth (Barlow 1969), cell size, nucleus size, RNA content (Clowes 1956b) and in DNA content (Clowes 1959a,b, le Coq and Guervin 1968, Thompson and Clowes 1968). Labelling showed less DNA synthesis (Clowes 1956a, Thompson and Clowes 1968) but the labelling can pass through these cells demonstrating that their inactivity is not due to isolation and starvation (Clowes 1970b).

This group of cells has been called the quiescent centre. It has been found in the embryo (Byrne 1970, Guignard and Mestre 1967, Guignard et al. 1968) and also in cultured roots (Phillips and Torrey 1970). The size seems to depend on the size of the root (Clowes 1961). Just as the méristème d'attente in the stem, the quiescent centre is necessary to prevent chaos and keep the form of the apex in the root (Clowes 1961, 1964, Jensen 1959, Thompson and Clowes 1968). It acts as a reserve for cells to repair the apex when the apex is damaged, especially after irradiation (Clowes 1963, 1970a). The cells of the quiescent centre are less active and therefore less sensitive to irradiation which even stimulates them to divide (Clowes 1970a). Indirect evidence for a quiescent centre comes from polyploidy experiments in which, even after prolonged treatment, diploid cells can still be found (Levan 1938) which revert the apex back to the diploid state (Němec 1961).

Apart from the quiescent centre other differences in cell division are found in the root apex (Socher and Davidson 1970). Synchronization of cell division in the root seems possible and has been done for mutation studies (Wagenaar 1966). Experiments have however shown that the root and shoot apex may react differently to mutagenic treatment so that results cannot be directly compared (Künzel and Maluszyniski 1966).

2.2.5 *Development of the apex*

Studies of the embryogenesis of plants have revealed that some cells may be inactive at a very early stage (Guignard et al. 1968, Vallade 1970). This may be seen at the 16-cell stage (Guignard and Mestre

1967), or at the 30-60 cell stage of the proembryo (Pritchard 1964). The quiescent centre develops later when the embryo is discontinuous with the suspensor (Rondet 1965).

The cotyledons develop from surrounding cells as the leaves do on an older apex (Guignard et al. 1968, Mestre and Guignard 1967). At germination some dedifferentiation occurs (Guignard et al. 1968), and the embryo itself usually lacks zonation (Buvat 1955, Lance 1952, 1957, Millington and Fisk 1956, Nougarède 1967, Rougier 1955) which reappears when the apex grows after germination (Fardy et al. 1950, Nougarède 1967).

The layering in the apex of the embryo is usually not yet very clear and stable (Philipson 1949, Rauh and Reznik 1953, Senghas 1957, Varossieau 1940). It consists of an $L_1(T_1)$ and deeper tissue (L_3 or C) (Gifford 1954, Miksche and Brown 1963) from which the $L_2(T_2)$ develops (von Guttenberg 1960, Lance 1954). At first some periclinal divisions still occur in the L_1 (Roth 1956, Senghas 1957) and in the L_2 (Klopfer 1965c, Reeve 1948a,b, Senghas 1957, Souéges 1914). In the older embryo (Reeve 1948b) or in the seedling the layers are eventually stabilized.

Changes in the older apex have been reviewed in the foregoing and will here be briefly mentioned.

The first leaves develop near to or on the apex, including a large part of the apex, later leaves develop further from the summit and the primordia are relatively smaller although the primordium size stays constant (2.1.2). The apex size increases (Bersillon 1955, Hagemann 1963, Lance 1957, Philipson 1949, Popham 1964, Popham and Chan 1952), cell size decreases (Rösler 1928, Stein and Fosket 1969), leaves are formed faster (Senghas 1957) and axillary buds develop nearer to the apex (Gifford and Tepper 1961, Hagemann 1963, Schüepp 1966) as the apex approaches the generative stage.

The zonation in the apex changes, the méristème d'attente becoming active, when the physiological prefloral stage is reached (Buvat 1952, 1955, Corson 1969, Fardy et al. 1950, Lance 1952, 1957, Loiseau 1962, Pédurand 1969b). The last products of the anneau initial are the sepals (Buvat 1952, 1955) and the petals (Plantefol 1948). The older generative apex itself may however have an active "flank" meristem (Nougarède 1967, Taillandier 1969, Vaughan 1955, Vaughan and Jones 1953). The different floral parts represent different physiologic morphogenic stages of the floral apex and therefore occur in a certain sequence (Cusick 1956, 1959).

The generative apex changes during the season (Phelonzat 1970), decreasing in size towards the end of the inflorescence (Senghas 1957),

and the tip may degenerate (Hagemann 1963, Pédurand 1969a) or return to the vegetative stage (Hagemann 1963). The generative apex resembles the vegetative apex at the end of its growth (Newman 1965) and may develop from it in the next season (Raju 1969) or together with it at the end of the season (Monet and Bastard 1969). Layering is often less clear, or lost in the generative apex (Popham and Chan 1952, Rauh and Rappert 1954, Rauh and Reznik 1953).

2.3 CHANGES IN THE APEX

2.3.1 *Natural variation*

The apex size, shape and zonation is different in different species (Gifford 1954, Newman 1961, Popham 1951) and may even differ within a species (de Nettancourt and Devreux 1969). Polyploidy increases cell size and therefore, at a constant cell number, also apex size (Cross and Johnson 1941, Randolph et al. 1944) without affecting layering (McGowan and Bishop 1953). Very small apices may have no zonation, or it may be difficult to discern (Buvat 1955, Catesson 1953, Loiseau 1962) but the size does not affect layering (Rouffa and Gunckel 1951a). Apices of terminal buds may be narrower than those of axillary buds (Popham 1960). The developmental gradient found in axillary buds along the stem (Chouard and Aghion 1961, Fardy et al. 1953, Heslop-Harrison and Heslop-Harrison 1967, Rivière 1968) may influence their size and zonation.

Sections including the leaf primordium often show clearer zonation or layering (Gifford and Tepper 1962, Thielke 1954). The stages of the plastochron do not influence layering (Millington and Gunckel 1950) but make it less stable (Gifford 1954, Rouffa and Gunckel 1951b). When the leaf is formed nearer to the summit it more clearly affects the size, shape and orientation of the apex (Abbe et al. 1951, Catesson 1953, Gifford 1954, Kaplan 1970, McCoy 1940, Popham 1960, Rauh and Rappert 1954, Rösler 1928, Rouffa and Gunkel 1951a, Rougier 1955, Schüepp 1966, Schwabe 1959, Senghas 1957, Tucker 1962). The leaf primordium and its initiation influence the position (Edgar 1961, Soma and Ball 1964) and direction (Lyndon 1970a,b, Majumdar 1942) of cell divisions in the apex. These divisions may show diurnal periodicity (Edgar 1961, Karsten 1918, Lance 1952, Popham 1958) or not (Savelkoul 1957), possibly depending on the temperature (Edgar 1961).

A seasonal effect on apex size, activity and structure has also been found (Popham 1960, Rouffa and Gunckel 1951a, Taylor 1965) though

this does not seem to affect layering (Millington and Gunckel 1950), but may influence the initial cells (Bain and Dermen 1944). Seasonal changes often go together with developmental changes in the plant which greatly influence apex size and zonation (2.2.3, 2.2.5). The apex size increases till flowering, though the final size may vary (Hussey 1963), and may then decrease again (Hagemann 1963, Senghas 1957). Cell size changes during the vegetative stage (Iqbal 1969), is small at the beginning of the floral stage (Rösler 1928, Stein and Fosket 1969) and then increases again (Bernier et al. 1967).

2.3.2 *Induced changes*

Many external factors have been shown to influence the development of the plant. Those inducing the reproductive stage have been studied most, giving great changes in the apex. It is not always possible to distinguish between a direct effect on the apex and an indirect effect through floral induction.

Light may influence apex growth (Husain and Aspinall 1970) through its intensity (Aspinall and Paleg 1963, Cutter 1965, Hussey 1963) which affects the zonation (Buvat 1952), rate of leaf formation (Aspinall and Paleg 1963) and the inflorescence size (Devlin 1969). Total darkness gives smaller apices (Loiseau 1969) or only slower development (Butler and Lane 1959). Weak light may cause a reversal from the generative to the vegetative state (Diomainto-Bonnand 1969, 1970).

The photoperiod affects the size and shape of vegetative organs (Lance 1957), the number of buds which develop (Arnal et al. 1969), and the induction of the generative stage (Devlin 1969, Heslop-Harrison and Heslop-Harrison 1967, Jacqmard 1965, Pédurand 1969b). In a photoperiod, unfavorable for flower induction, expansion of the apex may continue much longer before the generative stage is reached (Besnard-Wibaut 1966, 1967). Interruption of the proper photoperiod may revert the apex to the vegetative stage (Arnal 1966). Long day and short day plants are differently affected. The photoperiod probably acts through its effect on DNA synthesis (Bernier et al. 1967, Bronchart and Nougarède 1967, Zeevaart 1962) or RNA synthesis (Bronchart et al. 1970).

Temperature. Some plants need low temperature, vernalization, to induce flowering. This induction may be lost in subsequent high temperatures. Water and oxygen is required for these processes (Devlin 1969). During the cold period the apex continues to develop (Besnard-Wibaut 1970) though the plastochron may be longer (Schwabe 1959). Continuous light replaces vernalization in some cases (Blondon 1970).

High temperature may also induce flowering (Harada 1969). It has already been mentioned that temperature affects the diurnal periodicity of cell divisions in the apex (Edgar 1961).

Apical enlargement is slower at high temperature (Cutter 1965, Hussey 1963), yet low temperature gives fewer floral parts (Cutter 1965) which is associated with smaller apex size (2.4). Temperature shocks influence helix number which increases with a drop in temperature and vice versa (Tort 1967). With a longer plastochron at low temperature the ratio primordium size: apex size may change allowing an extra helix.

Chemicals. Water stress influences apex growth (Husain and Aspinall 1970), and nutrition (Hussey 1963), especially a high C/N ratio, favours floral induction (Deltour 1967, Heslop-Harrison and Heslop-Harrison 1967) even under unfavorable photoperiod. This effect is usually indirect through hormones. These regulate normal development and may also be responsible for induction after decapitation (Harada 1969) and defoliation (Hussey 1963). The former effect can be increased by various hormones (Harada 1969). The most used hormone, GA, induces flowering in long day plants (Devlin 1969) even with an unfavorable photoperiod (Besnard-Wibaut 1967) or temperature (Sarkar 1958). GA causes cell division leading to elongation which may be related to flowering (Jacqmard 1967, Michniewicz and Kamiénska 1965). In weak light, GA may cause elongation without flowering (Jacques 1969). CCC suppresses flower induction and internode elongation. These can be restored by GA but the concentration necessary to restore elongation is higher than that required to restore flowering (Zeevaart and Lance 1963). Auxin and kinetin also influence flowering through their effect on RNA and DNA synthesis respectively (Helgeson 1968, Lang 1966) or indirectly by influencing the GA in the plant (Michniewicz and Kamiénska 1965) which influences DNA synthesis (Lang 1966). Other compounds, e.g. geranylvalerate (Bonzi and Napp-Zinn 1967) have also been used to induce flowering.

Some thymidine analogues induce flowering (Brown 1962, Hirono and Rédei 1966) probably by inhibiting DNA synthesis (Brown 1967) in the actively dividing cells and thus inducing the resting summit cells to divide (2.2.3). Thiouracil inhibits RNA synthesis and floral induction, at the stage when RNA synthesis takes places (Bronchart et al. 1970).

Hormones also influence apical growth by breaking dormancy (Naylor 1958, Sachs and Thimann 1964). They may even induce loss of flowering potential in axillary buds (Sankhla 1969) and affect the layering in the apex (Ball 1944).

In tissue cultures light, temperature, medium and hormones influence the organ formation (Skoog 1944, Torrey and Shigemura 1957, Wirth 1960).

EMS (ethyl methane sulphonate) which is used to induce mutations leads to decreased growth but may stimulate at low doses (Müller 1966).

X-rays and wounding. Decapitation and defoliation have been mentioned for floral induction. The latter gives an increase in apex size (Hussey 1963). Wounding may affect phyllotaxis (Tort 1969a) probably through its effect on the active apex size as well as on the visible apex size. X-rays often disorganize the apex (Miksche et al. 1962), disturbing the layering (Crockett 1957, 1968, Howard 1967, Iqbal 1969, Kuehnert 1962, Lapins and Hough 1970, Pratt 1960, 1963, 1967, Sagawa and Mehlquist 1957) or the zonation and leaf formation (Crockett 1968, Gunckel 1957, Lapins and Hough 1970). This may lead to forking (Kress 1953, Lapins and Hough 1970, Mericle and Mericle 1957, Reichardt 1955, Reinholz 1959, Remussi and Gutierrez 1964, Sankewitsch 1953), multiple branching (Ivanov 1967), fasciation (Grober 1959, Gunckel 1957, Haccius and Reichert 1963) or the appearance of vegetative organs on a floral apex (Gunckel 1957).

After irradiation one may find cell death through toxic substances (Gunckel 1957) or general damage (Kuehnert 1962) and precocious cell maturation (von Wangenheim 1970, von Wangenheim et al. 1970) or blocked differentiation (Haccius and Reichert 1963). Other cells may be stimulated to displace damaged cells or to form a new apex (Pratt 1963) and may change the phyllotaxis (Stein and Sparrow 1966). Fasciation leading to forking is the result of damage as after wounding (Loiseau 1962), or of an outsized apex due to blocked differentiation (Haccius and Reichert 1963). Cell death in the apex also causes forking or multiple branching. When no new apex is formed, axillary or adventitious buds may take over (Pratt 1959, 1963, 1967).

Sometimes X-rays stimulate germination (Reinholz 1968) or flowering (Samuel and Kamra 1968). The latter may be due to damage of the active cells so that the resting summit cells divide. In roots X-rays stimulate the resting quiescent centre cells to divide (Clowes 1959d, 1963).

2.4 CORRELATIONS

With increasing apex size the rate of organ (mainly leaf) production, usually increases (Abbe and Phinney 1951, Hagemann 1963, Hussey 1963), but it may also stay constant (Berg and Cutter 1969) or even decrease (in the embryo; Abbe et al. 1951). The apex also influences organ size and shape. It produces larger leaves as it increases in diam-

eter (Cutter 1965, Millington and Fisk 1956, Stant 1954, Sunderland 1960, Wardlaw 1957c), or continues to form small leaves when it is inhibited in its growth (Röbbelen 1957a). Near the inflorescence leaf size may, however, decrease, but bud size stays constant (Schüepp 1966). Rate of leaf production and leaf shape are also related (Njoku 1957). The apex influences the number of organs: more floral organs (Grégoire 1938, Stebbins 1967) or cotyledons (Stebbins 1967), more helices (Plantefol 1947a) and a higher phyllotaxis (Priestley and Scott 1933, Thomas et al. 1970) are associated with a larger apex.

Conversely, the leaves may influence apex size by competing for assimilates so that defoliation causes an increase in apex size (Husain and Aspinall 1970). Apex shape is related to leaf shape (Stant 1954) and to phyllotaxis, curved apices having spiral, and flat apices decussate or whorled phyllotaxes (Koch 1893).

Movement of the apex is more pronounced in plants with spiral phyllotaxis (Schüepp 1966). The number of initial cells in the apex may be related to the phyllotaxis, 4 in decussate and 3 in spiral phyllotaxes (Frandsen 1968). A wounded apex sometimes forms two new apices, usually with different phyllotaxis, spiral instead of decussate (Snow and Snow 1935) or fewer helices (Loiseau 1959). This may be the result of a change in apex shape, size or initial cell number.

In summing up

The foregoing permits a few conclusions to be drawn on plant development and structure which may be important for an understanding of chimerism.

The apex consists of (3) cell layers and a few initial cells which will determine where the derivatives of a mutated cell are most likely to go, e.g. only cells from the L_2 are represented in the gametes. The cells in the summit of the apex hardly divide (méristème d'attente), and therefore act as a buffer for the initial cells. In the root this is still more pronounced.

Leaves are initiated according to a very regular and stable pattern (phyllotaxis). This is also reflected in the vascular bundles which develop very early as a network connecting the leaves. The axillary bud meristem originates from the apical meristem though the bud often starts developing further from the apex. The first divisions for the bud primordium are found in the L_3 (C).

Zonation changes during plant development, often together with apex size. Small apices and young seedlings often have no méristème d'attente. Apex size may also affect leaf size, leaf shape, phyllotaxis and development. Towards flowering the apex also changes, the méristème

d'attente becomes active and the apex increases in size. In certain types of inflorescences zonation is re-established and the flowers appear, as the leaves, in a phyllotaxis.

2.5 ARABIDOPSIS AND SUNFLOWER

Arabidopsis and the sunflower are the plants that were used in the experiments to be presented.

2.5.1 *Arabidopsis*

Arabidopsis thaliana (L.) Heynh. is a small crucifer common as a weed on sandy soils in many parts of the world. It has low nutritional requirements and some ecotypes have a very short life cycle (30 days). It is a self-fertilizer with a large seed production (Laibach 1943a,b). Different races have quantitatively different photoperiodic (Laibach and Kribben 1954, Langridge 1957) and cold requirements (Laibach and Zenker 1954). It has ten small chromosomes (Laibach 1943a, Steinitz-Sears 1963). Cultures from various plant parts can be grown to flowering (Yokoyama and Jones 1965), detached leaves may sometimes form roots and even plantlets (Napp-Zinn and Berset 1966) and seedlings easily form new roots when the root has been removed (Balkema 1969).

A full biography has recently appeared (Rédei 1969).

Morphology. Arabidopsis is a rosette plant with a 3/8 or 5/13 phyllotaxis (Müller 1965a) and 8-13 vascular bundles (Adams 1964). The rosette sometimes has a twisted appearance (Balkema 1971, Reinholz 1947). The number of rosette leaves and cauline leaves varies according to the variety and the duration of the vegetative phase. Plants from old seed (Napp-Zinn 1964), vernalized plants (Zenker 1956) and plants grown in the greenhouse (compared to growth chamber; Velemínsky et al. 1968) have fewer leaves. Axillary buds develop into side shoots in the axils of the cauline leaves and some of the rosette leaves, depending on the variety (Napp-Zinn 1967), the season (Barthelmess 1967) or the light regime (Arnal et al. 1969). Vernalization, CCC and decapitation promote, and GA inhibits branching (Napp-Zinn 1958, 1967, Sankhla 1970). X-rays may influence branching (Buiatti and Lorenzoni 1963) and cause abnormal branching, e.g. multiple branching (many thin shoots) (Bouharmont 1965a, Ivanov 1967) or forking (Haccius and Reichert 1963). Branching and days to flowering are positively correlated and the heritability depends on the varieties crossed (Barthelmess 1964). Different major genes for days to flowering are known (Hussein 1968).

The flowers are of the crucifer type (Müller 1961b) and are arranged on an unbranched inflorescence which flowers from the base upwards. In crucifers the leaves bearing the flowers are probably degenerated and fused with the flower stalk (Hagemann 1963). Abnormal "flowers" with two or three flowers on one stalk (Buggert and Röbbelen 1970, Usmanov 1970) may be reversions to a more primitive state (2.1.5). Green and abnormal flowers at the end of the inflorescence may be caused by greenfly (Lehmann 1969) but are also found as a mutant phenotype (Röbbelen 1965a). Maybe they are caused by hormone disbalance in both cases. Polyploidy has been induced, but is not quite stable, especially not above 4n (Bouharmont 1967b). Polyploids are later flowering and less fertile than the corresponding diploids (Bouharmont 1965b, 1967a).

The apex. Arabidopsis has a very small apex. In the mature embryo there are two cell layers (Gerlach-Cruse 1969), and the primordia of the first two leaves (Miksche and Brown 1963). The apex may be merely 6 cells in diameter (Miksche and Brown 1965). Only after five days can the three tissue layers be distinguished. X-rayed seed sometimes gives forked plants, because the inhibited cell differentiation leads to a broad apex which forks. The cotyledons and first two leaves are normal, the next two leaves may be double, demonstrating that the first two leaves were, at least, predetermined in the seed (Haccius and Reichert 1963). Development in other crucifers shows that the first leaves reduce the apex size, e.g. from 9 to 2 or 3 cells in diameter. The apex grows during development (from 9 to 20 cells diameter) (Senghas 1957) and the leaf base occupies a progressively smaller part of the circumference (Hagemann 1963).

Different cell numbers and measurements have been given for the diameter or height of the (arabidopsis) apex. The differences may be due to the variety and plant age, but also to the shape of the apex, which is long and narrow at first. Reported diameter sizes vary: 28μ and 12 cells (Brown et al. 1964), 30μ (Besnard-Wibaut 1966), 40μ (Brown 1967), 45μ and 12 cells (Bouharmont 1965a) and 54μ and 15 cells (Vaughan 1952) for the vegetative apex, and for the generative apex 45μ (Besnard-Wibaut 1967, Wibaut 1966) and 90μ (Vaughan 1955). The tetraploid apex is a little larger (55μ) with fewer (11) cells (Bouharmont 1965a). The apex size may influence leaf size and shape (Röbbelen 1957a).

The central cells of the vegetative apex divide less (Besnard-Wibaut 1966, Vaughan 1952) and have less DNA synthesis (Besnard-Wibaut 1967, Brown 1967, Brown et al. 1964, Miksche and Brown 1963, Wibaut 1966) than the cells at the sides and they form the méristème d'at-

tente which becomes active when the plant goes into the intermediate (Besnard-Wibaut 1966) or generative stage (Besnard-Wibaut 1967, Brown 1967, Wibaut 1966). When the plant is in the generative stage zonation is re-established (Besnard-Wibaut 1967, Wibaut 1966) so that the apex seems similar in the vegetative and the generative stage (Vaughan and Jones 1953).

Gametogenesis (Misra 1962, Rédei 1965a,c, Vandendries 1909) and embryogenesis (Gerlach-Cruse 1969, Reinholz 1959, Souéges 1914) have been studied. The ripe dry pollen is oval, 27 × 15μ (Müller 1961b), becoming round when hydrated, with a diameter of 21μ in diploid and 26μ in tetraploid plants (Bouharmont 1965b). Fertilization takes place from the base of the siliqua upwards (Bouharmont 1965b) but all embryos in a siliqua are at the same developmental stage (Gerlach-Cruse 1969, Reinholz 1959). Semigamy can be induced (Arnold and Cruse 1968).

The young proembryo shows layering which is partly disturbed when the cotyledons bend over (Souéges 1914). X-ray treatment of proembryos leads to abnormalities such as twinning, which resembles forking after seed treatment, and which is more complete when younger proembryos are treated (Reinholz 1959). X-raying pollen also gives abnormalities demonstrating that these are not necessarily due to direct damage (Röbbelen 1960, 1962c).

Physiology. Usually long day promotes flowering, but short day does not prevent flowering (Besnard-Wibaut 1966, 1967). These (short day) plants are often stronger due to the longer vegetative growth (Hirono 1964). When applied at the right time GA promotes flowering (Besnard-Wibaut 1967, Langridge 1957, Napp-Zinn 1963), at the wrong time it may give stalked rosettes (Besnard-Wibaut 1968). This shows that elongation and flowering both caused by GA are different processes (Napp-Zinn and Bonzi 1970). Kinetin and vitamin E also promote flowering, possibly through their effect on GA (Michniewicz and Kamiénska 1965).

Usually cold treatment promotes flowering, and some varieties have been reported (Laibach and Zenker 1954) to require cold treatment. Vernalization, devernalization and revernalization have been studied at different plant stages (Napp-Zinn 1957a, 1959a,b,c, Zenker 1956). Water and oxygen are required (Napp-Zinn 1957b), and light may help during vernalization (Napp-Zinn 1959a,b,c). GA can replace vernalization (Langridge 1957, Sarkar 1958) but does not affect leaf number (Sarkar 1958).

DNA base analogues can promote flowering (Hirono and Rédei 1966) probably by releasing the cells in the méristème d'attente (Brown 1967). Also geranylvalerate (Bonzi and Napp-Zinn 1967) and even irradia-

tion (Samuel and Kamra 1968) can promote flowering. Tetraploids flower slightly later, and higher polyploids much later than diploids (Bouharmont 1965b, 1967a).

Ripe seed has a certain degree of dormancy (Laibach 1956) which can be broken by cold (Laibach 1956) and by GA (Kribben 1957). Some varieties require light for germination but injury to the testa (Kugler 1951) or X-rays (Reinholz 1968) may replace the light requirement. Some mutants show no dormancy but lose their power to germinate after prolonged storage (Röbbelen 1966a, Velemínsky and Gichner 1967). After-ripening conditions (Rehwaldt 1966) and even the filter-paper used may influence germination (Rehwaldt 1968). Unripe (green) seed sown directly has no dormancy yet (Balkema 1968). This has also been recorded in other plants (Morgan and Berrie 1970).

Cell division is reported to start 25 to 40 hours after sowing (Jacobs and Bonotto 1968), or after 30 hours in the root and after 45 hours in the epicotyl (Müller 1967a).

Buds sometimes show dormancy, which can be broken by decapitation (Laibach and Kribben 1954). Vernalization and CCC promote bud development and GA inhibits it (Napp-Zinn 1967).

2.5.2 *The sunflower*

Helianthus annuus L. is a composite, consisting of a main stem with leaves and a terminal flower which is an inflorescence. The phyllotaxis changes from decussate to spiral (Esau 1945, Priestley and Scott 1936) having 2 or 3 helices (Plantefol 1947a). Each leaf has 3 leaf traces and the stem has 24 vascular bundles (Priestley and Scott 1936).

The apex in the embryo has two leaf primordia while two others are being formed (Senghas 1957). The first leaves include most of the apex, leaving only a few cells (Guliaev 1963, Rauh and Reznik 1953). In the embryo the cells organize an L_2 when the cotyledons start forming (von Guttenberg et al. 1955). The embryo itself has two outer layers (L_1 and L_2) (Senghas 1957) or only an L_1 (Gifford 1954) and shows zonation (Senghas 1957).

The sunflower is indifferent to daylength (Pinthus 1959). The flower, a capitulum, starts flowering from the outside and, depending on the weather, 1 to 4 rows of florets open per day (Putt 1940). It is a cross-pollinator, often self-incompatible with a low seed set after selfing (Hamilton 1926, Pinthus 1959).

The mitotic cycle time is 9 hours (van 't Hof and Sparrow 1963) and the first cell divisions in the root are in anaphase 28 hours after sowing (Brunori et al. 1970).

2.6 EXPERIMENTAL PART

2.6.1 *Arabidopsis: growing methods*

Different varieties were tried and the cultivar Landsberg erecta was eventually used. It requires little space and flowers early (3 weeks after sowing). Since M_2 embryo mutations can be scored in the siliquas two weeks later (3.7.1) and, when directly sown, the M_2 seedlings germinate within the next two weeks, the generation interval is less than 7 weeks. Some pests occasionally occur, but the only real problem is greenfly which is difficult to combat in the greenhouse and may cause floral abnormalities and sterility.

Generally dry ripe seed is given a cold pretreatment (5 days at 3° C on wet filterpaper in the dark) to break dormancy, and then redried. The seed is sown on agar with a trace of KNO_3, to synchronize germination, placed in the light till germination starts ($\pm$ 1 day), then put in the dark for two days to etiolate (the seedlings) and replaced in the light for recovery (2 or 3 days). The seedlings are transplanted with agar, into pots with a sandy soil in the greenhouse. In winter additional light is given continuously (van der Veen 1965).

Different variations were used: The cold pretreatment was sometimes left out in old seed, which has no dormancy. Etiolation was left out or reduced to get shorter sturdier seedlings. Green seed has no dormancy yet but germinates irregularly (Fig. 2-4). GA promotes and cold retards germination (of green seed). Green seed was used in some experiments, mainly with colchicine treatments. When seedlings were treated (EMS) they were transplanted, with or without their roots, onto perlite to recover before transplanting into the greenhouse. On perlite new roots developed so easily that the removal did not affect flowering date. By removing the roots differential root-damage, due to various factors, could be avoided.

In some experiments part of the germinated seeds or seedlings were vernalized for 2 or 4 weeks (3° C, dark). Last winter daylength differences were introduced in an attempt to obtain stronger plants: 10 hours gave vigorous plants which, however, had many sterile flowers. 16 hours gave slightly stronger (fertile) plants than continuous light. Daylength, etiolation and vernalization affect days to flowering and leaf number. The magnitude of these differences varies, probably due to natural daylight differences and due to the distance from the lamps to the plants. The position in the greenhouse, even in the rows, affected plant growth (Plate ID) but this could not be quantitatively expressed.

EMS and colchicine were used as mutagens to induce mutations and

chimerism (see 3.6.2, 3.6.3). Green seed, ripe seed with or without cold pretreatment, and seedlings were treated with EMS or colchicine or simultaneously with both. These treatments affected germination, flowering date and branching.

The effect of EMS on branching also appears from the correlation between cauline and rosette branches which is influenced by EMS and apparently even by the time of the EMS treatment (seed or seedling).

2.6.2 *Arabidopsis: observations on morphology*

Ripe seed (after cold pretreatment) germinates within 24 hours of sowing, or a few hours later when it has been subjected to an EMS treatment. Ripe seed and green seed, sown directly after harvesting, germinate after 10 to 14 days depending on the ripeness of the seed (Fig. 2-4). EMS, colchicine, and combined treatment of green seed promote germination (Fig. 2-5). This is in contrast to the EMS effect on ripe seed.

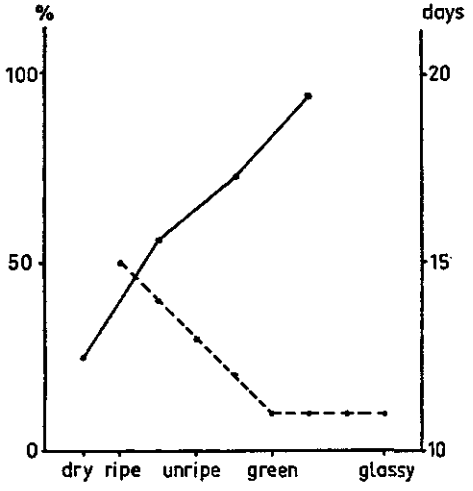


Fig.2-4. Germination of seed sown at different developmental stages (arabidopsis) ---days to 50% germination (25 seeds). — % germination after 14 days (200 seeds).

Landsberg erecta develops a small rosette (6 or 7 leaves) before it elongates (1 to 3 cauline leaves) and starts flowering. The cauline and some of the rosette leaves have axillary buds which may develop into side shoots. (The topmost cauline leaf sometimes bears only a flower,

Table 2–1. The effect of various treatments on plant development (arabidopsis, Exp. IV and V)

germination group[1]	daylength[2]	etiolation[3] (E) vernalization[4] (V)	number of plants	days to flowering[5,6]	cauline leaves (side shoots)	rosette leaves[7]	rosette buds at flowering	rosette side shoots 4 weeks later
IV. Green seed treated								
			EMS					
C	24		69	20,8	2,6	5,7	1,6	1,8
C	16		230	25,5	3,1	6,6	1,1	1,2
			colchicine					
B	24		56	21,3	1,8	5,9	1,5	2,4
B	16		41	25,4	1,8	6,8	1,9	1,9
B	16	E	48	27,7	2,0	7,1	2,1	1,5
			colchicine + EMS					
First flower diploid								
A	24		88	25,7	2,6	(5,8)	1,3	1,8
A	16		77	32,2	2,7	(6,7)	1,3	1,5
A	16	E	82	30,2	2,5	(7,3)	1,7	1,7
First flower polyploid								
A	24		19	28,7	2,4	(6,0)	1,1	0,9
A	16		17	35,2	2,9	(6,4)	0,8	0,8
A	16	E	24	36,6	2,5	(7,0)	1,0	1,2
V. Ripe seed treated								
			EMS					
	24		271	22,8	3,0	5,2	1,0	
	24	E	267	24,2	2,6	5,5	1,3	
	24	V	249	22,3	2,5	5,9	1,5	

1. A, B, C = early, medium and late germinating group (green seed).
2. 24, 16 = 24 or 16 hours daylength.
3. 48 hours in the dark at 24° C.
4. 16 days in the dark at 3° C.

number of plants	days to flowering[5,6]	cauline leaves (side shoots)	rosette leaves[7]	rosette buds at flowering	rosette side shoots 4 weeks later
control (water)					
122	20,3	1,9	5,9	1,8	1,9
213	25,2	1,9	6,7	1,6	1,8
control (water)					
27	21,8	1,9	5,9	1,6	2,4
27	25,0	2,0	7,0	2,1	1,9
colchicine					
54	25,7	1,9	(5,9)	1,3	2,1
48	31,7	1,8	(6,6)	1,2	1,4
43	30,6	1,9	(7,0)	1,6	1,4
10	32,0	1,9	(5,6)	0,6	1,1
15	39,5	2,0	(6,8)	0,8	1,1
24	39,5	1,9	(6,9)	0,9	1,0
control (water)					
243	22,8	2,1	5,5	1,3	
262	23,0	2,0	5,5	1,4	
264	22,9	1,7	6,4	2,0	

5. days after germination + one day.
6. same as 5, minus 14 days for vernalized seeds.
7. ( ) less reliable because of irregular growth.

the first flower of the inflorescence). The number of cauline leaves is influenced by the treatment, EMS clearly giving more cauline leaves (Table 2-1). Their axillary buds all develop into side shoots.

