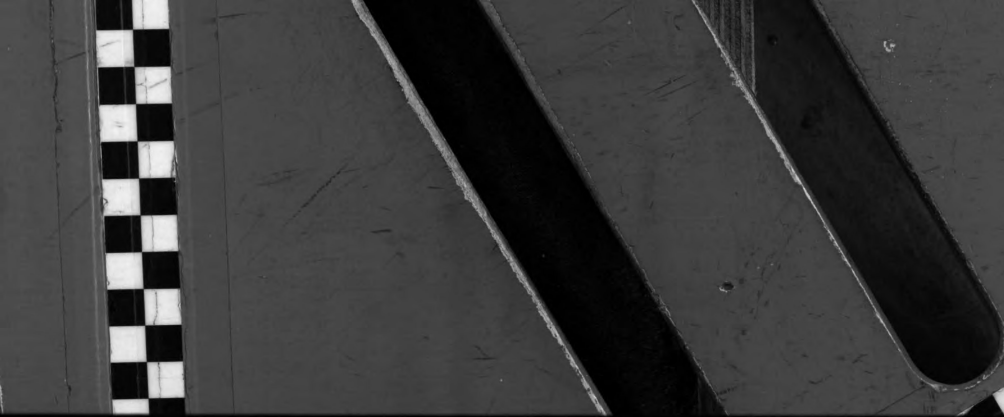

LEE CHRISTIANSON

Ph. D.

A CHARACTERIZATION OF SOMATIC SECTORING IN TRADESCANTIA

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
MICHAEL LEE CHRISTIANSON
1975

This is to certify that the

thesis entitled

A Characterization of Somatic Sectoring in Tradescantia

presented by

Michael Lee Christianson

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in **The Genetics Program and Department of Botany and Plant Pathology**

H. W. Mericle

Major professor

Date **July 27, 1976**

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ABSTRACT

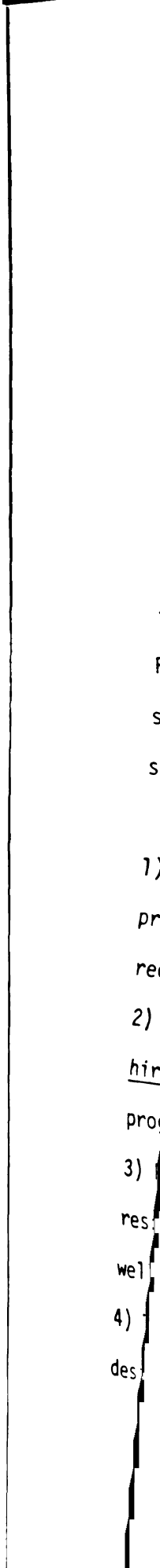
A CHARACTERIZATION OF SOMATIC SECTORS IN TRADESCANTIA

By

Michael Lee Christianson

Somatic sectoring in Tradescantia is the most sensitive biological indicator of radiation known, and has been suggested as an admirable test system for the study of chemical mutagens as well. The radiobiology of Tradescantia, as induced chromosome aberrations, is described in an enormous literature, but the investigation of the mechanism(s) of production of somatic "mutations" has just begun. Unless or until the mechanisms of "low-level sectoring (spontaneous and few-fold enhancement)" in Tradescantia are well described and shown to operate in similar relative frequency and at similar efficiency in human beings, the extrapolation of relative biological effectiveness (RBE) or the transfer of "mutation rate" from Tradescantia sp. to Homo sapiens cannot be valid.

This dissertation makes a major contribution to the first process, i.e., the characterization of spontaneous and slightly-enhanced somatic sectoring in Tradescantia. Section I of the dissertation describes the chemogenetics of a diploid, purple-flowered T. hirsuticaulis stock and the mutant-colored (red and blue) cells found upon close inspection of the pigmented floral parts. Section II considers the mutant sectors in the stamen hairs, monofiliform chains of large, anthocyanin-pigmented cells, in more detail. The red and blue mutant cells are analysed with respect to their



distribution within and among the hairs on a stamen, as well as with respect to their frequencies and associations with putative chromosome fragments both spontaneously and after exposure to relatively low amounts of ^{60}Co gamma radiation. These analyses support an argument for somatic crossing-over as the major mode of production of spontaneous sectors as well as an important mode of production of sectors after exposure to as much as 60 R of radiation. Section III considers some conceptual analogies in the genetic behavior of facultative apomicts and somatically sectoring Tradescantia flowers. From this consideration arises a new method of treating somatic sectoring data and the introduction of a previously undescribed somatic genetic parameter, A, the apomictoid fraction.

Major findings of this study may be summarized as follows:

1) Purple flower color of the T. hirsuticaulis stock is due to the presence of approximately equal amounts of blue (delphinidin) and red (cyanidin) pigments.

2) The total amount of anthocyanin pigment in purple-flowered T. hirsuticaulis is twice that in blue- or red-flowered T. clone 02 and progeny.

3) Purple-flowered T. hirsuticaulis possesses an allele, E^+, which is responsible for both #1 and #2 above. Action of the E^+ allele may well have a simple molecular explanation.

4) The genotype of the purple-flowered stock is D^+E^-/D^-E^+. This designation was arrived at through an analysis of the occasional red

and blue mutant cells produced in the purple floral tissues and is supported by breeding data.

5) Purple-flowered T. hirsuticaulis exhibits three classes of somatic sectors: red-only, red/blue twin spots, and blue-only. The twin spots are shown to be the results of single events.

6) As in T. clone 02, deletion, as evidenced by the presence of micronuclei (chromosome fragments), is indicated to be one mechanism producing certain kinds of sectors (red-only) in response to ionizing radiation.

7) The predominant, if not exclusive, mechanism of spontaneous sectoring and an important mechanism even for 60 R-induced sectoring in this T. hirsuticaulis (and by inference, in other Tradescantia) is argued to be mitotic crossing-over.

8) Some conceptual parallels between the genetic behavior of facultative apomicts and sectoring flowers of Tradescantia are described. This results in a description of new somatic genetic parameters, A, the apomictoid fraction, and 1-A, the mictoid fraction, those fractions of the cell divisions that are typical mitoses, or atypical mitoses where the chromosomes are amenable to exchange, respectively.

9) A genetically accurate method of using somatic sectoring data for the calculation of mitotic map distances and coincidence is described.

10) Mitotic crossing-over through failure of some canalized gene of mitosis is shown to predict a "log response - log dose" plot which simulates that found for somatic sectors in T. clone 02.

A CHARACTERIZATION OF SOMATIC SECTORING IN TRADESCANTIA

By

Michael Lee Christianson

A DISSERTATION

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Genetics Program

and

Department of Botany and Plant Pathology

1975

This Dissertation is dedicated:

To the memory of

Walt Whitman,

To the actuality of

Dr. George Landon,

To the future of

gay women and men,

and

To Diana Ross.

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ACKNOWLEDGEMENTS

This bound volume would never have appeared but for Phyllis Robertson who typed the final manuscript. The color plate, Figure 3, was printed by Dr. L. W. Mericle. The layout of Figures 6 - 12 was done by Dr. R. P. Mericle.

During my long career at Michigan State University, I have had many significant interactions with faculty and staff. Prominent among these are the guidance and encouragement of my committee members, Drs. J. H. Asher, jr., P. S. Carlson, W. G. Fields, L. W. Mericle, R. P. Mericle, and Wm. Tai. A particular expression of gratitude goes to the Drs. Mericle, chairman and non-faculty member of my committee, respectively, not just for all the time spent in editorial suggestion, nor the long discussions, but for the combination of freedom, responsibility and respect that I found in their laboratory. I am grateful, as well, to Dr. A. H. Sparrow for the opportunity to be associated with his group at Brookhaven National Laboratory during the summer of 1973.

Had it not been for my friends and members of my living groups, I might have finished sooner, or might not have finished at all. For their intellectual and emotional support, I thank them: L. E. Murry, M. A. Mead, D. E. Clark, K. O. Hoffman, P. H. deZeeuw, M. W. deZeeuw, J. S.

Lee, S. L. White, S. J. Risch, P. Hartz, S. George, J. W. Enders, S. Itzkowitz, and G. D. Starks.

The financial support came from a National Science Foundation Predoctoral Fellowship.

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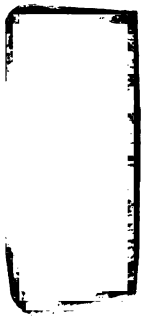

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INTRODUCTION

Strasburger first described the division of young, living stamen hair cells in Tradescantia in 1875 (45,134). The existence of somatic sectors, cells of a "mutant" color, in the stamen hairs of Tradescantia was first reported in 1958 (32). The study of radiation-enhanced sectoring in plants, however, dates back to at least 1935 (93), and the interest in spontaneous somatic sectors, to the very beginnings of the science of genetics as exemplified in studies of flaking by de Vries (146), patching, by Punnet (112), and unstable alleles, by Demerec (35).

Somatic sectoring in Tradescantia is the most sensitive biological indicator of radiation known (76,77), and has been suggested as an admirable test system for the study of chemical mutagens as well (141). However, the conversion of these slightly increased rates of sectoring into meaningful estimates of hazards to human beings is not, I believe, possible at present. Unless or until the mechanisms of low-level sectoring in Tradescantia are well described and shown to operate in similar relative frequency and at similar efficiency in human beings, the extrapolation of relative biological effectiveness (RBE) or the transfer of "mutation rate" from Tradescantia sp. to

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This dissertation makes a major contribution to the characterization of spontaneous and slightly-enhanced somatic sectoring in Tradescantia. Section I of the dissertation describes the chemogenetics of the purple-flowered T. hirsuticaulis stock and the mutant-colored (red and blue) cells found upon close inspection of the pigmented floral parts. Section II considers the mutant sectors in the stamen hairs in more detail and argues for a mechanism, somatic crossing-over, as their major mode of spontaneous production, as well as an important mode of their production at enhanced sectoring rates following exposure to as much as 60R ^{60}Co gamma radiation. Section III considers some analogies between the genetic behavior of facultative apomicts and somatically sectoring Tradescantia flowers. From this consideration arises a new method of treating sectoring data, applicable to all organisms exhibiting somatic crossing-over, and the introduction of a previously undescribed somatic genetic parameter, A, the apomictoid fraction.

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Section I

Chemogenetics of a diploid, purple-flowered, Tradescantia hirsuticaulis

General considerations

Flowers of the Anthophyta exist in an incredible range and variety of colors caused by the presence of just a few classes of compounds. These compounds can be "diagnostic characters" for the taxonomist: for example, all plants with betalins belong to the order Centrospermae (51,71). More often, variant pigmentation is of horticultural or genetic interest. Indeed, Mendel's first paper (73) records the results of crosses between violet-red and white flowered Pisum.

In Pisum and Tradescantia, floral pigmentation is due mainly to the presence (or absence) of various anthocyanin pigments contained in the vacuoles of the epidermal cells of the floral parts. The anthocyanin molecule is more properly a substituted 2-phenyl-benzopyrilium molecule: sugar residues attached to the A ring, and 1,2, or 3 hydroxyl groups on the B ring (Figure 1). The anthocyanin molecule is colored, of course, because it absorbs certain wavelengths of visible light. The quanta of energy are absorbed by the electrons in conjugated π molecular orbitals. These electrons make "permitted transitions" to excited energy states and subsequently return to the

Figure 1. The anthocyanin chromatophore.

Substitution with sugar residues at the 3 and 5 positions (arrows) of the anthocyanidin converts it to the corresponding, water-soluble, anthocyanin. Each named anthocyanin or anthocyanidin has a characteristic hydroxylation pattern for the B-ring; pelargonidin is 3,5,7,4'-tetrahydroxy-benzopyrilium, cyanidin, 3,5,7,3',4'-pentahydroxy-benzopyrilium, and delphinidin, 3,5,7,3',4',5'-hexahydroxy-benzopyrilium. These pigments are associated with orange, red, and blue flower color, respectively.

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"ground state", releasing their energy as non-visible quanta. The reflected or transmitted light, then, has differing amounts of various wavelengths removed and thus appears colored to the human eye.

The electron-donating or electron-withdrawing properties of the substituent groups on the phenyl ring change the electron density in the π molecular orbital. Molecules with different substituent groups on the B ring then differ in which transitions to excited states are permitted; they have different colors to the human eye. Readers interested in further exploration of this topic are referred to some of the papers I consulted in arriving at the above summary: for historical perspective (69,107,108) for evidence from visible spectroscopy (115, 116,127,132), from UV spectroscopy (37), and from electron paramagnetic resonance (EPR) studies (46,103,126).

Most of the English-speaking world follows the method of Harborne and coworkers for the study of anthocyanins, i.e., extraction into acidified (1-2%) alcohol (48,49,50,62,139,140). Extracts of the various Tradescantia stocks prepared in this way exhibit single peaked absorption curves (Figure 2). While Stevenson (132) says

"In plants, the flower pigments usually exist in an environment that is chemically nearly identical to the acidified methanol...", this is clearly not so; the anthocyanin in the petal changes color dramatically upon contact with the acid methanol. This is the in vivo expression of the long known in vitro effect of pH on the color of anthocyanin extracts (26,38,110,116,127) (Figure 3). In contrast,

Figure 2. Visible absorption spectra of 2% HCl-methanolic extracts of flowers of blue T. clone 02, a red-flowered progeny, and purple T. hirsuticaulis.

The three variously colored stocks give smooth single peaks, with rather sharply defined maxima at 532, 548, and 540 nm for red, blue, and purple stocks, respectively, when measured with a Beckman DBG spectrophotometer.

Absorbance

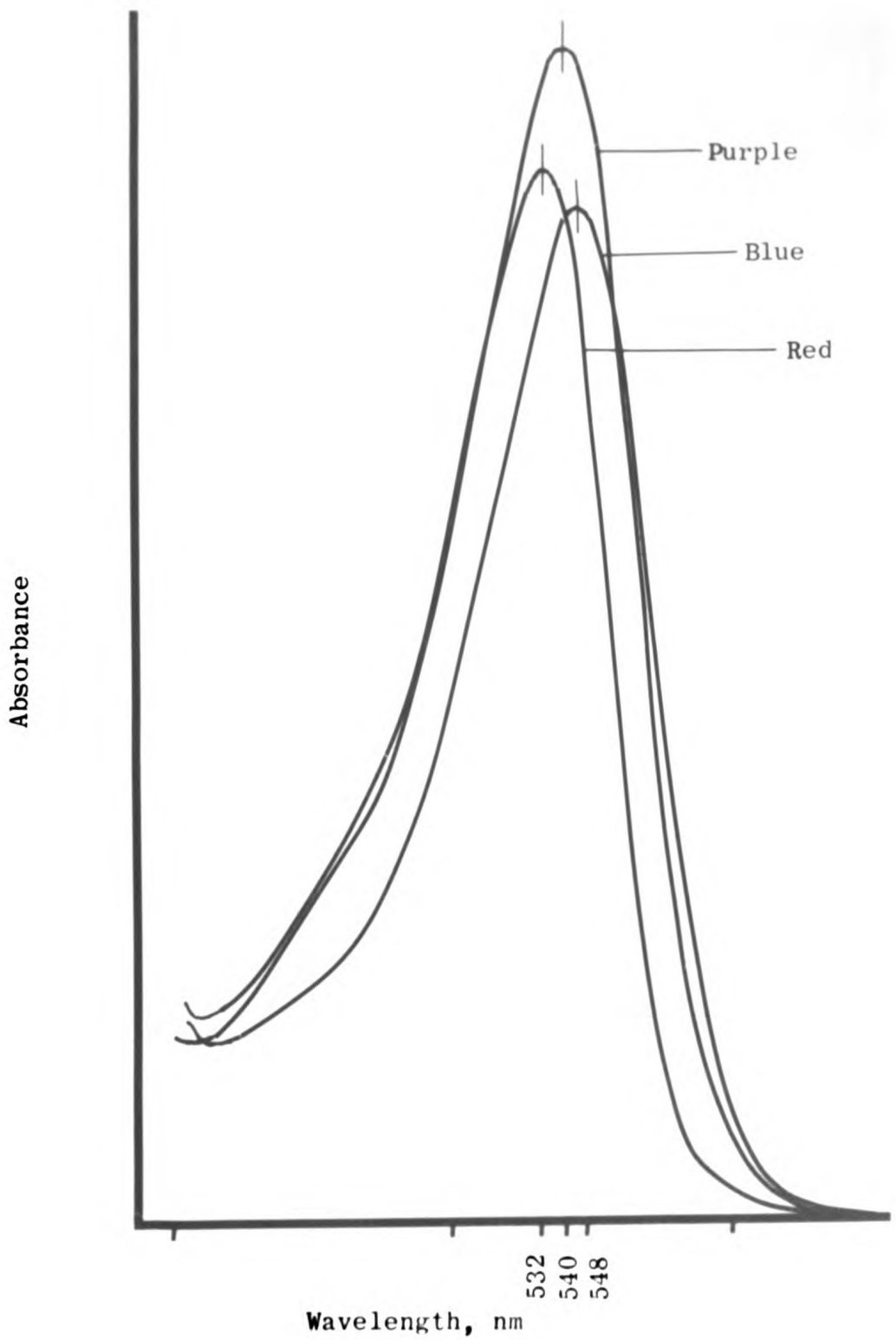

Figure 2

Figure 3. The color of anthocyanin extracts at various pH.

Shifts in the pH of aqueous extracts prepared from petals of blue-flowered T. clone 02 and a red-flowered progeny were effected through the addition of small amounts of dilute HCl or NaOH; each vial is labeled as to the pH of the solution within. Notice that the color of the extract of red flowers (series labeled "C") at pH 5.0 and 5.6 is nearly identical to the color of the extract of blue flowers (series labeled "D") at pH 2.8 and 3.1, respectively.

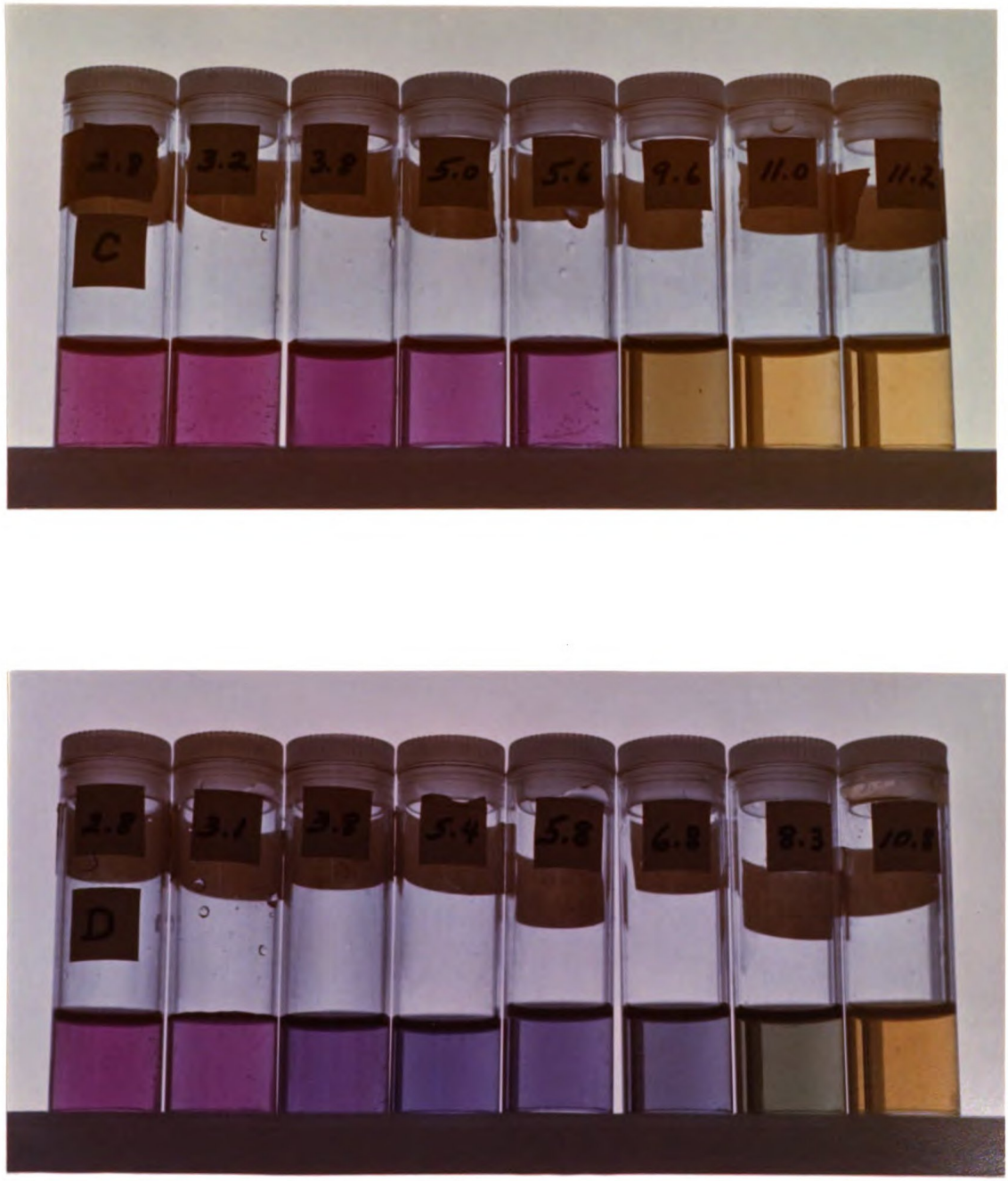

Figure 3

extraction of petals in distilled water gives a preparation of pH 5.6 - 5.8 (75,135) whose color and visible absorption spectra do not differ from those of intact, living petals or individual living stamen hairs (82,88). (The routine extraction into acidified water most likely explains the differences observed by Stewart et al. (133) between spectra of intact tissues and anthocyanin extracts of those tissues.)

While anthocyanins are reported to be unstable in non-acidified solution (139), that is not the case with extracts from Tradescantia. Simple aqueous extracts of Tradescantia stocks have kept their original color during more than 6 months of refrigeration. While homogenates of certain stocks of Tradescantia are, indeed, very unstable, for reasons not yet known, a new technique serendipitously discovered by Mericle and Mericle (in preparation) gives stable, non-acidified, aqueous extracts of them as well. Initial studies of the visible spectroscopy of simple aqueous extracts were carried out during 1967 - 1968 by Mericle and Mericle. While these workers had expected to find single-peaked absorption curves (75,87), they observed that both the aqueous extracts and living materials exhibited multi-peaked absorption curves within the visible wavelengths. This type of spectrum, they discovered, had just been reported in the botanical literature (119).

Multiple-peaked absorption curves, however, were not without precedent, and interpretation, in the physical-chemical literature. Adams and Rosenstein (1) investigated the spectrum of crystal violet, a well

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known dye, in solutions at various pH. They concluded that each chemical species (ionic form) of the dye had a single-peaked absorption curve (a "fundamental") and that the multimodal absorption curve seen at any given pH was the sum of each molar "fundamental" times the respective partial molar fraction of each chemical species. Subsequent investigation by others with different dyes (reviewed, 69) led to the same conclusions.

While the English language literature considers equilibria between one colored form and various non-colored species of anthocyanins (e.g., 139,140) other work (38,116) considers a red cationic form and a blue anionic form of any pure anthocyanin. Whether a single cationic and a single anionic form actually describes all the ionic species of, say, the gylcosylated 3,5,7,3',4',5' -hexahydroxy-2-phenyl-benzopyrilium molecule (delphinidin) is not yet known.

Theoretics and physical chemistry aside, the non-acidified aqueous extracts of various Tradescantia stocks give distinctive, multipeaked spectral "fingerprints." Figure 4 shows the spectral curves of the anthocyanin from red-flowered and blue-flowered Tradescantia. The red flowers, from S-62, a red-flowered segregant from T. clone 02, contain cyanin, the blue flowers of T. clone 02, a pigment mixture of mostly delphinin with a trace of cyanin (79). Increases in pH are known to cause "blueing" of anthocyanins in vivo (8,18) and in vitro (26). While the human eye may see delphinin and cyanin at different pH as the same color (Figure 3), a spectrophotometer detects differences.

Figure 4. The effect of pH on visible absorption spectra of blue T. clone 02 and a red-flowered progeny.

Aqueous extracts of the blue and red stocks, initially pH 5.85 and 5.60 respectively, had their pH altered by the addition of small amounts of hydrochloric acid. The visible absorption spectra at these new pH were measured with a Beckman DBG spectrophotometer; a) blue stock, b) red stock. The dotted line at 550 nm is to facilitate comparison of the two sets of curves.

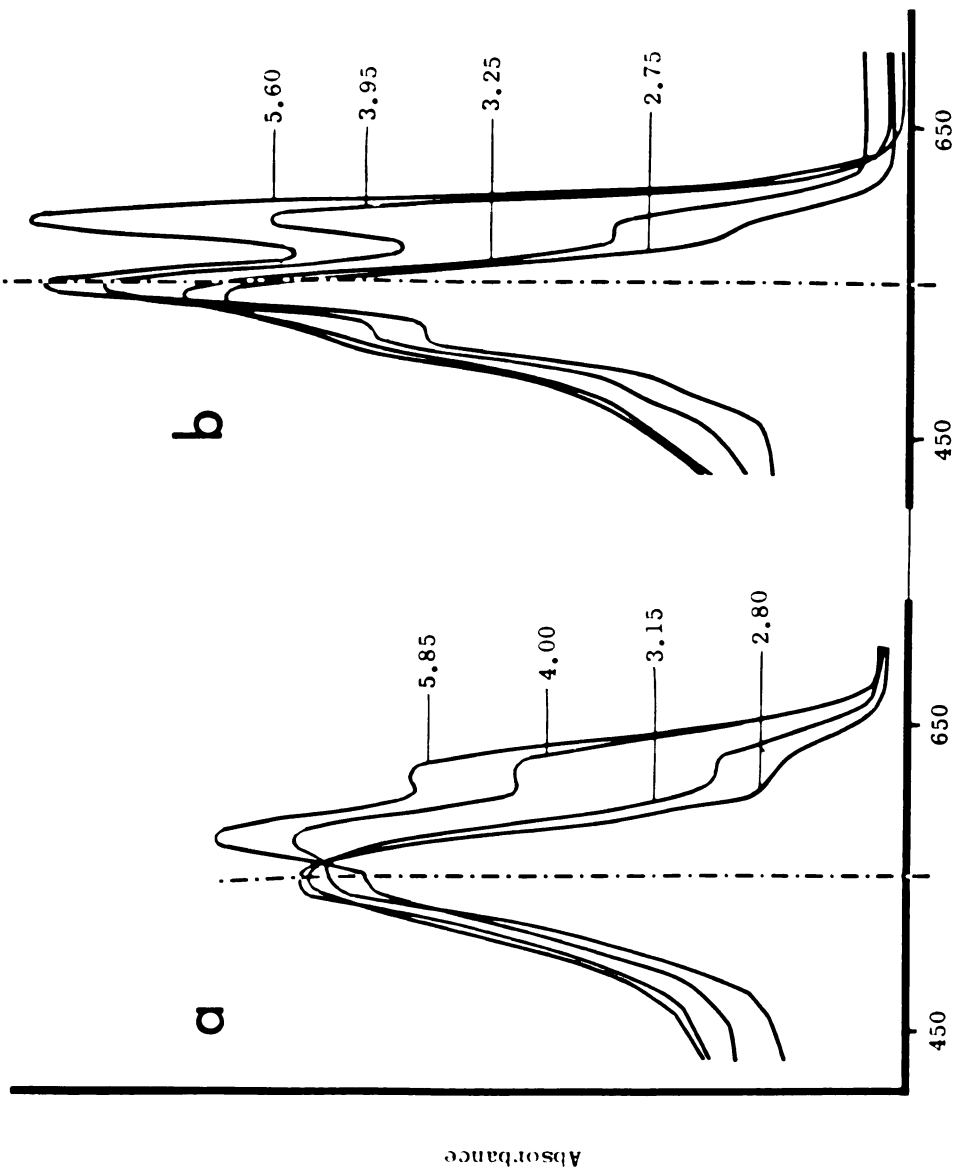
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Figure 4 shows the results of changing the pH of the blue and red extracts. Note that the spectral curves change in shape, but the curve of one pigment is never converted into the curve of the other pigment. In highly acidic aqueous solution, each pigment does give a single-peaked absorption curve; these, however, possess a distinct, characteristic wavelength of maximum absorbance (λ_{max}).

Purple pigmentation: qualitative nature

The various spectral properties of anthocyanins, and dyes, in general, give a means of identifying the nature of the purple pigmentation in a purple-flowered T. hirsuticaulis stock (#2091, obtained from Dr. A. H. Sparrow, Brookhaven National Laboratory). The flower color, purple, could be obtained through any one or a combination of several routes: through the presence of one, purple, pigment, via the simultaneous presence of approximately equal proportions of red and blue pigments, or via a shifting of visual color through pH effects on a red or a blue pigment. This latter is ruled out since simple aqueous extracts of purple petals as well as the petals themselves or individual stamen hair cells show a tetramodal absorption curve unlike the maximally trimodal curves seen for cyanin or delphinin at any pH (compare Figures 6,7 with Figure 4).

While chromatography of an extract of the purple petals could clearly show whether a single purple pigment, or both red and blue pigments were the cause of the purple flower color, Tradescantia

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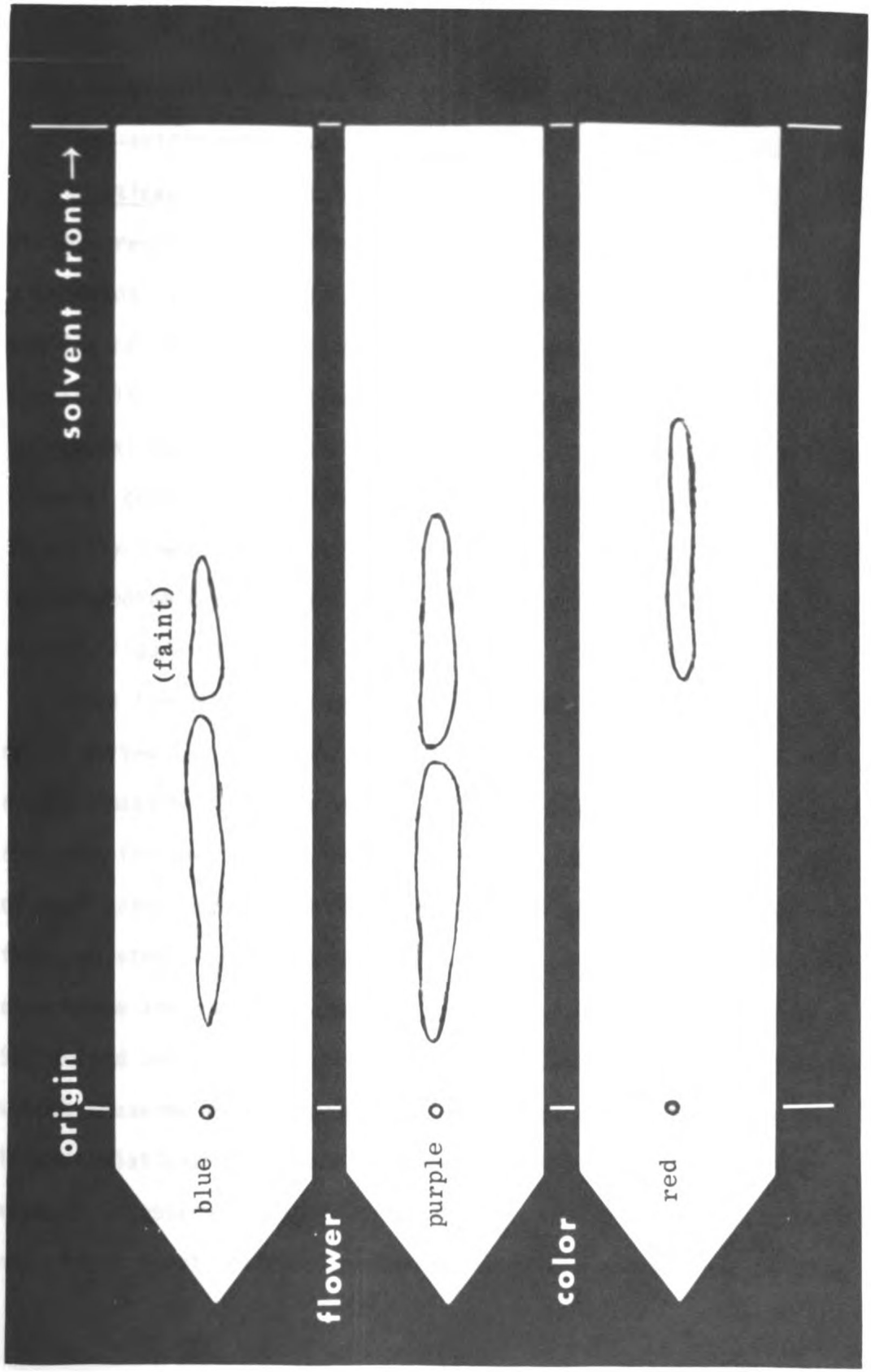
pigments do not separate well under most standard chromatographic conditions. Progressive hydrolysis and subsequent chromatography gave rise to "extra spots", identified as partial breakdown products and unhydrolyzed pigments (40). My chromatography of anthocyanidins after the methods of several workers (7,49,94,102) did not give a separation (or, of course, the retention of natural color in the acid solvents). Finally, I attempted chromatography of aqueous extracts of Tradescantia flowers containing anthocyanins: Whatman #1 filter paper strips with development in mixtures of isopropanol, ethanol, and water (drawn from the methods of Osawa 103, and Alvarez 2). Proportions of 1:1:1 gave the best separation and spot definition, (Figure 5). This method preserves the natural color of the pigments; the cyanin spot is red, the delphinin, blue. It proved impossible, however, to elute the pigments from the dried chromatogram in order to quantitate the amounts of pigment.

