SINGLE-LOCUS MODIFICATION OF POSITION-EFFECT VARIEGATION IN *DROSOPHILA MELANOGASTER*. II. REGION 3C LOCI[1]

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THE extent to which and ways in which the organization of the individual pieces of genetic information within chromosomes in higher organisms contributes to the regularities of developmental patterns is a problem whose importance is gaining increasing recognition. Position-effect variegation provides a phenomenon whose study can be expected to yield insight into this problem. Especially, genetic suppressors of this variegation deserve study. In bacterial systems, characterization of the type of specificity of broad-range suppressors of mutants at numerous loci has provoked insights into the mechanism of gene expression at the level of translation (CAPECCHI and GUSSIN 1965). The hope sustaining the present series of inquiries is that similarly fruitful insights into mechanisms operating at the chromosomal level to govern gene expression—at least in Drosophila—may be provoked by the adequate characterization of the type of specificity of broad-range suppressors of position-effect variegation.

The two alleles at the Suppressor-of-Variegation *(Su(var))* locus on chromosome 3 of *Drosophila melanogaster* were first distinguished and then characterized by their effects on the amount of eye pigment produced in the genotype w/w; $Dp(1;3)w^{m264-58}$/III. The only white locus allele effective in pigment production in this genotype is in the 20-band insertion including region 3C from the *X* into the proximal heterochromatin of the left arm of chromosome 3. The *Su(var)* locus has two modes of action on the white-variegation associated with this duplication: direct and maternal. Flies homozygous for the *Su(var)* allele have pigment in larger areas of the eyes than do heterozygotes, which in their turn have pigment in larger areas than do flies homozygous for the + allele. Flies of a given genotype have more pigment in their eyes if their mothers were *Su(var)/Su(var)* than if their mothers were *Su(var)*/+, or more if their mothers were *Su(var)*/+ than if their mothers were +/+ (SPOFFORD 1967, in which the locus is designated "*Su-V*"). Other variegating loci in the same duplication appear to respond similarly.

If the *Su(var)* locus, unlike extra *Y* heterochromatin, is not a universal modifier of position-effect variegation, those features defining its range of specificity may be identified. *A priori*, specificity may arise from limitation of *Su(var)* action in any or some combination of the following ways: (1) in location in the organism—limited to derivatives from a single embryonic region or imaginal

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disk; (2) in time—limited to tissues undergoing determination during a particular period; (3) in the chromosomal region containing the loci susceptible to its action; or (4) in the heterochromatic component of the rearrangement evincing variegation—limited to rearrangements involving particular heterochromatic regions.

Hence, a survey of the effects of the *Su(var)* locus on rearrangements juxtaposing loci from various parts of the genome, with various phenotypes, to various regions of foreign heterochromatin was undertaken. A preliminary report of the findings has already appeared (SPOFFORD 1963). The present paper analyzes the results for two *X*-chromosome inversions bringing parts of region 3C next to the hC region of the proximal *X* heterochromatin (COOPER 1959), thus providing a test of the fourth conceivable limitation on *Su(var)* action.

MATERIALS AND METHODS

Stocks employed. For descriptions of mutants and chromosomes, see LINDSLEY and GRELL (1968). Five *X* chromosome inversions have been used to date: *In(1)w^{m4}; In(1)rst^{3}, y rst^{3} car bb; In(1)y^{3P}; In(1)sc^{4}, y sc^{4};* and *In(1)sc^{8}*. Their breakpoints are indicated in Figure 1. The results for the first two are presented here. Each was introduced opposite an attached-*X* marked with *y* (yellow) and *w* (white) into companion stocks, one homozygous for *Su(var)* and the other for its + allele. To accomplish this, a series of three crosses first replaced the inversion stocks' third chromosomes by a *Ly Sb* (Lyra, Stubble) chromosome and then replaced the latter with the *Su(var)* or + chromosome from the originally coisogenic stocks described by SPOFFORD (1967). Chromosomes *2* and *4* were unmarked and uncontrolled in origin. Both *Su(var)* and + females taken from stock for these crosses were certified for *Su(var)* locus genotype by later matings to *Dp (1;3)w$^{m264-58}$* males that produced appropriately identifiable progeny from the second mating.

Design of experimental crosses. For each inversion, all sixteen possible crosses between +/+, *Su(var)/Su(var)*, +/*Su(var)* and *Su(var)*/+ (the maternal chromosome preceding the slash in the genotype) were set up as five replicated pair matings on at least two dates. Matings at one time were made in vials of a single batch of Carpenter's medium, kept together in one tray at

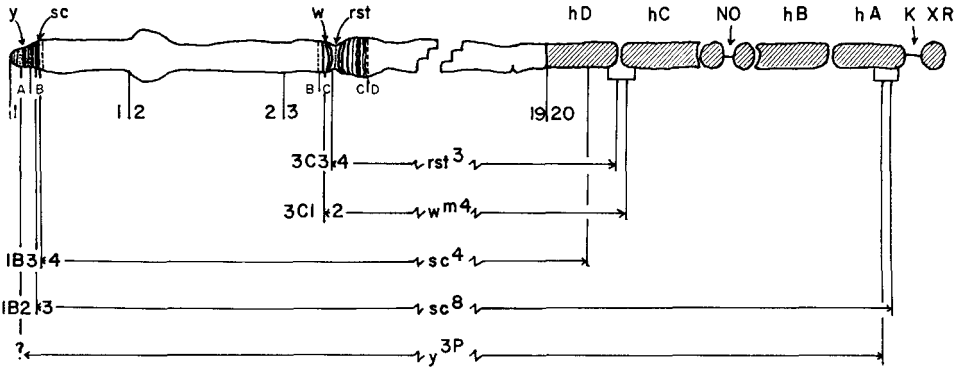

FIGURE 1.—Diagram of *X* chromosome showing breakpoints of *Ins(1)rst^{3}*, *w^{m}*, *sc^{4}*, *sc^{8}*, and *y^{3P}*. Only the salivary banding patterns of euchromatic regions near the breakpoints are shown. Heterochromatic regions are cross-hatched, the subdivisions hA, hB, hC, hD and XR as distinguished by COOPER (1959). Right-hand breakpoints of *rst^{3}* and *w^{m4}* may be in either order; the same is true for *sc^{8}* and *y^{3P}*. K = centromere, NO = nucleolus organizer. Salivary bands redrawn from LINDSLEY and GRELL (1968).

