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**SPATIAL ANALYSIS OF THE FORMATION OF  
ADVENTITIOUS SHOOT MERISTEMS**

A Thesis Presented

by

**HUI-CHENG TIAN**

Submitted to the Graduate School of the  
University of Massachusetts in partial fulfillment  
of the requirements for the degree of

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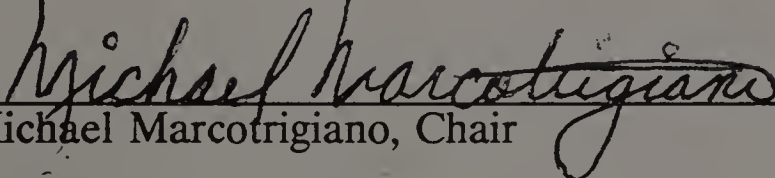
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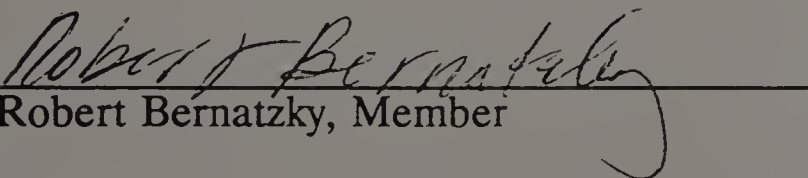
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ABSTRACT

SPATIAL ANALYSIS OF THE FORMATION OF  
ADVENTITIOUS SHOOT MERISTEMS

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Most studies concerning the formation and development of shoot apical meristems have been performed on shoot apices that were ultimately derived from an embryo. Little is known about the early events and subsequent organization of adventitious shoot meristems. Adventitious shoots were regenerated in situ from leaf axils in a series of six interspecific periclinal tobacco chimeras by decapitating the plants and removing all of the axillary buds and any adventitious buds arising from roots. Eighty four of the 413 shoots regenerated were chimeric. Many of the shoots were complex chimeras which possessed axillary buds with a variety of periclinal arrangements. The adventitious shoots arose from LII and/or LIII apical descendants of the source plant, while the LI descendants were not involved in the adventitious shoot formation, as shoots arose from regions internal to the scar tissue of the excised axillary buds. With time, nearly all shoot apices

became non-chimeric or stabilized as periclinal chimeras. I describe a method which can be used to create 1) small genetically distinct sectors analogous to radiation-induced sectors and 2) a complete series of periclinal chimeras; both of which can be used to determine tissue-tissue interactions. Results also indicate that the first one to three leaves of adventitious shoots may not arise from the shoot apical initials of a meristem proper.

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# CHAPTER I

## INTRODUCTION

### Structure of the Shoot Meristem

In many higher plants, the shoot meristem is composed of three superimposed cell layers. As proposed by Schmidt (1924), the organized meristem structure is maintained by the predominant anticlinal cell divisions (i.e. divisions perpendicular to the surface of the apical dome) in the two outer cell layers, or the tunica. Therefore, each layer of the tunica tends to persist as an independent cell layer during development. The outermost tunica layer, which eventually gives rise to the plant's epidermis, is usually designated as the LI, while the second tunica as the LII. Beneath the tunica is the corpus or the body of the meristem, which contains in its uppermost cell layer (the LIII) the cells that proliferate in any direction and therefore perpetuate the inner cylinder of the meristem.

Within each meristem cell layer, there are cells commonly called apical initials that reside at the center of each layer. The apical initials are generally considered as the ultimate source of shoot growth (Esau, 1965). Some authors, however, hold that there are no permanent apical initial cells in the shoot meristem (see e.g., Newman, 1965). They consider all the apical cells as temporary inhabitants in the shoot meristem since they appear to be frequently displaced by their daughter cells or adjacent cells from within or between the

layers (as reviewed by Newman, 1965 and Klekowski, 1989). Therefore, any apical cell can be left behind from the shoot meristem and no cell can be permanently assigned to any constant position in the meristem during growth. Several lines of evidence, however, suggests that this may be an oversimplification of the meristem cell behavior. Observations on many plants have confirmed that there is an apparent zonation of cells in the shoot meristem. Cells in the central zone of the meristem are usually larger in size, more highly vacuolated and less active than cells of the peripheral region (as reviewed by Steeves and Sussex, 1989). This suggests that the apical initials, which are supposedly within the central zone, may be a group of relatively stable residues in the shoot meristem. By studying genetic mosaics, Stewart and Dermen (1970a) found that shoots of some species had persistent and regular variegated sectors, which led them to conclude that there is a certain number of apical initials existing in each apical cell layer during growth.

### **Concept of Plant Chimeras**

Chimeras can be defined as plants which possess cells of different genotypes coexisting in the shoot meristem (Poethig, 1987) and thus generating genetically different cell lineages in tissues and organs. Plant chimeras which possess distinct phenotypic markers are valuable for use in developmental studies, because the markers allow the observation of the lineage pattern of shoot meristem cells as

they generate tissues and organs. Dicots usually have three apical cell layers while monocots, depending on species, may have two or three (Stewart and Dermen, 1979; Tilney-Bassett, 1963). However, the number of apical cell layers can vary between species (e.g., Poethig, 1984; Stewart et al., 1974; Stewart and Dermen, 1975; Tilney-Bassett, 1963) or in a particular shoot (Reeve, 1948; Romberger, 1963). Unless numerous shoots are studied, this fluctuation can cause difficulties in interpreting chimeric structure by phenotypic analysis (Marcotrigiano, 1990).

There are basically two types of chimeras, both of which are distinguished by possessing at least two types of genetically distinct cells within the shoot apical meristem. Mericlinal chimeras contain sectors of genetically different cells in at least one apical cell layer, and periclinal chimeras possess an entire apical cell layer or layers genetically different from other layers. Periclinal chimeras are stably maintained by the predominant anticlinal cell divisions in each tunica layer. Mericlinal chimeras are often greatly variable in variegated pattern because of the shift of meristem cells during growth. However, the axillary meristems of mericlinal chimeras frequently display periclinally chimeric structure generated within a sector wide enough to include an entire axillary meristem.

## **Origin of the Shoot Meristem**

Plant chimeras with distinct phenotypic markers allow the investigations on the single- and/or multicellular origin of shoot meristems. Broertjes and Van Harten (1978) observed a great number of non-chimeric shoots regenerated in the mutation breeding of many species. They then concluded that the adventitious shoot meristem originated from a single cell because only non-chimeric shoots were recovered (Broertjes and Van Harten, 1985).

In some cases, however, the shoot meristem can be multicellular in origin. This is evidenced by the formation of chimeras which are composed of genetically distinct cell populations (Stewart and Dermen, 1970b; Marcotrigiano and Gouin, 1984b). Because of the flexible behavior of meristem cells during development (Dermen, 1960, Newman, 1965) as well as cell interactions (Yeoman, et al., 1978), non-chimeric plants may arise even though the early event of meristem formation is multicellular in nature. The possible fluctuation in the number of cells involved in meristem formation from different tissue sources or under different conditions (e.g., *in vivo* and *in vitro*) may make this issue more complicated.

## **Generation of Tissues and Organs from the Shoot Meristem**

Most of the information on the tissue lineage of organs derived from the meristem cell layers was obtained through the investigation of plant chimeras with

distinct phenotypic markers (Satina, 1944, 1945; Satina and Blakeslee, 1941, 1943; Stewart and Burk, 1970; Stewart et al., 1974; Stewart and Dermen, 1975; Dermen, 1960). Consistent results were obtained on tobacco (Burk et al., 1964; Stewart and Burk, 1970), poinsettia and carnation (Stewart, 1965), as well as many other species (as reviewed by Tilney-Bassett, 1963 and Neilsen-Jones, 1969). In general, the LI gives rise to the plant's epidermis; the LII to the palisade parenchyma, the lower spongy parenchyma and all of the spongy parenchyma of the leaf margin. The LII is usually the layer responsible for the formation of male and female gametes. The LIII gives rise to the upper and middle layers of the spongy parenchyma of the leaf as well as the pith of the stem. The LIII generally makes no contribution to the leaf margin.

Many observations indicate that cells of the shoot meristem do not possess fixed developmental fate. Instead, plant cells are most likely to follow different paths of differentiation according to the final location regardless of the lineage origin. For example, by studying peach cytochimeras, Dermen (1953) observed that in a stem the boundary between the LII and LIII lineages, which were derived from a stable periclinal chimeric shoot meristem, was extremely irregular due to the temporal difference in the rate of cell divisions during the development of the stem. Stewart and Burk (1970) found that the LI cells of a stable periclinal chimera, which normally differentiated as epidermal cells, formed normal chlorophyll-containing mesophyll or cortical tissues when occasionally displaced into these tissues. Further studies have confirmed this observation in other

species. This indicates that derivative cells of an apical cell layer have the potential to form structures normally derived from other apical layers. In some cases, the ultimate morphology of an organ is not changed even though the organ is composed of atypical quantities of derivatives from each apical cell layer (Stewart et al., 1974; Stewart and Dermen, 1975).

### **Formation of Plant Chimeras**

There are many ways of generating plant chimeras. In general, chimeras are produced by either mutation or experimental synthesis (as reviewed by Marcotrigiano, 1990 and Tilney-Bassett, 1986).

**By Spontaneous or Induced Mutations.** Mutations occurring in the shoot meristem cells of higher plants may generate chimeric meristems and therefore chimeric plants if the meristem cells with such mutations generate the lineage(s) of different genotypes. Spontaneous mutations occur at low rate in nature. Numerous physical and chemical mutagens (e.g., X-ray, ethyl methane sulphonate, etc.) have been used to induce mutations to occur at high frequency (Broertjes and Van Harten, 1978). While in a few cases the mutations are dominant and are discernible in the  $M_1$  generation, most of the mutations are recessive (as reviewed by Tilney-Bassett, 1986). The segregation of recessive mutations from the heterozygous  $M_1$  generation can be observed in the  $M_2$  after crossing.

**By Experimental Syntheses.** The early known synthesized chimeras were those arising from the graft union of plants of different genotypes via adventitious shoot formation. Such chimeras are now generally termed as "graft chimeras". They are composed of cells from both scion and rootstock. Theoretically, graft chimeras can be formed from the union of any two graft-compatible plants. However, the success in generating chimeras has been largely limited to Solanaceae species (e.g., Fucik, 1960; Heichel and Anagnostakis, 1978; Junker and Mayer, 1974; Marcotrigiano and Gouin, 1984b).

Some chimeras can be synthesized via the coculture of genetically different cell populations (Carlson and Chaleff, 1974; Binding, et al., 1987). In these experiments, heterogeneous callus tissues, which were formed by a mixed culture of genetically distinct cell lines, were utilized to regenerate chimeric shoots. Carlson and Chaleff (1974) regenerated interspecific chimeras of Nicotiana tabacum and the amphiploid hybrid of N. glauca X N. langsdorfii from the mosaic callus tissue induced by coculturing the pith slices of the two genotypes. Marcotrigiano and Gouin (1984a) experimentally synthesized tobacco chimeras by mixing in culture the cells of the wild-type, Su/Su, and the semi-dominant sulphur mutant, Su/su. Four chimeras were identified from among the 1317 regenerated shoots, but the possibility of spontaneous mutation causing them could not be eliminated. On the other hand, the two investigators failed to obtain any chimeras from the mosaic callus of Nicotiana tabacum and N. glauca (Marcotrigiano and Gouin, 1984b).

By coculturing the protoplasts of Solanum nigrum and S. tuberosum, Binding and his co-workers (1987) obtained both periclinal and mericlinal chimeras with the aid of morphological and cytological markers. However, the competition between genetically distinct cells in culture may significantly reduce the recovery of chimeric shoots (Bayliss, 1977; Marcotrigiano and Gouin, 1984a).

### **Variation of the Chimeric Structure**

Many events (e.g., cell competition, cell displacement, disadvantageous mutations, etc.) occurring in the shoot meristem during development can result in the instability in chimeric associations.

**Change of the Chimeric Structure via Cell Displacement.** The stability of a chimeric structure is dependent on the spatial arrangement of genetically different cells in the shoot meristem (e.g., whether the chimera is periclinal or mericlinal). The mericlinally chimeric structure can be converted into a periclinal or non-chimeric structure if cells of one genotype in a cell layer are completely displaced by cells of another genotype during growth. On the contrary, a periclinal chimera can occasionally become a mericlinal chimera if cells of one genotype in a cell layer are displaced by cells of another genotype from an adjacent cell layer through periclinal cell divisions (Dermen, 1960). In addition, when cell displacement occurs, the change of a chimeric structure will be more pronounced

if the cells in question reside close to the summit of the shoot meristem than if at the peripheral part of the meristem.

Shift of meristem cells between apical layers is mostly caused by the occasional periclinal or oblique divisions of cells in an apical layer which further displaces cells of the "invaded" layer (Sawhney and Sekhar, 1985; Stewart and Burk, 1970). Variation of the cell division pattern in tunica layers can be of periodic occurrence in woody plants (Pillai, 1963; Reeve, 1948). Some environmental factors can also affect the cell division pattern and therefore the chimeric structure of the meristem (Balkema, 1972; Popham, 1951).

**Dissociation of Chimeric Components.** Chimeric components are usually dissociated via the formation of adventitious shoots arising from the leaf, root or stem cutting (Dermen, 1948; Miedema, 1973; Stewart and Dermen, 1970b; Burk, 1975; Broertjes and Van Harten, 1978).. The adventitious shoots are usually non-chimeric, or may not come "true to type". Adventitious shoots which are produced from various vegetative organs usually come from the inner tissues which are mostly of the LIII lineage, or in some cases of the LII lineage (Dermen, 1948, 1951; Asseyeva, 1927; Bergann and Bergann, 1959, 1982). From leaf cuttings of Peperomia, Bergann and Bergann (1982) obtained a number of chimeric adventitious shoots composed of cells of both the LII and LIII lineages. In other species, only non-chimeric shoots were regenerated from leaf cuttings (Burk, 1975;

Broertjes and Van Harten, 1978; Marcotrigiano, unpublished data). Roots generally give rise to shoots of the LIII genotype (Tilney-Bassett, 1963).