The chromatogram (Figure 5) shows that the anthocyanin pigmentation of the purple T. hirsuticaulis is not the result of a single purple pigment, but instead due to the presence of perhaps equal amounts of red and blue pigments. Furthermore, the co-chromatogram confirms previous reports (79) that the blue-flowered T. clone 02 contains mainly blue pigment plus a trace of red pigment, but by chromatography of the anthocyanins, not the anthocyanidins.

Concurrent with initial, unsuccessful, attempts at elucidating the nature of the purple pigmentation via chromatography,

Figure 5. Co-chromatograms of anthocyanin extracts of blue T. clone 02, a red-flowered progeny, and purple T. hirsuticaulis.

Ascending chromatography on Whatman #1 filter paper strips with development in the solvent Isopropanol:Ethanol:Water::1:1:1 of equal amounts of similarly prepared aqueous extracts of blue, red, and purple stocks. The red, cyanin, pigment has a higher R_f than the blue, delphinin, pigment.



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Non-acidified aqueous extracts of petals from the purple-flowered T. hirsuticaulis have essentially the same absorption spectra in the visible region as the living intact petals (Figure 6); this phenomenon was previously reported for the blue flowered T. clone 02 and its red-flowered progeny (82). The purple pigment, in intact petals, living stamen hairs, or in aqueous extracts, exhibits a tetramodal absorption spectrum which corresponds to neither of the trimodal curves obtained for blue or red flowers. This difference can be easily seen in a comparison of absorption curves obtained by micro-spectrophotometry of individual, living, stamen hair cells of the three stocks (Figure 7).

When the petals are extracted in acidic methanol (2% HCl), multimodal curves are not seen. Instead, single peaks with rather sharply defined maxima are obtained; the wavelength at maximum absorbance is specific for each of the variously colored stocks (Figure 2). Mixtures of such acidified methanolic extracts of petals from blue- and red-flowered stocks also show single peaks whose wavelengths at maximal absorbance are intermediate between those of the unmixed extracts, 535 nm and 546 nm, respectively, for red-flowered and blue-flowered stocks measured with a Beckman DBG spectrophotometer. Indeed, a linear relationship between λ_{max} and proportion of red (or blue) extract is observed (Figure 8). The λ_{max} of the purple stock is 541 nm, almost exactly midway between those of the unmixed red or blue

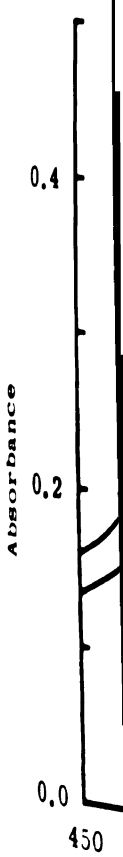
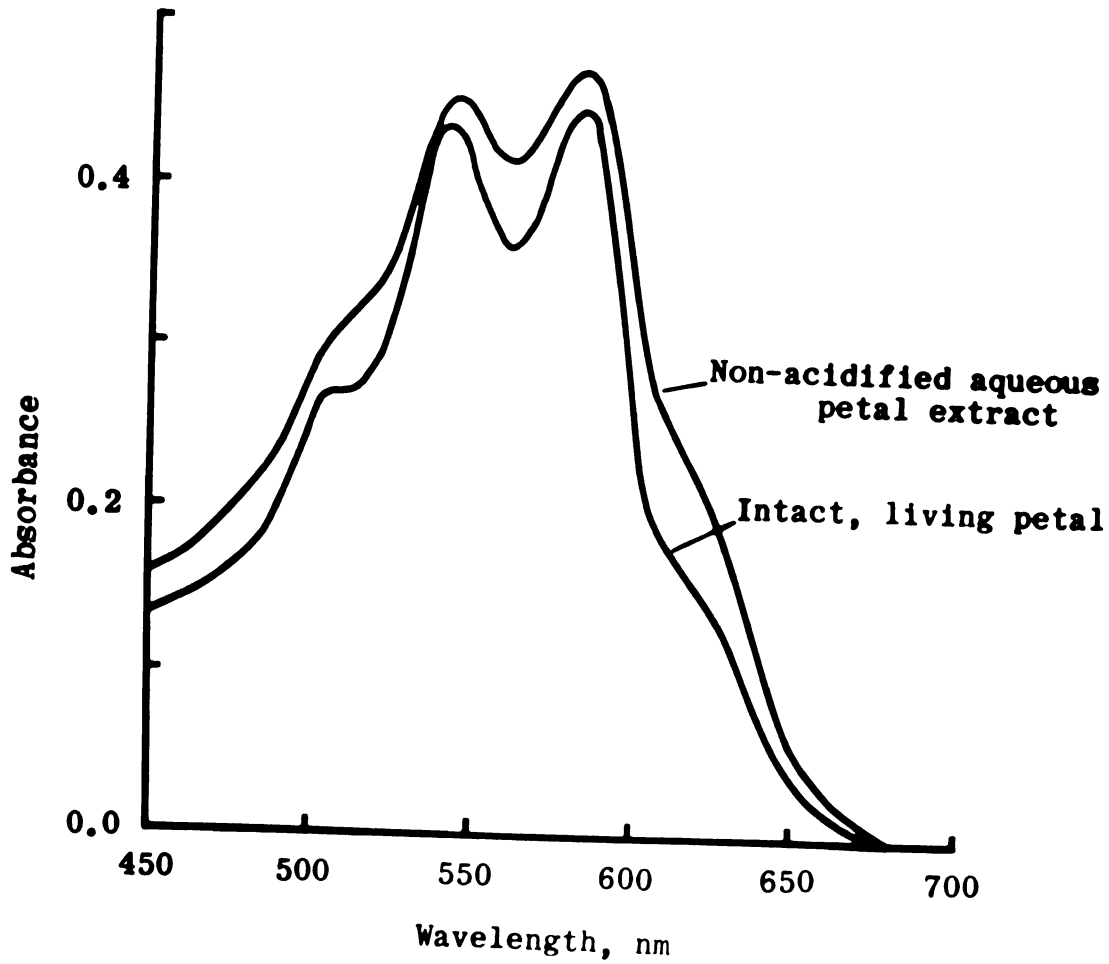


Figure 6. Absorption curves of petals and aqueous extracts.

Absorption curves obtained by macrospectrophotometry of living, intact petals and non-acidified aqueous extracts of petals from purple flowers of T. hirsuticaulis. The spectrophotometer was a Cary 15.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Wavelength, nm
Non-acidified aqueous petal extract
Intact, living petal

Figure 6

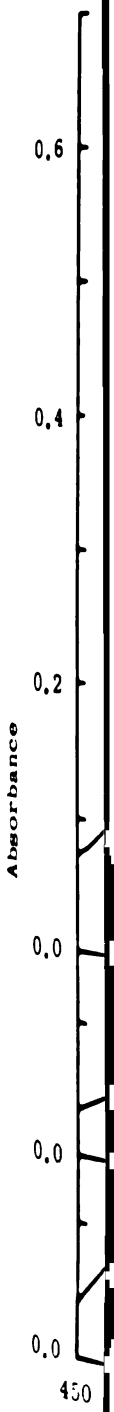

Figure 7. Absorption curves of stamen hair cells.

Absorption curves obtained by microspectrophotometry of individual, living stamen hair cells from blue-flowered T. clone 02, its red-flowered progeny, and from purple-flowered T. hirsuticaulis. From "plug" measurements uncorrected for cell size or pigment concentration, measured with a Zeiss 01 microspectrophotometer.

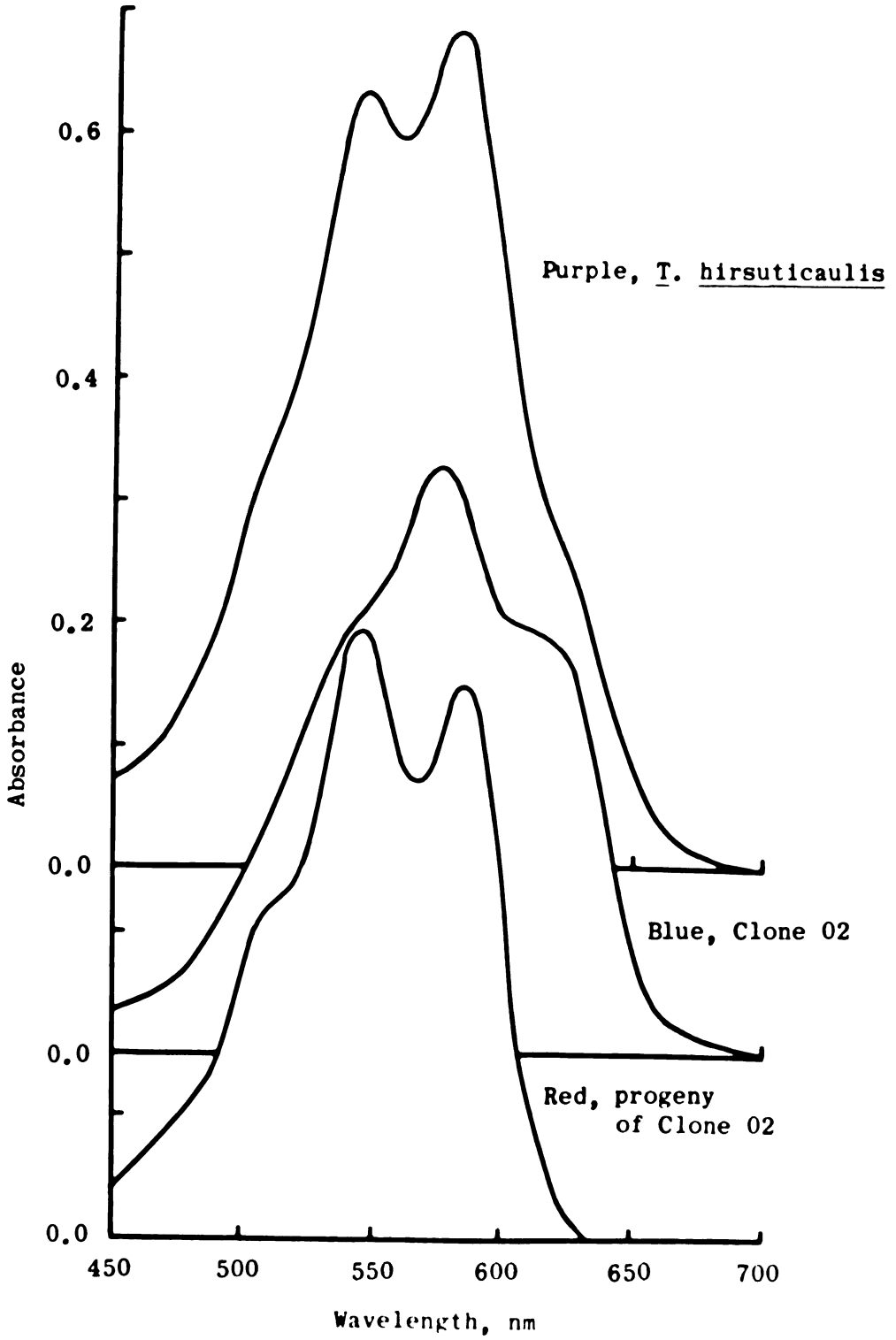
0.6
0.4
0.2
0.0
0.0
0.0
Absorbance
Purple, T. hirsuticaulis
Blue, Clone 02
Red, progeny
of Clone 02
450
500
550
600
650
700
Wavelength, nm

Figure 7

Figure 8. Wavelengths at maximum absorbance of various mixtures of red and blue extracts compared with that of purple extract.

Wavelengths at maximum absorbance for acidified-methanol extracts prepared from equal numbers of petals from blue-flowered T. clone 02 and its red-flowered progeny, mixtures of the two in various proportions, and comparison with that of an acidified-methanol extract prepared from half the number of petals from purple-flowered T. hirsuticaulis. The spectrophotometer was a Beckman DBG.

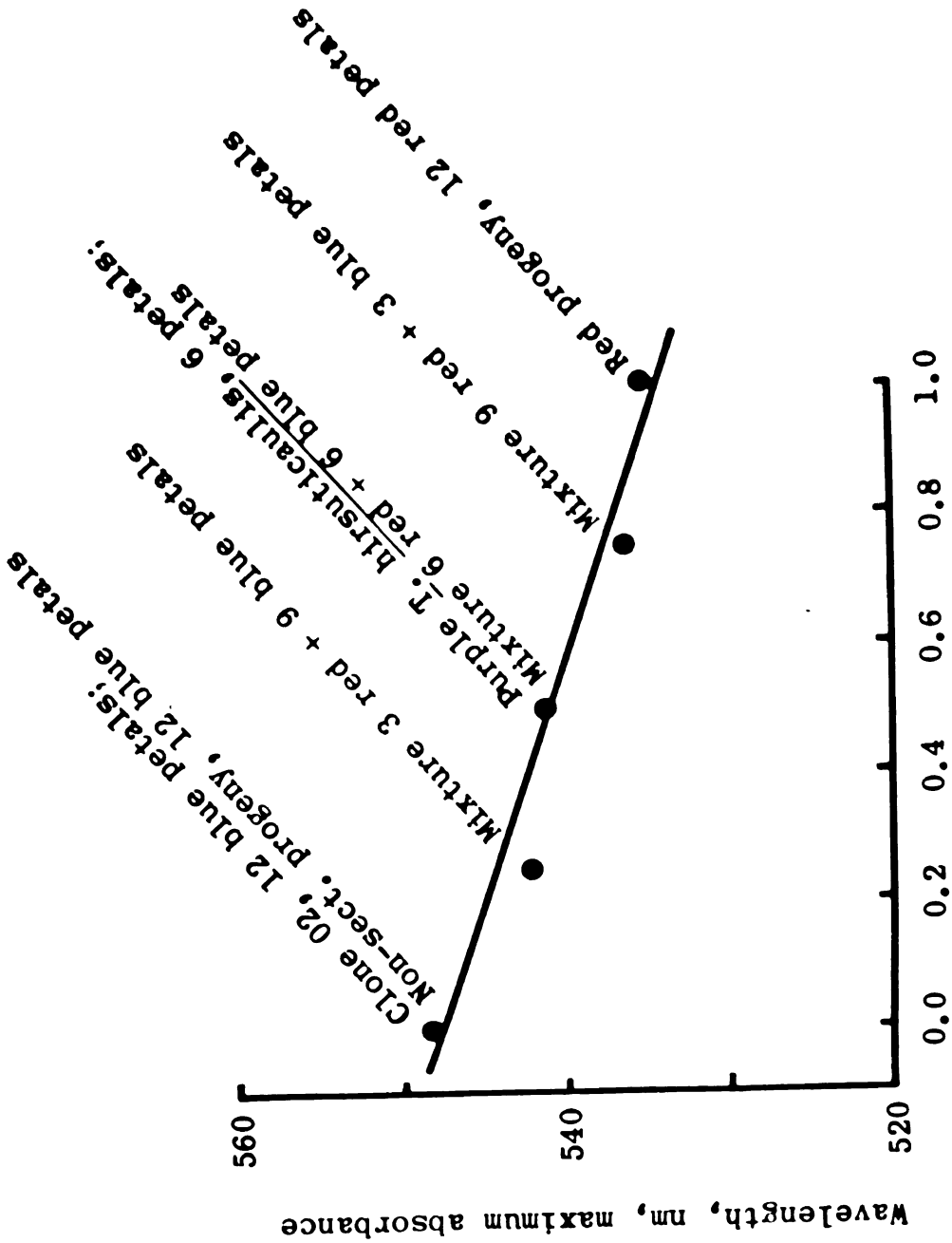
Wavelength, nm, maximum absorbance
560
540
520
0.0
0.2
0.4
0.6
0.8
1.0
Proportion red petals of total petals
Clone 02, 12 blue petals; Non-sect. progeny, 12 blue petals
Mixture 3 red + 9 blue petals
Purple F_1 hirsuticaulis, 6 petals; Mixture 6 red + 6 blue petals
Mixture 9 red + 3 blue petals
Red progeny, 12 red petals

Figure 8

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extracts, and corresponds exactly to that observed for a mixture of equal amounts of red and blue extract (541 nm). On the other hand, the unique, purplish pigments, petunidin and malvinidin or their glycosides, as observed by Stevenson (132), have their respective λ_{max} near but higher than the midpoint of the interval from the λ_{max} of cyanidin to that of delphinidin. I interpret these experiments as strongly supporting the suggestion from the chromatographic work that the purple color may well be the result of the simultaneous presence of approximately equal amounts of delphinin and cyanin. There is further support for this interpretation in the spectra of aqueous extracts of the red, blue and purple stocks.

Mixtures of non-acidified aqueous extracts prepared from equal numbers of red and blue petals give tetramodal absorption curves similar to the tetramodal spectrum of the purple stock; the location of the peaks and shoulders, as well as their relative heights, depend on the proportions of the mixture (88). Indeed, simple mathematical addition of the trimodal absorption curves for non-acidified aqueous extracts of equal numbers of red and blue petals gives a tetramodal curve "closely resembling" that from an aqueous extract of purple petals (Figure 9). If the purple color were due to a unique purple pigment, this observed duplication of the "spectral fingerprint" of the purple-flowered stock by addition of the fingerprints of the red and blue stocks would not be expected. Thus, even without the co-chromatograms of the three stocks (Figure 5), the purple color of

Figure 9. Simulation of purple spectral "fingerprint".

Simulated "purple" absorption curve derived from mathematical addition of the curves obtained by macrospectrophotometry (Beckman DBG) of non-acidified aqueous extracts prepared using equal numbers of petals from blue-flowered T. clone 02 and its red-flowered progeny.

Absorbance

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Beckman
numbers
d progeny.

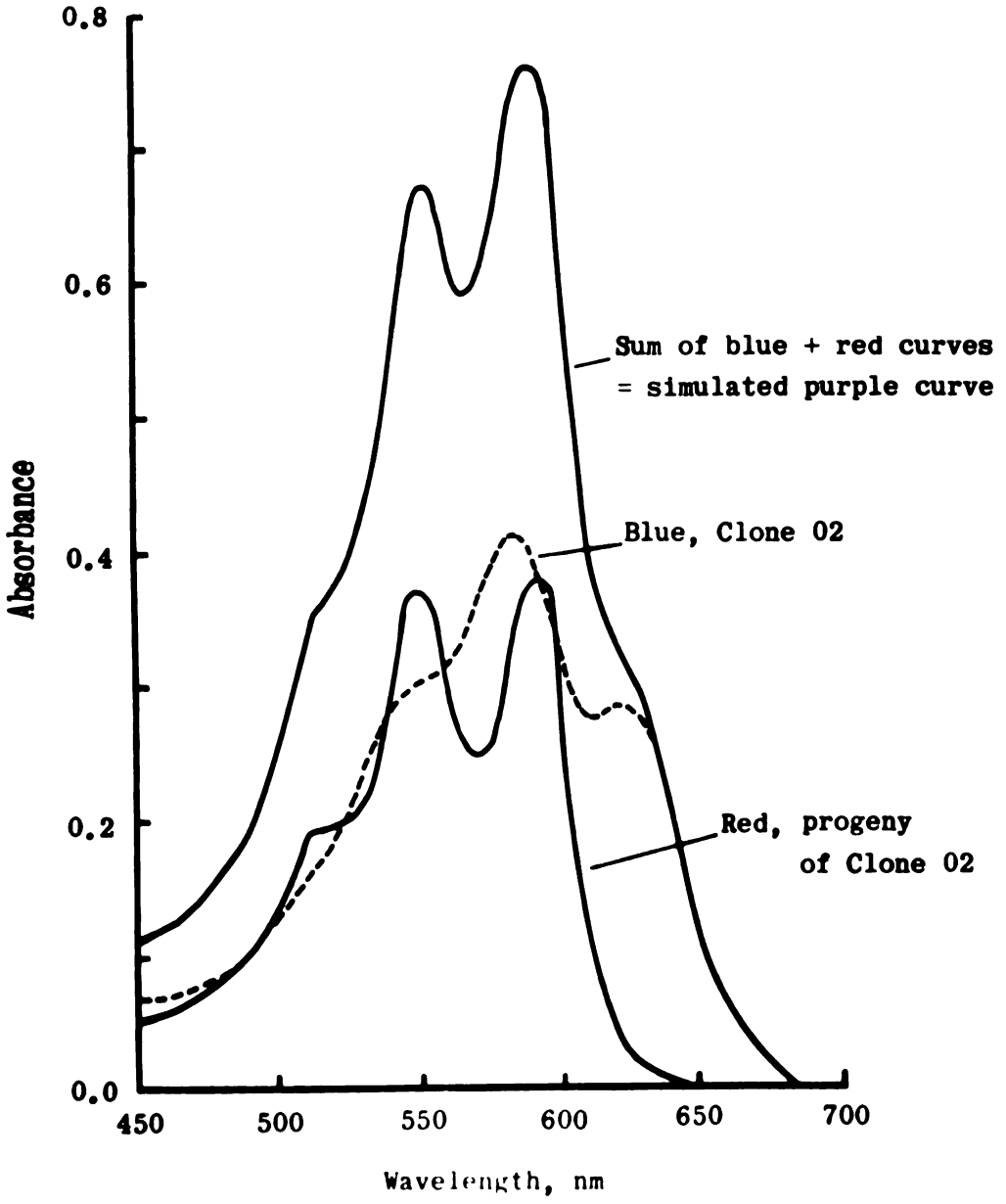

Figure 9

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the T. hirsuticaulis stock can be assigned to the simultaneous presence of both red and blue pigments in approximately equal amounts.

Purple pigmentation: quantitative nature

In addition to showing the probable anthocyanin composition of the purple-flowered stock, the spectrophotometric data also provide information on the total amounts of pigment produced by purple flowers of T. hisuticaulis and blue or red flowers of T. clone 02 and its progeny. Maximum absorbance can be used to give a rough quantitative measure of anthocyanin content (50); this has been demonstrated as applicable to these stocks of Tradescantia (88).

Non-acidified aqueous extracts of equal numbers of petals (which are nearly matched for size, Table 1) from blue and red flowers exhibit similar levels of maximum absorbance (see Figure 10 as an example) as well as similar total area under the spectral curve. (Weights of cut-out tracings of spectral curves, 700-450nm, are 0.56 and 0.55 g, respectively, for red and blue extracts.) These results agree with aforementioned estimates, based on visual intensity and spot size in chromatograms, that blue and red flowers contain approximately equal amounts of delphinin and cyanin, respectively. However, extraction of only half as many (or fewer) petals of the purple T. hirsuticaulis is sufficient to give a comparable maximum absorbance value (Figure 10) or area under the spectral curve. (Weight of cut-out tracing of spectral curve, 700-450nm, is 0.66 g, for purple extract) even

Table

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Stock
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red

[a]Area

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[b]Weig

[c]Dens

Table 1. Petal area, weight, and density of purple *T. hirsuticaulis*, blue *T. clone 02* and its red segregant, S-62.

Stock	Area[a]	Weight[b]	Density[c]
purple	1.17	0.0193	0.0164
blue	1.36	0.0208	0.0153
red	1.33	0.0212	0.0159

[a]Area determined by the weights of a cut-out tracing of the projected image of a water-mounted, flattened petal of each stock relative to that of a similarly projected 1 cm^2.

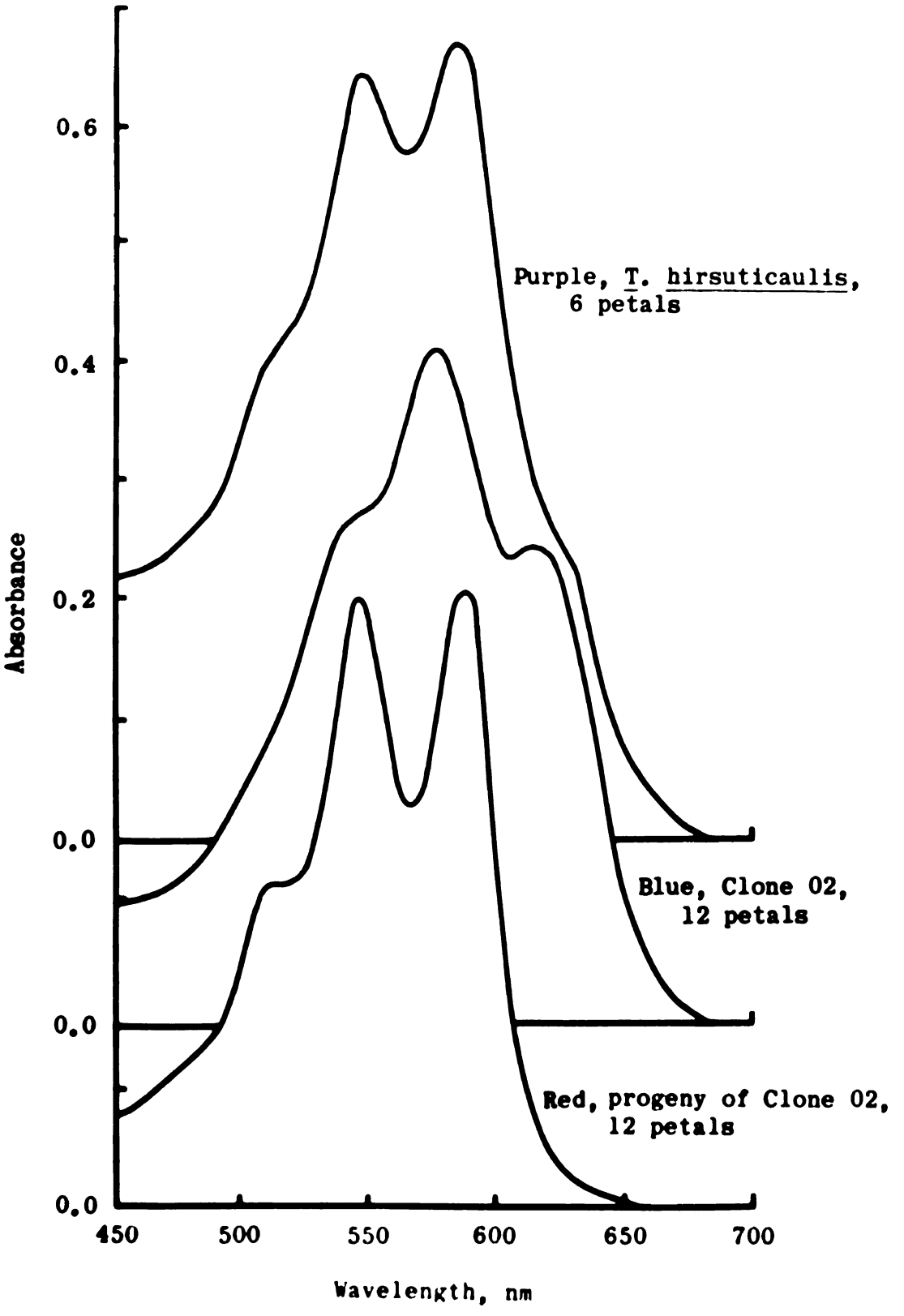
[b]Weight = average fresh weights of two petals of each stock

[c]Density = Weight/Area

Figure 10. Spectral comparison of pigment intensity.

Absorption curves obtained by macrospecrophotometry of non-acidified aqueous extracts prepared from equal numbers of petals from blue-flowered T. clone 02 and its red-flowered progeny, and half as many petals from purple-flowered T. hirsuticaulis. The spectrophotometer was a Beckman DBG.

Absorbance


0.6
0.4
0.2
0.0
0.0
0.0
Absorbance
Purple, T. hirsuticaulis,
6 petals
Blue, Clone 02,
12 petals
Red, progeny of Clone 02,
12 petals
450
500
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Wavelength, nm

Figure 10

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though the petals of T. hirsuticaulis are comparable in size, weight, and density to those of red and blue flowered T. clone 02 and progeny (Table 1). The latter implies that the flowers of purple T. hirsuticaulis contain twice as much anthocyanin as flowers from T. clone 02 or its progeny, and explains why the red and blue flowers of these latter match the lighter, "/1", tones of the Royal Horticultural Society Colour Chart (RHC) (52) while the purple flowers of the former match the "full" tone (29,88).

The co-chromatograms (Figure 5) show parity in size of the cyanin spots between the red and purple stocks, as well as parity in size of the delphinin spots between the blue and purple stocks. Equal amounts of extracts from equal numbers of petals of the three stocks were applied to the chromatogram; the purple stock, then, must have as much blue pigment as the blue stock and as much red pigment as the red stock, a total amount twice as much as either stock. Therefore, any proposed genotype for the diploid (75), purple-flowered. T. hirsuticaulis must take into account a phenotype that differs from both the blue and red phenotypes of diploid T. clone 02 and its progeny, not only in regard to the kinds and proportions of anthocyanin pigment produced, but also with respect to the total amount of pigment synthesized in each cell.

Genetics of flower color in Tradescantia

While the inheritance of flower color has been established for many plant species (summarized by Paris et al. 106), very little is known about flower color in the genus Tradescantia; it is of minor horticultural importance (e.g., the cultivars Iris Pritchard and Purple Dome), despite its extensive use for cytological studies (e.g., 67, 121). Anderson and Diehl (4) suggested that blue flower color was probably inherited, in diploids, as a simple Mendelian factor "dominant" to both red and white, or, in tetraploids, as a co-dominant. Their interpretation stemmed from observations of discrete (blue, red, white) classes of flower color in populations of diploid Tradescantia and a more continuous range of colors including intermediate tones observed in a tetraploid population of T. reflexa. (As yet unpublished observations of Mericle and Mericle on Tradescantia populations in Texas and Colorado, however, reveal that diploid populations of at least five species exhibit "intermediate" colors.)