23° ± 1°C. Parents were transferred after six days to fresh medium and discarded six days later. The flies used as parents were derived from pair-matings within and between the companion stocks. Parents for the crosses made on the later date were taken from among the early-hatching offspring of the appropriate four crosses made earlier; their later-hatching brothers were scored phenotypically. Equal numbers of sons (10 for w^{m4}, 15 for rst^3) were picked randomly from each brood from each pair mating and were classified as to extent of variegation. When fewer than ten eclosed, as was often the case for certain crosses, all were classified.

Measurement of extent of variegation-suppression. (1) w^{m4}. The inversion brings the *w* locus (1–1.5) next to region hC of the proximal *X*-heterochromatin. The amount of drosopterin (red eye pigment) was taken as the index of *w* locus variegation (for justification, see SPOFFORD 1967), since ommatidia are either fully pigmented or white. The procedure for measuring drosopterin per fly was similar to that described in more detail previously. In brief, heads from males 3 or more days old were squashed on large sheets of Whatman No. 3 filter paper at 1 cm intervals; the sheets were developed for 5 hrs by descending chromatography in butanol-water-acetic acid (4:4:1) after a three-hour equilibration. The intensity of each drosopterin spot was measured by a Densichron model 451–4 densitometer. The optical density readings themselves serving as basic data. (For comparison, wild-type Oregon-R males yield averages of approximately 55.)

(2) rst^3. The heterochromatic break of $In(1)rst^3$ is very close to that of $In(1)w^{m4}$. Variegation at the *rst* locus (1–3.0), thus brought next to proximal heterochromatin, was measured as the fraction of tissue that was wild type, with facets in regular hexagonal array, as opposed to *rst* in phenotype, with facets irregularly jumbled. The wild-type fraction was estimated to the nearest tenth for each eye. Values for the two eyes were averaged for each fly. Thus, a completely mutant fly would be rated as 0% and a completely wild-type fly, as 100%. For single-genotype progenies, the correlation between the two eyes of the 20 offspring of a single pair of parents ranged from + 43 to +.98 (average +.642), unless the eyes were virtually fully wild type.

RESULTS

$In(1)w^{m4}$: The main effects of the pattern of *Su(var)* on white-locus variegation are displayed in the histograms in Figure 2. As can be seen from the upper three graphs, *Su(var)/Su(var)* males have the most pigment and +/+ males, the least. Heterozygotes are intermediate. Each histogram pools the progeny of both broods from the replicated pair matings of two separate two-generation series. For many of the crosses, the divergence between the mean values for the separate vial cultures was too great to be attributed to sampling. The two broods from the same pair of parents were as likely to differ as two cultures from separate replication series. Thus both between- and within-culture non-genetic variance is reflected in the upper three graphs, and the significance of differences in means among crosses was tested against the between-vial variances.

Histograms for the reciprocal F_1's are plotted on a single set of axes. The F_1's with +/+ mothers have much less pigment ($P < .001$ that the two groups sample the same population).

Genotypic diversity for *Su(var)* appears in the greater variances in the backcross and F_2 progenies plotted in the lower three graphs. The averages for the three kinds of crosses agree closely with those predicted on the assumptions that (1) *Su(var)* genotypic ratios are Mendelian—the genotypes are equally viable; (2) the average phenotype for a given genotype from a given maternal genotype is unaffected by other genotypes in the same culture—or at least, the effects of the genotypes on each other are equal in magnitude and opposite in direction; and (3) the maternal "contribution" of a heterozygous mother is exactly intermediate

