3cep

COO-1400-41

RESOLVING THE ENIGMA OF MULTIPLE MUTANT SECTORS IN STAMEN HAIRS OF TRADESCANTIA¹

L. W. MERICLE AND R. P. MERICLE

Department of Botany and Plant Pathology, and Biology Research Center

Michigan State University, East Lansing, Michigan 48823

ABSTRACT

Mutant sectors in stamen hairs of Clone 02 *Tradescantia* are designated as "multiple sectors" when two or more occupy the same hair, separated by non-mutant cells. Statistical analyses show that most multiple sectors do not arise as chance associations of independent events: when the frequency of stamens with two or more sectors is lowest, the probability that the sectors will be located in the same, rather than in different, hairs is highest. Ontogenetically, the ratio of sector pairs in different hairs to pairs in the same hair is highest in that period of response to acute irradiation prior to the appearance of entire-hair sectors; thereafter, the ratio subsides, approaching that of spontaneous mutations. No classical concept of mitotic chromosomes will account for the production of multiple mutant and non-mutant sectors, dispersed along a linear structure, following a single mutational event. However, by postulating a minimal bichromatid chromosome structure, with mutation or breakage in not more than one DNA molecule, then, with continued mitotic activity, possibilities are provided for either the immediate segregation of mutant and non-mutant cells, or for the perpetuation in daughter nuclei of a "heterozygous" chromosome capable of segregation at some later mitosis.

¹ Auspices of US AEC, Tech. Rpt. COO-1400-41

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Stamen hairs of the genus *Tradescantia* typically are linear multicellular appendages arising from the lower two-thirds of the stamen filament. In Clone 02, which carries the D gene for delphinidin pigment in single dose (D^+D^-),² the cells of the stamen hairs normally exhibit the same purplish-blue color as cells of the filament, style, and petals. However, when the D gene is inactivated, mutates to an amorphic allele, or is lost by deletion or mitotic recombination, prior to 3'-hydroxylation of the anthocyanin precursor, "mutant", D-deficient cells (D^-D^-) are formed which develop purplish-red pigmentation (MERICLE and MERICLE 1971b). These mutations occur spontaneously and with increased frequency in response to ionizing radiation (MERICLE and MERICLE 1965a, 1965b, 1967a, 1969; SPARROW *et al.* 1972) and certain environments (MERICLE and MERICLE 1965a, 1965b, 1967a, 1971a; SPARROW *et al.* 1971). Once a D-deficient "red" cell is created, all mitotic derivatives of that cell are likewise red in color (no proven instance of reverse mutation from D^- to D^+ is known, MERICLE and MERICLE 1967b, 1971b). Therefore, the product of a single mutational event may be expressed at anthesis as an individual, isolated red cell, or as a multicellular sector composed of two to hundreds or thousands of contiguous red cells, depending upon how early in development the D-deficient cell originates. Stamen sectors are designated (MERICLE and MERICLE 1969) as "single sectors", if comprised of only one red cell or a portion of one hair; as "entire-hair sectors", if all cells of a hair are included in the sector, and as "multiple-entire-hair sectors" if two or more entirely mutant hairs are connected by a vertical file of mutant cells in the filament. In addition,

2 Whether Clone 02, a diploid with $2n = 12$, is heterozygous or hemizygous at the D-locus (with D^- representing a recessive, amorphic allele, or the absence of the D-locus, resp.) is still unresolved (MERICLE and MERICLE 1971b).

some stamen hairs exhibit more than one red sector, separated by normal-appearing blue cells (MERICLE and MERICLE 1967b, 1971b). It is the origin of these "multiple sectors" with which we are here concerned. Do they arise as a consequence of chance, coincident occurrence of two or more independent events in the same hair, or is their origin, as a group, traceable instead to some common, single initiating event? And if the latter, by what cytogenetic mechanisms are they induced and how is their spacial dispersal within a linear, single file structure accomplished?

Origin of multiple sectors as multiple vs. single mutational events: In Clone 02 each of the six stamens per flower possesses 30-70 (avg ~50) hairs of 10-30 (avg ~20) cells in length. The occurrence of two or more sectors of spontaneous origin within the same stamen is not common, averaging only about once for every 100 stamens examined. Therefore, on the basis of chance alone, one would expect, by overwhelming odds, that any two or more spontaneous sectors arising as independent mutational events would occupy different hairs within a stamen. This is not at all the case, as became apparent earlier in our *Tradescantia* work (MERICLE and MERICLE 1967b), and has been reinforced now by additional data.