A. H. Sparrow's group at Brookhaven National Laboratory inferred that the diploid, blue-flowered T. clone 02 was heterozygous for flower color from the observation that red-colored somatic sectors arose in petals and stamen hairs, just as mutant sectors arose in the petals of heterozygous Antirrhinum (32); these red-celled sectors were interpreted as results of mutations from a dominant blue allele to a recessive red allele (100). Subsequent chemogenetic studies by Mericle and Mericle (79,82) have established that T. clone 02 is blue

flowered due to the presence, in single dose, of a dominant genetic factor, D^+, which gives delphinidin production.

Indeed, D^+ behaves as a completely dominant Mendelian factor: no visual or spectrophotometric differences are apparent between the parent, D^+D^-, plant and its D^+D^+ progeny. The latter are recognized by the fact that their flowers do not show the typical, occasional red (D^-D^-) mutant sectors, even following relatively high levels of radiation (79,82). Those progeny from selfing T. clone 02 which segregate for the absence of this D^+ factor, D^-D^-, lack the capability to synthesize delphinidin. They are found to be red-flowered, rather than white-flowered, because they can produce cyanidin, presumably due to the presence of another genetic factor, C^+. As both D^+D^- and D^+D^+ stocks, however, produce similar, small amounts of cyanidin, revealed by chromatography of floral extracts (79), the C^+ factor is indicated to be at a different locus and not simply an allelomorph of D^+. No C^-C^- (white-flowered) plants have been recovered from selfing T. clone 02 or various red x red intercrosses; clone 02 is therefore presumed to be homozygous C^+C^+ (88). While blue flower color might appear as a dominant to red flower color, the production of delphinidin is epistatic, not dominant, to the production of cyanidin.

In purple-flowered T. hirsuticaulis, the genes producing delphinidin and cyanidin are not epistatic and incompletely hypostatic, respectively, but rather, isostatic. It is not that the D^+ allele in T. hirsuticaulis has no epistatic capacity. As will be shown later,

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occasional, mutant, blue cells in the stamen hairs have absorption spectra identical to those of cells in blue-flowered T. clone 02, i.e., containing mostly delphinin with traces of cyanin. Perhaps T. hirsuticaulis has a genetic factor that specifically releases the epistatic suppression of C^+ in the presence of D^+, or, perhaps the stock carries an allelomorph of C^+ insensitive to the action of D^+. As the purple flower color in T. hirsuticaulis is the result of the enhancement of the trace of cyanidin produced in other D^+ bearing stocks, or the release of the epistatic suppression of C^+ in the presence of D^+, the genetic factor responsible is given the tentative designation E^+ (29,83,88).

The action of E^+ most likely has a simple molecular explanation, perhaps similar to the molecular basis elucidated for the order of dominance in an allelomorphic series at a locus responsible for the glycosylation of flavones in Melandrium (19,20,21,23,24) and a variant, co-dominant, allele at that locus (22). In Melandrium, the completely dominant allele, g^G, which gives glucosylation of the flavones, produces an enzyme with a V_{max} ten-fold greater than the enzyme produced by the allele g^X, which gives xylosylation of the flavone. In the heterozygote, $g^G g^X$, both enzymes are present in equal amounts; however, the enzyme with the higher V_{max} consumes all the available flavone, the common precursor, before the other enzyme can form detectable amounts of xylosylated product. In one plant, however, the genes appeared to be co-dominant; the heterozygote, $g^G g^{X'}$, produced equal

amounts of glycosylated and xylosylated flavone. The $g^{X'}$ allele produced an enzyme whose V_{max} was similar to that of the enzyme from the g^{G} allele, and some ten-fold greater than the enzyme from the g^{X} allele. The "bottom" recessive, the g allele, was found to produce an enzyme with 6% of the glucosylating activity of the g^{G} allele.

The allele E^{+} in T. hirsuticaulis may well prove to be $C^{+'}$, an allele of C^{+}, which codes for an enzyme molecule which is insensitive to feedback inhibition by delphinidin, has a higher K_m, or exhibits a higher V_{max} (to give three likely possibilities). If this were so, stocks such as $D^{+}D^{+}C^{+'}C^{+}$ and $D^{+}D^{-}C^{+'}C^{+'}$ could be obtained through appropriate breeding schemes. If the $C^{+'}$ enzyme had, for example, a V_{max} comparable to the D^{+} enzyme, while the C^{+} enzyme did not, then the first named stock would have blue-purple flowers. While the C^{+} enzyme, with its low V_{max}, would produce little product, the enzymes from the two D^{+} and one $C^{+'}$ alleles would compete equally well for the common substrate, giving, finally, delphinidin and cyanidin in a 2:1 ratio and a flower color intermediate between purple and blue. As will be mentioned later, a blue-purple flowered stock has been obtained; its spectrum and that of a mixture of aqueous extracts of blue T. clone 02 and of a red-segregant in proportions of 2:1 are remarkably similar (Figure 13d).

The other stock $D^{+}D^{-}C^{+'}C^{+'}$, by a similar competition of enzymes from one D^{+} and two $C^{+'}$ alleles would have red-purple flowers. Such a plant has not yet appeared among the very small number of progeny

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derived from breeding experiments with T. hirsuticaulis materials. Continued breeding and biochemical studies will be needed to completely describe the nature of the E^+ factor.

Prediction of flower color genotype by somatic cell analyses.

While Anderson and Diehl maintain that Tradescantia "grows easily from seeds" (4), that has not been the experience in previous genetic studies on Tradescantia by Mericle and Mericle (79,82). In these studies, however, the plants being utilized, T. clone 02, were at least self-fertile, whereas the purple T. hirsuticaulis is self-sterile. Although Dr. A. H. Sparrow kindly offered a blue-flowered T. hirsulticaulis for my use in making crosses, subsequent examination of his collection revealed that the stock plant had died (98). Thus, as described below, elucidation of the genotype of the purple stock is based on its capacity to produce somatic sectors and on the pigment chemistry of those sectors. As will be shown later in this Section, the rare progeny which have been obtained to date from an extensive series of self-pollinations fully support the postulated genotype.

When stamen hairs and petals of the purple-flowered stock of T. hirsuticaulis are examined under the same conditions used in this laboratory for mutation scoring of T. clone 02 (76,81), occasional, non-purple cells are seen. As with T. clone 02, exposure of this purple-flowered stock to ionizing radiation increases the frequency of these "mutant sectors" -- a subject which will be treated in more

detail in Section II of this dissertation.

Some of these non-purple mutant cells are red. Microspectrophotometry of individual, living, red mutant cells in stamen hairs of the purple stock yields absorption curves identical with those of both red mutant cells from the blue T. clone 02 and non-mutant cells of its red-flowered progeny (Figure 11). Since the latter are known from breeding experiments to be D^-D^- and to have only cyanidin as their anthocyanin pigment, an assignment of the same cellular pigmentation and genotype is made for the red-mutant cells in the purple flowers, as was done for their counterparts in the blue T. clone 02 (79,82). Therefore, as in T. clone 02, the purple T. hirsuticaulis must have a genotype of D^+D^-.

The genotype of T. hirsuticaulis differs from that of T. clone 02, however, by the presence of E^+ in the former. As was described above, the E^+ factor, whatever its molecular nature, affects the final phenotype by allowing the cells to synthesize, in addition to the "unit" amount of delphinidin produced in blue T. clone 02 cells, an additional "unit" of cyanidin, rather than simply a trace. This results in both an overall purple color and pigmentation that is twice as intense.

The procedure for determining whether the stock is heterozygous or homozygous for the E^+ allele follows that outlined above for the D^+ allele; a stock that is E^+E^- is expected to produce occasional E^-E^- mutant cells -- these cells being blue in color, as they contain a D^+ allele at the D locus.

Figure 11. Spectral "fingerprints" of red cells in several stocks.

Absorption curves obtained by microspectrophotometry of individual, living stamen hair cells consisting of red mutant cells from blue-flowered T. clone 02 and purple-flowered T. hirsuticaulis, and non-mutant red cells from T. clone 02's red-flowered progeny. From "plug" measurements uncorrected for cell size or pigment concentrations, measured with a Zeiss 01 microspectrophotometer.

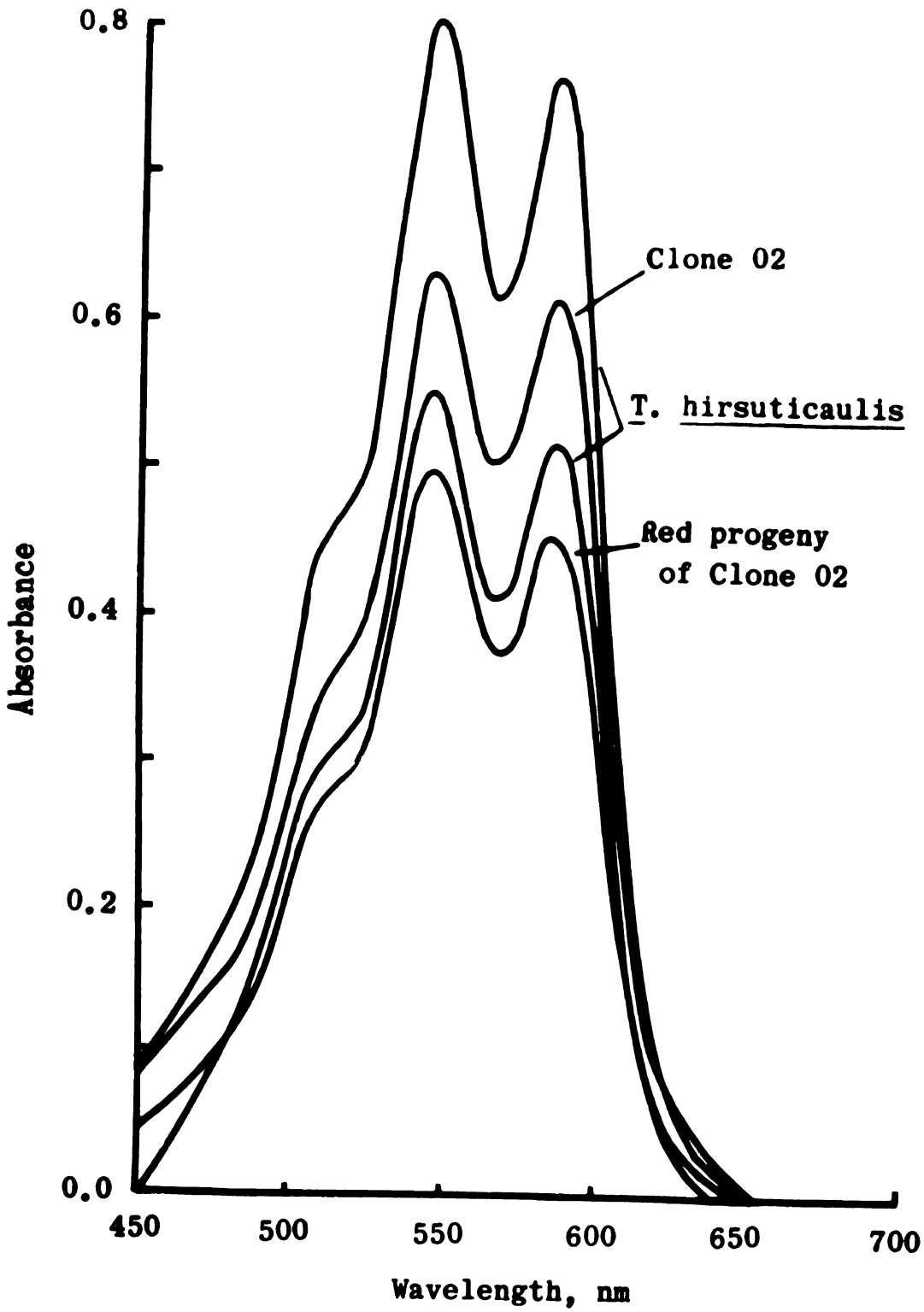
0.8
0.6
0.4
0.2
0.0
Absorbance
Clone 02
T. hirsuticaulis
Red progeny
of Clone 02
450
500
550
600
650
700
Wavelength, nm

Figure 11

Indeed, others of the aforementioned non-purple mutant cells are blue. Microspectrophotometry of individual, living, blue mutant cells in stamen hairs of the purple T. hirsuticaulis yields absorption curves identical to those found for non-mutant cells of the blue T. clone 02 (Figure 12). Therefore, the purple stock is assigned a genotype of E^+E^-.

Additional analyses of the sectoring data in purple T. hirsuticaulis, presented more appropriately in Section II of this dissertation, leads to the conclusions that the D and E loci are linked, that the D locus is the more distal, and that D^+ and E^+ are in a repulsed (trans) configuration.

Results of breeding studies

Although the purple stock typically sets no seed after self-pollination, persistence in making self-pollinations has led to the production of 22 seeds*, of which five have germinated over a period of three years and survived to flowering. One additional seedling was inadvertently lost. Although these data are certainly not "extensive", they do confirm some of the conclusions reached through analyses of somatic sectoring in the purple stock. If the purple stock is indeed D^+E^-/D^-E^+, progeny obtained from self-fertilization should form nine

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* It should be noted that these seeds are assumed to contain embryos which are the results of the sexual process; at present, I have no way of determining whether some -- or all -- of them might have arisen via some apomictic process.

Figure 12. Spectral "fingerprints" of blue cells in several stocks.

Absorption curves obtained by microspectrophotometry of individual living stamen hair cells consisting of blue mutant cells from purple-flowered T. hirsuticaulis and non-mutant blue cells from blue-flowered T. clone 02. From "plug" measurements uncorrected for cell size or pigment concentration, measured with a Leitz MPV-1 microspectrophotometer.

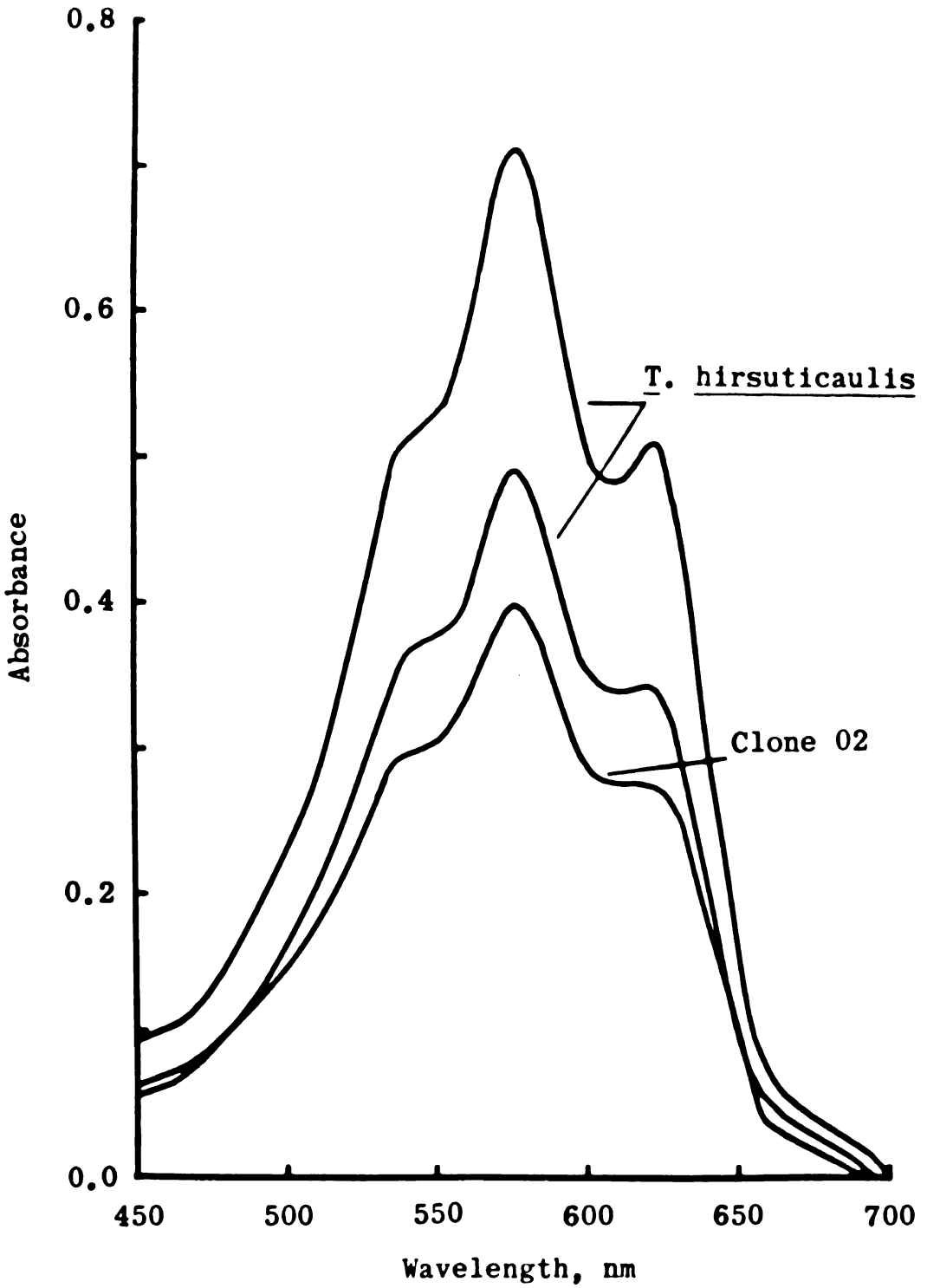
0.8
0.6
0.4
0.2
0.0
Absorbance
T. hirsuticaulis
Clone 02
450
500
550
600
650
700
Wavelength, nm

Figure 12

genotypic classes (Table 2). The red-sectoring capability of D^+D^- stocks would permit the separate identification of some genotypes having identical phenotypes, e.g., purple-flowered, $D^+D^+E^+E^+$ and $D^+D^-E^+E^-$, stocks or blue-flowered, $D^+D^+E^-E^-$ and $D^+D^-E^-E^-$, stocks. It was originally (88) proposed that $D^+D^-E^+E^+$ and $D^+D^+E^+E^-$ progeny might be purple-flowered as well, but identifiable by their ability to sector either red or blue, respectively, but not <u>both</u> types of mutant cells; I am now proposing that these two genotypes produce red-purple and blue-purple flower color, respectively, identifiable without recourse to an analysis of their distinctive sectoring capabilities.

The phenotypic characteristics of the five progeny plants are presented in Table 3 with their flower colors recorded as spectral fingerprints in Figure 13. The flowers of those plants listed as red-flowered in Table 3, S-1, S-2, and S-4, have visible absorption spectra very similar to red progeny of <u>T. clone 02</u> (Figure 13a). The plant recorded as purple-flowered in Table 3, S-5, exhibits a spectral curve very similar to the purple parent (Figure 13b). In contrast, plant S-3, scored as blue-purple-flowered* in Table 3, exhibits a spectral

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* This blue-purple plant was originally scored as being blue-flowered, its flower matching the RHC #41/1. Subsequently, it was recorded as being "purple", no notation of a match to the Colour Chart being made. As there was a possibility that the plant had somatically sectored, yielding two distinctly pigmented plants, it was subdivided into 5 parts. All parts have now bloomed; all produce flowers blue-purple in color, matching RHC #39/1. The match to RHC #38/0 is also very good. In some lights, the flowers of S-3 match RHC #40/1. Indeed, there are only small, (but significant), differences in the spectral fingerprints of purple, blue-purple and blue pigments. The original and second

(continued page 46)

curve (Figure 13d) intermediate between that of purple-flowered stocks (Figure 13b) and blue-flowered T. clone 02 (Figure 13c). The shoulder on the S-3 curve at 626 nm is larger than the similar shoulder on purple curves at 630 nm, and smaller than the peak at 614 nm in the blue spectral curve; the main peak of the S-3 curve at 582 nm is intermediate between the 586 nm peak of purple stocks and the 576 nm peak of the blue stock. It is, as noted earlier, very similar to the spectral fingerprint of a mixture of delphinidin and cyanidin extracts in proportions of 2:1, even though this latter curve was from a Beckman DBG spectrophotometer while the other fingerprints discussed here were from a Cary 15 spectrophotometer (Figure 13d).

That red, D^-D^-, progeny were produced from selfing T. hirsuticaulis confirms the heterozygosity of the purple parent at the D locus. It has also been advanced (88) that red progeny might fall into two intensity classes, "/0" and "/1"; however, RHC notations for S-1, S-2, and S-4 are either "/1" or "/3" (Table 3). Quantitative spectrophotometry suggests this latter difference may well be a result of differences in cell size, not in amount of pigment per cell. Thus, the E locus, in these plants at least seems to have no effect on cyanidin production, in the absence of D^+.

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notations are both thought to be errors due to the inability at these times to compare the flower color not only to the Colour Chart, but also to flowers of the other, intermittently blooming, T. hirsuticaulis stocks.

Table 2. Progeny expected from inbreeding of purple-flowered T. hirsuticaulis (predicted

Table 2. Progeny expected from inbreeding of purple-flowered *T. hirsuticaulis* (predicted genotype $D^+D^-E^+E^-$).

Phenotype	Genotype	Spectrophotometric pigment "units"			Mutant sectors
		Total pigment	Ratio Delphinidin:Cyanidin		
Purple	$D^+D^+E^+E^+$	2	1	1	no sectors
Purple	$D^+D^-E^+E^-$	2	1	1	red, blue
Red-purple	$D^+D^-E^+E^+$	1.5	1	2	red, purple
Blue-purple	$D^+D^+E^+E^-$	1.5	2	1	blue, purple
Blue	$D^+D^+E^-E^-$	1	1	trace	no sectors
Blue	$D^+D^-E^-E^-$	1	1	trace	red
Red	$D^-D^-E^+E^+$	1	0	1	no sectors
Red	$D^-D^-E^+E^-$	1	0	1	no sectors
Red	$D^-D^-E^-E^-$	1	0	1	no sectors

Table 3. Progeny obtained from self-pollination of purple *T. hirsuticaulis*.

CODE	COLOR	RHC#	SPECTRUM	RED-SECTORING
P	purple	37/0	Figure 13b	0.15/stamen (430/2780)
S-5	purple	37/0	Figure 13b	0.14/stamen (5/36)
S-1	red	34/3	Figure 13a	---
S-2	red	33/1	Figure 13a	---
S-4	red	34/3	Figure 13a	---
S-3	blue-purple	39/1	Figure 13d	0.00/stamen (1/233)[a]

[a]The one sector is thought to have arisen via a gene mutation early in floral development, giving a large $D^{+}D^{-}$ sector, within which a sector-producing mitotic crossover occurred.

RHC = Royal Horticultural Society Colour Chart (52).

Figure 13. Spectral "fingerprints" of progeny from purple T. hirsuticaulis compared to those of blue, red and purple stocks.

All curves are of non-acidified aqueous extracts of petals.

a) Spectral curves of a red segregant from T. clone 02, and the three red-flowered progeny from the parental purple T. hirsuticaulis, S-1, S-2, S-4.

b) Spectral curves of the parental purple plant, P, and a purple-flowered offspring, S-5.

c) Spectral curve of blue-flowered T. clone 02.

d) Spectral curve of S-3, an offspring of purple T. hirsuticaulis having blue-purple flower color, and of a 2:1 mixture of aqueous extracts of blue-flowered T. clone 02 and one of its red progeny. This latter curve was obtained with a Beckman DBG spectrophotometer, all others, with a Cary 15.

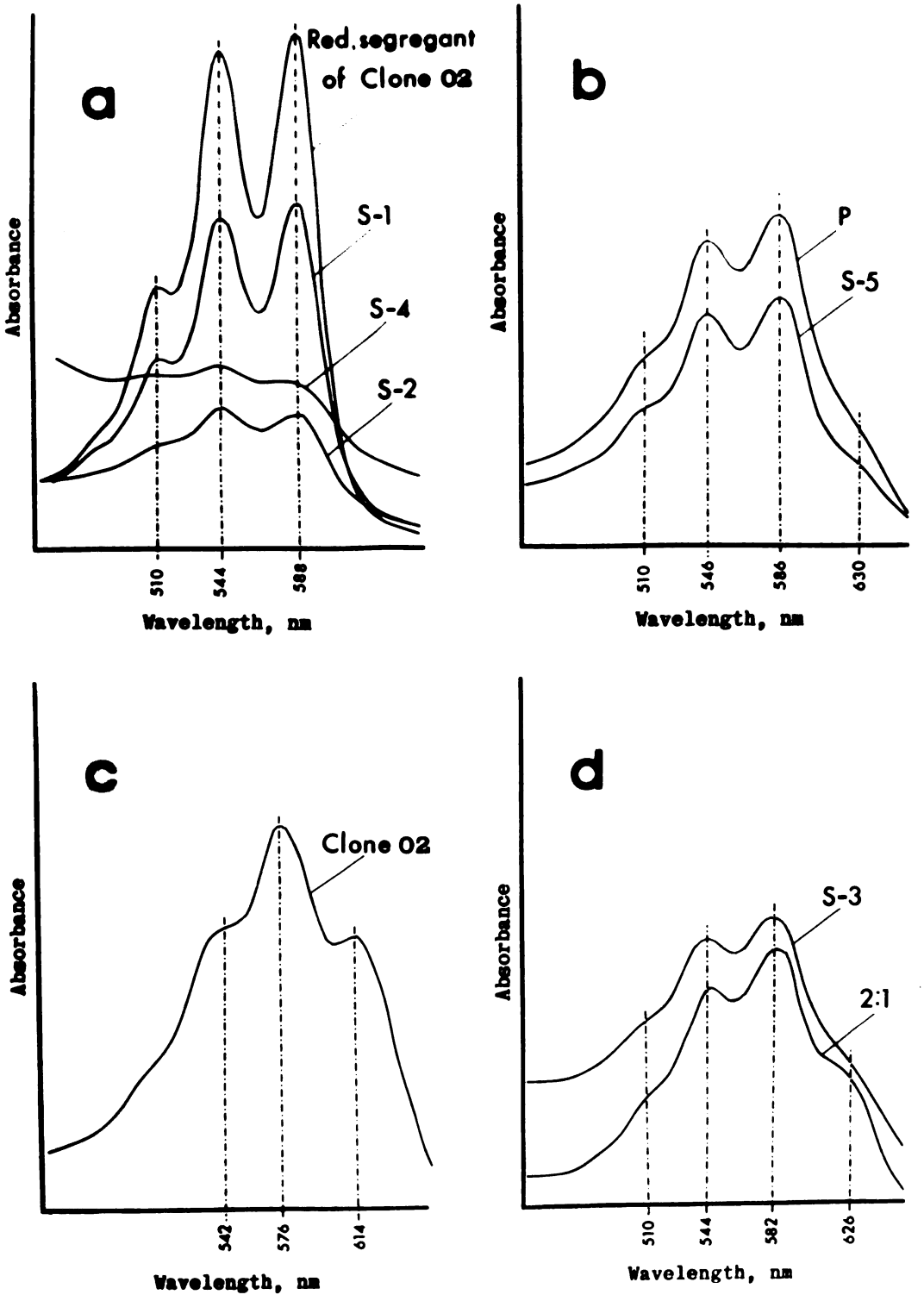
a
Red. segregant of Clone 02
S-1
S-4
S-2
Absorbance
510
544
588
Wavelength, nm
b
P
S-5
Absorbance
510
546
586
630
Wavelength, nm
c
Clone 02
Absorbance
542
576
614
Wavelength, nm
d
S-3
2:1
Absorbance
510
544
582
626
Wavelength, nm

Figure 13

While no blue, E^-E^-, plants have yet been recovered to directly confirm the E^+E^- composition of the purple parent, the existence of a blue-purple progeny still confirms the heterozygous E locus in the purple parent. If the purple parent were E^+E^+, then only three genotypes would be expected amongst its progeny: $D^+D^+E^+E^+$, purple, $D^+D^-E^+E^+$, red-purple, and $D^-D^-E^+E^+$, red. A plant with blue-purple flowers is expected only from interaction of two doses of D^+ with one dose of E^+. It follows then, that the blue-purple progeny is heterozygous at the E locus, and, barring gene mutation, so is the purple parental stock.

Blue-purple flowers, predicted as having a "unit" of delphinidin and half a unit of cyanidin, are expected to be less intensely pigmented than purple flowers with their two units of pigment, but more intensely pigmented than either red or blue flowers with their one unit of pigment. While S-3 is recorded as RHC #39/1, it is, however, noticeably more intense than the blue T. clone 02, recorded as RHC #41/1 (79). The Colour Chart was not a perfect match for either flower color.

The purple-flowered offspring, S-5, is closely similar to the purple parent. Flowers of both match RHC #37/0. They have identical spectral fingerprints and a red-mutant sectoring rate of 0.14 and 0.15 sectors per stamen, respectively (Table 3). These characters, then, certainly seem to be heritable through the germ line (but see footnote, page 42).

Five progeny plants do not permit calculation of an accurate meiotic map. It is possible to show, however, that, while these five plants are not at variance with a hypothesis of independent assortment of the D and E loci, neither are they at variance with the hypothesis of linkage between D and E to the extent (12.1 centimorgans) calculated mitotically in Section III of this dissertation (Table 4).

On the basis of either hypothesis, there is an excess of red progeny recovered, exactly contrary to the case in breeding results from selfing T. clone 02 (79,82). At present, the breeding data are not extensive enough to permit interpretation, although some aspects of the haplontic/diplontic-selection question will be discussed in Section II of this dissertation during a consideration of sizes and cellular genotypes of the various mutant somatic sectors in the parental purple T. hirsuticaulis stock.

Extensive self-pollination of the "self-sterile" purple stock only yielded 22 seeds over a two year period. I hoped that some of the five progeny produced from 19 seeds put to germinate would prove to cross readily with their parent. In addition, a cross to one of the three red progeny might be a testcross, i.e., $D^{+}D^{-}E^{+}E^{-}$ purple x $D^{-}D^{-}E^{-}E^{-}$ red; in any event, such crosses would reveal the E locus composition of the various red progeny. It was found, however, that the cross S-4 x P gave no seed set. In contrast, S-1 x P gave abundant seed set, an average of 3.32 (156/47) seeds per capsule, similar to the value reported by Anderson and Sax (5) for intracrosses with four species of

Table 4. Testing linkage of D and E loci in T. hirsuticaulis.

PHENOTYPE	GENOTYPE	#	EXPECTED NUMBERS	
			D-E ≥ 0.50[a]	D-E = 12.1[a]
purple,n.s.	$D^+D^+E^+E^+$	0	0.3125	0.0183
purple	$D^+D^-E^+E^-$	1	1.2500	1.9682
blue-purple	$D^+D^+E^+E^-$	1	0.6250	0.2659
red-purple	$D^+D^-E^+E^+$	0	0.6250	0.2659
blue,n.s.	$D^+D^+E^-E^-$	0	0.3125	0.9658
blue	$D^+D^-E^-E^-$	0	0.6250	0.2659
red	$D^-D^-E^+E^+$, $D^-D^-E^+E^-$, $D^-D^-E^-E^-$	3	1.2500	1.2500
calculated	X^2, 6 d.f.		4.60	6.49
			0.25<P<0.50	0.50<P<0.75

[a]map distance in centimorgans

n.s. = non-sectoring

Tradescantia. In my material, seeds occurred randomly distributed between the upper and lower three ovules in the capsule (29:31, X^2, 1 d.f., = 0.96, n.s.). The distribution of number of seeds per capsule fits a binomial distribution where each of the six seeds possible has a probablility of 0.5 of maturing (Table 5); it is not known at present whether this has any biological significance.