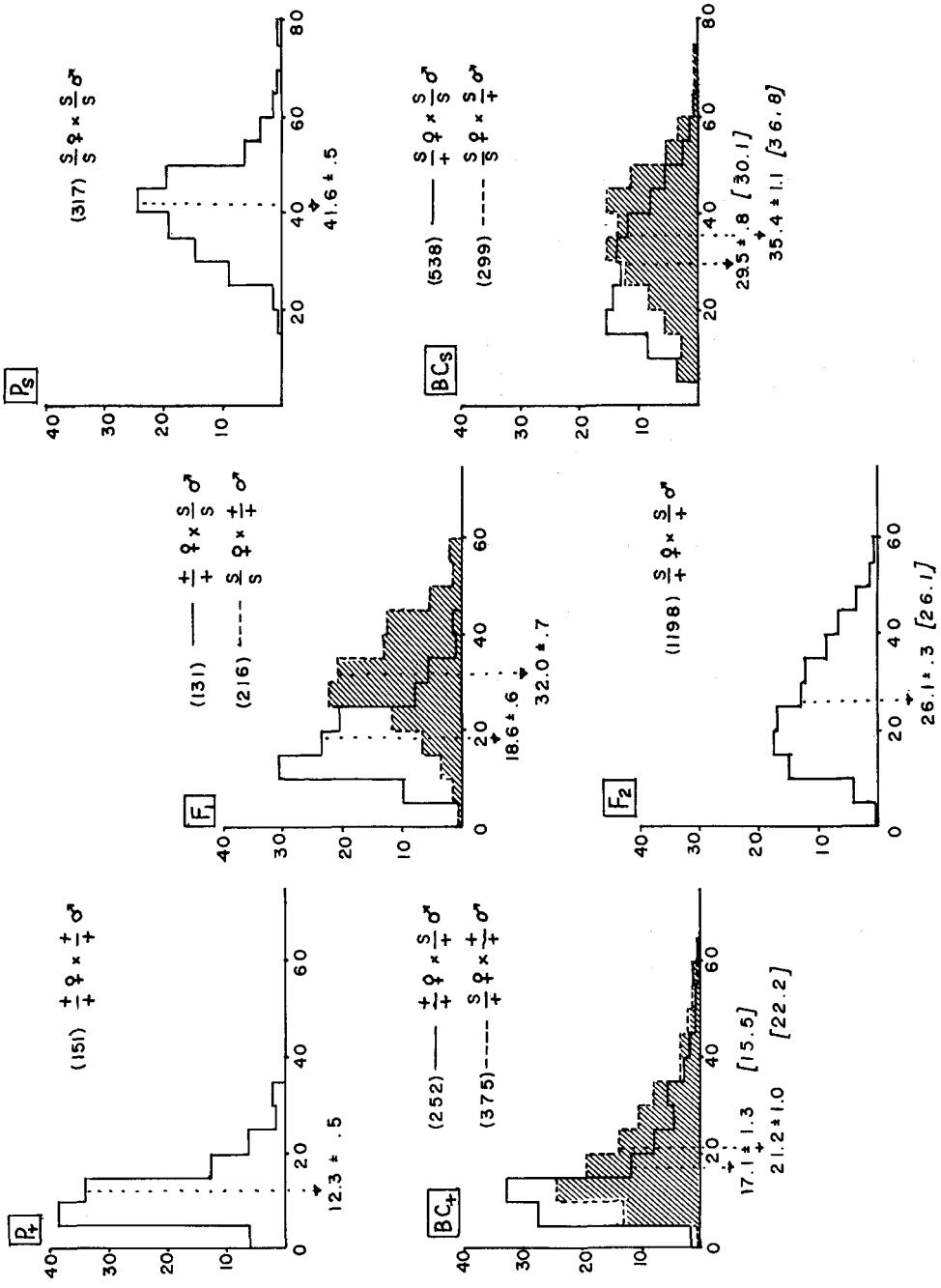

FIGURE 2.--Results for w^{m4}: Abscissa of each histogram: densitometer measurement of drosopterin eye pigment in head of single fly. Ordinate: percentage of offspring of indicated cross. The number of offspring measured is given in parentheses next to the parent genotypes; S = *Su(var)*. Below each histogram is the mean eye pigment value and the minimum standard error of the mean calculated without regard to the heterogeneity among the replicated sibships pooled in the frequency histogram. Estimated averages for crosses yielding two or more *Su(var)* genotypes, assuming Mendelian ratios, equal viabilities, and non-dominance of maternal effect, are bracketed to the right of the corresponding means. In three cases, F_1, BC_+ and BC_S, offspring of putatively identical arrays of genotypes from reciprocal crosses are plotted on the same axes. Broken outlines and cross-hatching indicate offspring whose maternal genome had more *Su(var)* alleles than those indicated by solid outlines.

between those of homozygous mothers. Thus the +/+ offspring of the backcross of F_1 females to +/+ males would be expected to average higher than 12.3 by the predicted maternal effect of ½(32.0–18.6) and the *Su(var)*/+ offspring higher than 18.6 by the same amount, yielding 22.2 for the overall average. Again in the backcrosses, the offspring of reciprocal crosses differed, though less strikingly. Pigment was always more intense in males whose mothers had more *Su(var)* alleles in their genotypes (for the reciprocal backcrosses to +/+, $F = 5.12$ and $.01 < P < .05$; to *Su(var)/Su(var)*, $F = 14.2$ and $P < .001$).

It is possible to rule out one interpretation of the difference between reciprocal crosses not associated with the *Su(var)* locus. The *Y* chromosome of an F_1 male in these attached-*X* crosses is derived from his mother and came initially from the same stock as did his mother's third chromosome. Should the *Y* chromosomes cause the difference between reciprocal crosses directly, rather than the *Su(var)* locus maternally, the sons of +/*Su(var)* mothers should have detectably less pigment than the sons of *Su(var)*/+ mothers in otherwise identical crosses. Table 1 presents the relevant information. There is no difference between the progenies of these two types of mothers, and thus no difference in the effectiveness of the *Y*'s of these stocks in suppressing variegation. The same data eliminate any importance of grandparental genotypes except as they contribute to parental genotypes. The progeny of crosses using +/*Su(var)* and *Su(var)*/+ parents are pooled in Figure 2.

The correlation between non-genetic within-vial variance and mean eye pigment is apparent in the first four lines of Table 2. This has a number of possible reasons, among which is the fact that the sector most likely to be pigmented (in the posterior border of the eye) is the smallest sector of cells derived from any one of the 20 presumptive eye cells in the first instar head anlage (BECKER 1959). The mosaic pattern has a strong cell-lineage basis, ommatidia of different sectors having different probabilities of being pigmented (BECKER 1961; BAKER 1963,

TABLE 1

Comparison of average red eye pigment measurements in w^{m4} *sons of* +/Su(var) *and of* Su(var)/+ *mothers*

Father	+/*Su(var)* mothers		*Su(var)*/+ mothers		Probability of greater difference‡
	Number measured	Mean pigment†	Number measured	Mean pigment†	
+/+	220	20.87***	155	21.68***	>.20
Su(var)/Su(var)	232	29.29***	306	29.57***	>.20
+/*Su(var)*	344	25.75***	270	24.96	>.20
Su(var)/+	284	25.60**	300	27.93	>.05

† Between-vial heterogeneity significant at 5% level indicated by (*); at 1% level, by (**); at 0.1% level, by (***).

‡ Analysis of variance performed on uncorrected measurements. Even with within-cross mean square not partitioned into between- and within-vial components, variance ratios were in all but the last case < 1. For the last row, when compared with the between-vial mean square, $F = 3.34$ with $P > .05$. For the last two rows combined—all F_2 progenies—$F < 1$ also even on the more stringent test.

1967), though pigmented and white ommatidia may lie side by side within a sector.

This correlation of variance with mean makes more difficult a comparison of within-vial variances of single-, two-, and three-genotype crosses, to exhibit the consequences of segregation for *Su(var)*. On the assumptions listed previously, the variance of a backcross sibship is expected to be

$$\sigma^2_{BC} = \frac{1}{2}[\sigma^2_1 + \sigma^2_2] + \frac{1}{4}(m_1 - m_2)^2 \quad (1)$$

where m = mean and the subscripts denote the two genotype groups as measured separately for the single mother genotype. The variance of the F_2 is expected to be

$$\sigma^2_{F_2} = \frac{1}{4}[\sigma^2_1 + m^2_1] + \frac{1}{2}[\sigma^2_2 + m^2_2] + \frac{1}{4}[\sigma^2_3 + m^2_3] - (\tfrac{1}{4}m_1 + \tfrac{1}{2}m_2 + \tfrac{1}{4}m_3)^2 \quad (2)$$

where subscript 1 denotes *Su(var)/Su(var)*, 2 denotes heterozygotes, and 3 denotes +/+; or to be

$$\sigma^2_{F_2} = \frac{1}{2}[\sigma^2{}_{BC_S} + \sigma^2{}_{BC_+}] + \frac{1}{4}(m_{BC_S} - m_{BC_+})^2: \quad (3)$$

Since *Su(var)*/+ and +/*Su(var)* parents gave indistinguishable progeny, these were pooled, reducing the number of distinct crosses from 16 to 9. Table 2 records the pooled within-vial mean squares of pigment measurements for each of the nine crosses. These are estimates of the variance attributable to the combined effects of segregation when present and of non-genetic factors. Estimates of variance for segregating progenies, calculated on the basis of the above equations, in all cases fall short of the observed variances. This discrepancy may indicate that the basic variance:mean relationship is concave downward instead of being linear as assumed in assigning variances to single genotypes with heterozygous mothers. Besides *Su(var)*, whether any other variegation-affecting loci on the uncontrolled and unmarked autosomes also segregated in these progenies can be neither confirmed nor disproved; however, one would expect the increment in variance due to segregation, given the individual P_1 and F_1 means, to be inversely related to the number of relevant loci.

