GENETIC STUDIES ON SOMATIC INSTABILITY IN CULTURES
DERIVED FROM HYBRIDS BETWEEN NICOTIANA
LANGSDORFFII AND N. SANDERAE¹

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THE studies described below are concerned with plants showing a phenomenon that has been variously termed mottling, mosaicism or variegation. The unifying descriptive characteristic of such phenomena is an altered content, appearance or structure of a cell or group of cells. The patches of altered tissue must have an apparently random spatial distribution in order to be classified here as a *variegation* rather than as a *pattern*. Particular cases of variegation have been attributed in the literature to virus disease, to mutable genes, to unequal plastid or gene-part segregations, to unstable interactions between genes and cytoplasm or among two or more chromosomal loci, as well as to various chromosomal aberrations. Specific references to the literature are contained in later sections of this paper.

One of the major interests in variegation is concerned with the mechanism of differentiation of any cell or tissue, whether normal or abnormal. What is the nature and the mechanism of action of the system, genic and physiological, which controls development? Why is the system generally so stable and effectively buffered against environmental disturbance? What elements of the system are altered when it first becomes spontaneously unstable? What point in the genic-physiological system is the site at which each subsequent altered expression is initiated? What inherent and environmental factors influence the morphological distribution, the statistical frequency and the developmental timing of the instability?

Penetrating answers to such questions may not be easy to attain, but a promising avenue of research in which to seek them is through study of a variegation culture in which departures from stable developmental expression are frequent.

Analysis is begun below for a case of nonuniform spatial distribution of anthocyanin production in flower petal cells. In this *variegated* phenotype, the presence of adjacent epidermal regions differing in degree or distribution of pigmentation resembles the color *patterns* of certain petals and leaves which have margins or proximal areas differing from the rest of the tissue. Each case implies a problem in *tissue differentiation*. However, in the *variegation* there is no obvious correlation between pigmentation and such morphological regions as petal margins or leaf petioles. Other correlations must be looked for as clues to the cause of differential development.

Data are presented to show that the spontaneous somatic instability described is

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present in cultures which are altered from stable types at *one, major* chromosomal locus critical to their anthocyanin differentiating system. This primary thesis is developed further to show genetic differences present also *among* the variegated plants.

MATERIAL AND METHODS

A plant showing flower-color variegation arose in our *Nicotiana* culture in 1939. It appeared in an F_2 generation of the interspecific cross *Nicotiana langsdorffii* ($n = 9$) $\times$ *N. sanderae* ($n = 9$), and was the origin of a line called variegated-1 which has been maintained by pedigree culture to the present. The strains used of these two species are highly homozygous and have been described and illustrated in previous publications (SMITH 1937, 1943).

The breeding program followed has included: (1) A series of five backcrosses to *N. sanderae* var. Sutton's Scarlet each followed by selection for the instability, which has brought the variegated-1 character into a genotype close to the *sanderae* parent; and (2) appropriate crosses among the major distinct phenotypes of variegated-1 which have revealed a basically simple mode of inheritance, as well as complicating ramifications.

In other cultures derived from the same interspecific cross there have originated more than six lines displaying heritable flower-color variegations of types different from that of variegated-1 and at least that many which resemble variegated-1 in appearance. In the study reported here emphasis has been placed upon certain aspects of the phenomena evidenced by variegated-1 and variegated-3, a phenotypically somewhat different type.

Description of flower color phenotypes

The corolla of *N. sanderae* is solid red in color due to the presence of anthocyanin pigmentation in the cell sap of the epidermis. Plants of variegated-1 bear flowers which fall into one or more of the following four classes: 1. Speckled, *s*—scattered, small clusters of red cells on a background of colorless cells. 2. Sectorial, *S*—frequent, clearly defined sectors of speckled tissue on a background of solid red tissue. 3. Rare sectorial, *rare-S*—very infrequent sectors of speckled tissue on a background of solid red tissue. 4. Dark speckled-Light speckled-Sectorial, *DsLsS*—frequent sectors of light speckling on a background that is dark speckled.

Corollas showing the solid color typical for *N. sanderae* var. Sutton's Scarlet and each of the main phenotypes of variegated-1, in pedigrees genetically close to *sanderae*, are shown in figure 1.

Although many plants of variegated-1 show only one phenotype of flower color, it is not uncommon for a plant to show two types, and individuals have been observed bearing all four major classes of flowers. The pairs which most commonly occur together on chimeral plants are: (1) *S* and *s*, (2) *rare-S* and *S*, and (3) *DsLsS* and *s*.

The sectoring of variegated-1 may occur over a wide range of time during plant or flower development. Thus a sector may involve only a few petal cells or it may include half a flower, a whole flower, a branch, several branches, or perhaps even

FIGURE 1.—Photographs of *Nicotiana* flowers. A. *Nicotiana sanderae*, solid red; B. Variegated-1; speckled; C. Variegated-1, rare sectorial; D. Variegated-1, dark speckled with light speckled sectors, and E. Variegated-1, sectorial.

the petal cells of all the flowering branches in one plant. Such early sectoring events may account for speckled flowers occurring on plants which also bear sectorial flowers. In such cases the plants were classified as sectorial in the data tables. A similar convention in tabulation was followed for the other chimeral plants.

Plants of variegated-3 bear flowers which resemble those of variegated-1 except for a dramatic difference in the timing of the sectoring events. In this line the sectors are frequent but medium to small in size, giving a flecked appearance to the petals. Chimeral plants, bearing more than one major phenotype of flower, are rare presumably because sectoring events occurring early in development are rare. Figure 2 shows successive flowers harvested from such a rare chimeral branch of variegated-3. Such chimeral branches are more common in variegated-1.

GENETIC RESULTS

The V locus

In the cross of variegated-1 $\times$ *sanderae*, or its reciprocal, it has been invariably true that all F_1 progeny bear uniformly solid red, nonvariegated flowers. A primarily simple genetic basis for the variegation phenomenon is shown by the segregations obtained in seven families of F_2 progeny from this cross (table 1). Although family 51107 gave a segregation heterogeneous to the others, the remaining six families are statistically homogeneous and support the hypothesis of an F_2 progeny segregation of three solid to one variegated.

Segregations are available from testcrosses of two general types: (1) those involving the cross of a heterozygous diploid F_1 with variegated-1, and (2) those involving the cross of a heterozygous trisomic F_1 with variegated-1. Crosses with the trisomic type containing an extra chromosome 2 (SMITH 1943) have been found generally to give segregations different from those of the other testcrosses. Data for this latter group, omitting trisomic-2 families, are presented in table 2. The segregation is clearly in the proportion of one solid to one variegated.

FIGURE 2.—Photographs of *Nicotiana* flowers from a single branch of a variegated-3 plant. The flowers are numbered from 1-8 in order of bloom and are placed in angular positions according to the branch phyllotaxy. The figure illustrates the qualitative (speckled or sectorial) and quantitative (size and frequency of sectors) variation in variegation which may occur among flowers from one plant. The phenotypes of flowers 3, 4, 5, 7, and 8, in conjunction with their respective positions, show a pattern suggestive of a speckled sector from 144° to 360° in the apical meristem of the branch.

