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Paramutation at the *sulfurea* Locus of *Lycopersicon esculentum* Mill.

VII. Determination of the Time of Occurrence of Paramutation by the Quantitative Evaluation of the Variegation

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Summary.

1. In tomato plants heterozygous for a mutant allele of the *sulfurea* (*sulf*) locus paramutation may take place: under the influence of a paramutant *sulf* allele, the paramutable wild type allele $sulf^+$, which is present in the same nucleus, is heritably altered with a definite frequency to a *sulf* mutant allele, either of the $sulf^{pura}$ or the $sulf^{vag}$ group.

2. A number of the $sulf^+sulf$ heterozygotes remain entirely green during their whole ontogenetic development (type I plants, without paramutation). However, others of the plants become variegated: these variegated plants contain – apart from green sectors – only yellow-green speckled $sulf^{vag}$ sectors (type II plants), or only pure yellow $sulf^{pura}$ sectors (type III plants) or both $sulf^{vag}$ and $sulf^{pura}$ sectors side by side (type IV plants).

3. For all variegated plants (types II, III and IV) we determined the sizes of the green and of the paramutant $sulf^{vag}$ and $sulf^{pura}$ sectors and made a statistical analysis of the values obtained.

4. We conducted observations over a period of three years and obtained following findings: type II plants (with $sulf^{vag}$ sectors) have an average size of the paramutant sectors of 27.9% (the whole plant being 100%). Type III plants (with $sulf^{pura}$ sectors) have an average sector's size of 25.7%, whereas the size of the paramutant sectors in type IV plants (with both $sulf^{vag}$ and $sulf^{pura}$ sectors) amounts to 54.4% (35.7% $sulf^{vag}$ and 18.7% $sulf^{pura}$). Thus, the occurrence of tissues of both phenotypes in one plant has, on the average, been found to be correlated with a doubling of the proportion of paramutant sectors in that plant.

5. Within $sulf^+sulf$ heterozygotes there is, in general, a positive correlation between the frequency of paramutant plants and the proportion of paramutant sectors within the plants. This is mainly due to the fact that there is a significant positive correlation between the frequency of type IV plants and the frequency of paramutant plants, i.e. the more plants within a progeny variegated, the greater the frequency of type IV plants containing both $sulf^{vag}$ and $sulf^{pura}$ sectors.

6. These findings (mathematically analysed and compared with the consequences of several models) may result in the following concept: the paramutation processes in $sulf^+sulf$ heterozygotes are restricted to a small group of cells (16 cells at the most) during a short period of about three cell generations after seed germination and expansion of the cotyledons. In the course of which, the probability for the occurrence of paramutation decreases rather quickly from one cell generation to the next. These characteristics of paramutation processes mentioned cause the occurrence of rather large and well defined sectors of paramutant tissue.

Key words: Genetic instability – Paramutation – Tomato – Sectors

Introduction

As compared to other cases of genetic instability, the paramutation found in higher plants is characterized by the following phenomenon: particular (paramutagenic) alleles of a gene induce, when they are heterozygous with other (paramutable) alleles, genetically stable or metastable genetic changes of the paramutable alleles (reviews: Hagemann 1969a; Brink 1974; Hagemann and Berg 1977). These induced and partially directed genetic changes in heterozygotes occur either in whole plants (for the *R* and the *B* loci in *Zea mays*) or in individual sectors of the plants (for the *sulfurea* locus in tomato and the *cruciata* gene of *Oenothera*). Of particular interest is the idea of Brink (1974) that paramutation is due to abnormal actions of chromosomal components which normally may control reactions of metabolism and ontogenetic differen-

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tiation. The finding that in most examples of paramutation the genetic inactivation of the paramutable allele occurs in somatic cells is in accordance with this hypothesis.

The paramutation system at the *sulfurea* (*sulf*) locus of the tomato is particularly suitable for determining the time in ontogenesis in which paramutation takes place because the genetic change of the wild type allele *sulf*$^+$ (for green plant colour) into a mutant *sulf* allele (determining yellow or yellow-green speckled colour) is directly visible in the changed plant sectors.

Part of the *sulf*$^+$*sulf* heterozygotes remain entirely green throughout their development (type I plants) whereas others become variegated; on these plants, apart from green heterozygous (*sulf*$^+$*sulf*) tissues, there also occurs mutant sectors with a pure yellow (*sulf*^{pura}) or a yellow-green speckled phenotype (*sulf*^{vag}). Such variegated plants may contain – besides green sectors – only *sulf*^{vag} sectors (type II plants) or only *sulf*^{pura} sectors (type III plants) or both *sulf*^{vag} and *sulf*^{pura} sectors (type IV plants). From these findings the question arises: in what sizes and in what proportions are *sulf*^{vag} and/or *sulf*^{pura} sectors formed by paramutation in heterozygous plants?

The percentage of variegated heterozygous plants (= the frequency of paramutation) differs in various lines depending upon the paramutation activity of the particular paramutagenic *sulf* allele present in this line. The *sulf*^{pura} group includes alleles with all possible degrees of paramutation activity between 0.5 and 100%; on the average it is between 3.6 and 92.9% (within several years) (comp. Hagemann 1969b). The *sulf*^{vag} alleles have a lower paramutation activity, the maximum being about 12%. (Paramutation activity of e.g. 90% means that 90 plants out of 100 *sulf*$^+$*sulf* heterozygotes are variegated, and 10 are entirely green.) It seemed reasonable that the paramutation activity of a paramutagenic allele does not only influence the frequency of variegated plants but also the amount of mutant sectors in variegated plants. Therefore, we studied the connection between the frequency of variegated plants and the proportion of *sulf*^{vag} and *sulf*^{pura} sectors.