In summary, the effects of the *Su(var)* locus on variegation of the white locus in *In(1)w^{m4}* were: (1) The effect of substituting one *Su(var)* allele for its + allele in offspring of +/+ mothers was to add 6.3 units of pigment to the eyes, as measured densitometrically; the effect of a second *Su(var)* allele, detectable in the offspring of *Su(var)/Su(var)* mothers, 9.6 units more. Thus on the scale employed there is a slight dominance of the + allele in production of the immediate phenotype.

(2) The effect of substituting a *Su(var)* for a + allele in either +-containing maternal genotype was to add 6.7 units of pigment to the eyes of the sons. There is no detectable dominance of either allele in its maternal effect on phenotype. The maternal constitution is virtually as important as the individual's own genotype in suppressing white variegation here.

(3) The between-generation influence of the *Su(var)* locus is limited to mother-to-progeny; there is no "grandparental" effect.

TABLE 2

Within-vial variance of red eye pigment for w^{m4} *males*

Mother	Genotypes expected		Mean eye pigment	Probability of homogeneity† of variances	Within-vial variance‡	Estimated variance*
+/+	+/+	(1)	12.32	.10	21.51	23.39
+/+	+/*Su(var)*	(2)	18.59	.01–.02	28.20	31.80
Su(var)/*Su(var)*	*Su(var)*/+	(3)	32.04	.05–.10	64.16	49.84
Su(var)/*Su(var)*	*Su(var)*/*Su(var)*	(4)	41.59	.20–.30	53.73	62.66
Su(var)/+ or +/*Su(var)*	+/+	(5)	*19.05*§	.....	....	*32.41*
Su(var)/+ or +/*Su(var)*	*Su(var)*/+ or +/*Su(var)*	(6)	*25.32*	.....	....	*40.82*
Su(var)/+ or +/*Su(var)*	*Su(var)*/*Su(var)*	(7)	*34.87*	.....	....	*53.64*
+/+ *Su(var)*/+ or +/*Su(var)*	1/2 (1):1/2 (2)		17.08	< .001‖	80.13	37.43
Su(var)/+ or +/*Su(var)*	1/2 (5):1/2 (6)		21.20	.001–.01¶	70.41	46.44
Su(var)/+ or +/*Su(var)*	1/2 (6):1/2 (7)		29.45	< .001††	87.96	70.05
Su(var)/*Su(var)* *Su(var)*/+ or +/*Su(var)*	1/2 (3):1/2 (4)		35.40	.05–.10	91.40	79.05
	1/4 (5):1/2 (6):1/4 (7)		26.08	.30–.50	101.59	73.90 (96.20‡‡)

† Based on Bartlett's test for homogeneity of variances (SNEDECOR 1946); within-vial mean square pigment measurements calculated separately for the two generations in each series for each cross producing the specified genotypes.

‡ Obtained by pooling residual sums of squares and degrees of freedom between the four replications for the indicated crosses.

* For single-genotype progenies, estimated $\sigma^2 = 6.85 + 1.34 \times$ mean, parameters were estimated by the method of least squares on the assumption that a straight line adequately related mean and variance in the obtained range. For multiple-genotype progenies, formulae (1) and (2) given in the text were applied to single-genotype estimates.

§ Estimates based on the assumption that the heterozygous maternal effect is exactly intermediate between the homozygous maternal effects.

‖ Range: 18.54–258.65.

¶ Range: 21.80–135.57.

†† Range: 14.10–102.66.

‡‡ Using formula (3) applied to the measured, as opposed to estimated, variances of the two backcrosses.

In(1)rst^3: Figure 3 presents the *rst*^{3} phenotypes for the six kinds of *Su(var)* genotypic arrays. The single-genotype progenies in the upper three graphs display the same kind of direct and maternal effects encountered for w^{m4}–wild-type action of the *rst* locus in the highest number of ommatidia when both mother and sons are *Su(var)*/*Su(var)*, with dominance not complete. Average values for the multiple-genotype progenies in the lower three graphs are close to those predicted (in brackets) on the assumptions of Mendelian ratios and non-dominance of maternal effects; i.e., since heterozygous progeny of *Su(var)*/*Su(var)* mothers average 16.2% more wild-type tissue than if their mothers had been +/+, progeny of *Su(var)*/+ mothers were assumed to average roughly 8% more than