Segregations in testcrosses of trisomic-2 plants are shown in table 3. Since the first six of the nine families deviate significantly from a 1:1 proportion there can be no doubt that trisomic-2 families require separate consideration. They also differ among themselves, falling into three groups: (1) in which the variegated individuals clearly exceed the solids, (2) in which the solids predominate, and (3) in which the two classes of progeny are about equal.

The 1:2 and 2:1 proportions of groups 1 and 2 can be explained on the basis of trisomic inheritance. Each ratio is expected among diploid progeny when, respectively, the simplex and duplex trisomic heterozygote is involved in a testcross with

TABLE 1
F₂ segregation from self-pollinating the hybrid, variegated-1 × N. sanderae

Culture	Progeny segregation		Total progeny	3:1 Hypothesis		
	Solid	Variegated		Chi square	d.f.	P
51105	51	14	65	0.42	1	0.51
51107	72	39	111	6.08	1	0.015
51109	58	12	70	2.31	1	0.12
S5338	32	16	48	1.78	1	0.17
S5439	58	21	79	0.11	1	0.74
S5441	49	15	64	0.08	1	0.78
S5443	55	15	70	0.48	1	0.47
Total	375	132	507	0.29	1	0.58
Heterogeneity	—	—	—	10.96	6	0.09
Total, omitting 51107	303	93	396	0.49	1	0.47
Heterogeneity, omitting 51107	—	—	—	4.68	5	0.48

TABLE 2
Segregation among diploid progeny of the testcross, heterozygous F₁ × variegated-1

Culture	F ₁ type	Diploid progeny segregation		Total diploid progeny	1:1 Hypothesis		
		Solid	Variegated		Chi square	d.f.	P
S5339	2n	15	25	40	2.50	1	0.11
S5440	2n	24	23	47	0.02	1	0.88
S5442	2n	37	37	74	0	1	0.99
S5444	2n	45	32	77	2.19	1	0.17
50107	2n + 1	35	37	72	0.06	1	0.82
51112	2n + 1	27	29	56	0.07	1	0.78
51113	2n + 1	30	30	60	0	1	0.99
51117	2n + 1	29	28	57	0.02	1	0.89
S5345	2n + 3	12	7	19	1.32	1	0.25
S5346	2n + 4	23	13	36	2.78	1	0.09
S5221	2n + 5	30	27	57	0.16	1	0.69
50108	2n + 7	22	19	41	0.22	1	0.65
51119	2n + 7	29	31	60	0.07	1	0.79
S5347	2n + 8	5	10	15	1.67	1	0.19
51120	2n + C	22	27	49	0.51	1	0.46
Total	—	385	375	760	0.13	1	0.71
Heterogeneity	—	—	—	—	11.45	14	0.65

a diploid recessive. The consistent excess of variegated individuals above the expected two thirds among diploids in the group 1 cultures may indicate preferential synapsis of v_s/v_s bearing chromosomes in the simplex trisomic parents. This interpretation is possibly substantiated by the excess of solid colored segregants over

TABLE 3

*Segregation among diploid and trisomic progeny of the testcross, heterozygous trisomic-2
× variegated-1*

Group	Culture	Gener- ation*	Diploid progeny segregation		Total diploid progeny	1:2 Hypothesis			2:1 Hypothesis			Trisomic progeny	
			Solid	Var.		Chi square	d.f.	P	Chi square	d.f.	P	Solid	Var.
1	S5216	BC ₁	6	25	31	2.72	1	0.10	—	—	—	1	3
	S5219	BC ₁	9	31	40	2.11	1	0.15	—	—	—	2	0
	S5340	BC ₂	7	17	24	0.19	1	0.66	—	—	—	3	2
	S5452	BC ₃	13	35	48	0.84	1	0.36	—	—	—	3	0
	S5453	BC ₃	10	24	34	0.23	1	0.64	—	—	—	5	0
2	S1118	BC ₁	38	18	56	—	—	—	0.04	1	0.85	4	0
	S5218	BC ₁	9	3	12	—	—	—	0.38	1	0.54	2	1
3	S5217	BC ₁	29	23	52	—	—	—	—	—	—	6	0
	S5344	BC ₂	10	9	19	—	—	—	—	—	—	4	0
Group 1 total			45	132	177	4.98	1	0.03				14	5
Group 1 heterogeneity						1.11	4	0.89					

* BC₁, BC₂, and BC₃ designate first, second, and third backcrosses, respectively, to diploid speckled plants ($v_s v_s$) of solid-colored trisomic-2 plants heterozygous (VVv or Vvv) for variegated-1.

expectation in the pooled trisomic progeny of this group, although the numbers are small.

The 1:1 proportions in the families of group 3 are not expected. They cannot be explained as having come from diploid parents, for both families contained trisomic progeny. Since these trisomics are all solid-colored the preferred inference is that the two families belong to group 2.

Comparison of the phenotypic segregation among the trisomic-2 progeny of group 1 with the pooled phenotypic segregation among the trisomic progeny of non-2 trisomics is instructive. The former fit closely to a 2 solid:1 variegated ratio while the latter approximate a 1:1 ratio.

On the basis of the data presented it is proposed that individuals of variegated-1 phenotype possess a genetic locus, v . *N. sanderae* is homozygous for V , which behaves in our genetic tests as a dominant allele of v . Individuals homozygous or heterozygous for V invariably bear flowers which are solid-colored, nonvariegated. The single-factor mendelian segregations, which support the involvement of one locus, are supplemented by trisomic ratios which indicate that the locus is situated in chromosome 2 of the complement.

Since *sanderae* is homozygous for a basic color factor, P , necessary for the production of anthocyanin pigmentation (SMITH 1937), it is of interest to investigate possible relationships between P and V . The homozygous recessive, p , has white flowers whereas homozygous recessive v , is expressed as a restriction of anthocyanin coloration giving a white background with red spots. Data, obtained from a rather

limited number of test progeny, are consistent with the hypothesis of independent assortment of P and V ; and, it is clear that, if linked, P and V at least do not lie close together on the same chromosome.

Allelic forms and segregational differences

Although it is shown that the variegated-1 phenotypes, considered as a pooled class in crosses with *sanderæ*, are controlled in inheritance by a basically simple genetic mechanism, there is abundant evidence that the variegated plants possess at least two allelic forms of v .

Evidence for alleles of v is contained in genetic data from self- and cross-pollinations among the major variegated-1 phenotypes: speckled (s), sectorial (S), and rare sectorial (*rare-S*). These data, grouped according to parental phenotype, are shown in table 4. Segregant progeny scored as $DsLsS$ phenotype are omitted from the tabulation. The frequency of this class is low and can be determined for each family as the difference between the total number of progeny and the sum of the frequencies of the three recorded phenotypes.