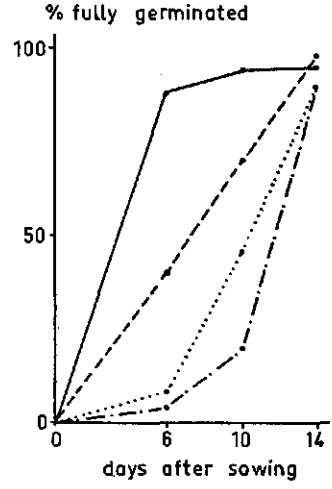


Fig. 2–5. Germination of green seed after various treatments (arabidopsis, Exp. II). ---- colchicine, —— colchicine + EMS, ···· EMS, –·– water.
% = 100 × transplantable seedlings/seeds sown (cumulative). 360 seeds per treatment.

The number of rosette leaves is affected by daylength (fewer with continuous light) but also by etiolation (more), vernalization (more) and, possibly, indirectly by EMS (fewer) (Table 2-1). The number of rosette shoots that develop, depends on the vigour of the plant, but also on the number of cauline shoots, with which it is negatively correlated in plants from EMS treatments. This correlation may depend on the stage treated (Table 2-2).

Colchicine disturbs the development of the seedling and causes irregular development of the rosette so that some leaves hardly grow. The recorded number of rosette leaves is therefore an estimate. The axillary buds in the rosette were counted when the first flower (of the plant) opened and again a few weeks later. Usually buds are seen in the axils of the top (youngest) leaves and these develop into side shoots. After colchicine treatment, fewer buds are found or fewer develop into side shoots. The effect of colchicine is clearest in the rosettes of plants in which polyploidy was found (first flower polyploid) and seemed to be absent in the treatments or items, e.g. early germination (B in Table 2-1), in which no polyploidy occurred (Table 2-1).

Table 2–2. Correlations (in %) between branching and mutations after EMS treatments (arabidopsis, Exp. VI)

		unvernalized			vernalized		
		seed		seedling	seed		seedling
		EMS	control	EMS	EMS	control	EMS
n		528	60	460	290	40	108
Z	with R	–6*	26*	–29**	–25**	3	–33**
Z	with Z+R	62**	74**	30**	53**	65**	30**
R	with Z+R	67**	84**	82**	69**	78**	80**
Z	with M	24**	11	11*	20**	10	9
R	with M	15**	–4	–6	4	–22	–5
Z+R	with M	30**	1	1	19**	–11	1

Z, R, Z+R = cauline, rosette and total side shoots, M=mutations (sectors) per plant, n=number of plants.

* 5% level of significance.

** 1% level of significance.

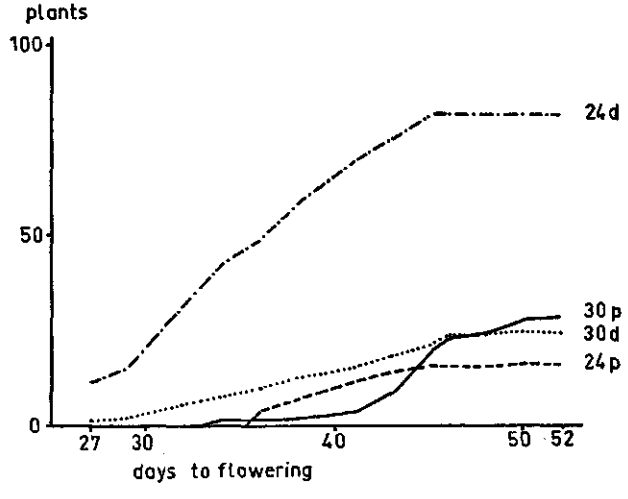


Fig. 2–6. Days to flowering after colchicine treatment (arabidopsis, Exp. II). 24, 30 = duration (h) of treatment (0,2% colchicine, 24° C). d = diploid and p = polyploid (first flower), cumulative graph.

Decapitation promotes the development of side shoots. On the stem it induces secondary axillary shoots, between the cauline shoot and the leaf. In the rosette it induces more buds to develop into shoots, but it also

induces secondary and higher order shoots so that a rosette may produce 20 rosette shoots which, on closer examination, can be reduced to 5 or 6 primary shoots and their derivatives (Fig. 5-2).

The main stem is occasionally fasciated or forked, especially after extreme treatments (Table 3-1). This is probably due to an outsized apex as was recorded after irradiation (Haccius and Reichert 1963).

Normally the first flower opens about 3 weeks after sowing but this may be modified by various factors (Table 2-1). Shorter days (16 vs 24 h) give later flowering; etiolation (2 days) gives later flowering only when the days in the dark are included; and vernalization (16 days regarded as 14 days + 2 days etiolation effect) has no effect when 14 days are subtracted but seems to promote flowering when 16 days are subtracted.

Colchicine retards flowering especially in those plants in which polyploidy was found (Fig. 2-6) and seems to have no effect in treatments which gave no polyploidy (Table 2-1). This corresponds to the effect of colchicine on plant development (rosette shoots).

EMS does not change the flowering date nor the effect of colchicine on flowering date.

In an inflorescence one or 2 flowers open per day till 30 or 40 flowers have been produced. The number of flowers and rate of flowering depend on the vigour and fertility of the plant which produces more flowers if it is a vigorous plant or when it is sterile. The elongation of the inflorescence axis depends on the developing fruits and removal of the flowers, e.g. for scoring, leads to a short thin axis.

Sometimes the first flower(s) seems to have a lower point of insertion than the topmost side shoot. This can only be due to differential growth and fusion with the stem, because floral primordia develop later than foliar primordia. Unexpected positions of flowers (fruits) on the inflorescence can usually be explained in the same way.

2.6.3 *Arabidopsis: phyllotaxis*

The cotyledons are opposite and the next leaf pair too, perpendicular to the cotyledons. The next leaves are often directly above the cotyledons but sometimes three more or less equally spaced leaves are found (Plate I A, B). This then is the beginning of the spiral phyllotaxis which can scarcely be discerned in the 8 or 9 leaves present under the first flower. The flowers themselves are usually arranged in a 3/8 phyllotaxis, which may not yet be clear in the first few flowers and may change in the last flowers (Fig. 4-1, 4-2).

This compares quite well with the number of vascular bundles found

(Table 2-3). The inflorescence usually has 8 vascular bundles corresponding to the 3/8 phyllotaxis found. Lower in the stem, there are too few leaves to establish a phyllotaxis but the number of vascular bundles suggests a 2/5 or a 2/4 (decussate) phyllotaxis. If phyllotaxis and number of vascular bundles are related to apex size the observed differences (Table 2-3) may indicate differences in apex size within the plant (rosette, stem and inflorescence) and between plants (strong, normal and weak plants).

Table 2-3. Number of vascular bundles in different parts of the plant (arabidopsis)

	plant appearance		
part of plant	strong	normal	weak
inflorescence	8	8	5 or 4
stem below inflorescence	8	5	5
stem above rosette	8 or 7	5	5 or 4
base of rosette	2	2	2

At the insertion of the flower stalk or leaf base three vascular bundles join those in the stem and fuse with two or three stem vascular bundles. The former case (with 2) gives asymmetrical fusion so that the vascular bundles will gradually deviate from the growth axis of the plant (Fig. 2-1). This deviation is related to the phyllotaxis. The vascular bundles were used to make a topography of the plant. The positions of the flowers (or fruits) and side shoots relative to a certain vascular bundle were drawn giving vertical topographies of the plant (Fig. 4-1, 4-2). This could sometimes be done directly but usually the stem was so thin that it was found easier to disect the dried, and then presoaked, stem along a vascular bundle under a disection microscope and then to establish the relative positions.

Another type of twisted growth was found, independent of the phyllotaxis, which may influence the relative positions of the flowers or fruits. The apex seems to twist in a clockwise direction as could be seen in a variety (Limburg 2) with many rosette leaves grown in a sunny autumn (Plate IC). This could be due to the influence of the sun and may be a temperature or light effect. The former has been observed to influence diurnal periodicity for cell division (Edgar 1961). If this slightly twisted growth of the apex is common it may explain, together with deviations caused by the vascular bundles, the occurrence of twisted sectors in chimeric plants (Fig. 4-1, 4-2, 4-3). It has not yet been possible to ascertain whether this is indeed the case.

2.6.4 *Arabidopsis: apex*

The development of the cauline apex (Fig. 2-7) and the effect of various treatments on it (Table 2-4) has been studied. The seed or seedling was disected under a disection microscope and then studied in glycerine under an ordinary microscope. In this way it was possible to get an impression of the three dimensional apex. Immediate observation (within 10 minutes) revealed the most details. Drawings were made directly from the material.

Very young seed has a pale glassy appearance due to the endosperm which surrounds the bright green embryo. When the endosperm has been absorbed the seed appears green till the testa covering it turns brown and the embryo itself turns white. The apex increases in size till the endosperm is completely absorbed. Further development only occurs at germination. In the seed the apex, consisting of small cells, lies at the base of the cotyledons (Fig. 2-7). Seen from above it is long and narrow, slightly oval in shape. From the side, longitudinally, it is flat, about 12 cells long (Fig. 2-7: 1).

At germination a small dome is formed at one end followed by one at the other end. These are the first leaf primordia (Fig. 2-7: 2,3). A narrow group of cells, appearing elongated, remains between the two primordia (Fig. 2-7: 2a). The primordia increase in height and large-celled stipules develop at their base (Fig. 2-7: 4). The apex also increases in size. When the first leaf primordia have developed a certain height, the primordium for the next leaf can be seen at one end of the oval apex (Fig. 2-7: 4). The next primordium may develop opposite it or more to the side in which case another leaf develops to the other side (Fig. 2-7: 5) giving a more spirally arranged phyllotaxis (Plate I A, B).

Roughly the development of the apex can be divided into a few stages. Stage 1 is found in the seed, stage 2 at germination, stage 3 in the young seedling (leaf 3 and 4 are visible microscopically only), stage 4 in the older seedling (leaf 3 and 4 are visible, 5 and sometimes 6 only visible microscopically), and stage 5 when the apex starts to produce floral primordia (Fig. 2-7: 5).

In stage 4 the apex is no longer flat and in stage 5 it is clearly dome shaped. Usually the plants are in stage 3 when they are transplanted to the greenhouse. The first flower primordium develops opposite the top leaf (flower primordia are larger than leaf primordia), and at this stage the stem starts to elongate.

The cotyledons and the first leaf pair (leaf 3 and 4) enclose the apex with their (leaf) bases, while later leaves progressively have relatively

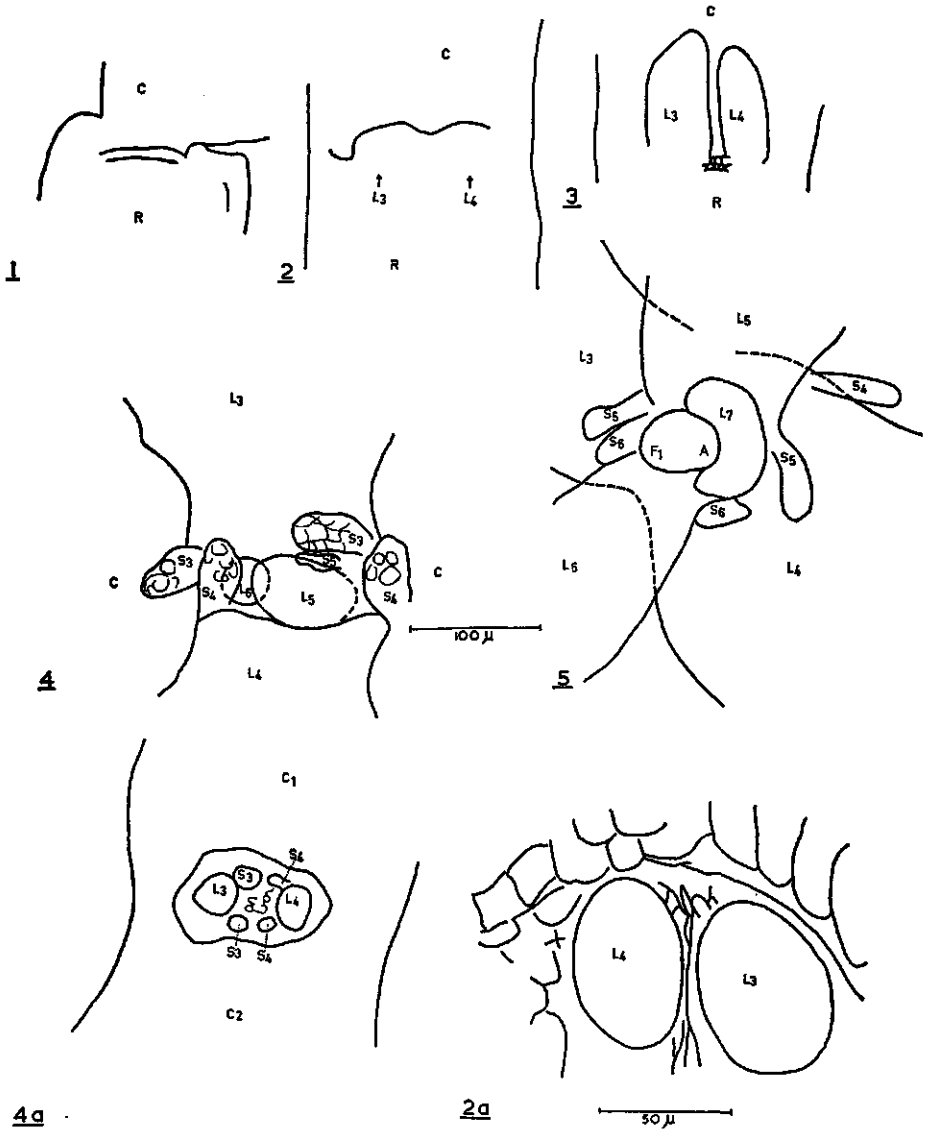


Fig. 2–7. Apical development in arabidopsis. 1 = stage 1 in mature embryo, 2 = stage 2 in germinating seed, 2a = stage 2 from above, 3 = stage 3 in young seedling, 4 = stage 4 in older seedling from above, 4a = same age as 4 after colchicine treatment of green seed, 5 = stage 5 in (mature) plant.
R = root, C = cotyledon, L_3–L_7 = leaves (primordia), s_{3-7} = stipules of L_{3-7}, A = apex, F = flower (primordium).
All the same scale except 2a.

smaller bases. Axillary buds develop at the leaf bases. They are first observed when the inflorescence primordium develops. The buds in the axils of the younger leaves are more advanced (Fig. 2-8), and the oldest leaves (leaf 3 and 4) and cotyledons usually have no axillary buds.

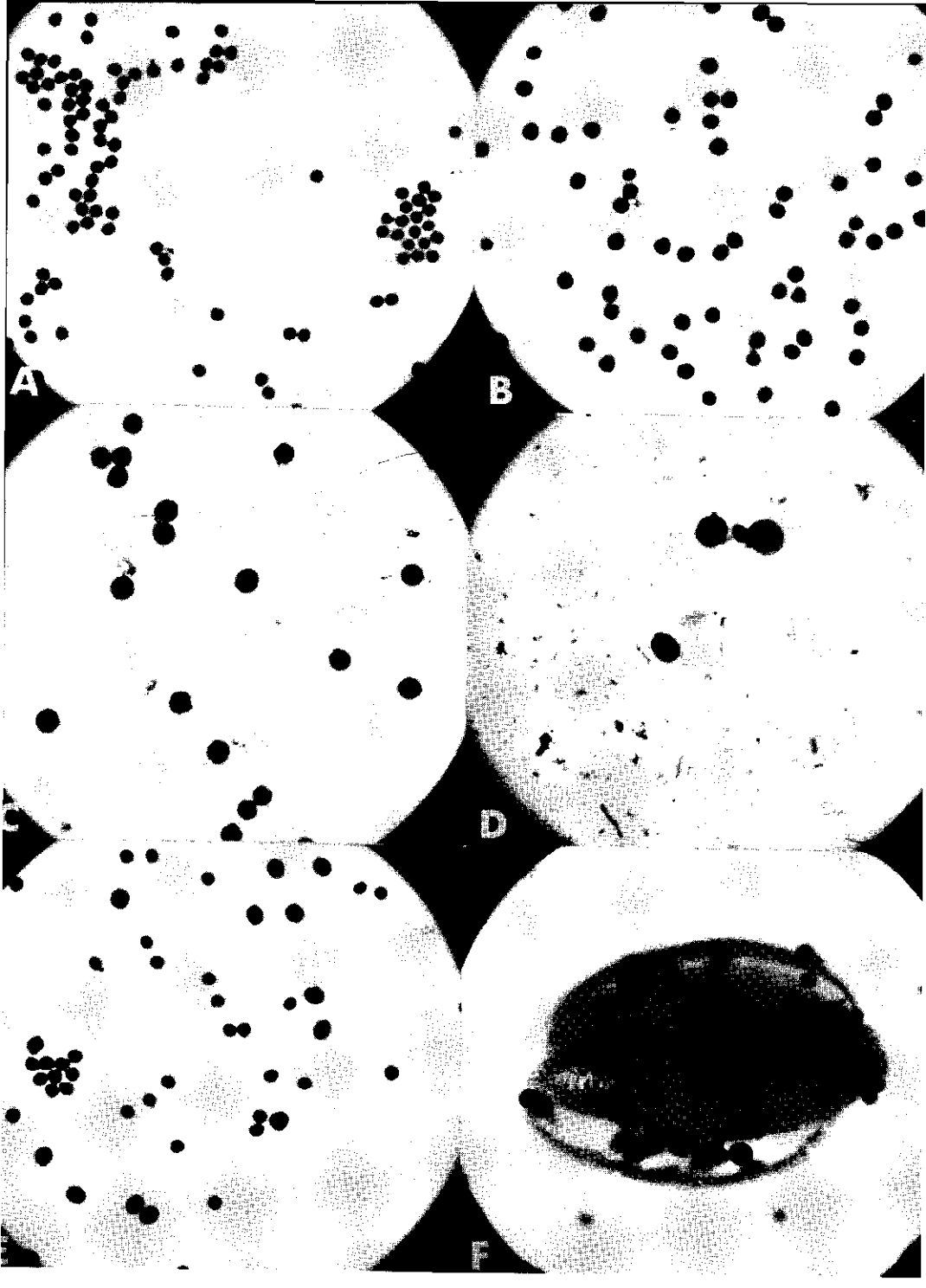
Various treatments may influence the development in the apex (Table 2-4) usually retarding it. Due to the shape of the apex and variability within a treatment it was not possible to assess differences in size. The appearance of the apex was strikingly changed only by colchicine treatment of green seed (Fig. 2-7: 4a). The plants themselves were short with a swollen hypocotyl, the apex seemed tightly enclosed between the cotyledon bases, and the first leaf primordia often consisted of only a few giant cells as if no cell division and only expansion had occurred. Many of these plants usually die after transplanting and the survivors often flower late and show growth abnormalities, such as apparently-absent leaves or forked stems (Table 2-1, Fig. 2-6).

Table 2–4. Apical development after various treatments (arabidopsis)

treatment	number of days after sowing				
	3	4	5	6	7
sown directly					
control (diploid)	2*	2	2+3		4
tetraploid (M_2)	2	2	2+3		4
etiolated (48h)	2	2	2		3
vernalized (16d)			4		
sown after 24 hours with:					
water	2	2		3+4	
colchicine	2	2		3+4	
EMS	2	2		3	
colchicine + EMS	2	2		2+3	
colchicine (green seed)	1	1+2		2	2+3

* stage reached: 1 = undifferentiated, 2 = primordia leaf 3 and 4 appear, 3 = leaf primordia longer than wide, 4 = primordia leaf 5 (and 6) appear (see Fig. 2–7).

Plate I. A B. Phyllotaxis in arabidopsis plants. A has 2 cotyledons and 2 pairs of leaves. B has 2 cotyledons, 1 pair of leaves and 3 spirally arranged leaves. C. Twisted growth of an arabidopsis (variety Limburg 2) plant. The apex appears to twist in a clockwise direction. D. Two rows of arabidopsis (variety Limburg 2) plants which show differences for plant size and development depending on the position in the row.


A
B
D
F

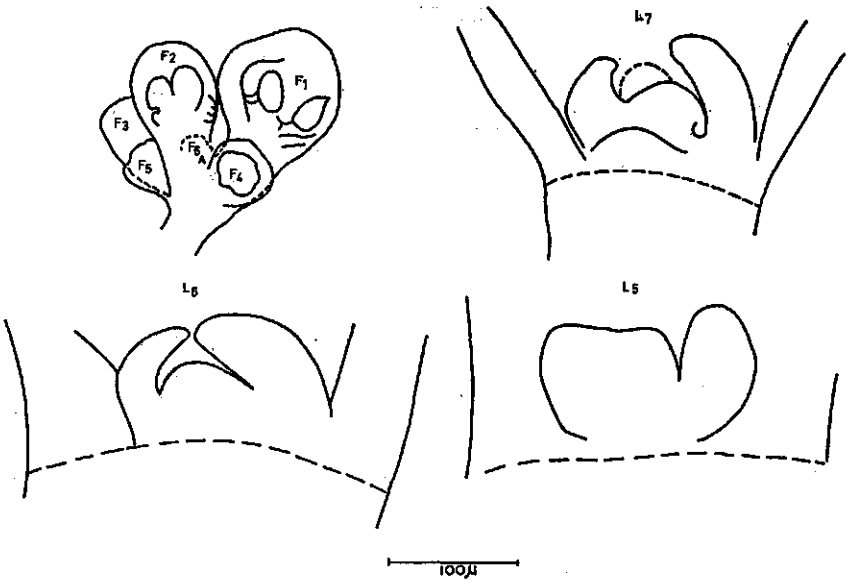


Fig. 2–8. Inflorescence and all the visible axillary buds from one plant (arabidopsis). L_{567} = leaf 5, 6, 7 with axillary buds 3, 2, 1 respectively, F_{1-6} = flower 1–6, A = apex.

2.6.5 *The sunflower*

The sunflower has not been morphologically studied. The apex in the seed appears undifferentiated as in arabidopsis. Phyllotaxis soon settles at 2/5 or occassionally 3/8. The plant material (supplied by the Department of Plant Breeding, Wageningen), was very heterogeneous in growth habit. The seed was sown dry or after treatment. The young plants were transplanted in the greenhouse in the winter, or to the field in the summer. Plants grown in the greenhouse in the winter were often smaller with less branching than those in the field (Table 5-2). Branching was promoted by removing the flowers.

In one experiment half the plants were decapitated well before flowering. An attempt to root the tops failed but the plant bases developed axillary shoots in the axils of the top 2 to 4 leaves.

Plate II. Pollen from arabidopsis flowers differing in ploidy. A = a diploid, B = a tetraploid, C = an octoploid, D = a 16?-ploid, E = a chimeric flower, F = a chimeric anther (× 100).

CHAPTER 3

MUTATIONS

A mutation is a sudden genetic change. Different types of mutation are known depending on the level at which they take place. This can be in the genetic material of the cytoplasm, plastids or nucleus. In the nucleus it may involve a doubling of the chromosome number, a change in the chromosome number, a change in the chromosome structure or a change at the gene level. A small change in the chromosome may resemble a change at the gene level.

In practice only those mutations which can be observed by the technique used in an experiment are recorded while the rest of the material, possibly carrying a load of unidentified mutations is usually classified as normal. The terms mutated and normal are therefore not absolute and refer only to the type of mutation under observation in a particular experiment.

In plants mutations are most readily recognized in genetically homogeneous, heterozygous material such as a clone or different parts of the same plant. A fruit mutation was first recorded in 1741 by Collinson (Shamel and Pomeroy 1936). In many vegetatively propagated plants mutations may be present for a long time before they are revealed by a change in the plant growth (Asseyeva 1927).

Muller (1927) showed that X-rays induce mutations in Drosophila. This was confirmed for plants by Stadler (1928). Since then other types of irradiation, and chemicals have been shown, and used, to induce mutations. Colchicine induces a very special type of mutation viz. polyploidy.

3.1 COLCHICINE

Colchicine affects the mitotic spindle so that a normal mitosis is prevented from taking place, sometimes even in cells which had already reached anaphase (Eigsti and Dustin 1957) though some cells may re-

main unaffected (Davidson 1969). There is a short (15 or 30 min.) lag before the colchicine acts (Eigsti and Dustin 1957, Neary et al. 1959) but the colchicine itself may inhibit cells from entering mitosis (Evans et al. 1957) or retard subsequent cell divisions (Eigsti and Dustin 1957). When the treatment is stopped it takes a few days for the plants to recover and normal cell division to be resumed. In cells with many chromosomes multipolar spindles may form giving several, not necessarily balanced, nuclei (Eigsti and Dustin 1957).

Colchicine may also cause cell death, retarded growth, tumors, aneuploidy or, apparently, gene mutations. The latter were observed and studied in *Sorghum*. Colchicine sometimes induces diploidy in tetraploid *Sorghum* and the "mutations" may arise when the diploids revert to tetraploidy and heterozygous genes appear in homozygous pairs. This could be demonstrated for heterozygous chromosome markers (Simantel et al. 1963).

Different methods have been devised for the treatment because the roots are often very sensitive to colchicine (Eigsti and Dustin 1957). IAA (Glotov 1939), DMSO (Sanders and Hull 1970) and X-rays (Broertjes 1969a) affect survival and the frequency of polyploidy though probably through very different pathways. Other chemicals or physical methods have been used to induce polyploidy but not one with as much success as colchicine.

3.2 IRRADIATION

Irradiation causes (gene) mutations and chromosome aberrations. Fractionated doses show that a few distinct changes, some of which are reversible, lead to the chromosome breaks (Evans 1967). Chromosome aberrations may escape detection because the cell dies or stops dividing (Gunckel 1957), or, in the case of fragments, they may be lost. Chromosome aberrations do not, however, necessarily lead to (direct) cell death (von Wangenheim 1970). They do sometimes cause spotting on the leaves of treated plants (Kaplan 1954) or sterility in the mature plant (Kaplan 1949, Matsuo et al. 1958). Somatic exchange (Hirono and Rédei 1965a,b) and polyploidy (Sosna 1963) are occasionally found after irradiation.

Plant morphology may be affected by irradiation through its effect on physiological processes or by cell death. Cells in different positions or different layers vary in sensitivity so that disorganized growth and changed layering may be induced. Stimulation of cells will also cause disorganized growth and lead to abnormalities.

Irradiation may inhibit cells from entering mitosis (Gunckel 1957, Iqbal 1969), stimulate cells to divide (Clowes 1963, 1970a) or slow down cell division itself (Neary et al. 1959). The cytoplasm continues differentiating (von Wangenheim 1970) so that delayed mitosis results in premature differentiation of cells (Evans 1965, Iqbal 1970, Kuehnert 1962, von Wangenheim 1970, von Wangenheim et al. 1970). These cells may thereby lose their capacity to divide leading to the above-mentioned growth abnormalities.

3.2.1 *Sensitivity to irradiation*

The sensitivity of a plant depends on many morphological and physiological aspects of the cells in the material treated and on the treatment itself.

The cell undergoes various changes during mitosis especially in the interphase. The interphase can be divided into three stages G_1, S (when DNA synthesis takes place) and G_2, during which the DNA content changes from 2C (G_1) to 4C (G_2). Irradiation in G_1 gives chromosome breaks and in G_2, which is more sensitive (Evans 1967, Mikaelsen 1969), chromatid breaks (Brunori et al. 1966). Sometimes the chromosomes are multi-stranded and mutations or aberrations occur at a lower chromosome level (Bruns 1954). The different sensitivity of the G_1 and G_2 stage may explain differences in sensitivity found for different plant stages or organs (Miah and Brunori 1970).

Meiosis has been reported to be very sensitive (Kawai and Inoshita 1965, Sparrow and Woodwell 1962). Gametes, zygotes and proembryos vary in sensitivity.

Different pre- and posttreatments have been used with various results. Colchicine pretreatment generally seems to increase the mutation rate (D'Amato and Gustafsson 1948, Gaul 1957b) possibly because it increases the number of (sensitive) metaphases (Brumfield 1943b).

3.3 RADIOMIMETIC CHEMICALS

Many chemicals can induce mutations. They are often referred to as radiomimetic because their action resembles that of irradiation. The most important group, the alkylating agents, probably acts on the DNA bases (Loveless 1966). In contrast to irradiation the G_1 and early S are the most sensitive stages of the mitotic cycle (Bacq and Alexander 1961, Moutschen 1964) yet aberrations are produced mainly at the chromatid level (Loveless 1966) and appear one or two cell divisions later if they

are induced in the G_2 stage. A potential lesion is formed which may form a stable lesion or be repaired (Dubinin et al. 1968). This repair may be influenced by the temperature during (Rieger and Michaelis 1965) or after (Satpathy and Arnasson 1969) the treatment and by storage after the treatment (Müller 1965a) or during the (fractionated) treatment (Loveless 1966, Moutschen 1964).

The action of chemicals appears to be delayed, especially in comparison with irradiation. This has been observed with regard to the effect on cell division (Bacq and Alexander 1961), and the mutations occur at a lower chromosome level though the G_1 was found to be more sensitive. As a result chemicals are generally reported to give more chimerism. Chemicals may act less directly, e.g. on a DNA precursor (Moutschen 1964) or the potential lesions may remain "open" longer than after irradiation. On the other hand killing by irradiation or DNA synthesis during (often long) chemical treatment (Lindgren et al. 1970) is probably responsible for at least some of the observed difference in chimerism.

Chemical mutagens also cause various side-effects such as cell death, retarded growth (Gichner 1966), retarded cell division (Loveless 1966, Moutschen 1964), or sterility due to chromosome aberrations. EMS shows few side-effects and produces many (gene) mutations so that it may be called an efficient mutagen (Heslot et al. 1958, Loveless 1966).

3.3.1 *EMS*

EMS causes mainly gene (or small chromosome) mutations but under certain circumstances also causes chromosome aberrations (Loveless 1966, Moutschen 1964). Death after EMS treatment may be due to protein alkylation (Osterman-Golkar et al. 1970). Different developmental stages can be, and have been, treated with EMS but seed treatment is easiest and therefore most generally applied.

Presoaking the seed may affect the results, probably because it promotes EMS uptake (Jacobs 1969, Natarajan and Shivasankar 1965), or, especially in the presence of oxygen, because it promotes germination (Müller 1967b). The temperature during (Jacobs 1969), or after the treatment (Satpathy and Arnason 1969) and washing and drying after the treatment (Bender and Gaul 1967, Froese-Gertzen et al. 1964, Müller 1966) also affect the results.