Non-chimeric shoots are frequently regenerated from the culture of chimeric tissues from chimeric plants (Cassells and Minas, 1983; Kasperbauer et al., 1981; Kameya, 1975).

As mentioned previously, germ cells normally originate from the LII layer of the shoot meristem. Therefore, the genotype(s) of seedlings derived from self-pollination will be identical with that of the LII cells. However, seedlings may possess the LI phenotype as a result of an infrequent displacement of the LII cells by LI cells (Chittenden, 1926; Neilson-Jones, 1969).

In addition, high level of BA (6-benzylaminopurine) sprayed onto intact plants may induce the recovery of nonchimeric adventitious shoots. Radiation treatments often destroy the chimeric structure (see Marcotrigiano, 1990 and Tilney-Bassett, 1986).

### **Application of Plant Chimeras**

Plant chimeras have been cultured by gardeners and plant collectors for hundreds of years. Numerous economically important plants are chimeras which possess unique and desirable traits.

For research purposes, plant chimeras have been widely used to study many essential problems, such as developmental relationships, cell autonomy (Hake,

1986), self-incompatibility (Gunther, 1961), the function of leaf epidermis in the perception of and the response to light (Mayer et al., 1973; Junker and Mayer, 1974; Heichel and Anagnostakis, 1978) as well as insect resistance (Clayberg, 1975). This thesis, however, concerns itself with the cellular pattern of adventitious meristem formation.

### **Introduction of the Experiment**

As mentioned previously, plant chimeras can be experimentally generated via adventitious shoot formation if genetically different cells are involved in the formation of a single shoot (e.g. Jorgensen and Crane, 1927; Clayberg, 1975, and Marcotrigiano and Gouin, 1984b). Although most periclinal chimeras can be maintained by propagating stem cuttings which possess axillary meristems (see review by Marcotrigiano, 1990), adventitious shoot formation is frequently associated with the dissociation of chimeric components into their component genotypes (Dermen, 1948; Miedema, 1973; Stewart and Dermen, 1970b; Burk, 1975; Broertjes and Van Harten, 1978).

Most investigations on adventitious meristem formation have been limited to determining whether meristems are of single- or multiple-cell origin or from which apical cell layer they originate (Broertjes and Van Harten, 1978; Broertjes and Keen, 1980; Marcotrigiano, 1986a; Stewart and Dermen, 1970a, 1970b). For example, Dermen (1948) forced periclinal cytochimeras (i.e., ploidy chimeras) in

apple trees to produce adventitious shoots by decapitating young trees and removing all the lateral buds. He found that genetically uniform adventitious shoots with the ploidy level of the L-III arose from the phloem region of axillary shoots. With the same technique, Stewart and Dermen (1970b) found that adventitious shoots arising from nodal regions of chrysanthemum periclinal chimeras gave rise to periclinal chimeras. However, utilizing cytochimeras does not allow temporal developmental analysis because tissues must be fixed for observation. In addition, determining the chimeric pattern of the adventitious shoots whose only markers are in terminally borne flowers, provides little information on the spatial pattern of meristem initiation, growth dynamics of the shoot meristem or on cell competition prior to flowering.

When a complete set of periclinal chimeras is available, accurate interpretations regarding the role of each apical layer in the development of an organ can be made. For example, Stewart et al. (1974) obtained all possible periclinal chimeras from Pelargonium plants possessing green and chlorophyll-deficient cells. By comparing the relative proportion of white and green tissues in the mature leaves of different periclinal chimeras, they were able to conclude that this chimera did not possess "disadvantaged" cells and could therefore be utilized to study normal leaf ontogeny. However, when some periclinal arrangements are not available, assigning developmental control of an organ to a given cell layer becomes problematic. Clayberg (1975) and Goffreda et al. (1990) came to opposite conclusions when studying aphid resistance in periclinal chimeras

Lycopersicon pennellii epidermis covering L. esculentum LII and LIII. The former claimed that subepidermal factors were responsible for aphid resistance while the latter attributed the epidermis itself to resistance. Access to the "reciprocal" chimeric arrangement (i.e., an L. pennellii plant possessing an L. esculentum epidermis) could have resolved this contradiction.

In this experiment, I have utilized a complete set of six phenotypically-marked periclinal chimeras to investigate the origin of adventitious shoot meristems which developed in situ. I report patterns in the origin of adventitious shoots as related to the chimeric composition of the source plant and determine whether or not competition exists between genetically different cells during the initiation and growth of the shoot meristem. I also report a technology for obtaining a complete set of periclinal chimeras from a single chimeric plant, and for generating small sectors of genetically dissimilar tissue in leaves and stems.

## CHAPTER II

### MATERIALS AND METHODS

#### Terminology

In this thesis, shoot apical meristem composition will be letter-designated. For example, a periclinal chimera with an LI of Nicotiana tabacum (T), and an LII and LIII of N. glauca (G) will be designated as a TGG chimera.

The group of cells which begins to divide and organize to form an adventitious meristem will be called the "early cell mass" to distinguish it from a mature meristem which would possess a tunica-carpus organization. This group of cells, when observed histologically, displays features consistent with meristematic tissue and should not be confused with wound callus.

#### Plant Material

A complete set of six periclinal interspecific tobacco chimeras composed of Nicotiana tabacum (Su/su) (Burk and Menser, 1964) and N. glauca were used. With phenotypic markers, the apical organization of periclinal interspecific tobacco chimeras could be deduced based on the knowledge of the derivatives of the apical cell layers to the plant body (Burk, et al., 1964; Satina, 1944, 1945;

Satina and Blakeslee, 1941, 1943; Stewart and Burk, 1970). Early experiments utilized the four available periclinal chimeras, i.e., TGT, TGG, TTG, and GTT (Marcotrigiano, 1986a; Marcotrigiano and Gouin, 1984b). The other two periclinal arrangements (i.e. GTG and GGT) which were obtained during this experiment were vegetatively propagated and used to repeat the experimental procedure described below. Leaf and stem markers, which were utilized to analyze the composition of shoots, are summarized in Table 1. The markers allowed differences in genotype to be observed with high resolution. Thus, sectors terminating on a leaf could be distinguished from those which persisted for many nodes. Floral markers which have been previously described (Marcotrigiano, 1986b) were used for additional verification of final apical composition of the terminal shoot apex. The spatial relationship between genetically dissimilar meristem cells of adventitious shoots at different times of growth was deduced from the leaf composition.

### **Growth Conditions**

All plants were maintained in a glass-covered greenhouse in Amherst, MA with a minimum temperature of 18°C. Chimeric plants were propagated vegetatively from single node cuttings and once rooted were potted in 15 cm wide 2.6 l pots filled with Pro-Mix BX (Pro-Mix, Stamford CT). Fertilization was applied as a constant liquid feed of [20N - 4.3P - 16.6 K (12% NO<sub>3</sub>-N, 8% NH<sub>4</sub>-N)]. High

Table 1. Genotype-specific phenotypic markers used for the identification of apical composition in chimeral shoots.

Marker position		<u>N. tabacum</u>	<u>N. glauca</u>	Marker
				for
Leaf	epidermis	hairy	glabrous	LI
	margin	yellow	green	LII
	central region	yellow	green	LIII
	petiole wing	present	absent	LII
	petiole base	no anthocyanins	anthocyanins	LII
Stem	epidermis	hairy	glabrous	LI
	cortex	light green	green	LII
	pith*	yellow	green	LIII

\* only visible when freehand cross-sections are examined.

pressure sodium lamps were used to extend the day-length to 16 hours when the natural day-length was less than 16 hours. Pest control was employed as necessary.

### **Adventitious Shoot Formation**

To induce adventitious shoot formation, periclinal chimeras with 25 to 30 fully expanded leaves were decapitated leaving the basal 15 nodes on the stem. After 14 days, all activated axillary buds were removed. Because Nicotiana can have more than one axillary bud per node (Seltmann and Kim, 1964), all subsequently activated axillary buds (as determined by the time of appearance, position of initiation, and early leaf orientation) were also removed, as were adventitious shoots arising from roots or any region outside of the nodal region.

The distinct markers in these genotypes enabled the adventitious shoots to be readily recognized at an early stage as being either non-chimeric or chimeric. The original chimeric arrangement of adventitious shoots could be easily determined by observing phenotypic markers on all parts of young adventitious shoots. All of the non-chimeric shoots were recorded as to genotype and removed as soon as they could be identified. Chimeric adventitious shoots were allowed to grow in situ until they flowered. As chimeric shoots developed, diagrams were constructed to document node to node changes in the apical composition or the relative

position of both genotypes in the shoot meristem by observing the composition of leaves and stem (see Appendix).

## CHAPTER III

### RESULTS AND DISCUSSION

#### Shoot Origin

Following the removal of all axillary buds from a source plant, adventitious shoots were produced from the cut stem surface, roots and in leaf axils. Only 1 to 3 nodes (usually the most apical) were active in adventitious shoot formation. While most of the shoots forced from axils were non-chimeric, 84 of total 413 shoots were mosaic (Table 2). Axillary buds in the nodes of mericlinally chimeric adventitious shoots were frequently periclinal chimeras and their apical arrangement reflected the chimeric structure of the leaf in the axil from which they arose. For example, GTT, GGT, GTG, TGT and TGG axillary buds were present on adventitious mericlinal chimeras derived from TTG source plants (see Fig.1 and Table 3). TTG, GTT, TGT and GTG axillary buds were present on GGT source plants, and TGG and TTG were present on TGT (Table 3). However, from the axils of GTT source plants only N. tabacum shoots arose and from TGG only N. glauca shoots arose (Fig.2). This indicates that only the inner tissues which were the derivatives of apical layer LII and/or LIII were involved in the adventitious shoot formation and that epidermal cells (of LI origin) were not involved. Observations through a dissecting microscope revealed that adventitious

Table 2. Influence of source plant composition on the number and composition of adventitious shoots forced from nodal regions of decapitated and disbudded plants.

Source plant	No. plants analyzed	Mean no. shoots/plant	Shoot composition of adventitious shoots				Total
			TTT	GGG	Mericlinal	Periclinal	
TTT	8	0.5	4	-	-	-	4
GGG	7	2.6	-	18	-	-	18
TGG	16	1.0	0	16	0	0	16
GTT	16	0.7	11	0	0	0	11
TTG	15	7.8	19	68	29	1	117
GGT	27	3.3	51	26	11	0	88
TGT	15	1.9	20	1	5	2	28
GTG	18	7.3	24	71	33	3	131
Total	122	-	129	200	78	6	413