Table 1 includes spontaneous mutation data pooled from ~40 Clone 02 experiments (carried out with variously aged materials under a wide variety of environments). Among the 13,466 stamens scored, 136 (1%) contained two or more non-entire-hair sectors. If these sectors are analysed on a pair-by-pair basis as to whether any two sectors are located in different hairs (d) or in the same hair (s) of a given stamen, the ratio of d:s should be highly in favor of d (approximating 50:1 if all cells of a hair had equal potential for mutation induction and expression, and all hairs of a stamen contained

the same number of cells). Instead, the d:s ratio is only 0.7:1! This means that the sectors are not distributed at random among the ~50 hairs of a stamen, but are actually localized preferentially within the very same hair (even the χ^2 for a 1:1 ratio has a p of <0.02). Preferential localization is also supported by our occasional observation of five or six discontinuous red sectors within a single hair of a stamen exhibiting no other mutations.

The data from spontaneously mutated hairs are essentially duplicated in stamens receiving very low levels of chronic radiation (<100 mR/da from ^{60}Co or terrestrial thorium and uranium emanations), Table 1. Here, the frequency of stamens with two or more sectors is also low (0.5%) and the d:s ratio, 0.9:1.

When the incidence of mutation is increased (e.g., by 2-3 R/da chronic ^{60}Co γ -radiation, chronic diurnal temperature regimes of 80/40 F, or the presence of genetic modulators for high mutability), the frequency of stamens with two or more sectors rises, Table 1. This should increase the likelihood of chance association of any two sectors within the same hair. Instead, the reverse is true! So long as the frequency of stamens with two or more sectors remains relatively low ($<5\%$) the d:s ratio is not more than 2:1. But as the frequency approaches unity, reaching 97%, the d:s ratio increases markedly until, at 47.1:1, it approximates random distribution of sector pairs among all hairs of a stamen.

The above data demonstrate that, especially at low mutation frequencies, the occurrence of multiple sectors within a stamen hair is not a chance, multiple association phenomenon, but should be looked upon, instead, as the product of a single mutational event. Further substantiation in this regard is shown in the data from acute radiation experiments presented below.

Time of origin of multiple-sectored hairs: Following an acute exposure

of developing floral buds of Clone 02 to X- or γ -radiation, a latent period of several days ensues during which there is no change in mutation frequency from background levels. Then mutation frequency begins to rise sharply, reaches a maximum within a few days, and thereafter gradually returns to pre-response levels over a period of several weeks. During the time of maximum mutation production, almost all mutant sectors encompass less than entire hairs. As the mutation frequency declines, entire-hair sectors are present in their greatest proportional incidence, and finally, as the mutation response curve once again approaches pre-response levels, multiple-entire-hair sectors make their appearance (MERICLE and MERICLE 1969).

If multiple-sectored hairs are the result of chance associations of independent events, then their highest proportional frequency should coincide with the peak of overall mutation production in the stamen response curve. That this does not occur has been pointed out previously (MERICLE and MERICLE 1969), and can be seen, also in Table 2, which contains the pooled data from a number of acute radiation experiments. Pair-by-pair analysis of stamen sectors yields a far higher d:s ratio in that period of mutation response prior to the appearance of entire-hair sectors than during or after that time. Just as in the cumulative mutation data in Table 1, the more frequent the occurrence of stamens with two or more sectors, the more nearly the d:s ratio expresses random distribution of the sectors amongst all hairs of a stamen. However, once the entire hair sectors have begun to make their appearance, the d:s ratio dramatically subsides to levels equal to or approaching that of spontaneous mutations.

The data in Table 2 supplement our previous observations (MERICLE and MERICLE 1969) that hairs containing only two sectors may occur in relatively

high frequency during or immediately after the period of maximum mutation production (so some of these hairs may truly represent associations of independent events). But most hairs exhibiting three to six sectors appear considerably later in the mutation response curve (corresponding to the time of hair initiation and early hair development). The mutational event responsible for triggering the formation of such highly multiple-sectored hairs must take place, therefore, at a time in stamen ontogeny when the hair consists of only one or a very few cells (or coincident with its origin from the filament).

Yield and dispersal of multiple sectors: Classical concepts of chromosome behavior during mitosis can readily account for the formation of a D-deficient red sector following deletion of the D-locus at the chromosome or chromatid level, or inactivation or mutation of the D-gene. Mitotic recombination between the D⁺ and D⁻ homologs could also give rise to a D-deficient sector, although there is still no positive proof of somatic crossing-over in higher plants (MERICLE and MERICLE 1967b, 1971b). But to account for the production of multiple mutant and non-mutant sectors dispersed along a linear structure such as a stamen hair, consequent to a single initiating mutational event, forces consideration of other schemes, since none of the above mechanisms will yield more than one sector per event.