It can be seen from the pooled averages that progeny from self-pollinations of speckled, sectorial, and rare sectorial give widely different segregations; thus, demonstrating that these three phenotypes possess different genotypes. Speckled parents give a preponderance of speckled progeny, rare sectorial parents a preponderance of rare sectorial progeny, and sectorial parents tend to have all three phenotypes well represented among their progeny from self-pollinations. Certain families which are starred in table 4, are heterogeneous to their phenotypic group and give segregations more nearly like those typical for the breeding behavior of another phenotype. If these families are removed and totals calculated from the remainder, modal segregations are obtained as shown in table 5 for each of the cross- and self-pollinations.

Further insight is attained by comparing these segregations with mendelian ratios which they most nearly approximate. A working hypothesis, suggested by the data, is that there are two allelic forms of the variegated locus, namely: v_s for speckling and v_S for unstable solid, i.e., sectorial. The three major phenotypes of variegated-1 are interpreted as being of the following genotypes:

Phenotype	Abbreviated designation	Genotype
speckled	s	$v_s v_s$
sectorial	S	$v_S v_s$
rare sectorial	<i>rare-S</i>	$v_S v_S$

The allele v_S is dominant over v_s and is characterized in expression by solid-color tissue until the occurrence of a somatic sectoring event which results in loss of the solid color and assumption of a typical speckled appearance. The nature of this somatic event, by which unstable v_S is distinguished from the stable V of *sanderæ*, has all the appearance of a mutation from v_S to v_s . In a developing epidermis the cell-lineage derivatives of a daughter cell resulting from the change of v_S to v_s (solid tissue) to $v_s v_s$ constitute a speckled sector in the mature petal. It follows that a

TABLE 4

Segregation among progeny from self- and cross-pollinations of the main phenotypes of variegated-1

Culture	Phenotype of parents	Total progeny	Progeny segregation		
			Speckled	Sectorial	Rare sectorial
*41144	<i>s</i> (X)	31	10	12	9
41154	<i>s</i> (X)	6	5	1	0
*41155	<i>s</i> (X)	17	1	5	6
41163	<i>s</i> (X)	25	24	1	0
42041	<i>s</i> (X)	98	96	2	0
43014	<i>s</i> (X)	12	11	0	0
*43027	<i>s</i> (X)	11	5	3	3
43033	<i>s</i> (X)	9	9	0	0
4689	<i>s</i> (X)	58	52	5	0
*46105	<i>s</i> (X)	18	5	9	4
46112	<i>s</i> (X)	13	13	0	0
46113	<i>s</i> (X)	37	35	2	0
4783	<i>s</i> (X)	13	10	3	0
48019	<i>s</i> (X)	25	23	2	0
49175	<i>s</i> (X)	36	35	1	0
49196	<i>s</i> (X)	52	46	5	0
50103	<i>s</i> (X)	56	46	8	1
51104	<i>s</i> (X)	64	61	2	0
*51114	<i>s</i> (X)	56	19	21	16
S521, S2079B	<i>s</i> (X)	69	56	10	1
S537, S547	<i>s</i> (X)	131	117	13	0
*S538, S546	<i>s</i> (X)	179	103	71	2
S539, S5413	<i>s</i> (X)	117	100	16	0
*S5310, S543, 4	<i>s</i> (X)	157	51	77	29
S5311, S548	<i>s</i> (X)	122	93	26	1
S5327, S541, 2	<i>s</i> (X)	110	100	10	0
S549	<i>s</i> (X)	26	21	4	1
S5411	<i>s</i> (X)	77	62	12	0
S5412	<i>s</i> (X)	80	69	8	1
Total	<i>s</i> (X)	1,705	1,278	329	74
Percent	<i>s</i> (X)		76.02	19.57	4.41
S5312	<i>s</i> X <i>s</i>	41	33	5	1
*S5313, 14	<i>s</i> X <i>s</i>	109	50	47	10
*S5315	<i>s</i> X <i>s</i>	19	7	12	0
S5341	<i>s</i> X <i>s</i>	32	26	3	2
S5342	<i>s</i> X <i>s</i>	22	21	1	0
Total	<i>s</i> X <i>s</i>	223	137	68	13
Percent	<i>s</i> X <i>s</i>		62.84	31.19	5.97
41046	<i>S</i> (X)	17	7	6	4
41138	<i>S</i> (X)	27	13	12	2
41139	<i>S</i> (X)	28	8	11	9
41143	<i>S</i> (X)	30	7	17	6
42040	<i>S</i> (X)	78	27	39	12

TABLE 4. *Continued*

Culture	Phenotype of parents	Total progeny	Progeny segregation		
			Speckled	Sectorial	Rare sectorial
43026	<i>S</i> (X)	34	11	19	4
*43035	<i>S</i> (X)	24	21	3	0
4627a	<i>S</i> (X)	46	12	24	10
*4631a	<i>S</i> (X)	18	17	1	0
4695	<i>S</i> (X)	56	13	31	12
46106	<i>S</i> (X)	43	10	23	10
48115	<i>S</i> (X)	37	7	27	3
48118	<i>S</i> (X)	85	19	56	10
*49199	<i>S</i> (X)	48	34	10	0
*50105	<i>S</i> (X)	8	8	0	0
51108	<i>S</i> (X)	7	3	4	0
*52078, S526, 7	<i>S</i> (X)	86	47	33	5
*S5322A, B; S5433, 4, 5, 6	<i>S</i> (X)	252	134	62	16
S5323, 4, 4A; + S5429, 30, 1, 2, 7	<i>S</i> (X)	290	99	132	58
Total	<i>S</i> (X)	1,214	497	510	161
Percent	<i>S</i> (X)		42.55	43.66	13.79
*S531C	<i>Rare-S</i> (X)	80	74	6	0
S5318	<i>Rare-S</i> (X)	18	0	1	17
S5454	<i>Rare-S</i> (X)	49	0	0	49
S5455	<i>Rare-S</i> (X)	36	0	2	34
S5456	<i>Rare-S</i> (X)	43	2	4	37
S5424	<i>Rare-S</i> (X)	23	1	9	13
Total	<i>Rare-S</i> (X)	249	77	22	150
Percent	<i>Rare-S</i> (X)		30.92	8.84	60.24
43024	<i>s</i> X <i>S</i>	36	19	17	0
4788	<i>s</i> X <i>S</i>	29	16	13	0
48116	<i>s</i> X <i>S</i>	52	27	25	0
*48119	<i>s</i> X <i>S</i>	93	58	35	0
*49197	<i>s</i> X <i>S</i>	41	30	4	0
51110	<i>s</i> X <i>S</i>	52	23	23	5
*51115	<i>s</i> X <i>S</i>	56	22	24	10
*S5426	<i>s</i> X <i>S</i>	57	53	4	0
43025	<i>S</i> X <i>s</i>	28	13	15	0
*49198	<i>S</i> X <i>s</i>	41	36	4	0
49200	<i>S</i> X <i>s</i>	56	30	26	0
49201	<i>S</i> X <i>s</i>	55	21	30	0
*51111	<i>S</i> X <i>s</i>	67	37	26	4
*51116	<i>S</i> X <i>s</i>	64	38	23	2
S5214, S5215	<i>S</i> X <i>s</i>	74	38	33	1
*S5220	<i>S</i> X <i>s</i>	46	35	10	0
S5330	<i>S</i> X <i>s</i>	26	12	12	2
S5349	<i>S</i> X <i>s</i>	8	6	2	0
S5427	<i>S</i> X <i>s</i>	29	17	12	0
Total	<i>s</i> X <i>S</i> and <i>S</i> X <i>s</i>	910	531 59.46	338 37.84	24 2.70