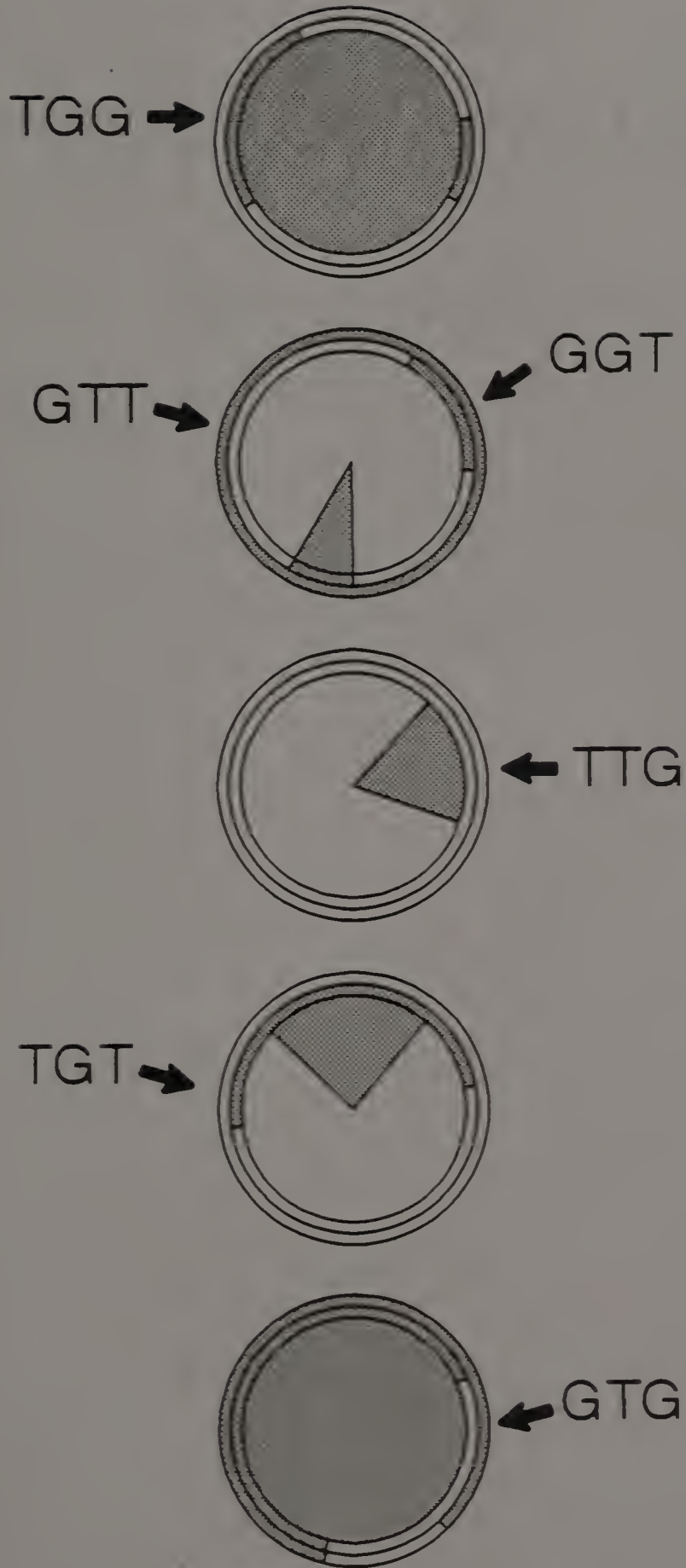
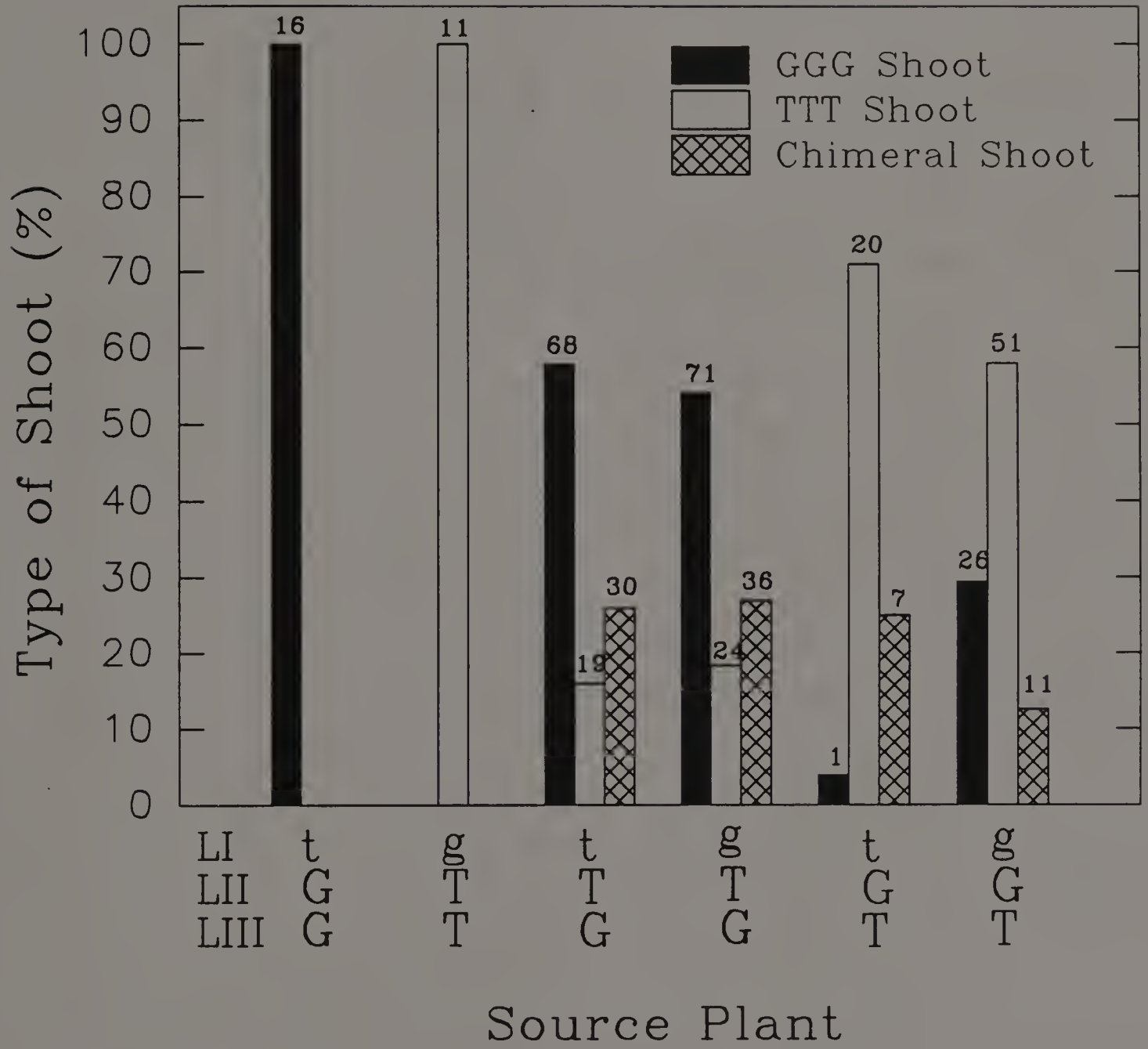


Table 3. Axillary buds that were periclinal chimeras although present on mericlinal chimeras.

Total number and type of periclinal axillary buds present on adventitious mericlinal shoots						
Source Plant*	TGG	GTT	TTG	GGT	TGT	GTG
TTG	4	5	19	2	2	2
GGT	1	4	3	0	2	2
TGT	4	0	1	0	0	0
GTG	17	2	11	0	0	2
Total	26	11	34	2	4	6

\* GTT and TGG source plants produced only non-chimeral adventitious shoots.





shoots originating in the axils were produced from the center of the wound surface where axillary buds had been removed as they appeared over a four month period. The quantity of shoots produced depended on the relative arrangement of N. tabacum and N. glauca in LII and LIII layers. Thus, an interaction between the LII and LIII derivatives in source plants affects shoot production. The majority of the non-chimeric shoots originated from the LIII descendants, while the remainder of the shoots were of either of LII cell lineage or chimeric (i.e., of both LII and LIII lineages) (Fig.2). Patterns in shoot population were distinct only when the LII/LIII composition was reversed. TTG and GTG both possess the same LII/LIII composition, so do TGT and GGT. Shoot populations within these pairs were of strikingly similar distribution (see the bar graphs of Fig.2).

The multicellular origin of some adventitious shoots derived from periclinal chimeras does not necessarily indicate that different tissue types were involved in the formation of a single shoot. Cross-sections of fresh stems taken from source plants revealed differences in chlorophyll content between genotypes making possible the observation of the fate of the apical cell layers in young TTG and GTG stems. In our periclinal chimeras, and in others (Dermen, 1953), it appears that in inner stem tissue the boundaries of the LII and LIII derivatives are quite irregular due to temporal differences in LII and LIII tangential and periclinal divisions as the stem thickens. Therefore, it is reasonable to assume that if all adventitious shoots arose near or from vascular tissue, some would arise from the

region where the two cell lineages contact, thereby giving rise to chimeric shoots (Fig.3). It should be noted that, while an apical origin for adventitious shoots can be deduced from the data, a tissue origin for each shoot cannot be inferred unless histological observations are made.

## Organization

Statistical analysis indicates that the formation and composition of meristem cell layers of adventitious shoots was not random, but genotype-dependent (Table 4). Even in the early stages of development, the epidermal cell layer of most chimeric adventitious shoots possessed only N. tabacum cells. Mosaic epidermis was found on only 20% of the chimeric shoots and in most cases, in just a few nodes, the entire epidermis became N. tabacum. The cell layers beneath the epidermis were sometimes genetically homogeneous but more often contained sectors of the two genotypes (Fig.4). The relative proportion of cell mass of each genotype in an early meristem was variable. However, within the mosaic layers of most shoots, larger N. tabacum sectors were present during the early development regardless of the composition of the source plant (Table 4 and Table 5). This indicates that the relative contribution of cells to the early meristem was genotype-dependent and not position-dependent.



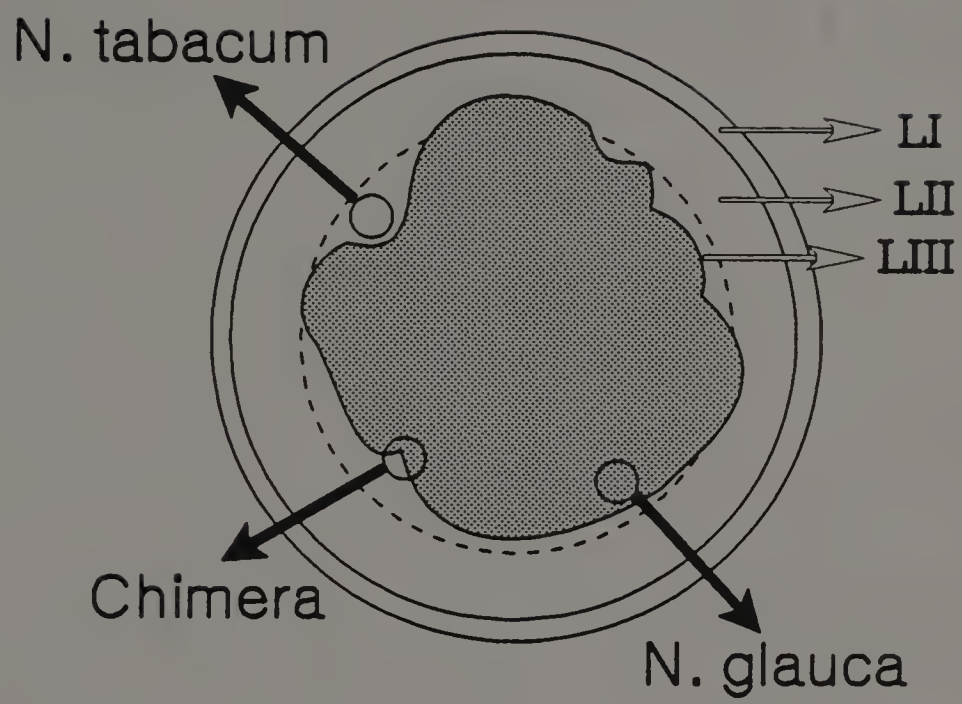


Table 4. Chi-square analysis to determine if the distribution of genotypes in mosaic cell layers of 84 genetic mosaics is random in the early cell mass.

Cell layer	Layer > 50% <u>N. tabacum</u>	Layer > 50% <u>N. glauca</u>	probability*
LI	Observed : 72 Expected: 42	Observed : 12 Expected : 42	< 0.001
LII	Observed : 68 Expected : 42	Observed : 16 Expected : 42	< 0.001
LIII	Observed : 61 Expected : 42	Observed : 23 Expected : 42	< 0.001

\* Yates correction factor used because there is only one degree of freedom.



Chimeral shoots possessing cells of the designated genotype(s)  
in the designated apical layers (%)

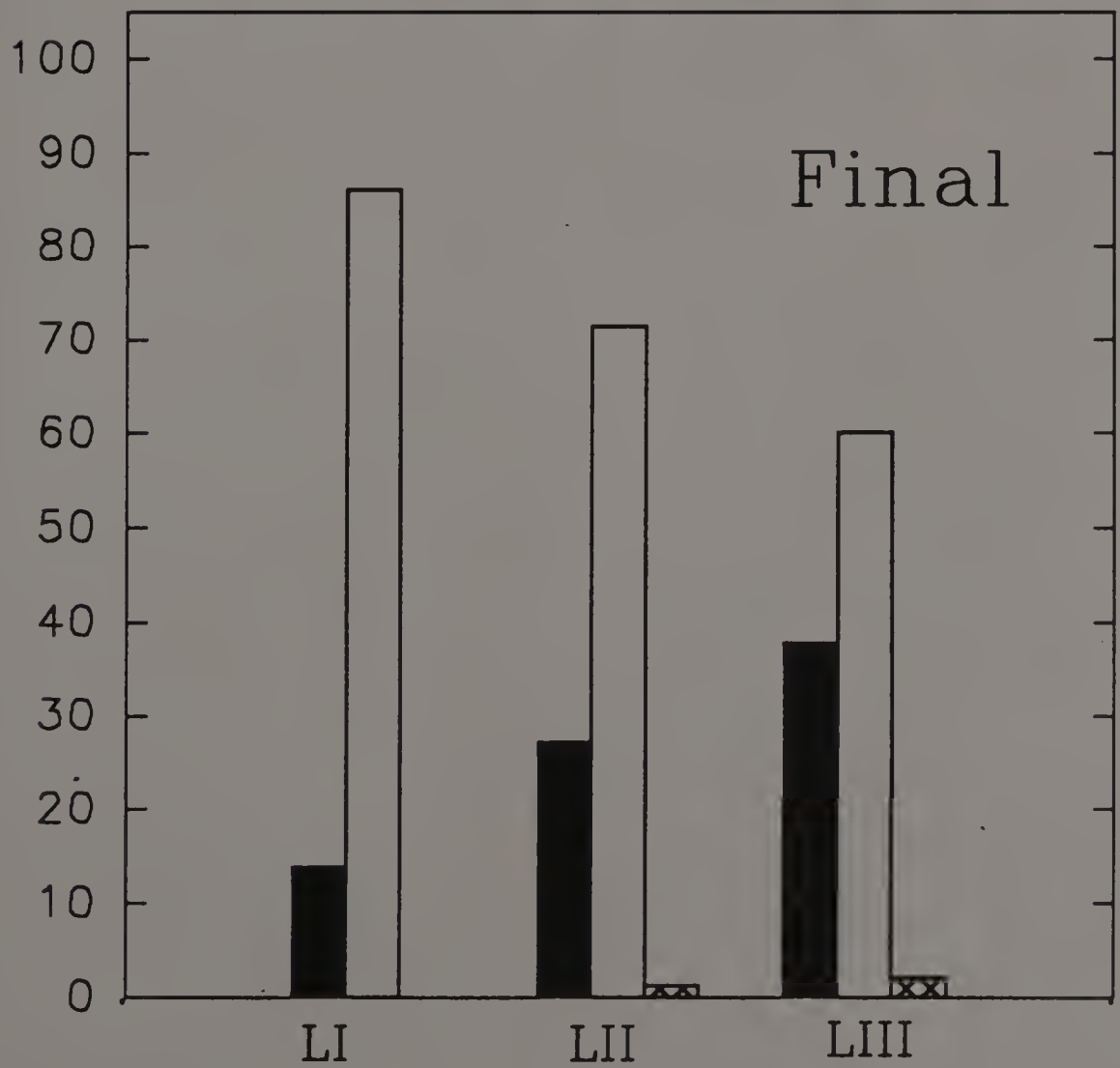
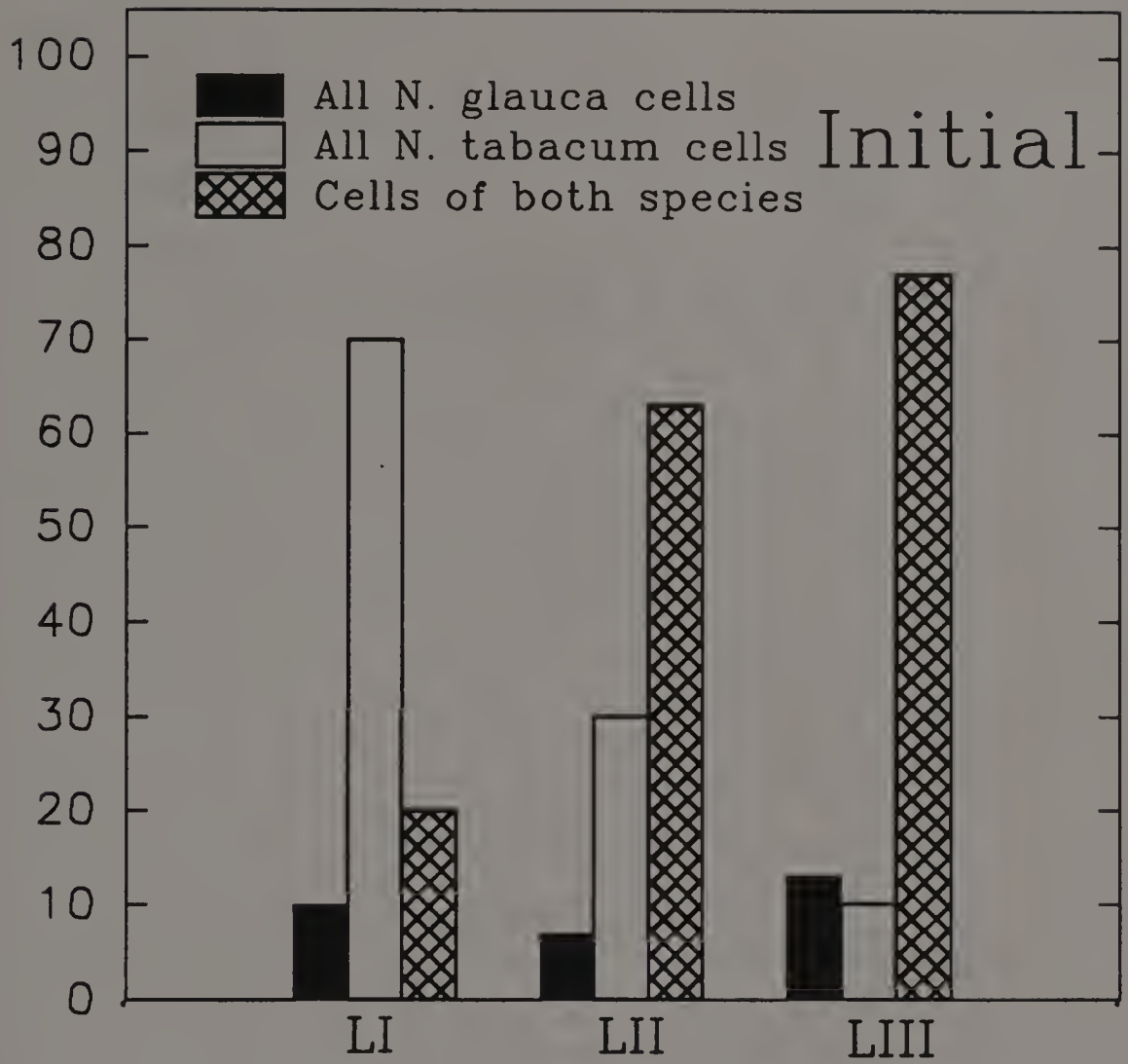