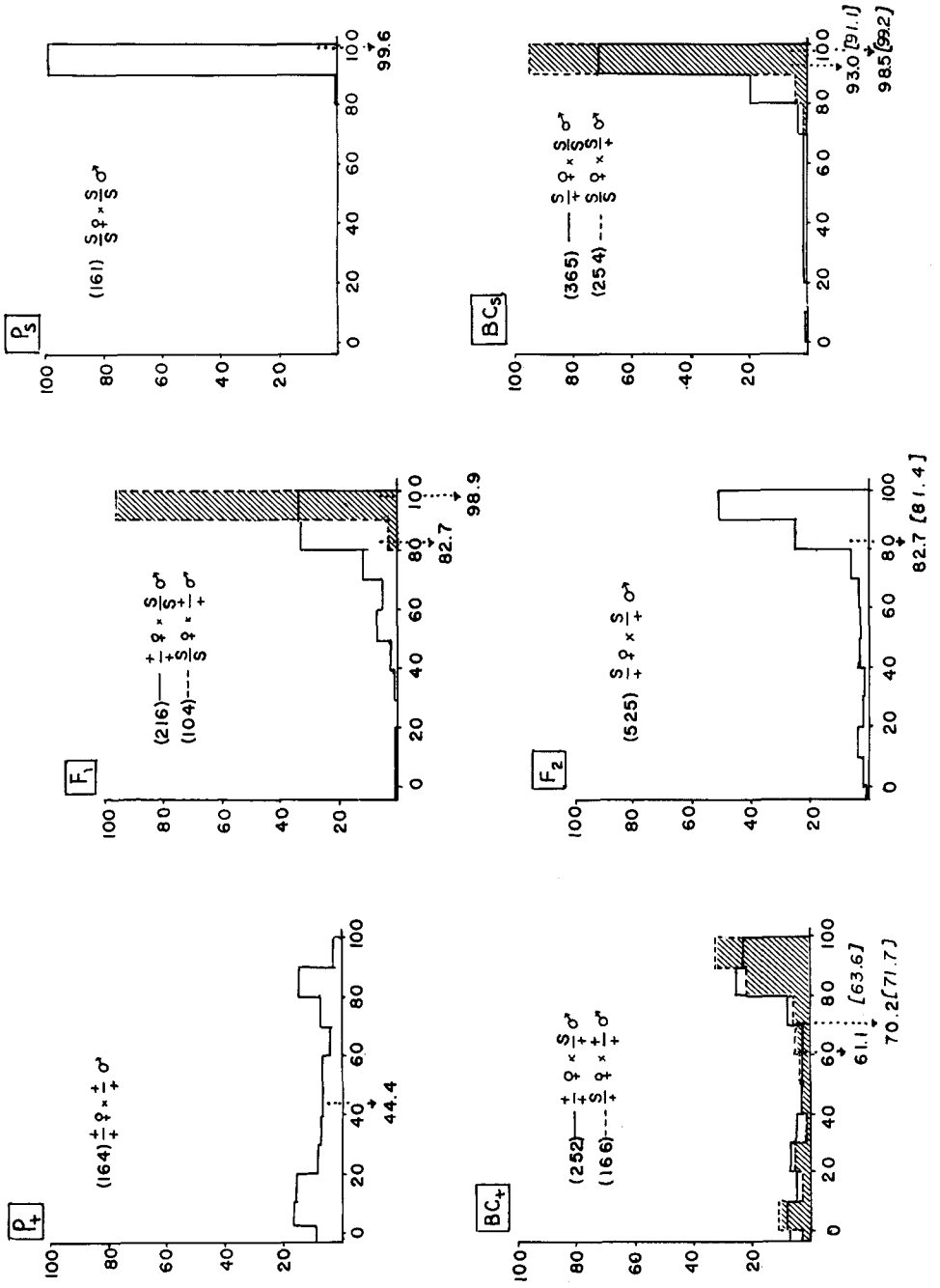

FIGURE 3.—Results for *rst*[3]: Abscissa of each histogram: Fraction of eye area wild type (with facets regularly arranged) in percent. Ordinate: percentage of offspring of indicated cross. The number below each histogram is the unweighted mean of the culture means. Other symbolism as in Figure 2.

those of a reciprocal cross using +/+ mothers, or 8% less than those of a reciprocal cross using *Su(var)/Su(var)* mothers. The latter difference in mean was too small to be detectable as statistically significant for the *Su(var)*/+ vs. +/+ mother comparison, but was highly significant for the *Su(var)/Su(var)* vs. *Su(var)*/+ mother comparison ($F = 17, P < .001$), using culture averages rather than individual values as units in both cases because of a high between-culture variance for each kind of cross.

It should be pointed out that although there appears to be no dominance in the maternal effect of *Su(var)*, there is a slight dominance (on the scale employed) of *Su(var)* over its + allele in its direct effect. This is reflected in the expected value for the F_2 being lower than that of either type of F_1.

Progeny of *Su(var)*/+ mothers can be compared with those of +/*Su(var)* mothers in Table 3. Again no systematic nor significant difference is apparent. The same kind of comparison for progeny of the two kinds of heterozygous fathers had similar results.

For each cross and replication date, the total phenotypic variance was sorted into between-culture and within-culture components. The between-culture component was too high to be ascribed solely to sampling in 13 out of the 32 instances. The two cultures (broods) from the same pair of parents were positively and significantly correlated only for the cross *Su(var)/Su(var)* × *Su(var)/Su(var)*, so that the culture means served as units for statistical comparisons between crosses.

The pooled residual within-culture variances for each of the nine genotypically distinct crosses are listed in Table 4. For the single-genotype progenies they quite closely approximate the simple binomial form $\sigma^2 = \alpha pq$. The coefficient α was estimated to be 0.16455 by the method of least squares. The expected within-culture variances for genotypes with heterozygous mothers were calculated from the previously-estimated phenotype values for these flies. These were then sub-

TABLE 3

Comparison of average percentage of regularly-arranged facets in rst[3] *sons of* +/Su(var) *and of* Su(var)/+ *mothers*

Father	+/*Su(var)* mothers			*Su(var)*/+ mothers			Probability‡
	Number		Area smooth† (in %)	Number		Area smooth† (in %)	
	Flies	Cultures		Flies	Cultures		
+/+	159	13	72.4**	154	12	63.9***	> .05
Su(var)/Su(var)	199	19	92.8***	166	13	93.7	> .20
+/*Su(var)*	151	13	85.7	134	9	80.7*	> .05
Su(var)/+	103	12	82.2	132	11	82.0*	> .20

† Mean proportion of wild-type tissue in eyes, estimated to nearest 5% for each fly. Between-vial heterogeneity significant at 5% level indicated by (*); at 1% level, by (**); at 0.1% level, by (***).

‡ Analyses of variance performed on vial means without further transformation of scale, since the means and thus also the variances to be compared were similar in size. For the last two rows combined, the F_2, $F = 1.95$ with $.05 < P < .20$.

TABLE 4

Within-vial variance of size of smooth areas in eyes of rst^3 *males*

Mother	Genotypes expected		Mean area smooth† (in percent)	Within-vial variance‡,§ (in percent^2)	Estimated variance‖ (in percent^2)
+/+	+/+	(1)	44.41	441.30	406.25
+/+	+/*Su(var)*	(2)	82.68	176.08	235.75
Su(var)/*Su(var)*	*Su(var)*/+	(3)	98.90	6.99	18.00
Su(var)/*Su(var)*	*Su(var)*/*Su(var)*	(4)	99.59	8.25	6.00
Su(var)/+	+/+	(5)	*52.55*¶	....	*410.25*
Su(var)/+	*Su(var)*/+	(6)	*90.79*	....	*137.75*
Su(var)/+	*Su(var)*/*Su(var)*	(7)	*91.48*	....	*128.25*
+/+	1/2 (1):1/2 (2)		61.11	1044.15	686.75
Su(var)/+	1/2 (5):1/2 (6)		70.16	971.10*#	639.50
Su(var)/+	1/2 (6):1/2 (7)		92.96	209.82***††	133.12
Su(var)/*Su(var)*	1/2 (3):1/2 (4)		98.50	10.94***‡‡	12.15
Su(var)/+	1/4 (5):1/2 (6):1/4 (7)		82.74	597.50	481.00

† Mean of culture means.

‡ Obtained by pooling residual sums of squares and degrees of freedom between the two replications of all crosses in category.