TABLE 4. *Continued*

Culture	Phenotype of parents	Total progeny	Progeny segregation		
			Speckled	Sectorial	Rare sectorial
*S5316	$s \times \text{Rare-}S$	17	1	8	8
S5317	$s \times \text{Rare-}S$	20	3	17	0
S5428	$s \times \text{Rare-}S$	51	9	34	7
*52074	$\text{Rare-}S \times s$	24	17	6	1
S5458	$\text{Rare-}S \times s$	39	4	27	8
Total	$s \times \text{Rare-}S$ and $\text{Rare-}S \times s$	151	34	92	24
Percent			22.67	61.33	16.00
S5321	$S \times \text{Rare-}S$	28	1	15	12
S5459	$\text{Rare-}S \times S$	38	2	16	20
Total	$S \times \text{Rare-}S$ and $\text{Rare-}S \times S$	66	3	31	32
Percent			4.56	46.96	48.48

(X) = Selfed.

 s = speckled. S = sectorial. $\text{Rare-}S$ = rare sectorial.

*—heterogeneous within phenotype group.

TABLE 5

Modal segregations in selected families from self-pollinations and intercrosses among the main phenotypes of variegated-1

Parental phenotype	Progeny totals				Progeny frequencies			Mendelian ratios obscurely approximated			Calculated frequency v_s gametes
	s	S	$\text{Rare-}S$	Total	s	S	$\text{Rare-}S$	s	S	$\text{Rare-}S$	
s (X)	1084	131	5	1220	0.889	0.107	0.004	1.00	0	0	0.942
$s \times s$	80	9	3	92	0.870	0.098	0.032	1.00	0	0	—
S (X)	236	401	140	777	0.304	0.516	0.180	0.25	0.50	0.25	0.562
$\text{Rare-}S$ (X)	3	16	150	169	0.018	0.095	0.887	0	0	1.00	0.066
$s \times S$ & $S \times s$	222	208	8	438	0.507	0.475	0.018	0.50	0.50	0	—
$s \times \text{Rare-}S$ & $\text{Rare-}S \times s$	16	78	15	109	0.147	0.716	0.137	0	1.00	0	—
$S \times \text{Rare-}S$ & $\text{Rare-}S \times S$	3	31	32	66	0.046	0.470	0.484	0	0.50	0.50	—

rare sectorial, $v_s v_s$, plant would require two such events in a single cell lineage to originate a speckled sector. The expectation, on the assumption of independence of the events, would be the square of the frequency of the single event and thus would account for the lower frequency of sectors observed.

The phenotype of speckled suggests (by the presence of occasional small sectors of solid tissue as in figure 3A, and perhaps also by the very presence of the pigmented cells which constitute the speckling) that the v_s allele may "back mutate" to v_s .

If events having the characteristics described for somatic tissue were to occur

FIGURE 3.—Photomicrographs of *Nicotiana* petals. A. Light speckled variegation: infrequent small clusters of red-pigmented cells scattered among colorless cells; and a larger sector of pigmented cells; and B. Dark speckled variegation: more frequent and larger clusters of red-pigmented cells. Diffusion is evident into the region surrounding each pigmentation center in both A and B.

also in germinal or pregerminal tissue, this would provide a mechanism for explaining the modal segregations shown in table 5.

If their genetic loci were stable, the three main types of zygotes proposed would be expected to produce respectively the following proportions of gametes: speckled, 100 percent v_s ; sectorial, 50 percent v_s and 50 percent v_{ss} ; and rare sectorial, 100 percent v_s . The zygotic proportions obtained in progeny from self-pollinations are, however, not the typical mendelian ratios characteristic of stable loci. Instead the

progeny segregations are as if the respective frequencies of the v_s gametes are those shown in the last column of table 5. As a working hypothesis, the aberrant ratios are attributed to altered gamete frequencies which are the net product of interallelic mutation rates. A study of such a culture takes on many of the aspects of a study in population genetics.

Estimation of sources of segregational variability

Sources eliminated. In material which gives ratios as unfamiliar as those presented here, there is particular merit in obtaining assurances that the segregations are repeatable. Limited data are available from duplicate samplings of each of two seed lots which indicate homogeneity of the duplicate families at probability levels of 0.22 and 0.64. Differential temperature treatment of duplicate samples grown at 50°F and 80°F during 23 days from germination through early seedling development was ineffective in producing significantly different segregations. It was conceivable that competition among segregants in a crowded seed pan, accompanied by unconscious selection of those seedlings grown to maturity, could be a source of bias in the data. However, sparsely and abundantly sown samples of the same seed lot do not yield significantly different segregations. In addition, two tests have indicated that synthetic mixtures of two seed-lots in known proportions result in segregations which are proportional syntheses of the segregations obtained for samples of the two seed-lots grown independently. Thus, evidence available shows that the segregations, odd as they may be, are obtainable repeatedly from the same seed-lot under the conditions employed. Sample differences and cultural environment evidently are not serious sources of variation in segregation.

It was also conceivable that deviations of the observed segregations from mendelian expectation might be due, not to inherent gametic proportions, but to competition among pollen tubes bearing the different gametes. If such a competition exists it should be removable, or at least reducible, by sparse pollination. Several sparse and abundant pollinations were made, using only one bloom as male on each of two sectorial plants. There was no indication of a segregational difference due to sparse pollination. Thus, the preferred interpretation is that *gametic frequencies* dictate the genotypic constitution of the resulting seed-progenies which, in turn, are affected by *developmental events*; so that both phenomena, acting in conjunction, determine the phenotypic segregations observed among flowering plants.

Differences among major phenotypes. The data presented in tables 4 and 5, showing that the three major phenotypes breed differently, were obtained largely from individuals in different families. It is of interest to know if sister plants, representing the different phenotypes, also breed differently. Seeds from unprotected pollinations were harvested from six groups of plants in one family, two groups for each of the three phenotypes. Each group was made up of bulked seed from a number of capsules (at least 24) from four or more sister plants of the same phenotype. The segregations were distinctly different among progeny of different phenotypes and consistent within phenotype groups of sister plants.

Differences among plants of the same major phenotype. Two main types of observations have been used to demonstrate differences among plants belonging to the

speckled phenotype. In the first, speckling intensity was estimated by counting the frequency of pigmented epidermal cells per microscopic field of view, which encompassed approximately 200 cells at the magnification used. This is, essentially, a measure of the *density* of pigmented cells, an important element of phenotypic speckling intensity.