Table 5. Influence of source plant on the proportion of *N. tabacum* cells within apical cell layers of adventitious shoots at the early\* stage of development.

Source plant**	n	Percentage of chimeral shoots with the designated composition		
		LI > 50%T	LII > 50%T	LIII > 50%T
XTG	66	86	77	71
XGT	18	83	77	67

\* determined on the basis of observation of the 1st three to five leaves.

\*\* Because LI is not involved in shoot formation, data is pooled for source plants TTG and GTG and for source plants TGT and GGT.

Following meristem initiation, there is clearly a shift of cells within the shoot apex during growth. In all cases, the epidermis stabilized so that it contained only cells of the genotype which originally made up over 50% of the surface (Table 6). This indicates that for the epidermal cell layer of the early cell mass, it was the relative proportion of cells of a given genotype and not the genotype of the cells that mostly influenced the final composition. However, for the cell layers beneath the epidermis of adventitious meristems, the final composition of a layer did not always correspond to the relative area of tissue of a given genotype within an early cell mass. Ten out of 84 chimeric adventitious shoots which initiated from the early cell mass composed of less than 50% N. glauca in the inner cell layers, became entirely N. glauca after several nodes had formed (Table 6). In these ten shoots, it is possible that the "selection" of the apical initials in the inner cell layers was from a few cells which were of the minority within the mosaic cell mass.

Some shoots were generated which possessed cells of both genotypes in more than one apical layer. This allowed us to determine if shifts within one apical layer occurred independently from shifts in another. I could also determine if certain apical layers stabilized faster than others. Clearly, chimeric epidermis was transient and within a few nodes became genetically homogeneous, while in most cases it took more nodes for inner chimeric layers to "sort" (Table 6). In addition, the shift of cells in one apical cell layer could occur independently of the other apical layers, i.e. in some shoots, the complete 'sorting out' of either

Table 6. The number of nodes to "sort" and final composition of individual "apical layers" in all mericlinal chimeras as influenced by the "early" composition of the layer.

Apical layer*	Composition**		n	Mean number of nodes to sort $\pm$ S.E	Minimum : Maximum nodes to sort
	Early	Final			
LI	<50% G	100% T	14	3.57 $\pm$ 0.47	1 : 7
	<50% G	100% G	0	-	
	<50% T	100% G	4	2.50 $\pm$ 0.96	1 : 5
	<50% T	100% T	0	-	
LII	<50% G	100% T	37	4.38 $\pm$ 0.51	1 : 12
	<50% G	100% G	3	10.33 $\pm$ 2.33	8 : 15
	<50% T	100% G	10	3.40 $\pm$ 0.76	1 : 8
	<50% T	100% T	0	-	
LIII	<50% G	100% T	41	6.88 $\pm$ 1.15	1 : 34
	<50% G	100% G	7	12.86 $\pm$ 1.70	5 : 20
	<50% T	100% G	13	5.08 $\pm$ 0.89	1 : 12
	<50% T	100% T	1	3.00 $\pm$ 0.00	3 : 3

\* For the first 1-3 nodes, LI, LII, and LIII are operational terms since at this stage a true "tunica-carpus" meristem may not exist.

\*\* Percentage indicates fraction of layer occupied by the designated genotype, G = *N. glauca*, T = *N. tabacum*. Mericlinal layers of the three shoots which did not stabilize (see Table 5) are not included in the data.

genotype within each apical cell layer took place over a different number of nodes (Fig.5). In most cases, wider sectors were more persistent, especially if they persisted past the first 5 nodes. There is evidence that the stability of mericlinal chimeras is enhanced by the size of the meristem, which may in fact determine the apparent number of potential apical initials within a layer (Klekowski, 1988). Therefore, one could expect longer lasting sectors after the meristem had reached its mature size.

An analysis of the final disposition of the terminal apex indicated that there was not a random sorting into any one of the six possible periclinal arrangements or either one of the genotypes. For example, the terminal meristems in 39 of the 84 chimeric adventitious shoots (mericlinal or mosaic during initiation) ultimately became non-chimeric N. tabacum while only 4 chimeric shoots stabilized as non-chimeric N. glauca (Table 7). Clearly, the fact that the initial events of shoot formation favored N. tabacum in the early meristem cell layers, biased the final outcome (Table 4), and the possible competition between the cells of different genotypes during further development might not significantly affect this outcome. It is also worth noting that 15 chimeric adventitious shoots were stabilized and in some cases directly regenerated as TGG periclinal chimera (Table 7 and Table 2). In a previous study, adventitious meristems regenerated in culture from chimeric leaf discs also formed a majority of TGG shoots regardless of the position of the genetically



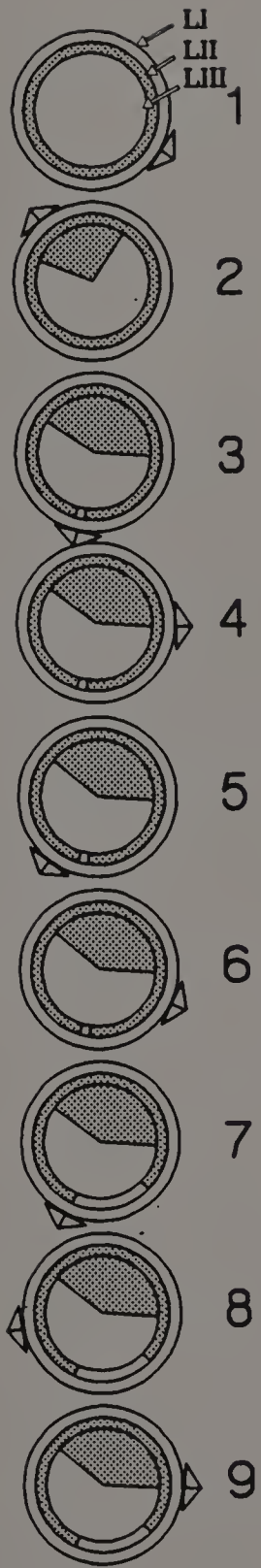


Table 7. Types of periclinal chimeral or non-chimeral shoots obtained after apical stabilization of the terminal meristem of mericlinal chimeras.

Source plant**	Final apical composition of the terminal shoot apex of "once mericlinal" adventitious shoots*								Still mericlinal***
	TTT	GGG	TGG	GTT	TTG	GGT	TGT	GTG	
TTG	16	3	4	3	2	1	1	0	0
GGT	5	0	0	2	1	0	2	0	1
TGT	2	1	3	0	1	0	0	0	0
GTG	16	0	8	2	8	0	0	0	2
Total	39	4	15	8	12	1	2	0	3

\* Six of the 15 TGG shoots were periclinal at the earliest visible stage (i.e., did not appear to be mericlinal)

\*\*GTT and TGG source plants produced only non-chimeral adventitious shoots

\*\*\*At the time of flowering

dissimilar cells in the explant (Marcotrigiano, 1986a). While the interaction between cells of different genotypes appears to play a significant role in the formation of the mosaic cell mass, the organization and final disposition of a mosaic shoot apical meristem can be influenced by stochastic process.

### **Spatial Analysis**

The chimeric adventitious shoots obtained were usually mericlinal when they were initiated. Many adventitious shoots were complex chimeras composed of several small sectors originating within a single apical layer. The first few leaves of most adventitious shoots were atypical in shape, had a poorly defined vascular network, and did not follow the normal phyllotaxy on extremely short internodes. Subsequent leaves appeared normal, were larger when fully expanded and were arranged in a consistent phyllotactic pattern. On 25% of the chimeric adventitious shoots, chimeric sectors were observed in only the first 1 or 2 leaves of the shoots with subsequently generated leaves being genetically homogeneous. Could it be possible that the first leaves of chimeric adventitious shoots may not originate as descendants of shoot apical initials, which may not exist at the time basal cells are committed to form the first leaves? Since apical initials do not divide as frequently as their descendants, sectors originating from genetically dissimilar apical initials should persist for many nodes as their daughter cells continue to make the major contribution to new tissue (Stewart and Dermen,

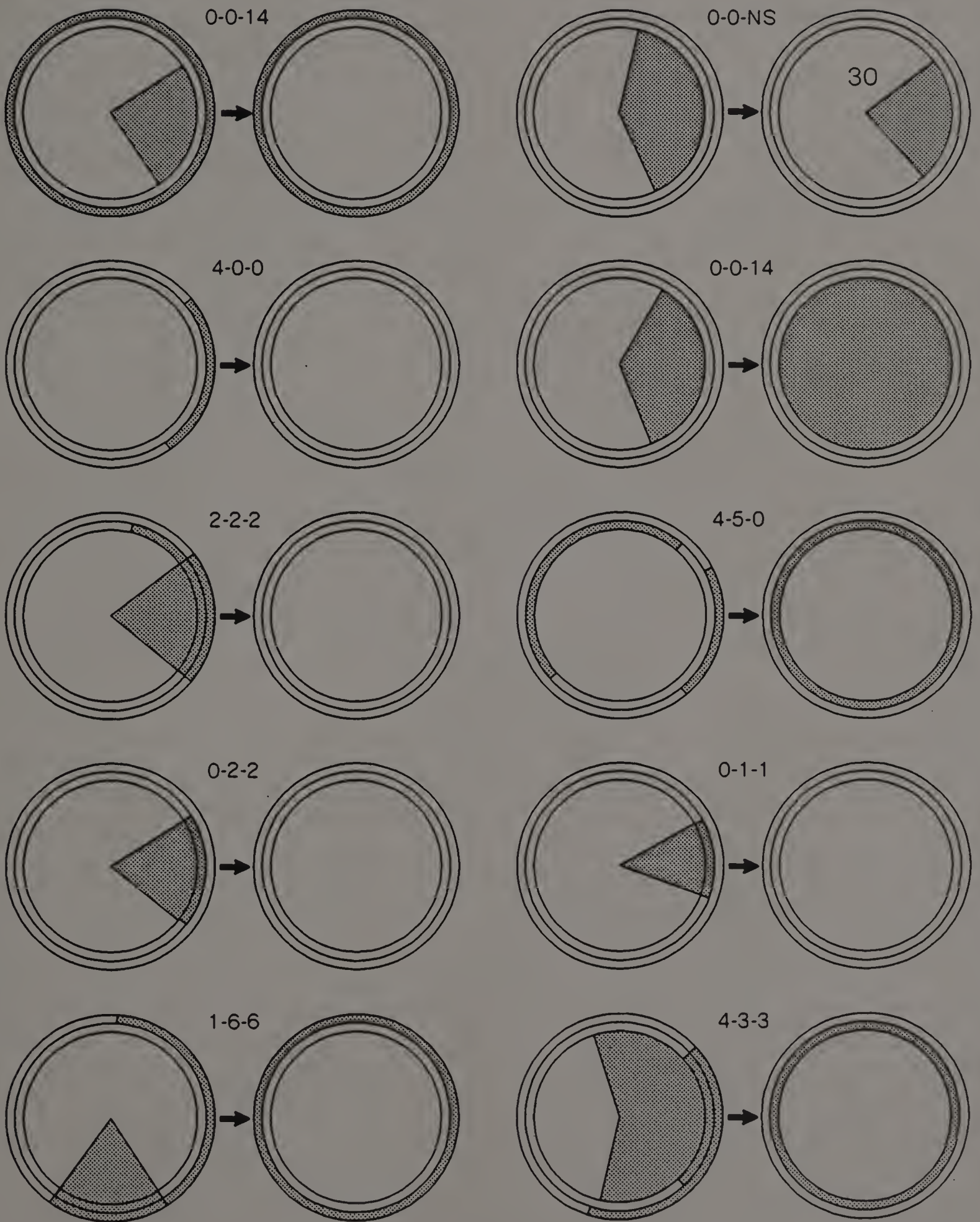
1970a). It is unlikely that one to two node sectors originate as the product of genetically dissimilar apical initials. Such small sectors are more likely derived from cells basal to the terminus of the apex. Christianson obtained similar ephemeral sectors on chimeric shoots regenerated from tissue-cultured leaf discs (Christianson, 1985). He induced phenotypically marked cell clones by irradiating heterozygous leaf tissue of a semi-dominant chlorotic mutant of tobacco prior to the initiation of adventitious shoot meristems. In Christianson's experiment, all of the "within leaf chimeras" (i.e., mosaics which had genetically unique cell clones in a single leaf) occurred in the lower 3-5 leaves of developing shoots. In contrast, chimeric tissue never extended down into the first 3-4 nodes on shoots which were periclinal or sectorial chimeras in their upper nodes. He concluded, therefore, that the lower and upper portions of the shoot arose from different groups of cells in tissue-culture generated shoots. I agree that different group of cells which give rise to the first few leaves may exist prior to the establishment of a well-defined apical meristem and extend this conclusion to adventitious shoots generated in situ.