Self-sterility in various Tradescantia has been investigated and found to be of the Nicotiana (stylar incompatibility) type (5,6,92). Plants are heterozygous for self-sterility alleles, and consequently produce two types of pollen, A_1 and A_2, neither of which will grow in stylar tissue of an A_1A_2 genotype, thus, their self-sterility. It is my hypothesis that the plant, S-1, arose from a self-fertilization involving a mutant, A_3, pollen grain, perfectly capable of growing in the A_1A_2 style. The A_3 bearing sperm united with an A_1 egg, to produce the plant, S-1. The A_1A_3 stylar tissue of S-1 does not inhibit the growth of the purple parent's A_2-bearing pollen, so the cross S-1 x P sets many seed. The S-4 plant and, since, preliminary trials indicate the S-3 x P cross to be incompatible, the S-3 plant as well, probably arose through a rare lack of penetrance of either the A gene in the pollen or the appropriate A gene in the style. Identification of an A_1A_1 plant through continued intercrosses (i.e., none of the pollen grows in the A_1A_3 style of the S-1 plant) would give support to the above hypothesis concerning the origin of the S-3 and S-4 plants.

At this time, 2 of the 156 seeds from the S-1 x P backcross have

Table 5. Seeds per capsule from the cross S-1 x P.

Number of mature seeds	0	1	2	3	4	5	6
Capsules observed	-	4	4	11	6	3	2
Binomial Expectation	0.5	2.9	7.1	9.5	7.1	2.9	0.5
Binomial Coefficients	(1)	(6)	(15)	(20)	(15)	(6)	(1)

X^2, 5 d.f. = 8.81, n.s.

germinated but neither of these plants, B-1 nor B-2, has as yet flowered.

SECTION II

Somatic sectoring behavior in stamen hairs of purple-flowered *T. hirsuticaulis*

General considerations.

Stamen hairs of *Tradescantia* flowers are monofiliform (single file) chains of large anthocyanin-pigmented cells derived from single-celled initials on the filament (e.g., Figure 15). Just as a fungal ascus contains an ordered array of meiotic events, a mature stamen hair, the result of a series of terminal, subterminal and intercalary divisions (53, 84, 105), consists of the products of an ordered set of mitotic events.

Somatic sectoring of *Tradescantia* flowers was first reported by Cuany *et al.* (32). Later work by Mericle and Mericle (79,82) established the genetic nature of the mutant color sectoring in *T. clone 02* and began the investigation into the nature of the mechanisms producing spontaneous sectors. The purple-flowered *T. hirsuticaulis* whose genetics has been described in Section I and previously (29,83, 88), appears to hold particular promise for extending the investigation of the mechanisms of floral sectoring in *Tradescantia*.

This stock, as described in Section I, has markers at two loci

involved in the production of anthocyanin pigments. As in T. clone 02, the D^+ allele at the D locus gives the capability to produce blue pigment, delphinidin, with the suppression of the production of red pigment, cyanidin (79,88). Unlike T. clone 02, the presence of an E^+ allele at the E locus confers the release of this suppression. Thus the T. hirsuticaulis stock, which is $D^+D^-E^+E^-$, produces large amounts of red and blue pigments, resulting in its purple color (88, Sect. I).

Close inspection of the pigmented floral parts, however, reveals the presence of "somatic mutations", single cells or groups of cells of a variant hue -- some red, due to the "mutational" loss of D^+ ability, some blue, due to the loss of E^+ ability.

The presence of two sector colors permits certain analyses that are not possible in T. clone 02. Unfortunately, T. hirsuticaulis is not amenable to some analyses possible with T. clone 02 which have provided much information. For example, it is possible to follow individual clusters (inflorescences) of T. clone 02 throughout their substantial period of flower production (80). This permits the detection and selection of inflorescences with high or low mutation rates (79). The ability to pre-score clusters, and thereby subsequently assign materials with similar mutation rates to different experimental treatments, allows for great precision in detecting small differences (76,77,86). Additionally, the mutation rate in T. clone 02 has been seen to change with age of the cluster, i.e., how long it has been blooming (78,80,81, 91). Work by Mericle and Mericle shows that this is partially due to

the decline in the average number of stamen hairs per stamen (78,80). T. clone 02 produces inflorescences prolifically throughout the year in growth chambers, with each inflorescence averaging some 140 flowers (80). The plant is also vigorous enough to continue to bloom even when kept in total darkness for some 3 weeks (75).

T. hirsuticaulis may have an additional genetic marker, but it has many disadvantages as an experimental organism. Regardless of the various growth chamber environments tried, it produces small inflorescences, typically near or below soil level, with a tendency for the later buds to blast. The recorded maximum number of flowers produced by one inflorescence is 16, while many "inflorescences" produce only one flower. Not only does T. hirsuticaulis produce small inflorescences under standard growth chamber conditions (82) used in this laboratory, but the plants produce inflorescences only sporadically, a most annoying peculiarity. Furthermore, while T. clone 02 sets seed readily upon self-pollination (79,82), purple T. hirsuticaulis, like many other Tradescantia (5,6,92) is self-sterile, typically producing no seed after self-pollination. Moreover, when a concerted self-pollination program produces a few seeds, they are just as difficult to germinate as those of T. clone 02 (79,82).

Just as in T. clone 02, the spontaneous mutant-sectoring frequency in T. hirsuticaulis can be enhanced above the spontaneous level by exposing the plants to ionizing radiation. Acute ^{60}Co gamma exposures of 34 and 60 R delivered at a rate of 26 R/minute were used in the

experiments reported here. The cymose inflorescences on potted plants were positioned immediately behind a 6 mm thick Lucite shield to help achieve electron equilibrium and maximum absorbed dose (60). For purposes of this study, the first seven days of increased frequency of sectoring were used as the radiation-response period.

"Somatic sectoring" is evidenced by those cells of the stamen hair which exhibit differences from the typical purple hue (hue as opposed to intensity). These cells are recorded as to their number, color, and position in the hair through the use of a standard set of abbreviations (Table 6), together with sketches of particularly interesting sectors, (see Appendix A), or cases of red and blue cells in the same hair (Figure 14). Data on sector distribution among the stamens of a flower, and among flowers produced any given day or of any experimental treatment are retrievable through the use of a standardized "scoring sheet" (Figure 14). These scoring sheets provide space for recording additional comments, as well as data on the numbers of hairs on antisepalous and antipetalous stamens and the number of cells per hair for two hairs each chosen from the top, middle, and bottom thirds of the two aforementioned stamens (Figure 14).

Mericle and Mericle have described "multiple-sectored" hairs in T. clone 02, i.e., individual hairs containing more than one red sector separated by typically pigmented (non-mutant) cells, and concluded that they are the result of a single mutational event (79,81,84). Accordingly, I have tabulated multiply-sectored red or

Table 6. Notation system used for recording somatic sectors in stamen hairs of T. hirsuticaulis.

Notation	Description of the sector
xT	a terminal sector, x = number of contiguous red mutant cells which encompass the terminal cell of the hair
x-y	a subterminal sector, x = number of contiguous red mutant cells with y number of non-mutant cells more terminal in the hair
xE	an entire hair sector, a hair composed of x number of red mutant cells, it has no non-mutant cells
(x_1E, x_2E)	a multiple-entire hair sector, two or more hairs composed entirely of red mutant cells, one with x_1 number of cells, one with x_2 number of cells, etc., and connected by a file of red mutant cells in the epidermis of the filament
$(x_1\text{-}y_1, x_2\text{-}y_2)$	a multiple-sectored hair, one hair containing two or more red mutant sectors separated by non-red cells; one sector comprising x_1 number of cells, the other, x_2 number of cells, with y_1 and y_2 cells more terminal in the hair, respectively
z-Blue	a sector of blue mutant cells, the preceding notation (z) is composed of blue, not red, mutant cells
z*, z**, etc.	a sector of red and blue cells, a red/blue twin, the preceeding red sector (z) is associated with blue mutant cells; a labeled sketch of the sector is always "footnoted" on the scoring sheet (see Figure 1)

Figure 14. The standard scoring sheet.

This is a facsimile of the standard scoring sheet.

a) radiation exposure

b) day post-irradiation

c) date flower sample taken

d) plant identification number

e) experimental material identification (species, clone,...)

f) location of the plant when sampled

g) flower from which inflorescence (cluster), and, if it produced more than one flower that day, which flower

h) stamen color mutants, recorded as in Table 6

i) sectors contained in each of the 6 stamens, individually

j) I and II are antisepalous and antipetalous stamens; the first entry is the number of hairs on each stamen, the sets of two entries which follow are the numbers of cells in each of two hairs chosen from the top, middle, and lower thirds of each stamen, respectively.

k) space for drawings of exceptional sectors and all sectors involving both red and blue cells; B, R, denote blue and red cells, respectively, others are understood to be purple.

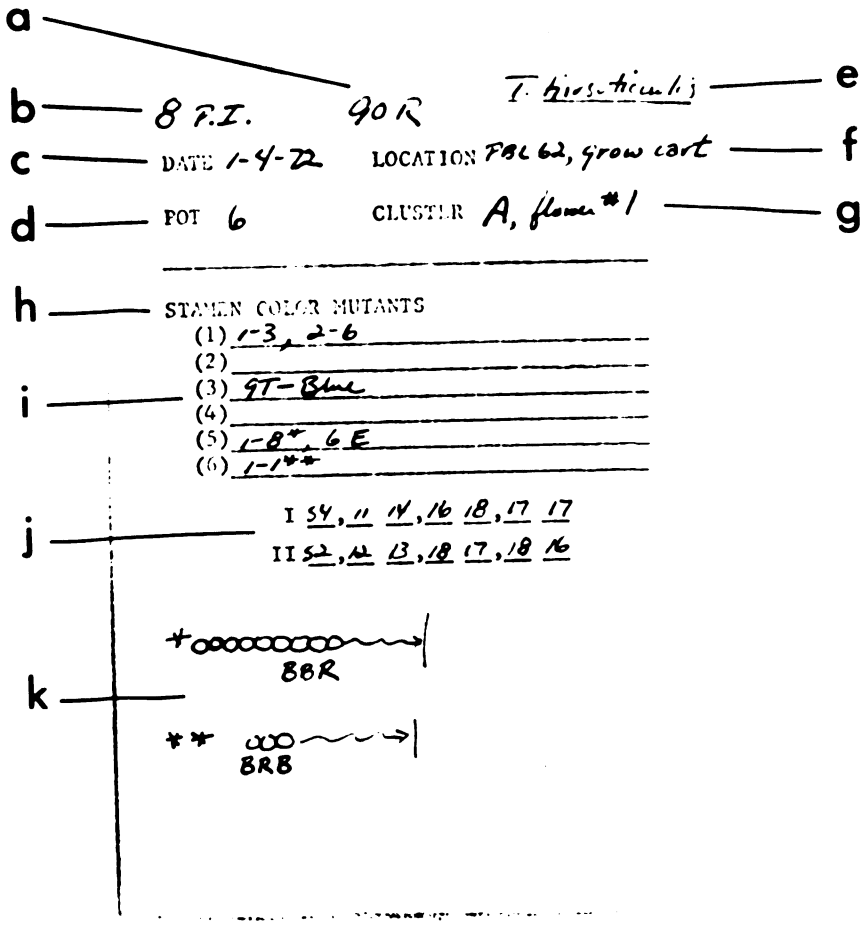
a
90R
T. hirsuticulis
e
b
8 F.I.
c
DATE 1-4-22
LOCATION PBL 62, grow cart
f
d
POT 6
CLUSTER A, flower #1
g
h
STAMEN COLOR MUTANTS
(1) 1-3, 2-6
(2)
(3) 9T-Blue
(4)
(5) 1-8**, 6E
(6) 1-1**
i
j
I 54, 11 14, 16 18, 17 17
II 52, 12 13, 18 17, 18 16
BBR
k
** BRB

Figure 14

multiply-sectored blue hairs as single mutational events (Table 6). This decision materially affects the numerical value calculated for mutation rate inasmuch as 7.9, 8.0, and 6.2 percent (Table 7) of the total red-only sectors of the spontaneous, 34 R and 60 R response materials, respectively, are multiple-sectored hairs which would otherwise be counted as two (minimally) sectors. (See also Table 4, ref. 79).

Red/blue twin spots: their existence.

As this stock of T. hirsuticaulis exhibits both red and blue somatic sectors, a kind of "multiple sector" -- a hair with red and blue sectors -- was theoretically possible. Indeed, red and blue mutant cells were often observed in the same stamen hair. As a matter of fact, in 89% (304/343) of the cases of a red and a blue sector in a hair, (Table 8), they were adjacent in the file of cells, i.e., without a purple cell separating the red and blue sectors. This suggested that adjacent red and blue mutant cells might represent the result of a single event, rather than be the coincident, independent, occurrence of a red and a blue mutation during hair ontogeny.

Accordingly, all stamens (the biological grouping of stamen hairs next larger than individual hairs) containing both red and blue sectors were scored for numbers of red - blue pairs whose members were in the same hair and numbers of red - blue pairs whose members were situated in different hairs (Figure 15). A random distribution of red and blue

Table 7. Contribution of multiple sectors to total sectors in T. hirsuticaulis.

Treatment	Number[a]				Percent		
	total	S.S.[b]	M.S.[c]	E. + M.E.[d]	S.S.[b]	M.S.[c]	E. + M.E.[d]
Spontaneous	430	367	34	29	85.3	7.9	6.7
34 R-response	100	87	8	5	87.0	8.0	5.0
60 R-response	273	251	17	5	91.9	6.2	1.8

[a]red-only sectors, the hair contains no blue mutant cells.

[b]single sectors, the hair does not contain more than one group of contigous red mutant cells. Excludes hairs entirely composed of red mutant cells.

[c]multiple sectors, the hair contains two or more groups of contigous red mutant cells separated by non-mutant, purple cells.

[d]entire plus multiple-entire sectors, the hair is composed entirely of red mutant cells. In the case of multiple-entires, two or more entire red mutant hairs are connected by a file of red mutant cells in the epidermis of the filament.

Table 8. Red/blue twin sectors with and without intervening purple cells.

	Twin Sectors[a]		
Subtotal[b]	Without[c]	With[c]	Total
A	94	18	112
B	55	5	60
C	85	10	95
D	70	6	76
Total	304	39	343

calculated value of homogeneity X^2, 3 d.f., 3.98, $p > 0.05$

[a]Red/blue twin sectors comprised of a single red and a single blue sector within the hair were used for the analysis.

[b]The subtotals comprise sectors from the following experiments

A, Spontaneous, 34 R-preresponse and 60 R-preresponse,

B, 34 R-response,

C, 60 R-response,

D, 34 R-postresponse and 60 R-postresponse.

[c]with or without non-mutant, purple cells separating the red and the blue sector in the hair.

Figure 15. A schematic stamen.

A schematic depiction of a stamen with red and blue mutant sectors distributed amongst its hairs to illustrate pair-by-pair analysis. The crosshatched areas represent blue mutant cells, the stipled areas, red mutant cells. All other cells are understood to be purple. This stamen, then, has one red-blue pair in the same hair, and three red-blue pairs involving sectors in different hairs, a s:d ratio of 1:3.

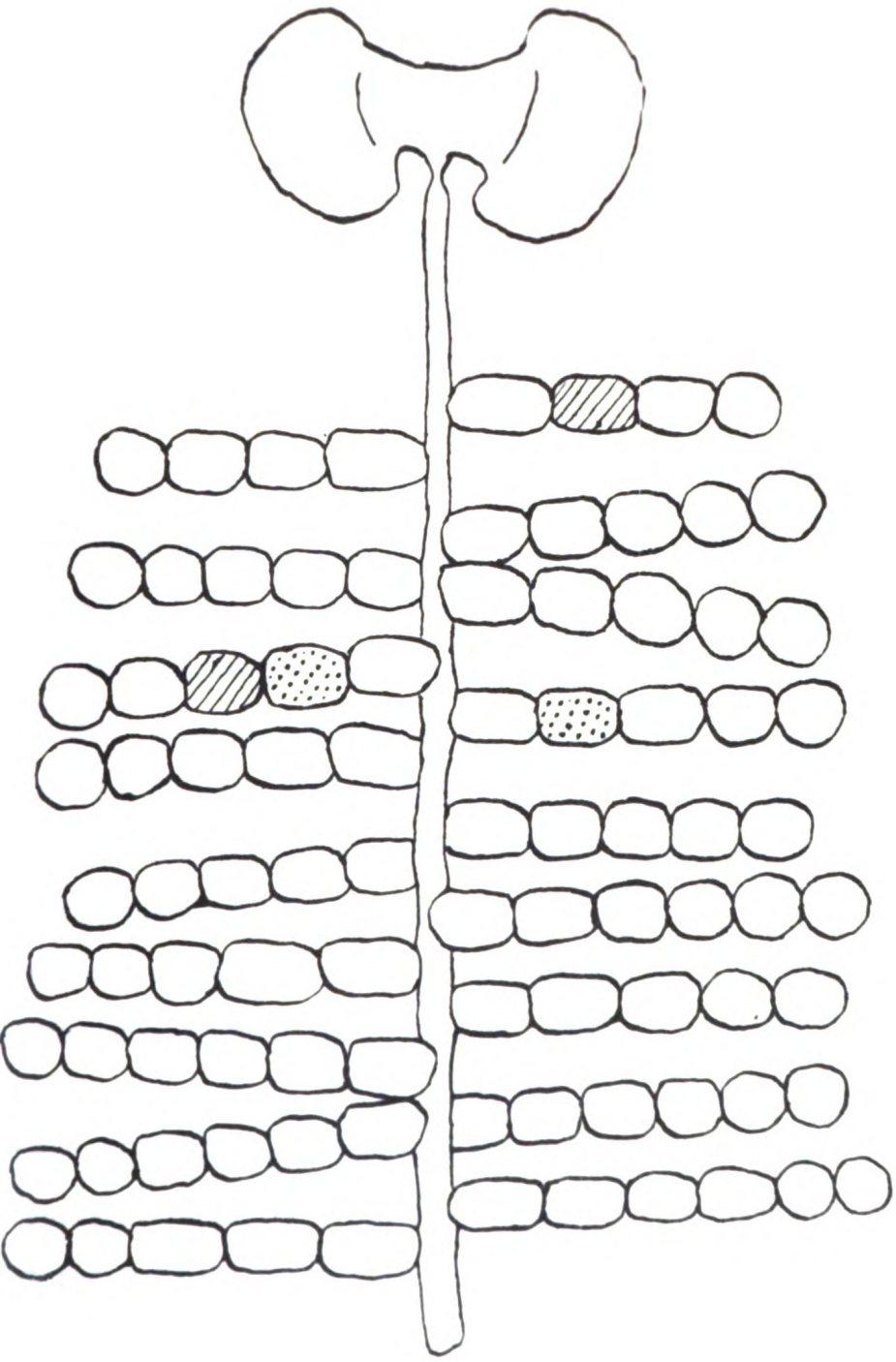

Figure 15

events in stamens containing some 54.5 (30) to 55.4 (Table 21) hairs would be expected to result in a same:different (s:d) ratio of 1:53.5 - 54.4; instead, the s:d ratios found were 94:42, 71:83, and 109:193 for spontaneous (0 R) and responses to 34 R and 60 R ^{60}Co irradiation, respectively (Table 9). Each of these ratios is very significantly different, ($p < 0.001$), from that expected on the basis of random association.

It might be argued that either a red- or blue-sector-producing event made the other more likely, (i.e., facilitated its occurrence). Such would account for the demonstrated non-random distribution of sectors without supposing that red and blue sectors in the same hair were the result of a single event. The most extreme case would be that of a red or a blue event requiring the other to occur at the next cell division. However, as a consequence of the random segregation of mitotic chromosomes at that division, at most only 50% of the hairs containing both a red and a blue sector could have those sectors without an intervening purple cell. Yet 89% (304/343, Table 8) of those instances in which a red and a blue sector occurred in the same hair were without an intervening purple cell. This 89% value is very significantly different ($p < 0.001$) from 50%. I interpret this to mean that red and blue sectors occurring in a single hair constitute a red/blue twin spot (r/b twin), i.e., each such twin spot does indeed constitute a single entity -- the result of one initiating event which is distinct from those events that produce red-only (ro) or

Table 9. Red/blue twin sectors: Pair-by-pair analysis of all stamens with red and blue mutant sectors.

experiment	numbers		calculated value X^2		P
	Same[a]	Different[b]			
5/72 Spontaneous	75	36			
10/72 Spontaneous	7	3	homogeneity	1.6037	> 0.500
6/72 33 R-preresponse	3	0	vs. 1:53.5	3411.6	< 0.001
10/72 34 R-preresponse	9	3	vs. 1:54.4	3483.8	< 0.001
0 R, TOTAL	94	42			
6/72 33 R-response	46	60	homogeneity	1.0245	> 0.750
10/72 34 R-response	25	23	vs. 1:53.5	1672.8	< 0.001
34 R, TOTAL	71	83	vs. 1:54.4	1704.9	< 0.001
11/72 60 R-response	109	193	vs. 1:53.5	1968.2	< 0.001
60 R, TOTAL	109	193	vs. 1:54.4	2003.6	< 0.001

[a] red sector(s) and blue sector(s) in the same hair, see Figure 15.

[b] red sector(s) in one hair, blue sector(s) in another hair of the same stamen, see Figure 15.

blue-only (bo) sectors*.

Red-only and blue-only sectors: their existence.

A stamen hair, being a single file of cells, has no underlying tissue in which to hide "half-a-twin". Therefore, red-only and blue-only sectors cannot simply be "partially seen" r/b twins -- a possible interpretation of single spots that occur in the same tissue as twin spots (131,145). Moreover, if the initiating event for a r/b twin required more than one cell division to become expressed (i.e., minimally two rounds, producing four cells), thus permitting half a sector to be hidden as an unexpressed spot, then at most 25% of the r/b twin sectors could be without an intervening purple cell (50% would have at least one intervening purple cell, and 25%, at least two). The experimental value of 89% for r/b twins without an intervening purple cell is very significantly different from 25% ($p < 0.001$). Thus, I conclude that a ro or a bo sector could not have a "half-twin" hidden

---

*Admittedly, some rare cases of red and blue sectors in the same hair, (especially when the mutation rate is high), could be the result of separate, coincident ro and bo events during hair ontogeny. These can be corrected for by solving the following set of simultaneous equations:

observed ro sectors/hair = ro events/hair(1 - bo events/hair)
observed bo sectors/hair = bo events/hair(1 - ro events/hair)
observed r/b twins/hair = r/b twin events/hair + (ro events/hair x bo events/hair)

Inspection of Table 18 shows that the resultant correction would be very small.

as an "incipient sector".

It seems clear that while there are two mutant colors, red and blue, there appear to be three distinct classes of sectors: red/blue twin, red-only and blue-only.

Red/blue twin spots: position and orientation in hairs.

If indeed r/b twins are the result of a single event, then, as a consequence of the random segregation of chromosomes at that cell division, the red sector would be expected to be more terminal 50% of the time, and the blue sector more terminal, the other 50% of the time. Analysis was carried out on all hairs containing a single red sector and a single blue sector, although these might or might not be contiguous in the hair, i.e., included the 11% of the r/b twins with intervening purple cells. These 389 sectored hairs were tabulated as to whether the red or the blue component was terminal, i.e., encompassed the terminal cell of the hair, or simply more terminal in the hair. Summing these two ways, some 206 instances of the red component and 183 instances of the blue component being the more terminal were found (Table 10). This is not significantly different from the 1:1 ratio expected if the distribution were random; the calculated value of X^2 with 1 degree of freedom is 1.36, $p > 0.90$.

The data in Table 10 do show a significant difference between the red and blue component in the manner of being the more terminal component of the twin. Red components most often encompass the terminal

Table 10. Red/blue twin sectors: Orientation of red and blue component in the hair.

	T[a]	MT[b]	Total
Red	127	79	206
Blue	63	120	183
Total	190	199	389

[a]T, the mutant sector encompasses the terminal cell of the hair.

[b]MT, the mutant sector is merely more terminal within the hair; it does not encompass the terminal cell of the hair.

cell when they are the more terminal constituent of the twin spot; blue components do not (Table 10). Statistically, the difference in the behavior of the two is highly significant; the X^2 value with 1 degree of freedom is 28.79, $p < 0.005$. The biological significance of this is unknown at the present time.

There might be a tendency for the blue component to be more terminal after a mitosis with a red/blue mutant event when that mitosis is in a subterminal cell, and a corresponding tendency for the red component to be the terminal daughter cell when the mutant division is in a terminal cell. Non-random, preferential segregation of chromosomes has been observed in certain special, meiotic, cases, i.e., heteromorphic chromosomes (114), "affinity" loci (41), however, it seems most prudent simply to point out this curious situation in the r/b twin sectors and venture no explanations until some further experiments are designed and completed.

Sector sizes.

The above is not the only curious phenomenon that involves the red and blue components of the twin spot. An analysis of the number of cells comprising the red and blue components of twin spots, red-only sectors and blue-only sectors is presented in Table 11. The effect of

Table 11. Sizes of non-multiple, non-entire hair sectors in T. hirsuticaulis.

a) Analysis of Variance

Source	d.f.	s.s.	m.s.	F	p
Total	1553	9808.72			
Radiation	2	22.75	11.37	2.11	> 0.20
Sector type	3	683.71	227.90	42.28	< 0.01
R x S	6	792.41	132.07	24.50	< 0.01
error	1542	8309.85	5.39		

b) Duncan's Multiple Range Test

Color	b,r/b	b,r/b	b,r/b	r,r/b	ro	r,r/b	r,r/b	ro	ro	bo	bo	bo
Radiation	60	0	34	0	60	60	34	0	34	34	60	0
Mean # cells	1.34	1.39	1.66	2.27	2.42	2.58	2.71	2.77	2.78	3.36	3.42	5.41

Any two means connected by the same line are not significantly different from each other ($p > 0.05$) using Duncan's Multiple Range procedure.

ro = red-only, bo = blue-only, r,r/b = red component of red/blue twin, b,r/b = blue component of red/blue twin

"radiation"* on the average size of sectors is found to be non-significant, the calculated value of $F_{2,1542} = 2.11$, $p < 0.20$. The small number of buds per inflorescence in T. hirsuticaulis mentioned earlier is reflected in the relatively short time elapsing between the initiation and completion of stamen hair development, paralleling the behavior of old inflorescences in T. clone 02 (82); this is evidenced by the appearance of many entire-hair sectors only 5 to 7 days after the initial rise in mutation rate elicited by ^{60}Co irradiation (Figure 16, Table 12). Thus the first 7 days of increased sectoring rate taken as the radiation response period in these experiments encompasses essentially the entire ontogenesis of the stamen hairs, which is, of course, the same period over which spontaneous sectors are induced.

The R x S interaction (Table 11a) is found to be significant, the calculated value of $F_{6,1542} = 24.50$, $p < 0.01$. I can detect no pattern in this interaction (Table 11b). The mean size of 34 R and 60 R bo, 0 R and 34 R ro, 0 R and 60 R b,r/b, and 34 R and 60 R r,r/b sectors are not significantly different from each other ($p > 0.05$) by Duncan's Multiple Range Test: that list includes all the possible pairings of radiation exposures. (This is not unexpected since, for example, if all four had 34 R and 60 R means non-significant from each

---

*This is not a measure of the effect of radiation, per se, but of the selection of sectors from seven days of the response curve; i.e., the radiation-induced sectors on the first days are smaller in size than those on later days. See Table 12 for data in Tradescantia hirsuticaulis.

Figure 16. Mutation response curves.

The average frequency of red-only sectors at various times before and after the radiation-induced rise in mutation rate. Raw data are found in Table 12.

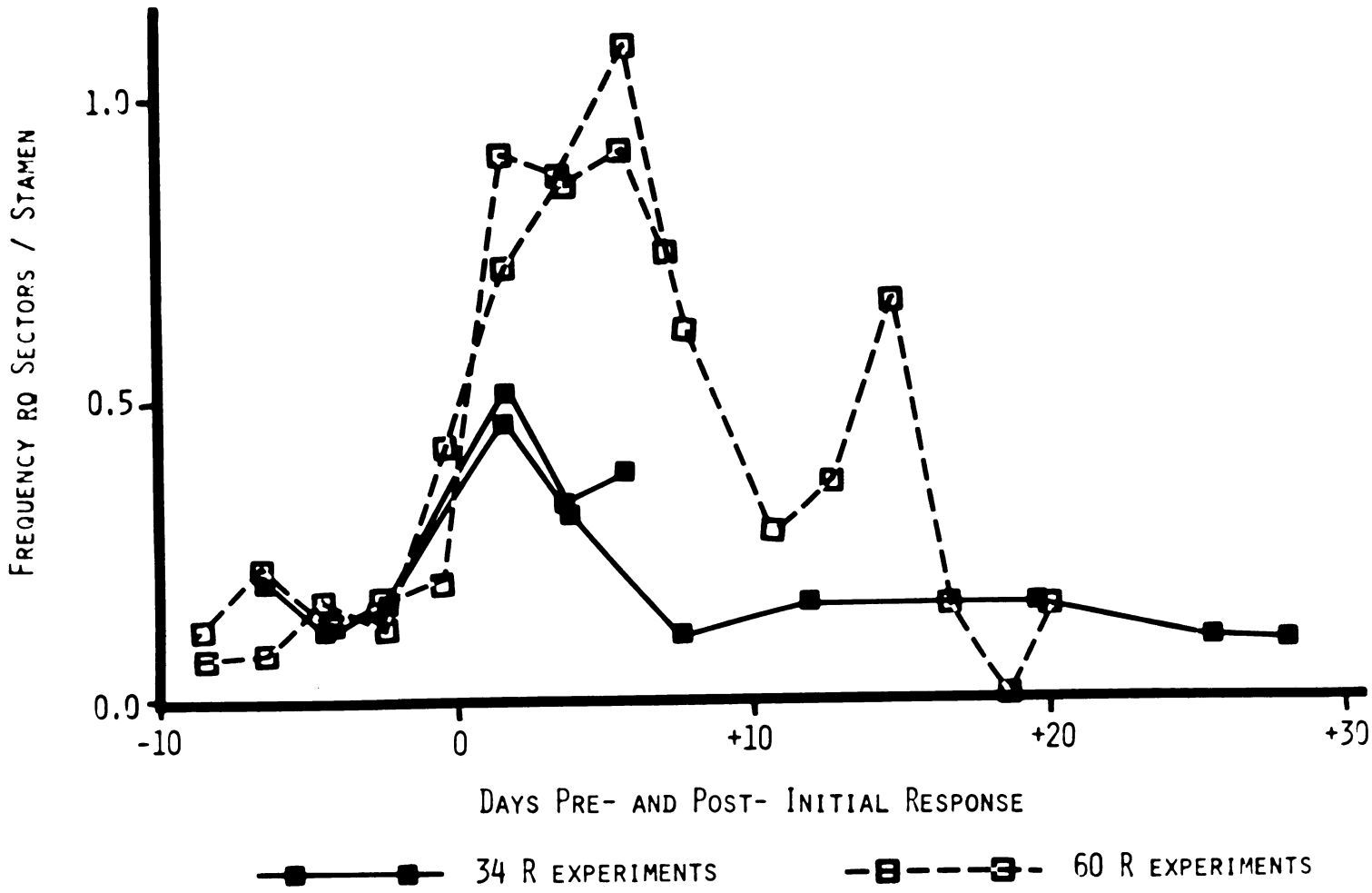
FREQUENCY RQ SECTORS / STAMEN
1.0
0.5
0.0
-10
0
+10
+20
+30
DAYS PRE- AND POST- INITIAL RESPONSE
34 R EXPERIMENTS
60 R EXPERIMENTS

Figure 16

Table 12. Numbers and types of sectors in T. hirsuticaulis after exposure to ^{60}Co gamma radiation.