During a period of six days in the summer of 1952 20 speckled plants (self-progeny of one speckled individual) were scored. Three flowers were harvested from each plant and ten fields of view were counted on one lobe of each flower. The plant means

FIGURE 4.—Regression of progeny mean speckling intensity on parental mean speckling intensity in *Nicotiana* flowers. The scale of measurement is proportional to the frequency of pigmented cells in the petal epidermis. The circles represent families from self-pollination; while the crosses are for families from cross-pollination plotted against their mid-parental speckling intensity. The points do not deviate significantly from a linear regression.

for number of pigmented epidermal cells per field of view ranged from eight in the very light-speckled to 33 in the darkest speckled. There were peaks at 13 and 23. It is clear that the 20 sister plants scored in this family fall into at least two, and probably more, phenotypic categories with respect to their speckling intensity. Further data would be needed to determine if this pattern of distribution holds, in general, for all speckled families. The photomicrographs in figure 3 show the difference in frequency of pigmented cells in the epidermis of a light-speckled and a dark-speckled flower petal.

Progeny tests have been carried out to investigate the extent to which the phenotypic difference in speckling intensity among plants is heritable. For this purpose self-progeny were grown from seven speckled plants along with three families resulting from cross-pollinations. The parents were cultured as clones and were grown in the same field with their offspring. Flowers from parents and speckled progeny were scored for speckling intensity by counting numbers of red cells per microscope field of view, as described above.

Analysis of the data revealed a linear regression of progeny mean speckling intensity on parental mean speckling intensity. The regression is shown graphically in figure 4. It is highly significant when tested against the mean square for deviations from regression. Thus it is concluded that hereditary factors contribute to differences in speckling intensity among plants.

Still another method was employed to demonstrate heritable differences among individuals of the speckled phenotype. The Hunter Color Difference Meter was used to rate the speckling intensity of a parental generation. The scores recorded are measures of absorption of light for petals of each parent plant on a scale in which white equals zero and solid-red equals 100. In the self-progenies of nine selected speckled plants and four crosses between plants, a value was calculated which is a measure of the frequency of dominant v_s alleles in each family. This quantity, the percentage of v_s alleles, was arrived at by expressing the sum of the rare sectorial progeny ($v_s v_s$) and one half of the sectorial progeny ($v_s v_s$) as a percentage of the total progeny. In some families a correlation was observed between speckling intensity of the parent and frequency of v_s alleles in the progeny, as summarized below:

Hunter Meter score of parent		Percent v_s in progeny	
		1953	1954
self	22.6	7.6	2.9
	52.8	20.5	21.4
	82.7	38.0	44.4
cross	52.7	22.6	
	52.7	28.4	
	67.8	31.6	

In the remaining families, six self-progeny and one cross-progeny, there were fewer than ten percent v_s alleles and no apparent correlation with speckling intensity of their parental plant. Repeated tests (1953 and 1954) showed definitely, however, that individuals of the speckled phenotype may differ consistently in the frequency of the dominant v_s allele appearing in their progeny.

The dark-speckled plant which gave 38 to 44 percent v_s alleles in its progeny

actually closely approximated the proportion of segregants expected for a sectorial plant. However, it is unlikely that this plant is a periclinal chimera in view of the fact that 17 shoot cuttings and 16 root cuttings have all had dark-speckled flowers. If one accepts this plant as genotypically speckled it then appears that certain speckled plants undergo a sufficiently high frequency of reversion of v_s to v_S so that their gamete proportions approximate those of sectorial plants.

The complexity of phenotypic expression of speckling intensity, in the families studied here, would be expected to contribute to the difficulty in establishing a direct correlation with frequency of v_S alleles among progeny. Speckling intensity is not a simple variable. It reflects differences in the proportion of colored to white cells (speckling density) as well as differences in intensity of pigmentation within the colored cells. Furthermore, when pigment intensity is great there is apparently more extensive diffusion from the heavily-colored cells into adjacent areas of colored cells.

It is tentatively concluded that speckling density may provide a phenotypic measure for predicting the frequency of v_S alleles among progeny of speckled plants. However, a clear demonstration of this point, at least if the Hunter Color Difference Meter is to be used, will require that the families are homozygous for genetic factors affecting pigment intensity as a component of speckling intensity.

Genetic differences between sectorial plants are shown in table 6 where data are presented for two sister sectorial plants, the breeding behaviors of which have been tested over a period of two years. The segregations in their progeny are compared in terms of the frequency of recessive alleles calculated as the sum of the number of speckled progeny ($v_s v_s$) and one half the sectorial progeny ($v_S v_s$) and expressed as a percentage of total progeny. The segregations are highly significantly different when tested by homogeneity chi square. The parental phenotypes are also quantitatively different, as measured by their frequency of sectoring. This frequency is expressed as the number of sectors per angular degree of petal epidermis (360 degrees constituting a whole flower).

The data show correlation between the frequency of sectors of recessive somatic tissue and the frequency of recessive v_s alleles obtained in self-progeny of sister sectorial plants.

Difference between flowers of the same phenotype, plant, or clone

It is shown in table 5 that the average breeding behavior of speckled-flowered plants is to give progeny that are 88.9 percent speckled, 10.7 percent sectorial, and 0.4 percent rare-sectorial. However, as shown in table 4, seven families out of 29 bred as though they were of a genotype different from speckled. At least four of these (41144, 46105, 51114, and S5310-S543,4) gave offspring frequencies essentially the same as self-progeny of sectorial plants. It is concluded that the layer of tissue giving rise to the germ cells was of sectorial ($v_S v_s$) genotype whereas the epidermal layer was, as a result of early mutation ($v_S \rightarrow v_s$), speckled throughout the entire plant.

Similarly, although the modal frequency for self-progeny of sectorial plants is 30.4 percent speckled, 51.6 percent sectorial, and 18.0 percent rare-sectorial (table

5); six families out of 19 bred as though they were of a different genotype (table 4). Three of these (43035, 4631a, and 50105) gave segregational ratios typical for self-progeny of speckled plants. This was also true of one family the phenotype of which was rare-sectorial (S531C, table 4). It is concluded, therefore, that mutational events may occur independently in the tissue layers of the plant so that the epidermal layer (by which the phenotype is classified) may be different genetically from the layer that gives rise to the sex cells. More detailed information on this point can be obtained by a study of progeny from single flower pollinations on different parts of one chimeral plant and by use of clonal material.

Seven tests of the consistency of the breeding behavior of speckled plants were made by using seed produced from pollinating the same plant in a different year. In only one test was the segregation significantly different from that obtained in the original test. On the other hand, in three out of four comparisons involving sectorial plants, the segregation in the repeated test was significantly different from that of the original. The implication is that different flowers employed in producing seed from the same plant, particularly if it is a sectorial plant, may give different progeny segregations.

During the process of studying the variegation problem over 500 cuttings from 50 different plants have been cultured and observed at flowering. Some of the clones have been maintained for two and three years by care during the winter in the greenhouse. The material is capable of indefinite culture in this manner.

Of the 50 clones 21 have contained one or more cuttings which gave flower phenotypes different from those observed on the respective parent plants.