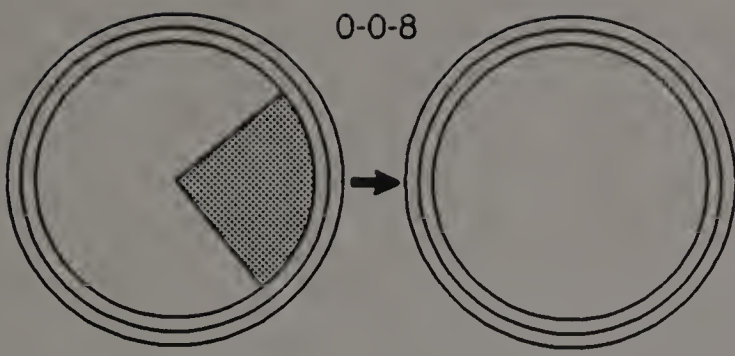
## APPENDIX

### MERISTEMS OF ADVENTITIOUS SHOOTS AT INITIAL AND FINAL STAGE

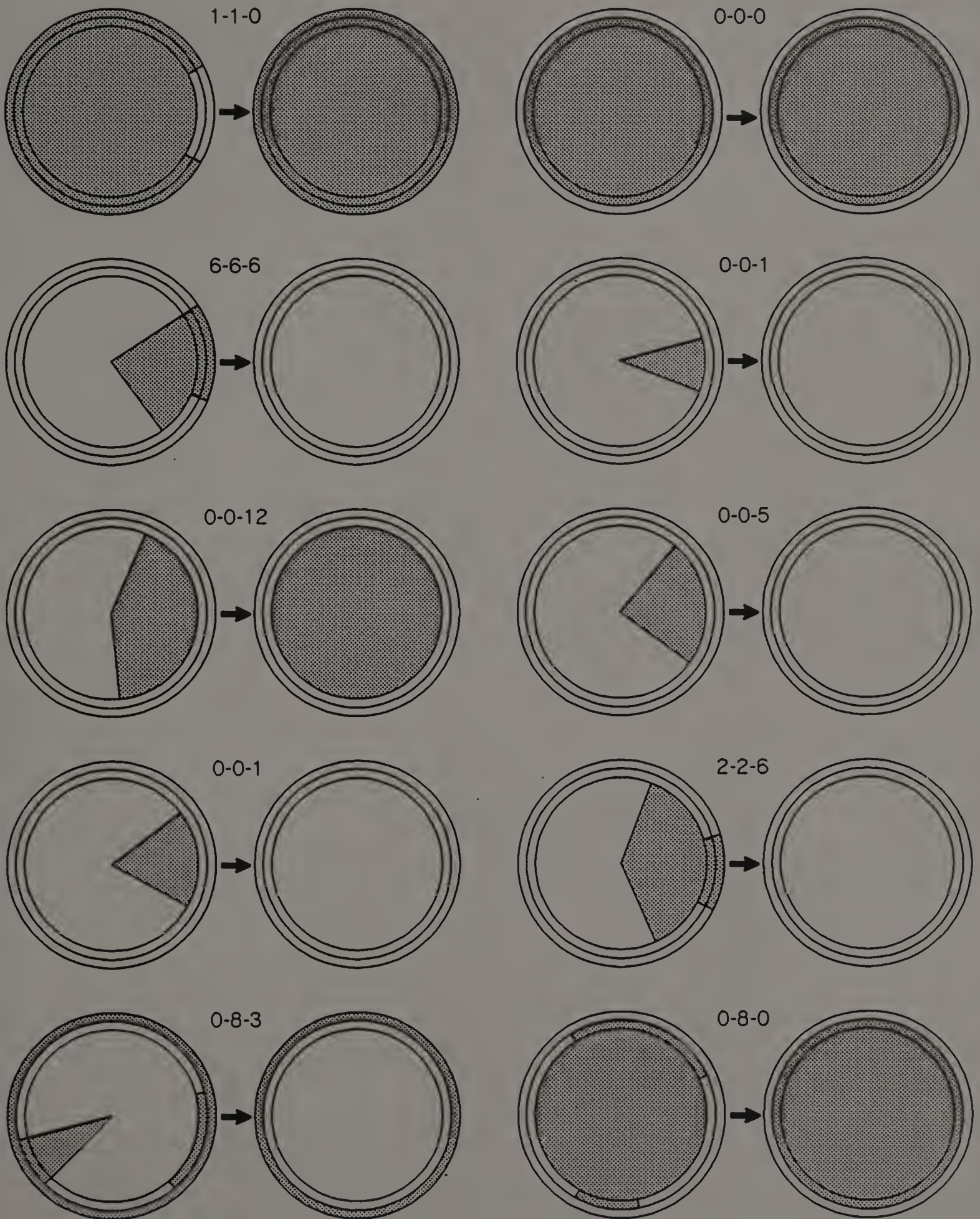
Diagrams represent the initial and final disposition of the apical cell layers of adventitious meristems for all 84 adventitious shoots regenerated in the experiment. The numbers between the circle diagrams represent the number of nodes a mosaic cell layer took to become homogeneous in composition. For example a 4-5-0 would indicate that the LI took 4 nodes and the LII took 5 nodes to become homogeneous. The "0" indicates that the LIII was homogeneous at the earliest detection possible. If a layer remained heterogeneous even in the flowering plant, the letters "NS" (not sorted) appear and a number is placed near that layer in the circle diagram to the right of the arrow. The number represents the number of nodes that this layer remained heterogeneous. The shaded areas represent Nicotiana glauca tissue while the unshaded areas represent Nicotiana tabacum Su/su.

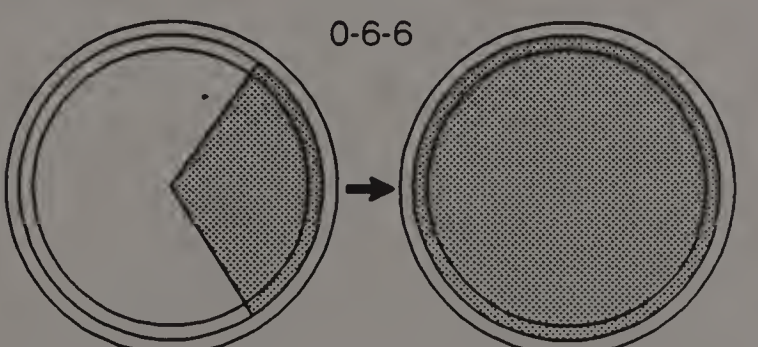
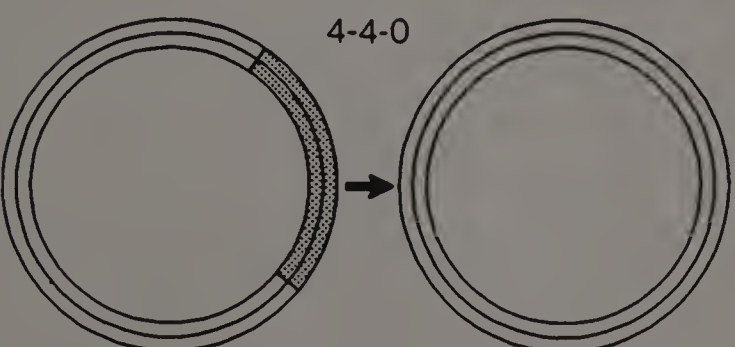
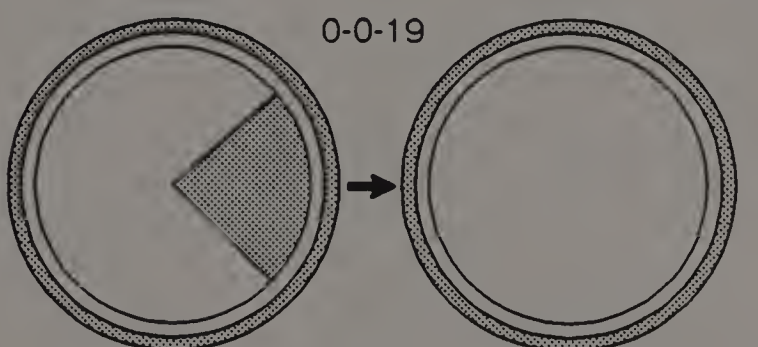
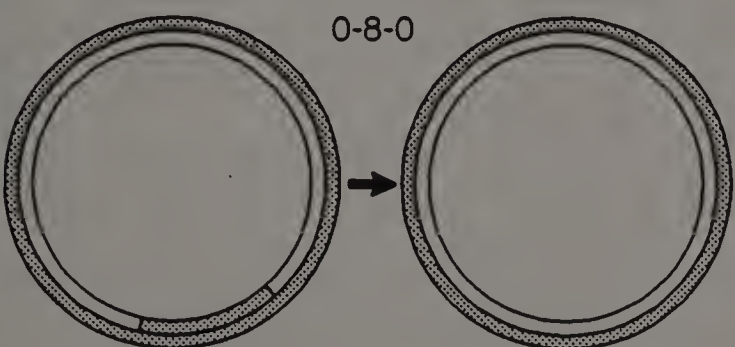
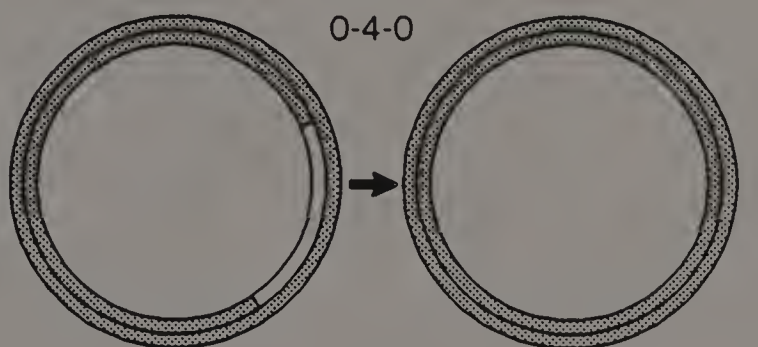
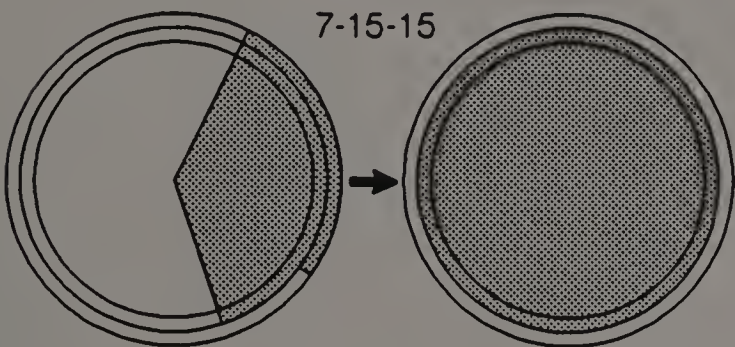
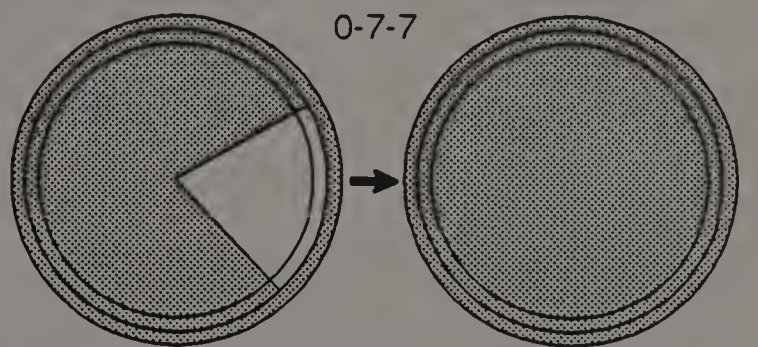
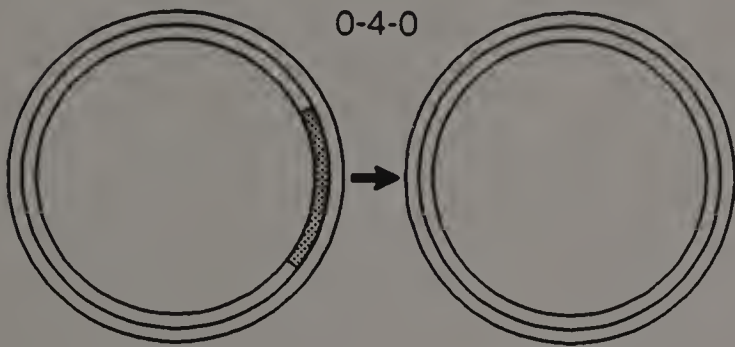
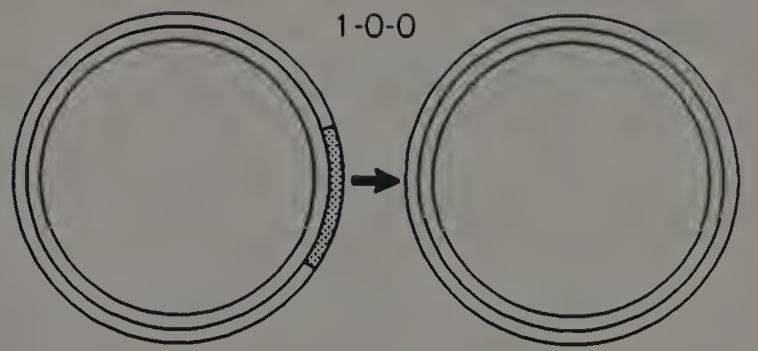
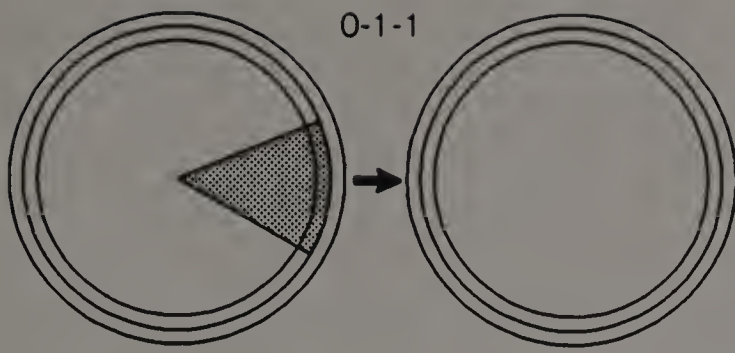
# Adventitious Shoots from GGT Chimeras