§ Variance ratio tests were performed when two crosses were pooled; Bartlett's test was applied to the four crosses pooled in the last row. Heterogeneity significant at 5% level indicated by (*); at 1% level, by (**); at 0.1% level, by (***).

‖ For single-genotype progenies, estimated $\sigma^2 = 1615.52 \cdot p\ q$, where p = the fraction of eye phenotypically wild type (smooth) and q = the fraction phenotypically *rst*^{3}, and the numerical coefficient provided the least-squares fit for such a binomial relationship to the four observed pairs of means and variances. For multiple-genotype progenies, formulae (1) and (2), given in the text, were applied to single-genotype estimates.

¶ Estimated on the assumption that for each maternal *Su(var)* allele an additional 8.11% of the eye becomes smooth rather than rough-faceted.

783.45 from +/*Su(var)* mothers, 1162.68 from *Su(var)*/+ mothers.

†† 111.40 from +/*Su(var)* mothers, 325.58 from *Su(var)*/+ mothers.

‡‡ 6.72 from +/*Su(var)* fathers, 14.65 from *Su(var)*/+ fathers.

stituted into equations (1) and (2) to yield estimated variances for the multiple-genotype progenies. Although the obtained variance was nearly always larger than the estimate, the two are clearly in the same size range, indicating that the *Su(var)* chromosome region was the only one segregating that is of major importance to *rst*^{3} variegation.

In summary, the effects of the *Su(var)* locus on variegation of the *rst* locus in *In(1)rst*^{3} were:

(1) The effect of substituting one *Su(var)* allele for its + allele in +/+ was to permit wild-type expression in an additional 38.3% of the eye; the effect of a second *Su(var)* allele, merely in an additional 0.7%. In both cases, the mutant area was reduced to approximately one-third of its former value by the substitution.

(2) The effect of substituting a *Su(var)* allele for a + allele in either +-containing maternal genotype was to permit wild-type expression in an additional 8.1% of the eye area in the sons. Thus, the maternal effect greatly outweighs the direct effect on the additive scale for near-wild-type phenotypes, but is outweighed by the direct effect for more mutant phenotypes.

(3) The between-generation influence of the *Su(var)* locus is limited to mother-to-progeny; there is no "grandparental" effect.

DISCUSSION

BECKER (1961) and BAKER (1963, 1967) have convincingly argued that the expression thresholds for the white locus juxtaposed to heterochromatin are established at the end of the first larval instar, when the primordium of the lower half of each eye consists of eight cells. From each of these cells, an area of contiguous facets develops, displaying topologically the cell lineage pattern. Estimation of the number of progenitor cells for the dorsal half of the eye by similar methods is precluded by cell migration continuing after that stage.

The discovery that the within-genotype variation in fraction of the eye expressing *rst* could be approximated reasonably well by a binomial model prompted the question whether variegation has the same clonal basis for the *rst* locus as for the *w* locus.

The non-genetic variance for *rst* variegation had been fitted reasonably well as for a binomial distribution, with $\sigma^2 = 0.1646\ pq$. There are several interpretations for α in the formula $\sigma^2 = \alpha\ pq$ for the variance of a fraction. The simplest is that there are $1/\alpha$ independent variable units contributing equally (i.e. sectors have equal areas) with equal probabilities of gene expression (i.e. p is constant throughout the eye). In this case, one would conclude that the thresholds for expression are set independently in 6 units (cells giving rise to clones), presumably three per eye. However, cell migration, intermingling clones, would continue until there are at least 8 progenitor cells for the lower half of each eye.

Other interpretations involve departures from the assumptions of complete independence of units, equality of resulting areas, or equality of resulting probabilities of gene expression. The total variance in expression is

$$\sigma^2{}_T = \sum_i^n \sigma^2{}_i + \sum_i^n \sum_{j \neq i}^{n-1} r_{ij}\sigma_i\sigma_j \qquad (4)$$

where there are n clonal sectors, of which the ith has the variance $\sigma^2{}_i$ and is correlated by an amount r_{ij} with the jth sector. If clonal areas, probabilities, and interclonal correlations are uniform, so that only the previous assumption of independence is removed, formula (4) reduces (see APPENDIX) to

$$\sigma^2{}_T = p\,q\,\frac{1 + r(n-1)}{n}, \qquad (5)$$

which retains the binomial form, but which yields a hyperbolic relation between r and n for any single value of α. Curve B in Figure 4 graphs this relation for the least-squares estimate of $\alpha = .1646$, and indicates the region within which the "true" curve most likely lies. A second relationship between r and n is provided by the correlation between rst^s values for right and left eyes among flies of presumably identical genotype developing in the same culture. It can be assumed as a simple working hypothesis that the correlation between the two eyes of a fly arises solely from the fact that each eye is composed of a set of clones and each clone is correlated to the same degree with every other clone, whether in the same

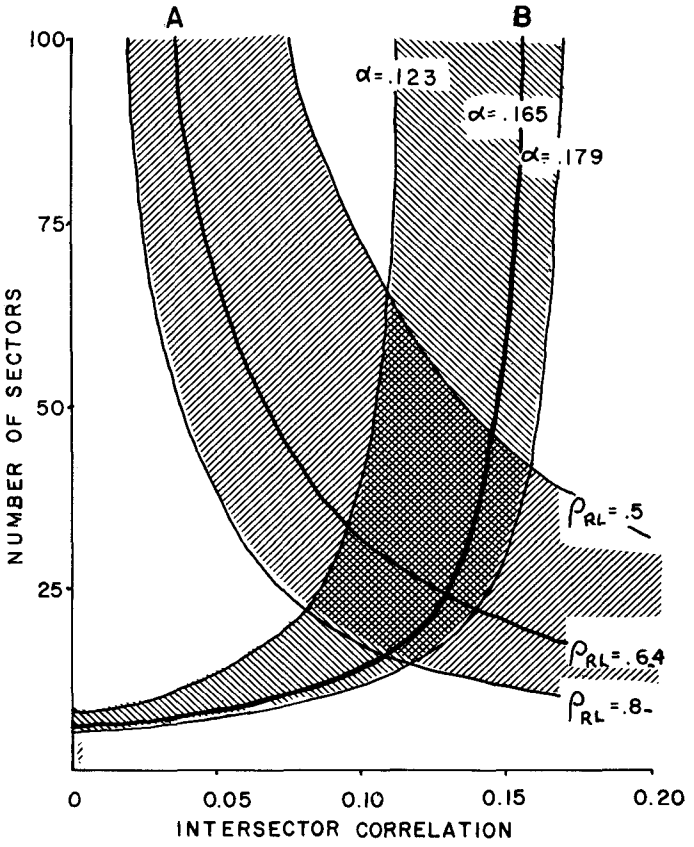

FIGURE 4.—*Curve A*: relation between number of eye sectors and average intersector correlation compatible with the observed mean right-left eye correlation = .642 for *rst*[3] variegation; cross-hatched region is bounded by the curves for right-left correlations of .5 above and .8 below. *Curve B*: relation between number of sectors and average intersector correlation on assumption that within-culture variance for single genotypes is binomial and arises solely from variation within sectors of equal area with equal probability of *rst*[3] expression, for the least-squares estimate of $\alpha = .1645525$; cross-hatched region is bounded above by the curve corresponding to the smallest obtained α (for +/*Su(var)*) and below, to the largest obtained α (for +/+).