One clone, particularly worthy of note, produced only dark-speckled, light-speckled sectorial flowers in the field in 1952. Yet it has yielded 36 stem cuttings nearly equally divided among the parental phenotype (*DsLsS*), speckled, sectorial, and rare sectorial phenotypes. Thus all four of the major phenotypes obtainable among sexual progeny have been derived by asexual propagation from a single stock plant.

Twelve single-step one-directional changes are conceivable among the four major phenotypes, and of these, all have been observed at least once with the exception of that from speckled to rare sectorial. Even this change has been observed in a single clone occurring in two steps from speckled through sectorial to rare sectorial.

It should be mentioned that among the variegated-1 phenotypes that of speckled appears to be the most stable. Also, no gross phenotypic alterations have been observed in a large clonal culture of the flecked variegated-3.

Comparative genetic tests, employing progeny of phenotypic chimeral plants and

TABLE 6

Correlation between frequency of sectors in sectorial plants and the frequency of v_s alleles in their progeny

Sectorial plant selfed	Parental sectors/degree	Segregation homogeneity				No. progeny
		Progeny % v_s	Chi square	d.f.	P	
S527-34	0.55 ± 0.08	57.3	—	—	—	289
S527-36	0.74 ± 0.04	73.0	—	—	—	252
			28.33	2	0.0001	

clones, have been used to investigate the variegation problem further. In table 7 the results of eight such tests are presented, the last three of which indicate significantly different segregations.

In general the procedure was to self-pollinate flowers of two different phenotypes appearing on chimera branches or clones of one plant. However, in next to the last test shown in the table two sectorial flowers were pollinated, one by a sectorial and the other by a speckled flower from this same plant. Also, in the last test shown in table 7 one sectorial and one speckled flower, each on different branches of a sectorial plant, were pollinated with a single flower from another plant which was entirely speckled.

It is to be noted that two of the three tests showing significant differences in table 7 involve single flower pollinations. The first six tests in the table establish that significant differences in average breeding behavior between flower phenotypes are not necessarily demonstrable when capsules from several pollinations are bulked. Such differences do exist, however, between single flowers of the same plant as shown by the last two tests in table 7. In general, the particular sectoring events which alter the proportions of somatic tissue in the epidermal layer of the flowers are

TABLE 7
Comparative segregations from two-membered chimera plants and clones

Culture	Parentage	Phenotype	No. of capsules	Progeny segregation			Homogeneity hypothesis		
				<i>s</i>	<i>S</i>	<i>Rare-S</i>	Chi square	d.f.	P
S537	S521-48 (X)	<i>s</i> branch (X)	4	62	12	0			
S536	S521-48 (X)	<i>S</i> branch (X)	3	21	3	0	0.19	1	0.66
S547	S521-48 (X)	<i>s</i> clone (X)	3	55	1	0			
S5419	S521-48 (X)	<i>S</i> clone (X)	4	56	6	0	3.28	1	0.07
S5420	S5314-30 (X)	<i>DsLs S</i> branch (X)	4	59	6	0			
S5421	S5314-30 (X)	<i>s</i> branch (X)	2	65	2	0	2.26	1	0.13
S5422	S5317-14 (X)	<i>S</i> branch (X)	4	7	23	6			
S5423	S5317-14 (X)	<i>s</i> branch (X)	3	8	28	19	3.50	2	0.18
S5424	S5321-23 (X)	<i>Rare-S</i> branch (X)	2	1	9	13			
S5425	S5321-23 (X)	<i>S</i> branch (X)	1	0	4	6	0.002	1	0.99
S5433	S527-36 (X)	<i>S</i> clone (X)	4	60	25	1			
S5418	S527-36 (X)	<i>s</i> clone (X)	3	22	34	5	13.60	1	0.0002
S526	51105-30 (X)	<i>S</i> branch (X)	1	33	9	2			
S527	51105-30 (X)	<i>S</i> (X) <i>s</i> branch	1	12	18	1	11.11	1	0.001
S5214	51107-52 X 51105-36	<i>S</i> branch X <i>s</i>	1	23	10	0			
S5215	51107-52 X 51105-36	<i>s</i> branch X <i>s</i>	1	15	23	1	6.48	1	0.011

TABLE 8
Comparative segregations from different phenotypes within two chimera clones

Culture	Parentage	Phenotype	No. of capsules	Progeny segregation			
				<i>s</i>	<i>S</i>	<i>Rare-S</i>	<i>DsLsS</i>
S531C	S526-6 (X)	<i>Rare-S</i> (X)	5	74	6	0	0
S5327	S526-6 (X)	<i>s</i> (X)	2	26	4	0	0
S5326	S526-6 (X)	<i>Rare-S</i> × <i>s</i>	2	34	4	0	0
S5328	S526-6 (X)	<i>s</i> × <i>Rare-S</i>	3	31	7	0	0
S5416	S5210-1 (X)	<i>s</i> (X)	4	50	3	1	1
S5415	S5210-1 (X)	<i>S</i> (X)	5	24	10	53	2
S5414	S5210-1 (X)	<i>Rare-S</i> (X)	5	8	14	32	0
S5417	S5210-1 (X)	<i>DsLsS</i> (X)	3	23	2	27	12

apparently relatively independent of the similar events which are considered to cause the demonstrated alterations in germinal tissue in different flowers.

More intensive tests are available for two chimera clones, genetic data for which are shown in table 8. The parent plant of the first clone, S526-6, was of rare sectorial phenotype but cuttings from it were of two types: rare sectorial and speckled. Self-pollinations of the two types and reciprocal crosses between them reveal a consistent speckled breeding behavior for all families. This represents our best documented case for the existence of periclinal chimeras in the variegated-1 culture. Although the parent plant and more than half its cuttings were of rare sectorial phenotype, not a single rare sectorial offspring was observed among 186 progeny from 12 seed capsules.

The parent plant of the second clone, S5210-1, was of *DsLsS* phenotype but cuttings from the parent represented four phenotypes: *s*, *S*, *rare-S*, and *DsLsS*. Self-pollinations of each of these phenotypes gave the segregations shown in the second part of table 8. The segregation among progeny of speckled selfed, *s* (X), is clearly different from the other three families which are themselves statistically heterogeneous. The segregation for *DsLsS* selfed is significantly different from that for both *S* selfed and *rare-S* selfed, although the latter two families differ from each other only at the five percent level. Thus the clone contains members of at least three categories of breeding behavior and, by inference, members of at least three genotypes. This is the best documented case favoring the interpretation that the phenotypic sectoring events observed in the variegated-1 cultures represent alterations in the genotype.

PRELIMINARY CYTOLOGICAL STUDIES

A limited number of cytological observations have been made on microsporocytes, root tips, and young lateral shoot meristems prepared by the aceto-orcin smear method. Sufficient data have not yet been obtained from a large enough sample of plants with adequate controls to permit more than a preliminary evaluation of the cytological characteristics of the material.