Materials and Methods

For the quantitative evaluation of the degree of variegation we used progenies of heterozygous *sulf*$^+$*sulf* plants (after selfing) during the years 1970 to 1972. The frequency of paramutation (= percentage of variegated heterozygotes) was determined as follows: out of segregating progeny, 100 plants with green cotyledons (i.e. homozygous normal and heterozygous plants) were grown in the experimental field. From these plants, two thirds were expected to be heterozygotes. We thus determined the frequency of variegated plants (which showed paramutation) and calculated their frequency in relation to two thirds of the total amount of plants of this line (comp. Hagemann 1969b).

The heterozygous plants were divided into four classes: type I – plants entirely green, type II – paramutant tissue exclusively *sulf*^{vag}, type III – paramutant tissue exclusively *sulf*^{pura}, and type IV – paramutant tissue partly *sulf*^{vag} and partly *sulf*^{pura}. (This classification was also used by Uth 1972).

The proportion of paramutant tissue within a variegated plant was determined for fully developed plants in the experimental field. Each plant was taken to be 100% and the proportion of paramutant tissue was determined at 10%-intervals. If, e.g., half of a plant was green, three tenths were paramutant *sulf*^{vag} and two tenths were paramutant *sulf*^{pura}, we recorded for this plant: 50% green, 30% vag (= *sulf*^{vag}) and 20% pura (= *sulf*^{pura}). In order to determine the average proportion of the different sectors we added the individual values for all plants of the progeny and divided it by the number of variegated plants. The statistical evalutation was performed with the χ^2 test (test of homogeneity) and with Spearman's rank correlation coefficient (comp. Rasch et al. 1973).

Results

The frequency of the different types of paramutant plants (types II, III, IV) differed considerably during the years 1970, 1971, 1972. While in 1970 and 1972 about half of the paramutant plants contained both *sulf*^{vag} and *sulf*^{pura} tissue (= type IV), this group was almost lacking in 1971. (This was mainly due to the fact that in the summer of 1971 we had very bad weather conditions – at the beginning it was rainy and cool and afterwards, very dry, so that plant growth was very poor.) The frequency of variegated plants is given in Table 1. Table 2 shows the average proportions of paramutant tissues in types II-IV. In these results differences can also be seen between different years. In 1970 a higher proportion of tissues was paramutant than in the following two years. (Later experiments testing the influence of different temperatures showed that indeed the proportion of paramutant tissue differs under various temperatures.)

However, it has to be emphasized that within each plant type (II, III and IV) the proportion of paramutant tissues is statistically homogeneous (Tab. 3, lines 1, 2, 4, 5, 6). Even when adding the results of all three years, no

Table 1. Frequency of different types of paramutated plants. Type II: green + sulf^{vag}. Type III: green + sulf^{pura}. Type IV: green + sulf^{vag} + sulf^{pura}

Year	n	Type II (%)	Type III (%)	Type IV (%)
Diploids				
1970	154	42.9	15.6	41.6
1971	398	93.5	2.7	3.8
1972	588	45.3	3.1	51.6
Tetraploids				
1970	996	90.9	1.3	7.8

n = number of paramutated plants

Table 2. Proportion of paramutant tissues in the three types of paramutated (= variegated) plants

Year	Type II		Type III		Type IV			
	n	$sulf^{vag}$ %	n	$sulf^{pura}$ %	n	$sulf^{vag}$ %	$sulf^{pura}$ %	sulf %
Diploids								
1970	66	40.3	24	30.0	64	47.3	22.2	69.5
1971	372	29.5	11	22.7	15	16.6	12.7	29.3
1972	262	22.2	18	22.2	298	34.1	18.1	52.2
Sum	724	27.9	54	25.7	377	35.7	18.7	54.4
Tetraploids								
1970	905	41.1	13	29.2	78	39.5	23.1	62.6

n = number of variegated plants per type; sulf = the sum of $sulf^{vag}$ and $sulf^{pura}$ tissue

Table 3. Tests of homogeneity (x^2) for the proportions of paramutated sectors in single progenies (P) or lines (groups of progenies, L)

Progenies or lines tested			n		p
1	1970	type II, L 1,2 $sulf^{vag}$	82	+	0.1
2		type IV, L 1,2	64	+	0.05
3		type II vs. type IV, all L	146	−−	
4	1971	type II, L 1-3	372	+	0.1
5	1972	type II, all P with more than 20 plants	160	+	0.9
6		type IV, all P with more than 11 plants	288	+	0.99
7		type II vs type IV with more than 13 plants	500	−−−	
8	1970-72	type II, all P with more than 18 plants	496	+	0.5
9		type II sum	724	−−	
10		type III sum	54	+	0.7
11		type IV sum	483	+	0.1

n = number of paramutated plants
+ = no significant heterogeneity with the probability p
−−, −−− sample heterogeneous at 0.01 and 0.001 levels, respectively

statistically significant differences within each type are revealed (with only one exception; Tab. 3, lines 8, 10, 11). The total sum for type II seems to be heterogeneous (Tab. 3, line 9); but if one takes into account only larger progenies with more than 18 type-II-plants, then significant differences cannot be found (Tab. 3, line 8).

In contrast to the homogeneity within type II and type IV plants, there is a highly significant difference between type II and IV with regard to the proportion of paramutant tissues (Tab. 3, line 3 and line 7): the paramutant sectors of type IV have, on the average, double the size as those of type II (Tab. 2); two thirds of the paramutant sectors are of *$sulf^{vag}$* phenotype and one third is of *$sulf^{pura}$* phenotype (Fig. 1).