Analyses of Feulgen-positive micronuclei (chromosome fragments) in single-sectored stamen hairs indicate that the mechanism for mutation at the D-locus can, but usually does not, involve chromosome breakage when the mutations are of spontaneous or low-level radiation origin (MERICLE and MERICLE 1967b, 1971b). Yet in multiple-sectored hairs of the same origin, micronuclei are present approximately twice as often, showing that, here, chromosome aberration serves more frequently as the invoking mechanism

(MERICLE and MERICLE 1967b). MC CLINTOCK's (1951) "breakage-fusion-bridge" cycle, with its reliance on chromosome breakage as the initiating event, and its provision for repeated production of either genetically deficient or non-deficient cells, would offer a tempting explanation for multiple sectors. In *Tradescantia*, however, unlike some plants, broken chromosome ends apparently do not fuse after replication, but remain separate (SAX and MATHER 1939): a condition which would preclude operation of a "b-f-b" cycle. Furthermore, even though chromosome or iso-chromatid breakage could yield, de novo, two D-deficient cells following a single event, those cells would lie adjacent to one another in the hair and so be indistinguishable from a single D-deficient cell which had undergone one additional mutation.

At the present time, consideration of a polyneme chromosome structure seems to provide the only reasonable basis for explaining the development of multiple-sectored stamen hairs. The best evidence for the existence of a polyneme state in *Tradescantia* chromosomes is that of SPARVOLI et al.'s (1965) tetraspireme reconstructions of anaphase chromosomes from electron micrographs of dividing stamen hair cells. But even if we assume a minimal bineme structure — with the G_1 chromosome comprised of two DNA double helices, as in PEACOCK's (1963) model, and with mutation or breakage occurring in either one or both strands of one of the DNA molecules — then we can readily derive a scheme for both the production and the dispersal of multiple sectors.

Letting $\underline{D^+D^+}$ represent the genetic information contained in the two DNA molecules comprising one homolog in Clone 02, and $\underline{D^-D^-}$, the other homolog, stamen hair cells in G_1 would have a "DNA-genotype" relationship of $\underline{D^+D^+}/\underline{D^-D^-}$. By deletion or mutation in both strands of one DNA molecule, the $\underline{D^+D^+}$ chromosome would be converted to $\underline{D^+D^-}$, and contain at G_2 , after semi-conservative

replication, two $\underline{D}^+$ and two $\underline{D}^-$ DNA molecules; whereas, the $\underline{D}^+D^-$ homolog, after synthesis, would contain four $\underline{D}^-$ DNA molecules. Mutation or deletion in only one strand of one DNA molecule of the $\underline{D}^+D^+$ homolog would simply postpone creation of the $\underline{D}^+D^-$ chromosome through an additional mitotic cycle.

PEACOCK's model seems to assume in G_2 either a random two-by-two sorting of the four DNA molecules into the two chromatids of a metaphase chromosome, or that all combinations are possible even though some associations might occur preferentially. On the basis of random association, the probability would be $1/3$ for the two mutant DNA molecules ending up in the same chromatid. This would give rise to two daughter cells, one of which would become red in color because of DNA-genotype $\underline{D}^+D^-/\underline{D}^+D^-$, and the other, blue, with DNA-genotype $\underline{D}^+D^+/D^-D^-$. The probability would be $2/3$ for both daughter cells being blue, but each carrying one of the mutant DNA molecules in a "heterozygous" $\underline{D}^+D^-$ chromosome which, along with its homolog $\underline{D}^+D^-$, would then possess the potential for segregating red and blue cells at some later mitosis. Any degree of preferential association of "sister" DNA strands (ones contained in the same DNA molecule prior to synthesis) into the same chromatid, would increase the probability for red-blue cell segregation above $1/3$. Should within-chromatid reassociations be the rule, perpetuation of a $\underline{D}^+D^-$ chromosome could only be accomplished by invoking new mutational events or interchromatid exchanges; while if the converse be true, red-blue cell segregation would require additional events or exchange. PEACOCK's (1963) data from Vicia faba shows a high frequency (>50%) of interchromatid exchange in mitotic chromosomes. If a similar situation obtains in Clone 02 Tradescantia, the net result would be some attenuation of whatever degree of preferential assortment might be present, without any need for resorting to additional mutation events.

Postulation of a bineme structure (two double-helix DNA molecules) for G_1 chromosomes in Clone 02 will provide for the immediate production of a D-deficient cell and its D-containing counterpart, as well as for the delayed segregation of such cells later in hair ontogeny. The only other requisite for multiple-sectored hair formation would seem to be a sufficiently high mitotic capability by all cells during early hair ontogeny to allow for repeated segregations. This proviso is probably met by our observation that in young stamen hairs comprised of as many as six cells, all cells may be in some stage of mitosis at the same time.