A summary of the data available on mitotic anaphase aberrations is presented in

TABLE 9
Summary of observations on the frequency of mitotic anaphase aberrations

Culture	Mitotic late anaphase cells scored			% Aberrant anaphases
	Normal	Bridge	Fragment	
Variegated-1	1538	7	6	0.84
Variegated-3, flecked	276	3	1	1.43
<i>Sanderae</i>	123	0	0	0

TABLE 10
Summary of observations on the frequency of microsporocyte anaphase I aberrations

Culture	Anaphase I meiocytes scored			% cells with laggards	% cells with fragments
	Normal	Laggards	Fragments		
Variegated-1	795	187	149	16.53	13.17
<i>Sanderae</i>	220	46	6	16.91	2.21

table 9. There is unquestionably a low frequency of somatic bridges at late anaphase in the two variegated cultures. There is however a serious paucity of observations on *sanderae* and, furthermore, the other different variegated phenotypes have not been studied cytologically. While the possibility of a correlation between mitotic aberrations and the variegated phenotype may exist, or at least cannot at present be ruled out, the genetic data do not support an interpretation of loss of chromosomal material as the cause of variegation.

A summary of the data obtained from studying microsporocytes at first anaphase is presented in table 10. A relatively high frequency of bridge-like formations at early first anaphase is characteristic in this material. It appears to be a manifestation of lack of synchronization in separation of the bivalents, as if a chromosome stickiness were present. This is characteristic of *sanderae* as well as of variegated-1. Cells were classified as containing laggards in table 10 if either this formation or lagging chromosomes were observed.

The frequency of meiocytes containing fragments may be higher in variegated-1 than in *sanderae*, as indicated by the data in table 10; but this may be of no pertinence to the variegation problem since the variegated cultures are of relatively recent hybrid origin. A control culture more appropriate than *sanderae* would be nonvariegated with a residual hybridity more nearly equivalent to that of the variegated cultures.

DISCUSSION

Evidence has been given that a particular genotype, differing from *sanderae* in only one locus (*V*), must be present to show the variegated-1 phenotype. Homozygosity for *v* insures that one of the variegated-1 phenotypes will be displayed.

Two alleles at the variegated locus, *v_s* and *v_S*, are necessary and sufficient to account genetically for different modal breeding behaviors of the three major variegated-1 phenotypes: speckled, sectorial, and rare sectorial.

Deviations from modal breeding behavior and from Mendelian expectation, demonstrably not due to sampling errors, suggest that v_s and v_s are unstable states of the variegated locus. However, despite changes in both directions, the variegated locus appears constant in its instability, in the sense that it remains v without giving rise to detected V in the form of stable red or stable white individuals.

Evidence obtained from favorable clonal material has indicated that the phenotypic sectoring events are correlated with alterations in breeding behavior. Since reciprocal crosses give the same progeny segregations, it appears untenable to consider the phenotypic sectoring as an expression of physiological instability of a gene influenced cytoplasmic system. Rather, the phenotypic sectoring phenomenon is interpreted as an expression of primary events occurring at the gene or chromosomal level of cell organization.

Since the mutant cells ($v_s \rightarrow v_s$) in speckled epidermis are spatially distributed more evenly than the mutant cells ($v_s \rightarrow v_s$) in sectorial epidermis, there is greater phenotypic variation among flowers of a sectorial plant than among flowers of a speckled plant. The greater variation in progeny segregations from different flowers of the same sectorial plant thus becomes interpretable in terms of a similar difference in spatial distribution of mutant cells in the *germinal* tissue of sectorial versus speckled.

A system of alternative expression of the v locus in somatic tissue is proposed, whereby changes between the v_s and v_s alleles are correlated with changes between the potential absence and presence of pigmentation. Since genetic tests indicate that the change occurs in both directions, it is strongly implied that the change is reversible in germinal tissue. Reversible changes in somatic tissue, with that from v_s to v_s (to produce sectors) occurring earlier in development than the reverse change (to produce speckling within the sectors), are consistent with the variegated-1 phenotypes. In addition, colder temperatures increase speckling intensity but decrease sectoring frequency (SAND 1955), which is an effect consistent with the two phenomena being reversals of each other. Since this concept of reversal in expression integrates the available facts and is subject to further test, it is entertained as a working hypothesis.

The reversal indicates that there is inhibition and release from inhibition of a locus critical to pigment synthesis. If either condition had ever been observed to be permanent, somatic segregation or a loss mechanism conceivably could be proposed on the basis of chromosomal aberration. But in the absence of evidence for stable derivatives of v , the behavior of the material is more readily reconciled with that expected of a reversibly "mutable gene" or of a variegated-type position effect (LEWIS 1950). Critical evidence is not now available to permit distinction between these two models.

However, certain information about the origin and behavior of the variegated culture may bear upon its nature. Nonvariegated F_1 's result from crosses of the variegated culture with *N. sanderae* and with very rare exceptions from crosses with *N. langsdorffii*. It is reasonably clear from this observation in conjunction with segregations obtained in F_2 and test cross generations that the mendelizing unit, v , correlated with variegation is unique to the unstable culture or at least is neither

homozygous nor heterozygous in *sanderæ* or in *langsдорffii*. The tests indicate that if *v* is present in either *sanderæ* or *langsдорffii* its frequency is very low. It thus appears more likely that *v* arose, and may be repeatedly generated, in the F_1 hybrid of *N. langsдорffii* by *N. sanderæ*.

If this be its origin, it is conceivable that *v* could come into existence in the F_1 by (1) point mutation in an element of either the *sanderæ* or the *langsдорffii* genome, or by (2) crossing over or translocation to produce an interspecific chromatin association. In the former case, *v* should be considered a *mutable gene*; whereas in the latter case *v* should be considered the result of a chromosomal rearrangement showing a *variegated-type position effect*.

If the variegated *Nicotiana* cultures contain such a chromosomal rearrangement showing position effect, the rearrangement is likely homozygous due to selection for *v* which is genetically recessive. Thus it should maintain itself unaltered, in crosses within the culture, without giving meiotic products in which the normal arrangement is restored. This expectation is consistent with the failure to detect stable derivatives of the variegated locus. The origin of a rearranged chromosome is conceivable in the interspecific hybrid from which the variegated cultures derived. Since considerable meiotic chromosome pairing occurs in the hybrid (SMITH 1937), crossing over might occasionally produce a particular interspecific chromatin association giving a position effect.

Interpretation of variegation phenomena and their relation to gene action and mutation is at present in a state of heightened interest and speculation owing to significant experimental results obtained mainly in *Drosophila* and in maize. LEWIS (1950) has brought some order to a complex situation in *Drosophila* by distinguishing between stable and variegated type position effects. The latter has been interpreted in terms of intrachromosomal action between euchromatic genes and heterochromatin, beginning with the work of SCHULTZ (1936) and including such examples as DEMEREC (1940), BELGOVSKY (1946), PROKOFYEVA-BELGOVSKAYA (1947), and BAKER (1953).

In maize different controlling elements and systems of interrelated elements, associated with but accessory to the genes, have been adduced which control gene action and mutation (McCLINTOCK 1951, 1953, 1956). Their most characteristic phenotypic effect is to give distinctive types of variegation. A number of such systems have now been described: the *Dt-a₁* system (RHOADES 1941), the *Ac-Ds* system (McCLINTOCK 1951), the *P^{RR}-Mp* system (BRINK 1954), the *Pg-En* system (PETERSON, 1953, 1955), and the *Spm-a₁^{m-1}* system (McCLINTOCK 1956).