Day Post Response	# Stamens	red-only sectors a/b	c	d	e	r/b twins a	d	blue-only sectors a/b	c	d
34 R experiments										
pre	234	28/ 70	1	5		6	4	2/ 5		
1	48	17/ 34				9		2/ 6		
2	48	17/ 34				10		1/ 1		1
3	42	15/ 67		3		16	5	3/ 6		1
4	30	15/ 64		2		8	1	1/ 4		
5	42	11/ 47		1		9	1	4/17		1
6	18	4/ 7	2	2		3			1	
7	24	6/ 27	3			3	1	1/ 1		
>7	108	10/ 41	3		2	6	2	2/10		1
60 R experiments										
pre	354	48/106	1	4	1	38	3	4/28		1
1	54	38/ 90		4		17	2			
2	42	30/ 64		1		21	2	2/ 3		
3	60	54/119		3		22	5	5/19		
4	48	31/ 66		2		19	5	1/ 1		
5	54	50/102	1	2		29	3	8/19		1
6	36	21/ 90	2	4		13	4	5/16		1
7	36	27/ 74	4	1		11	7	5/31		1
>7	144	44/199	22	7	5	24	10	6/50	2	

a = single sectors, b = total number cells, c = entire hair sectors, d = multiple sectors, e = multiple entire hair sectors

other, but significant from the 0 R means, the "radiation" effect in the analysis of variance would have been significant, not non-significant.) Neither the two types of red sectors, ro and r,r/b, nor the two types of blue sectors, bo and b,r/b, show similar patterns of significance of means, e.g., 34 R and 60 R bo are non-significant, while 0 R and 60 R b,r/b are non-significant.

The average sizes of the red components of twins are found to be larger than those of the blue components (Table 11b). Moreover, it can be seen that the average sizes of the red components are essentially the same as the average sizes of red-only sectors (Table 11b). On the other hand, the average sizes of the blue-only sectors are significantly larger than either the average sizes of red components of r/b twins or of red-only sectors, and, in contrast to the parity in sizes of these latter, is also significantly larger than the average sizes of blue components of r/b twins (Table 11b).

Part of this is probably due to the difficulty of distinguishing small blue-only sectors against a background of purple cells. The distribution of sizes of red-only and blue-only sectors is different, and statistically distinct, (Table 13); the difference between the two appears to be a deficiency in observed one- and two-celled blue-only sectors. While it is possible to estimate the number of one- and two-celled sectors that should have been observed if they were distributed exactly as are the red-only sectors, this latter could well be an unwarranted assumption. The average size of a blue-only sector may well

Table 13. Distribution of sizes of non-multiple, non-entire red-only and blue-only sectors.

Sector		1	2	3	4	5	6	7	8	9	10
Red-only	#	317	211	38	22	16	19	23	15	21	7
	%	45	30	5	3	2	3	3	2	3	1
Blue-only	#	25	11	3	6	6	6	3	3	6	2
	%	32	14	4	8	8	8	4	4	8	3
Total	#	342	222	41	26	22	25	26	18	27	9

		11	12	13	14	15	16	17	Total
Red-only	#	8	5	1		1	1		705
	%	1	1						
Blue-only	#	3		2	2			1	79
	%	4		3	3			1	
Total	#	11	5	3	2	1	1	1	784

calculated homogeneity X^2, 16 d.f., = 76.05, $p < 0.005$

be truly larger than that of a red-only, and the distribution of sector sizes for the two sector classes really may be different. Certainly questions of "detection" do not apply to the components of r/b twin spots, yet the average size of the red component, here, is almost twice as large as the average size of the blue component.

The discussion above excluded consideration of entire hair sectors. A comparison of the number of cells in red-entire, blue-entire, and non-mutant purple hairs is presented in Table 14. The effect of "radiation" (see footnote, page 76) is found to be non-significant; the calculated $F_{2,567.5} = 2.30$, $p = 0.20$. The R x C interaction is significant, the calculated $F_{4,567.5} = 31.66$, $p < 0.01$, but, as in the analysis of non-entire hair sectors above, I can distinguish no pattern in the interaction (Table 14b). The mean sizes of 0 R and 60 R blue-entires, 0 R and 34 R non-mutant purple, and both 0 R and 60 R and 0 R and 34 R red-entire hair sectors are not significantly different from each other ($p > 0.05$) by Duncan's Multiple Range Test.

The average sizes of red-entire hair sectors are significantly different from those of blue-entire hair sectors -- the 34 R blue-entire "mean" represents a single observation (Table 14b). When compared with the average length of all-purple hairs, 16.61 to 17.13 cells, both red-entire and blue-entire hairs exhibit a substantial and significant reduction in length. The 24.1 to 19.3% and 15.4 to 6.9% reduction in cell number of red-entire and blue-entire hairs, respectively, in T. hirsuticaulis is similar to the 18.3 to 18.4% reduction reported by

Table 14. Sizes of non-multiple entire hair sectors and non-mutant entire hairs in T. hirsuticaulis.

a) Analysis of Variance

Source	d.f.	s.s	m.s.	F	p
Total	575.5	5110.47	---		
Radiation	2	28.71	14.36	2.30	0.20
Color	2	759.08	379.54	60.92	< 0.01
R x C	4	788.98	197.24	31.66	< 0.01
error	567.5	3533.70	6.23		

b) Duncan's Multiple Range Test

Color	R	B	R	R	B	B	P	P	P
Radiation	60	34	0	34	0	60	34	60	0
Mean #	13.00	13.00	13.21	13.40	14.50	14.50	16.61	(16.69)	17.13

Any two means connected by the same line are not significantly different from each other ($p > 0.05$) using Duncan's Multiple Range procedure. The mean in parentheses is of a "missing entry" in the analysis of variance.

R = red, B = blue, P = purple

Mericle and Mericle (79) for entire-hair sectors in spontaneous and 1.5 - 6.0 R/day irradiated plants of T. clone 02. This phenomenon, the smaller size of entire mutant hairs compared to entire non-mutant hairs, has been termed "loss of reproductive capacity" or "integrity" (54,79), terms also used to describe the shortening of non-mutant hairs after exposure to large or very large doses of radiation (3,34,53,55, 100,142,143). Inasmuch as the phenomenon seems to be the result of meristematic cells of the stamen hair not undergoing as many mitoses in experimental material as in control material, it seems perhaps more appropriate to term it loss of "mitotic fitness."

Sparrow and coworkers interpret the deficiency of cells in "stunted" but typically pigmented hairs from heavily irradiated Tradescantia material as the result of a combination of induced mitotic lag and a temporary lengthening of the mitotic cycle time (53,54,55). Mericle and Mericle point out additionally that mutant hairs, even those of spontaneous origin show a reduction in average length although the mitotic fitness of mutant cells does not seem to be sufficiently depressed to regularly cause clefts and indentations in mutant petal sectors (79). Diplontic selection against genetically red tissue has, however, been advanced by Mericle and Mericle to explain the deficiency of red progeny derived from inbreeding the heterozygous T. clone 02 (82), as well as to explain the reduction in the average length of red entire hairs in this material (79,82).

If a genetic constitution of $D^{-}D^{-}$, with a putative associated small

homozygous deficiency (82), has a reduced mitotic fitness, then the red progeny from the selfing of T. clone 02 should show this same depression of mitotic fitness and a correspondingly small average length of their stamen hairs. Accordingly, as a part of the present study, an analysis was made of the stamen hairs from flowers of S-42 and S-62, the two red progeny from T. clone 02 which remain in this laboratory's collection. The average length of stamen hairs seems to be a relatively conservative parameter; average hair length is not significantly different even between materials that do show significant differences in mean number of stamen hairs (Table 21). However, the flowers used for the analysis were, indeed, picked from young, vigorous inflorescences, thus roughly matching them for age. The results of the analysis do not support the hypothesis of a smaller average hair length in red progeny (Table 15). Instead the average hair length of S-42, 18.28 ± 0.54 cells, and S-62, 23.70 ± 0.67 cells, are very significantly different from one another and are both below and above the value of 20.20 ± 0.35 cells reported as the average length of blue hairs in T. clone 02 (79). Seemingly, the mitotic fitness of the red, $D^{-}D^{-}$, genotype can be modified by other factors in the genome, putative "vitality genes" (87).

The chromosomes of Tradescantia are well known to possess many inversions, (Swanson, 136); the recombination of chromosomes or chromosome segments in progeny as a result of the sexual process, or in some somatic cells as a result of mitotic recombination might well result

Table 15. Comparison of stamen hair length (number of cells) in S-42 and S-62, two red-flowered progeny from T. clone 02 inbreeding.

a) Summary of Data

	S-42	S-62	total
Σx	658	856	1514
Σx^2	12398	20922	33320
n	36	36	72

b) Analysis of Variance

Source	d.f.	s.s	m.s.	F	p
Total	71	1483.9444			
Stock	1	678.6111	678.6111	58.99	< 0.001
error	70	805.3333	11.5048		

c) Average Hair Lengths

T. clone 02 = 20.20 ± 0.35 cells (ref. 79)

S-42 = 18.28 ± 0.54 cells, a 9.5% reduction from T. clone 02

S-62 = 23.78 ± 0.67 cells, a 17.7% increase from T. clone 02

in genotypic differences in "fitness" known to accompany different combinations of inverted chromosomes in many organisms (39).* As evidence for inversions in these particular stocks, I should like to point out the high percentages of pollen abortion in these diploid species, some 39% in T. clone 02 (128) and 12%**, 203/1680, in purple T. hirsuticaulis, which is well within the range of values reported by Swanson (136) for Tradescantia stocks known to have inversions.

Obviously, as their phenotypes differ, the genotypes of red, blue and purple cells in T. hirsuticaulis are different; as will be explained later, the cellular genotype of red-only sectors differs from the cellular genotype of the red components of r/b twins, $D^-D^-E^+E^-$ and $D^-D^-E^+E^+$, respectively (Figure 18). Similarly, the cellular genotype of a blue-only sector differs from that of the blue component in a r/b twin, $D^+D^-E^-E^-$ and $D^+D^+E^-E^-$, respectively (Figure 18).

The two types of red cellular genotypes, $D^-D^-E^+E^-$ and $D^-D^-E^+E^+$, exhibit a similar reduction in "mitotic fitness"; sectors of each have essentially the same average size (Table 11). The cells of both these sectors are also indicated as being homozygous for the distal segment of the chromosome, which is the presumptive location of the D^-

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* Indeed, mitotic crossing-over between chromosomes heterozygous for small subterminal inversions might well be producing at least some of the micronuclei associated with spontaneous sectors in T. clone 02 (79, 90) and T. hirsuticaulis (Table 17). That spontaneous sectors are indeed largely the result of mitotic exchange will be shown later.

**Pollen abortion measured by failure to stain with I_2KI (58).

allele, as well as that part of the Tradescantia chromosome indicated to carry small, inverted segments (136). Cells of the blue component of the r/b twin are also homozygous, but for the distal segment of the other, D^+, chromosome tip. As the two kinds of red cells do not differ significantly in mitotic fitness (Table 11), the more proximal segment of the chromosome is indicated as having relatively little, or only secondarily, important effect on mitotic fitness. On the other hand, entire blue hairs, $D^+D^-E^-E^-$, are seen to be significantly shorter, by 2.68 cells, than non-mutant hairs, $D^+D^-E^+E^-$, (Table 14), although they are significantly longer, 1.08 cells, than entire red hairs, $D^-D^-E^+E^-$. This seems to indicate that the proximal segment of the chromosome effects mitotic fitness almost as much as the distal segment.

Such contra-indications lead to no firm conclusions. A complete understanding of the various sector sizes must await further observations and experiments on both somatic sectors and progeny of this purple T. hirsuticaulis. As was mentioned in Section I, in contrast to the breeding results with T. clone 02 (79,82), at present, the red progeny plants of T. hirsuticaulis appear to be diplontically selected for.

Mechanisms of sector production.

The discovery of "twin spots" in Tradescantia hirsuticaulis immediately suggests mitotic crossing-over as a mechanism of sectoring

in Tradescantia. Mitotic crossing-over was advanced as a theoretical possibility by Mericle and Mericle (79). Until recently, no one could disagree with Sinnott and Dunn (125) who, after describing somatic crossing-over in Drosophila, went on to say,

> "Somatic crossing over has not been demonstrated in other organisms, although in maize Jones (61) has found neighboring spots of different genetic constitution in the aleurone and endosperm. Some of these appear to be due to exchanges between homologous chromosomes; but whether these occur by crossing over or by some other mechanism is not certain."

Recent reports of genetic and cytogenetic characterization of somatic sectors in cotton (15) and tobacco (27) leave no doubt but that somatic exchange between homeologues and homologues, respectively, does occur in higher plants. However, an examination of 12 pairs of twin sectors in tobacco showed one to have been produced as the result of mitotic non-disjunction (27); there is evidence that in soybean too, mitotic non-disjunction may be the cause of at least some of the somatic sectors (144). Similarly, mitotic crossing-over cannot be the only mechanism of sector production in Tradescantia. Mericle and Mericle demonstrated that at relatively "high" levels of radiation (60 R), deletion (evidenced by the presence of Feulgen-positive fragments or micronuclei), is an important mechanism of sector production in T. clone 02 (79,82). They also point out the converse, that many 60 R and most spontaneous and lower radiation level sectors were not so indicated to be results of deletion. Their suggestion that the deletions are terminal is supported by Swanson's observation of 886

terminal versus no interstitial deletions after ultraviolet and X-ray treatment of mitotic Tradescantia chromosomes (137).

In T. hirsuticaulis, at 60 R, some 58% of the red-only sectors have an associated micronucleus, suggesting that these sectors were produced by deletion (Table 16). However the twin spots in T. hirsuticaulis, even at 60 R, are not found associated with micronuclei (Table 16). The two classes of sectors are statistically distinct, the calculated value of X^2, 1 d.f. = 3.9, $p < 0.05$. This is in agreement with Auerbach (12), who, in a general consideration of the mechanism of somatic sectoring in Drosophila, maintains that twin spots cannot arise from simple deletion. I have previously indicated that each twin spot in T. hirsuticaulis is probably the result of a single initiating event; this is also in accord with her observation that, for appropriate genotypes, mitotic crossing-over is the only mechanism that will produce a twin spot as the result of a single event.

A consideration of the association of micronuclei with spont neous (0 R) sectors leads to similar conclusions. Mericle et al. (90) report that some 10% of the spontaneous sectors in T. clone 02 under typical growth conditions are associated with micronuclei. Initially it appeared as if the red-only sectors in T. hirsuticaulis, in spite of the similarity to sectors at 60 R in T. clone 02, were much different in their association with micronuclei at 0 R (Table 17). The data, however, can be readily separated into two groups: data from flowers with

Table 16. Micronuclei association in 60 R-response hairs.

Sector Class	With[a]	Without	Total
red-only	23	17	40
red/blue twins	0	29	29
blue-only	0	2	2

[a]Feulgen-positive micronucleus (putative chromosome fragment) present in the mutant, or, for ro and bo sectors, immediately adjacent "sister" cells.

Table 17. Micronuclei association in O.R (Spontaneous) hairs.

Sector Class		Incidence of mutations in 6 stamens of flower								
		1	2	sub	3	4	5	7	sub	TOTAL
red-only	with[a]	1	0	1	1	1	1	0	3	4
	without	5	3	8	1	1	0	0	2	10
				11%					60%	29%
red/blue twins	with	0	0	0	0	0	0	0	0	0
	without	2	6	8	1	1	0	1	3	11
				0%					0%	0%
blue-only	with	0	0	0	0	0	0	0	0	0
	without	0	1	1	0	0	0	1	1	2
				0%					0%	0%

calculated homogeneity X^2, red-only sectors, 1 d.f., 24.67, $p < 0.001$

[a]Feulgen positive micronucleus (putative chromosome fragment) present in the mutant, or, for ro and bo sectors, immediately adjacent "sister" cells.

a typical spontaneous mutation rate, 1 or 2 mutant stamen hair sectors per flower, and data from flowers with an atypical high, mutation rate, 3 or more stamen hair sectors per flower. The latter is an incidence of sectors comparable to that in flowers exposed to 60 R or more of radiation, (Figure 16, Table 12). The micronuclei associations with red-only sectors in the two groups, in spite of the small number of data entries, are statistically distinct, the calculated value of X^2, 1 d.f., = 24.67, $p < 0.001$. The ro sectors from the highly sectored flowers were some 60% associated with micronuclei (Table 17), a figure of the same magnitude as that for association in 60 R-response sectors (Table 16). The ro sectors from flowers with an incidence of sectors typical of spontaneous material showed an association with micronuclei of some 11% (Table 17), a value similar to that mentioned above for spontaneous sectors in T. clone 02 (90).

The twin spots, by contrast, are not found to be associated with micronuclei, even in flowers with a high incidence of sectors atypical of spontaneous material (Table 17). This reinforces the conclusions reached above on the basis of their unassociation with micronuclei in 60 R-response material: twin spots are only produced by some non-deletory mechanism.

The use of association with micronuclei as a measure of deletion is a procedure fraught with difficulties (79). It has just recently become clear that the use of minimally-sized sectors leads to lower, not higher, values for micronuclei association than the use of 2 or 4

celled sectors (85); however, as only relative comparisons were to be made, as the data previously published by Mericle and Mericle (79) consisted mostly of single-celled sectors, and, most importantly, as twin sectors containing exactly 2 red and 2 blue cells are so rare as to be virtually non-existent, I chose to use minimally sized (1-celled, or in the case of twins, "1+1"-celled) sectors.

The frequency of micronuclei itself is an inefficient indicator of the absolute frequency of breakage (42,129), but, as long as comparisons are kept to the relative importance of deletion, micronuclei can be a valid measure (79). It is now becoming increasingly apparent that micronuclei can also be produced in ways other than gross deletion (57,89). As this latter phenomenon in Tradescantia stamen hairs seems to be mostly related to environmental differences (89,90), relative comparisons of material in the same environment, particularly of different classes of sectors in the same flowers, do not appear to be contraindicted.

More evidence of the differential behavior of the various classes of sectors can be seen in a "dose"-response curve (Figure 17). While the r/b twins and bo sectors increase linearly with radiation exposure, the ro sectors do not and are, instead, markedly "elevated" at 60 R. This suggests the existence of an extra mechanism at 60 R, producing ro sectors, in addition to a mechanism which, at 0 R or 60 R, produces all three classes of sectors. I have already mentioned that 58% of the ro sectors produced in response to 60 R are associated with

Figure 17. "Dose"-response curve.

Frequency of mutant sectors in stamen hairs of purple-flowered T. hirsuticaulis during "response period" following various ^{60}Co gamma radiation exposures.

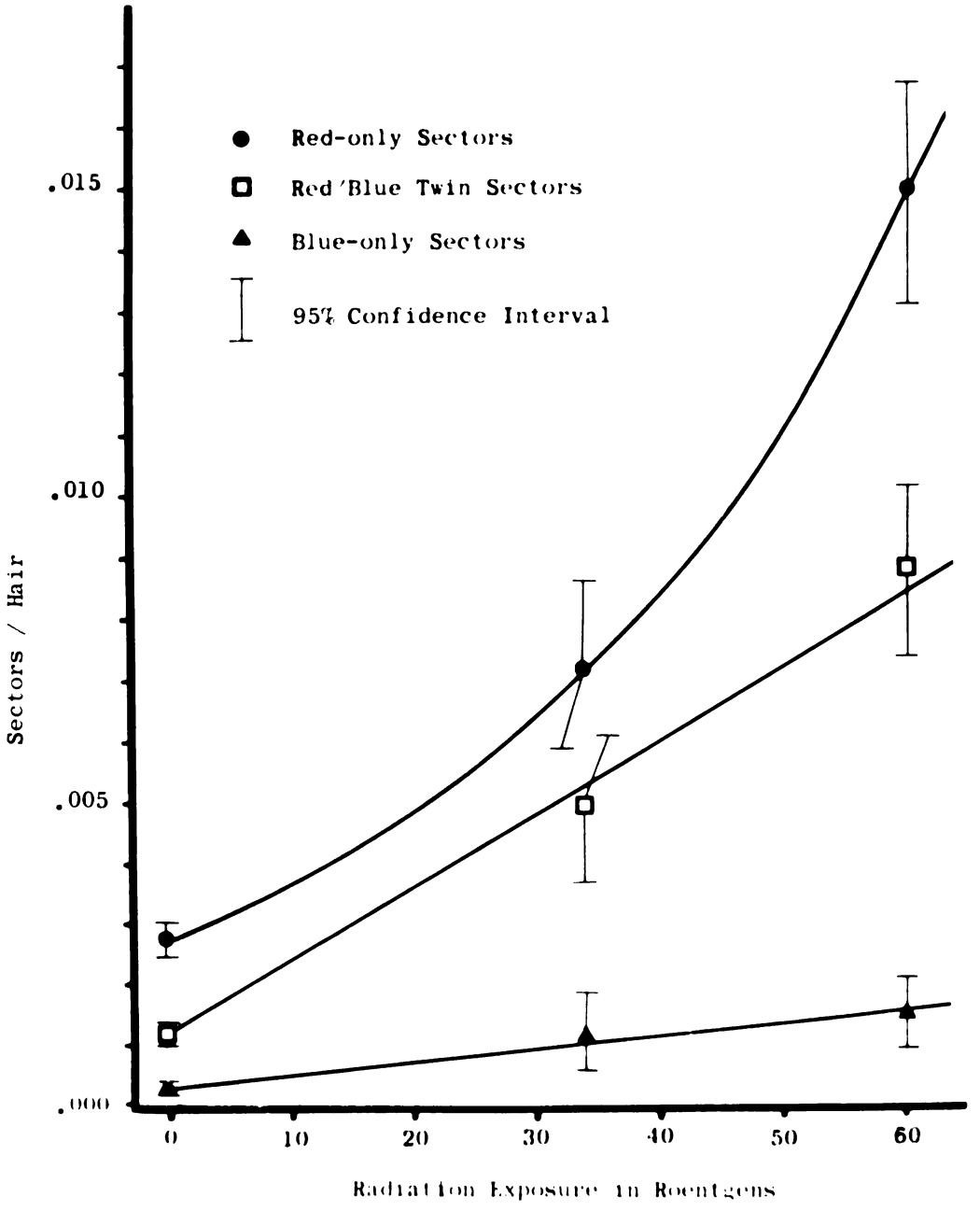

Red-only Sectors
Red/Blue Twin Sectors
Blue-only Sectors
95% Confidence Interval
.015
.010
.005
.000
Sectors / Hair
0
10
20
30
40
50
60
Radiation Exposure in Roentgens

Figure 17

micronuclei (Table 16), indicating that, as in T. clone 02, the extra mechanism must be deletion. The other mechanism(s) then, non-deletory and responsible for most, if not all, of the spontaneous and the majority (63%) of even the 60 R sectors, shows a linear response with the radiation doses used. Mitotic crossing-over in Drosophila, a reciprocal exchange process, also exhibits such a linear response to radiation exposure (16,43,68) (although the exposures employed are much higher than those used for Tradescantia in these experiments; this difference is discussed in Section III).

The assumption that mitotic crossing-over is the mechanism of fragment-unassociated sector production leads to several genetic predictions. Since the r/b twin spot is shown to be the result of a single initiating crossover event, the D and E loci must be on the same chromosome arm. Furthermore, the dominant alleles must be in a repulsed (trans) configuration, since this arrangement yields a r/b twin spot as the result of an appropriate crossover, while a coupled (cis) configuration yields only ro or bo sectors (barring the occurrence of a 4-strand triple crossover). Further, the D locus is indicated to be the more distal since ro sectors are the more frequent class among spontaneous "mutations" (61) (Table 18). This positioning of the D locus* is supported by the finding of fragment associated ro

*Testing the linkage arrangment of alleles at the D and E loci by breeding experiments is hampered by the self-incompatibility present in this Tradescantia. To date only heterozygosity at both loci has been confirmed. See Section I.

Table 18. Frequency of somatic sectors in stamen hairs of *T. hirsuticaulis*.

Treatment	Hairs		Number of Sectors			Frequency per Hair		
			ro	r/b	bo	ro	r/b	bo
0 R	154,135	observed	430	183	47	.002790	.001187	.000305
		corrected[a]	430.1	182.9	47.1	.002790	.001187	.000306
34 R	13,972	observed	100	69	16	.007157	.004938	.001145
		corrected	100.1	69.0	16.1	.007164	.004938	.001153
60 R	18,297	observed	273	161	28	.014920	.008799	.001530
		corrected	273.4	160.6	28.4	.014942	.008777	.001552

[a]Correction for chance coincident occurrence of a red-only and a blue-only event during the ontogeny of a hair. See text for equations.

sectors at 60 R in large numbers, as one would expect via the deletion of a more terminal locus (Table 16). At this level of radiation, not only are bo sectors seemingly not produced in large numbers (Table 18), but those few bo sectors scored were found to be unassociated with fragments (Table 16), as was the one sector found in spontaneous material with an atypically high incidence of sectors (Table 17).

That portion of somatic sectoring in T. hirsuticaulis which is unassociated with fragments is completely self-consistent with mitotic crossing-over as the invoking mechanism; all the classes of sectors are predicted, and in the proper order of frequency. A genotype of D^+E^-/D^-E^+ would produce all three types of sectors* as the consequences of single or double crossover events (Figure 18). The more frequent classes would result from single exchanges (plus a possible small contribution to one class from putative three strand double exchange), the least frequent, from double exchange. Fifty percent of the actual exchanges are recovered as mutant sectors (131), the three-strand doubles being exceptions, recovered 50% as one class, 50% as another class of sector. Accordingly, ro sectors are the products of single exchange in region I (Figure 18), r/b twins, a single exchange in region II, and bo sectors, a double exchange involving both regions I and II. Three strand double exchanges would also produce a few ro

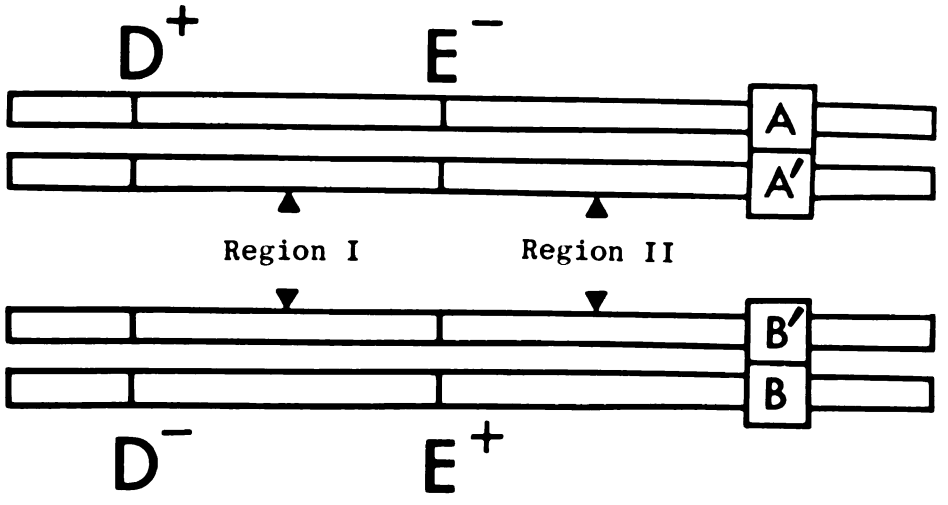
*Red-only and blue-only sectors may really be red/blue-purple and blue/red-purple twin spots, respectively. Microspectrophotometric experiments are being planned to test this. See Section I for a discussion of the possible color of $D^+D^+E^+E^-$ and $D^+D^-E^+E^+$ genotypes.

Figure 18. Somatic exchange and its results.

a) Scheme of D^+E^-/D^-E^+ chromosome pair showing regions of possible crossing-over.

b) Genotype and phenotype of daughter cells produced following crossover events and centromere assortment.

a)



b)

Daughter Cells After Mitosis With Crossover Event In				Region
Centromere Assortment				
A/B' & A'/B		or A/B & A'/B'		
D^+E^-/D^+E^+ & purple	D^-E^-/D^-E^+ red	D^+E^-/D^-E^+ & purple	D^+E^+/D^-E^- purple	I
D^+E^-/D^+E^- & blue	D^-E^+/D^-E^+ red	D^+E^-/D^-E^+ & purple	D^+E^-/D^-E^+ purple	II
D^+E^-/D^-E^- & blue	D^-E^+/D^+E^+ purple	D^+E^-/D^-E^+ & purple	D^+E^+/D^-E^- purple	I & II

Figure 18

sectors.

The sectoring data of T. hirsuticaulis are expressed as "crossover events" in Table 19. The "coefficients of coincidence" calculated from these data are within the range encountered for mitotic recombination in Drosophila: less than a value of 0.63 calculated from the data of Auerbach (12) on chemically induced somatic segregation in the eye, and very similar to the values of 0.35 to 0.23 from the data of Table 3 of Garcia-Bellido (43) on radiation-enhanced sectoring of the abdomen. A more adequate method of treating the data is described in Section III of this work.

Since those somatic sectors in the stamen hairs of T. hirsuticaulis which are unassociated with fragments behave exactly as if they were the result of mitotic crossing-over, I propose that these sectors are predominantly the result of mitotic crossing-over. Presumably, those similar somatic sectors in other floral parts and other Tradescantia are as well.

Table 19. Sectors presented as representing crossover events.

Treatment	Crossover Events in Region					
	I only		II only		I & II	
	#	%	#	%	#	%
0 R	2 x 430.1	65.2	2 x 182.9	27.7	2 x 47.1	7.1
34 R	2 x 100.1	54.0	2 x 69.0	37.3	2 x 16.1	8.7
60 R	2 x 116.2[a]	38.1	2 x 160.6	52.6	2 x 28.4	9.3

	Total Events		Expected I & II	"Coincidence"
	#	%	%	
0 R	2 x 660.1	100.0	25.2	0.28
34 R	2 x 185.2	100.0	28.8	0.30
60 R	2 x 305.2	100.0	29.3	0.32

[a] Red-only sectors without associated micronuclei

SECTION III

Towards a theoretically sound method of treating data from mitotic exchange

Parallels between facultative apomicts and somatic sectoring in Tradescantia.

Mendel described the characteristics of a good experimental organism for genetic studies with three rules (73), and then promptly ignored them when he began to study Hieracium. His experiments with Hieracium "hybrids" (74) led to no firm conclusions other than that these hybrids might well posess some "special condition" that made them deviate so from the orderly genetic laws he had described as the result of experiments in the genus Pisum (73).

And indeed, they are "special", for Hieracium sp. are now known to be notorious apomicts (17,118, cited in 72; 59). While apomicts have some interesting genetic properties and uses which have been described (e.g., 9,10,95), they also present knotty problems for the geneticist who desires to make crosses and observe segregation of genes (discovering and mapping genes being endemic to geneticists).

Of course, obligate apomicts can never be hybridized by ordinary sexual means. Facultative apomicts offer more hope, but still present a host of insurmountable problems. One can construct an F_1 by having

a marker in each parent in the cross and then selecting only those progeny exhibiting both markers. By counting the other progeny as well, one can even arrive at an estimate of the percent apomixis. However, no easy genetic trick is available for getting an F_2 which does not include progeny which resulted from an apomictic process. Doing test-crosses will not solve the problem either. There will always be excesses scored in certain classes due to the apomictic progeny being assigned into those classes. A plant with somatic apospory, for example, will show an excess of non-crossover strands. This will result in a distortion in the calculation of map distances from the data.

As can be seen in Figure 19, the effect is that the apparent map distance is less than the true map distance. The effect becomes more pronounced as the percentage of apomixis increases, and, as an upper limit, an obligate apomict will show absolute linkage of all loci. This relationship between apparent map distance, true map distance and the apomictic fraction, may be described mathematically* as:

$$MD_a = MD_t\,(1-A)^{\frac{1}{2}} \qquad (I)$$

where MD_a is the apparent map distance, MD_t is the true map distance, and A is the fraction of apomictic progeny.