EMS degrades faster in a phosphate buffer, especially at high pH (Wickham et al. 1969). Hydrolized EMS has no mutagenic (Müller 1966) or chromosome breaking properties (Moutschen-Dahmen and Moutschen 1963).

3.4 SCORING OF MUTATIONS

Some of the effects induced by mutagenic treatment are positively (or negatively) correlated. When this is a correlation between treatments, e.g. retarded growth and mutations for a certain mutagen, it may be useful for estimating a practical dose. When there is a correlation within a treatment is may help early selection. Sterility is usually not correlated with mutation rate (Gaul 1958) so that partly sterile plants can be discarded without loss of mutations. M_1 and M_2 fertility are positively correlated (Mesken and van der Veen 1968). Chromosome aberrations (Gaul 1958, Gaul and Mittelstenscheid 1960) and visible sectors on M_1 plants (Blixt 1965) are not correlated with (gene) mutation frequency.

Plastid mutations (Röbbelen 1963b), mutations in heterozygous plants (Fujii 1964, Gröber 1962, Mericle and Mericle 1967b) and some back mutations (Röbbelen 1965c) can be directly scored. Monosomics (Douglas 1968), polyploids (Eigsti and Dustin 1957), sterility (Anderson et al. 1949) and some (special) mutations (Eriksson 1965) can be scored from the pollen. Chromosome aberrations can be observed only in certain stages of the cell cycle, the stage scored even influencing the results (Conger 1965). The rate of cell division (Gaul 1957c) and the time after the treatment (Miller and Colaiace 1970) are also important. Killing (Mericle and Mericle 1967a, 1969b) or selection against mutant tissue within the plant (Gaul 1957a,c, 1960, Gaul and Mittelstenscheid 1960) are said to influence the number of chromosome aberrations or gene mutations found.

Most gene mutations are recessive and only found in the next generation so that selection against mutant gametes may take place. Differential aging in the seeds may influence observed segregation ratios (Röbbelen 1966a, Velemínsky and Gichner 1967) and environmental conditions may influence the expression of some mutations (Holm 1954, Jacobs 1969).

The index used to express the mutation rate is important. Gaul compares three indices, mutations per 100 M_1 plants, mutations per 100 M_1 spikes (shoots) and mutants per 1000 M_2 plants, and comes to the conclusion that mutants per 1000 M_2 plants gives the best index. Chimerism in the M_1 plants and recessive deficit, commonly found in the M_1 plant progenies, can be corrected for, but within-plant selection against the mutation (diplontic selection) still influences the results (Gaul 1960, 1963b, 1964, Gaul and Mittelstenscheid 1960).

When multiple mutations occur, i.e. especially at high mutation rates, this index will still underestimate the real mutation rate (Hänsel 1968) as was found for mosaics in Drosophila (Epler 1966). This can be partly

corrected for by differentiating between different mutations. Another index taking the genome number into consideration has been suggested as it would enable comparison with micro-organisms (Li and Rédei 1969).

In summing up

Colchicine induces mainly polyploidy. It acts on the mitosis which it requires to induce polyploidy.

Cells in G_2 are more sensitive to X-rays and those in G_1 are more sensitive to EMS, yet EMS gives more chimerism. Both cause potential lesions which, when they are not repaired, give mutations. Repair or delayed mutations after EMS may be responsible for part of the chimerism found. Stimulating and inhibiting or retarding effects have been observed with both mutagens, and often lead to growth abnormalities.

3.5 ARABIDOPSIS AND SUNFLOWER

3.5.1 *Arabidopsis*

Arabidopsis has been extensively used for mutation research. See Rédei for a review (Rédei 1969).

X-rays have been found, besides inducing mutations and chromosome aberrations, to cause plastid mutations (Röbbelen 1962b); polyploidy (Sosna 1963); somatic recombination (Hirono and Rédei 1963, 1965a,b) sometimes with twinspots and usually with chromosome abnormalities (Hirono 1965); semigamy (Arnold and Cruse 1968); unstable mutations which revert to normal in later generations (Röbbelen 1958, Sosna 1962); and phenotypic changes in mutants (Rédei 1967c). Malformations (Reinholz 1959, Röbbelen 1960, 1962c), changed branching (Bouharmont 1965a, Buiatti and Lorenzoni 1963, Ivanov 1967) and stimulation effects (Reinholz 1968) are also found. Apart from X- and γ-rays, mutations have also been produced with UV (Fujii 1965a, 1966b) and heavy ionizing particles (Fujii et al. 1966).

Colchicine has been successfully used on young plants to produce polyploids (Arnold 1964, Bouharmont 1965a, Bronckers 1963), which are usually chimeric (Arnold 1964). Tetraploid pollen is larger than diploid pollen (Bouharmont 1965b, Bronckers 1963). Polyploidy, especially above 4n, is not stable and chromosomes are lost, which results in aneuploidy (Bouharmont 1967a,b). Embryosac aberrations are more common in polyploids (Bouharmont 1965c).

EMS has been used with different pre- and posttreatments. Presoaking was shown by means of labelled EMS, to promote EMS uptake (Jacobs 1969, Walles and Ahnström 1965) which gives a higher effective dose and more mutations (Müller 1967a,c, Röbbelen 1964, 1965d). (Changes in the seedcoat permeability due to presoaking remain, after redrying, and promote water uptake and germination in tomato and oat seed; Berrie and Drennan 1951). Presoaking (5 days 3° C) to break dormancy, followed by redrying (van der Veen 1965) may therefore influence subsequent EMS uptake. During long treatments, especially under aerobic conditions, germination may start and change the sensitivity (Müller 1965d, Röbbelen 1965d). Postsoaking compared with direct sowing on agar, showed little effect, diffusion from the seed into the agar probably being equal to diffusion into water. Postsoaking before redrying and storage did decrease the damage (van der Veen and van Heemert 1968). The temperature during postsoaking influences the damage (Müller 1966).

Hydrolized EMS gives no mutations but may affect germination (Müller 1966). Fractionated doses show fewer mutations if the interval is very long (Müller 1969b). DMSO promoted EMS uptake in plants giving more mutations (Bhatia 1967) while fewer mutations were found after seedtreatment (Müller 1969a). EDTA had no effect (Gichner and Velemínsky 1965). Copper and zinc ions and high pH increase the mutation rate (Bhatia and Narayanan 1965a,b). pH within the seed (pH 4,4-5,2) is independent of the buffers used (Velemínsky and Gichner 1970). The concentration used has been observed to affect the mutation spectrum (Jacobs 1964). Changes in spectrum may, however, be due to different survival (McKelvie 1962).

Other alkylating agents resemble EMS but may differ in optimal presoaking time (Müller 1965d, Röbbelen 1964) or react differently to anaerobic conditions (Velemínsky et al. 1965). Pre- and postsoaking (Müller 1965e) or different mutagens (Jacobs 1969) have given different mutation spectra.

Differences between EMS and X-rays were found for the mutation spectrum (Jacobs 1969, Röbbelen 1962a), for the optimal presoaking time (Röbbelen 1963a) and for the number and size of mutated sectors (Müller 1965b). Attempts to obtain back mutations clearly showed differences between EMS and X-rays (Röbbelen 1968, 1969).

Various stages have been treated with X-rays: seed, zygotes and gametes (Röbbelen 1960, 1962c), pollen (Müller 1965c), embryosacs (Arnold and Cruse 1968), proembryos (Gerlach-Cruse 1969, Reinholz 1959, 1965) and plants (Bouharmont 1965a,c). Colchicine (Bouharmont

1965a, Bronckers 1963) and UV (Fujii 1965a, 1966b) have been used in plant treatments. Seeds, but also seedlings (Balkema 1969), plants (Bhatia 1967) and inflorescences (Röbbelen 1962a, 1965b) have been treated with EMS.

Scoring for mutations has been done in many specialized ways. The embryotest, developed by Müller, makes use of the transparent seed-coat of the unripe seed to score the embryos directly from the mother-plant. Not only chlorophyll mutations affecting the cotyledons can be scored, but also the unfertilized ovules or those that die at an early stage (the embryonic lethals) can be recognized and scored (Müller 1961a, 1963a, 1966, Usmanov and Müller 1970). Embryonic lethality was shown to be partly a maternal effect (van der Veen 1967a,b).

Seedlings can be scored as with other plants, but give less information on fertilization and early lethality, and differential dormancy or aging may influence the segregation ratio found (Röbbelen 1966a, Velemínsky and Gichner 1967). A combination of the embryo and seedling test is possible. The unripe scored seeds can be sown on agar and the young seedlings scored for chlorophyll and morphological mutations (Balkema 1968).

Heterozygotes have been used to enable quick scoring of visible homozygous sectors (Fujii 1964, 1965a, 1966b, Fujii et al. 1966, Gichner and Velemínsky 1963, Hirono and Rédei 1964). The number of sectors found may, however, depend on the heterozygous factor used (Gichner and Velemínsky 1963) and mutagenic treatment induces some sectors in homozygous plants too (Hirono 1965) at rather variable rates (Röbbelen, see Jacobs 1965). Plastid mutations also give sectors (Röbbelen 1963b, 1965c).

Biochemical mutants may be found in ways similar to those used in micro-organisms (van der Berg and Feenstra 1968, Langridge 1955, 1958a, Rédei 1968). Physiological mutants, mutants for flowering time and leaf number (Hussein 1968), morphological mutants (Reinholz 1947) and flower structure mutants (Röbbelen 1965a) have been found.

Pollen size and chromosome counts are used to identify polyploids (Bouharmont 1965a).

Mutations may disappear in the M_1 due to loss of chimerism (Müller 1963b, 1965a), plastid mutations may be lost as a result of slower division of the mutated plastids (Röbbelen 1963b) and other mutations have sometimes been lost after a few generations (Röbbelen 1958, Sosna 1962). The latter was also found in certain higher polyploids where chromosomes are lost, often till the 4n number is reached (Bouharmont 1967a,b). An interesting phenotypically sectored mutant has been found, which can be modified to a normal phenotype (Rédei 1967a,b,c,).

Some indication of the expected mutation rate at a certain treatment can be obtained from observed growth retardation (Gichner 1966, Müller 1966). Growth retardation and visible chimerism also give an indication of M_1 sterility (Gichner 1966, Müller, see Jacobs 1965).

Within the treatment mutations are not related to fertility (Mesken and van der Veen 1968) and sterility occurs in (different) sectors (van der Veen and Gerlach 1965). Visible chimerism and mutant segregation are not correlated (Müller, see Jacobs 1965). M_2 fertility is correlated with M_1 fertility so that less fertile M_1 plants can be discarded (Mesken and van der Veen 1968). Late flowering M_1 plants have been observed to yield a lower mutation frequency (McKelvie 1963).

The dose response rate may depend on the mutagen used (Jacobs 1969) or on the way mutation rate is expressed. A curve is found when embryonic lethals are included (Müller 1969b), but a lineair increase with dose is found when the chlorophyll-mutation rate is expressed as a % of only the non-lethal embryos (van der Veen 1968). A recessive deficit is usually found giving a segregation ratio of 21% (Müller 1963a). Chimerism reduces this still further to almost 12% (Langridge 1958b, McKelvie 1961). The latter ratio may give an indication of the number and size of the sectors (Jacobs 1969).

3.5.2 *The sunflower*

The sunflower has been less extensively used for mutation research. It is a cross-fertilizer and therefore less practical for mutation experiments. Spontaneous mutations were already mentioned in 1917 (Cockerell 1917). X-rays have been used giving malformations (Remussi and Gutierrez 1964), chromosome aberrations (Brunori et al. 1970) and gene mutations (Beletzky and Liashchenko 1968). β- and γ-rays have also been tried (Sanduljak 1965). Colchicine treatment of seedlings gave chimeric plants with larger pollen, seed and ray florets (Rybin 1939).

3.6 EXPERIMENTAL PART

3.6.1 *Scoring methods*

Colchicine was used to induce polyploidy in arabidopsis and the sunflower, and pollen size was scored. For this the florets of the sunflower were picked and the pollen directly shaken or rubbed into drops of Bellings grycerine (1 Acetocarmine 1% : 1 glycerol) on slides, and scored under the microscope the next day.

In arabidopsis the flowers were picked and placed in drops of Bellings glycerine. Under a disection microscope the anthers were then opened and the rest of the flower removed. The pollen was scored (measured) the next day. In some cases the anthers were individually scored. Occasionally chimeric anthers were found (Plate II F). Polyploid pollen is larger, more irregular and often more sterility is found (Plate II). Chromosome counts were made on a few plants confirming that small pollen belonged to the diploid, larger pollen to tetraploid and still larger to octoploid inflorescences. Occasionally even larger pollen was found (Plate II D), but no chromosome counts could be made due to lack of suitable material. Fruit size also gives an indication of polyploidy in arabidopsis due to the lower fertility at higher polyploidy (Plate III A).

The first flower was always scored in arabidopsis while later flowers were scored only in those cases where the first flower showed polyploidy. Experiments where more flowers were scored showed that the gain was small compared with the extra work entailed, especially at low polyploidy frequencies, as more than 80% of the polyploidy was detected in the first flower, the rest being found in the second or only in the third flower. Similarly the side shoots, when scored, occasionally revealed polyploidy not found in the first flower (of the plant).

In the sunflower 4 florets, equally spaced round the circumference were scored for every flower that opened. Sometimes, on chimeric flowers, scoring was continued, many more points per circumference being scored daily (Fig. 4-4). Usually the flower was removed, after the first florets had been scored, to induce axillary buds, on the stem below it, to develop.

Enough pollen is usually available to make a clear distinction between diploid, tetraploid, octoploid and chimeric, but in polyploid flowers irregular pollen sometimes complicates the classification. This may be caused by the periclinal chimeric structure of the plant if differences between the layers influence pollen fertility and regularity.

EMS was used to induce mutations in arabidopsis and it sometimes produced visible sectors on the M_1 plant. These sectors usually included half of one, rarely two leaves (Plate III B). The exact background of this chimerism (gene, chromosome or plastid mutation) is not known and the occurrence is irregular and relatively rare (Table 3-1). It was only studied as a side-effect.

M_2 mutations were scored mainly by means of the embryotest (3.5.1). By this test chlorophyll mutants, embryonic lethals and unfertilized ovules can be scored in the unripe fruit directly from the M_1 plant

Table 3–1. Frequency of visible M_1 effects of mutagenic treatments (arabidopsis, Exp. IV)

treatment	M_1 forked	M_1 chimeric	n	n_1
EMS	0	11	458	
EMS + colchicine	13	17	382	307
colchicine	1	0	443	194

n = number of plants, n_1 = number of plants in the germination group (early) in which forking occurred.

(Plate IV A). Certation may still affect the results. Usually only one side of the replum, i.e. half the fruit, is scored for two consecutive fruits, but sometimes both sides were scored. Fruits 5 and 6, or 6 and 7, are the most practical to score as the right stage is easy to determine when fruits 1 and 2 turn brown. Often fruits 1 and 2, or 2 and 3, were scored, the stage being estimated from the flowering date. Higher fruits give practical difficulties especially when the plants are grown close together or are weak, producing only few fruits. The position of the scored fruits affects the fertility and mutation rate found (Fig. 3-1).

Green seeds can be directly sown (Fig. 2-4) and similarly seed scored in the embryotest can be sown. The seedling mutants may have a different phenotype from that scored in the embryos (Fig. 3-2). In this way more differentiation is possible. Very young embryos which appear

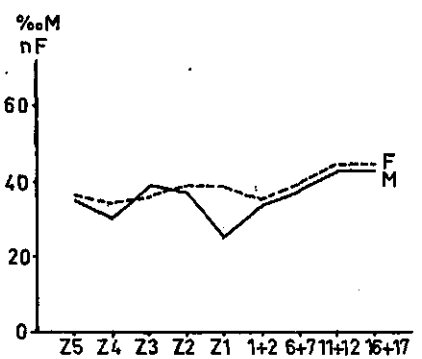


Fig. 3–1. Mutation frequency and fertility at different scoring points on the same plants (2 fruits per point, 48 plants, arabidopsis, 480 mM EMS, ½ hour, ripe seed) ‰ M = ‰ M_2 chlorophyll mutants, nF = number of fertilized ovules per fruit.
1–17 = the position (from bottom to top) of the fruits scored on the main inflorescence. Z 1–5 = position (from top to bottom) of the side shoots.

glassy may also be sown but give weak seedlings which occasionally show white sectors on one or both of the cotyledons, probably due to some sort of damage.

The whole fruit may also be sown. Germination is slower than for separate green seeds, but after 10-14 days the seedlings push the fruit open and can be scored (Plate IV B). When many fruits from one plant must be simultaneously scored this method is easier and enables comparison of the mutants, when necessary, at different ages. Usually more seed can then still be harvested from the M_1 plant, for further or later observations. Fruits that are very young when sown sometimes give weak and aberrant seedlings.

Ripe fruit may be harvested, sown and scored, but this entails more administration with a possibility of errors. In one experiment (Exp. VI) seed was harvested per inflorescence from chimeric plants. In this way large M_2 populations per inflorescence for several inflorescences per plant could be compared.

In another experiment the embryos from non-segregating M_1 fruits of chimeric plants were sown directly after the embryotest. M_3 embryos on the M_2 plants were scored to detect heterozygous M_2 plants. These did occur and indicate escape from detection in the M_1 fruits probably due to within-fruit chimerism.

Simultaneous EMS and colchicine treatment was given in some experiments (Exp. IV). The plants were scored for polyploidy in the first flower and for chlorophyll mutations in the next fruits. In plants which showed polyploidy in the first flower, fruits were scored only in those inflorescences which were found to be not, or no longer, chimeric for polyploidy. Tetraploid and octoploid fruits usually had less seed, fewer embryonic lethals and sometimes some chlorophyll mutations (Table 3-2). The chlorophyll mutations seem to indicate immediate action of the EMS, at the chromosome level, before polyploidy is induced. Col-

Table 3-2. Scoring results for different sectors of polyploid plants (arabidopsis, Exp. IV, EMS + colchicine)*

ploidy of sector	n	$\bar{x}$	%M	%E
diploid	27	40,4	12,1	26,4
tetraploid	91	14,5	1,6	3,9
octoploid	5	5,3	(7,7)	

n = number of inflorescences scored, $\bar{x}$ = number of fertilized ovules per fruit, %M = % M_2 chlorophyll mutants, % E = % embryonic lethals.

* inflorescence no longer (not) chimeric for polyploidy.

Table 3–3. Summary of the main experiments

no.	season	stage(s) treated	mutagen(s) used	pre- and post-treatment	scored	table	fig.
Arabidopsis colchicine (only 0,2%, 24 or 30 h), EMS (only 240 mMh 24°C)							
I	spring	seed, seedling	colchicine	—	pollen	3–5, 4–5	
II	winter	seed	colchicine	decapitated	pollen (rosette)	3–5, 5–4, 5–5	2–6, 5–2
III	summer	seed, green seed	colchicine, combined	various	pollen	3–5, 4–3, 4–5, 6–2	
IV	winter	green seed	colchicine, combined, EMS	various	pollen, embryotest	2–1, 2–4, 3–1, 3–2, 3–6, 5–3, 5–6, 6–1, 6–2, 6–3	2–5
V	autumn	seed	EMS	various	embryotest	2–1, 3–6, 5–6	
VI	spring	seed, seedling	EMS	vernalized	seedlings (whole plant)	2–2, 3–7, 3–8	4–6, 4–7, 4–8
Sunflower only colchicine (0,2%, 20 h)							
I	1969	seedling	colchicine	—	pollen	4–6, 5–1, 5–2	
II	1970–'71	seed, seedling	colchicine	decapitated	pollen	3–4, 5–2	

chicine alone gave no chlorophyll mutations. EMS + colchicine appears to give more visible chimerism than EMS alone (Table 3-1).

3.6.2 *Treatments tried and used*

In arabidopsis a variety of different treatments, pretreatments, combined treatment, posttreatments and treatment durations were tried, with several developmental stages and varieties.

Buffering and DMSO were tried but rejected showing no great advantage but adding another variable. For the same reason, after some initial trials, only variety Landsberg erecta was used. Treatment of older plants or inflorescences and presoaking were not further pursued so that the main stages used were green seed, ripe seed and seedlings. Vernalization was done only on these stages; 24 hours after treatment for 2 or 4 weeks at 3° C.

The EMS dose eventually used was 10 m Mol for 24 hours (24° C in the dark) or a higher concentration with shorter duration giving the same dose (dose = concentration × duration i.c. 240 mMh). The results indicate some influence of duration of treatment (Fig. 3-3). Shorter treatment was necessary for seedling treatment even when the EMS was buffered.

The colchicine dose was 0,2% for 24 or 30 hours. Simultaneous EMS and colchicine treatment for 24 hours, was first done to see whether cell division occurs during a 24 hour EMS treatment. The results were interesting enough to continue with this treatment (Table 3-1, Fig. 2-5).

Etiolation and different photoperiods were introduced as variables when a causal relationship between growth and chimerism was suspected (Table 5-6, 6-1, 6-2, 6-3).

The sunflower was treated only with colchicine 0,2% for 20 hours as seed or as seedling.

A summary of the main experiments is given (Table 3-3).

The aim of the treatments was not to induce a maximum of mutations, mutants or polyploids, but to induce sectorial chimerism.

3.6.3 *Methods of treatment*

Seed treatment. For EMS treatment dry seed that has had a cold pretreatment (to break dormancy) is treated submersed in an EMS solution for 24 hours at 24° C in the dark. The seeds are then washed with 1 liter tapwater and sown directly on agar, placed in the light for 24 hours (or till germination starts) and then etiolated or vernalized as described (2.6.1). This is an anaerobic treatment. Seeds can also be

treated on filterpaper in a petridish with a few ml EMS solution, resulting in an aerobic treatment which was used, unless otherwise stated, in all experiments.

The duration can also be varied or fractionated, a 1/4 hour EMS, $23^1/_2$ hours water and 1/4 hour EMS being used in this case. Ripe seed can also be treated without cold pretreatment; or without drying after the pretreatment (Exp. V, Table 3-6).

For combined treatment equal quantities of double concentration solutions of EMS and colchicine were mixed. For the combined and for the colchicine treatment the same variations were possible. Here only aerobic treatments were used.

In the sunflower (seed treatment, Exp. II) different presoaking times were tried, but so little polyploidy was obtained that the results were pooled. The frequency of polyploidy after seed treatment was not lower than in some arabidopsis experiments, but fewer plants can be grown. Seedlings were treated just after germination, with a piece of cotton wool soaked in colchicine and placed on the epicotyl for 20 hours (Table 3-4).

Table 3-4. Polyploidy in the sunflower after colchicine treatment (seed and seedling, Exp. II)

stage treated:	seed			seedling		
ploidy:	T	C	D	T	C	D
main flower (untopped)	2	1	74	1	3	22
side shoots 1–3 (untopped)	1	1	36	6	2	29
side shoots 1–3 (topped)	1	2	57	2	3	32

T = tetraploid, C = chimeric, D = diploid flower.
Untopped and topped refer to decapitation of the main stem well before flowering. Side shoots = only the first three primary side shoots.

Arabidopsis seedlings are too small to treat with cotton wool. For EMS treatment the best results were obtained when the seedlings were grown on filterpaper, slightly etiolated (1 day in the dark) and then placed in the EMS solution so that only the roots and hypocotyls are in contact with the EMS. The EMS concentration could be corrected for the water still in the filterpaper. Long treatments are lethal so that a high concentration and short duration were used. The pH of fresh solutions drops below pH 3 within a few hours, but this was not the only reason for the lethality as buffered solutions also gave no survival after long treatments.

Plate III. Chimeric arabidopsis plants.
A. chimeric for ploidy with (l to r) a tetraploid, an octoploid and a diploid shoot. B. visible chimerism on the rosette leaf (front).

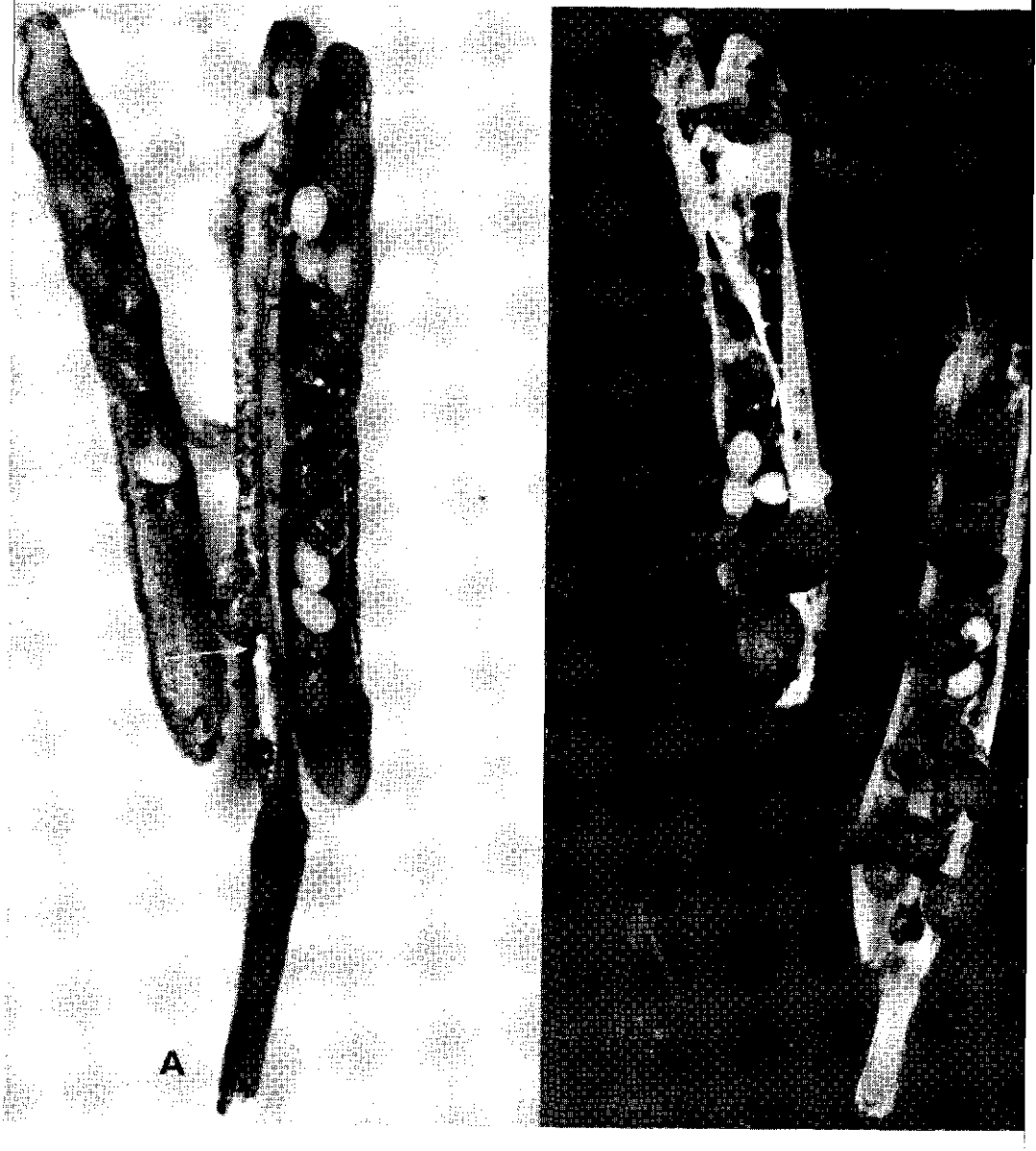


Plate IV. M_2 chlorophyll mutants (albinas) *in arabidopsis*.
A. as seen in the embryotest (the arrow points to an unfertilized ovule).
B. as seen when the (whole) fruits are sown (all seeds have not yet germinated necessitating a later, second, scoring).

The best dose was found to be 240 mMhour given as 480 mM for half an hour. After treatment the filterpaper with the seedlings is submersed in 2 liter tapwater. The roots are already attached to the paper so that removing the plants for transplanting causes variable root damage. To standardize the damage the seedlings were cut from their roots, transplanted to perlite to recover and form new roots, and then transplanted to pots in the greenhouse. New roots are so easily formed that the removal did not affect flowering date. Seedling treatment gives a lower mutation frequency than seed treatment (Exp. VI, Table 3-7, Fig. 4-6, 4-7).

Seedling treatment was also done with colchicine. As extra sensitivity of the roots was expected drops of the solution were placed on the plants or on filterpaper laid over the plants, 24 hours before transplanting to the greenhouse. The polyploidy rate was higher than after seed treatment (Exp. I, Table 3-5).

Sometimes the seedlings were also vernalized (30 days) 24 hours after the end of the (EMS) treatment (Table 3-7).

3.6.4 *Presentation of the results*

Mutation frequency can be expressed in different ways. For chlorophyll mutants the normal method is to express the M_2 mutants as % or ‰ of the non-lethal M_2 embryos (Table 3-2, 3-5, 3-6, Fig. 3-1, 3-3) or, when scoring M_2 seedlings, as a % of the scored seedlings. The embryonic lethals are given as a % of the fertilized ovules (Table 3-2). Sometimes the % mutated M_1 plants is used, and it gives almost the same results as ‰ M_2 mutants (Fig. 3-3). In one experiment (Exp. VI) where all the inflorescences (main stem, primary side shoots; 2 fruits per inflorescence) were scored by the seedling method, mutation frequency per inflorescence position is given as % of all scored fruits of these inflorescence (Fig. 4-6, 4-7), excluding plants without mutations. Mutation frequency for the treatments, replications, etc., was given as 100 × the mean number of separately distributed mutations (i.e. mutated sectors) found per plant (Table 3-7) (0-4 mutated sectors could be distinguished in a plant).

With colchicine (arabidopsis) the mutation (polyploidy) frequency is the frequency of polyploidy in the first flower of the main inflorescence of all plants scored (Table 3-5). For comparisons of chimerism the frequency is given of complete polyploidy contrasted to chimerism in the main inflorescence (Table 6-2) or, in axillary inflorescences of chimeric plants, the frequency of complete polyploidy contrasted to chimerism *and* diploidy (Table 6-2).

In the sunflower polyploidy refers to the frequency of polyploidy among all flowers at that order of branching in all plants (Table 3-4, 5-2) or to the frequency of polyploidy in only the chimeric plants (Table 5-2).

In arabidopsis and sunflower comparison between polyploid and diploid sector size is sometimes required. In these cases the chimeras were taken to be half polyploid half diploid, and added to the polyploid and diploid numbers respectively (Table 5-1, 5-2, 5-4, 5-5, Fig. 5-1).

3.6.5 *Discussion of the results*

Colchicine induces polyploidy by affecting the spindle during mitosis. Thus mitosis is a prerequisite for the action of colchicine. Different treatments have been used to induce polyploidy (Table 3-5) mostly with 24 hour treatment of seed, though the first mitoses in seed were only recorded after 25 to 45 hours and then usually first in the root (Jacobs and Bonotto 1968, Müller 1967a). Dry seed with no pretreatment indeed gave no polyploidy. Wet seed treated directly after the cold pretreatment gave some polyploidy, and green seed, or seedlings gave still more polyploidy. It is possible that some mitoses do normally occur, but too rarely to have been detected within the first 24 hours. The after-effects of colchicine were reported to last a few days (Eigsti and Dustin 1957) so that polyploidy may possibly still be induced after the treatment, or some colchicine nay remain in the seed (after the treatment).

A slight increase in duration of the treatment gives a relatively large increase in polyploidy which suggests that the actual time of treatment is more important than after-effects and that the last hours of the treatment are probably the most effective (Exp. II, Table 3-5).

Another possible explanation is that colchicine stimulates the seed, inducing cell-divisions. It was shown that colchicine has a stimulating effect on green seed (Fig. 2-5). A similar effect may occur in ripe seed. The influence of EMS in combined treatments may also be due to a stimulating effect (of the EMS) as was found in the green seed (Fig. 2-5). This effect is so strong that polyploidy is even found in ripe seed which has had no (cold) pretreatment (Table 3-5).