Almost the same values for the size of the paramutant sectors have been found for tetraploids as for diploids, when compared for the same year (Fig. 2); therefore, the same mechanism in principle seems to operate for the occurrence of the paramutant sectors in heterozygous diploids and tetraploids. However, a difference can be observed with regard to the frequency of type II- and type III-plants: in 1970, type II- and type IV-plants were equally frequent among the diploids (about 40% each); in contrast, among the tetraploids 91% of the variegated plants contained only *$sulf^{vag}$* tissue (= type II), whereas only 7,8% of the variegated plants contained both *$sulf^{vag}$* and *$sulf^{pura}$* tissue (= type IV).

Still more insight into the characteristics of paramutation can be obtained from the study of the distribution patterns of the sector size within the different types of variegated plants (II, III, IV). In Figure 3 a comparison is made between the sector sizes within types II and IV. For this analysis we used the data of 1972 (the data of other years show the same results in principle). The individual values for the sector size in both types vary considerably. The mean value for type II plants is 22 ± 13% paramutant tissue per plant and for type IV plants 52 ± 15%. Both mutant phenotypes (*$sulf^{vag}$* and *$sulf^{pura}$*) in one plant are

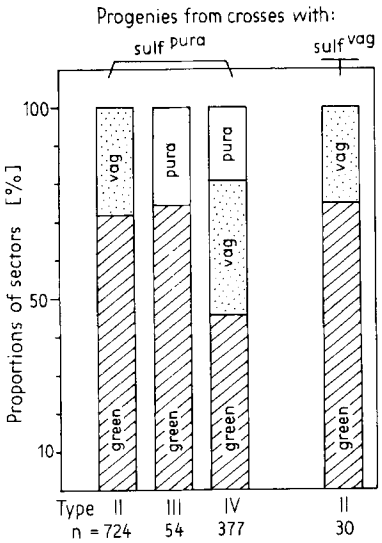

Fig. 1. Proportions of green (*sulf*$^+$.), yellow-green speckled (*sulf*^{vag}.) and yellow (*sulf*^{pura}) sectors in diploid plants heterozygous for a *sulf*^{pura} or a *sulf*^{vag} allele (plants evaluated 1970-72)

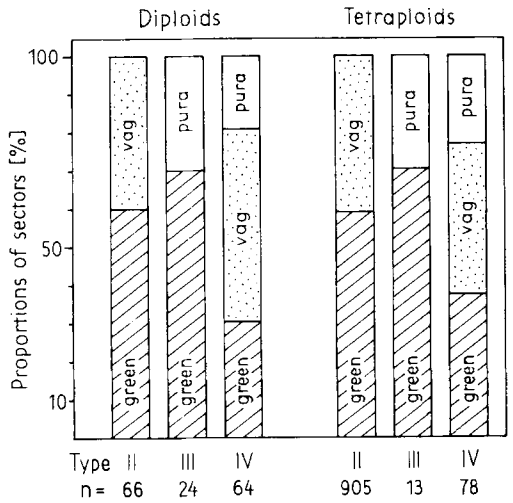

Fig. 2. Proportions of green, yellow-green speckled and yellow sectors in diploid and in tetraploid *sulf* heterozygotes (plants evaluated 1970)

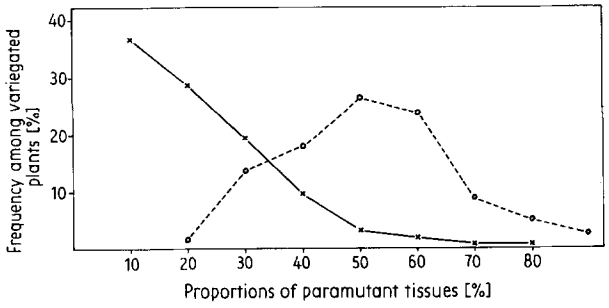

Fig. 3. Relative frequencies of variegated (= paramutated) plants with paramutant sectors of various sizes (1972)
(——— type II: green + *sulf*^{vag}
– – – – type IV: green + *sulf*^{vag} + *sulf*^{pura})

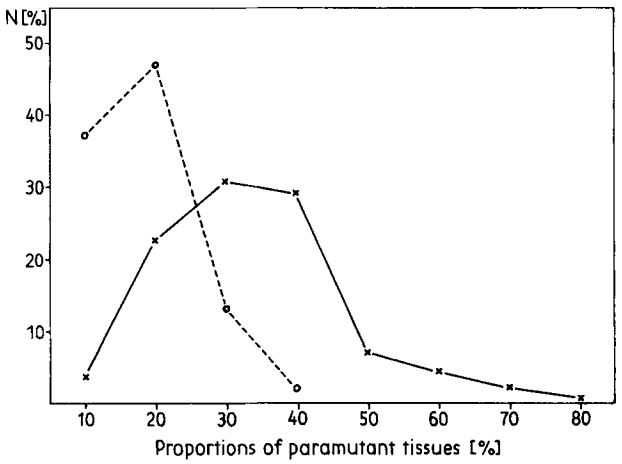

Fig. 4. Relative frequencies of *sulf*^{vag} sectors (——) and of *sulf*^{pura} sectors (– – –) among type IV plants (sectors evaluated separately) (1972)

Table 4. Spearman rank correlation coefficient r_s for frequency of paramutation and different paramutated phenotypes

Correlation between frequency of paramutation and	r_s	
1. average proportion of *sulf* tissue	0.6112	**
2. average proportion of *sulf*^{vag} tissue	0.4552	**
3. average proportion of *sulf*^{pura} tissue	0.5782	**
4. frequency of type II plants	0.5517	**
5. frequency of type III plants	0.4027	*
6. frequency of type IV plants	0.6032	**

*, ** significant correlation at 0.05 and 0.01 level

on the average only found in plants which have double the size of mutant tissue compared to plants with only mutant *sulf*^{vag} phenotype.