It is not possible at present with our *Nicotiana* material to make appropriate tests to distinguish between mutation and position effect (CATCHESIDE 1947; JUDD 1955) nor to permit critical comparisons with the maize systems. Specifically we lack known linked marker genes and a stable recessive allele of the *V* locus. However, dark and light speckling in the *Nicotiana* variegateds may be analogous to dark and light mottling in *Drosophila* and to *Ds* or *a₁^{m-1}* changes of state in maize.

If the assumption is allowed that the *Nicotiana* case, based on analogy with *Drosophila*, represents a position effect in which "temporary modifications" (GERSE 1952) or heterochromatization (PROKOFYEVA-BELGOVSKAYA 1947) may be specu-

lated, then the V locus may be visualized with a threshold value for genic inhibition midway between the maxima and minima of a heterochromatization cycle. Thus in somatic tissue a cell (giving rise by division to a sector) of potential mutant expression originates when heterochromatization exceeds the genic inhibition threshold. The sector resulting would be colorless except that the heterochromatization cycle later falls below the gene inhibition threshold in certain cells within the sector permitting genic action and resulting in pigmentation. Reversibility in phenotypic expression results. In germinal tissue also the reversibility may occur, but apparently both the heterochromatic and the euchromatic conditions of the locus have sufficient persistence in sexual reproduction to permit their genetic detection. Respectively they have been named the unstable alleles v_s and v_S . This scheme is a speculative concept of the reversible "mutation" shown by v .

SUMMARY

The origin, following an interspecific hybridization, of *Nicotiana* cultures showing flower-color variegation is described.

The variegated-1 phenotypes of speckled, sectorial, rare sectorial, and Dark speckled-Light speckled-Sectorial are described and compared with the phenotypes of variegated-3 and *N. sanderae*.

Genetic data are presented which support the hypothesis that variegated-1 individuals possess a *recessive, unstable* allele, v ; thus differing by one gene from *N. sanderae*, which contains a *dominant, stable* allele, V , at this locus. Trisomic ratios suggest that the position of the v locus is on chromosome 2 of the complement.

Further genetic data show that speckled, sectorial, and rare sectorial individuals have different genotypes. Two unstable alleles, v_s and v_S , at the v locus are adequate to explain the floral phenotypes and the modal breeding behaviors. These three variegated-1 genotypes are represented, respectively, as follows: v_s/v_s , v_S/v_s , and v_S/v_S . The phenotypic variegation is attributed to somatic mutation. The aberrant genetic ratios have a pattern which is plausible in terms of interallelic mutation in germinal tissue. These genetic events in both somatic and germinal tissue appear to be successively reversible between the allelic forms v_s and v_S .

Studies are designed to estimate the magnitude of several possible sources of the unusual variability in the observed segregations. It is concluded that there exists no detectable differential pollen-tube competition between the gamete types, and that the segregations are repeatedly obtainable from the same seed-lot under the cultural conditions employed. There is no evidence for differential seed pan competition among segregants. Two types of evidence are presented which demonstrate a correlation between a quantitative expression of the phenotype and its breeding behavior. Speckled, v_s/v_s , plants of different speckling intensity may show a *correlated difference* in the frequency of v_S bearing individuals occurring in their progeny. Also, two sister sectorial, v_S/v_s , plants have shown correlation between their phenotypic frequency of sectors of *recessive* (presumed v_s/v_s) somatic tissue and the respective frequency of recessive alleles, v_s , in progeny from self-pollinations.

Comparative genetic tests of flowers from chimeral plants and clones support the hypotheses that (1) periclinal chimeras exist in the variegated-1 culture, and (2)

the sectors observed in the variegated-1 phenotypes reflect alterations in the genotype which are genetically demonstrable if they occur early enough in development and in germinal tissue.

Preliminary cytological studies of variegated plants have shown chromatin bridges in about one percent of the somatic cells observed at late anaphase. Also, in microsporocytes at first anaphase the frequency of cells containing chromosome fragments is about ten percent greater than in *N. sanderae*. However, *sanderae* is not considered a valid control because of its lesser residual hybridity.

LITERATURE CITED

- BAKER, W. K., 1953 V-type position effects of a gene in *Drosophila virilis* normally located in heterochromatin. *Genetics* **38**: 328-344.
- BELGOVSKY, M. L., 1946 On the causes of mosaicism associated with heterochromatic chromosome regions. *Am. Naturalist* **80**: 180-185.
- BRINK, R. A., 1954 Very light variegated pericarp in maize. *Genetics* **39**: 724-740.
- CATCHESIDE, D. G., 1947 The *P* locus position effect in *Oenothera*. *J. Genet.* **48**: 31-42.
- DEMEREK, M., 1940 Genetic behavior of euchromatic segments inserted into heterochromatin. *Genetics* **25**: 618-627.
- GERSH, E. S., 1952 Pigmentation in a mottled white eye due to position effect in *Drosophila melanogaster*. *Genetics* **37**: 322-338.
- JUDD, B. H., 1955 Direct proof of a variegated-type position effect at the white locus in *Drosophila melanogaster*. *Genetics* **40**: 739-744.
- LEWIS, E. B., 1950 The phenomenon of position effect. *Advances in Genet.* **3**: 73-115.
- MCCLEINTOCK, B., 1951 Chromosome organization and genic expression. *Cold Spring Harbor Symposia Quant. Biol.* **16**: 13-17.
- 1953 Induction of instability at selected loci in maize. *Genetics* **38**: 579-599.
- 1956 Intranuclear systems controlling gene action and mutation. *Brookhaven Symposia Biol.* **8**: 58-74.
- PETERSON, P. A., 1953 A mutable pale green locus in maize. (Abstr.) *Genetics* **38**: 682.
- 1955 Dosage effect on the mutability of a mutable pale green locus in maize. (Abstr.) *Genetics* **40**: 589.
- PROKOFYEVA-BELGOVSKAYA, A. A., 1947 Heterochromatization as a change of chromosome cycle. *J. Genet.* **48**: 80-98.
- RHOADES, M. M., 1941 The genetic control of mutability in maize. *Cold Spring Harbor Symposia Quant. Biol.* **9**: 138-144.
- SAND, S. A., 1955 Heritable somatic instability in *Nicotiana* cultures derived from a hybrid of *N. langsdorffii* by *N. sanderae*. Ph.D. thesis, Cornell Univ. 72 pp.
- SCHULTZ, J., 1936 Variegation in *Drosophila* and the inert chromosome regions. *Proc. Natl. Acad. Sci. U.S.* **22**: 27-33.
- SMITH, H. H., 1937 Inheritance of corolla color in the cross *Nicotiana langsdorffii* by *N. sanderae*. *Genetics* **22**: 347-360.
- 1943 Effects of genome balance, polyploidy, and single extra chromosomes on size in *Nicotiana*. *Genetics* **28**: 227-236.