---

* Consider a facultative apomict (somatic apospory) of genotype Ab/aB. Let A = fraction asexual progeny and (1-A) = fraction sexual progeny. Of N seeds, NA, the asexual seed, will be Ab/aB, N(1-A), the sexual seed, will be distributed among 9 genotypic classes, AABB to aabb. The double recessives are most unambiguously identified, and can be used to calculate the map distance, as $\frac{\#aabb}{N(1-A)} = \left(\frac{MD_t}{2}\right)^2$. However, if (1-A) is not known, the calculation is $\frac{\#aabb}{N} = \left(\frac{MD_a}{2}\right)^2$. Combining these two expressions gives equation I.

Figure 19. Variation in apparent map distance with apomixis.

The two lines are smooth curves connecting map distances calculated for genetic lengths of 50 or 25 centimorgans at various percents apomixis. At 0% apomixis, apparent map distance equals true map distance.

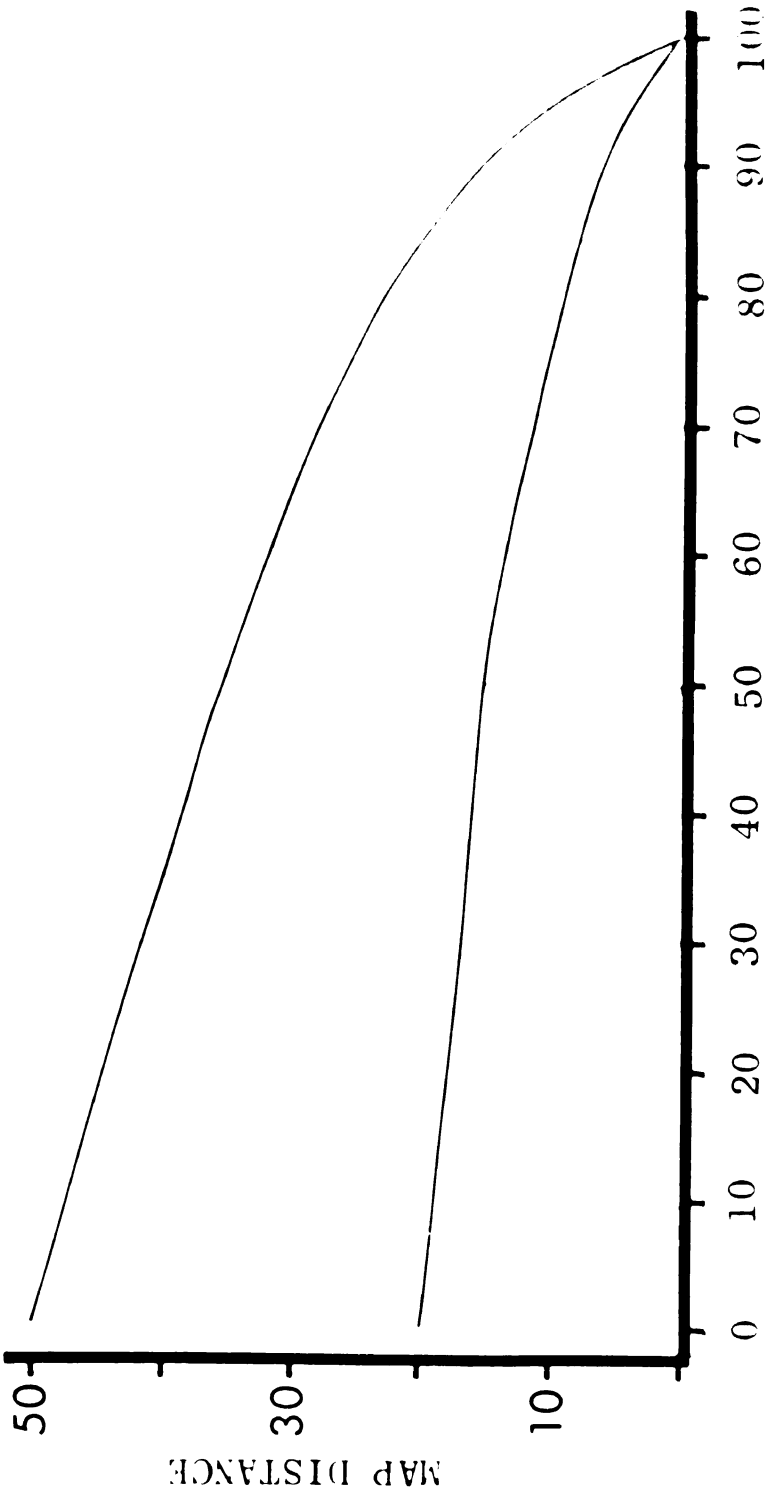


Figure 19

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tance.

Apomixis will produce effects on other genetic calculations as well; the coefficient of coincidence (the ratio of observed to expected double crossovers), a measure of chromosome interference, is one such affected genetic parameter. An increasing degree of apomixis results in an increasingly elevated estimate of chromosome interference (Figure 20); mathematically* this is:

$$c.c._a = \frac{1}{(1-A)}\, c.c._t \qquad (II)$$

where $c.c._t$ is the true coincidence, $c.c._a$ is the apparent coincidence, and A is the apomictic fraction. Indeed, for any given amount of positive chromosome interference, there is an apomictic fraction above which it will appear to be negative chromosome interference! This phenomenon, too, is extreme at high degrees of apomixis, an infinite amount of negative chromosome interference being approached as the apomictic fraction approaches unity.

Reports of negative chromosome interference are not frequent in the genetic literature. Negative interference has been found in

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* Considering N strands, let NA be from the asexual progeny, N(1-A) be from the sexual progeny of a faculatative apomict. Coincidence is given by $c.c._t = \dfrac{\frac{\#dco}{N(1-A)}}{\frac{\#\,coI}{N(1-A)}\,\frac{\#\,coII}{N(1-A)}}$. If (1-A) is not known, however, apparent coincidence is calculated as $c.c._a = \dfrac{\frac{\#dco}{N}}{\frac{\#\,coI}{N}\,\frac{\#\,coII}{N}}$. Combining these two expressions gives equation II.

Figure 20. Variation in apparent coefficient of coincidence with apomixis.

The dotted line represents no chromosome interference, below it is the region of positive (+) chromosome interference, above it, of negative (-) chromosome interference.

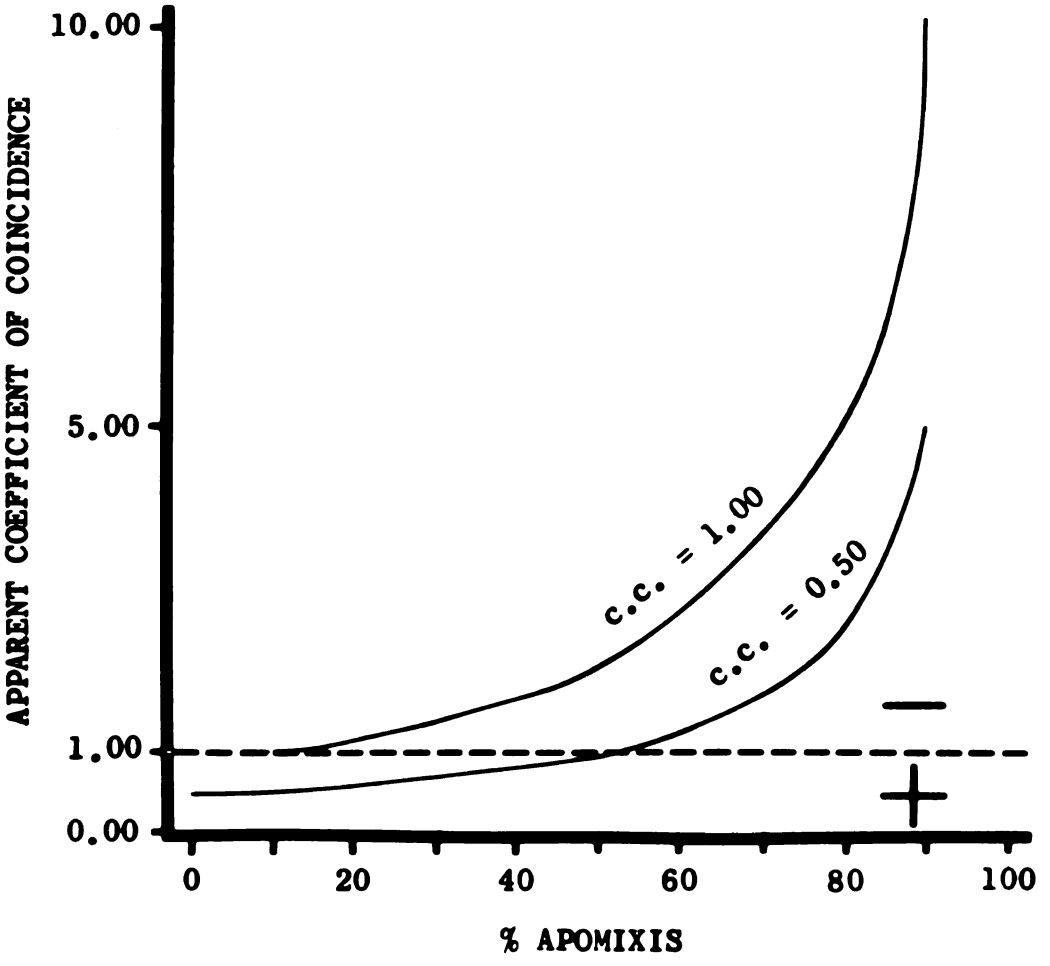
10.00
5.00
1.00
0.00
APPARENT COEFFICIENT OF COINCIDENCE
c.c. = 1.00
c.c. = 0.50
0
20
40
60
80
100
% APOMIXIS

Figure 20

Aspergillis (111), in a study of mitotic recombination, in phage (28), as well as in meiotic recombination in fungi (several references in ref. 41). It has also been reported for somatic crossing-over in Drosophila; e.g., Garcia-Bellido (43) found a 10- to 20-fold excess of double crossovers in his material. He, however, seems content to say "the appearance of a high frequency of spots, due to double CO (crossover) even in both irradiated and control experiments remains unexplained."

Similar to Garcia-Bellido's results, I find an excess of double crossover sectors in the spontaneous somatic sectoring of Tradescantia hirsuticaulis if the calculation is done in the traditional way, i.e., basing the frequencies on a population of all the cells. I believe this to be a result of the inadequacy of that traditional way of treating sectoring data. Indeed, I suggest there are strong parallels between somatic sectoring and the case of the facultative apomict described above: that this apparent excess of double crossovers is spurious, and that it may be understood as arising out of the same conceptual roots as the negative chromosome interference in apomicts. This approach not only provides an insight into the processes involved in somatic sectoring, but also permits the development of a method which, I think, more adequately treats sectoring data, therefore resulting in the calculation of more valid mitotic maps, as well as the introduction of new, not heretofore defined, somatic genetic parameters.

Theory of somatic exchange.

Traditionally, somatic cells have been treated as if they were all equally amenable to somatic exchange; I would suggest rather that there exist two subpopulations of cells: one whose chromosomes will undergo typical mitosis, and the other whose chromosomes are amenable to somatic exchange.

By "amenable to somatic exchange" I do not mean to imply that somatic chromosomes must pair intimately, as at meiosis; neither, do I intend to exclude this possibility. Just as intimate pairing does not necessarily mean exchange must or will occur, effective intimate pairing over long cytological distances may not be necessary for exchange to occur. In Drosophila, pairing in somatic cells is known to occur at certain stages of ontogeny; there are even reports of chiasma-like structures in these mitoses (31,64). There is a suggestion that Kitani (66) observed somatic pairing in two species of Tradescantia; persons in this laboratory (75) have remarked that they feel there is a tendency for the mitotic chromosomes of Tradescantia to lie with homologues closer than one would expect if they were just randomly distributed in the cell. My own observations leave me with the same feeling: metaphases in root tips of T. clone 02 and a colchicine-produced tetraploid tissue of that clone show a tendency for the chromosomes to lie in "twos" or even "fours" (see e.g., Figure 21). The metaphase of a 4N cell in the center of the filament of a 2N/4N chimeric T. clone 02, pretreated for some 11 hours with 1% colchicine, shows this tendency

Figure 21. Cytological indications of somatic pairing.

a) diploid, T. clone 02 root tip, 2032x, final magnification

b) interpretive drawing of above

c) tetraploid, T. clone 02 root tip, 2672x, final magnification

d) interpretive drawing of above

e) tetraploid, T. clone 02 filament, 3100x final magnification

f) interpretive drawing of above

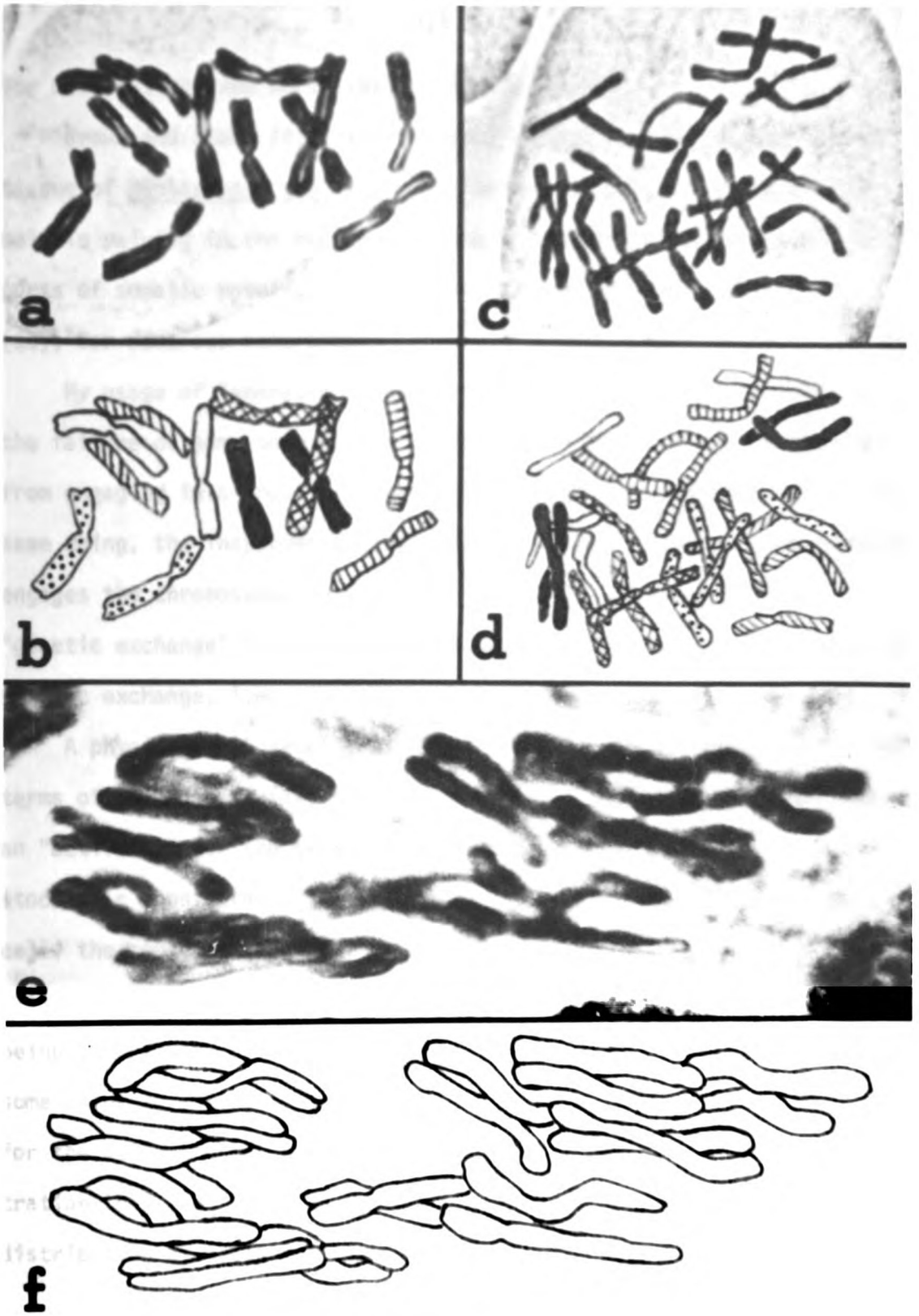

Figure 21

for association even more clearly (Figure 21e,f).

Brown and Stack (25) have reported somatic pairing in the floral tissue of Haplopappus gracilis as extensive as the considerable premeiotic pairing in the microspore mother cell divisions; there are reports of somatic pairing in many other plants (see Brown and Stack, (25), for numerous references).

My usage of "amenable to somatic exchange" is best understood as the failure of some gene active in mitosis which keeps the chromosomes from engaging in a process of genetic exchange (or, what amounts to the same thing, the inappropriate calling into play of a meiotic gene which engages the chromosomes in a process of genetic exchange). This "genetic exchange" is most conveniently thought of in the same terms as meiotic exchange, i.e., including pairing.

A physiological genetic basis for all this can be described in the terms of Goldschmidt (44) and Rendel (113). In a cell, there will be an "activator" for the gene. Due to metabolic fluxes, and just the stochastic considerations of compartmentalization and of mixing in a cell, the concentration of this substance in the immediate, "sensitive", vicinity of the gene on the chromosome will vary from cell to cell, being distributed around a mean value (Figure 22a). There will be some value at which the gene is no longer kept active, its affinity for the activator not being strong enough to give binding at a concentration that low. This is the threshold value, and it divides the distribution of cells into two subpopulations: (a) cells with an

Figure 22. Canalization of the mitotic gene which keeps chromosomes from pairing.

a) Typical distribution of somatic cells, showing the normal distribution around a mean. $\bar{M}_0$, of gene activator.

b) Distribution of somatic cells after the imposition of some stress which changes the mean to $\bar{M}'$.

c) Distribution of somatic cells after the imposition of some greater stress which changes the mean to $\bar{M}''$.

M_t is a threshold value of activator, beyond which, the gene fails to function.

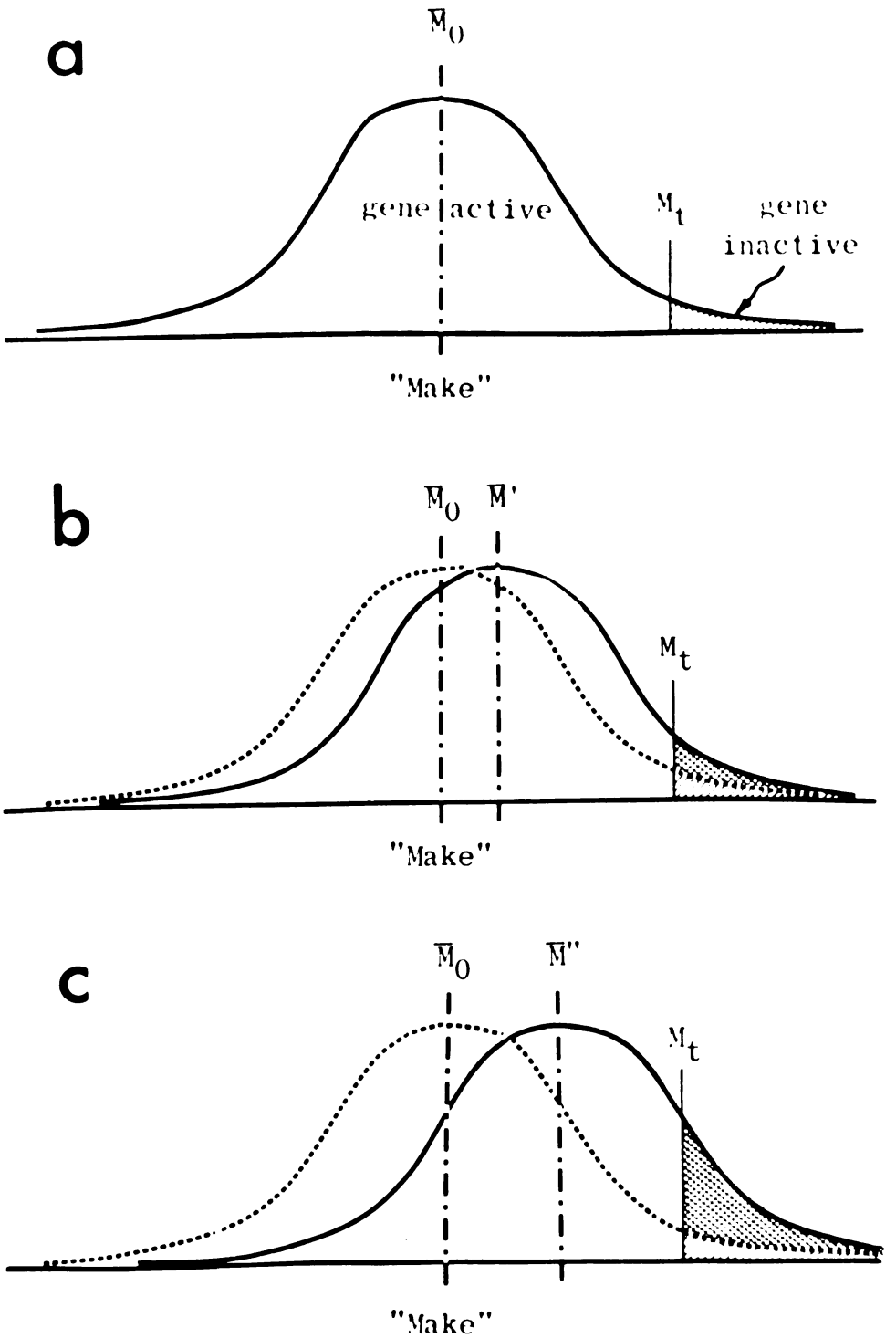
a
$\bar{M}_0$
gene active
M_t
gene inactive
"Make"
b
$\bar{M}_0$
$\bar{M}'$
M_t
"Make"
c
$\bar{M}_0$
$\bar{M}''$
M_t
"Make"

Figure 22

"active gene", and therefore undergoing a typical mitosis, and (b), cells with an "inactive gene", and therefore undergoing an atypical mitosis during which the chromosomes are amenable to exchange (Figure 22a). The gradient of somatic pairing observed by Brown and Stack (25) might well be interpreted on a similar basis: a change in the mean concentration of this "activator", a quantity Rendel (113) terms "make", M, would result in an ever increasing fraction of the cells being amenable to exchange (Figure 22,a,b,c).

Similarly, an increased rate of somatic sectoring in Tradescantia could be thought of as occurring through an environmental perturbation of cellular metabolism resulting in a small shift of $\overline{M}$, the mean value of "make".

Selection pressure operates to increase the penetrance of the gene at the cellular level, that is to maximize the difference between the mean value of "make" and the threshold value. As this distance increases, however, a larger and larger fraction of the cells in a tissue tend to have the gene active. This tends to make the gross differences from a tissue in which all the cells have the gene active (a theoretical impossibility, given the shape of the normal distribution) negligible, and undetectable at the whole organism level where selection must act. Thus an equilibrium distance will be reached where $\overline{M}$, the mean "make", is sufficiently separated from the threshold value so that only a small, negligible fraction of the cells will not have the gene functioning. If however, this is a tissue where each and every

individual cell may be examined, an investigator may observe this small fraction of the cells. Such is the case with the cells of the stamen hairs in Tradescantia.*

Comparable to the gradient of somatic pairing observed by Brown and Stack (25), there have been shown to be variations in the frequency of various kinds of somatic sectoring with both position and developmental stage. In Tradescantia clone 02, with spontaneous sectors, the upper, middle, and lower thirds of the stamens have characteristically different mutation rates (55). Sand (120) reports different mutation rates at various flower nodes in Nicotiana; Vig and Paddock (145) report statistically distinct sectoring rates in the ontogenetic series of leaves of young soybean plants. Although not suggesting any mechanism for the sectoring, Demerec (35,36) describes somatically sectoring stocks of Delphinium whose sectoring is limited to certain stages in ontogeny, some whose permissive time is short, others, long.

A canalized-gene model for somatic sectoring can also account for the observed genetic variation of spontaneous mutation rate in Tradescantia (79). A series of structurally mutant alleles are theoretically possible which would differ in their association constant for the activator molecule. Thus, although the mean physiologic

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* The hypothesis might be advanced, then, that somatic mutations are most simply understood as the failure of a "canalized" pigmentation gene to operate. While this possibility may not be discounted in T. clone 02, such a mechanism could not account for the twin spots in T. hirsuticaulis, which have been shown above (Section II) and previously (30) to be the result of one, single event, not the two coincident events this hypothesis would require.

concentration of the activator could be the same from stock to stock, the threshold value, a property intrinsic to the allele and a function of the association constant, might vary, permitting large differences in spontaneous sectoring rate between stocks. Recently, Nauman et al. (99) have suggested that differences in specific activity of DNA-repair enzymes might account for the different spontaneous "mutation" rates of various Tradescantia stocks. Perhaps a DNA-repair enzyme is the product of the canalized gene; repair-deficient strains are well known to exhibit increased frequencies of mitotic crossing-over (56,123). Additionally, "modifiers", genes distinct from the canalized gene, could well exist. Minute genes, for example, are well known for their action of enhancing the frequency of somatic sectors (mosaics) in Drosophila (63,131). These modifiers would function by effecting slight alterations in the cellular metabolism which would result in small shifts of $\overline{M}$, the mean value of "make" (Figure 23a). Different stocks with the same allele of the canalized gene would show differences in spontaneous sectoring rate due to small differences in $\overline{M}$, the mean "make". Alternatively, these modifiers might well broaden the distribution of cells around the same mean value of "make". This also would result in a higher fraction of the population beyond the threshold value (Figure 22b).

Differences from the spontaneous sectoring rate caused by externally applied forces can also be explained with this model, as was alluded to earlier. Obviously, increases in the sectoring rate

Figure 23. Differences in spontaneous mutation rate explained as the action of modifiers on cellular physiology.

a) The modifiers shift the population mean "Make".

b) The modifiers broaden the distribution of somatic cells around the same value of mean "Make".

$\overline{M}_0$ is the original mean "Make", M_t, the threshold value, $\overline{M}'$, the value of mean "Make" in a stock with modifiers, δ, the average effect of the modifiers.

ained as the

cells and

lue, $\bar{M}'$, the

erage effect

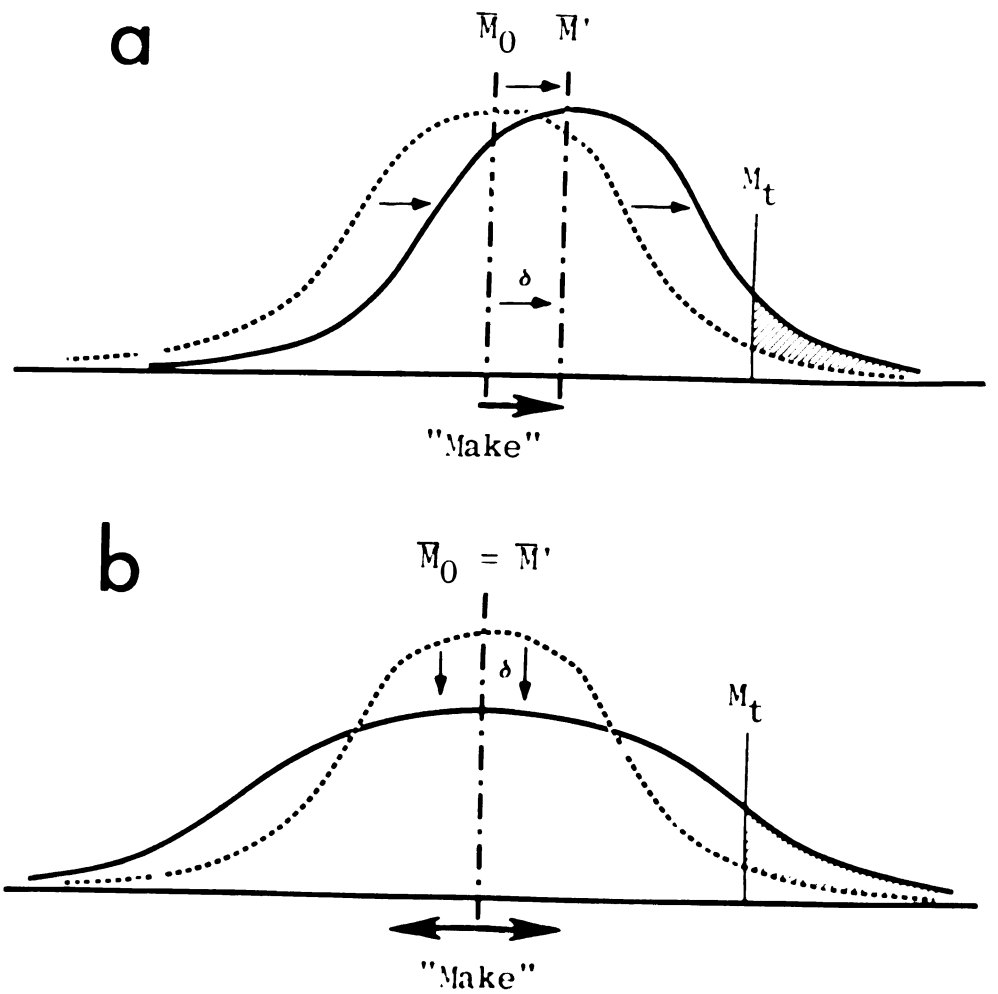


Figure 23

Figure 23. Differences in spontaneous mutation rate explained as the action of modifiers on cellular physiology.

a) The modifiers shift the population mean "Make".

b) The modifiers broaden the distribution of somatic cells around the same value of mean "Make".

$\bar{M}_0$ is the original mean "Make", M_t, the threshold value, $\bar{M}'$, the value of mean "Make" in a stock with modifiers, δ, the average effect of the modifiers.

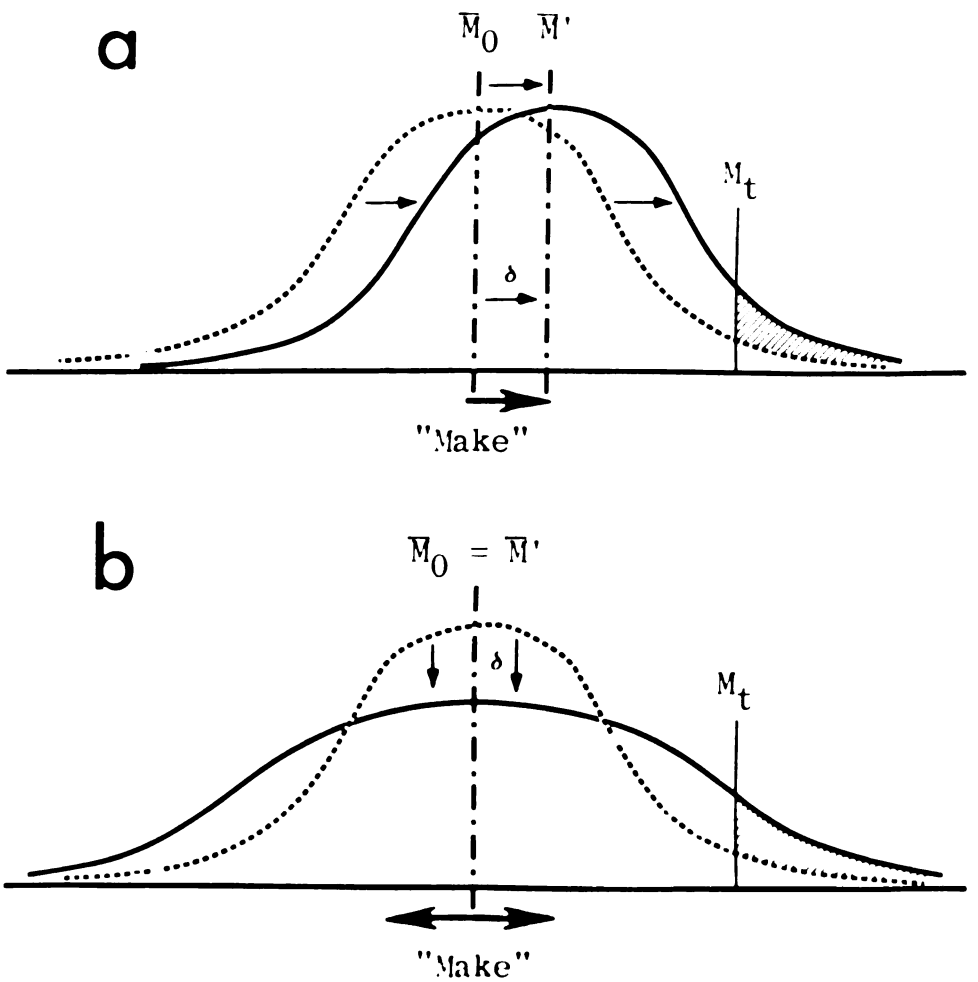


Figure 23

after exposure to large amounts of mutagenic agents will occur through deletion (30,79) and other mechanisms, but some agents might well exert at least part of their effect by flattening the distribution of gene activator around the mean (Figure 23b) or by causing a small shift in mean "make" due to an imposed metabolic stress (Figure 23a).