The difference between *sulf*^{vag}- and *sulf*^{pura}-tissue in type IV plants reveals that a type IV plant cannot merely be considered to be the sum of type II and type III. For the relatively few type III plants (with only paramutant *sulf*^{pura} tissue) the correspondence with the *sulf*^{pura}-sector in type IV cannot be disputed (Table 2). But the amount of *sulf*^{vag} sectors in type IV plants differs significantly from the percentage of *sulf*^{vag} sectors in type II plants (Fig. 3, 4). This refers both to the mean values (type II: 22%; type IV: 34% *sulf*^{vag}) and to the distribution of the values (one-sided vs. one peak). These differences in distribution are statistically highly significant (χ^2 = 113, 6 degrees of freedom, $P < 0{,}1\%$).

Having characterized the quantitative distributions of paramutant tissues in variegated plants, the question arises whether there is a connection between the frequency of paramutation (in a number of heterozygous plants) and the amount of paramutant sectors in these plants. For

that purpose we compared the frequency of paramutant (variegated) plants separately in several progenies with the average sector size (Table 4, lines 1-3) and the frequency of the different plant types (I-IV) (Table 4, lines 4-6).

Spearman's rank correlation coefficient clearly demonstrates a positive correlation between the frequency of paramutant plants in a progeny and the proportion of paramutant sectors (both *sulf*^{vag} and *sulf*^{pura}). The general positive correlation is due to the fact that there is a significant positive correlation between the frequency of type IV-plants (with *sulf*^{vag} and *sulf*^{pura} sectors) and the frequency of paramutant plants (in contrast type II is negatively correlated with the frequency of paramutant plants; type IV seems to increase at the cost of type II). Moreover, there is also a slight positive correlation between the number of type III plants and the frequency of paramutation.

The frequency of paramutation, i.e. the frequency of paramutant plants, does not directly increase the size of the paramutant sectors within variegated plants; rather, the more plants within a progeny variegated, the greater is the frequency of type IV plants. Very important for the interpretation of these results is the fact that the occurrence of tissues of both phenotypes (*sulf*^{vag} and *sulf*^{pura}) in one plant is, on the average, connected with a doubling of the proportion of the paramutant sectors in that plant.

Discussion

In heterozygous *sulf*$^{+}$ *sulf* plants (*sulf* meaning *sulf*^{vag} or *sulf*^{pura}) paramutation takes place regularly: the paramutable wild type allele *sulf*$^{+}$ is heritably altered under the influence of a paramutagenic *sulf* allele which is present in the same nucleus. The *sulf*$^{+}$ allele is altered with a definite frequency to a *sulf* mutant allele, either of the *sulf*^{vag} or the *sulf*^{pura} group. These changes cause the occurrence of sectors with mutant phenotypes in a heterozygous plant. In all cases the cotyledons of heterozygotes are still green. Sectors with *sulf* mutant phenotype can be earliest seen on the primary foliage leaves (Hagemann 1958, 1965, 1966, 1969a; Hagemann and Berg 1977).

Formerly, we tended to suppose that the change of *sulf*$^{+}$ to a *sulf* allele can take place with equal probability in many, or even in all, somatic cells of *sulf*$^{+}$*sulf* heterozygotes, beginning with the formation of foliage leaves. However, the results of the analysis of the sector size of paramutant tissues made the idea of a somatic and cell-autonomous change over a long ontogenetic period rather improbable.

In order to study this problem more thoroughly we formulated three hypotheses, worked out the mathematical consequences of these models and compared them with the experimental results. This analysis has been worked out in detail by Berg (Ph.D. thesis 1975); a short version of it is given in the Appendix of this paper. The hypotheses are:

Hypothesis I: Paramutation occurs in many somatic cells independent of each other: 'multiple autonomous events'.

Hypothesis II: Paramutation occurs in somatic cells depending upon a single primary change in early plant ontogenesis: 'early switch-on mechanism imprinting the later events'.

Hypothesis III: Paramutation can take place only in a very short period of early plant ontogenesis (during about 3 cell generations) in few somatic cells (16 at the most) independent of each other; the probability of the occurrence of paramutation decreases during this period rather quickly: 'independent events during a restricted sensitive stage'.

The comparison of the mathematical consequences of these three hypotheses with the experimental results clearly showed that the expectations developed from hypothesis III are in full accordance with the experimental findings. The paramutation processes in *sulf*$^{+}$*sulf* heterozygotes are obviously restricted to a small group of cells during a rather short period of about three cell generations after seed germination and expansion of cotyledons. During this period the probability for the occurrence of paramutation decreases rather quickly from one cell generation to the next. At first the paramutation alters the wild type allele mostly to a *sulf*^{vag} allele (partial inactivation of the *sulf* gene) and only afterwards to a *sulf*^{pura} allele (full inactivation). (Details of the calculations are given in the Appendix of this paper.)

A narrow limitation of independent paramutation events to an early ontogenetic period of somatic plant development like that of the *sulfurea* system of the tomato has not been shown for any other paramutation system.