Colchicine also affects plant growth and flowering date (Table 2-1). This effect is however only found in the treatments giving polyploidy and especially in the plants showing polyploidy (in the first flower). This points to an immediate action of colchicine. It is also found after combined treatment, again only in the treatment (germination date) giving polyploidy, suggesting that the EMS does not affect the colchicine, i.e. the two chemicals simultaneously administered act indepen-

Table 3–5. Polyploidy and chimerism after various colchicine treatments (arabidopsis, Exp. I, II and III)

stage treated with colchicine 0,2 %	duration of treatment (hours)	cold pretreatment (without redrying)	etiolated (48 h, 24° C)	vernalized (30 days, 3° C)	number treated	flower 1 diploid	flower 1 polyploid	% polyploidy	inflorescences chimeric	inflorescences (partly) octoploid
Experiment I										
seedling (8 day old)	24				320	3	38	93	13*	9
ripe seed	30	v			160	15	2	12	3*	0
Experiment II										
ripe seed	24	v			124	82	18	18	7**	1
ripe seed	30	v			89	25	33	57	9**	11
Experiment III										
ripe seed	24				200	159	0	0		
				v	75	37	0	0		
		v	v		250	161	15	8	13	2
		v			100	77	6	7	6	
		v		v	225	163	6	3	6	
ripe seed + EMS	24	v	v		150	105	1	1	1	
		v		v	75	40	1	2	1	
					100	71	7	9	7	2
			v		75	67	0	0		
green seed	24				100	74	19	20	14	4
				v	50	14	7	33	5	2

v = treatment applied.
* includes inflorescences with flower 1 diploid.
** flower 1 itself chimeric.

dently. Octoploids are found after a 24 hour treatment, indicating 2 c-mitoses within that time.

Treatments inducing more polyploidy generally show more octoploidy and less chimerism. More polyploidy will be found when more mitoses occur. The chance for 2 mitoses in the same cell (octoploidy) or

in adjacent cells (less chimerism) will be higher when more mitoses occur. Combined treatment of green seed seems to be an exception (Table 5-3) in that it gives a very low number of octoploids. This is ascribed to the inhibitory or retarding effect of EMS after some initial cell division (Bacq and Alexander 1961).

The apparently increased number of visible chimeras after combined treatment (Table 3-1) may be similar to the observed visible chimeras after colchicine treatment of heterozygotes (Gröber 1962) but differences in sensitivity and in germination (date), or chance, may also explain the results. The increased forking may be comparable to that reported after X-ray treatment, which was ascribed to retarded cell differentiation giving an outsized apex which reorganizes by forking (Haccius and Reichert 1963). Here initial "cell division" may lead to polyploidy, subsequent inhibition, due to EMS, prevents these cells from dividing. Later division gives an unstable outsize apex which forks. The changed branching (Table 2-1, 2-2) after EMS and combined treatments may be caused by a similar though smaller, change in apex size. Combined treatment of dry seeds leads to an unusual type of chimerism, polyploidy being found only in the main inflorescence (Table 4-5). This suggests an inhibition in the apex cells lasting till the generative apex.

In the sunflower treatment of seedlings gave more polyploidy than treatment of seeds probably again due to more cell division (Table 3-4). Different presoaking times were tried but gave no clear effect.

EMS. A number of EMS induced mutations was scored by the embryotest and subsequently by the seedling-test (Fig. 3-2) and seems to suggest a greater rate of detection with the embryotest. To test this a representative number of "normal" embryos would also have to be sown because they may contain morphological mutations which cannot be scored in the embryotest, and chlorophyll mutations with late expression. A combination of the two tests does however allow for better differentiation between different mutations, so that the three groups (Fig. 3-2) in the embryo show a total of 16 different types of expression from the embryo to the older seedling stage.

Different germination dates were found after green seed treatment with EMS and EMS + colchicine (Fig. 2-5). In both cases the earlier dates showed higher chlorophyll mutation rates (Table 3-6). In the combined treatments this is not caused by the polyploids because these were not included in the score given. Polyploidy was scored on the first flower and occurred only in the early group and escape from detection would have decreased the mutation rate. It might also be argued that similarly to the higher rate of visible chimeras (Table 3-1) the colchicine

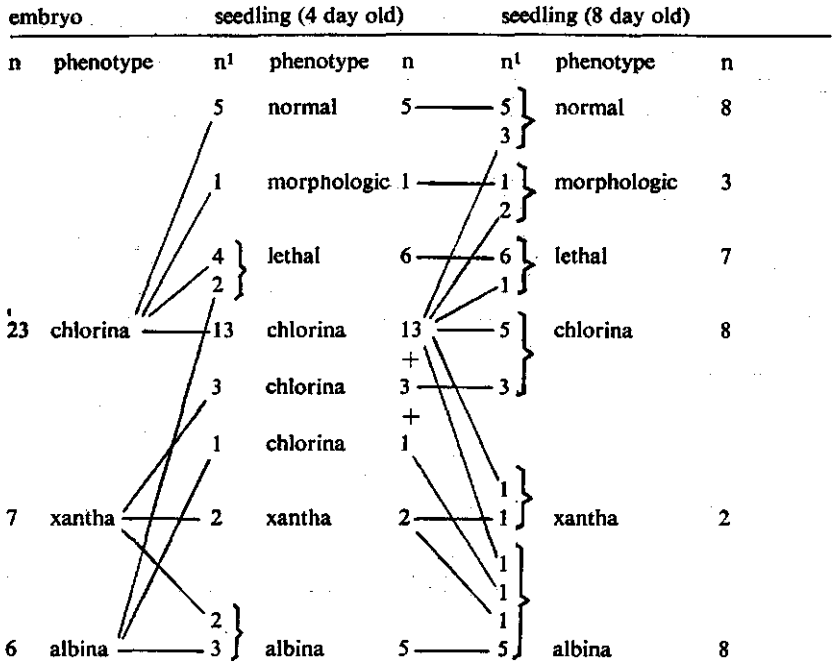


Fig. 3–2. The same mutations scored in the embryotest, as young seedlings and as older seedlings (arabidopsis). n = number per phenotype per (scoring) stage, n^1 = the number and origin of the phenotype at a seedling stage. The lines connect the mutants through the different stages.

may have increased the rate of M_2 mutations. The differences observed for the EMS treatment are however parallel to those for the combined treatment. Therefore early germination was probably caused by the EMS simultaneously with the higher mutation frequency, or seed more easily induced to germinate, is also more sensitive to EMS.

The effect of the different pretreatments (Table 3-6) on the action of the EMS is not easy to explain. From literature, cold and wet was expected to give the highest mutation frequency (similar to presoaking in general), cold but dry to give a lower (only a changed seed coat effect), and no pretreatment a still lower mutation frequency. The results do not fit the expectations. They can only be explained by assuming physiological differences, due to the pretreatments, which affect the sensitivity and therefore the mutation rate, and not just by assuming differential penetration of EMS.

Table 3–6. Mutation frequency after various pre- and posttreatments (arabidopsis, Exp. IV and V)

IV. Green seed: EMS	posttreatment					
	control 24		etiolated 16		control 16	
germinated	%M	n	%M	n	%M	n
late	6,6	69			5,5	227
medium	9,5	79			8,2	50
medium*			8,3	50		
early*	11,1	87	14,9	77	10,7	68

V. Ripe seed: EMS	posttreatment					
	control 24		etiolated 24		vernalized 24	
pretreated	%M	n	%M	n	%M	n
cold, redried	9,7	86	10,9	78	12,3	78
cold (wet)	8,1	84	9,7	85	10,8	92
no cold (dry)	7,6	85	11,8	87	13,8	78

24, 16 refer to daylength (hours). $\%M = \%M_2$ chlorophyll mutants. n = number of plants scored.
* EMS + colchicine

The posttreatments affect the mutation frequency (Table 3-6). Generally it is accepted that the time between treatment and scoring allows loss of mutations to take place. In that case a decrease of mutation rate with vernalization (16 days), and with 16h daylength is expected. The results contradict this, and will be explained later (Chapter 5 and 6).

After different concentrations and durations similar results are found for short acute, fractionated acute, and long treatments (Fig. 3-3, see also Table 3-8). With a lag before EMS action the long treatment is expected to give more mutations and the fractionated treatment to give the least. In the case of after-effects the fractionated acute treatment should show more mutations. The observed results seem to indicate no lag for the acute treatments, though the long treatment suggests a lag or a duration (of treatment) effect. (In the fractionated treatment the lag effect may have been present but countered by an after-effect). The observed results seem to indicate a very direct action of the EMS.

The occurrence of mutations in the induced polyploids (after combined treatment) which can be scored in the M_2 also suggests that the EMS induces at least some mutations very directly i.e. before cell divisions or c-mitoses occur. Tetraploids in which chlorophyll mutations

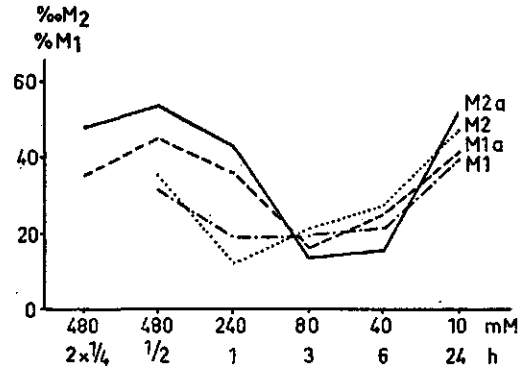


Fig. 3–3. Mutation frequency after various seed treatments (arabidopsis, 120 plants per treatment, EMS 240 mMh, 24° C, dark). ‰ M_2 mutants, % mutated M_1 plants, aerobic and anaerobic (a) treatment. mM = EMS concentration, h = hours (duration of treatment).

were induced after the chromosomes have become double-stranded would not segregate in the M_2 unless double reduction occurs or the same mutation was induced on two chromosomes simultaneously, both expected to occur at a very low frequency only. Mutations in the octoploid M_2 lead to the same conclusion.

The observed decrease in mutation frequency at intermediate concentrations and durations seems to be found after a shorter duration in the aerobic treatment (all the same dose, Fig. 3-3). This suggests that the explanation may again lie in the physiological constitution of the seeds which, affected by (the duration of) the treatment, influences sensitivity. Possibly cell division or, more probably, DNA synthesis occurs in the first hours of the treatment. The very short treatments are too short, in longer treatments chromatid mutations occur, due to the DNA synthesis or to double-stranded chromosomes, and in very long treatments these cells have divided and others are inhibited so that mutations again occur at the chromosome level. Mutations at chromatid level will only be found in one of the two cells after cell division and therefore give an apparently halved mutation rate after one cell-generation. This may explain the differences found (Fig. 3-3).

Sensitivity for alkylating agents has been reported to vary for the different stages of the cell cycle (Bacq and Alexander 1961). During longer treatments more cells will be (temporarily) in an EMS sensitive stage so that duration (of treatment) may affect the mutation rate or the number of (different) mutations found (Table 3-7).

Table 3–7. Mutations and branching as influenced by various factors (arabidopsis, Exp. VI)

EMS treatments (240 mMh)

	seed		
	2 × ¼ h	½ h	24 h
% mutations*	96 ± 11	96 ± 10	133 ± 12
n side shoots	4,11 ± 0,24	4,34 ± 0,13	4,51 ± 0,25

Unvernalized seed treatments only

	sowing dates		
	3/2	10/2	17/2
% mutations	71 ± 17	99 ± 18	88 ± 16
n side shoots	3,65 ± 0,18	4,09 ± 0,19	3,96 ± 0,20
	total number of side shoots		
	3	4	5
% mutations	75 ± 17	83 ± 12	121 ± 15
	number of cauline shoots		
	2	3	4
% mutations	72 ± 13	110 ± 11	126 ± 19

2 × ¼, ½ and 24 h = duration of EMS treatment with 480, 480 and 10 mM resp.
E, 0 = etiolated or not before EMS (½ h, 480 mM).
% mutations = 100 × average number of different mutations (sectors) per plant (all inflorescences scored).

A relationship is found between mutations and branching (Table 3-7, 3-8, 2-2). In these tables "mutations" indicates the average number of separately distributed mutations (0-4) found in a plant by scoring seedlings from 2 fruits per inflorescence (main shoot and first order side shoots). These results compare reasonably with those for only the main inflorescences but, in addition, give an indication of chimerism.

Branching and mutations seem to be positively correlated, but the results after vernalization show that it is not necessarily a causal relationship. It is not only a result of better detection either, because the number of rosette branches shows a weaker relationship, though more rosette branches should have the same effect on detection. The relationship is clearly seen in a closer analysis of two groups of plants, the early

			Seed treatments only (pooled)	
seedling		control	vernalization	
E	0		0 days	30 days
41 ± 6	44 ± 14	3 ± 3	104 ± 8	111 ± 10
4,23 ± 0,10	3,96 ± 0,24	3,75 ± 0,19	4,53 ± 0,11	3,96 ± 0,11

24/2	3/3	10/3
143 ± 18	119 ± 18	87 ± 27
5,96 ± 0,20	4,91 ± 0,20	4,15 ± 0,20

6	7
146 ± 21	144 ± 33

	number of rosette shoots			
5	0	1	2	3
144 ± 44	80 ± 19	95 ± 13	112 ± 14	127 ± 22

n side shoots = the average number of side shoots.
* % mutated M_1 plants (main inflorescence only) was 27, 28, 37, 9, 10 and 1 respectively.

and the later sown replications, between which differences in vigour had been observed (Table 3-8). It is seen that the stronger plants had more branches and more mutations but also had more sterile (0 seedlings) and semifertile (less than 10 seedlings) fruits so that the extra branches cannot affect detection very much, certainly not enough to explain the higher number of mutations. Both branching and mutation rate may have been influenced by the same factors, probably factors stimulating growth (e.g. time of year, position in greenhouse).

The differences between seed and seedling treatment (Table 3-7) may be explained by differential sensitivity of the cells (4.7.3). The treatment itself may however have induced different effective EMS concentrations. Seedlings were treated indirectly through the root, which was removed

Table 3–8. Comparison of replications of the same EMS treatments sown at different dates (arabidopsis, Exp. VI, seed treatments pooled)

	sowing date*	
	3/2, 10/2, 17/2	24/2, 3/3
plants (vigour)	weak	strong
number scored	277	205
n side shoots/plant	3,9	5,4
mutations/plant	0,87	1,31
HS fruits/mutated plant	0,8	2,2
S fruits/mutated plant	0,2	0,7

n side shoots = the average number of side shoots.
HS = 1–9, S = 0 seedlings per fruit (all inflorescences scored, 2 fruits per inflorescence).
Mutations = different mutations (sectors).
* sowing dates were confounded with position in the greenhouse.

immediately after the treatment. In this way the EMS dose, effective in the shoot apex, may have been very low. Direct shoot treatment, as with colchicine was not possible.

Differences in effect between colchicine and EMS are clearest after seedling treatment (high polyploidy frequency, low chlorophyll mutation frequency) but this may be due to the method of treatment. Seed treatment also shows differences which are clearly related to different prerequisites for mutagenic action of the two chemicals, colchicine needing mitosis, EMS not.

CHAPTER 4

CHIMERISM

4.1 TYPES OF CHIMERISM

In plants, individuals with genetically different cells, cell groups or tissues are generally called chimeras. Different types are distinguished ranging from mixed, with no apparent system (often referred to as mixochimeras), through partly stable sectors in one or more cell layers (mericlinal chimeras), to fairly stable differences between the layers (periclinal chimeras). As a result of cell divisions the chimerism can change from one type to another or be lost (Bergann and Bergann 1959).

Baur (1909b) observed that there must be a system to plant growth allowing chimerism to exist and to persist. Here periclinal chimerism was involved, which can now be understood as due to the layered structure of the plant. Dermen (1945) was surprised at the persistence of some mutated sectors which again indicates the presence of some system. This may be determined by the initial cells, but their existence is not universally accepted.

Mixed chimerism can usually be explained by plastid segregation from mixed cells, or chromosome loss (Hegwood and Hough 1958, Monti and Saccardo 1969). In either case the "unstable" cell gives different types of daughter cells.

Another type of chimerism, sometimes reported, is one in which the top of the plant consists mainly, or only, of one "sector", and the base of another sector (Bateson and Pellew 1915, 1920, Brotherton 1919, Hildering and Verkerk 1964). There may be different explanations, e.g. the sympodial growth habit (in the case of the tomato), the apex forming the first truss and the top axillary shoot forming the rest of the plant (Hildering and Verkerk 1964). A comparison of the first truss (which is at the base of the plant) with subsequent trusses (from the axillary shoot or its derivatives) is not a comparison between the base and the top of a plant, but between the main inflorescence and the derivatives of the top

side shoot. The observed differences can thus be interpreted in the same way as for sectorial chimerism.

Strictly speaking the term sectorial implies all layers, and therefore the term mericlinal was introduced for sectors in only one layer (Jørgensen and Crane 1927). However, in genetic studies on chimerism, based on gametes, etc., which derive from one layer (the L_2), the term sectorial should give no confusion as it necessarily implies mericlinal chimerism of the L_2.

4.1.1 *Graft chimeras*

The term chimera was introduced by Winkler (1907) who had grafted different *Solanum* species onto each other and found that adventitious buds induced on the plane of union sometimes differed from both donors. One case reported had equally large sectors of *Solanum lycopersicum* and *Solanum nigrum* tissue. Baur (1909b) suggested that other known "graft hybrids" were also graft chimeras with layers of different origin. He could also show that even variegated plants are often periclinal chimeras (Baur 1909a). Since then more, and more complex, graft chimeras have been constituted (Guillaumin 1949, Heuer 1910, Jørgensen and Crane 1927, Neilson-Jones 1934, 1969, Swingle 1927).

4.1.2 *Spontaneous chimerism*

Spontaneous chimerism is mainly found in vegetatively propagated (Dahlgren 1927) and (other) heterozygous plants (Patch 1930, Lamprecht and Svensson 1949), because it can be observed in the plant in which it originates. In vegetatively propagated plants periclinal chimerism may exist for a long time and is only detected when it changes to mericlinal in the same or in another layer (Baur 1909a). The frequency of this change can be artificially increased (Asseyeva 1927, 1931, Bateson 1916, 1921, Robinson and Darrow 1929). A special terminology has been developed for chimerism in the different layers of vegetatively propagated plants.

Chimerism for caryotype, especially chromosome number, may also be detected on the plant itself (Müntzing and Prakken 1941). Spontaneous recessive mutations with very low segregation ratios observed in the progeny from single plants may indicate chimerism if a normal segregation is found in later generations (Hallqvist 1924).

Plastid mutations usually lead to directly visible chimerism. They may give variegated plants through periclinal chimerism, but also sometimes sectors i.e. mericlinal chimerism. In the latter case seeds from the

sector border (L_2) may transmit chimerism to the "next generation" (Anderson 1923, Correns 1922).

Unstable chromosome aberrations may lead to chimerism. When loss of a chromosome or a fragment has a visible effect, variegation (Gupta 1968, Monti and Saccardo 1969, Scarascia-Mugnozza 1969) or frequent sporting (Stewart and Dermen 1970b) will be seen. Twin spots may be caused by unstable chromosomes (Stino 1940); or by somatic crossing over after X-rays (Hirono 1965, Hirono and Rédei 1963, 1965a,b) and in certain hybrids (Menzel and Brown 1952).

Abnormalities at fertilization may prevent fusion of the two nuclei, leading to an embryo chimeric for maternal and paternal tissue. The position of the two tissues depends on the position of the male gamete, which often forms only the suspensor (Arnold and Cruse 1968, Battaglia 1963, Turcotte and Feaster 1967). Similar abnormalities may explain ploidy chimerism found in the embryo of a variety which frequently produces unreduced gametes (Pratassenja 1939). Chimerism in hybrids (Kostoff 1930, Schwanitz 1955) may have a similar origin or may be due to loss of chromosomes during irregular mitoses.

Mutations may cause phenotypic chimeras (Rédei 1965b, 1967a,b,c) or maternally inherited variegation (Stroup 1970).

In animals exchange of cells between two embryos may also give chimerism (Basrur and Kanagawa 1969).

4.1.3 *Natural chimerism*

Endomitosis leading to endopolyploidy is sometimes observed in roots (Clowes 1961). Cells with higher DNA complements are common in the root (Clowes 1959a, McLeish and Sunderland 1961) but these cells usually do not divide so that the chromosomes can seldom be counted. Similarly other plant tissues may often contain polyploid cells which become apparent when these cells are stimulated, by auxins or wounding, to divide (Coleman 1950, D'Amato 1952, Partanen 1959a, Tschermak-Woess 1956). This may be a type of cell-differentiation (Coleman 1950, Partanen 1959b.)

B-chromosomes, found in some plants, divide less regularly than the normal (A) chromosomes so that chimerism for them may occur. As they are usually genetically "empty" no effects are seen.

4.1.4 *Induced chimerism*

Together with the artificial induction of mutations came the problem of chimerism which is mentioned in most publications on mutation work.

Colchicine induces polyploidy in some cells, which by division will give rise to cell lineages (Dermen 1945). In sterile intergeneric (or interspecific) hybrids where polyploidy restores fertility, these cell lineages may be seen as fertile sectors (Howard 1938 pers. comm.). Leaf colour and size, stomata size, plastid number, pollen size, cell size in general and chromosome counts give indications of polyploidy and are used to distinguish sectors after heat shock (Randolph et al. 1944) or colchicine treatment (Dermen 1945, Nebel and Ruttle 1938).

X-rays, EMS and other mutagens vary in the degree of chimerism induced. EMS and other chemicals (Chatterjee et al. 1965, D'Amato 1964, Ficsor 1965, Lindgren et al. 1970, Scarascia-Mugnozza et al. 1963), and UV (Ficsor 1965, Fujii 1965a), give more chimerism than X-rays, probably due to the chromosome level at which they act or to less direct action. The mutagen dose also influences chimerism, high doses usually giving less (Bergner et al. 1940, Monti and Scarascia-Mugnozza 1964, Yamagughi 1962), but sometimes more chimerism (Julén 1954). The latter may be due to other complications.

The stage treated determines the number of cells or the constitution of the chromosomes or their DNA, and therefore influences chimerism. Similarly the method used to detect chimerism will influence the results; e.g. chimerism induced in the roottip usually does not reach the M_2. Even the genetic constitution of the material may influence the appearance of chimerism (Mericle and Mericle 1967b).

4.2 OBSERVATIONS ON CHIMERISM

4.2.1 *Direct observation*

When chimerism results from a mutation in the plastids or the cytoplasm it can often be directly observed (Röbbelen 1962b, 1963b, 1965c, 1966b, Stadler 1930, Stroup 1970). Lower vitality of mutant plastids can be overcome by studying revertants in a background with a chlorophyll mutation (Röbbelen 1965c). Other maternal factors may be observed in the next generation (Lamprecht 1960, van der Veen 1967a,b) but possibly also occur in sectors or layers (Mesken and van der Veen 1968).

Dominant mutations or mutations in heterozygous material often allow direct observation of chimerism (Åkerman 1920, Fujii 1964, 1965a, 1966b, Fujii et al. 1966, Gichner and Velemínsky 1963, Granhall 1953, Mericle and Mericle 1967a, 1969a, Stadler 1928). They may occur only in the outer layer, not reappearing in the M_2 (Kress 1953). Chimerism

involving sterility is also easy to observe (Howard 1938 pers. comm., van der Mey 1970, van der Veen and Gerlach 1965).

Direct observations on pollen for sterility (Anderson et al. 1949), mutations (Eriksson 1966, Lindgren et al. 1970) and polyploidy; or observations on cell size (Dermen 1945, Némec 1962a,b, Avery et al. 1959) or on chromosome number and aberrations (Hegwood and Hough 1958, Kachidze 1932, van der Mey 1970), also give an impression of chimerism.

Often, however, chimerism has to be observed indirectly from differences between the mutations or segregation ratios in different parts of the plant (Müller 1963b, 1965a, Stadler 1928, van der Veen and Gerlach 1965, Yamagughi 1962).

For determining and comparing mutation rates it is important to know the average number of cells from which the M_2 derives (Gaul 1963b, 1964) or the genetically effective cell number (Li and Rédei 1969), which are expressed as the number of initial cells or as the number of sectors found.

4.2.2 *Initials*

Initial cells are those cells to which the observed cell lineages can be traced back. Their number need not correspond with the original (initial) number of cells at the time of treatment. The deduced number is usually lower but may also be higher, e.g. with chimerism after pollen treatment. The position and number of initial cells depends on the time, place and method of both detection and treatment. Observation directly after the treatment will give an initial cell number identical to the number of cells observed. Generally only one cell layer, often the L_2 which gives the M_2, is studied and the initial cell number refers to that layer only.

The term, initial cell, is easily confused with that used by botanists to indicate the cells, descendents of which form a certain organ. Thus a plant with genetically two initial cells may have leaves originating from more than two initial cells. When the same organs are studied the numbers should correspond. However, even the botanical use of the term "initial" is not uniform.

Sector number is often deduced from sector size and directly translated into initial cell number, although only average sector size is inversely related to sector number, and sector number and initial cell number only correspond when one layer of a chimeric plant part is observed some time after the treatment.

4.2.3 *Sector size*

Observations on sector size may be made directly on visible sectors or indirectly through the M_2. Sometimes the two methods can be combined to show the relationship between two layers (Akerman 1927, Kress 1953). The expected M_2 segregation, with two equal sectors is 1/8, for self fertilization within the sectors, and 1/16 for random fertilization between the sectors. Recessive deficits, often caused by certation, cause further deviations. The ratio 7 : 1 seems to be a realistic expectation as within-flower chimerism is less common. In chimeric flowers non-simultaneous ripening of anthers may give an effect resembling certation.

Commonly found segregation ratios of 12% (Gottschalk and Weiling 1960), 1/8 (Langridge 1958b, Stadler 1928) and 15% (Nybom et al. 1956) for chimeric plants, or 12-16% (D'Amato 1964, Scarascia-Mugnozza et al. 1963) for all plants, indicate two sectors. Often clearly different M_2 ratios show that the M_1 population consisted of chimeric and non-chimeric plants (Bergner et al. 1940, Gottschalk and Weiling 1960, Monti and Scarascia-Mugnozza 1964, Nybom et al. 1956, Stadler 1928, Weiling and Gottschalk 1961, Yamagughi 1962).

Visible chimerism or differences between the mutations from different positions on the plant give (mutated) sector sizes of 1/3 (Mason 1930, Shamel 1930), 25-30% (Müller 1963b, 1965a, van der Veen and Gerlach 1965) and 1/4 (Breider 1953), or sector numbers of 2 (van der Mey 1970, Reitz 1949), 2 and 3 (Hildering and Verkerk 1964), 1-4 (Lindgren et al. 1970) and 1-5 (Anderson et al. 1949). Higher numbers or smaller sectors may be found when the observation is made shortly after the treatment or near to the point of treatment, or when different parts of the plant, deriving from different parts of the treated material, are observed (Dulieu 1969, Hildering 1971 pers. comm.).

Chimerism may be found within the plant, within the inflorescence (Bruns 1954, Lindgren et al. 1970, MacKey 1954, Reitz 1949), within the flower or fruit (Gaul 1959, Hekstra and Broertjes 1968, Howard 1938 pers. comm., van der Veen and Gerlach 1965) and even within the anthers (Hill and Myers 1944, Nebel and Ruttle 1938).

In leaves the sector usually covers 1/4 (3/4) or 1/2 of the leaf area (Breider 1953, Bruns 1954, Fujii 1964, Howard 1961b, 1965). Sectors appear as stripes in monocotyledons (Ichikawa and Ikushima 1967).

Through branching chimerism, as seen in the branches, decreases and is often lost (Gladstones 1958, Monti 1965a,b, Monti and Scarascia-Mugnozza 1964). This is to be expected as the branch only includes part of the tissue from the stem. Persistence of chimerism for eleven vegetative generations (Hill and Myers 1944) seems more surprising.

4.3 FACTORS INFLUENCING CHIMERISM

Different explanations have been given for the presence, and also the absence, of chimerism. Usually it results from a mutation in a cell from which only part of the observed tissue derives (Fujii 1966a, Stadler 1928, 1930). When a mutation occurs at the chromatid level, even cells deriving from one cell will differ.

Reverse mutation (Weiling and Gottschalk 1961), repair or delayed mutation (Lindgren et al. 1970) may cause chimerism in derivatives from one cell (at the time of treatment) but still the chimerism originates from more than one cell at the time of (final) mutagenesis. Delayed mutation, when it results from an instability, may possibly occur in more than one daughter cell from one affected cell and then cause sectors which have the same mutation (Ehrenberg, see Hildering and Verkerk 1964). Cell exchange between two layers is rare (Stewart and Burk 1970) but may cause chimerism to appear in the layer under observation (Stewart and Burk 1970, Weiling and Gottschalk 1961).

Lack of chimerism after heavy treatments may be due to cell death so that one cell gives rise to all the tissue subsequently studied. Of course, the same mutation may have been induced in more than one cell but the chance for this to happen is small, except in the case of polyploidy. Sterility or semisterility may prevent detection of other mutations but then sterility sectors can sometimes be recognized.

Chimerism is an expression of mutated tissue and therefore dependent on all factors influencing mutagenesis, e.g. the type of mutagen and pretreatment (Ichikawa and Ikushima 1967, Nishiyama et al. 1966, Yamagughi 1969). In their effect mutagens may differ due to the chromosome level at which they act (Bruns 1954, Chatterjee et al. 1965, Kreizinger 1958, Lindgren et al. 1970), due to delayed action or repair processes (Drake 1969, 1970, Green and Krieg 1961, Kreizinger 1958, Lindgren et al. 1970, Nasim and Auerbach 1967), and possibly due to the duration of the treatment. The latter is often long for chemicals so that DNA synthesis or even cell division may occur during the treatment (Lindgren et al. 1970, Yamagughi 1969), and result in one cell producing two different daughter cells. If this occurs in an apical initial cell two consecutive (partly different) sectors will be formed.

The stage which is treated determines sensitivity and also the maximum number of cells, or at the one cell stage, the number of chromosome strands or DNA complements, and therefore the potential chimerism. Chimerism does occur after treatment at the unicellular level, e.g. pollen (Devreux and Saccardo 1968, Ficsor 1965, Shapiro 1966) or zygotes (Mericle and Mericle 1969b, Röbbelen 1960) but is rare (Röbbelen

1960) and often absent (Donini et al. 1970). It probably depends on the stage of the gametophyte (or zygote) treated (Scarascia-Mugnozza 1969). Chromosomes may be double stranded in the gamete (Ficsor 1965) and multistranded in the zygote (Mericle and Mericle 1967a, 1969b, 1970).

Whether chimerism is found (Kirk and Tilney-Bassett 1967, Mericle and Mericle 1961, 1962, Singleton 1962) or not found (Mericle and Mericle 1961, 1962, Stadler 1930) after proembryo treatment, depends on the (proembryo) stage exposed. Usually the embryo in the ripe seed is treated because it is the easiest stage to handle. The embryo may, however, be differentiated and contain the primordia for one or several leaves. In barley axillary shoots (tillers) associated with these leaves are referred to as preformed, and differ, in their chimerism, from later tillers (Frydenberg et al. 1964, Stadler 1930).

In vegetatively propagated crops, highly differentiated tissue often has to be treated and chimerism may be a problem. Treatment of small buds and tubers or young bulbs (Hekstra and Broertjes 1968) gives less chimerism, but forcing side shoots to develop may be a simpler method to eliminate sectorial (mericlinal) chimerism.

Flower buds or inflorescences have also been treated, mainly to study flower development from the differences in number, size and position of the induced sectors (Gupta and Samata 1967, Mericle and Mericle 1967a, 1969a).

