A VARIABLE PHENOTYPE ASSOCIATED WITH THE FOURTH CHROMOSOME OF *DROSOPHILA MELANOGASTER* AND AFFECTED BY HETEROCHROMATIN*

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ORIGIN

THE character called "sparkling" (*spa*) was first seen in some of the scute bobbed (*sc bb*) females of a stock of closed X (X^{cl}) heterozygous for *sc bb*. Two *sc bb* virgin females having rough eyes were mated separately each to three *sc bb* males from the same mass culture of *sc bb*/X^{cl} (the males of the stock, as is usual, did not show *bb* because of the presence of its wild-type allele in the Y chromosome). One female produced sparkling females, all of which were also *sc* and *bb*, and males which were all *sc* and not-*bb* and were not-*spa*.

The second female produced *sc bb; spa* females, and *sc* not-*spa* females, the latter all *bb* except one which was rated as an XXY female. The males were *sc* not-*bb* not-*spa* except one in which both *bb* and *spa* were manifested to an exaggerated degree. The exception judged by the exaggeration of *bb* was undoubtedly an XO male.

In the first culture the manifestation of sparkling accompanied that of the recessive sex-linked character bobbed, but in the second culture though all of the F$_1$ females were bobbed many of them were not-*spa*. The mother had been *spa* so that if *spa* were a recessive character, it had not been carried in the X chromosome of her mate. The two classes of females, wild-type and *spa*, in F$_1$ suggested that the father might have been heterozygous for a recessive autosomal gene whose expression in the F$_1$ males was suppressed in the presence of a Y chromosome. The exceptional XXY female (not-*bb* and not-*spa*) and the exceptional XO male (exaggerated *bb* and exaggerated *spa*), were in accord with the interpretation.

Females homozygous for *sc bb; spa* were mated to males carrying the dominant mutant Curly (*Cy*) in chromosome 2 and Stubble (*Sb*) in chromosome 3, and others were mated to males that were shaven naked (*sv^n*) a recessive mutant in chromosome 4; *spa* did not appear in the F$_1$ generation. In F$_2$ females, *spa* showed independent assortment with *Cy* and with *Sb*, but none of the F$_2$ *sv* females was *spa*; *spa* was thus shown to be a recessive character associated with a differential in the largely heterochromatic fourth chromosome. The region associated with the expression of *spa* was determined by use of the translocation T(2; 4)b in which the break has been found by E. B. LEWIS (unpublished) to be distal to the proximal bands of 102C (BRIDGES' salivary chromosome map, 1935). In crosses between *spa* and the translocation, males and females survived that carried a normal second chromosome from each

parent, one fourth chromosome from the parent that was *spa* and the 4-2L fragment which was composed of the centromere and proximal region of chromosome 4, as far as to include the proximal region of 102C, and of the tip of 2L. The hyperploid (for 2) is recognized as a short plump fly with small scutellum, broad thorax, small spread wings and small sometimes knotty legs and bulging eyes. Dp(2; 4)b flies both female and male that carried the single fourth chromosome showed a very high degree of *spa*, indicating that the altered region of the chromosome which is in some way associated with *spa* is distal to nearly all of region 102C. In salivary glands of larvae heterozygous for *spa* there appears a disturbance in the distal range of 102C, proximal to 102D1. The character and the extent (perhaps variable) of the disturbance have not been determined.

By genetic methods *spa* is found to be related to the dominant mutant Cataract (*Cat*) in the fourth chromosome discovered by BELGOVSKY (unpublished). *Cat* is recognized as a roughness and thickening of the posterior rim (or half) of the eye visible in both males and females. In flies heterozygous for *Cat* and *spa* the anterior part of the eye is slightly *spa* in XX females and decidedly *spa* in XO males.

From the first matings of *spa* it had been found that its manifestation was in some way correlated with the presence and absence of the Y chromosome but not in the sense that *bb*, which was present with *spa*, is suppressed by Y. The normal allele of *bb* in Y accounts for the latter whereas *spa* was found to be associated with a differential located in the fourth chromosome. The effect of Y on *spa* suggested rather the suppression of mosaicism in the class of position effects which are known as variegations, such as Plum (*Pm*) in the second chromosome, the white variegated series in the X and numerous others.

The eye of sparkling presents no visible mosaic pattern as is seen in roughest (*rst³*, KAUFMANN 1942) or in eye color variegations, but the grade of roughness in individuals varies among the progeny of pairs of flies as does the extent of mosaicism shown by individuals in populations of variegations. With this lead experiments were made to test *spa* for other properties of variegations, namely, differences in the extent of expression at different temperatures and reaction to differing amounts of heterochromatin in the nucleus; also incidental observations were made bearing on the detection of a possible maternal effect of heterochromatin.

The eyes of homozygous sparkling females are often somewhat bulging. The ommatidia appear to vary in convexity and to vary in size so that their boundaries form uneven lines giving a disturbed appearance to the surface of the eye. The posterior rim of a slightly *spa* eye may be somewhat angular ventrally rather than smoothly curved. In low grades of *spa* the rough effect varies if the eye is turned to receive the light at different points. A sparkling effect may result from uneven illumination of the irregular ommatidia and from reflection of light from the hairs between, which seem to be longer than normal; with increased degree of *spa* the glistening hairs may project well beyond the surface of the eye when viewed from above. In extreme *spa* no dark fleck or rays are seen, and the eye is often small and condensed (ommatidia

sometimes unevenly crowded); although a high degree of *spa* may occur also in large eyes (differences characteristic of different lines).