The paramutation at the *B* locus of *Zea mays* takes place, according to Coe (1968), in one step shortly before meiosis or even during meiosis, but before anaphase I. In contrast, all studies of the paramutation systems at the *R* locus of maize demonstrate that the paramutation occurs gradually in many somatic cell generations (Brink, Styles and Axtell 1968; Brink 1974; McWhirter and Brink 1963; Sastry, Cooper and Brink 1965). A very similar interpretation is applicable to the paramutation processes in heterozygous rogue peas. In rogue heterozygotes the rogue phenotype gradually increases from node to node during the ontogenetic development of the plants; in accordance with this phenotypic change the proportion of rogue homozygotes from flowers originating at different nodes increases simultaneously (Bateson 1926; Dodds 1963). The changes at the *cruciata* locus of *Oenothera* have not been studied in detail with regard to this question but they also

seem to occur gradually. Renner (1937, 1959) described, partly on the same plant, normal, intermediate and cruciate flowers. But these three types do not reflect the whole intra-individual variability since Oehlkers (1938) distinguished between 7 different phenotypic classes with normal and fully cruciate flowers as the extremes.

The limitation of the paramutation events at the *sulfurea* locus to a short ontogenetic period can perhaps be related to the idea of Brink (1974) that paramutation may be due to the abnormal action of chromosomal components which normally control metabolic or differentiation processes. In the present case we might assume a connection between the paramutation processes and the differentiation of meristems for the formation of the first foliage leaves.

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Appendix
Mathematical Formulation of Alternative Hypotheses for the Occurrence of Paramutation during Tomato Ontogeny

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1 Hypothesis I: Paramutation Occurs in Many Somatic Cells Independent of Each Other: 'Multiple Autonomous Events'

The size of a paramutated tissue patch is related to the number of cell generations between the paramutation event and the final state of the plant. Therefore, it depends on the period in which paramutation is possible during the ontogenetic development and the paramutation probability of the cells.

1.1 Expectation Values

The following symbols are used:
p – probability of changing *sulf*$^+$ to *sulf* in a cell;
n – the number of cells in which genetic changes are possible independent of each other.
The probability of totally green plants without any paramutation event is

$$W = (1 - p)^n. \quad (1)$$

This can be determined experimentally by the frequency of paramutation (1 – W)

1.1.1 Number of Paramutation Events per Variegated Plant

Since in some progenies more than 90% green plants (W > 0,9) have been found, the paramutation probability p must be very low supposing many paramutable cells. Therefore, it is possible to assume the POISSON distribution

$$P_k = \frac{\lambda^k}{k!} e^{-\lambda} \quad (2)$$

to calculate the probability P_k observing a (paramutation) event k-times in a plant. Thereby,

$$\lambda = np$$

is the average frequency of events and can be determined by the experimentally estimated part of green plants (k = 0)

$$P_0 = e^{-\lambda}. \quad (3)$$

According to equation (2), at low frequencies of paramutation one can expect only one paramutation event per plant at least in 95 or 90% of all variegated descendants (Fig. 5). At high frequencies of paramutation (i.e. 90%), however, the most frequent number of events per plant increases to three but more than six would be very unlikely.

On the other hand, assuming the number of elements n is small, the frequencies of independent events must be calculated by the binomial distribution

$$P_k = \binom{n}{k} p^k (1 - p)^{n-k} \quad (4)$$

The number of plants with k = 1, 2 ... paramutation events is estimated for (n =) 1, 2, 4, and 10 paramutable cells, respectively, (Berg 1975) using a recursion formula. It is shown that from n = 10 the values calculated by

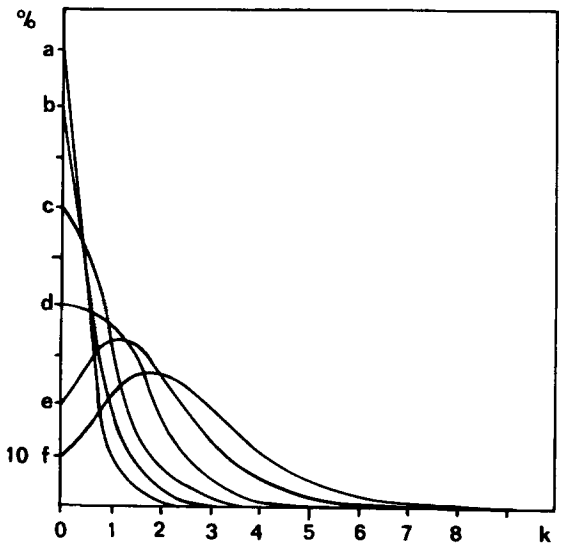

Fig. 5. Plants with 0, 1, 2 ... k paramutation events in percent of the total of paramutable plants using equation (2). A comparison is made for different frequencies of paramutation: 10% (a), 20% (b), 40% (c), 60% (d), 80% (e) and 90% (f) variegated plants per offspring

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the binomial distribution correspond approximately with those by the POISSON-distribution.