A method has been devised to bypass chimerism. Petioles are treated and then induced to form adventitious buds, which usually originate from one cell (Arisumi and Frazier 1968, Broertjes 1969a). These cells may also be physiologically less sensitive to mutagenic treatment (von Wangenheim et al. 1970).

Synchronizing cell division may decrease chimerism when colchicine is used to induce polyploidy (Clarke 1969).

4.4 INFERENCES FROM CHIMERISM

Chimerism, especially periclinal chimerism, can act as a genetic reserve in vegetatively propagated plants but may be a handicap when genetically homogeneous material is desired. Sometimes, as in variegated plants, chimerism itself is desired. Chimerism is also a useful aid in studying plant histogenesis and morphogenesis.

4.4.1 *Chimerism and layers*

Periclinal chimerism for polyploidy, and later also for chlorophyll mu-

tations (Dulieu 1967b) has been used extensively to prove the existence of stable layers and to deduce the origin of different organs or tissues (Avery et al. 1959, Dermen 1947, 1951, 1960, Dermen and Bain 1944, Satina 1945, Satina and Blakeslee 1941). The L_2 producing the pollen and embryo (Dermen 1947, Dermen and Bain 1944) is important for mutation research in generatively reproducing plants while the layer(s) responsible for leaf shape, tuber colour, etc. may be important in vegetatively reproducing plants (Howard 1969, Klopfer 1965a,c). Special techniques have been developed to expose (genetically) these layers (Asseyeva 1927, 1931, Bateson 1916, Howard 1964, 1970). No anatomic or chimeric layering could be found in some *Saccharum* species (Thielke 1959).

The stability of the layers is not absolute and may, in periclinal chimeras, be decreased by the mutation (Dulieu 1967b, Thielke 1948, 1954) or by wounding (Dulieu 1967b). Cell exchange between different layers is rare, e.g. 1/3000 (Stewart and Burk 1970), and only exchange or displacement in the initial cells will lastingly affect chimerism.

The layers may differ physiologically, e.g. in sensitivity to X-rays, and in mutation rate (Asseyeva 1931, Bateson 1921, Satina et al. 1940). Periclinal chimerism causes variegation when the layers participate differentially in different tissues, e.g. the leaf edge (Dulieu 1968); or when periclinal divisions occur in the (mutated) L_1, giving sectors or stripes (dependent on when this division occurs) (Thielke 1951, 1957). In *Tradescantia* variegation is caused in this way, opposite leaves and oriented cell division allowing the appearance of parallel stripes (Thielke 1954, 1955).

4.4.2 *Sectorial chimerism and cell lineages*

Sectorial chimerism is based on continuous cell lineages and will be similar in the L_1 and L_2 which are only one cell deep. The L_3 (corpus) and the root may be different as cell divisions in various planes occur so that another relation to the initial cells exists. Usually the L_2 is studied and then "sectorial" refers only to that layer.

In animals, cells can move about, giving discontinuous cell groups derived from the same initial cell. Grafting together two genetically different cells resulted in 34 different sectors on the skin of the mouse (Mintz 1967).

In plants however, the cell walls form a common framework for all cells so that the only possibility to change relative positions is by differential cell growth and division (Meeuse 1942, Sinnott and Bloch 1939). Intrusive growth may occur, for some specialized cells, when a

cell pushes between two other cells or grows through intercellular spaces (Foster 1945, Meeuse 1942, Schüepp 1966). A common framework implies that growth and division in one cell will influence the other cells around it. Similarly cell death will influence surrounding cells and possibly even their plane of division, because the dead cell cannot grow or divide.

Sectorial chimerism confirms the continuity of cell lineages. Sometimes foreign cells appear to occur in a mutated sector. This was observed in a ploidy chimeric root (Juhl 1953). Incomplete observation and spontaneous polyploidy may have influenced the results, and a complete "three dimensional" observation may still have shown continuous cell lineages perpendicular to the sections observed.

Cell exchange *between layers* may give groups or whole sectors of different cells. Yet even here continuity of the displacing cell with the cells from its layer of origin remains. The displaced cell may move to the next layer (Stewart and Dermen 1970a) or it may have been damaged and killed. This exchange is, however, rare.

Plastid mutations and genetic variegation may give the impression of discontinuous cell lineages. Plastid lineages need not be continuous as the pastids can move about within the cell.

Chimerism allows the cell lineages to be identified and, therefore, can be used to study the origin of different tissues and organs, also within the layers. This offers methodological possibilities for studies on development and may be important for mutation research.

4.4.3 *Sectors and the apex*

Visible chimerism usually appears as a sector apparently originating from one central cell. This led to the idea that the number of cells in the centre (flowers) or at the top (stems and tubers) is very limited. With induced mutations enough chimerism became available to study this, generally as a sideline to mutation research. Some confusion has arisen due to the stage treated. When a potato with several buds is treated the buds are expected to mutate independently. Similarly bud primordia present in the seed at the time of treatment must be expected to mutate independently, and cannot be used to get an impression of the situation in the stem apex. Usually, however, the main apex has been studied, long enough after the treatment to obtain an impression of the normal situation in the apex.

In most studies the average sector size is determined (4.2.3) and from this the sector number or initial cell number is deduced. A sector can be identified by its colour (chlorophyll mutation), type of M_2 mutants,

Table 4–1. Reported number of sectors or initial cells for various plants (dicotyledons and monocotyledons)

number of initials or sectors	plant(s) studied	method used	author and date
2	arabidopsis	M_2 segregation	Langridge 1958b
	arabidopsis	M_2 segregation	McKelvie 1961
2 or 3	arabidopsis	M_2 segregation	Jacobs 1969
	arabidopsis	M_2 mutations	De Boelpaepe 1968
1	coffee	M_2 mutations	Moh 1961
1–3	cranberry	sector size (polyploidy), cells seen in the apex	Dermen 1945
5	crucifers	sector size (pollen size)	Howard 1961a
4	epilobium	sector size, cells seen in the apex	Bartels 1960
	epilobium	sector size	Michaelis 1967
1, 2, more	flax	calculated	Beard 1970
	lupins	calculated	Gladstones 1958
	pea	calculated	Monti 1965b
often 1	pea	calculated	Gottschalk and Weiling 1960
1 or 2	pea	sector size	Blixt 1960
1–3	pea	M_2 mutations	Monti 1965b
6(L_1)	potato	sector size	Howard 1961b
2(L_2)	potato	sector size (pollen size)	Howard 1961a
	potato	sector size	Howard 1965
2 or 3	tomato	M_2 mutations	Hildering and Verkerk 1964
	tomato	sterility	Van der Mey 1970
1–4	various	sector size	Stewart and Dermen 1970a
often 1	barley	chromosomes	Caldecott and Smith 1952
1 or 2	barley	M_2 mutations	Jacobsen 1966
2	barley	mutations (pollen)	Eriksson 1965
1, 2, more	barley	calculated	Monti 1966
1–4	barley	calculated	Gaul 1961, 1963a
1–7,3	barley	calculated	Lindgren et al. 1970
3–5	maize	chromosomes	Randolph 1950
5 or 6	maize	calculated, cells seen in the apex	Steffensen 1968
7–8	maize	sterility	Anderson et al. 1949
1–3	phalaris	M_2 mutations, chromosomes	Prasad and Godward 1969
1–10	phalaris	calculated	Prasad and Godward 1969
5 or 6	rice	calculated	Osone 1963
1–4	wheat	calculated	D'Amato 1962

chromosome number, chromosome aberrations or pollen abnormalities. It can also be calculated or estimated from the segregation ratios (Gaul 1960, 1961, Weiling 1962). The results are summarized in Table 4-1 which shows that the number of sectors is usually small and that high numbers occur mainly when they have been calculated or directly deduced from sector size. Maize seems to be an exception but the chromosome aberrations may not have been independent and they are often not stable, the sterility may result from these aberrations and may have been influenced by other layers.

Only one sector will be found when chimerism is unstable, due to the structure and development in the apex itself or due to disturbed growth as a result of the treatment. Two or three sectors, and apical initial cells, seems anatomically reasonable, while four may occur in a large stable apex.

The sectors often seem slightly twisted (Balkema 1970, Kaukis and Reitz 1955, Michaelis 1967, Steffensen 1968).

The deeper (L_3 or C) tissue of the stem (Dermen 1945) and the root may have more sectors or initial cells. This was suggested after anatomic observations of the root (Clowes 1950), yet chimeras may indicate 2 (Němec 1962b), 3 (Brumfield 1943a, Rickard 1952 see Clowes 1961), 1-6 (Davidson 1959), 8 (Nawaschin 1926) or 16 initials (O'Dell and Foard 1968).

Often fewer initials are inferred for side shoots (Monti 1965a,b, Monti and Scarascia-Mugnozza 1964) because there are fewer sectors (Gladstones 1968) as side shoots are less chimeric. Here the number of initials at the moment of treatment should not be confused with the number of side shoot initials which is probably the same as for the main stem (Michaelis 1967).

4.4.4 *Sectors and the origin of leaves and buds*

Studies on induced chimeras led to a new theory on the origin and development of the leaf. According to this theory the leaf originates from several cell files, next to each other, which fan out to form the leaf blade (Balkema 1965 unpublished, Bartels 1960, Dulieu 1967c, 1968, Dulieu and Bugnon 1966, Dulieu et al. 1967) (Fig. 2-2). This corresponds to observations on the primordia of compound leaves (Weberling 1956, Williams 1970). The vascular bundles develop parallel to the cell files except when they develop late (Bugnon et al. 1969, Guédès 1969). In compound leaves the top leaflet seems to include the derivatives of almost half the cell files. This was calculated from the frequency of chimerism between various points of observation on leaves,

chimeric for polyploidy in the epidermis (Balkema 1965 unpublished). Chimeric leaves are usually half mutated (4.2.3), sometimes only the top is mutated (Schwanitz 1955) or the sector includes half the leaf at the base, but more (or less) at the top (Gröber 1962, Michaelis 1967). With regard to their chimerism, axillary buds usually resemble the top of the leaf more closely than the base (Michaelis 1967). The mutation may influence the shape of the chimeric leaf (Michaelis 1967). Stipules often retain the sector longer than the leaves themselves (Dulieu 1969).

In monocotyledons leaves originate in the same way though not the whole base is represented in the blade (Turlier 1968). This is confirmed by chimerism as seen in *Tradescantia* (Thielke 1955). The stripes in the centre appear narrower than those at the sides (Ichikawa and Ikushima 1967) showing that here too the centre includes relatively more cell files. This can be explained if the centre originates first and near to the apex, the rest later, by secondary extension, further from the apex. If this is the correct explanation, leaves with asymmetric lateral extension (Catesson 1953) should have narrow stripes or sectors on one side (the original centre) instead of in the centre.

Axillary buds usually contain the same mutation as the leaf and often are also chimeric (Howard 1961b, 1965). Correlation for chimerism or mutations between different leaves or side shoots reflects the phyllotaxis of the plant (Bain and Dermen 1944, Bartels 1960, Dermen 1945, Dulieu 1970, Howard 1965, Osone 1963). Yet the leaf primordia (Michaelis 1967) and helices, develop independent of the cell lineages.

The length of mutated sectors often seems to be correlated to phyllotaxis when it is expressed in number of nodes or plastochrons (Dulieu 1969, 1970). This may be due to apex size relative to leaf size. Long sectors have also been found in plants with decussate phyllotaxis (Michaelis 1967) probably because here too the plastochron does not alter the symmetry or the balance in the apex.

In *Vitis* chimerism has been used as an argument against sympodial growth (Breider 1953, Reichard 1955). Sympodial growth implies that an axillary shoot takes the function of main shoot when the latter forms an inflorescence or a tendril. It follows that chimerism, being lost by branching, cannot occur as regular sectors. Sectors are however found in *Vitis* but they do not reflect the phyllotaxis (Breider 1953). Probably the axillary shoot originates so near to the (original) apex that chimerism is retained. In the tomato, which grows sympodially chimerism is rapidly lost, a clear discontinuity being found between the original apex and subsequent apices (Hildering and Verkerk 1964). The last branching in a bunch of grapes appears, from the pattern of chimerism on the berries, to be dichotomous (Breider 1953).

Adventitious buds develop from differentiated tissue that dedifferentiates, and will therefore derive from only a few, or only one cell (Broertjes 1969a). When they derive from more than one cell they may be chimeric when they develop on periclinally chimeric leaves (Clowes and Juniper 1968), periclinally chimeric stems (Stewart and Dermen 1970b) or chimeric roots (Robinson and Darrow 1929).

4.4.5 *Sectors and the ontogeny of the plant*

Usually seed is treated to induce mutations. The shoot meristem here consists of a few central cells which will eventually become apical initial cells, and a number of cells which may form leaf or bud initials for the first leaves and buds of the stem. A few leaf primordia may even be present in the seed. A mutation will have little effect (on chimerism) if it occurs in a cell of a leaf primordium and much effect if it occurs in one of the apical initial cells.

After seed treatment the first few leaves often have (visible) mutated spots or thin stripes, while later leaves show larger sectors or fewer but wider stripes (Blixt and Gelin 1964, Blixt et al. 1964, Dulieu 1965, 1967a, 1969, 1970, Nishiyama et al. 1966, Randolph 1950, Stein and Steffensen 1959). The position of the first striped leaves may vary with the pretreatment (Nishiyama et al. 1966). The first sectors will be narrow and short extending over a few leaves only (Bain and Dermen 1944, Dulieu 1969, 1970, Michaelis 1967, Stewart and Dermen 1970a). Later they will be wider as they originate in the apical initials. At this stage no new sectors appear but sectors may disappear by shifts in the apical initials.

This stage is reached later in plants with a high phyllotaxis (Dermen 1945, Dulieu 1969, 1970) probably because in these plants more primordia are formed before the cells from the circumference are used up and replaced by daughter cells from the apical initials. Two sectors (at the base) may join to form one sector higher in the plant (Dulieu 1969).

Changes in sector size, and appearance or disappearance of sectors, usually occur abruptly at a leaf insertion, ending on the lower surface or starting on the upper surface of the leaf (Dulieu 1967a). Sometimes a new sector starts just above the leaf but includes the axillary bud (Michaelis 1967). The abrupt ending, change or beginning of a sector which involves many cells when it is observed, must have taken place in one cell which must at first have divided mainly to the sides. This is probably what happens when a leaf primordium originates in the anneau initial. The stipules, placed vertically above each other, retain the "sector" longer than the leaves (Dulieu 1969). This may also reflect the

activity in the anneau initial as fewer cells are probably required for the stipules than for the leaves so that the supply of mutated cells lasts longer. Chimerism often disappears abruptly after dormancy (Bain and Dermen 1944), at the base of the inflorescence (Michaelis 1967), or after a shift within the apex (Dulieu 1967a), which all reflect the activity of the anneau initial.

The first leaves show more and smaller sectors than later leaves and these sectors are usually independent from those in later leaves. Similarly the first side shoots usually show more chimerism and more mutations (Frydenberg and Jacobsen 1966, Gaul 1959, 1961, Osone 1963) and the mutations are independent from those found in later shoots (tillers) (Blixt et al. 1958, Jacobsen 1966, Nishjmura and Kurakami 1952, Osone 1963, Stadler 1930). The observed independence may be affected by the dose (Blixt et al. 1960), probably because other branches (e.g. later shoots only) develop after a higher dose.

Proembryo treatment gives larger sectors (Mericle and Mericle 1961, 1962, Michaelis 1967), because the derivatives of the apical initials form most of the plant, and, in very early treatments, the initials themselves may all derive from the same cell.

It has been suggested that spontaneous mutations at an incompatibility locus could easily be scored by the observed seed set, and give information on the developmental stage at which the mutations occur (Lewis 1948).

Size and number of induced sectors have been used to study floral development. For this purpose plants were treated at different intervals after floral induction (Gupta and Samata 1967). Similar studies have been done with sectors on petals, stamens and stamenhairs (Mericle and Mericle 1967b, 1969a, Nayar and Sparrow 1967). The sector size was found to depend on the stage treated, the age of the inflorescence, the genetic background and on the position of the sector on the petal (Mericle and Mericle 1967b, 1969a). In heterozygous leaves sector size was also dependent on the mutagen used (Fujii 1965a, Röbbelen 1969).

The size of mutated sectors in petals has also been used to deduce cell number at the time of treatment (Buiatti et al. 1964, Buiatti and Ragazzini 1965), but the position of the sectors which was not considered, possibly influenced the results.

4.5 COMPLICATIONS

Complications may occur due to mixed sexual and asexual reproduction (Julén 1954), mutable genes (Brink et al. 1968) and the plastids

(Anderson 1923, Correns 1922, Demerec 1927, Karper 1934, Michaelis 1957, Potrykus 1970, Stroup 1970). Often only one type of plastid develops in a cell while the other degenerates and is lost (Burk et al. 1964, Michaelis 1957). Variegation is found in pollen cultures showing that pollen does have plastids but usually cannot transmit them through fertilization (Nilsson-Tillgren and von Wettstein-Knowles 1970). When both parents contribute plastids to the next generation only one type of plastid remains, apparently the first type to divide, and variegation only occurs when both types divide simultaneously (Tilney-Basett 1970). Under certain circumstances genetically white tissue can develop normal plastids (Rick et al. 1959) or genetically normal tissue may give only white plantlets from tissue cultures (Gamborg et al. 1970). Genes giving a variegated phenotype add another complication (Rédei 1965b, 1967a,b,c).

Spontaneous reverse mutations (Weiling and Gottschalk 1961) and delayed mutation (D'Amato 1964, Eriksson 1965, Frydenberg and Jacobsen 1966) have been suggested as extra complications. When they occur late in the development of the plant they may indeed give rise to an unexpected extra sector.

Embryonic lethality which may be maternally determined (van der Veen 1967a,b) is also found in sectors (Mesken and van der Veen 1968) which need not be in the same layer as the mutation studied (L_2). Sectors in different layers are not necessarily parallel (Åkerman 1927) so that other layers may distort the results seen in the L_2.

Sterility also occurs in sectors (Burdick 1959, van der Veen and Gerlach 1965), is probably also partly maternal and may be in another layer giving the same effect.

Different branches of a plant may vary in their fertility, partly due to their position on the plant. This is found in untreated plants and may influence observations on chimerism in treated plants (Iqbal Khan and Doll 1968, Osone 1963).

In summing up

Chimerism usually occurs as clear layers and/or sectors because the cell walls form a common framework which keeps cell lineages together. Complications may occur due to the plastids, chromosome instabilities and growth irregularities, or when chimerism is studied soon after or close to the point of treatment so that a pattern has not yet established itself.

The structure and development of the plant help to understand chimerism, similarly, chimerism may help to understand the plant and has been used to identify the layers or initial cells and to determine the

origin of various organs a.o. the leaf. The number of apical initial cells of a stem determines the number of sectors that can be found. The reported number varies and depends on the method used to determine it, but the actual number probably lies between 2 and 4. Branching affects the average number of sectors found in the branch (chimerism decreases), though the number of apical initial cells may be assumed to be the same in branches as in the main stem.

4.6 ARABIDOPSIS AND SUNFLOWER

4.6.1 *Arabidopsis*

Sectors have been studied in the main inflorescence and were found to have an average size of 25% (van der Veen and Gerlach 1965) to 30% (Müller 1963b, 1965a). They are correlated with the phyllotaxis but show a slight twist (Balkema 1970). Chimeric fruits have been found (van der Veen and Gerlach 1965). M_2 populations showed segregation ratios deviating from 25%, suggesting 2 or 3 sectors (Jacobs 1969, Langridge 1958b, McKelvie 1961), and also showed different mutations from the same plant suggesting 2 or 3 sectors (de Boelpaepe 1968).

Visible sectors may be found on the 7th or higher leaves of heterozygous plants (Fujii 1964, 1965a) which is in agreement with results from other plants as the character studied (hairlessness) cannot be observed in the "small sector" stage. Usually the sector size on the leaf is 1/2 or 1/4 of the leaf area but this depends on the mutagen used.

Chlorophyll sectors, visible in the M_1 plants, are usually due to maternally inherited factors. Plastid mutations may give variegation but cytoplasmic factors or directed division usually causes plastid sorting out to take place faster than expected (with 30 plastids) and results in clear sectors. In plants with a genic chlorophyll deficiency more visible sectors are found. This may indicate that mutated plastids have more opportunity to form sectors in this case (Röbbelen 1962b, 1963b, 1965c).

Genetically caused variegation has been found and can be modified by environmental conditions. The white sectors appear at random but subsequently form continuous cell lineages. The progeny from white and green sectors is the same (Rédei 1965b, 1967a,b,c).

Embryonic lethality (Mesken and van der Veen 1968) and sterility (van der Veen and Gerlach 1965) often occur in sectors. These may be in another layer when they are of maternal origin, and will then influence the segregation ratios and the detection of mutations in the L_2.

This may lead to an underestimation of sector size or an overestimation of sector number.

4.6.2 *The sunflower*

Sectorial chimerism has been observed in the flower a.o. after colchicine treatment (Rybin 1939). Spontaneous polyploidy giving natural chimerism has not been found in *Helianthus tuberosum* and possibly does not occur in other composites either (Partanen 1959b).

4.7 EXPERIMENTAL PART

4.7.1 *Chimeric structure of the plant*

Direct observation of visible chimerism (Plate III B) gives little information as this type of chimerism is relatively rare, usually unstable and its origin (genetic, plastid, L_1, L_2 or L_3) uncertain. The position of chimeric leaves, the size of the mutant sector and the relationship with the axillary bud were recorded for a number of plants which derived from various treatments. There were few visibly chimeric plants per treatment and no clear differences between the treatments so that the results were pooled (Table 4-2). These show that sectors are most frequent on leaf 7 and 8. This may be partly due to better detection on these (larger) leaves. These leaves are probably the first leaves formed wholly by derivatives from the apex as leaf 3 and 4 are predetermined in the embryo apex (Fig. 2-7), and 5 and 6 are probably partly predetermined (the back or underside of the leaf forming from cells already present in the embryo apex during the treatment).

The frequent correspondence (for chimerism) between the leaf and its axillary bud (Table 4-2) indicates that the bud meristem is almost the same size (chimerically) as the leaf primordium. This agrees with morphological observations on the axillary buds (Fig. 2-8). Usually half the leaf was mutated but sometimes more (or less), or only the middle of the leaf was mutated. This agrees with the quoted observations on the origin of the leaf (4.4.2).

These sectors seldom continued into the inflorescence.

Scoring pollen also allowed very direct observation of (ploidy) chimerism at various levels, e.g. in arabidopsis chimerism could be observed within anthers (Plate II F), within flowers (Table 4-3, Plate II E) within inflorescences (Table 4-4, Fig. 4-1) within side shoots (Fig. 4-2

Table 4–2. Visible chimerism in leaves and axillary buds (arabidopsis)

	position of the leaf (bottom to top)						
leaf	4	5	6	7	8	9	10
chimeric n	1	3	3	9	11	5	2
mutated area %	50	40	30	35	36	25	50
	mutated area (% of leaf area)						
axillary bud	0	25	50	75	centre (25)		Σ
normal n		3	2				5
chimeric n	1	8	8	2	3		22

n = number, % = % of each type.

BC) and within the plant (Fig. 4-2 AD, Plate III A). A disadvantage of colchicine, compared to EMS, is that usually only two tissue types can be distinguished.

Results show that the mean sector size in flowers is usually 50% and that flowers with "missing" anthers seem to have smaller tetraploid sectors (Table 4-3). The "missing" anthers may be absent or degenerated and apparently this happens more frequently to tetraploid anthers. Whether the position of the stamens in the flower influences sector size is not clear. Three adjacent flowers usually suffice to detect the presence of (ploidy) chimerism in an inflorescence (Fig. 4-1,4-2).

Table 4–3. Frequency of different sizes of tetraploid sectors in chimeric flowers scored per stamen (arabidopsis, Exp. III)

sector size	tetraploid vs diploid			tetraploid vs octoploid	
	6s	5s	4s	6s	5s
½	4	1			
1	5	2			
1½	3		1	1	
2	3	4	2		
2½		1	1		
3	9	3		2	1
3½	6				
4	4	1		2	
4½	1			1	
5	3				
5½	2			1	

6s, 5s, 4s = flowers with 6, 5, 4 stamens.

Short sectors on the inflorescence, which affect only a few flowers usually appear very narrow when drawn in a topography. This may either be an illusion caused by the drawing itself or be real if they are the end of a (3rd) sector. Long sectors which last more than 40 flowers, or till the end of the inflorescence, are more easily delimited in the drawings. They can be seen to include approximately half the circumference (Fig. 4-1, 4-2) and the average sector size is also approximately 50% (Table 4-4). The degree of within-flower chimerism shows that only flowers on the sector borders are chimeric (2 out of 8 with a 3/8 phyllotaxis).

Table 4–4. Frequency of different flower (fruit) types in the chimeric section of inflorescences (arabidopsis)*

type	n	%	type	n	%	type**	n	%
T	50	32	0	15	41	M	10	39
C	38	24	C	7	19	C	5	19
D	68	44	T	15	41	N	11	42

C = chimeric, O, T, D = octo-, tetra-, diploid flowers.
M, C, N = mutated, chimeric and normal fruits.
n = number of flowers (fruits), % = % of each type.
* last 4 flowers (fruits) excluded.
** from one chimeric inflorescence, M_2 and M_3 scored for chlorophyll mutations.

The polyploid tissue is seen to occur in a continuous sector on the topography drawn from the plant, but the sector seems clearer when a theoretical topography is drawn from the phyllotaxis 3/8 (Fig. 4-1, 4-2). This is not surprising since phyllotaxis itself is very stable and originates very early and near to the summit of the apex (2.1.1). Later differential growth may disturb this symmetry so that the topography no longer shows the exact phyllotactic regularity, yet the cell lineages enter the floral primordia at their origin i.e. at the origin of phyllotaxis.

As with very short sectors, the ends of the sectors may be difficult to delimit. It is clear however from observations that the sectors do not end abruptly but, rather, gradually (Fig. 4-2D). This is in contrast to chimerism in leaves, which usually ends abruptly at leaf insertion. This difference may be explained from the difference between foliar and floral primordia. The former originates as a small primordium on the still expanding circumference of the apex, and shows secondary lateral extension. The latter originates as a much larger rounded primordium which probably includes all the genetic material, in the proportion in which it will be scored (Fig. 2-7).

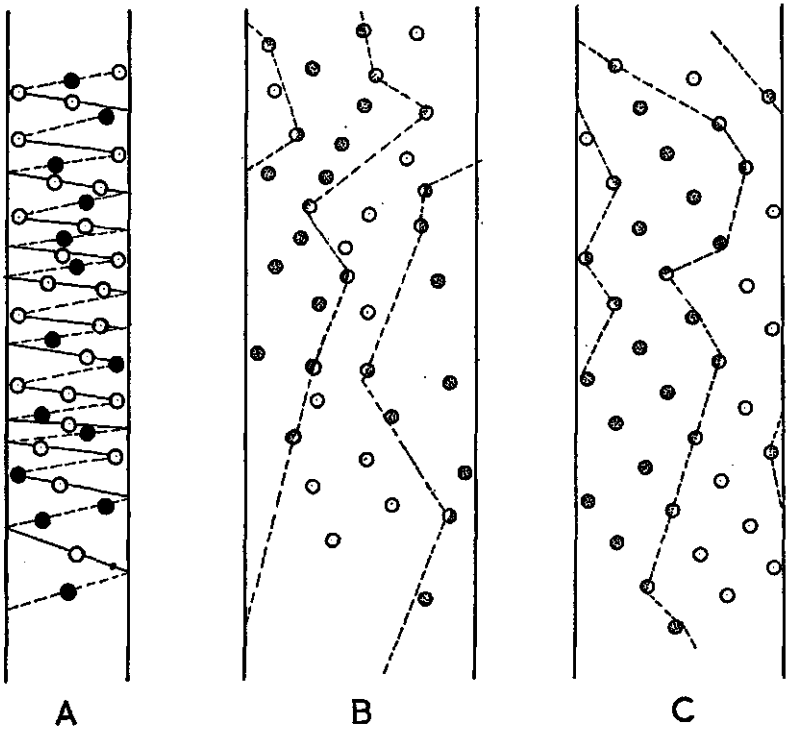


Fig. 4–1. Vertical topography of a (polyploidy) chimeric inflorescence (arabidopsis).
A. The positions of the flowers (along a spiral) on the inflorescence. ○ = front, ● = backside of stem.
B. The positions of the flowers drawn in one plane, the vertical topography, from A. ○ = diploid, ◍ tetraploid, ◑ chimeric.
C. The (theoretical) positions of the flowers drawn according to a 3/8 phyllotaxis (probably the top had a lower phyllotaxis). ○, ◍, ◑ as for B.

On the vertical topography the sectors show a slight twist, even when the phyllotaxis is used (Fig. 4-1).

This twist is still more pronounced when the topography is projected on a horizontal plane, but seems to vary for different plants, sometimes hardly occurring (Fig. 4-2). It is also seen in the stem below the inflorescence (Fig. 4-3). The reason for this twist is unknown, but probably the factor which causes the, before mentioned (2.6.3, Plate I C), torsion in the rosette may also affect growth in the inflorescence. Together with the phyllotaxis this may cause the apparent twisting of the sector. To study this, sectors from plants grown under controlled con-

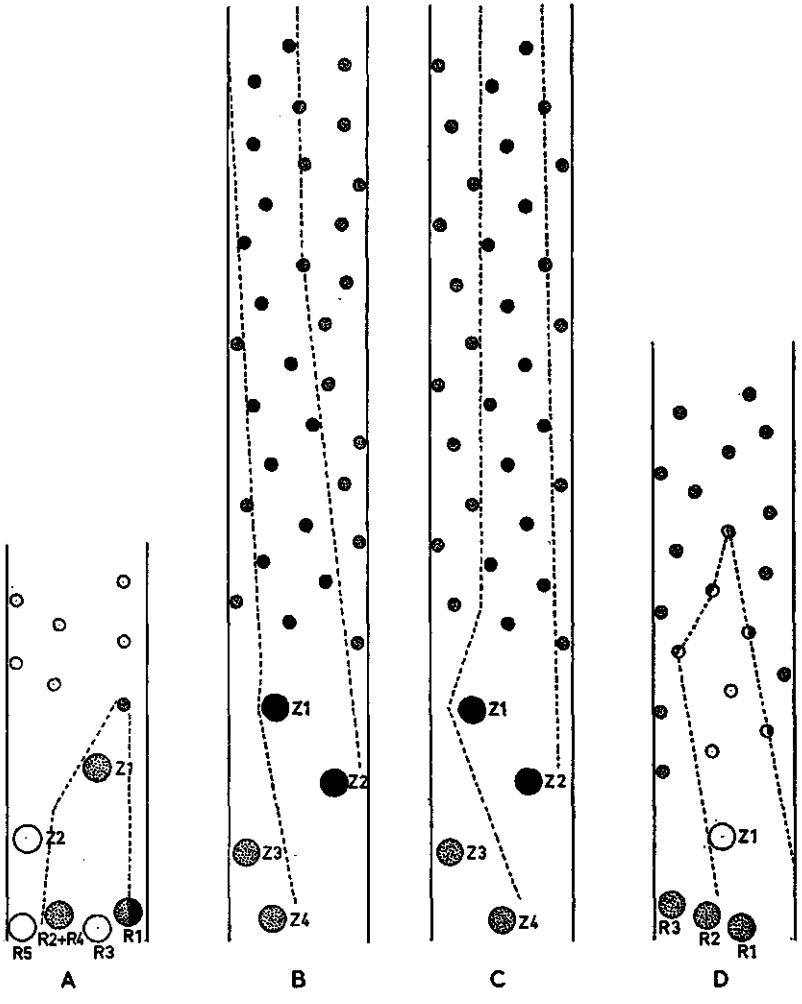


Fig. 4–2. Vertical topographies of arabidopsis

A. Topography of the main stem of a ploidy chimeric plant. Z_1–Z_2 = cauline, R_1–R_5 = rosette side shoots, ○ = diploid, ◍ = tetraploid, ● = octoploid, ◍, ● = chimeric.