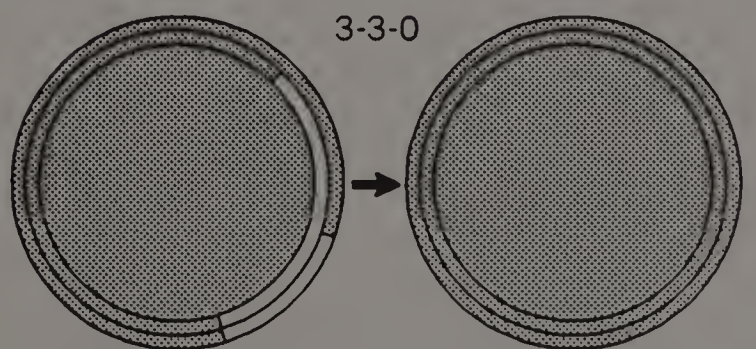
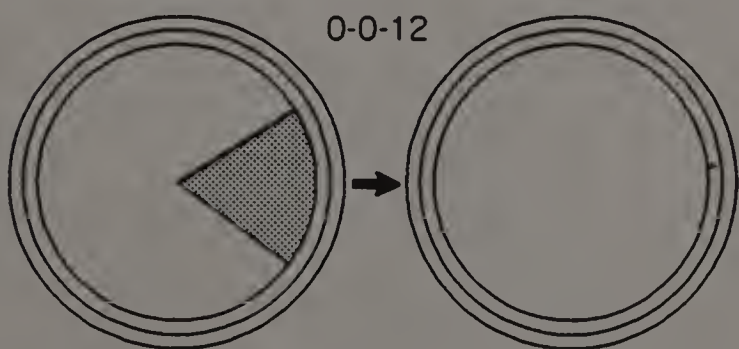
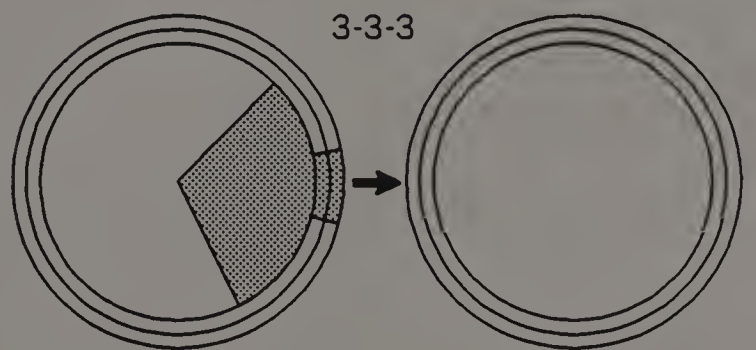
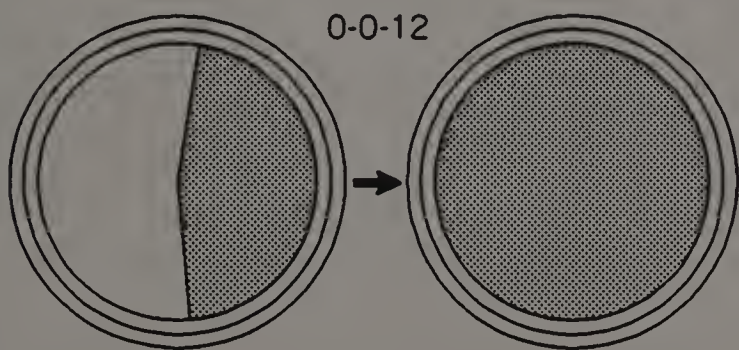
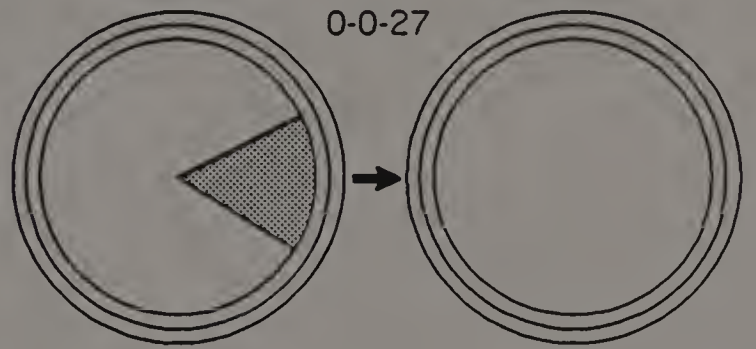
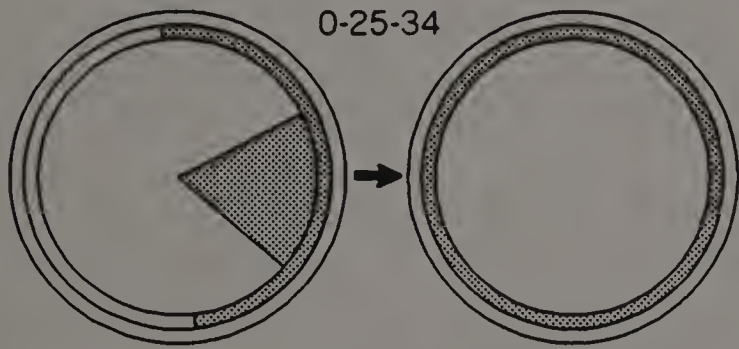
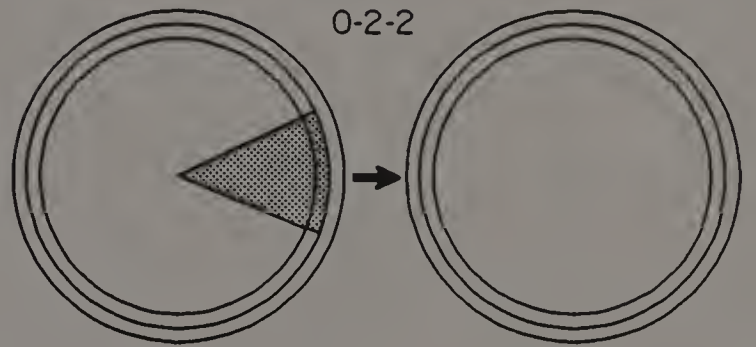
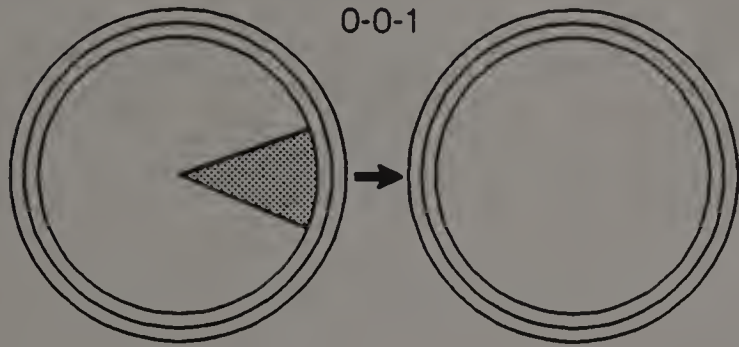
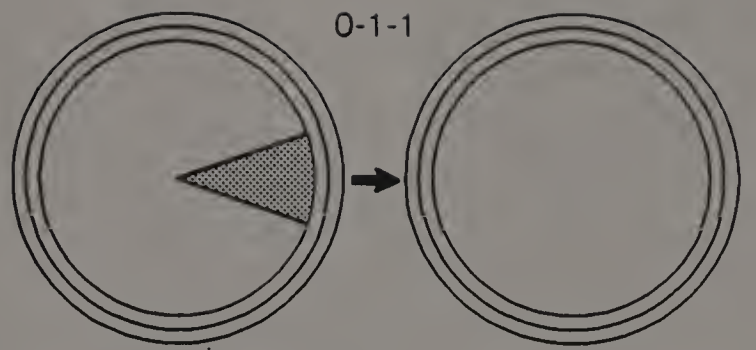
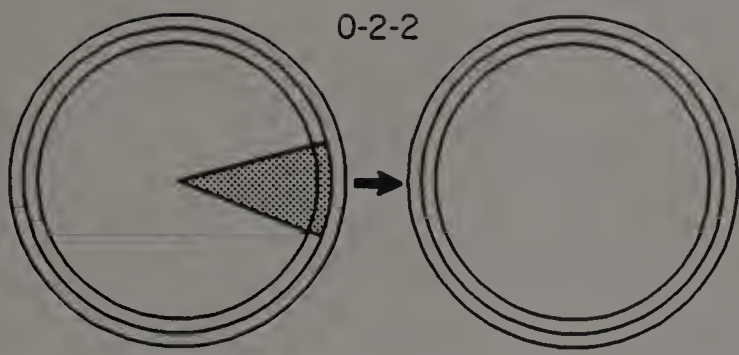