1.1.2 The Paramutation Frequencies and the Extent of Variegation

The time of paramutation determines the extent of the variegation:

(A) An unequivocal connection is made if all paramutable cells can paramutate only at a single developmental stage. The sum of paramutated tissues of the variegated heterozygotes of a whole offspring is

$$F = \sum_{k=1}^{i} \frac{P_k}{1 - P_0} \, kf \tag{5}$$

(f = the average of paramutated tissue belonging to one paramutation event; $\uparrow P_{k/(1-P_0)}$ = the relative part of plants with k paramutation events in respect to all paramutated plants). Equation (5) is transformed with Equation (2) to

$$F = f \frac{1}{1 - P_0} e^{-\lambda} \sum_{k=1}^{i} k \frac{\lambda^k}{k!} \tag{6}$$

The quotient

$$\frac{F}{f} = \frac{1}{1 - P_0} e^{-\lambda} \sum_{k=1}^{\infty} \frac{\lambda^{k-1}}{(k-1)!} \tag{7}$$

gives after summation

$$\frac{F}{f} = \frac{1}{1 - P_0} \tag{8}$$

and because of Equ. (3) with

$$\lambda = \ln P_0 = \ln [1 - (1 - P_0)] \tag{9}$$

Equ. (8) becomes to

$$\frac{F}{f} = \frac{-\ln [1 - (1 - P_0)]}{1 - P_0}$$

and

$$\frac{F}{f} = -\frac{\ln (1 - x)}{x} \tag{10}$$

if $1 - P_0 = x$ (the frequency of paramutation) is substituted.

The dependence of the sum of paramutated tissues from the frequency of paramutation is shown in Figure 6 (upper curve) in arbitrary units for f.

The difference between distinct paramutation frequencies x_1, x_2 can be estimated by

$$\frac{F_{x_1}}{F_{x_2}} = \frac{x_2}{x_1} \frac{\ln (1-x_1)}{\ln (1-x_2)} \tag{11}$$

which gives for $x_1 = 0{,}9$ and $x_2 = 0{,}1$ (90% against 10% variegated plants per offspring) $F_{0,9}/F_{0.1} = 2.43$.

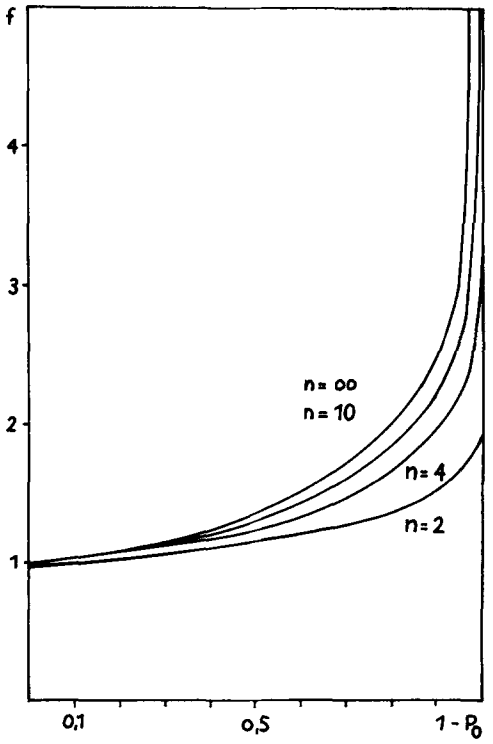

Fig. 6. The sum of paramutated tissues of a whole offspring depending on the frequency of paramutation ($1\text{-}P_0$) (f = arbitrary units). Equations (10) and (13) were used to compute the values for 2, 4, 10 and very many (POISSON) cells paramutable independent of each other

To calculate F by means of Equ. (5) the binomial distribution Equ. (4) can also be used:

$$\frac{F}{f} = \frac{1}{1 - P_0} \sum_{k=1}^{n} k \binom{n}{k} p^k (1 - p)^{n-k} \tag{12}$$

and

$$\frac{F}{f} = \frac{n (1 - P_0^{1/n})}{1 - P_0} \tag{13}$$

A calculation is presented in Table 5 to compare the quantity of paramutated tissues computed by the binomial and the POISSON-distribution, respectively. One would expect about 1.5 to 2.2 times more paramutated tissue in an

Table 5. Paramutated tissues (arbitrary units) for high ($P_0 = 0.1$) and low ($P_0 = 0.9$) paramutable offsprings comparing few and many paramutable cells per plant (binomial vs. POISSON-distribution) using equations (10) and (13). The last row gives the relation after equation (11)

	n = 2	n = 4	n = 10	POISSON
$P_0 = 0.1$	1.52	1.94	2.28	2.56
$P_0 = 0.9$	1.03	1.04	1.05	1.05
$F_{0.9}/F_{0.1}$	1.48	1.87	2.17	2.42

n = number of cells paramutable independenct of each other

offspring with 90% against one of 10% variegated heterozygotes assuming that only a few cells (two to ten) are independently paramutable (according to binomial distribution), whereas the difference would be greater assuming much more paramutable cells (according to POISSON-distribution).

(B) There is an essential difference if paramutation is possible during a long time interval. In this case one paramutation event can lead to a larger tissue patch than two or three events occurring later in development. In this case, therefore, an offspring would be expected essentially more heterogeneous than in case (A). While the average of the variegated tissue parts is the same in principle (and also the connections between the average values derived above), the individual plants must show very different portions of paramutant tissue because the rare paramutation events are distributed over a long time interval.

1.2 Rejection of Hypothesis I by Reason of Experimental Results

(A) The connection between variegated tissues and frequency of paramutation points to a lower number of independent paramutable cells. The offspring average of yellow tissue is only 1.81 times greater at 94% variegated plants per offspring compared to 13%. The χ^2-test demonstrates that the experimental values fit the expected relation (cf. Fig. 6) at $n = 4$ ($\chi^2 = 14.1$, d.f. = 13, $p > 0.3$) and at $n = 10$ ($\chi^2 = 18.1$, $p > 0.1$), but they differ significantly at $n = 2$ and $n = \infty$ ($\chi^2 = 28.3$, $p < 0.01$).