B. Topography of the chimeric rosette shoot in A. Z_1–Z_4 = (secondary) side shoots. ◍, , ● ◍ as for A.

C. Topography according to a 3/8 phyllotaxis for the same shoot as B.

D. Topography of a chimeric inflorescence showing the end (disappearance) of a sector. ○, ◍, ◑ as for A.

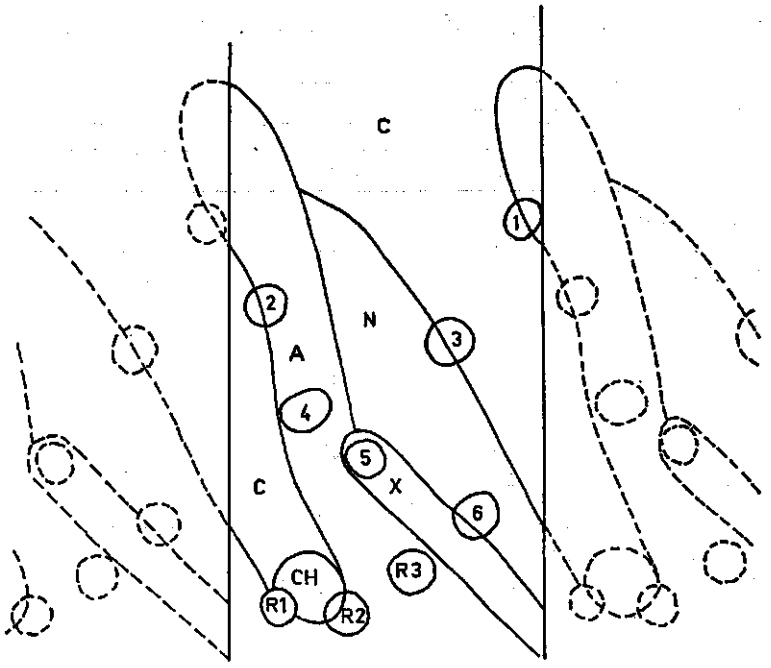


Fig. 4-3 Vertical topography of a chimeric main stem of arabidopsis after EMS seed-treatment.
1–6 = cauline, R1–R3 = rosette side shoots.
A = albina, X = xantha, C = chlorina, CH = half sterile chlorina, N = no mutation found.

ditions with upright inflorescences would have to be observed and compared.

Chimeric side shoots resemble the main shoot but are less frequent (Table 4-5). The sectors may be small and disappear very soon, or long and clearly delimited (Fig. 4-2) ending in the same way as in the main inflorescence. Secondary, tertiary and higher order side shoots give the same picture. Sometimes a secondary side shoot is formed in the same axil as the primary side shoot. Even when the primary side shoot is chimeric the secondary shoot is not chimeric demonstrating that, though in the same axil, it derives from a much smaller meristem.

In the sunflower chimeric flowers had sectors ranging from less than 1/4, to more than 3/4 of the circumference (Table 4-6). The sectors usually continue to the centre of the flower suggesting that the flower originates centrally on the apex (Fig. 4-4).

Table 4–5. Chimerism (in %) after different colchicine treatments (arabidopsis, Exp. I and III)

position*	treatment		
	I seedling n = 22	III ripe seed n = 46	III ripe seed + EMS n = 10
main inflorescence			
flower 5–8	35	22	0
flower 1–4	72	85	100
side shoot			
first (top)	29	36	0
second	27	30	0
rosette shoot			
first (top)	39	20	0
second	14	7	0
third		12	

n = number of polyploid plants.
* chimerism between or within 4 adjacent flowers per position.

Chimerism along the stem can be deduced from the presence or absence of polyploidy in flowers on side shoots and secondary side shoots. Often the flower at the end of a stem shows no chimerism while flowers

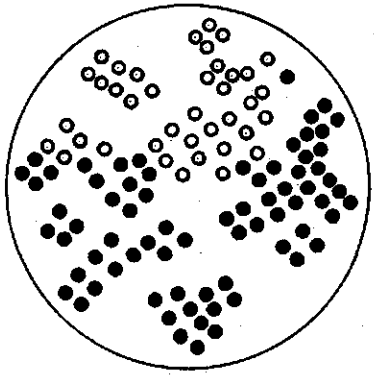


Fig. 4–4. Topography of a chimeric flower (sunflower). Only the scored florets are drawn.
○ = diploid, ● = tetraploid floret.

Table 4–6. Frequency of various sizes of the tetraploid sector in chimeric flowers on shoots of different orders (sunflower, Exp. I)

sector size	greenhouse			field				total		
	H	Z	ZZ	H	Z	ZZ	ZZZ	H	Z	ZZ
1/8–1/4				5	7	3		5	7	3
1/4	2	1		1	8	9	1	3	9	10
1/2	1	8	2	3	15	7	1	4	23	10
3/4		2			7	2			9	2
3/4–7/8					3				3	

H = main flower, Z, ZZ, ZZZ = flowers on primary, secondary and tertiary axillary shoots.

from axils below it show that chimerism is present in the stem (Fig. 4-5). At the base of the main stem this might still be a direct effect of the (seedling) treatment but higher on the main stem or on side shoots it has to be explained by loss of chimerism below the main flower on that stem or shoot. In a side shoot this may be partly due to chimerism occurring in the axillary meristem but not in the apical initial cells of that meristem so that only the base of that axillary shoot will be chimeric. This will be further discussed in chapter 5.

The sectors observed on the stem are more difficult to delimit than in the inflorescence of arabidopsis because the observation is less direct. (Similar problems are encountered in studying the stem of arabidopsis below the inflorescence, or even in the rosette, after colchicine treat-

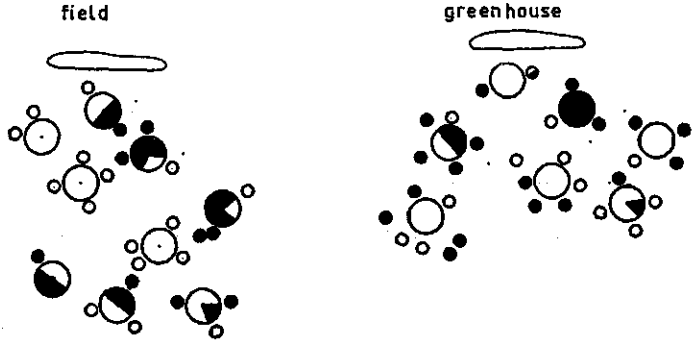


Fig. 4–5. Vertical topographies of chimeric sunflower plants grown in the field or in the greenhouse. Main flower and side shoot first order. Side shoots second order arranged, as observed, around the side shoot first order. ○ = diploid, ● = tetraploid, ◐ = chimeric (end) flower.

ment). Yet observations seem to suggest twisted sectors for the sunflower too (Fig. 4-5).

EMS induces many different chlorophyll and morphological mutations and therefore enables a better distinction between sectors. The scoring method, usually M_2 embryos or seedlings, seldom enables detection of chimerism within flowers (fruits), and gives less clearly delimited sectors in the inflorescences. These are however basically similar to those for polyploidy and can sometimes, when sterility or lethality is involved, be clearly seen on the M_2 plant.

The main advantage of EMS lies, however, in its wider mutation spectrum. Large M_2 populations per inflorescence allow a clear distinction between the inflorescences of different side shoots so that the number of distinct sectors on the stem and their position can be more readily determined. For this purpose, seed was harvested per inflorescence (from all the inflorescences of mutated plants, Exp. VI). 100 or more seeds per inflorescence could be sown and the seedlings scored. Mutants from different inflorescences could be compared at various (seedling) stages to determine whether they represented different mutations.

It was possible in this way to identify maximally 4 clearly different sectors in the main stem below the inflorescence (Fig. 4-3), but generally, only one or two in the inflorescence itself. The exact point of disappearance of the other sector(s) could not be determined. Sterility often occurs in sectors which may lie in a layer other than the L_2. Sometimes phenotypically the same chlorophyll mutant appears in two adjacent "sectors", of which one is fertile, the other semisterile. This may be caused by an underlying sterility sector partly overlapping a single chlorophyll sector. Other explanations are: delayed mutation and repair, or mutations at different chromosome levels in the same cell, all leading to differences between daughter cells from one cell, for some but not all mutations. If this occurs in an apical initial cell the so formed different sectors will be vertically above each other as is sometimes found (Fig. 4-3).

The rosette shoots usually show the same mutations as the stem, but sometimes also different mutations, e.g. when the stem had so few side shoots that not all the mutations present (in the stem) were detected. However later rosette shoots (from the first leaves) may have mutations which were induced in cells predetermined to form buds.

This occurs less often than expected from comparison with the position of visibly chimeric leaves. Buds develop from meristems which originate nearer to the apex than the leaves and may therefore originate

from derivatives of the apical initial cells when the leaves still develop from (primary) meristematic tissue of the embryo.

Chimerism was studied in the rosettes (Exp. II). After colchicine treatment of the seeds the rosette shoots were induced to develop by decapitating the plant (when the first flower opened). In this way rosettes could be induced to form up to 20 apparently independent rosette shoots. On closer examination, under a disection microscope, it appeared that they belong to maximally six groups which derive, by branching, from six primary rosette shoots. Macroscopically adjacent shoots do not necessarily derive from the same primary shoot. Examination of the origin of each shoot usually revealed clear sectors for the mutated (polyploid) tissue (Fig. 5-2).

Combined treatment allows simultaneous use of both methods (pollen and embryos) of scoring and, theoretically, still better differentiation. However, with ripe seed, combined treatment was found to influence chimerism (Table 4-5, see 5.5.3) and, with green seed, to affect plant growth (Table 3-1). The embryotest did reveal that the diploid "sector" often consisted of genetically different sectors.

Sometimes the same mutation seemed to occur both in the diploid and in the tetraploid sector but to confirm this more observations, and progeny tests would be needed.

4.7.2 *Average chimerism*

An index for average chimerism within a group of plants is necessary for comparisons of chimerism after different treatments or in different parts of the plants (Exp. IV and V). As 3 flowers suffice to detect chimerism in an inflorescence, three fruits for the embryotest should give a good indication. The general practice is, however, to score two fruits a.o. because they can be scored at the same time. In this way a number of mutations may escape detection as was observed when plants were scored for a second time.

Two adjacent fruits may be both mutated, one may be mutated, or both may be normal and, in that case, the whole plant may be normal. When only one appears to be normal this may be due to chimerism within the fruit or to chance so that, with a low segregation and small progeny size, the mutation was not detected; or the fruit may be normal in a normal sector on a chimerical inflorescence. Normal as used here describes the phenotype (no segregation seen) and not the genotype (escape from detection). The ratio between chimeric (1 fruit normal)

and fully mutated (both fruits segregating) plants gives an impression of the degree of chimerism.

The mutation frequency can also be used. The average M_2 segregation of a specific M_1 depends on mutation rate, fertility, recessive deficit and chimerism. It deviates from 25% and can be only approximately estimated from the M_3. The best estimate is probably obtained from the M_2 itself. This can be done by calculating the segregation for only the mutated fruits (pooled). The difference between this ratio and the ratio calculated for the mutated plants (1 or 2 segregating fruits) will be due to chimerism.

The two indices used are then:

$$C = \frac{MO}{MM + MO} \times 100 \text{ and } \% C = \frac{\% Mf - \% Mp}{\% Mf} \times 200$$

where MO = chimeric plants, MM = fully (2 fruits) mutated plants, % Mf = segregation ratio in mutated fruits, % Mp = segregation ratio in mutated plants (2 fruits).

Generally the two indices correspond quite well though C will be more sensitive to population size. Comparison between the base and the top of inflorescences (Table 5-6) shows the expected decrease in chimerism. The indices however do not reach zero, probably due to chance escape which will cause an overestimation of chimerism. 100 % is seldom reached because two mutated sectors in a plant will cause an underestimation of chimerism. The indices do, however, allow an estimate of chimerism and comparison of chimerism after different treatments or in different parts of the plant (Fig. 4-7, Table 5-6).

4.7.3 *Vertical chimerism*

Vertical chimerism can be studied quantitatively by comparing mutation rate and chimerism for different inflorescences in a group of mutated plants. To do this an experiment (Exp. VI) was set up with three different seed treatments (dose 240 mMh EMS given in 1/2 hour, 2 × 1/4 hour separated by 23 1/2 hour water, and 24 hour), and two plant treatments (with, and without etiolation before 240 mMh EMS given in 1/2 hour). Half of each treatment was grown directly, the other half was vernalized (30 days, 3° C, dark).

2 fruits per inflorescence were sown on agar, one petridish per plant, and scored for chlorophyll, and morphological, early seedling mutations. Mutants that occurred together were classified as one mutation (sector) and those which were separately distributed, as different mutations (sectors). In this way 0-4 mutations (sectors) were found per plant,

besides the normal (non-segregating) sector(s). The fruits of mutated plants were scored for fertility in three classes viz. fertile (at least 10 seedlings), semifertile (1-9 seedlings) and sterile.

The inflorescences of the side shoots were numbered according to their position, from top to bottom, on the stem (Z1 – 7), and according to their size, which corresponds reasonably with their position from top to bottom, on the rosette (R1 – 6). Some plants developed slowly and had to be scored a week later. The experiment was repeated weekly for six weeks giving six sowing dates.

The results were analysed, partly with the help of a computer. First the effects of different variables were studied (Table 2-2, 3-7, 3-8). Most variables, e.g. treatment, position in the greenhouse, sowing date, vernalization, branching, and scoring date showed some relationship with each other and with the number of mutations found, and for some variables interactions seemed to exist, e.g. scoring date and vernalization on branching, so that the effects could not be separately assessed.

The plants in which mutations had been found were analysed for mutation frequency per inflorescence (100 × mutated fruits/scored fruits). As the number and distribution of side shoots (inflorescences) varied and possible differences between first and last, or top and lower, or stem and rosette shoots had to be considered, calculations were done separately for the stem and rosette side shoots numbered from the top downwards, or from the root upwards, and also for all side shoots together, numbered from the top down or from the root up. The number of mutations per fruit, was totalled for the various (inflorescence) positions, expressed as a percentage of all fruits at the same position and presented in a graph to give an impression of the average distribution of mutations over the different side shoots of the plants. Different combinations of treatments, sowing dates, branch numbers, scoring dates and numbers of mutations were compared for their distribution (of mutations).

Differences between the seed (or seedling) treatments were small. Sowing date and branch number showed some effect but no real qualitative differences.

Scoring dates gave real differences but the distinction between early and late had been subjective and the effect was probably confounded with the treatment effects and therefore early and late were pooled again. The number of mutations was found to affect qualitatively the distribution (of the mutations) so that plants with one, two, and more mutations were grouped separately.

Eventually six groups remained, viz. seed treatment (s) with (v), or without (o) vernalization having one (1) or two (2) mutations, and seed-

ling (plant) treatment (p) with (v), or without (o) vernalization having one (1) mutation. Groups with more mutations were small and will be ignored. The distribution of mutations over the plant was found to differ for these six groups (Fig. 4-6, 4-7, 4-8).

In the unvernalized seed-treatments, plants with two mutations showed a distribution (of the mutations) differing from that for plants with one mutation. As results were expressed as percentages the latter could be subtracted, per position, from the former thus revealing (Fig. 4-6) the (average) distribution (of the mutations) for the "second" mutation. Similar comparisons made separately for plants (HM) with a mutation in the main inflorescence and plants (HO) without a mutation in the main inflorescence showed that the "second" mutation (so(2-1)HM) resembled the "first" mutation (so1HO) (Fig. 4-6). Apparently the "sec-

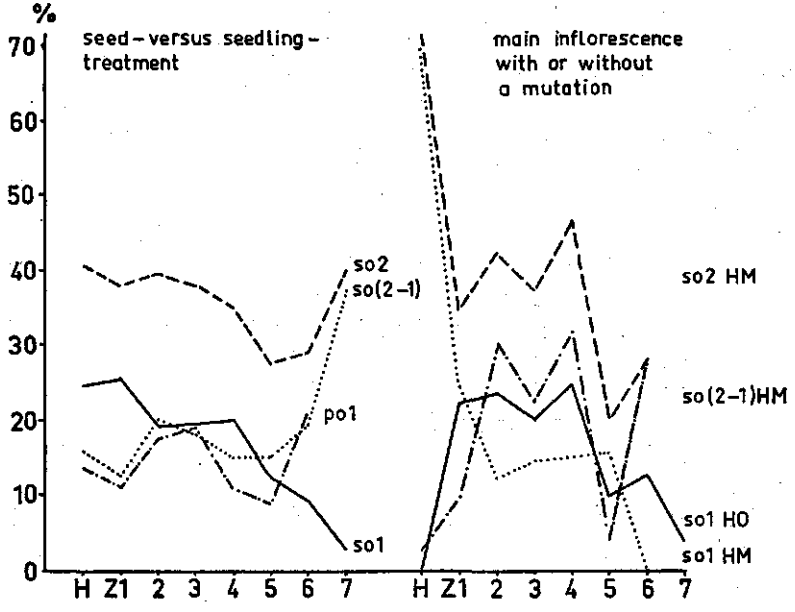


Fig. 4–6. The average distribution of mutations in arabidopsis (Exp. VI). H = main inflorescence, Z1–7 = side shoots from top to bottom. % = % of the fruits (2 per inflorescence) that segregated. so = seed treatment: 1, 2 and 2–1 = 1 mutation, 2 mutations and the difference i.e. the "second" mutation. po = seedling treatment. HM = with, HO = without a mutation in the main inflorescence. Maximum number of inflorescences (per position): so1 = 211, so2 = 120, po1 = 116, so1 HM = 77, so1 HO = 134, so2 HM = 65.

ond" mutation does not have the same chance to appear in the main inflorescence as the "first" mutation. This may be the reason for the differences in distribution (of the mutations). As mentioned up to 4 different sectors could be recognized in the stem but usually not more than 2 in the main inflorescence. The chance for both (in the inflorescence) to be mutated is apparently small. They probably derive from adjacent cells (in the seed) which are in different stages at the time of treatment.

When seed treatment (so1) is compared with seedling treatment (po1) their mutations seem to be differently distributed, but the "second" mutation resembles (in distribution) the mutation after seedling treatment (Fig. 4-6). In the case of seedling treatment the lowest side shoots cannot be included in the comparison as they may be directly affected by the treatment. Apparently certain cells exist which will reach the main inflorescence and one of which is more easily mutated during seed treatment but not easily mutated during seedling treatment; and other cells one of which mutates in the "second" place during seed treatment or (in the first place) during seedling treatment.

Vernalization slightly affects the (average) distribution (of the mutations) but the same similarity is found between the "first" mutation (when the main inflorescence is apparently normal) and the "second" mutation (when the main inflorescence is mutated); and between the "second" mutation and seedling treatment. Comparisons in which only fertile fruits were considered showed the same similarities. These similarities were also found when the inflorescences were numbered from the base upwards or separately for stem and rosette.

Chimerism within inflorescences (the two fruits differ) could be calculated per inflorescence expressed as a % and presented in graphs which show differences between the treatments (Fig. 4-7). Again the "second" mutation resembles seedling treatment. Vernalization affects mainly the "first" mutation.

Theoretically, observed chimerism depends on sector size and on the size of the part of the meristem relative to sector size from which the inflorescence of the side shoot develops. If sector size stays constant but the leaf primordium and the bud meristem develop further from the summit (retaining the same absolute size) their relative size will decrease and they will be less chimeric. This may be the reason for the decreased chimerism in the top side shoot after vernalization. Decreased chimerism in the main shoot may be due to faster loss of chimerism after vernalization (Table 5-6) (usually fruit 5 and 6, and not 1 and 2, were scored). However, the occurence of more chimerism for the "second" mutation remains unexplained.

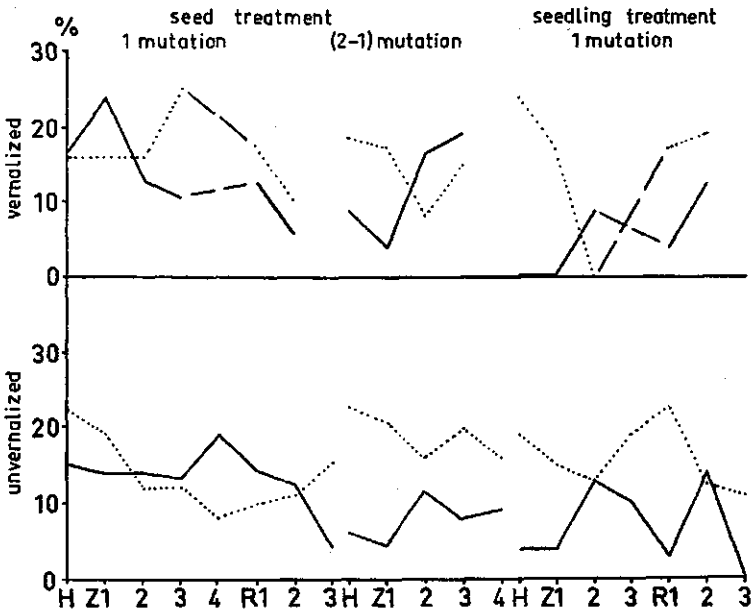


Fig. 4–7. Frequency (%) of chimeric and completely mutated inflorescences (arabidopsis, Exp. VI).
—— = completely mutated i.e. both fruits per inflorescence segregate for the same mutation.
···· = chimeric i.e. only one fruit segregates for the mutation under observation.
H = main, Z1–4 = cauline, R1–3 = rosette inflorescences (top to bottom). Maximum number of inflorescences per position: sv1 = 116, sv2 = 68, pv1 = 25, so1 = 207, so2 = 120, po1 = 116; where s = seed, p = seedling treatment; o = un–, v = vernalized; 1 = one, 2 = two different mutations (sectors).

The three main regions of the plant viz. main inflorescence (H), stem (Z) and rosette (R) were compared per mutation to find concordance or discordance for the different regions after different treatments. Two fruits were taken per region viz. 2 from the main inflorescence, 1 from each of the two top side shoots and 1 from each of the two top rosette shoots. In plants with more than one mutation the mutations were studied separately and then added together. Each region could be classified as mutated (M) or non-mutated (no segregation observed) (O), for the mutation under observation. In this way eight different combinations were possible. The frequency per combination was calculated and the results represented in such a way that all the plants with a muta-

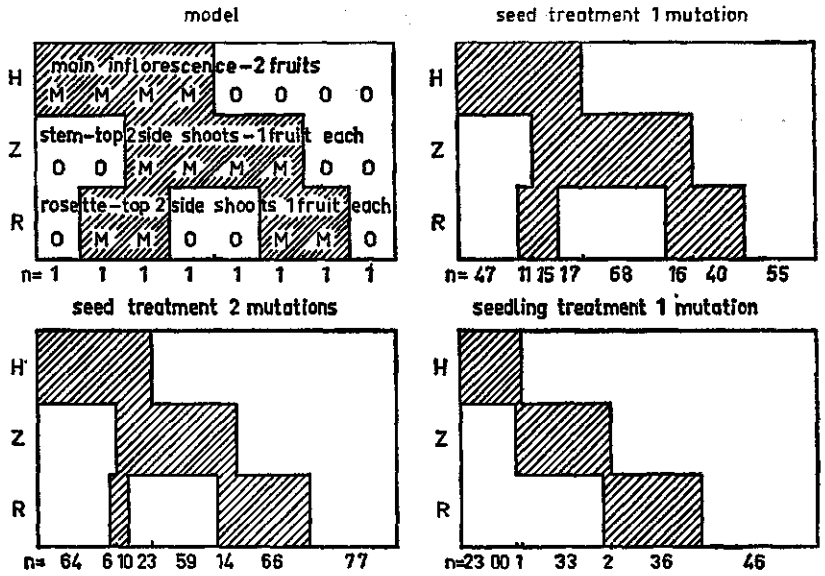


Fig. 4–8. Distribution of separate mutations (sectors) on the plant (arabidopsis, Exp. VI).
H, Z, R = the plant regions, /// = M = mutation found, O = not found in that region (see model).
H Z R = MOO, MOM ···· OOO = the 8 possible distributions (OOO = the sector under observation was not found in the fruits used for the comparison). n = number (mutated sectors) per distribution type.

tion in the main inflorescence are grouped together and all those with a mutation in the stem were grouped together (Fig. 4-8).

Here too, clear differences can be seen for the distribution, i.e. concordance and discordance, of mutations within plants after seed and after seedling treatment. Here too, plants with two mutations differ from those with one mutation and show a similarity with plants from seedling treatment though the frequencies for the "second" mutation were not calculated.

The "second" mutation cannot be phenotypically distinguished from the "first" and is therefore only a differently distributed mutation. Yet this seems to imply that mutations are not randomly induced in the apex and that the distribution is also dependent on the stage treated. This may be due to the cells and their mitotic stage, during the treatment. Two adjacent cells will usually not be in exactly the same mitotic stage and cells nearer the summit may differ from those further from the

summit, especially in seedlings where the beginning of a méristème d'attente may be present. Whether the other cells stay absolutely unmutated or give mutations of a different type that have to be scored by other techniques, cannot be said.

CHAPTER 5

DIPLONTIC SELECTION

5.1 SELECTION

"Selection is the non-random differential reproduction of genotypes" (Lerner 1958). Haplontic selection is selection at the gamete level, best known in the form of certation, i.e. pollen with different genotypes participates differentially in fertilization. Pollen with chromosome aberrations is often at a disadvantage (Gaul 1957). Selection may also occur in the female gametophyte (Rédei 1965a) or result from the genotype or cytoplasm of the female parent as with incompatibility.

Diplontic selection, or intrasomatic or intra-individual selection, is selection at the diploid or, preferably, somatic level during the ontogeny of the plant. It results from selective differences between two (or more) genotypes within one individual (plant) i.e. with chimerism. Usually it implies a selective advantage of normal over mutated cells (or tissue) and is used to explain differences between the expected and the observed mutation rate, the former being calculated from observations on other parts of the same (or other) plants.

5.1.1 *Differential viability*

For selection to take place differences in viability must exist. X-rays (Ehrenberg and Nybom 1954, Freisleben and Lein 1943) and also EMS (Dulieu 1965) and other mutagens often cause lethality in the treated material. Sometimes only a few cells will be affected. These may be observed microscopically (Lapins and Hough 1970), Némec 1962b, Stein and Steffensen 1959), but cell death is usually inferred from mutation data and plant lethality within a treatment (Drake 1969, Gaul 1957a, Gottschalk and Weiling 1960, Weiling and Gottschalk 1961). It often occurs directly or after a few cell divisions (von Wangenheim 1970) and may result from chromosome damage though such cells may continue dividing for a few cycles (Curtis 1966, von Wangenheim 1970).

Cell death may be due to toxicity of the mutagen and not to the genotype of the cell, but even when it is due to the changed genotype, at this early stage there has been no competition yet, and the cell dies through its own lethality. However, this may be included as a case of diplontic selection in the widest sense.

5.1.2 *Cell competition*

Competition is difficult to observe as most mutations cannot be identified at the cell level. Polyploidy and chromosome aberrations are exceptions, but cells with several chromosome defects have been seen to divide (von Wangenheim 1970). Division of abnormal cells, even when many chromosomes are missing, may be quite normal and is probably supported by surrounding normal cells (Koller 1947, Sachs 1952, Vaarama 1949). Such cells may, however, be unable to form complete tissues.

Dead cells have been observed in leaf primordia not yet present during the treatment, so that these cells must have been carried along by the surrounding active cells (Lapins and Hough 1970).

Gene mutations may have heterotic effects in the heterozygous condition (Röbbelen 1957b) in which case mutated cells might even have a selective advantage. After decapitation mixaploid roots form new mixaploid apices which demonstrates that the ploidy level caused no selective advantage for either cell type (Němec 1962a).

5.1.3 *Polyploidy*

Sometimes polyploidy seems to disappear in the root (Levan 1938). This may be due to somatic reduction after colchicine treatment but probably the initial cells or the cells in the quiescent centre have remained diploid so that new cells that are formed are diploid.

Roots have been observed to cast off polyploid cell groups when these occurred on the outside of the root. This may be a reaction to "foreign" tissue in which case diploid cells should also be cast off by mainly tetraploid roots. Mixaploid roots may fork or form a new lateral, often diploid, apex. Yet decapitation results in new mixaploid apices (Němec 1961, 1962a,b, 1963, 1965). These observations may indicate some diplontic selection.

In the shoot, apical cells may remain diploid during colchicine treatment, so that the developing plants seem to revert back to the diploid state (McGowan and Bishop 1953).

Sorghum is an exception, colchicine apparently induces diploidization of the whole apex which may subsequently revert to tetraploidy or, under certain circumstances, remain diploid (Chen and Ross 1963, 1965, Ross and Atkinson 1965, Simantel et al. 1963).

Progressive chromosome loss, observed in some polyploids (Bouharmont 1967a,b) may give the impression of diplontic selection in favour of the lower chromosome number, but usually results in aneuploids and often takes a few generations.

Diploid and tetraploid tissue have been observed to form relatively stable chimeras, both periclinal and sectorial (Bain and Dermen 1944). Later flowering, often found with tetraploidy, may cause a difference in ploidy between early and later tillers (Müntzing and Prakken 1941).

An interesting complication is the changed size of the tetraploid cells leading either to larger sectors or to fewer cells, *both* of which can be interpreted as diplontic selection, respectively for and against the tetraploid tissue.

5.1.4 *Chromosome aberrations*

Chromosome aberrations can, from an early stage, be followed in the developing plant. They can, however, be seen only in dividing cells and this may influence the results of observations, especially shortly after the treatment (Gaul 1957c). They often lead to cell death (5.1.1).

Observations on chromosome aberrations are usually done on roots (Gaul 1957c, van der Mey 1970). These have a quiescent centre, more resistent to X-rays, which starts dividing when the root is damaged forming a new less damaged population of root cells. Dividing cells scored directly after the treatment will then belong to a different cell population, with different sensitivity or mutation rate, from cells scored some time after the treatment. To determine diplontic selection a sample from the same population would have to be scored at a later stage but this is often impractical as the cells have then started differentiation.

In the shoot apex chromosome aberrations may be associated with sterility (Freisleben and Lein 1943, Kaplan 1951, 1953, Osone 1963) or weak plants (Gaul 1957a), but they are usually independent of the chlorophyli mutations (Gaul 1958, 1963a). Selection against chromosome aberrations is often mentioned (Ehrenberg and Nybom 1954, Kawai and Inoshita 1965, Marquardt 1949, Swaminathan 1961). Sometimes the aberrant chromosome itself is lost (McClintock 1932).

Diplontic selection was initially inferred from different mutation rates in different tillers in barley (Gaul 1957a), yet chromosome aberrations

were found to occur with the same frequency in plants with many or with few tillers (Sarvella et al. 1962). Some mutations scored in the pollen (Lindgren et al. 1970) or even in the M_2 (Röbbelen 1957b), are probably the result of chromosome aberrations. Here too no diplontic selection could be detected (Lindgren et al. 1970).

Chromosome aberrations, often have very deleterious effects yet they give no clear evidence for diplontic selection.

5.1.5 *Chlorophyll mutations*

Chlorophyll and flower colour mutations are sometimes visible in the M_1 but especially the former are often due to mutations in the plastids or cytoplasm and give special problems. Plastids often sort out faster than expected (Hagemann 1964, Röbbelen 1965c), though not necessarily through faster division of the normal plastids (Hagemann 1964). When mutations were induced in plants which already had mutated chlorophyll, more white sectors were found (in light green plants) (Röbbelen 1965c), because of less selection or because the already changed chlorophyll metabolism was more easily blocked by mutation. Normal sectors (revertants) on chlorophyll deficient plants were usually shorter than mutant sectors on normal plants (Röbbelen 1966b).