or contralateral eye. Note that this assumption is incompatible with the hypothesis of a fixed amount of "exhaustible" determinative material per fly, which would generate a negative correlation between eyes (Professor R. C. LEWONTIN, personal communication). As derived in the APPENDIX, the correlation generated between right and left eyes by interclone correlation,

$$\rho_{RL} = \frac{r\,n}{2 + r(n-2)}, \tag{6}$$

gives a hyperbolic relation between r and n for fixed values of ρ_{RL}. This relation is plotted as curve A in Figure 4 for $\rho_{RL} = .642$, the average of obtained right-left correlations, again with an indication of the region within which the "true" curve most likely lies. Curves A and B intersect at $r = .129$ and $n = 24.3$ clones, a value

in the range anticipated (2×8 plus an undetermined number for the dorsal half of the eye) if *rst* variegated expression is subject to control at the same developmental time as for *white*.

It is, of course, unrealistic to assume constancy of so many parameters. In the APPENDIX is given a brief examination of the sensitivity of this estimate to variation in these parameters. Variation either just in strength of interclonal correlation or just in ultimate area of clones does not impair the binomial form of the variance (see APPENDIX formulae (5′) and (6′)). Variation in area, if neglected, leads to underestimation of the number of clones. Variation in probability of expression, however, or in more than one parameter at a time, can lead to departure from binomial form of the variance (APPENDIX formula (7′)). Neglect of variation in p would lead to an overestimate of the number of clones.

A realistic degree of variation in these parameters would not seriously affect total variance and, through "α", the estimate of the number of separately influenced clones. This can be crudely gauged from the data for w^m in flies heterozygous for $T(1;4)w^{258-18}$ (graphed in Figure 5 of BECKER's 1961 paper). For the lower half of one eye, the following values can be calculated from this figure: $n = 8$, $\bar{a} = .125$, $\sigma^2_a = .001738$, $\bar{p} = .58$, and $\sigma^2_p = .03023$. If the corresponding parameters for rst^3 varied by a comparable amount, the estimated number of clones would be in error by no more than one or two.

The data thus are consistent with the hypothesis that rst^3 expression is subject to a determinative episode at much the same time as for the two white-variegating systems whose sector patterns were studied. A determinative episode for rst^3 as much as 2 or 3 cell divisions earlier—leading to non-contiguous regions of single clones—than for w^{m4} remains a possibility. Both loci are active in the same clone, but the *rst* locus undoubtedly acts earlier than the *w* locus. The final number of ommatidial precursors is present as regularly arranged clusters of cells at the time of puparium formation (KRAFKA 1924; STEINBERG 1941), while the earliest effects of the white locus on pigment granule morphology are apparent in the two-day-old pupa (SHOUP 1966).

There are several steps in gene expression at which a variegation-suppressor might intervene—at some to minimize the initial variegation mechanism and at others to compensate for the resulting lower gene activity. Variegation may entail the inhibition of a locus juxtaposed to a chromosome region undergoing a different replication cycle (RUDKIN 1965; BERENDES and KEYL 1967). PROKOFIEVA-BELGOVSKAYA (1938), RUDKIN (*loc. cit.*) and SCHULTZ (summarized, 1965) described differences in nucleic acid content and activity of a single salivary chromosome band in its normal position and in a variegation-causing rearrangement to heterochromatin in the same nucleus. Whether a locus adopts the replication phase and cycle of the neighboring heterochromatin, or retains those characteristic of its normal position, may affect its later ability to produce messenger RNA. The heterochromatic cycle might well depart from the euchromatic cycle at different times in the development of different tissues—it has been clocked only for larval brain and salivary glands (RUDKIN 1965; BERENDES and KEYL 1967). The *Su(var)* allele products might diminish, or its + allele products extend, the region

entrained by the adjacent heterochromatin, the more effectively, the more of its product present at the time the heterochromatic specialization occurs within the cell lineage.

The *Su(var)* locus is not concerned with the function of a single specific heterochromatic region. However, it may be concerned with the preparation of a single specific euchromatic region (3C) for later activity.

As a compensatory factor, the *Su(var)* allele might amplify the signal to begin messenger-transcription, might augment the amount or activity of one of the components of the translation process, or improve the activity of the final gene product—although it would not be expected to have this last effect for very many different variegating loci.

Whether *Su(var)* acts on the initial variegation process or as a compensatory factor might be distinguishable by its time of action. One hypothesis consistent with the information presently in hand is that the *Su(var)* locus products act at two different times in the genesis of both variegated phenotypes. Products of the locus in the maternal genome, formed during oogenesis and stored in the egg, may act at the end of the first larval instar. The non-dominance exhibited in the maternal effects of the *Su(var)* locus on both variegating systems is consistent with this. Products of the fly's own *Su(var)* locus would act later, perhaps at the time of messenger production by the *w* and *rst* loci. This supposition would be consistent with the inconsistent dominance relations of the *Su(var)* locus alleles in their immediate effect on phenotype.

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SUMMARY

The *Su(var)* (Suppressor-of-Variegation) locus had previously been found to have similar effects on variegation of several loci included in $Dp(1;3)w^{m264-58}$. A study of the range of specificity of *Su(var)* effects was begun with two *X* chromosome inversions in which variegation of the same euchromatic region, 3C, was induced by proximity to a different heterochromatic region—distal to the nucleolus organizer in the *X*, rather than in the proximal part of the left arm of the third chromosome. The effects were the same as before:—For both white variegation with $In(1)w^{m4}$ and roughest variegation with $In(1)rst^{3}$, the maternal genotype *Su(var)/Su(var)* decreased and the maternal genotype +/+ increased the extent of mutant eye tissue in offspring. Offspring of *Su(var)*/+ mothers were approximately intermediate. This maternal effect was superimposed on the directly expressed effect of this locus. The smallest portion of the eye was mutant in *Su(var)/Su(var)* flies; the largest, in +/+ flies. *Su(var)*/+ flies were phenotypically more similar to +/+ for white variegation, and more similar to *Su(var)/Su(var)* for roughest variegation.—The variegation pattern seems to be laid down for the roughest locus at roughly the same time as for the white locus, namely, at the end of the first larval instar.—It is proposed as plausible that the

Su(var) locus effect on both variegating systems occurs at two times: the maternal effect at the end of the first larval instar during the setting of threshold levels for later gene action in the several progenitor eye disk cells, and the direct effect at some time during or immediately after the message transcription from these genes —probably before the end of the 3rd larval instar for the *rst* locus and by mid-pupa for the *w* locus.