(B) In the experimental part of this paper it is shown that the three variegation types (II, III, IV) are statistically homogeneous. This means that the few paramutation events per plant demanded by the POISSON – as well as the binomial distribution are not distributed over a long time interval. It is impossible (without additional assumptions) to understand how one cell or also six cells will build up a quarter (22% in type II) or one half (52% in type IV) of the whole plant tissue even if the number of somatic, independent paramutable cells per plant were not more than hundred.

2 Hypothesis II: Paramutation Occurs in Somatic Cells Depending Upon a Single Primary Change in Early Plant Ontogenesis: 'Early Switch-on Mechanism Imprinting the Later Events'

After this, the realization is that the change of *sulf*$^+$ to *sulf* follows at any time in the whole development at relative constant rates ($\frac{1}{4}$ and $\frac{1}{2}$ of the whole tissues, respectively).

Here different mechanisms are hypothetically assumed for the decision over the possibility (switch-on mechanism) and the realization of paramutation. The former would be expressed in the frequency of paramutation and the latter as the percentage of paramutated tissues. It is impossible to explain the connection between such different processes without additional suppositions. Moreover, one would expect a continuous variation of the paramutated tissues mass on the whole range as on the contrary two variegation types differing significantly in this respect.

There is only indirect evidence against this hypothesis:

– If the realization of allele changes is possible in a quarter (or a half, respectively) of the whole vegetable cell mass (after positive decisions in the primary step), one would expect a more or less uniform variegation pattern similar to some kind of position effect variegation. However the real variegation caused by paramutation is variable (compare Hagemann 1958, 1966): on the same plant one can find smaller yellow or yellow-green stippled patches, but also large paramutated sectors, branches with periclinal structure as well as whole yellow or yellow-green speckled branches. Nevertheless, the percentage of paramutated tissues is substantial.

– In some cases plants remain green over a long time period but develop yellow tissue at the end of the vegetation period. Such late paramutation events never occur in distal parts of differentiated branches, e.g. above the first flowers, but only on new side shoots from the basis. This agrees well with the different fate of a little paramutated cell population by mixing and separation in a meristem as with continuous paramutation in all the cells of a plant.

Further experiments (e.g. cloning of green branches of heterozygous plants) may decide finally whether paramutation is possible only in a short period (hypothesis III) or at all stages after a switch-on mechanism (hypothesis II).

3 Hypothesis III: Paramutation can Take Place Only in a very Short Period of Early Plant Ontogenesis (During About 3 Cell Generations) in Few Somatic Cells (16 at the Most) Independent of Each Other; the Probability of the Occurrence of Paramutation Decreases During this Period Rather Quickly: 'Independent Events During a Restricted Sensitive Stage'

In this model, paramutation is realized in the following way: (i) it occurs in a few-cell-meristem of an early stage of the seedling's development (e.g. in the meristem for the first foliage leaves); (ii) complete inactivation is possible only following incomplete inactivation in a cell generation before (i.e. *sulf*$^+$ to *sulf*^{vag} to *sulf*^{pura}); (iii) the paramutation probability decreases quickly from one to another cell generation (e.g. in a geometric series, $\frac{1}{2}n$, corresponding to the reciprocal series of the cell propagation, 2^n).

Table 6. Derivation of hypothesis III model (cf. text)

gen.	$1 - (vag + pura)$	*vag*	*pura*
1.	$1 - p_v'$	p_v'	–
2.	$(1 - p_v')(1 - \frac{p_v''}{2})$	$p_v'(1 - p_p'') + 1 - p_v') \frac{p_v''}{2}$	$p_v' p_p''$
3.	$(1 - p_v')(1 - \frac{p_v''}{2})(1 - \frac{p_v'''}{4})$	$\left[p_v'(1 - p_p'') + (1 - p_v')\frac{p_v''}{2}\right](1 - \frac{p_p'''}{2}) + (1 - p_v')(1 - \frac{p_v''}{2})\frac{p_v'''}{4}$	$\left[p_v' p_p'' + p_v'(1 - p_p'') + (1 - p_v')\frac{p_v''}{2}\right]\frac{p_p'''}{2}$

Variegation type II: $p_v' = 0 \quad p_v'' = p_v'''$
Variegation type IV: $p_v' = p_v'' = p_v'''$
$p_p'' = p_p'''$

The portions of $sulf^{vag}$ and $sulf^{pura}$ tissues are indicated as *vag* and *pura*, respectively; gen. = number of cell generation

In order to control the reasonable meaning of these assumptions another model was calculated (in analogy to table 6) assuming equal paramutation probabilities in the following generations: there were already clear deviations between expectation and experiment after three cell generations.

3.1 Expectation Values and Comparison with Experimental Results

The following symbols are used
p_v – probability of changing $sulf^+$ to $sulf^{vag}$ in a cell
p_n – probability of changing $sulf^{vag}$ to $sulf^{pura}$ in a cell
distinguished in the three different cell generations as p_v', p_v'' (p_p''), and p_v''' (p_p'''), respectively.
vag and pura – portions of $sulf^{vag}$ and $sulf^{pura}$, respectively, distinguished by indices as belonging to type II or IV.

In table 6 the portions of different tissues are calculated in the three cell generations without an assumption about the real number of cells involved.

The different variegation types arise depending on whether paramutation is possible in generation 1 or not. If paramutation occurs in generation 1 (i.e. $p_v' = p_v'' = p_v'''$ and $p_p'' = p_p'''$) then variegation type IV arises, but if not till generation 2 (i.e. $p_v' = 0$ and $p_v'' = p_v'''$) then variegation type II occurs.