Quantitation of theory

A gene-canalization model for somatic sectoring permits the development of a method to adequately treat the sectoring data. The subpopulation with typical mitoses corresponds to the apomictic progeny in the consideration of facultative apomicts above, thus it will be referred to as the "apomictoid" population. The other subpopulation, amenable to genetic exchange, is termed the "mictoid" population.

The relationship between coincidence and apomictic fraction partially shown in Figure 20 is replotted in Figure 24 in a form more convenient for this analysis and with the abscissa changed to read "Fraction Apomictoid Cells." It can be seen that at high apomictoid fractions, the curves for various "true coincidence" values become very close. Thus, determining the apparent coincidence value, if it is sufficiently large in magnitude, will permit a rough estimate of the apomictic or the mictic fraction. For the T. hirsuticaulis spontaneous sectoring data (values in Table 18 converted to a per cell basis by multiplication by 16.88), the apparent coincidence, calculated in the traditional way, i.e., 2(frequency bo)/(frequency ro + bo)

Figure 24. Apparent coefficient of coincidence versus fraction of apomictoid cells.

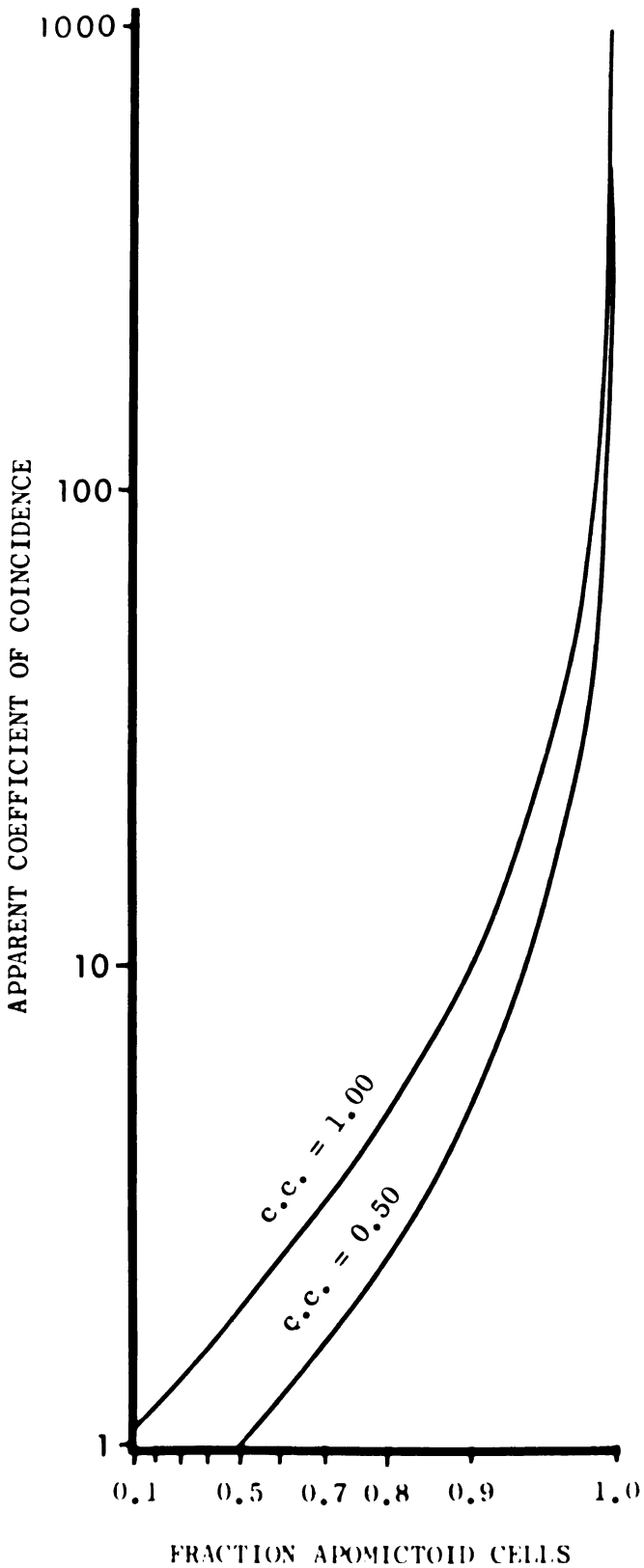

1000
100
10
1
APPARENT COEFFICIENT OF COINCIDENCE
c.c. = 1.00
c.c. = 0.50
0.1
0.5
0.7
0.8
0.9
1.0
FRACTION APOMICTOID CELLS

Figure 24

(frequency r/b + bo), is 2200: thus the mictoid fraction is indicated to be very small.

Calculation of the coincidence value in the traditional way is, I believe, genetically inaccurate. While previous investigators have to my knowledge treated mitotic-sectoring data as if it were meiotic-strand data (e.g. 43,131), it is clear to me that in reality it is "half-tetrad" data, (Figure 25), and so must be handled differently. The difference between meiotic exchange data and mitotic exchange data is illustrated in Figure 26.

Expanding on previous work by himself (9,11,95) and others (14,47, 104,117), Asher (10) has developed a mapping function which gives the frequencies of the seven tetrads formed in a trans-double heterozygote as a function of x_1 and x_2, the corrected map distances from the centromere to the proximal locus, and from the proximal to the distal locus, respectively. This mapping function assumes that the chromosome is "equally flexible and breakable at all points" (47) and that no chromatid interference occurs (14). There is some evidence that there is, perhaps, no chromatid interference during somatic crossing-over in Drosophila (122). While chromosomes are known to be heterogeneous in structure and propensity for exchange, similar mapping functions have been used with good fits to meiotic data from Drosophila, Neurospora, mouse, and several plant species (14).

From the two-locus mapping functions, a set of equations describing the probability of each sector type can be obtained: each mitotic tetrad will become one of two possible "half-tetrads" (Figure 25) with

Figure 25. The "half-tetrad" nature of somatic sectors.

While somatic exchange is a meiotic-like process, there is no evidence at present that the centromere disjunction is not typically mitotic. The two daughter cells, then, are analogous to half-tetrads given an organism whose equational division precedes the reductional division.

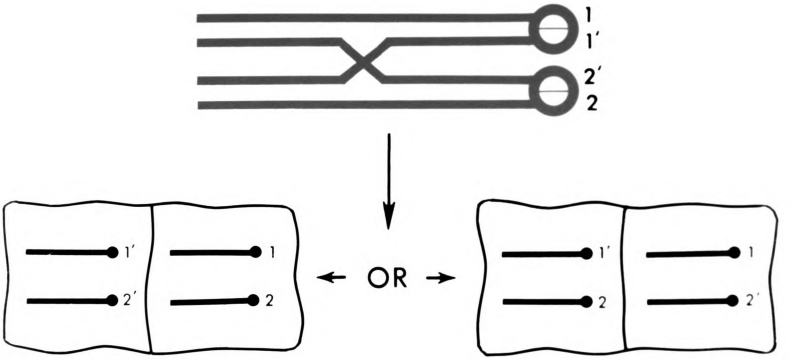
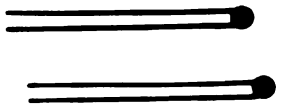
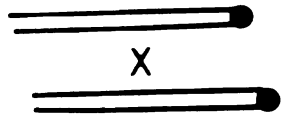
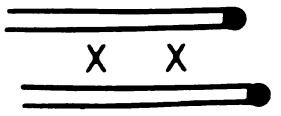


Figure 25

Figure 26. A comparison of meiotic and mitotic exchange.

E_0, E_I, and E_{II} stand for no, one, and two exchanges, respectively; nco, non-crossover; sco, single-crossover; dco, double-crossover. 2s, 3s, and 4s indicate 2-strand, 3-strand and 4-strand, respectively.

Exchange	Meiotic 4 Strands	Mitotic 2 Cells
E_0 	nco	no sectors
E_I 	$\frac{1}{2}$ sco $\frac{1}{2}$ nco	$\frac{1}{2}$ "sco" $\frac{1}{2}$ no
E_{II} 	2s E_{II} ; $\frac{1}{4}$ 3s E_{II} ; $\frac{2}{4}$ 4s E_{II} ; $\frac{1}{4}$	$\frac{1}{2}$ "dco" $\frac{1}{4}$ "sco" $\frac{1}{4}$ no

	nco	sco	dco
2s E_{II} ; $\frac{1}{4}$	2	0	2
3s E_{II} ; $\frac{2}{4}$	1	1,1	1
4s E_{II} ; $\frac{1}{4}$	0	2,2	0

Figure 26

the completion of mitosis. The equations for the probabilities of red-only, red/blue twin and blue-only sectors, P(ro), P(r/b) and P(bo), respectively, as well as for the unobservable purple sectors, P(p), are given by:

$$\begin{aligned} P(ro) &= \tfrac{1}{2} P(2) + \tfrac{1}{2} P(3) + \tfrac{1}{2} P(7) \\ P(r/b) &= \tfrac{1}{2} P(5) \\ P(bo) &= \tfrac{1}{2} P(3) + \tfrac{1}{2} P(3) \\ P(p) &= 1 - P(ro) - P(r/b) - P(bo) \end{aligned} \qquad \text{(III)}$$

where P(2), P(3), P(5), and P(7) are the probabilities of particular types of tetrads in a <u>trans</u>-heterozygote and are given by Asher (10) as:

$$\begin{aligned} P(2) &= 2\,(1 + 2e^{-3x_1})(1 - e^{-3x_2})/\,9 \\ P(3) &= 2\,(1 - e^{-3x_1})(1 - e^{-3x_2})/\,9 \\ P(5) &= (1 - e^{-3x_1})(1 + 3e^{-2x_2} + 2e^{-3x_2})/\,9 \\ P(7) &= (1 - e^{-3x_1})(1 - 3e^{-2x_2} + 2e^{-3x_2})/\,9 \end{aligned} \qquad \text{(IV)}$$

where x_1 and x_2 are the corrected map distances from the centromere to the more proximal locus (E) and from the proximal (E) to the distal locus (D), respectively. (See Figure 18.)

Substitution of equations IV into equations III gives a system of three independent equations in two unknowns, and is best solved by the method of maximum likelihood. Let n_1, n_2, and n_3 be the numbers of red-only, red/blue twin, and blue-only sectors observed, respectively. The likelihood of observing exactly n_1, n_2, and n_3 of those sectors is given by:

$$L = [P(ro)]^{n_1} \; [P(r/b)]^{n_2} \; [P(bo)]^{n_3} \; [P(p)]^{n_4} \qquad (V)$$

where n_4 is the unobservable number of purple sectors resulting after mitotic exchange. While the value of n_4 will be of interest later (as the sum, $n_1 + n_2 + n_3 + n_4$, is the number of cell divisions which were "mictoid"), it can be eliminated from the solution for values of x_1 and x_2 by writing the equivalent expression for equation V:

$$L = \left[\frac{P(ro)}{1 - P(p)}\right]^{n_1} \left[\frac{P(r/b)}{1 - P(p)}\right]^{n_2} \left[\frac{P(bo)}{1 - P(p)}\right]^{n_3} \qquad (Va)$$

The method of maximum likelihood selects those estimates for x_1 and x_2 which maximize L; calculation is simplified by maximizing ln L. The solution is effected by taking the partial derivatives, setting them equal to zero, and solving simultaneously. Substituting equations IV into equations III, and then equations III into equation Va and taking the partial derivatives gives:

$$\frac{\delta \ln L}{\delta x_1} = n_1 \frac{3e^{-3x_1}(4e^{-3x_2} - 3e^{-2x_2} - 1)}{6(1 - e^{-3x_2}) + (1 - e^{-3x_1})(4e^{-3x_2} - 3e^{-2x_2} - 1)} +$$

$$(n_2 + n_3) \frac{3e^{-3x_1}}{(1 - e^{-3x_1})} - \frac{(n_1 + n_2 + n_3)\, 3e^{-3x_1}(2 + e^{-3x_2})}{3(1 - e^{-3x_2}) + (1 - e^{-3x_1})(2 + e^{-3x_2})} \qquad \text{(VI)}$$

$$\frac{\delta \ln L}{\delta x_2} = n_1 \frac{18e^{-3x_2} + 6(1 - e^{-3x_1})(e^{-2x_2} - 2e^{-3x_2})}{6(1 - e^{-3x_2}) + (1 - e^{-3x_1})(4\, e^{-3x_2} - 3e^{-2x_2} - 1)} -$$

$$n_2 \frac{6(e^{-2x_2} + e^{-3x_2})}{(1 + 3e^{-2x_2} + 2e^{-3x_2})} + n_3 \frac{3e^{-3x_2}}{(1 - e^{-3x_2})} -$$

$$(n_1 + n_2 + n_3) \frac{9e^{-3x_2} - 3e^{-3x_2}(1 - e^{-3x_1})}{3(1 - e^{-3x_2}) + (1 - e^{-3x_1})(2 + e^{-3x_2})}$$

These can be solved simultaneously using a graphic method. Once values of x_1 and x_2 are obtained, n_4 is estimated by the expression:

$$\frac{n_4}{n_1 + n_2 + n_3 + n_4} = P(p) \qquad \text{(VII)}$$

The mictoid fraction, (1 - A), is now easily computed: it is simply the total number of mitotic tetrads divided by the total number of cell divisions*:

$$(1 - A) = \frac{n_1 + n_2 + n_3 + n_4}{\text{total number cell divisions}} \qquad \text{(VIII)}$$

The coefficient of coincidence may also be calculated.

---

* The estimation of the total number of cell divisions is described later in this Section.

The coefficient of coincidence is defined as the observed frequency of double crossover strands divided by the expected frequency of double crossover strands. The expected frequency of double crossover strands, is simply the product of the two decimal map distances, in this case, x_1x_2. What is observed after mitotic exchange, however, is half-tetrads. P(bo), then, is one half the probability of double exchange tetrads (Figure 25). Assuming no chromatid interference, there is a 1:2:1 ratio of 2-strand, 3-strand and 4-strand double exchanges (147). As shown in Figure 25, only one fourth the strands from all the double exchange tetrads are double crossover strands. Thus (ignoring contributions from higher order tetrads) the "observed" number of double crossover strands is $\frac{1}{4} \cdot 2 \cdot P(bo)$. Coincidence is then given by:

$$c.c. = \frac{P(bo)}{2x_1x_2} \qquad (IX)$$

Application of quantitated theory.

The numbers of sectors seen in stamen hairs of purple Tradescantia hirsuticaulis in experiments over a three year period are presented in Table 20. Two of the spontaneous experiments contributed some two-thirds (43.21/63.58) of the total X^2 value, affecting the frequency of blue-only sectors the most. Accordingly, the frequency of blue-only sectors was calculated with and without the "non-homogenous" rows. While the difference is large on a percentage basis, and this is what the X^2 values reflect, the actual difference is only some 0.0036 sector/stamen, a difference I judge here to be biologically

Table 20. Sectoring data on *T. hirsuticaulis* and X^2 homogeneity test.

	SECTOR CLASS							
	RED-ONLY		RED/BLUE		BLUE-ONLY		NUMBER	TOTAL
EXPERIMENT	#	X^2	#	X^2	#	X^2	STAMENS	X^2
5/72 spontaneous	166	0.53	75	1.02	13	1.00	1014	2.55
10/72 controls	19	0.76	2	6.28	10	21.91	150	28.95
6/72 pre-33R	30	0.00	7	2.52	1	1.56	192	4.08
10/72 pre-34R	4	0.96	3	0.02	1	0.12	42	1.10
11/72 pre-60R	11	0.09	13	12.07	3	2.14	78	14.30
12/72 pre-60R	34	0.07	20	2.76	2	0.68	210	3.51
11/73 spontaneous	20	1.38	7	1.49	3	0.01	168	2.88
11/73 pre-60R	8	0.48	8	3.09	0	1.12	66	4.69
1/75 spontaneous	138	0.19	48	1.31	14	0.02	860	1.52
TOTAL	430	4.46	183	30.56	47	28.56	2780	63.58**
6/72 33R-response	29	0.01	22	0.27	6	0.45	72	0.73
10/72 34R-response	71	0.00	47	0.11	10	0.18	180	0.29
TOTAL	100	0.01	69	0.38	16	0.63	252	1.02n.s.
11/72 60R-response	188	0.49	107	0.02	22	0.73	216	1.24
11/73 60R-response	85	0.92	54	0.05	6	1.39	114	2.36
TOTAL	273	1.41	161	0.07	28	2.12	330	3.60n.s.

** $p < 0.01$, X^2_{tab}, 24 d.f. = 43.0 at $p = 0.01$

n.s. indicates not significant, $p > 0.05$, X^2_{tab}, 3 d.f. = 7.81 at $p = 0.05$

inconsequential. Additionally, the higher frequency of blue-only sectors would render subsequent calculations (i.e., coincidence) more conservative, and might well be a more accurate estimate of the frequency of the "difficult-to-distinguish" blue-only sectors. So, although the homogeneity X^2 value for the series of spontaneous (0 R) experiments was highly significant, $p < 0.01$, according to the tabulated values of X^2 (124), I decided to retain the data from the two "non-homogenous" experiments.

In these experiments, the average number of hairs per stamen in T. hirsuticaulis was found to be 55.4444 (Table 21) and the average number of cells per hair over all experiments was found to be 16.8810 (Table 21). These sets of data show no significant differences in the length of hairs from radiation-response and non-radiation-response flowers; others (53,54,55,100,142,143) have demonstrated a significant reduction in stamen hair length after exposures to radiation, although of considerably higher doses than used in these experiments. In the analysis of variance for stamen hair number, the $F_{4,175}$ value was found to be highly significant. This is judged to be a reflection of the variation within the non-response data; the $F_{1,178}$ value is not highly significant. As the non-response means, 53.0, 49.3, and 57.7, bracket the response means, 55.8 and 53.2, all the data were combined, and a grand mean calculated.

Thus 2780 spontaneous stamens are seen to comprise the results of some 2,601,960.1 (2780 stamens x 55.44 hairs/stamen x 16.88 cells/hair)

TABLE 21. Numbers of hairs per stamen and cells per hair in *T. hirsuticaulis*.

		hairs / stamen			cells / hair		
SOURCE		n	Σx	Σx^2	n	Σx	Σx^2
10/72	controls	24	1271	68080	138	2421	43233
10/72	pre-34R	6	296	14758	36	560	8926
1/75	spontaneous	88	5077	296867	-	-	-
	subtotal	118	6644	386349	174	2981	52159
6/72	33R-response	14	781	44345	84	1382	23570
10/72	34R-response	48	2555	137929	78	1309	22513
	subtotal	62	3336	182274	162	2691	46083
TOTAL		180	9980	561979	336	5672	98242
MEAN			$\bar{X}$ = 55.4444			$\bar{X}$ = 16.8810	

ANOVA : RADIATION-RESPONSE VS NON-RADIATION-RESPONSE

SOURCE	d.f.	S.S.	M.S.	d.f.	S.S.	M.S.
Total	179	8643.4445	--	335	2493.2381	--
Tmt	1	253.7500	253.7500	1	22.7783	22.7783
Error	178	8389.6945	47.1331	334	2470.4598	7.3865
		$F_{1,178}$ = 5.38*			$F_{1,334}$ = 3.08, n.s.	

(continued)

Table 21 (cont'd.).

ANOVA : OVER ALL EXPERIMENTS

SOURCE	d.f.	S.S.	M.S.	d.f.	S.S.	M.S.
Total	179	8643.4445	--	335	2493.2381	--
Tmt	4	1054.6003	263.6500	3	140.0055	46.6685
Error	175	7588.8442	43.3648	332	2353.2326	7.0880
		$F_{4,175} = 6.08^{**}$			$F_{3,332} = 6.58^{**}$	

* $p < 0.05$, $F_{tab,1,178} = 5.02$

** $p < 0.01$, $F_{tab,4,175} = 3.72$, $F_{tab,3,332} = 4.28$

n.s., not significant, $p > 0.05$, $F_{tab,1,334} = 5.02$

stamen hair cell divisions. This counts the periclinal division in the filament epidermis, so the calculation of sector frequency per cell division will be slightly in error, as those sectors which are a single mutant cell at the base of a hair in the filament's epidermis have not been included. Since there are few mutations which encompass the basal cell of a hair, this error is judged to be relatively unimportant. Another very small, and disregarded, error arises from the inclusion of multiple-entire hair sectors among the sectors used in these calculations. Multiple-entire hair sectors arise from a mutant event occurring during the ontogeny of the filament itself, rather than during the some 2.6 million divisions producing the stamen hairs. These two small errors are in opposite directions, and thus tend to compensate for each other, making any final error tremendously small.

Tables 20 and 21 provide all the data necessary to calculate map distances, mictoid fraction, and coincidence using equations VI, VII, VIII and IX. The results of the solution of those equations for data from spontaneous (0 R), 34 R- and 60 R-response material are presented in Table 22.

The mitotic map presented in Figure 27 is the first genetic map in the genus Tradescantia. The distance from the centromere to the E locus is 5.6 centimorgans, that from the E locus to the D locus, 12.1 centimorgans. The addition of ionizing radiation affects those map distances. The more distal region appears to shorten -- linearly with exposure dose (Figure 28). The description of the behavior of the

Table 22. Somatic genetic parameters calculated for T. hirsuticaulis.

Radiation	0 R	34 R	60 R	60 R[a]
Cell Divisions	2601960.1	235861.1	308865.8	308865.8
N_T	4452	1262	3885	1760
(1-A)	0.001711	0.0053506	0.0125782	0.0056982
n_1	430	100	273	116
n_2	183	69	161	161
n_3	47	16	28	28
n_4	3792	1077	3423	1455
P(bo)	0.0105543639	0.0114421796	0.007193327853	0.0158352553
x_1	0.0565	0.0752	0.0527	0.1300
x_2	0.1211	0.0981	0.0834	0.0831
c.c.	0.7713	0.7755	0.8183	0.7329
"traditional" c.c.	2229.4	764.4	304.0	635.6
Fold Increase (1-A)	--	3.13	7.35	3.33

[a] ro sectors multiplied by 17/40 to "remove" those sectors associated with Feulgen-positive micronuclei.

Figure 27. Mitotic map of T. hirsuticaulis.

D is the delphinidin locus, E is the "enhancer" locus, c is the centromere. Map distances given in centimorgans, calculated after the method described in the text.

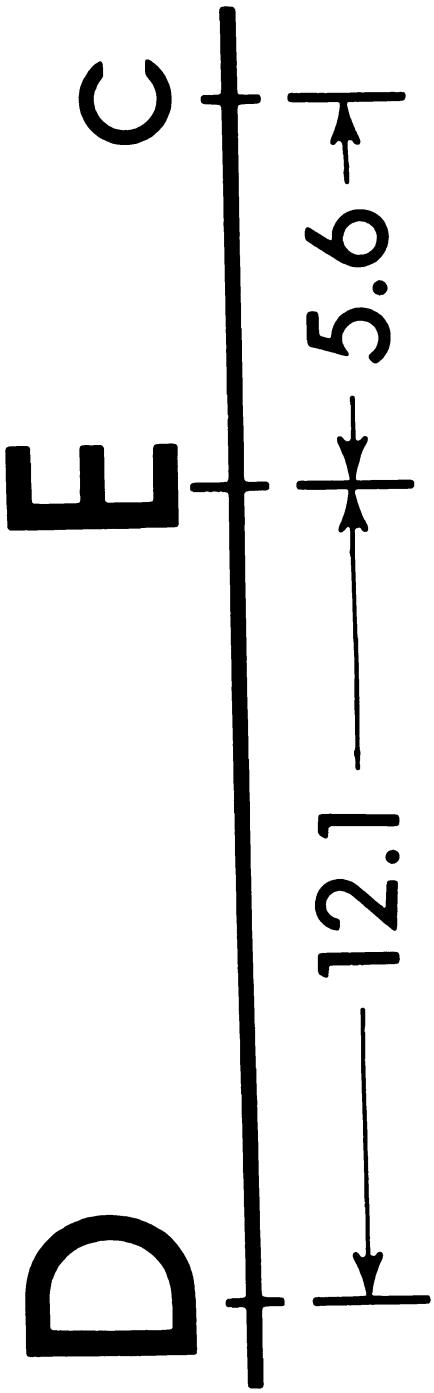


Figure 27

Figure 28. Somatic genetic parameters in T. hirsuticaulis after various ^{60}Co gamma radiation exposures.

The designation "$\bar{c}$ μn" means "with micronuclei", i.e., those sectors at 60 R associated with micronuclei have not been removed from the data; the designation "$\bar{s}$ μn" means the converse. The designations x_1 and x_2 represent the map distances from the centromere to the proximal (E) locus, and from the proximal (E) to the distal (D) locus respectively. The notation (1-A) denotes the mictoid fraction.

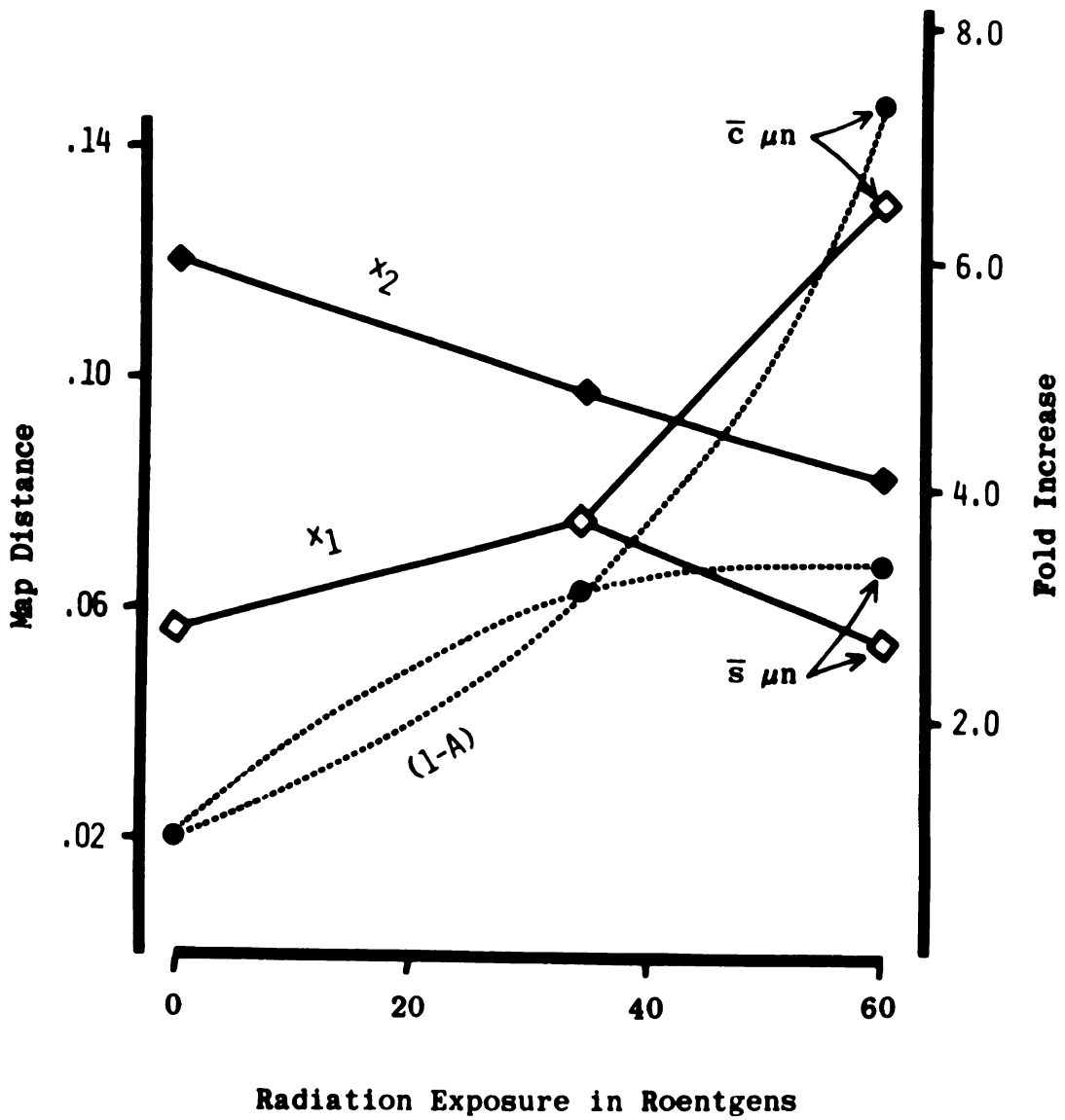

8.0
6.0
4.0
2.0
Fold Increase
.14
.10
.06
.02
Map Distance
$\bar{c}$ μn
x_2
x_1
$\bar{s}$ μn
(1-A)
0
20
40
60
Radiation Exposure in Roentgens

Figure 28

proximal region is complicated by the 60 R data. If the micronuclei-associated sectors are not removed from the data, the proximal region increases in apparent length at 34 R, then decreases to less than its 0 R length at 60 R. If all the micronuclei-associated sectors are removed from the data (as being putative products of deletion, not somatic exchange), a consistent increase in map length of the proximal region is seen -- the plot appears non-linear, with an upward trend. If some of the micronuclei-associated sectors were left included in the data (as some 10% of spontaneous red-only sectors were found to be associated with micronuclei), the map distance calculated for the proximal region at 60 R would lie between the two values for 60 R in Table 22. There is certainly some percent of micronuclei-associated sectors, which, if retained in the analysis, would complete a strict linear increase in proximal map length with exposure dose.

It is evident that radiation does not affect the frequency of recombination similarly in the two regions of the chromosome. If it had, the mitotic map would have increased (or decreased) uniformly. The significance of these changes in mitotic map length is not presently known.

The mictoid fraction, (1-A), also changes with radiation exposure (Figure 28). The observed increase in mictoid fraction is in accord with the previous discussion of the gene-canalization model for somatic sectoring. Just as with the mitotic map length of the proximal region, the removal of micronuclei-associated red-only sectors (to correct the data for the contribution made to total sectoring by deletion) affects

the exact value of (1-A) at 60 R. However, even removal of all the micronuclei-associated sectors results in a hyperbolic increase in mictoid fraction with exposure dose. The exact form of the increase -- linear or non-linear -- is not clear from these experiments.

The mictoid fraction in spontaneous Tradescantia hirsuticaulis material is 0.17% (Table 22). Thus, most of the stamen hair divisions, by far, are ordinary mitoses. This means that if a method can be perfected for obtaining well-squashed, well-spread, late-prophase to early metaphase mitotic figures in stamen hair divisions, perhaps some 0.2% of them will exhibit "mitotic tetrads".