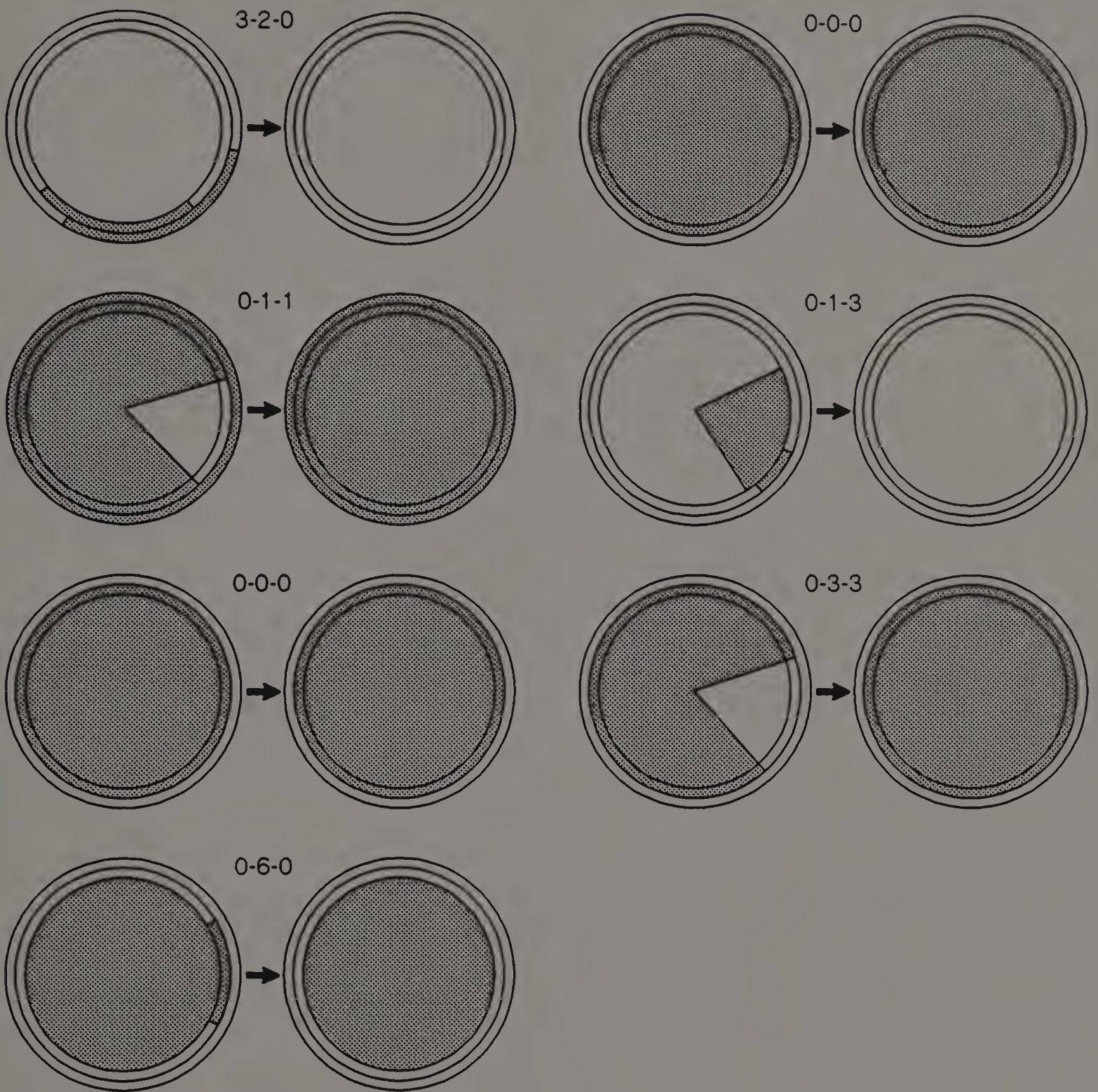
# Adventitious Shoots from TTG Chimeras



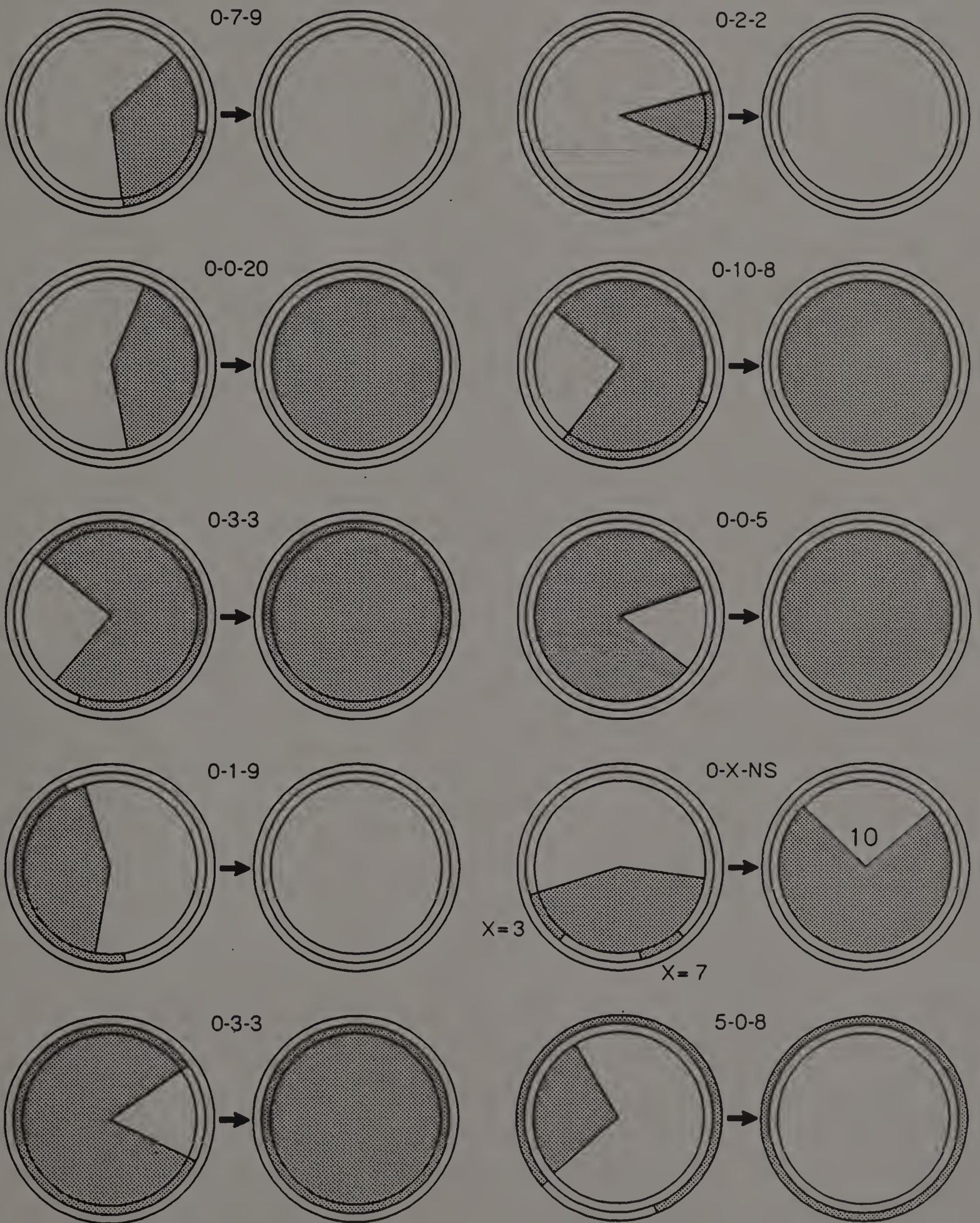


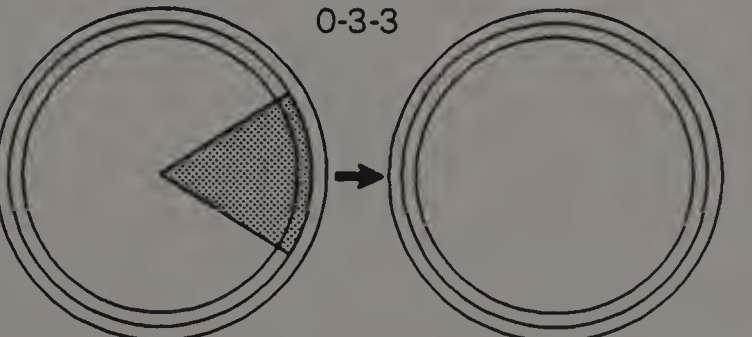
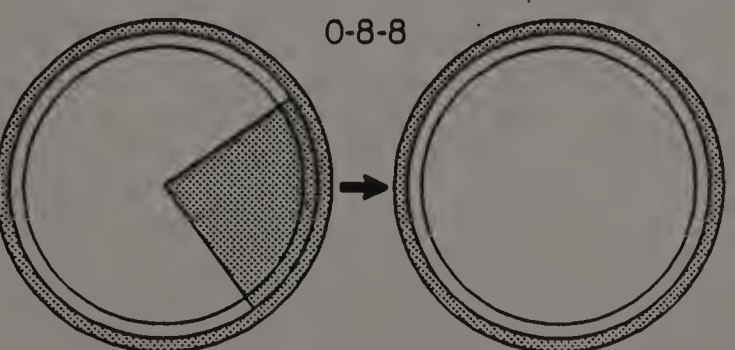
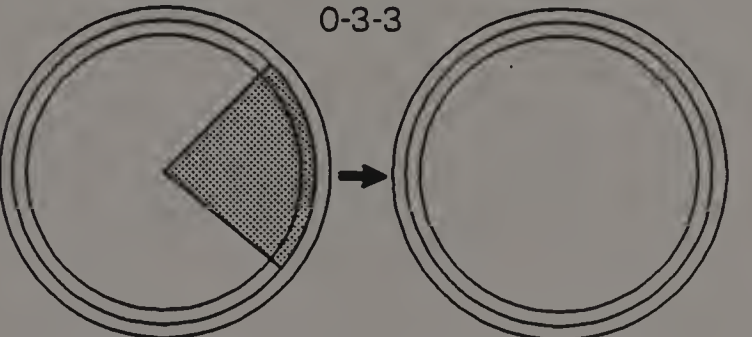
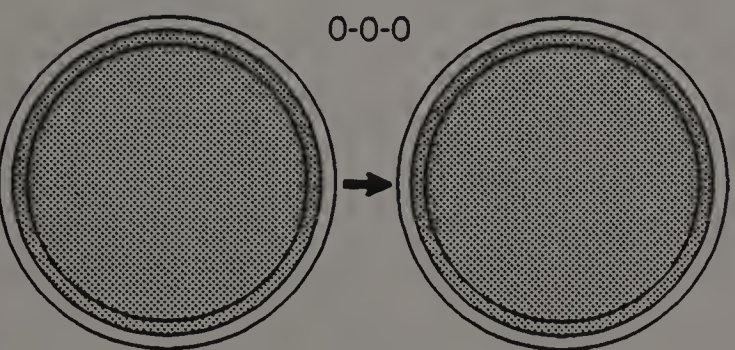
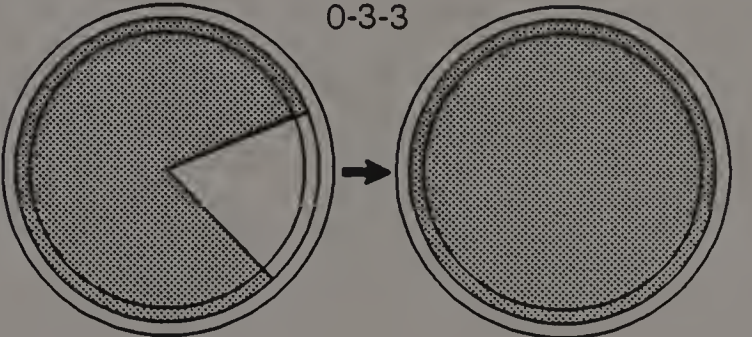
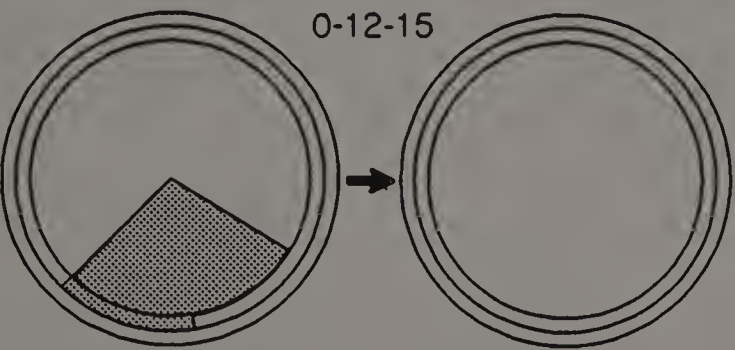
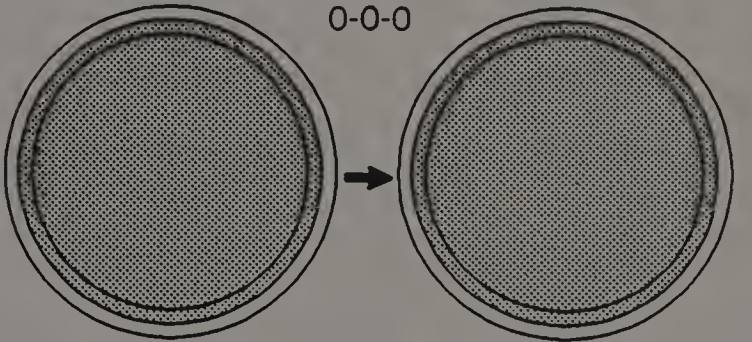
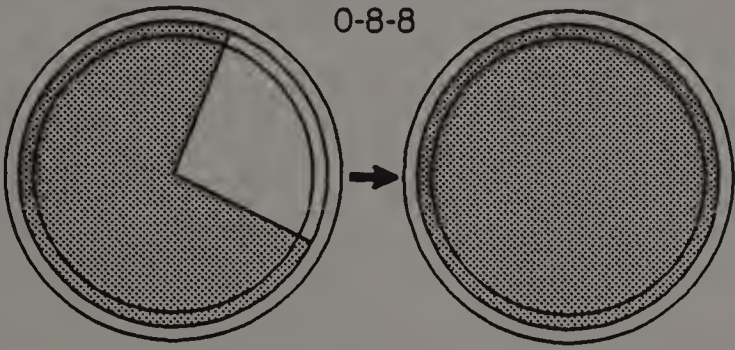
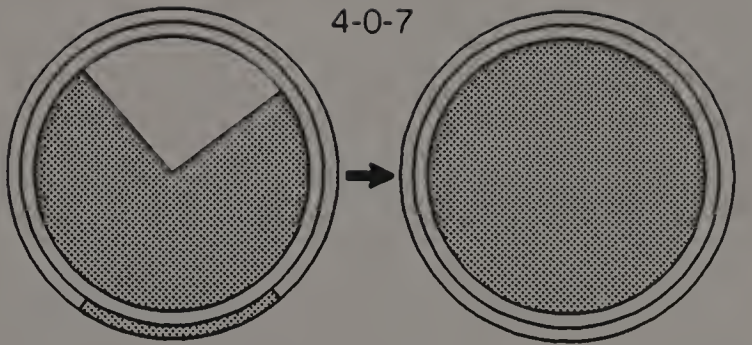
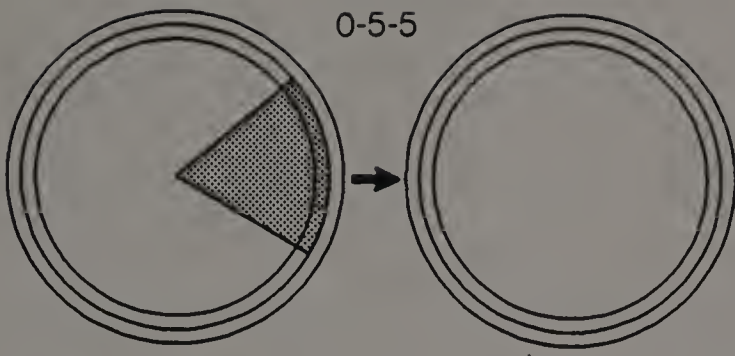