In variegation type IV, a remarkable portion of $sulf^{pura}$ tissue (p) arises because of $p_v' p_p''$ in generation 2. On the other hand, in variegation type II only $sulf^{vag}$ tissue (v) arises in generation 2 because of $p_v' = 0$, but a possible portion of $sulf^{pura}$ tissue is much lower than the 10% level (the experimental estimation unit) already in generation 3. Because of the quick decreasing paramutation probability one can neglect the following generations.

Therefore, Table 6 gives the proportions of paramutated tissues as follows:

$$\text{type II} \quad vag_{II} = 1 - (1 - \frac{p_v}{2})(1 - \frac{p_v}{4}) \tag{14}$$

$$\text{type IV} \quad (vag + pura)_{IV} = 1 - (1 - p_v)(1 - \frac{p_v}{2})(1 - \frac{p_v}{4}) \tag{15}$$

$$pura_{IV} = p_v p_p + p_v(1 - p_p) + (1 - p_v)\frac{p_v}{2}\frac{p_p}{2} \tag{16}$$

$$vag_{IV} = p_v(1 - p_p) + (1 - p_v)\frac{p_v}{2}(1 - \frac{p_p}{2}) + (1 - p_v)(1 - \frac{p_v}{2})\frac{p_v}{4} \tag{17}$$

Using experimental results it is possible to calculate the paramutation probabilities p_v and p_p. Equ. (14) gives, with experimental $vag_{II} = 0.236$, (nearly a quarter of paramutated tissue, cf. Table 2) $p_v = \frac{1}{3}$.

Computing the green part of type IV with Equ. (15), the result $(vag + pura)_{IV} = 0.49$ is consistent very well with the experimental value of 52% paramutated tissue (1972). The probability for changing $sulf^{vag}$ in $sulf^{pura}$ computed with Equ. (16) is approximented $p_p = \frac{1}{3}$ and using Equ. (17) the expected portion of $sulf^{vag}$ tissue of type IV is $vag_{IV} = 0.32$. Expectation values and experimental results are compared in Table 7.

The calculation gives about the same values for p_v and p_p. This suggests that the same mechanism can be postulated for incomplete and complete inactivation in the step-wise mechanism of paramutation.

3.2 The Real Number of Cells Involved

So far no assumption has been made about the actual number of paramutable cells in the hypothesis III model. It is given by probabilities only and leads to frequencies of paramutated leave tissue in good agreement with experi-

Table 7. Comparison of the expectation values from equations (14) to (17) with the experimental results. For 1972 (cf. Table 2) the calculated probabilities p_v and p_p are almost equal; they are approximated with 1/3. Using all (and considerable heterogeneous) material 1970-1972 different values of p_v and p_p as main difference

Assumption		Expectation values		Experimentally	
vag_{II}	= 0.22 (1972)	p_v = 0.35 (appr. 1/3)			
vag_{II}	= 0.28 (70/72)	p_v = 0.40			
$pura_{IV}$	= 0.18 (1972)	p_p = 0.37 (appr. 1/3)			
$pura_{IV}$	= 0.19 (70/72)	p_p = 0.32			
		p_v = 1/3 p_p = 1/3 (1972)	p_v = 0.40 p_p = 0.32 (1970-72)	1972	1970-72
vag_{II}		0.24	0.28	0.22	0.28
$(vag + pura)_{IV}$		0.49	0.57	0.52	0.54
vag_{IV}		0.32	0.38	0.34	0.36
$pura_{IV}$		0.18	0.19	0.18	0.19

(the theoretical possible value for $pura_{II}$ is 0.028 and therefore far below the experimental estimation unit of 10%)

mental results. But the arguments rejecting hypothesis I give further information on the number of independent paramutable cells in generations one of three (cf. p. 9 of section 1.2.).

Already if 16 cells are paramutable independent of each other, an offspring with 94% variegated plants frequency of paramutation compared with 13% would have on the average 2.05 times more paramutated tissue. The experimental value of 1.81 suggests that only 16 or fewer somatic cells are paramutable independently. This is in good agreement with the χ^2 test (1.2.A), which rather tends to four to ten cells.

Assuming a starting population of very few cells (or even cell clusters) in generation one, the mean values of a quarter (type II) and a half (type IV) paramutated tissues are easily understandable. The distribution of the probability p_k (Fig. 5) shows that two events are well expected at high paramutation frequencies, but at lower frequencies mainly one. This explains the fact shown in the previous paper that the general positive correlation between frequency of paramutation and the average of paramutated tissues is due to the significant positive correlation between the frequency of paramutation and the frequency of type IV plants (Table 4).

3.3 Additional Experimental Support of Hypothesis III

– So far no variegated leaves are observed arising late in the development of a plant from new paramutated cells (e.g. beyond the first flower meristems) (cf. hypothesis II).

– Cloning of green branches of heterozygous plants (species bastards) over several years never led to paramutation.

– Tests on temperature sensitivity of paramutation were negative if both the test plants and the controls grew under the same temperature up to the seedling stage and afterwards in different temperatures (Hagemann 1965). However, the opposite experiment (different temperatures for the seedlings but afterwards growing under the same conditions) gave a low but significant difference in the portion of paramutated tissues (Hagemann, unpubl.). This also gives a key argument for the difference of the paramutated tissues over several years (Tables 1, 2).

– In the last years it has become possible to differentiate not only between $sulf^{vag}$ and $sulf^{pura}$ but also between a strong green speckled $sulf^{vag\,1}$ and a strong yellow speckled $sulf^{vag\,2}$ phenotype. Because the distinction is very clear it supports and modifies the hypothesis: it suggests an inactivation mechanism step-wise in mainly three cell generations.