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APPENDIX

1. *Variance in fraction of wild-type tissue:* Let the total area be subdivided into n semiautonomous regions (clones), each capable of wild-type or mutant gene expression. The ith region

has variance $\sigma^2{}_i$ and is correlated by r_{ij} to the jth region. Most generally, the total variance (in decimal fractions of total tissue with wild-type expression) is

$$\sigma^2{}_T = \sum_i^n \sigma^2{}_i + \sum_i^n \sum_{j \neq i}^{n-1} r_{ij} \cdot \sigma_i \cdot \sigma_j \tag{1'}$$

whereas the ith component variance will depend on a_i = the fraction of total area comprising the ith region and p_i = its probability of wild-type gene expression

$$\sigma^2{}_i = a_i{}^2 p_i q_i \tag{2'}$$

leading to the cumbersome expression resulting from substitution of (2') in (1'):

$$\sigma^2{}_T = \sum_i^n a_i{}^2 p_i q_i + \sum_i^n \sum_{j \neq i}^{n-1} r_{ij} a_i a_j \sqrt{p_i q_i p_j q_j} \tag{3'}$$

which can be simplified by various assumptions about constancy of parameters.

(a) Constant a, p *and* r: When constant, $a = 1/n$, and expression (3') reduces to

$$\sigma^2{}_T = \frac{p\,q}{n} + \frac{r\,n\,(n-1)}{n^2}\,p\,q = \frac{p\,q}{n}\,[1 + r(n-1)]. \tag{4'}$$

Note that a constant r is necessarily non-negative.

(b) Constant a *and* p *and variable* r:

$$\sigma^2{}_T = \frac{p\,q}{n}\,[1 + \frac{1}{n} \sum_i^n \sum_{j \neq i}^{n-1} r_{ij}] = \frac{p\,q}{n}\,[1 + \bar{r}\,(n-1)]. \tag{5'}$$

(c) Constant p *and* r, *variable* a: Expression (3') becomes

$$\sigma^2{}_T = p\,q \sum_i^n a_i{}^2 + r\,p\,q \sum_i^n \sum_{j \neq i}^{n-1} a_i a_j = p\,q\,[\sum_i^n a_i{}^2 + r \sum_i^n a_i\,(1 - a_i)]$$

since $\sum_{j \neq i}^{n-1} a_j = 1 - a_i$,

$$\sigma^2{}_T = p\,q\,[(1-r) \sum_i^n a_i{}^2 + r \sum_i^n a_i] = p\,q\,[(1-r)(\{n-1\}\,\sigma^2{}_a + \frac{1}{n}) + r]$$

where $\sigma^2{}_a$ has the usual formula for the variance of a, or

$$\sigma^2{}_T = \frac{p\,q}{n}\,[\,1 + r\,(n-1) + (1-r)\,n\,(n-1)\,\sigma^2{}_a]. \tag{6'}$$

(d) Constant a *and* r, *variable* p: Expression (3') becomes

$$\begin{aligned}\sigma^2{}_T &= \frac{1}{n^2} \sum_i^n p_i\,(1-p_i) + \frac{r}{n^2} \sum_i^n \sum_{j \neq i}^{n-1} \sqrt{p_i p_j q_i q_j} \\ &= \frac{1}{n^2}\,[n\,\bar{p} - (n-1)\,\sigma^2{}_p - n\,\bar{p}^2 + r \sum_i^n \sum_{j \neq i}^{n-1} \tilde{p}_{ij}\,\tilde{q}_{ij} \\ &= \frac{p\,q}{n} - \frac{n-1}{n^2}\,\sigma^2{}_p + \frac{r}{n^2} \sum_i^n \sum_{j \neq i}^{n-1} \tilde{p}_{ij}\,\tilde{q}_{ij},\end{aligned} \tag{7'}$$

where $\tilde{p}_{ij}$ symbolizes the geometric mean of p_i and p_j, etc.

2. *Right-left correlation arising from inter-region correlations:* Assume that the n semi-autonomous regions are randomly sorted into two subsets (e.g., eyes) of $n/2$ apiece. In the notation used in the previous section, the variance for one subset is

$$\sigma^2{}_S = \sum_i^{n/2} \sigma^2{}_i + r \sum_i^{n/2} \sum_{j \neq i}^{(n/2)-1} \sigma_i \sigma_j$$

and if $\sigma^2{}_i$ is assumed to be constant and thus $= pq/n^2$,

$$\sigma^2{}_S = \frac{pq}{2n}\,[1 + r(\frac{n}{2} - 1)]$$

Letting ρ signify the correlation between subsets,

$$\begin{aligned}\sigma^2{}_T &= \sigma^2{}_{S_1} + \sigma^2{}_{S_2} + 2\,\rho\,\sigma_{S_1}\sigma_{S_2} \\ &= 2\,\sigma^2{}_S\,(1 + \rho) \\ &= \frac{pq}{n}\,[1 + r(\frac{n}{2} - 1)]\,[1 + \rho]\end{aligned} \tag{8'}$$

A comparison of (4′) and (8′) discloses that

$$1 + r(n-1) = [1 + r(\frac{n}{2} - 1)]\,[1 + \rho]$$

which can be solved to yield an expression for ρ:

$$\rho = \frac{rn}{2 + r(n-2)}$$

which approaches 1.0 as n increases without limit. Given a measured value for ρ, this yields the functional relation between n and r:

$$n = \frac{2\rho\,(1-r)}{r(1-\rho)}. \tag{9′}$$

Variation in a_i reduces the correlation between subsets corresponding to any given n and r. It can be shown that

$$\rho = r\,n/[2 + r(n-2) + 2(1-r)n(n-1)\sigma^2{}_a].$$

Thus, for a given value of ρ, use of formula (9′) leads to an underestimate of n when $\sigma^2{}_a > 0$.