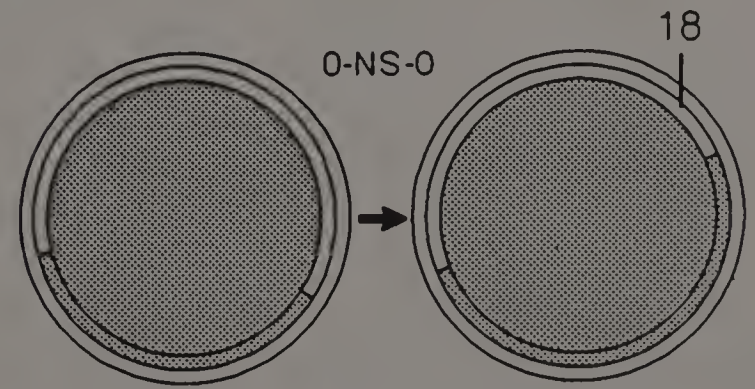
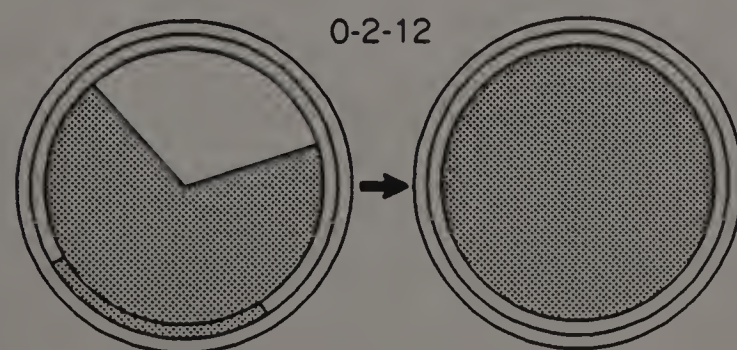
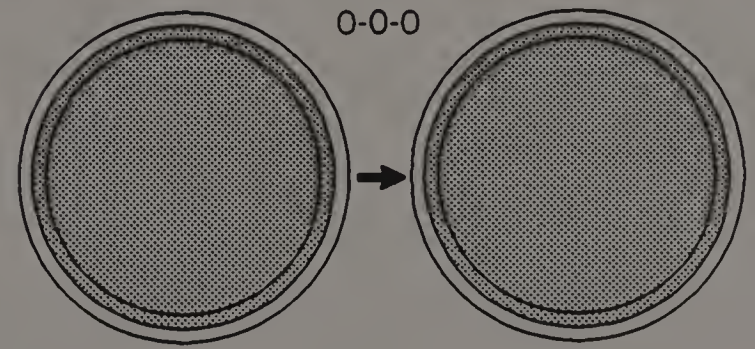
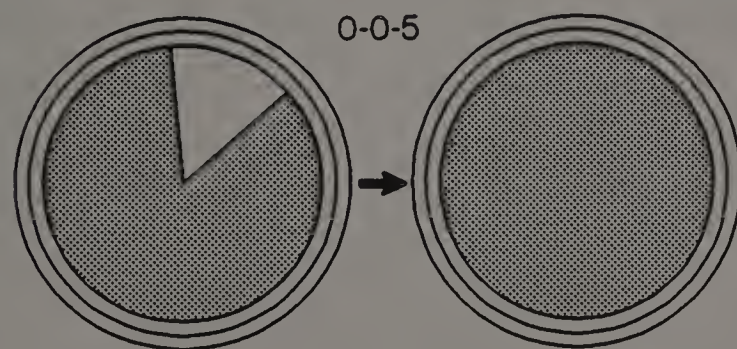
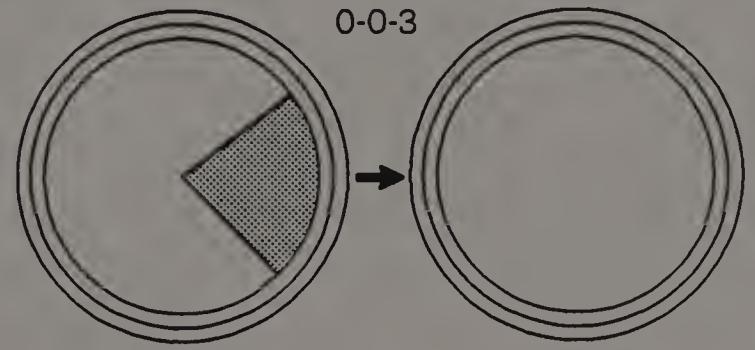
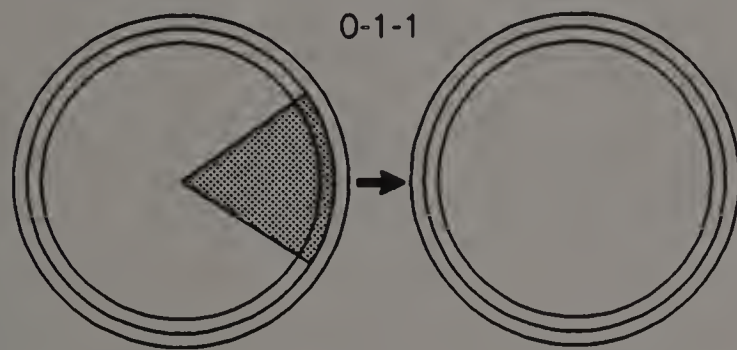
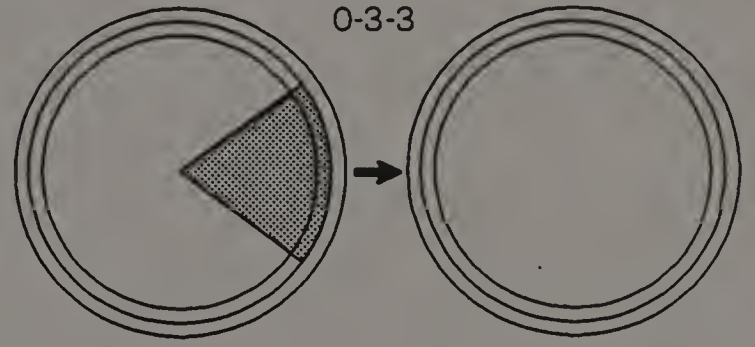
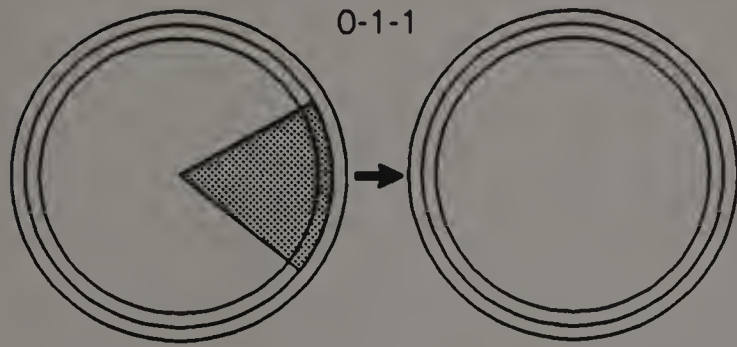
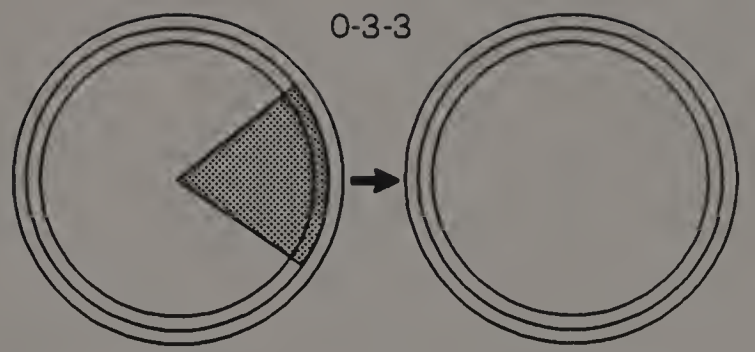
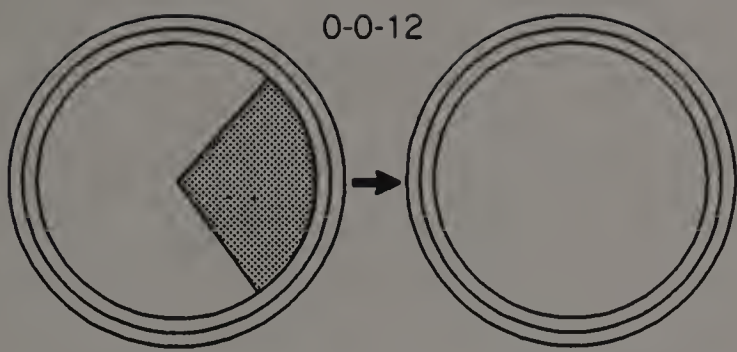
# Adventitious Shoots from TGT Chimeras

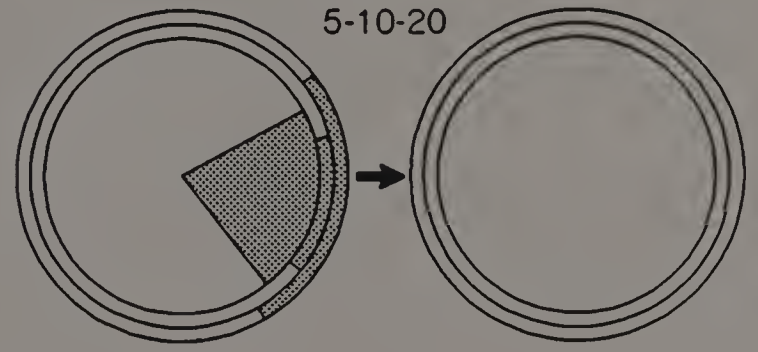
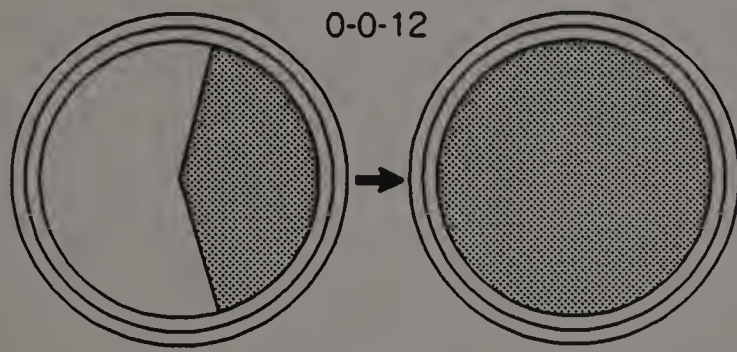
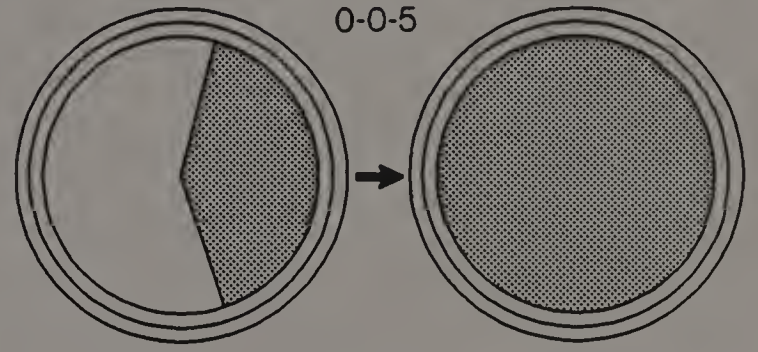
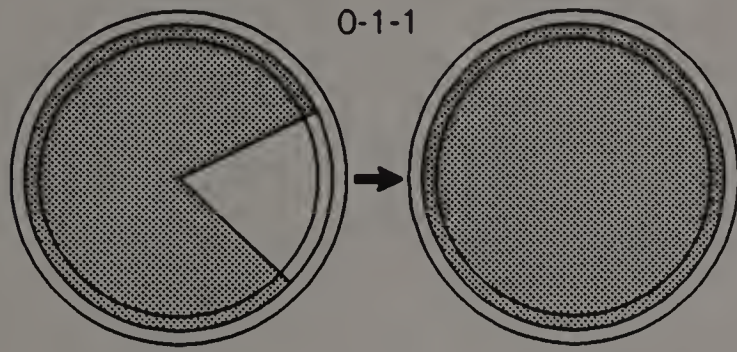
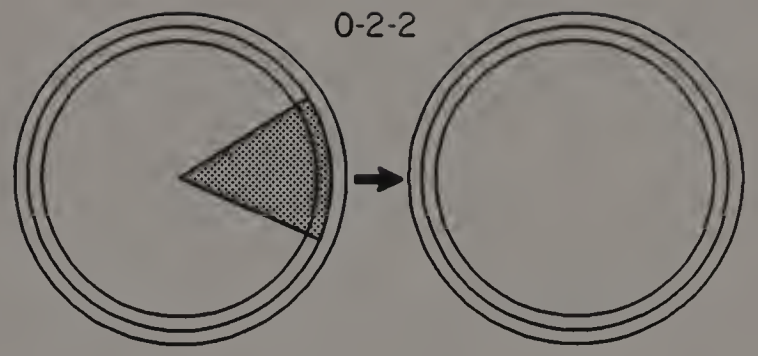
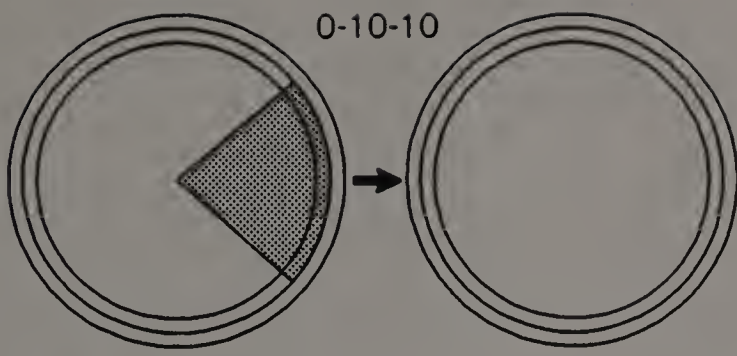


# Adventitious Shoots from GTG Chimeras









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