As expected, the coefficients of coincidence no longer indicate large amounts of negative chromosome interference. The values obtained -- 0.7713 to 0.8183 -- are close to the value assumed (1.00) in developing the mapping function. Inspection of the graph in Barratt et al. (14) shows that the effect of varying amounts of coincidence on mapping functions for distances of 10 centimorgans or less is very small.

The present system of equations may be applied to sets of data collected on somatic sectoring in other organisms by other investigators, and, within the limits set by the assumptions made in the derivations and the conditions of the experiments, solutions may be calculated.

Garcia-Bellido (43) has recently published an extensive study of somatic sectoring in Drosophila. He, like Auerbach (12), and unlike

Stern (131) and Schwartz (122), considered the possibility that some sectors might be the results of double exchanges, and that these were sufficiently important to consider. However, he, as I (30) previously, only seriously considers the double exchange contribution to one class, and neglects its tiny contribution to the single crossover class: this is the same as assuming that only two-strand double exchanges occur.

Garcia-Bellido (43) raises the question as to whether the spots he is assigning to a double crossover class are indeed produced as the result of double exchange. He tests, and finds that the mean sector size of his putative double crossover spots is the same as that of the single crossover spots. This places both sector-producing events at the same time in ontogeny, lending credence to the assertion that both are produced from somatic exchange at that time.

Table 23 shows both the data from three experiments reported by Garcia-Bellido (43), as well as the somatic genetic parameters calculated from those data by the method described previously in this Section.

The two experiments on the y f / + + genotype, at 0 R and 500 R, show the same pattern of interaction of radiation and mitotic map distance shown above for Tradescantia: the distal region decreases in apparent length, the proximal region increases in length. Comparison of the two experiments at 500 R, on y f / + + and y sn / + + flies, do not reveal any direct relationship between mitotic and meiotic map distances. While the meiotic map length of the proximal region was

Table 23. Somatic genetic parameters calculated for Drosophila.

EXPERIMENT[a]	y f/+ +	y f /+ +	y sn/ + +
Radiation	500 R	0 R	500 R
Cell Divisions	39520	13680	25440
N_T	4288	391	3502
(1-A)	0.108502	0.0285818	0.1376572
n_1	157	19	37
n_2	492	33	369
n_3	45	4	9
n_4	3594	335	3087
P(dco)	0.0104639947	0.0101468196	0.002545568447
x_1,mitotic	0.1572	0.1110	0.1305
MD_1,meiotic[b]	0.093	0.093	0.450
x_2,mitotic	0.0446	0.0586	0.0120
MD_2,meiotic[b]	0.567	0.567	0.210
c.c.	0.7462	0.7800	0.8128
"traditional" c.c.	21.9	128.6	26.3
Fold Increase (1-A)	3.77	--	4.82

[a]Data from Garcia-Bellido (43). [b]From (70).

P(dco) is the probability of "double-crossover sectors"

very different in the two genotypes, 9.3 and 45.0 map units, the mitotic map length was approximately the same, 15.7 and 13.0 units. The distal region, however, might indicate a correspondence between mitotic and meiotic maps. Regions of 21.0 and 56.7 meiotic units were found to be 1.2 and 4.5 mitotic map units in length. Obviously, further analysis of meiotic and mitotic recombination in the same organism is needed. However, the X-chromosome of Drosophila, with its large amount of proximal heterochromatin, may well turn out to be the worst possible material for such comparative studies, particularly when the frequency of sectoring has been enhanced by large amounts (500 to 1000 R) of radiation.

Garcia-Bellido (43) does note that there is a slight change in the distribution of the sectors among crossover classes between the control and irradiated experiments. While he talks of this as a "higher" amount of crossing-over in the distal region of the control flies, it seems to me to impart more biologic understanding to refer to this as an increase in the propensity of the proximal region to recombine under the influence of radiation. The work of Natarajan and Ahnstrom (96,97) has established the tendency of radiation-induced aberrations to be localized in heterochromatin; the proximal third of the X chromosome in Drosophila is heterochromatic. In fact, an examination of the data of Garcia-Bellido (43) shows that the proximal heterochromatin of the X chromosome undergoes an incredible amount of exchange. In three

experiments involving the genes y and f (exp 1, Table 4, exps. 1,2, Table 3 of Garcia-Bellido, (43)), the region proximal to "forked" is contributing some 59, 71 and 68 percent of the recombinations, for control, 500 R and 1000 R experiments, respectively, (even more if the contributions of three-strand doubles are correctly assigned to exchanges involving that region instead of being classed as single crossovers in the more distal region). Yet the genetic length of the proximal region is only 9.3 (that of the more distal region, 56.7)! Seemingly, the proximal heterochromatin (?) is participating in somatic exchange to a greater degree than it does in meiotic exchange.

Application of the equations described previously in this Section to data from two experiments of Garcia-Bellido (43) involving 500 R of radiation results in similar values for the mictoid fraction, 0.1085 and 0.1377, respectively (Table 23). Calculation of the mictoid fractions with data from others of the experiments of Garcia-Bellido, including the setting of some estimate of standard error on (1-A), will lead to the determination of the statistical "similarity" of mictoid fractions at various levels of radiation (and of the effect of fly genotype on the radiosensitivity of the mictoid fraction).

Mitotic exchange in Drosophila shows levels of coincidence -- 0.7462 to 0.8128 -- similar to those calculated for mitotic exchange in Tradescantia. There is no longer an unexplained "20-fold excess" of double crossovers. Instead, I hypothesize that only some of the tergite initials are competent to have pairing and exchange e.g., 2.9% spontaneously, and 10.9 to 13.8% in 500 R treated flies.

Further implications of the theory.

Garcia-Bellido (43), Lefevre (68) and Becker (16) all find, that, while radiation dose within a certain range increases the sectoring frequency (irradiated minus control) in a linear manner, extrapolation to zero dose does not give an accurate estimate of the spontaneous sectoring rate, but one far lower than that observed. Indeed, this latter is also true of sectoring in Tradescantia clone 02 (130).

This phenomenon has been interpreted (16,43,68,130) to indicate the involvement of several different mechanisms, and indeed, has led to much speculation as to what those mechanisms are on the basis of the slope of the curve in a plot of log (frequency irradiated - control) versus log (dose) (e.g., reference 65). It is my contention that in some cases these slopes can be simply products of the choice of method of analysis, and have little or no biologic significance. I suggest that a plot of log (irradiated - control) versus log (dose) is not an adequate way to treat Tradescantia and Drosophila somatic sectoring data, within ranges of low dose, although it is definitely the method of choice at much higher doses, and for certain other radiobiologic studies, e.g., the production of chromosome aberrations, or inactivation of enzyme molecules in solution.

I have advanced the hypothesis that somatic sectoring may be well understood as stemming from the "failure" of a canalized gene. If a plot of the normal curve (109), $\emptyset(x) = \frac{1}{\sqrt{2\pi}} e^{-\frac{x^2}{2}}$, is taken, and a

"threshold" value of x, the standardized deviate, is chosen, and a plot of log $\int_{x}^{x_t} \phi(x)dx$ versus log $(x_t - x)$ is made, the resultant curve (Figure 29) closely approximates that curve plotted from the experimental data collected on Tradescantia by Sparrow et al. (130)(or 65). It was suggested previously that the action of radiation on the sectoring rate could be understood to be effected through a disturbance of cellular physiology resulting in a shift in $\overline{M}$, the average value of "make" for the population of cells. This is, of course, mathematically equivalent to the process just carried out and plotted in Figure 29.

Referring specifically to Tradescantia sectoring, it has been shown, in this work (Section II, Table 15) and previously (79), that deletion is an important mechanism of sector production at "high" doses of radiation (60R). At high levels of radiation, a log-log plot of Tradescantia clone 02 sectoring data is the most theoretically sound method of plotting the data. At these high levels, the "contamination" by sectors produced as a result of somatic crossing-over is small, and almost eliminated by the subtraction of the spontaneous rate. In T. hirsuticaulis, however, where there are classes of sectors which cannot be produced except by somatic crossing-over, (barring rare, coincident events), a different kind of precision is possible.

I have long been uneasy about the assignation of the 1.4-hit kinetics indicated by the data of Sparrow et al. (130) to the "grab-bag" category of "mixed kinetics". I suggest that it is a result of the shape of the normal curve around a certain value (and a component,

Figure 29. A log-log plot of the normal curve of error.

$P(x_t) - P(x)$ is the area under the normal curve of error from x_t to x; x_t is the chosen "threshold" value of the standard deviate, $x_t = 3.24$, δ is $x_t - x$, the distance from this threshold value to some other value, x, of the standard deviate. The slope of a tangent to the log-log plot is denoted as n.

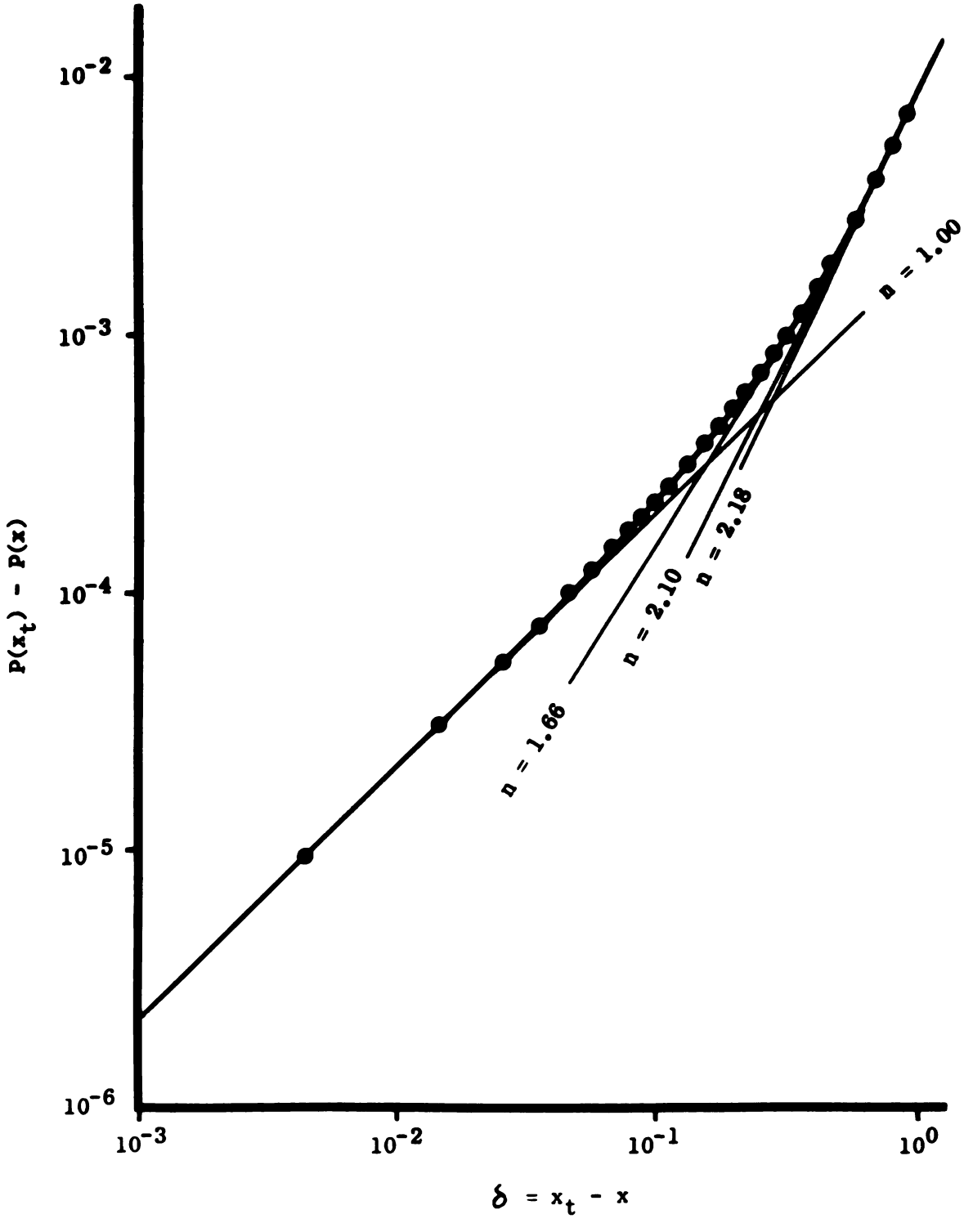

10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
$P(x_t) - P(x)$
10^{-3}
10^{-2}
10^{-1}
10^{0}
$\delta = x_t - x$
n = 1.00
n = 2.18
n = 2.10
n = 1.66

Figure 29

important at high doses, which is radiation-produced). Referring to Drosophila sectoring, Lefevre (68) and Becker (16) say that radiation does not merely increase the rate of spontaneous sectoring, an interpretation which stems from the large difference between the actual and expected rate of sectoring at D_0. Although Garcia-Bellido also suggests that induced exchange is different from spontaneous mitotic exchange he seems cautious; the ten - to twenty-fold excess of double crossovers above that expected on the basis of random association and the effect of Minute genes in increasing both spontaneous and irradiated sectoring-rates are mentioned just where a conclusion would typically appear. I suggest that, in Drosophila, at this (500 R) radiation level, a substantial part of the radiation effect does occur by increasing the spontaneous mechanism.

The mictoid fraction for unirradiated Drosophila, 0.029, (Table 23), is much higher than the spontaneous mictoid fraction in T. hirsuticaulis, 0.0017 (Table 22). In terms of the canalized gene model, this spontaneous mictoid fraction corresponds to that area beyond the threshold value of M, "make". Thus, referring to a Table of $\emptyset$ (x) (109), while the threshold for Tradescantia is indicated to be at

$M_t = \{x| \int_x^{\infty} \emptyset(x)dx = 0.0017\}$, x = 2.93, the threshold value for the canalized gene in Drosophila -- and it is not necessarily a homologous gene -- is at $M_t = \{x| \int_x^{\infty} \emptyset(x)dx = 0.029\}$, x = 2.31. This threshold is a full six-tenths of a standard deviation closer to the respective mean value of "make". I would suggest, without any

experimental evidence, that cellular physiology is much more buffered at that point, and that larger doses of radiation are therefore necessary to produce a perturbation which results in a substantial change in M. Mathematically, this is saying that the effect of a dose of radiation on changing the value of "make", is dependent on x as well as R, $dx/dR = f(x,R)$. To additionally mask response, the effect of some δx on area past the threshold is smaller on a percentage basis at $x_t = 2.31$ than at $x_t = 2.93$. Combining both these facts might mean that large doses of radiation are needed to get a small biologic effect with Drosophila (i.e., a 3.8--4.8-fold increase in (1-A) with 500 R (Table 23) as compared with a 3.1-fold increase in (1-A) after 34 R radiation exposure to Tradescantia (Table 22)). Describing the exact mathematics of all this is beyond the scope of the present work, as well as the informational content of the collections of data from other workers available to me at this time.

It seems to me, however, that the stamen hairs of Tradescantia will be some of the best material for use in further exploration of this subject. When appropriate corrections for the contribution to total sectoring by deletion are made, or when such a contribution is negligible, the change in mictoid fraction with radiation exposure can be used to characterize the above mentioned f(x,R) or to characterize the type of perturbation of "Make" that a stimulus (e.g., genetic background, radiation, diurnal temperature) produces. As the method here described for the treatment of data is presently being extended to treat

data from T. clone 02 and other one-locus systems, it will be possible to compare experimental behavior near several putative thresholds on the normal curve.

At present, while not "proven", the gene-canalization model is an alternative approach to the interpretation and explanation of somatic sectoring data. It can explain the high degrees of negative chromosome interference characteristic of somatic sectoring in Tradescantia and Drosophila, as well as the failure of log-log dose-response curves to extrapolate back to the observed spontaneous rate of "mutation". It further provides a hypothesis which is open to testing. Becker (16) may indeed have been right, but in perhaps a different sense than he intended, when he suggested that X-rays produce a physiological condition which is responsible for the production of somatic sectors. This insight may lead to a greater understanding of mitosis itself.

SUMMARY

1) Purple flower color of the T. hirsuticaulis stock is due to the presence of approximately equal amounts of blue (delphinidin) and red (cyanidin) pigments.

2) The total amount of anthocyanin pigment in the purple-flowered T. hirsuticaulis is twice that in the blue- or red-flowered T. clone 02 and progeny.

3) Purple-flowered T. hirsuticaulis stock possesses an allele, E^+, which is responsible for both #1 and #2 above. The action of the E^+ allele may well have a simple molecular explanation.

4) The genotype of the purple-flowered stock is D^+E^-/D^-E^+. This designation was arrived at through an analysis of the occasional red and blue mutant cells produced in the purple floral tissues and is supported by breeding data.

5) Purple-flowered T. hirsuticaulis exhibits three classes of somatic sectors red-only, red/blue twin spots, and blue-only. The twin spots are shown to be the results of single events.

6) As in T. clone 02, deletion, as evidenced by the presence of micronuclei (chromosome fragments), is indicated to be one mechanism producing certain kinds of sectors (red-only) in response to ionizing

radiation.

7) The predominant, if not exclusive, mechanism of spontaneous sectoring and an important mechanism even for 60 R-induced sectoring in this T. hirsuticaulis (and by inference, in other Tradescantia) is argued to be mitotic crossing-over.

8) Some conceptual parallels between the genetic behavior of facultative apomicts and sectoring flowers of Tradescantia are described. This results in a description of new somatic genetic parameters, A, the apomictoid fraction, and 1-A, the mictoid fraction, those fractions of the cell divisions that are typical mitoses, or atypical mitoses where the chromosomes are amenable to exchange, respectively.

9) A genetically accurate method of using somatic sectoring data for the calculation of mitotic map distances and coincidence is described.

10) Mitotic crossing-over through the failure of some canalized gene of mitosis is shown to predict a "log response - log dose" plot which simulates that found for somatic sectors in T. clone 02.

APPENDICES

APPENDIX A

Particularly interesting somatic sectors in *T. hirsuticaulis*

The world has Seven Wonders; I have chosen seven intriguing sectors to present here (Figure 30), which, I believe, demonstrate the complexity of the question of somatic sectoring.

The first sector (Figure 30a) is a multiple-entire, multiple-sectored hair. The two identical red-only multiple-sectored hairs were joined by two purple cells in the filament epidermis. I suggest that this sector is an example of the regularity and precision of the sorting-out of mutations after a mutant event, the process of the formation of a multiple-sectored hair.

The second sector (Figure 30b) illustrates the result of a spindle abnormality, an exceedingly rare event in this material, in conjunction with a red/blue twin mutant event. The blue cell, like a side-car on a motorcyle, must have been carried along as the red cell continued to divide. Eventually, the daughter cell to which the blue cell was attached was segregated subterminally and "left behind."

The third sector (Figure 30c) poses a problem. Red/blue twin events are explainable, but how did the terminal red cells arise from

Figure 30. Exceptional sectors in T. hirsuticaulis.

a) A multiple-entire, multiple-sectored hair, 22 days post-60 R

b) A curious red/blue twin, 15 days post-60 R

c) An entire hair red/blue twin, 16 days post-33 R

d) A multiple-sectored red/blue twin, 11 days post-34 R

e) A multiple-sectored red/blue twin, 17 days post-60 R

f) A multiple-sectored red/blue twin, spontaneous

g) A multiple-sectored red/blue twin, 19 days post-34 R

The wavy purple line represents a series of purple cells of unknown number.

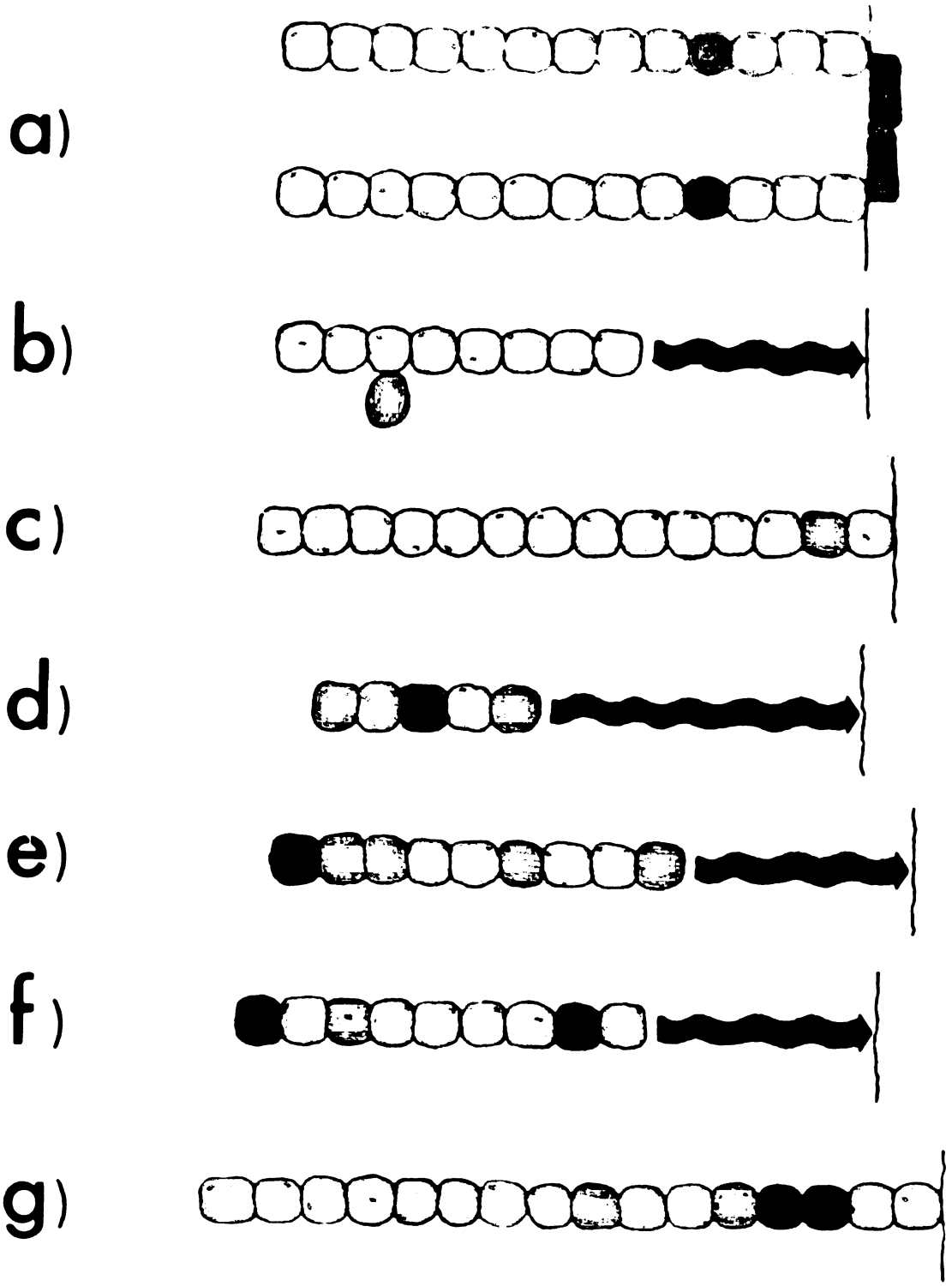


Figure 30

division of a blue cell? This is, I advance, a complex-multiple-sectored hair, the origins of which have not yet been described or hypothesized.

The fourth sector (Figure 30d) is a multiple-sectored red/blue twin spot. However, twin spots were argued previously (Section II) to arise immediately from a division including a red/blue mutant event; if this particular sector is not the result of chance occurrence of two red/blue mutant events in hair ontogeny, then it is evidence that r/b events, like red events in T. clone 02 (84), can exist occasionally in heterozygous states. The fact that it is from 11 days post-irradiation material, not the peak of mutation response, makes it unlikely that it is two coincident events; this material also exhibited entire-hair sectors, that time in the mutation response curve that produces the most multiple-sectored hairs in T. clone 02 (84).

The fifth sector (Figure 30e) is an immediately subterminal sector of alternating sectors of blue and red cells. It is worth noting that there are not two terminal purple cells; as the terminal cell "always divides" (53,54,100), presumably this string of red and blue sectors was segregated from one complex mutant event in a continually, terminally, segregated cell. How strange that after "throwing" all those mutants, the terminal cell is purple!

The sixth sector (Figure 30f) is similar to the last mentioned sector, except that purple cells are interspersed among the red and

blue cells.

The seventh sector (Figure 30g) is perhaps the most perplexing of all. Because this is an entire-hair sector, the original mutant event must have occurred in the periclinal division of the hair initial or in the 1-celled young hair. This one event sorted out to give multiple red, multiple blue and purple cells, and was not so disruptive as to stunt the hair to any great degree.

Mericle and Mericle (84) have proposed that the chromosome of young Tradescantia stamen hairs might be functionally minimally 2-partite; the exceptional sectored hairs presented here certainly do not indicate other than that the structure and behavior of the chromosomes in stamen hair cells may well be at least that complicated.

APPENDIX B

Amounts of nuclear DNA in a mature stamen hair of T. clone 02.

Attempts to quantify the size of micronuclei associated with mutant sectors in the stamen hairs of T. clone 02 generated a quantity of mature stamen hairs prepared for quantitative microspectrophotometry in the usual way: killing and fixation in 3:1, ethanol:acetic acid, Feulgen staining, and bleaching (101). I chose to practice my quantitative microspectrophotometric technique on a slide of this material, expecting to find the same 2C complement of DNA in each of the cells of the hair. What I found was a gradient of amounts of nuclear DNA (Figure 31). This is in agreement with unpublished observations of D. R. Davies (33) on the same material. Swift (138) working with three different species of Tradescantia found the expected 2C and 4C, as well as 8C nuclei in mature hairs; the 8C nuclei, however, were not observed until hair differentiation was completed. Data supporting this latter point, however, did not appear in the paper.

Mericle and Mericle (84) have proposed multineme chromosomes in the cells of young stamen hairs as one possible way that

Figure 31. Nuclear DNA in a mature stamen hair.

This Figure shows the amounts of nuclear DNA in the individual cells of a mature stamen hair of T. clone 02 killed and fixed in 3:1, ethanol:acetic acid and Feulgen-stained for quantitative microspectrophotometry. A Leitz MPV-1 microspectrophotometer was used for determining transmittances; the 2-wavelength method was employed for the calculation of relative DNA amounts. The DNA values given are in arbitrary units.

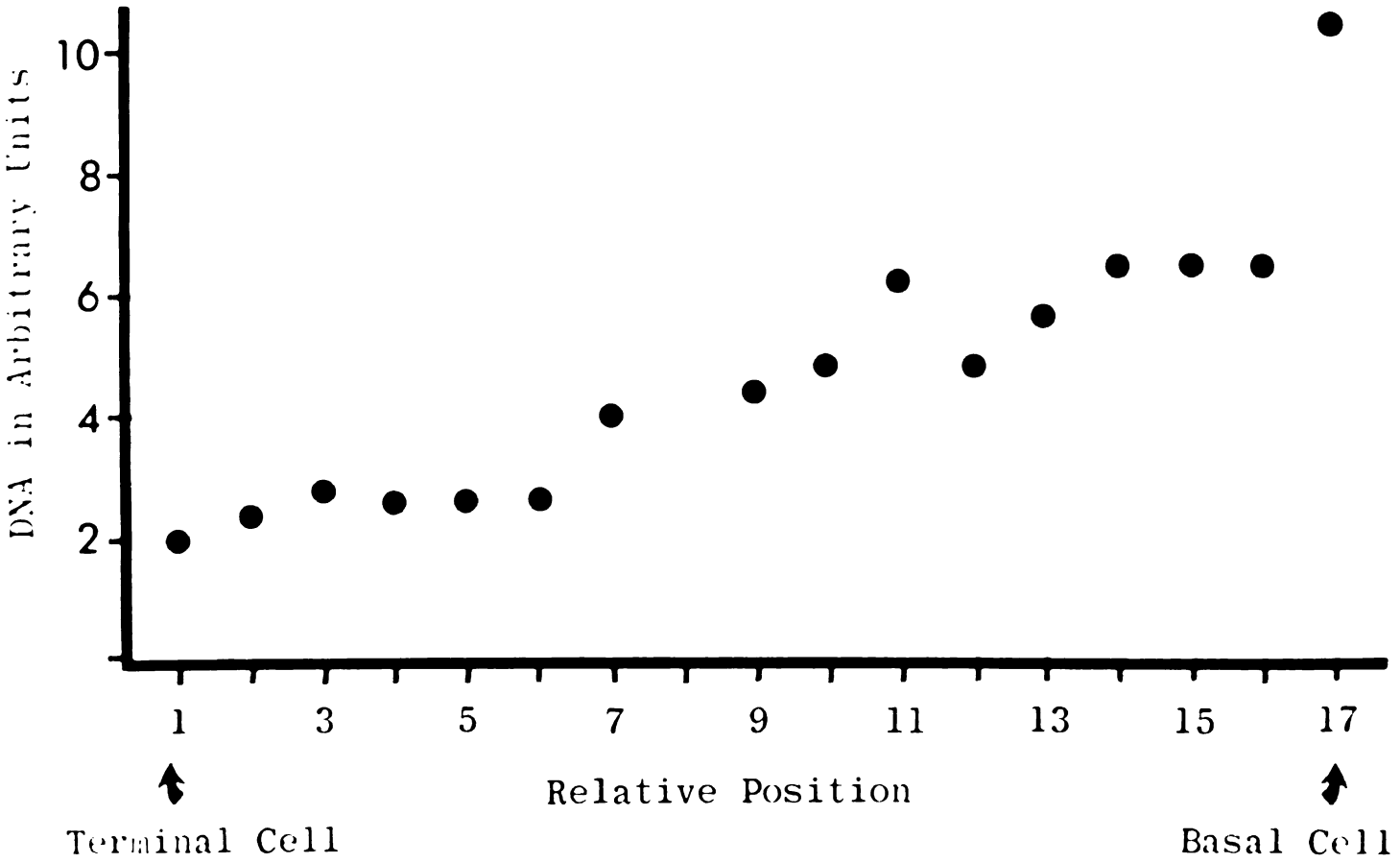

DNA in Arbitrary Units
10
8
6
4
2
1
3
5
7
9
11
13
15
17
Relative Position
Terminal Cell
Basal Cell

Figure 31

multiple-sectored hairs could originate. Certainly, the gradient of amounts of nuclear DNA shown in Figure 31 could be interpreted as polynemy of the chromosomes in the more basal cells. Polyneme chromosomes are not unknown in plants; the nuclei of suspensor cells in Phaseolus coccineus have amounts of DNA as high as 4096C, and yet are indicated to be diploid, 2n = 22 (13).

Swift (138) assumed that the 8C cells he observed in mature stamen hairs were the result of endopolyploidy. My investigation of the numbers and sizes of nucleoli in cells of mature stamen hairs does not lead me to the same assumption. While the most terminal cells in the stamen hairs of T. clone 02 tend to have 1 to 3 nucleoli, and the more basal cells, 3 to 6 nucleoli, the distribution of the numbers of nucleoli per cell in 135 cells from 11 stamen hairs does not differ markedly from the distributions in cells of root tips and young petals -- actively dividing, diploid tissues of this same material (101). Indeed, 100 cells from young petals showed a range of 1 to 9 nucleoli per cell, while the cells of the stamen hairs showed a range from 1 to only 7 nucleoli per cell. And in fact, the one cell with seven nucleoli was a terminal (presumptive 2C) cell in a hair. Were the more basal cells endopolyploid, as a consequence of their multiple sets of nucleolar organizers, I would expect to observe cells with far greater numbers of nucleoli than in cells of root tips or young petals. As I did not, I am inclined to ascribe the increased complements of DNA in basal cells to polyteny, rather than polyploidy.

Current investigations are aimed at determining whether the DNA complements of early ontogenetic stages are comparable to the DNA complements in the most terminal cells of mature hairs.

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