Chimera-studies using chlorophyll mutations showed that generally there was no selective advantage or disadvantage although the mutant tissue might be unable to exist on its own (Dulieu 1968, 1969, Michaelis 1967, Stewart and Dermen 1970a). In some cases the mutation did cause inferior growth giving asymmetric, chimeric leaves, in others it caused improved growth. A very weak mutant sector causes aberrant growth and eventually fasciation and forking of the stem during which process the mutant sector itself is lost (Michaelis 1967). This clearly is diplontic selection but the effects are also drastic, more drastic than any usually observed after mutagenic treatment.

Axillary buds of leaves chimeric for a "weak" sector often contain only normal tissue, the reverse occuring for "strong" sectors. It is not clear whether the buds are formed asymmetrically, secondary buds develop (Michaelis 1967), or the sector itself has changed just above the leaf (and under the bud) thus affecting only the bud. This is a case of diplontic selection of the type suggested by Gaul (1957a).

Sometimes sectors are found due to some genetic irregularity in the plant. These are often small and may occur as twin spots. It has been suggested that these are due to somatic crossing over (Hirono 1965, Hirono and Rédei 1963, 1965a,b, Menzel and Brown 1952). They are often associated with chromosome aberrations (Hirono 1965) and give

malformation of the flower or leaf (Menzel and Brown 1952). The spots stay small, probably due to their position. In twin spots there is often a difference in sector size between the components (and maybe single spots are twin spots one of which shows no phenotypic difference compared to the surrounding tissue or does not develop at all) suggesting differential growth due to genetic differences which could lead to diplontic selection if it occurred in a meristem.

5.1.6 *Gene mutations*

A problem arises when studying diplontic selection with gene mutations (or small chromosome aberrations which resemble gene mutations). With visible chromosome aberrations, polyploidy or plastid mutations a difference in viability may be expected to be related to the observed changes, but for gene mutations this relationship in less obvious, especially when the mutation is recessive and can only be seen in the homozygous recessive form in the M_2. Usually chlorophyll mutations, often in the seedling stage, are studied and the other cell lineages, having no chlorophyll mutations, are regarded as normal. Yet mutations may be found in these "normal" cell lineages when older seedlings are scored or other scoring techniques used, so that it is difficult to define which cells are normal and which are mutated after a treatment. Some mutated alleles cause heterosis in heterozygous plants and may therefore also induce heterotic effects in the mutated (heterozygous) cells. Interaction with surrounding cells or cell layers may give extra complications. Theoretically it should be possible to study the selective advantage or disadvantage of a specific well defined mutation in an equally well defined background.

5.2 SELECTION AND ONTOGENIC LOSS

The normal development of a plant follows a relatively fixed pattern. Cell death already causes complications; where are the dead cells left? and how does this affect the cells in contact with them? Dead cells may be carried along by the living cells and be found in primordia which were only formed after the treatment (Lapins and Hough 1970), or after wounding (Loiseau 1962, Soma and Ball 1964). They cannot simply be "eliminated" or "left behind". "Left behind" is an interesting expression suggesting that the cells stay somewhere at the base of the plant while they will in reality be found where their surrounding cells eventually go. A mutated cell that is "left behind" in the méristème d'attente may

remain there till the floral stage of the plant and then be included in a flower primordium thus reaching the M_2.

5.2.1 *Normal development of chimerism*

Mutagenic treatment of the seed may cause direct or indirect cell death precocious cell differentiation, mutations, or have no effect. In the root many cells already exist only a few of which, in the apical meristem, eventually form the adult root. A mutation in one of these will give chimerism with the possibility of diplontic selection.

In the epicotyl the situation is more complicated. There are again a few cells, the apical initial cells, which will eventually form the top of the plant, but the other cells may be partly organized as leaf primordia, the cells in the axils of which may form side shoots. These axillary cells are therefore also (potential) (apical) initial cells.

In the region, between the last leaf primordia and the apical initials, new leaf primordia will develop, so that some of the cells in this region may also be included in axillary meristems while the axillary meristems of the younger leaf primordia will derive from the apical initials themselves. In this way theoretically three preformed groups of apical initials or potential apical initials can be distinguished. The other cells of the seed apex are used in the formation of the first leaves and mutations in these cells are usually "lost".

The three (preformed) groups of initials may vary in sensitivity (Lapins and Hough 1970) and thus in their "expected" mutation rate. Those which develop into shoots will show essentially the same developmental pattern and the same potential sector number i.e. number of apical initials. Anatomically these shoots may be expected to have the same number of apical initials as there is no evidence from plant anatomy or from chimera studies of basic differences in apical structure due to the origin of the apices (main stem, side shoot). The origin may, however, affect chimerism.

If the mutation affects the vitality of the cells the mutated sector may be narrower than expected from the number of initials. The sector will become narrower (not shorter) because the lateral cell division will probably be most affected as vertical cell division has to concur with division in neighbouring "normal" cells. This has been suggested as the reason for gradual changes in sector size within some inflorescences (Eriksson 1965, Kaukis and Reitz 1955). Sometimes the mutation may affect the stability of the initial cells leading to a change, or loss, of chimerism though, not necessarily in favour of the (normal) neighbouring cell. Usually chimerism is lost somewhere during the ontogeny of the

plant, apparently (genotypically) at random (Dulieu 1969, Michaelis 1967, Müller 1963b).

Axillary buds from derivatives of the apical initials will be mutated if they develop in a mutated sector, normal if in a normal sector, and chimeric if on the border of two sectors (Michaelis 1967). The frequency of chimerism will depend on the number and size of the sectors and the relative size of the axillary meristem though the central (apical initial) cells of the axillary meristem are of main importance. The frequency of chimerism in axillary shoots is expected, and found, to be lower (D'Amato et al. 1964, Dommergues et al. 1967, Klopfer 1965b, Osone 1963) than in the three groups of primary preformed shoots (from the above-mentioned groups of initials).

Phyllotaxis is very regular in most plants, and the position of the axillary bud is determined by the leaf so that chimerism will hardly be expected to influence the position of the bud, especially in the case where the mutation is only apparent in the M_2. The development of bud primordia is usually initiated by cell divisions in the L_3, while the mutations studied in the M_2 occur in the L_2.

The position of the bud will only affect the results when it shifts to a, chimerically different, position. This may happen with axillary buds on sector borders (Michaelis 1967). In the case of primary preformed apices this can only be inferred from differences in mutation rate (Contant 1970, Gaul 1957a, 1959, 1963a) which may, however, also result from differential sensitivity.

Usually only some of the axillary buds develop (Gillet et al. 1969, Jacobsen 1966). Whether, and to what extent, an axillary bud develops depends on its position on the plant and nutritional, physiological and environmental conditions of the plant. Competition between developing buds, e.g. for available nutrients, has been found (Gillet et al. 1969).

Differences between mutation frequency in early and late tillers (Gaul 1961) or between plants with few or many tillers (Ehrenberg and Lundqvist 1957, Freisleben and Lein 1943, Gaul 1961, 1963a, Gillot and Dommergues 1965, Osone 1963) have been observed but the different origin of the tillers (preformed apices, axillary buds or axillary axillary buds), differences in fertility between different tillers (Iqbal Khan and Doll 1968, Osone 1963) and differences in chimerism related to tiller origin (Osone 1963) may have influenced these results and may cause contradictions. It seems possible that buds bearing a mutation have a disadvantage in competition with other buds and are excluded from further development. To test this, side shoots from the same position (and origin) should be compared for mutation frequency and for development.

When the M_2 is observed the segregation ratio may be distorted, by

meiotic drive (Rédei 1965a), pollination (certation), fertilization, seed development (van der Veen 1967a,b) and dormancy and germination (Röbbelen 1966a, Velemínsky and Gichner 1967) which are often influenced by environmental conditions or interaction with surrounding tissues. Differential pollination in a chimeric flower due to non-simultaneous ripening of the anthers is a form of diplontic selection only if this results from genotypic differences between the anthers.

Treatments at different developmental stages, allowing for more, or less, diplontic selection between the time of treatment and observation are usually confounded with sensitivity differences between the stages. Treatment of buds or sprouts, more differentiated than the seed miristem, shows different mutation rates for the different meristems present during the treatment, partly due to differences in sensitivity. When the apex is severely damaged it may be reconstituted from undamaged cells, a new apex may be formed, or axillary or adventitious buds may take over (Ferwerda 1964, Pratt 1963). When axillary buds take over, the least affected ones will probably be the first to develop and will show a low mutation frequency. This can be overcome by forcing the other buds to develop too, e.g. by cutting away developing buds (Bauer 1957, Jank 1957, Nayar 1969, Nayar and Dayal 1970, Zwintzcher 1955).

Some "mutations" are actually direct or indirect effects of the treatment, cell death being an extreme example. Phenotypic changes due to X-rays (Rédei 1967c), as well as sterility and recessive deficits (Gaul 1966, Fujii 1960) may be direct (physiological) effects. A few cases of mutations which, although homozygous, disappear in later generations (Mouli 1970, Röbbelen 1958, Sosna 1962) remain unexplained.

Sometimes adventitious buds are induced on treated leaf cuttings. The cells of the leaf cuttings are differentiated and probably more or less equal in sensitivity. Some of these cells will dedifferentiate and divide to form the adventitious buds, but many cells do not dedifferentiate. Here again the effect of the treatment or the induced mutation may determine whether a cell dedifferentiates or not. This seems to be confirmed by results from combined treatment with X-rays and colchicine which gave more tetraploid adventitious buds than when only colchicine was used (Broertjes 1969a,b).

The higher number of tetraploids after combined treatment may be due to differential sensitivity which gives the tetraploid cells a selective advantage (Broertjes 1969a,b). Whether this is diplontic selection or X-ray selection depends on the damage caused by the X-rays i.e. mainly genetic or mainly physiologic.

It might be possible to test this. In some plants adventitious buds are formed preferentially in certain positions on the leaf (Bigot 1970). These

cells may show differential sensitivity, but the mutation rate of buds from cells in different positions and the differences in position with various treatments might enable a conclusion on diplontic selection with the adventitious bud method.

5.2.2 *Diplontic selection?*

A critical analysis of reported diplontic selection shows little actual selection. There are many reports of mutations being lost (Bartels 1961, Bauer 1957, Kaplan 1953, Sherma and Rapoport 1965) or of the first leaves showing many spots or stripes while later leaves only occasionally show sectors (Contant 1970, Dommergues 1962, Kaplan 1954, Mertens and Burdick 1957, Natarajan and Shivasankar 1965, Nishiyama et al. 1964, 1966, Randolph 1950). This has been explained (4.4.2 and 5.2.1) to be a normal process in chimeric plants directly after treatment. It may indicate some difference in sensitivity but is independent of the genotype and not due to selection.

The mutation rate of the lower side shoots differs from that of the main shoot or higher side shoots (Frydenberg et al. 1964, Frydenberg and Jacobsen 1966, Gaul 1957a, 1963a,b, 1964, Gaul and Mittelstenscheid 1960, Kaplan 1951, Lindgren et al. 1970, Monti 1965a,b, Nayar 1969). This may be due to differences in fertility and germination which also occur in the control (Iqbal Khan and Doll 1968). It has been ascribed to diplontic selection (Gaul 1957a), but also as inherent to normal development (Frydenberg et al. 1964, Frydenberg and Jacobsen 1966, Lindgren et al. 1970; see also 5.2.1). Comparison of different parts of the plant for mutation rates gives some problems as these parts may differ in origin (5.2.1).

In roots the disappearance of mutations has also frequently been reported (Davidson 1959, 1969, Gaul 1957c, Levan 1938, van der Mey 1970, Němec 1961), though this too may be explained (5.2.1) as a normal aspect of root development.

Sometimes recessive deficits are included as diplontic selection (Blixt et al. 1960) but to ascertain this the reason for the deficit must be known.

Differences in mutation rate between X-rays and EMS (D'Amato et al. 1962, Monti 1965a, Müller 1965b) may be the result of more cell death with X-rays resulting in less chimerism, or delayed mutation and repair with EMS giving more chimerism. The former is sometimes included under diplontic selection (5.1.1).

Careful observation of chimerism on visible sectors (Dulieu 1967a,b, 1969, Michaelis 1967, Stewart and Dermen 1970a), on pollen (Lindgren

et al. 1970), on chromosome aberrations (Sarvella et al. 1962) or even on polyploidy (Bain and Dermen 1944) revealed no clear diplontic selection. Loss of mutations and different mutation frequencies in different parts of the plant could be explained as inherent to the normal growth of the plant and random loss of sectors from the apex.

When the M_2 from different positions on the inflorescence was studied the same conclusion was reached: Chimerism is lost, mean sector size increases, sector number decreases and the product stays constant for the first 5 fruits and for fruits 21-25. Plants with a longer vegetative period, more (25) leaves under the inflorescence, had fewer sectors which were again larger than in the control with few (7) leaves under the inflorescence (Müller 1963b, 1965a).

5.2.3 *Layers*

Chimerism can appear by cell exchange between 2 layers (4.3.1). Similarly it can disappear and this (complication) must be kept in mind when discussing diplontic selection. The mutation frequency found for the layers differs (Asseyeva 1931, Bateson 1921, Satina et al. 1940) through differences in sensitivity or in stability. Exchange between the layers has been observed especially the L_1 replacing the L_2 (Dulieu 1969, Fogle and Dermen 1969, Stewart and Burk 1970, Weiling and Gottschalk 1961) but also the L_2 replacing the L_3 (Burk et al. 1964) or the L_3 replacing the L_2 (Stewart and Burk 1970). Monocotyledons are often less stable (Popham 1964).

The presence of a mutation may affect the stability of the layers (Dulieu 1967b). In tobacco the exchange rates "L_1 to L_2" and "L_3 to L_2" were estimated to be 1/3000 in both cases (Stewart and Burk 1970), but more exchange between layers can be induced by wounding (Asseyeva 1927, 1931, Bergann 1967) while adventitious buds on leaves (Broertjes 1969a) or roots (Bateson 1916, Bergann 1967), consist mainly of the L_1 and L_3 respectively, and thus expose these layers.

In some plants these exchanges are so frequent that they are responsible for the variegated appearance (Hejnowicz 1959, Thielke 1954, 1955). In other plants they may be responsible for the sudden appearance or disappearance of a mutated sector and for differences between different organs on the same plant which derive from different layers, e.g. adventitious buds and axillary buds.

5.3 ENVIRONMENTAL INFLUENCES

The environment influences the mutation frequency by affecting seed

development on the M_1, germination, etc. by which expression and detection of mutations in the M_2 are affected; but also directly by its effect on the M_1 plant. This has been ascribed to diplontic selection resembling the effect in mammalian tissue where the fast dividing cells take over (Curtis 1966). In plants conditions favouring growth may give higher mutation frequencies (Buiatti et al. 1970, D'Amato 1964), while chimerism may be lost during dormancy (Bain and Dermen 1944).

The influence of the environment is often not clear. In *sorghum* stress promotes diploidy, the "mutation" induced by colchicine (Ross and Atkinson 1965). Differences in temperature at germination (Ehrenberg and Lundqvist 1957), planting date (Gaul 1963a) and in spike length (Kaplan 1951) have been recorded to influence mutation frequency, which shows that plant growth and factors influencing plant growth may influence observed mutation frequency. This may be through diplontic selection, but also through differential survival, different origin of the material eventually scored (main, axillary or adventitious shoot) or changes in chimerism.

In summing up

At the time of treatment, e.g. the seed, the meristem is usually multicellular but the destiny of most of the cells is already fixed, and mutations induced in cells predetermined to form leaves are "lost". Observations at the cell level show that mutated cells (even dead cells) are helped or carried along by the neighbouring cells. The mutated cells (tissue) will be at a disadvantage only in those cases where there are many potential organs of which only a few can develop, e.g. side shoots. Most other cases of loss of chimerism are probably independent of the genotypes involved.

5.4 ARABIDOPSIS

Observations on chimerism in the main inflorescence showed that, though sector size may change and some sectors may be lost or may take over the whole plant apex, this is a random process and average mutated sector size for the whole population remains constant. Similarly an increased number of leaves below the inflorescence increases the size of the sector and decreases the number of sectors so that the average again remains the same (Müller 1963b, 1965a). Sectors were larger after X-rays than after EMS probably due to the killing effect of X-rays (Müller 1965b). Abnormal segregation (Rédei 1965a), certation, differential seed development and differential germination (Röbbelen 1966a,

Velemínsky and Gichner 1967) may influence the observed mutation rate.

Modification by X-rays (Rédei 1965b) progressive chromosome loss in polyploids (Bouharmont 1967a,b) may confuse observations on mutation frequency and diplontic selection.

Chlorophyll mutations sometimes give heterosis (Röbbelen 1957b). Sectors for chlorophyll mutations were more frequent in chlorophyll mutated plants but even when the sector had the normal phenotype it was usually shorter than mutated sectors in normal plants (Röbbelen 1965c, 1966b). Some of these mutations are plastid mutations, showing other complications such as apparently directed sorting out (Röbbelen 1965c).

Twin spots and sectors, accompanied by chromosome aberrations have been found (Hirono 1965). They often differ in size, and single spots may be twins of which one does not develop or cannot be distinguished from the surrounding tissue. Difference in size due to retarded or stagnated development would clearly be diplontic selection.

Treatment at different unicellular stages viz. egg cell, pollen and zygote, gave different results for survival and mutation frequency which must be ascribed to differential sensitivity as diplontic selection can have very little effect because chimerism is rare (Röbbelen 1963a).

5.5 EXPERIMENTAL PART

5.5.1 *Sunflower*

In the sunflower (polyploidy) chimerism was often lost. No relationship seemed to exist between the flower and the stem below, scored indirectly through side shoots. A comparison between two experiments, one grown in the field and one in the greenhouse, suggested an explanation (Fig. 4-5). In the field the flowers were usually larger and more chimeric (Table 4-6, 5-1). Comparison of an end flower (side shoot 1st order) with its stem below (side shoots 2nd order) showed that although the frequency of chimerism in the stem (chimerism between shoots 2nd order) was almost equal (field and greenhouse) frequency of chimerism within the end flower was much higher in the field.

For this comparison side shoots were used as there were too few plants to use the main stem, though the difference is still larger there (Table 5-1). Besides, it was hoped to exclude direct treatment effects in this way. The side shoots observed were near to the top of the plant (most plants form side shoots from the top down).

The overall % of tetraploid tissue (a chimeric flower was counted as

Table 5-1. Chimerism and tetraploidy in various flowers on chimeric plants grown in the greenhouse or in the field (sunflower, Exp. I)

flowers scored	greenhouse					field				
	T	C	D	%C	%Tt	T	C	D	%C	%Tt
main flower	1	1	9	9	14	0	7	5	58	29
flowering order (date) versus position										
first 2 to flower	8	2	16	8	35	3	11	18	34	27
topmost 2 flowers	8	3	15	12	37	8	9	15	28	39
end flower versus subjacent axillary shoots*										
end flower	7	11	13	36	40	5	15	7	56	46
axillary shoots (pooled value)	4	23	4	74	50	2	22	3	82	48
end flower on short versus long shoots										
0–3 nodes	13	3	22	8	38	10	12	19	29	39
4-many nodes	19	7	26	14	43	14	21	51	24	29

T = tetraploid, C = chimeric, D = diploid, %C = % chimeric/all, %Tt = % tetraploid tissue/all = $(T + \frac{1}{2}C)/(T+C+D)$

* ploidy of subjacent axillary shoots was the pooled value for 2 or more shoots (2nd order) per shoot (1st order), i.e. T = all tetraploid, C = chimeric in or between the shoots (2nd order), D = all diploid.

half) in the end flowers and in the stem did not differ much and was almost the same for greenhouse and field plants, which shows that there was no pronounced diplontic selection. The side shoots on the plants in the field were usually longer (Table 5-2) so that progressive loss of chimerism cannot explain the difference in chimerism either. Average shoot length seems unrelated to chimerism and tetraploidy (Table 5-1, 5-2) though there may be a tendency for more diploid end flowers on longer side shoots in the field. A further discussion follows in chapter 6.

The difference between seed and seedling treatment (Table 3-4) was not caused by the number of nodes between treatment and scoring either. The plants had been divided into two equal groups forming pairs of the same developmental stage. One plant of each pair was topped (decapitated), the other grown untopped. Attempts to root the tops failed, but the top axillary shoots of the topped plants developed. Side shoots on topped plants were longer than on untopped plants. In both cases the top shoots developed first and these were higher on the main stem in the untopped plants. The total distance in nodes was 27,1 + 0,4 for the untopped and 10,3 + 7,9 for the topped plants, i.e. the side shoots on untopped plants were nearly 10 (Table 5-2) nodes further

Table 5-2. Branching and chimerism in sunflowers under various conditions (Exp. I and II)

	number of nodes	ploidy[1]				
		T	C	D	%C	%Tt
I. side shoot[4] – greenhouse	2,5	32	10	48	11	41
side shoot – field	3,5	24	33	70	26	32
II. main stem[2] – topped[3]	10,3					
main stem – untopped	27,1	3	4	96	4	5
side shoot[2] – topped	7,9	3	5	89	5	6
side shoot – untopped	0,4	7	3	75	4	10

T = tetraploid, C = chimeric, D = diploid, %C = % chimeric/all, %Tt = % tetraploid tissue/all = $(T + \frac{1}{2} C)/(T + C + D)$.

1. for all plants scored, in Exp. II, but for chimeric plants only, in Exp. I.
2. grown in the greenhouse.
3. topped refers to decapitation (main stem) well before flowering.
4. all untopped.

from the cotyledons. If distance was the reason that little polyploidy was found after seed treatment the "topped" branches of the seed treatment should approximate the "untopped" branches of the seedling treatment. This is not the case (Table 3-4). The results even seem to indicate less polyploidy, though more chimerism, in the longer side shoots of topped plants (Table 5-2).

The difference between seed and seedling treatment therefore seems to be due to a difference in sensitivity, rather than to a difference in detection. Similar sensitivity differences were found in arabidopsis and are easily explained by the action of colchicine which requires cell division to be able to cause polyploidy: more division is expected in the seedling apex than in the seed apex.

Comparison between the first flowers (on side shoots) to open and the topmost flowers (top side shoots) shows differences for chimerism, but especially for overall polyploidy in the field experiment (Table 5-1). This may indeed be an expression of differences which might mimic diplontic selection, e.g. if only the first flowers were harvested.

5.5.2 *Arabidopsis: colchicine treatment*

Colchicine affects the embryo and seedling development (Fig. 2-7). Plants that show polyploidy in the first flower differ from untreated plants in adult morphology, in flowering date and in the number and development of the rosette side shoots, and also differ from treated

plants where no polyploidy occurred in the first flower (though it may have been present in other flowers or other cell layers). The first rosette leaves are often malformed or apparently absent so that their number is probably underestimated (Table 2-1, Fig. 2-6). These differences clearly show that mutated (polyploid) plants have lower fitness, and seem to suggest that diplontic selection may occur against polyploid tissue within the plant.

When the first flower opens, axillary buds can be seen in the axils of some of the rosette leaves. A few weeks later the number of rosette side shoots that have developed can be scored. This number is influenced by environmental conditions and by the number of side shoots on the stem. In mutated (polyploid) plants it is apparently also influenced by the polyploidy itself. The effect of colchicine on rosette shoot development (fewer develop) and flowering (later) is not a direct effect because it is not found after colchicine treatments which give no polyploidy, e.g. the medium and late germinating groups after green seed treatment (Table 2-1). On the other hand tetraploid (M_2) plants are very regular in growth and flowering. Those seeds which germinate earlier and are sensitive to colchicine show polyploidy and disturbed growth probably due to temporarily disturbed mitoses and to chimerism within the plant. This results in later flowering and fewer rosette shoots.

The development of fewer rosette shoots might be an indication of diplontic selection if mainly polyploid (or diploid) shoots were inhibited. This is difficult to ascertain, also because of possible interactions with other layers. The polyploid tissue will be expected to be at a disadvantage, yet the percentage of polyploid shoots was usually higher in the rosette than on the stem suggesting that no diplontic selection has occurred among the rosette shoots (Table 6-2).

Tetraploid sectors in the flower vary in size, but the average size is half when all six stamens are scored. When one stamen is missing, the mean tetraploid sector size is smaller than half, indicating that the absent stamen was probably often tetraploid (Table 4-3). (Absent stamens are those which were missing or underdeveloped). This seems to indicate diplontic selection against the tetraploid tissue!

The stem has maximally four sectors, while usually not more than two sectors are found in the inflorescence. There are indications of a third sector at the base of the inflorescence, probably representing the end of a sector from the stem. Colchicine can induce the same mutation (e.g. tetraploidy) in two adjacent sectors so that they cannot be distinguished. This will happen more often at a higher mutation rate i.e. with certain treatments. With three sectors in the stem and base of inflorescence the expected ratios tetraploid : diploid will be 0:3, 1:2, 2:1, 3:0.

Table 5–3. Frequency of different degrees of ploidy after chimerism has been lost (arabidopsis, Exp. IV)

remaining tissue	chimeric for 0 or 1 flower treated with colchicine		chimeric for 2 or more flowers treated with colchicine	
	alone	+ EMS	alone	+ EMS
diploid	5	7	18	18
tetraploid	13	19	6	17
octoploid	12	4	2	0

The frequency of each ratio depends on the treatment and, in the absence of diplontic selection, will determine the ratio of tetraploid to diploid inflorescences after chimerism has been lost (Table 5-3) or the % of tetraploid tissue in different parts of a shoot (Table 5-5). The treatment may also influence the frequency of octoploidy (Table 5-3, see 3.6.5).

In the inflorescence (above a certain point) only two sectors occur and chimerism should end equally frequently for diploidy as for tetraploidy (or for tetraploidy vs octoploidy) but the point above which this should ocur is uncertain and may depend on the treatment (Table 5-3).

Polyploidy affects cell size so that interaction with other layers (only the L_2 is scored) may influence the loss of chimerism.

The first 7 flowers of the main inflorescence of 47 plants, showing tetraploidy in that inflorescence after green seed treatment, were compared (Fig. 5-1). The inflorescences were, however, scored till chimerism was definitely lost. Results show that the number of plants still chimeric at or above a certain flower (C) decreases from flower 1 to flower 7, while the number of plants diploid or tetraploid at and above that level increases. The number of chimeric flowers (c), consequently, decreases too, the number of tetraploid flowers (t) increases slightly but the amount of tetraploid tissue (Tt) (chimeric flowers counted as half) stays approximately constant. This demonstrates that the sharp decrease in chimerism is not due to diplontic selection for one tissue type but due to random loss of chimerism.

Chimerism in the rosette was studied on plants which were induced (by decapitation) to form many side shoots from the rosette (Exp. II). Plants formed up to 20 apparently independent rosette shoots which derive from not more than 6 primary rosette shoots (Fig. 5-2). Comparison of mutation rate in early and late rosette shoots shows a higher polyploidy rate for the first 10 shoots per plant than for the later shoots (Table 5-4). When, however, the primary rosette shoots viz. the main shoot in each axil, were compared according to their position on the

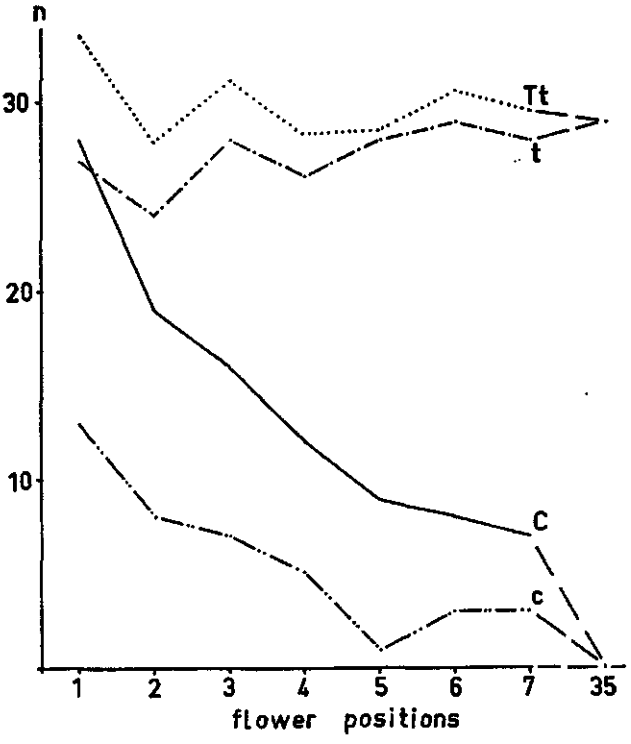


Fig. 5–1. Comparison of the first seven flowers in 47 mutated (polyploid) arabidopsis plants.
n = number of plants (for C) or flowers (for t, c and Tt).
C = plants, chimeric at and above that point. t = tetraploid, c = chimeric flowers at that point. Tt = tetraploid tissue (t + ½c) at that point.

plant (A = nearest to the top) no differences are found (Table 5-4), except an apparent oscillation between A and B, and C and D. When two rosette shoots were inserted at the same level the first one to flower was given the first letter. This occurred quite often as the rosette leaves usually have a decussate phyllotaxis. If, as was found on the main shoot (Fig. 2-6), and also in the sunflower (Table 5-1), the polyploid shoot flowers later, the method used to letter the shoots will show more diploidy in the first shoots of each pair. This was indeed the case (Table 5-4), and may be mistaken for a type of diplontic selection which will affect the results if only one rosette shoot, e.g. the first flowering, is scored.

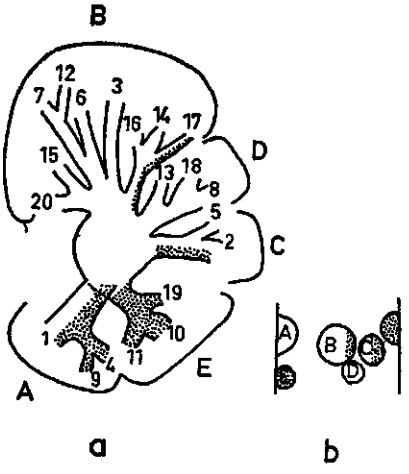


Fig. 5–2. Branching and chimerism in a rosette after colchicine treatment (arabidopsis, Exp. II).
a. from above, b. vertical topography.
Numbers (1–20) denote flowering order. Letters (A–E) denote position (top to bottom).
◍ = tetraploid, ○ = diploid.

Table 5–4. Polyploidy of inflorescences in the rosette of chimeric plants (arabidopsis, Exp. II, 24h colchicine)

ploidy[1]	flowering order, 1 to 20					
	1–5	6–10	11–15	16–20	1–20	
diploid	93	60	34	9	196	
chimeric	20	2	0	2	24	
tetraploid	72	51	16	4	163	
% tetraploid tissue[2]	44	47	32	27	46	
ploidy	growth order[3], top to bottom					
	A	B	C	D	E+F	A–F
diploid	25	17	17	10	6	75
chimeric	6	6	3	1	1	17
tetraploid	11	18	12	9	5	55
% tetraploid tissue[2]	33	51	42	48	46	43

1. 5 flowers scored per inflorescence to determine ploidy.
2. % tetraploid tissue = % (tetraploid + ½ × chimeric)/all inflorescences.
3. only the primary rosette shoots (maximally 6). Cf. Fig. 5–2.

Table 5-5. Comparison of primary and secondary inflorescences on branched chimeric rosette shoots (arabidopsis, Exp. II)

24 h colchicine 0,2%						30 h colchicine 0,2%					
Rp		Rs:	Ts	Cs	Ds	Rp		Rs:	Ts	Cs	Ds
3 Tp	→	4:	1	2	1	8 Tp	→	17:	10	5	2
11 Cp	→	20:	8	1	11	0 Cp	→	0:	0	0	0
10 Dp	→	28:	7	2	19	8 Dp	→	17:	5	2	10
24	→	52:	16	5	31	16	→	34:	15	7	12
% Ttp = 35			% Tts = 35			% Ttp = 50			% Tts = 54		

Rp = number, Tp = tetraploid, Cp = chimeric, Dp = diploid, % Ttp = % tetraploid tissue (T + ½ C)/(T + C + D) for primary inflorescences.
Rs, Ts, Cs, Ds, %Tts ditto for subjacent secondary inflorescences → connects inflorescences of the same shoots.