The above scheme for the formation of multiple sectors in linear stamen hairs will serve equally well to explain in petals: the occurrence of midline files of 20 or more alternating mutant and non-mutant sectors, or well-demarcated "mosaic" regions of 30 or more small red sectors, concomitant with midline files of contiguous red cells extending the entire length of the petal, or with the appearance of large, contiguous red cell sectors, resp. (MERICLE and MERICLE 1969).

Here we have presented but one possible scheme whereby a single mutational event can yield dispersed, multiple mutant products; other's ideas are welcome. We feel it is especially important to recognize that in any heterozygous or hemizygous system, mutant cell mosaicism need not mean the presence of a highly unstable, repeatedly mutable, gene state, but only requires the first order of chromosome polystructure above unineme, and continued mitotic capability of the daughter cells, following a single mutation. Such spacial dispersion of multiple mutant products may well play an important role in the distribution of mutations throughout plant populations which reproduce apomictically, and should be considered in any somatic mutation theory for etiology of cancer.

LITERATURE CITED

- MC CLINTOCK, B., 1951 Chromosome organization and genic expression. Cold Spring Harbor Symp. Quant. Biol. 16: 13-49.
- MERICLE, L. W., and R. P. MERICLE, 1965a Biological discrimination of differences in natural background radiation level. Radiat. Bot. 5: 475-492.
- , 1965b Reassessing the biological role of background terrestrial radiation as a constituent of the natural environment. Health Phys. 11: 1607-1620.
- , 1967a Mutation induction as influenced by developmental stage and age. In: Induzierte Mutationen und Ihre Nutzung, p. 65-77, Erwin-Baur-Gedachtnisvorlesungen IV, 1966, Abh. Deut. Akad. Wiss. Berlin.
- , 1967b Genetic nature of somatic mutations for flower color in Tradescantia, Clone 02. Radiat. Bot. 7: 449-464.
- , 1969 Induced somatic mutations for interpreting floral development and inflorescence aging. In: Induced Mutations in Plants, p. 591-601, IAEA, Vienna.
- , 1971a Diurnal temperature difference as a modifier of mutagenesis. Environ. Mut. Soc. Newsletter No. 5: 28-29.
- , 1971b Somatic mutations in Clone 02 Tradescantia — a search for genetic identity. J. Hered. 62: 323-328.
- PEACOCK, W. J., 1963 Chromosome duplication and structure as determined by autoradiography. Genetics 49: 793-801.
- SAX, K., and K. MATHER, 1939 An X-ray analysis of progressive chromosome splitting. J. Genet. 37: 483-490.
- SPARROW, A. H., L. A. SCHAIRER, M. M. NAWROCKY, and R. C. SAUTKULIS, 1971 Effects of low temperature and low level chronic gamma radiation on somatic mutation rates in Tradescantia. Radiat. Res. 47: 273-274.

SPARROW, A. H., A. G. UNDERBRINK, and H. H. ROSSI, 1972. Mutations induced in *Tradescantia* by small doses of X rays and neutrons: analysis of dose-response curves. *Science* 176: 916-918.

SPARVOLI, E., H. GAY, and B. P. KAUFMANN, 1965. Number and pattern of association of chromonemata in the chromosomes of *Tradescantia*. *Chromosoma* 16: 415-435.

TABLE 1

Pair-by-pair analysis of sectors located in different (d) vs. the same (s)
stamen hair when mutations are of spontaneous or enhanced origin

	Stamens scored	Mutant events	Stamens 2 or > sec	Sec prs		Ratio d : s
				d	s	
Spontaneous	13,466	0.093	0.010	83	120	0.7 : 1
Chronic radiation						
<100 mR/da γ	5,022	0.083	0.005	15	16	0.9 : 1
2-3 R/da γ	2,694	0.249	0.045	107	103	1.0 : 1
80/40 F diurnal						
beginning	1,122	0.273	0.043	55	28	2.0 : 1
after >1 yr	528	0.633	0.153	122	13	9.4 : 1
plateau	830	0.849	0.224	364	36	10.1 : 1
Genetic control						
highly mut- able subclone	78	5.269	0.974	990	21	47.1 : 1

TABLE 2

Pair-by-pair analysis of sectors located in different (d) vs. the same (s)
stamen hair relative to appearance of entire-hair sectors (E) in response curve

Radiation exposure	Period in curve	Stamens scored	Mutant events	Stamens 2 or > sec	Sec prs		Ratio d : s
					d	s	
25-30 R X or γ	prior to E	1771	1,013	0.307	1367	235	5.8 : 1
	during and after E	1550	0.388	0.074	135	133	1.0 : 1
60 R X rays	prior to E	144	2,931	0.757	868	55	15.8 : 1
	during and after E	330	0.818	0.209	164	85	1.9 : 1
125 R X rays	prior to E	84	3,107	0.789	359	10	35.9 : 1
	during and after E	108	0.889	0.222	53	11	4.8 : 1