Diploid rosette shoots often appeared to have more side shoots (Fig. 5-2), but when the ratio of diploid and polyploid shoots (R1-20) is compared with the ratio for only the primary rosette shoots (RA-F) the former does not have less polyploidy i.e. polyploid side shoots have no selective disadvantage (Table 5-4).

Comparison of chimerism in the inflorescence on branched chimeric rosette shoots (side shoot 1st order) shows that the treatment (24 vs 30 h) influences the frequency of chimerism in the rosette shoots (1st order) (Table 5-5). After heavy treatment (30 h) no chimerism is found in these shoots while inflorescences on the stem below (2nd order side shoots) indicate that chimerism often existed (e.g. tetraploid side shoots on a diploid primary shoot). The side shoot inflorescences (2nd order) themselves also show chimerism. The % tetraploid tissue (tetraploid + ½ chimeric) is the same for the inflorescences on the primary rosette shoots (1st order) and for their side shoots (2nd order). This seems to indicate that no diplontic selection occurs in the primary rosette shoots. The % tetraploid tissue however, differs for the two treatments, which indicates a different rate of induction for the two treatments.

5.5.3 *Arabidopsis: combined treatment*

After combined treatment with colchicine and EMS some interesting observations were made. All 10 mutated (polyploidy) plants after ripe seed treatment showed chimerism only in the first few flowers of the main inflorescence, while side shoots and secondary side shoots of the stem and the rosette had only normal pollen (Table 4-5, 6-2). This is a strange case of isolated chimerism in the top of the plant which may be

explained as diplontic selection against the polyploid tissue reducing it to a narrow sector below the inflorescence that escaped detection in the side shoots.

Another possibility is that the cells in the apex (some of which are tetraploid) are prevented from dividing, first by the EMS and then by being included in the méristème d'attente, so that no further division occurs before the floral stage. Tetraploidy has been mentioned as an aspect of differentiation (Coleman 1950), so that preferential inhibition of cell division in the tetraploid cells by EMS is theoretically possible.

With green seed, combined treatment gives (ploidy) chimerism throughout the plant demonstrating that chimerism *can* occur after combined treatment and is not directly prevented by the EMS (Table 5-3, 6-2).

EMS seems to stimulate chromosome division, enabling colchicine to induce polyploidy in ripe seed (Table 3-5) and promoting germination in green seed (Fig. 2-5); but subsequently EMS inhibits cell division causing isolated chimerism (ripe seed) (Table 4-5, 6-2) and less octoploidy or more forked apices (green seed) (Table 5-3, 3-1).

The degree of chimerism as scored by the embryotest is lower after combined treatment than after EMS treatment when only the plants in which the first flower had shown no polyploidy were considered. This may indicate that chimerism is lost sooner after combined treatment than after treatment with EMS only (Table 5-6). Chimerism (polyploidy) may also be lost sooner than after only colchicine treatment (Table 6-3) but these differences are not significant. This decrease of chimerism with the combined treatment may be due to the EMS enhancing the colchicine sensitivity of the cells. An effect of colchicine on EMS sensitivity was not found and seems to be absent, as the mutation (chlorophyll) frequency for combined treatment and for EMS alone is approximately the same in the germination group (medium) that gave no polyploidy (Table 3-3).

The loss of (chlorophyll) chimerism in the combined treatment (in which polyploidy occurred) suggests that the polyploidy has more influence on the stability of chimerism than other (chlorophyll) mutations (Table 5-6), even though only the "diploid" (first flower diploid) plants were studied. The (chlorophyll) mutation frequency was high in this combined treatment so that it seems unlikely that there was diplontic selection against chlorophyll mutations.

5.5.4 *Arabidopsis: EMS treatment*

Chimerism estimated by the chimeric index 4.7.2 decreases in the inflorescence i.e. is higher at the base than at the top, especially after certain posttreatments (Table 5-6). No clear relationship is found but the general tendency seems to be for mutation frequency (calculated for the whole population) to increase when chimerism (calculated for the mutated plants only) decreases. This shows that the decreased chimerism is not the result of diplontic selection against the mutations scored.

The increased mutation rate indicates that chimerism affects the observed mutation rate. This may be due to within-flower (fruit) chimerism. With random self-fertilization per flower this would reduce the segregation ratio (for an inflorescence with two equal sectors) from 1/8 (1/4 and 0/4) for no within-flower chimerism, to 1/16 (1/4 × 1/4) for only within-flower chimerism (half the sporogenic tissue mutated). In this way random loss of chimerism can considerably increase the segregation ratio, and therefore also the observed mutation rate. Diplontic selection affecting pollen shedding or stamen development, as seen for

Table 5-6. Chimerism and M_2 mutant frequency after different posttreatments (arabidopsis, Exp. IV and V)

treatment	day-length[1]	fruits 2 and 3				2 fruits between 6 and 12			
		n	C	C%	%M	n	C	C%	%M
V. Ripe seed EMS									
etiolated (48h)	24	121	82	60	10				
vernalized (16d)	24	137	74	64	12	138	43	35	20
control	24	104	87	77	9				
VI. Green seed EMS + colchicine									
early[2]	24	41	76	53	11	54	61	43	14
early	16	74	80	52	13	64	72	53	10
medium	16	25	80	71	8	35	66	63	9
green seed EMS									
medium and late	24	80	74	71	8	75	67	60	9
medium and late	16	104	82	75	6	106	70	65	7
strong plants[3]	16	20	80	84	6	21	67	68	6

n = number of mutated plants. C, C% = chimerism index for mutated plants based on fruits and mutant frequency resp. %M = M_2 mutant frequency for all the plants in the treatment.

1. 24, 16 = 24 or 16 hours daylength.
2. early, medium, late refer to germination group (Table 2-1).
3. plants with a higher phyllotaxis (Plate I).

polyploidy, may also occur and certation may have more effect in chimeric flowers.

With these factors influencing mutation rate in chimeric plants it is not possible to definitely exclude diplontic selection because the mutation rates observed at the different positions result partly from different segregation ratios.

When chimerism in various inflorescences of the plant is compared (Exp. VI, Fig. 4-7) it seems to be highest for the main inflorescence and the top axillary shoot. The main inflorescence is expected to be more chimeric than side shoots that derive from only part of the stem circumference. This was also found after colchicine treatment (Table 4-5).

Differences in chimerism of different side shoots (Fig. 4-7) do not reflect the degree of chimerism at that point of the stem. These differences result not only from the number and size of the sectors in the stem but also from the relative size of the meristem giving the side shoot. This again depends on the size of the leaf primordium relative to the apex and the size of the bud meristem relative to the leaf base. The latter seems to decrease in older leaves (Fig. 2-8). The former seems to decrease in younger leaves, the first leaf bases enclosing half the apex or stem and later leaves only a smaller part (Fig. 2-7).

The side shoots develop from the top of the plant down but the fruits scored were all harvested on the same day so that those from the higher side shoots derived from a higher position along the inflorescence. With progressive loss of chimerism they will therefore be expected to show less chimerism. The number of nodes on the side shoots was slightly lower for the higher side shoots and may in contrast lead to more chimerism.

Interaction of all these factors may explain the observed differences in degree of chimerism of the different side shoots.

The top side shoot opposite the first flower shows more chimerism probably because it originates very near to the apex. It is however also possible that the (main) inflorescence does not develop centrally on the apex but more to one side while the top shoot develops on the other side and therefore nearer to the summit but the concordance of mutations in the main inflorescence and top side shoot is higher than would be expected if this hypothesis was true.

Vernalization after seed treatment gives a different picture. Little chimerism is found in the main inflorescence and top side shoot. This may indeed be due to the position of the scored fruits as chimerism within the inflorescence is lost more quickly after vernalization (Table 5-6), but a different size relationship between apex, sector, leafprimordium and bud meristem may also be the explanation.

The frequency of completes (2 fruits per inflorescence mutated) and chimeras (only one fruit mutated) seems negatively correlated for the various scoring points (inflorescences) (Fig. 4-7). The sum of the two lines gives the number of mutated inflorescences, the difference shows the load of chimerism for the various inflorescences and the average [(2 × completes + chimeras)/total] gives the mutation frequency. The relation to the chimerism of the main stem is too complex to allow any conclusions concerning diplontic selection to be drawn from this.

CHAPTER 6

DIPLONTIC DRIFT

Sectorial chimerism and the persistence of sectors depends on the apical initial cells. If one of these loses its initial position it can never regain it and that sector ends. Developmental factors which affect apical stability may be expected to have more influence on this loss than genetic factors. In analogy to diplontic selection this, genotypically random, loss of chimerism may be called diplontic drift.

6.1 STABILITY OF THE APEX

The stability of the apex is demonstrated by the long sectors (Burk et al. 1964, Dermen 1945, Dulieu 1969, Heslot 1961, Steffensen 1968), the independence of the layers and the regular phyllotaxis. This stability is probably largely due to the buffer effect of the méristème d'attente. Environmental conditions may influence the number of leaves (Laibach 1943a,b, Müller 1963b, Schwabe 1959), the rate of leaf production (Schüepp and Chang 1938), the phyllotaxis (Loiseau 1959, 1970), the apex size and flowering (Laibach 1943a,b, Müller 1963b). Changes in the environment (Tort 1967), wounding and chemicals, e.g. mutagens, influence apex size and phyllotaxis, often causing abnormal growth (Dommergues 1962) or reducing layer stability (Iqbal 1969).

Mutations may affect growth and apex size directly (Röbbelen 1957a) or, in chimeras, by affecting the physiology of (Thielke 1948, 1955), or the divisions in other cells or cell layers (Chittenden 1927, Clowes 1957, Dommergues 1962, Dulieu 1967b, Klopfer 1965c, Renner and Voss 1942, Thielke 1948, 1954, 1955). Sometimes a correlation is found between mutation frequency and branching (Gillot and Dommergues 1965).

In the root chimerism may affect the origin of lateral roots and even the structure of the main root apex (Némec 1962b, 1965).

6.2 ENVIRONMENT, DEVELOPMENT AND CHIMERISM

Plant development may in turn affect chimerism (4.6.4, 5.3) or observations on chimerism as was explained for the sympodially growing tomato (4.1). Chimerism of the apical initial cells will be influenced by the behaviour and stability of these cells which again depends on the behaviour of the whole apex. If the apex is small (Dulieu 1969), disturbed by the treatment (Dommergues 1967, Heslot 1961) or unstable in size (Dulieu 1969) or layering (Clowes 1957), chimerism is not found or soon lost.

Changes in sector size often occur together with changes in plant development, e.g. fasciation (Michaelis 1967) and forking (Dommergues 1967, Heslot 1961), or at leaf initiation (Dulieu 1968, 1969, Gröber 1962) or in relation with dormancy (Bain and Dermen 1944). When chimerism appears late it is often more persistent (Dermen 1945, Dulieu 1969). This is often the case with a high phyllotaxis either because the leaf base is small (relative to the apex) so that more leaves are needed to sample the whole circumference and to use the cells from the anneau initial after which these are replaced by derivatives from the apical initials which form sectors; or it is because the apex is larger and more stable at higher phyllotaxes.

Monocotyledons with leaves which develop far apart, thus hardly affecting the apex, stay chimeric long too (Clowes 1957). Directed cell division in some of them allows chimerism to exist in the form of stripes in the apex and leaves (Thielke 1954, 1955).

The time between treatment and observation influences chimerism so that less is found with a longer time before observation, due to random loss of sectors (Müller 1963b). Favorable growth conditions favour apex size and often decrease the time to flowering (after which mutations are usually scored) but they increase the number of mutated sectors (Buiatti et al. 1970) or the number of mutations found (D'Amato 1964, Lapins et al. 1969). Probably other reports of environmental factors influencing mutation frequency (Ehrenberg and Lundqvist 1957, Gaul 1963a), or of tiller length affecting mutation frequency (Kaplan 1951), or even of differences in mutation rate between early and late plants, must also be understood in terms of the effect of changed development on chimerism.

Mutation frequencies observed in different branches may be influenced by the mutagen sensitivity, apex size and development, chimerism (as affected by branching) and sterility, so that differences found may be due to any or several of these factors. Mutation frequency is influenced by chimerism which in turn seems to be related to growth and to apical size and stability.

Different reasons for loss of chimerism have now been given viz. 1) random loss or diplontic drift, 2) directed loss due to genotypic differences or diplontic selection, and 3) directed loss due to the position of the mutation (and normal development) or ontogenic loss.

Most of the induced mutations are probably lost through ontogenic loss, occurring in cells which do not normally give rise to the gametes or M_2, e.g. cells in the root, leaf primordia and the L_1 and L_3. Loss through diplontic selection may be drastic (e.g. cell death) or differential (e.g. differential development of buds). In the former case the mutation is too weak to live even with the aid of surrounding tissue and therefore of little practical importance. In the latter case it can be partly prevented by inducing more buds to develop. Loss through diplontic drift is likely to be affected by the size and stability of the apex, which can be partly modified by environmental conditions. This may offer the most practical opportunity to influence chimerism and simultaneously the frequency of mutations detected.

In summing up

Sectorial chimerism depends on the apical initial cells, and the persistence of sectorial chimerism will depend on the stability of these cells. This may vary during the development of the plant, e.g. due to changes in the méristème d'attente, and can, to some extent, be influenced by environmental conditions. The loss of chimerism may affect observed mutation frequencies.

6.3 ARABIDOPSIS

Chimerism in arabidopsis plants which had a longer vegetative growth period or which were scored higher in the plant was found to be lost at random (Müller 1963b). After 20 flowers on the main inflorescence 62% of the chimerism had been lost, whereas 18 extra leaves (25 instead of 7, on plants grown under short day conditions) changed the frequency of segregating mutations in the first 5 fruits from 36% to 31% or the mean sector size from 44% to 52% (Müller 1963b). The results are expressed in different terms but the chimeric loss for 20 flowers seems much higher than that for 18 leaves. This may indicate that the "distance" measured in leaves (in contrast to flowers) is smaller so that less chimerism is lost. However, plants grown under short day conditions are usually stronger and more vigorous and this may also have affected chimerism.

6.4 EXPERIMENTAL PART

The experimental results give little evidence for diplontic selection, yet chimerism is not stable and is invariably lost. This then must be due to the imperfect stability of the apical initial cells. The first indication that the stability might be influenced by growth circumstances came from the sunflower experiments. A great difference was noticed between chimerism in the field and in the greenhouse (Table 5-1). The plants in the greenhouse were usually smaller and less vigorous with shorter branches (Table 5-2) and smaller flowers.

It can be argued that vigour and apex size and stability of the apical initial cells are positively correlated. Environmental conditions that influence vigour and the development of the plant might also influence the apex itself and therefore chimerism. This would explain the results from the sunflower experiments. Plants grown in the field were more vigorous and (therefore) had more chimeric flowers than those grown in the greenhouse.

The apex of arabidopsis is very small but increases in size during development (Fig. 2-7). The most sensitive stage of the apex is therefore probably early in the development of the plant. Little is known about changes of apex size due to environmental conditions. Microscopic observations on arabidopsis did not reveal much because the apex is so small, but it did reveal that etiolation retards the development of the seedling apex (Table 2-4). This means that a stage with a smaller apex is prolonged. If this is a critical stage, etiolation is expected to diminish chimerism. Vernalization of germinating seed probably has the same effect because during vernalization (in the dark) relatively little visible development takes place.

Observations on growing plants revealed a difference in phyllotaxis for the last rosette leaves, which consisted of two opposite leaves in some plants and three adjacent leaves in other plants (Plate I). Phyllotaxis is often related to apex size, and the occurrence of three leaves at the same level suggests a larger apex so that these plants were regarded as plants with, probably, a larger apex. In winter with continuous (artificial) light plants grow faster and often flower earlier than in summer, but they are usually also weaker especially if the lamps were rather high above the plants. In summer no artificial light is used, i.e. plants grow under natural daylight and daylength circumstances. Continuous light of a low intensity apparently promotes development but decreases vigour and probably also decreases apex size and stability.

These observations suggested various possibilities for influencing apex size and stability. Etiolation, vernalization and daylength could be va-

ried, and phyllotaxis could be observed in the flowering plants and used to select "naturally" vigorous plants. The posttreatments were applied after seed treatment with colchicine, colchicine + EMS, and EMS (Exp. IV). The plants were scored for polyploidy in the first flower; those not showing polyploidy were scored by the embryotest method in fruit 2 and 3, while those showing polyploidy were further scored for (ploidy) chimerism in the main inflorescence and in first order side shoots.

Table 6–1. Ploidy chimerism in different parts of plants subjected to various post-treatments after colchicine treatment (arabidopsis, Exp. IV, colchicine and colchicine + EMS)

posttreatment	daylength	position of the inflorescence(s) scored					
		main stem		side shoot Z1		shoots Z12, R12	
		%C1	n	%C2	n	%C2	n
etiolated	16 h	49	45	18	22	18	66
not etiolated	16 h	73	30	17	24	15	68
not etiolated	24 h	80	25	9	22	5	59

Z_{12} = cauline, R_{12} = rosette side shoots (top two). $\%C_1$ = chimeric/polyploid inflorescences. $\%C_2$ = chimeric/all inflorescences on chimeric plants only. n = number of inflorescences scored.

Table 6–2. Ploidy chimerism in chimeric arabidopsis plants (Exp. III and IV)

treatment	% chimeric			% completely polyploid			number scored		
	H*	Z	R	H*	Z	R	H*	Z	R
III daylight (12–16h)									
1,2 c	90	15	10	10	37	49	30	46	70
1,2 c + V	80	0	9	20	50	52	10	16	33
1 c + EMS	100	0	0	0	0	0	9	20	32
IV 16 h daylength									
2 c	89	24	24	11	33	52	19	34	21
2 c+ EMS	89	8	10	11	42	43	31	49	30
24 h daylength									
2 c	86	8	0	14	23	33	7	13	9
2 c + EMS	100	7	0	0	26	31	14	27	13

H = main, Z = cauline, R = rosette inflorescences; * = for H, % of polyploid inflorescenses only, for Z and R, % of all inflorescences on chimeric plants; 1 = ripe seed, 2 = green seed treated. V = 30 days vernalization. c = colchicine.

Table 6–3. Average sector length (minimum 3 flowers) in polyploid chimeric inflorescences (arabidopsis, Exp. IV)*

	number of flowers	number of sectors	average
24h, 16h daylength			
colchicine	250	25	10
colchicine + EMS	251	30	8,4
colchicine, colchicine + EMS			
24 h daylength	83	12	6,9
16 h daylength	418	43	9,7

* length of the sector that is lost i.e. length of chimerism.

The results for polyploidy (Table 6-1, 6-2, 6-3) and for the embryotest (Table 5-6) confirm these expectations. Etiolation and vernalization decrease chimerism (Table 5-6, 6-1, 6-2). Continuous light (24 h) gives earlier flowering and fewer leaves before flowering than 16 hours light (Table 2-1), yet the plants are *not* more chimeric than those with the longer vegetative period (Table 5-6, 6-1, 6-2).

If loss of chimerism is progressive, the greater distance in time and/or in leaf number between treatment and observation should cause a greater loss of chimerism in the slower and later plants. Even after the first scoring, loss of chimerism is seen to be more rapid under continuous light (Table 5-6) than under 16 hours light. This is seen from the sector length which is shorter with continuous light (Table 6-3), from chimerism (ploidy) in the side shoots which is less frequent with continuous light (Table 6-1), and from the degree of chimerism (M_2) in the top of the plant which is lower with continuous light (Table 5-6). The data on chimerism in the top of the plant may however be biassed because the top was scored five days after fruit 2 and 3, but with continuous light more fruits had been formed so that the position of the fruits scored was higher than with 16 hours light.

After etiolation or vernalization, both of which retard apex development at an early and small stage, less chimerism is found in the main inflorescence (Table 5-6, 6-1, 6-2). In the side shoots, (ploidy) chimerism (on chimeric plants only) equals that of unetiolated plants. This shows that etiolation directly affects only the main apex, which is present during etiolation (Table 6-2).

After vernalization chimerism in the apex (Table 5-6) and also in the side shoots (Table 6-2) seems to decrease very rapidly (Table 5-6). Vernalization shows the same effect as etiolation, but in addition it shows an after-effect on later chimerism (e.g. side shoots). This may be due to

a changed developmental pattern after vernalization. This is seen in other varieties in which vernalization leads to earlier flowering together with fewer leaves. In the (early flowering) variety used, however, these effects were not found (Table 2-1) though there were fewer side shoots (Table 3-7) possibly indicating some effect on plant vigour. If vernalization indeed promotes development of the whole plant it may have the same effect on chimerism as continuous light. This was found to be the case, and seems to indicate that vernalization affects plants in a lasting way, even when it does not noticeably affect flowering.

When the first flowers were scored, flowering date, leaf number, rosette shoot number and phyllotaxis were recorded. Colchicine affects plant growth, often to such a degree that the rosette leaves could not all be clearly discerned so that the phyllotaxis in the rosette could not be determined. Plants from the EMS treatment, however, occasionally showed a higher phyllotaxis. The mutation frequency and chimerism was calculated for all plants and subsequently only for those with higher phyllotaxis. There were only few of these within a comparable day-length treatment but the results show the expected higher degree of chimerism.

Loss of chimerism is not due to diplontic selection as can be seen from the mutation frequencies (Table 5-6). These increase with loss of chimerism, probably due to simultaneous loss of within-flower chimerism. Diplontic selection implies that the mutated tissue is weaker so that loss of chimerism should give stronger plants yet the stronger plants were the more chimeric plants. With (polyploidy) chimerism it was repeatedly observed that inflorescences producing 2 or even 3 flowers daily retain chimerism longer than inflorescences which produce one flower daily or every second day.

Lower chimerism and higher mutation frequency after combined treatment than after EMS (Table 5-6), seem to indicate that growth disturbances due to the colchicine, affect chimerism. This may be directly in the germinating seed and seedling, or indirectly through the effect of the induced polyploidy on cell size (within and between the layers).

The effect of combined treatment on polyploidy chimerism is more complicated because EMS influences cell sensitivity for colchicine. This affects the polyploidy rate, especially octoploidy and the appearance of chimerism.

Many of the results given here are based on data from relatively small numbers of plants and taken separately give only an indication but taken together they all give the same indication and clearly demonstrate that some relationship does exist between development or vigour and chimerism. This may explain the relationship between branching

and mutations (Table 3-7) or vigour and mutations (Table 3-8). In both cases "mutations" do not refer to mutant frequency but to the number of separately distributed mutations (sectors) per plant i.e. to the degree of chimerism. It may also explain apparent relationships found between sowing date, or position in the greenhouse, or position in the rows, or scoring date and mutation rate. These relationships were usually not repeatable in later experiments (with a.o. different natural daylight conditions) and therefore could not be quantified. It now seems probable that all these factors may influence growth and therefore chimerism (or mutations) which influences mutant frequency.

In analogy to diplontic selection this may be referred to as diplontic drift, random with regard to the genotype. The *amount* of drift, however, depends on various factors, especially those affecting vigour and plant development.

The techniques used to germinate mutagen-treated seeds and grow the M_1 are often varied according to the requirements of the plants but also according to other circumstances such as the season (summer or winter), the available space in the greenhouse, the available time for transplanting and even the day of the week (Sunday). The latter two may affect duration of etiolation and age at transplanting. In this way the same mutagen treatment may not give the same results when it is "repeated".

The effects, reported in the literature, of planting date, transplanting, temperature and even row distance on mutation rate may actually be effects on growth; and contradictory results may be due to small differences in growth circumstances and also to varietal differences in apex size or apex stability.

The relationship between growth and chimerism seems to indicate a possibility to induce loss of chimerism in a, with regard to the mutation, random way. The relationship between chimerism and mutant frequency demonstrates the possible value of losing chimerism when a high number of mutants is required. When many different mutations per plant are required it may, however, be preferable to retain chimerism.

CHAPTER 7

GENERAL CONCLUSIONS

The aim of this study was to obtain more information about sectorial chimerism within the plant, its occurrence, distribution and loss. To do this, mutations were induced and their distribution throughout the plant studied, and compared with expectations. These expectations were based on the present understanding of the development of the plant and of the action and effects of various mutagens, as deduced from the literature.

The plant apex consists of zones with differential activity, independent tissue layers from one of which the gametes derive, and probably a few apical initial cells (per layer) which allow the occurrence of sectorial chimerism. The leaves develop near the apex in a regular phyllotaxis while the axillary buds in their axils develop further from the apex, however, from a meristem directly derived from the apical meristem. Flowers develop from the generative apex itself as in the sunflower or around it in a regular phyllotaxis as in arabidopsis.

The apex size may affect the phyllotaxis which in turn may affect the number of vascular bundles, during normal development of the apex. Factors influencing the rate of cell division within the apex probably cause slightly twisted growth and sometimes forking of the stem. (Chapter 2).

An extensive literature exists on mutagenesis especially with X-rays which cause much physiological and gross chromosomal damage in addition to the induced (gene) mutations, whereas EMS induces mainly gene mutations. EMS resembles X-rays in many respects but acts earlier in the cell cycle. Differences in induced chimerism and duration of aftereffects, between EMS and X-rays have been explained in several ways.

Pre- and posttreatments may influence the results with EMS by affecting EMS uptake or diffusion out of the material i.e. by their effect on the effective EMS dose. It is possible that this, and the duration of the treatment are responsible for some of the differences (between EMS and X-rays).

The experimental results with arabidopsis (which has very small seeds) suggest a very direct action of EMS. (Chapter 3).

Cells in different parts of the apex or during different stages of plant development are known to vary in X-ray sensitivity. Similar differences in sensitivity were found here for EMS in which adjacent cells may even differ in sensitivity so that mutations seem to occur preferentially in some cells. (Chapter 4).

EMS initially stimulates cell division so that some division probably occurs during long (24 h) treatments, but afterwards it seems to inhibit (further) cell division. (Chapter 4).

Colchicine acts on cell division and this may explain the differential sensitivity found, (seed vs seedling, colchicine vs combined i.e. colchicine + EMS treatment). (Chapter 3). It seems to have a stimulating effect on the germination of green seed but causes abnormalities in the apex probably partly because of the size of the mutated (polyploid) cells. (Chapter 2). The seedlings from the treated (green) seeds which do *not* germinate soon after the treatment show no effect of the colchicine, develop normally and have no polyploidy which all shows that the action of colchicine is also very direct and ends with the treatment. (Chapter 3).

The literature reports various reasons for chimerism, partly ascribing it to the mutagen and the treatment, partly to the material treated. The experimental results show that mutagen, dose, duration of treatment and stage treated all influence chimerism. (Chapter 4).

Chimerism can be detected at various levels especially after colchicine treatment when even within anther chimerism was found (arabidopsis). Sector size varies but in arabidopsis the chimeric section of the inflorescences usually consists of two equal, slightly spiral sectors, while in the stem up to four different sectors could be distinguished and the rosette occasionally showed mutations not observed in the stem. The spiral course of the sectors is partly due to the method of assessing the positions of the scoring points but probably also results from the twisted growth mentioned above. (Chapter 4).

In the sunflower sectors were easy to detect in chimeric flowers but difficult to delimit along the stem. (Chapter 4).

Differences in mutation rate are often ascribed to chimerism and diplontic selection i.e. genotypically directed loss of chimerism. A careful analysis of recorded diplontic selection shows, however, that the loss of mutations or of chimerism is often inherent to normal development of the plant. The only evidence for diplontic selection is found when there are several potential (bud) meristems of which only a few can develop. This is often the case for axillary buds and for adventitous buds. Dif-

ferential development of cells within one meristem seems unlikely in view of the very regular developmental pattern of the plant and its organs. Where diplontic selection was observed it was accompanied by growth aberrations. (Chapter 5).

Observations on chimerism in sunflower and arabidopsis led to the same conclusions. Diplontic selection seems to occur when only some of the meristems develop into organs (i.c. stamens) though differences in the time or rate of development between diploid and polyploid organs may resemble diplontic selection or even lead to "harvest-induced" selection and will depend on the time of harvesting. (Chapter 5).

Most of the loss of chimerism seems to be independent of the mutation but is related to the growth of the plant itself. More vigorous plants may develop more slowly and have a longer vegetative phase but, when they reach the generative stage and can be scored, they are often found to be more chimeric, i.e. they average more sectors per plant and more flowers per sector, or have a higher chimerism index. (Chapter 6).

Plants grown under various conditions which affect their growth vary in the degree of chimerism found; and stronger plants, even within a treatment, were more chimeric. Loss of chimerism seemed correlated with increased mutant frequency so that diplontic selection (against the mutant genotype) could not be the main reason. The increased mutant frequency which is probably due to decreased within-flower chimerism, may be the reason for some of the contradictory results in the literature. (Chapter 6).

In analogy to diplontic selection based on genotypic differences, loss through development may be called diplontic drift. Diplontic drift is at random for the genotype but it depends on factors which influence the apex. Chimeric plants may yield more different mutations per plant but non-chimeric plants have the advantage of a segregation ratio which promotes the chance of detection. Manipulation of diplontic drift is possible by varying the growth conditions so that the desired degree of chimerism can be obtained.

SUMMARY

Chimerism is the concurrence of genotypically different tissues in one individual and usually results from a mutation early in the development of that individual. With the possibility to induce mutations came the problem of chimerism which gives heterogeneous plants and, allegedly, loss of mutations by diplontic selection, i.e. selection between genetically different tissues within an individual where the mutated tissue is assumed to be at a disadvantage.

Literature on anatomy, morphology and development, and on the mutation process gives the explanation for various aspects of chimerism: The appearance of chimerism depends on the constitution of the material treated and on the action of the mutagen used. Periclinal and sectorial chimerism are related to the structure of the apex i.e. the existence of independent cell layers and of a few central (apical) initial cells. The development of chimerism after a mutagenic treatment is determined by the differentiation already present in the material treated (usually seed), in which the destiny of most of the cells is already fixed.

The presented experiments (on arabidopsis and sunflower) indicate that the mutagenic action of the mutagens used (EMS and colchicine applied separately or simultaneously) was mainly confined to the duration of the treatment. In addition the mutagens influenced development, stimulating it at first and later (especially colchicine) retarding it, which has consequences for sensitivity with combined treatments, and which may affect chimerism. Differences in average distribution of mutations (from seed versus seedling treatment, "first" versus "second" mutation) were ascribed to differential mutagen (EMS) sensitivity of cells destined to form the various parts of the plant (main inflorescence, side shoots). Chimerism in the sporogenic tissue (pollen and M_2 were scored) occurred as sectors (usually 2 in the inflorescence and up to 4 in the stem of arabidopsis) which often seemed twisted probably due to the (twisted) growth of the plants. Generally chimerism was lost, apparently at random with regard to the observed mutations (polyploidy and M_2 chlorophyll mutants).

Although diplontic selection is often mentioned in the literature, most of the cases reported can be explained equally well, and often better, as aspects of normal development or as the result of differential (mutagen) sensitivity. Environmental conditions influence the development of the plant and also the (observed) mutation rate, but this influence may be indirect i.e. through the effect on chimerism. Chimerism depends on the stability of the apical initial cells which will be related to the stability of the apex and therefore to plant development and thus indirectly to environmental conditions.

Plants from experiments with various growth conditions (greenhouse vs field, daylength) differed in development and vigour, and simultaneously, in degree and persistence of chimerism, vigorous plants showing more chimerism. Treatments which retard apical development at an early stage (etiolation, vernalization) decreased chimerism. In both cases effects on the stability of the apex were probably responsible.

This loss of chimerism, at random with regard to the genotype, may be called diplontic drift. It depends on conditions affecting plant growth and may offer an opportunity to manipulate chimerism. Chimerism decreases the chance to detect a mutation but increases the number of (different) mutations that can be obtained from one plant, so that the degree of chimerism desired, may vary.

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