In order to facilitate classification an arbitrary scale of seven degrees of *spa* was chosen. An eye of grade 6 is exceedingly rough, has no fleck, and is often bulging, round, and small. At the other extreme, in grade 1, roughness is very slight, may appear not to involve all of the eye and is sometimes discernible only when the eye is seen in certain positions in relation to the source of light; it scarcely differs from grade 0 or smooth. Grade 2 is evidently sparkling and three more steps (grades 3–5) lead to the extreme, grade 6. When feasible, one parent of the matings was heterozygous for *spa* and for its wild-type allele in a chromosome marked by the dominant wing character, cubitus interruptus dominant (ci^D). Thus homozygous *spa* eyes could be compared in the same conditions directly with wild-type eyes which sometimes give the appearance of a surface which is granular rather than entirely smooth.

The counts of ci^D flies have not been entered in the tables. The ci^D flies have been found very useful for direct comparison in classification of grades 0 and 1 although even so the separation probably is not entirely accurate; in no case has there been any decided roughness in eyes of ci^D flies. In exp. 371 the eyes of ci^D looked granular and the sixteen $\underline{XX}$Y females classed as grade 1 may have been grade 0.

As there is no actual division between grades, the classification of borderline cases varies. Various factors may cause roughness of eye and for this reason normal controls were present whenever possible in the same cultures in which *spa* was rated in flies of altered genetic constitution, or sibs were used as P_1 flies.

The flies were raised at about 18°C except those in a few experiments where a higher temperature is noted in the tables. Three of these were controls for the effect of temperature.

In the description of matings the females are mentioned first. About three to five females were usually used in one culture, or in some experiments only one pair of flies, and the results of the separate cultures were summated.

The frequency distribution of grades of *spa* in eyes of flies of normal constitution is shown in table 2 and on the right of table 3. It may be seen on inspection that in every experiment except 131a, 131l (table 3), and 58bi (table 2) there is one mode of distribution and that the expression of *spa* was greater in females than in males. The percentage of females above grade 3 has been chosen as the distribution index of frequencies of grades of *spa* in females of normal constitution and the percentage over grade 1 as the index for normal males (table 5). The index for males is often lower than the index for females; although it is based on a frequency which includes two grades lower than the base of the female index. It is noticeable also that a shift in distribution is always in the same direction in both sexes and the modes of distribution vary correspondingly (table 5). In 1944 the smoothest flies and the most extreme *spa* of the two bimodal lines mentioned above were segregated, and in 1946 there are two nearly pure lines differing in the expression of *spa*, from each one of the original lines. There is also a third instance of a line (no. 102) of

high degree of *spa* which was separated in 1944 from smoother flies. These had been used in a test of effect of age on the expression of *spa*. The flies were graded daily for six days and they showed no effect of age. The distribution did not give two modes, but the high frequencies overlapped three successive

TABLE 1

F_1 *homozygous spa* (at $18°±$, $37l*$ at $22°±$). *All parental fourth chromosomes spa unless denoted as* ci^D. *119m*, X^-X^-Y, y sc $bb^{Df\ G}$; $ci^D×y$ sc $bb^{Df\ G}$. *53B*, X^-X^-Y, y sc $bb^{Df\ G}×y$ sc $bb^{Df\ G}$; ci^D. *87B, same. 53A*, $\underline{XX}Y$, $\underline{bb}×y$ sc $bb^{Df\ G}$. *105p*, $\underline{XX}Y$ $\underline{bb}$; $ci^D×y$ sc $bb^{Df\ G}$; ci^D. *37l*, $\underline{XX}Y$ $\underline{bb}$; $ci^D×y$ sc $bb^{Df\ G}$; ci^D. *37p*, $\underline{XX}Y$ $\underline{f}×y$ sc $bb^{Df\ G}$; ci^D. *114a*, $\underline{XX}O$ $\underline{bb}×(bb?)$. *114j*, $\underline{XX}O$ $\underline{bb}×sc$ bb. *114k*, $\underline{XX}O$ $\underline{bb}×bb$.

NO.	P_1♀	F_1	0	1	2	3	4	5	6
119m	X^-X^-Y	X^-X^-Y	42	48	2	—	—	—	—
		X^-Y	—	2	33	43	16	3	—
		X^-YY	64	—	—	—	—	—	—
53B	X^-X^-Y	X^-Y	—	—	20	21	3	—	—
		X^-YY	42	—	—	—	—	—	—
87B	X^-X^-Y	X^-X^-Y	19	28	—	—	—	—	—
		X^-Y	—	—	—	49	54	—	—
		X^-YY	66	—	—	—	—	—	—
53A	$\underline{XX}Y$	$\underline{XX}Y$	n	($\underline{XX}O$	—	—	—	17	12)
		X^-Y	—	—	—	26	34	—	—
105p	$\underline{XX}Y$	$\underline{XX}Y$	55	($\underline{XX}O$	—	—	1	5)	—
		X^-Y	—	—	1	8	27	7	—
		X^-YY	5	($\underline{XX}O$†	—	—	1	11)	—
37l*	$\underline{XX}Y$	$\underline{XX}Y$	24	16	($\underline{XX}O$	1	3	2)	—
		X^-Y	—	6	20	52	13	—	—
		X^-YY	16	($\underline{XX}O$†	—	—	12	4)	—
37P	$\underline{XX}Y$	$\underline{XX}Y$	62	($\underline{XX}O$	—	—	3	7	4)
		X^-Y	—	—	4	30	35	5	—
		X^-YY	3	—	—	—	—	—	—
114a	$\underline{XX}O$	$\underline{XX}Y$	14	—	—	—	—	—	—
		XO	(2XY)	—	—	—	—	14	10
114j	$\underline{XX}O$	$\underline{XX}Y$	40	—	—	—	—	—	—
		XO	—	—	—	—	—	6	22
114k	$\underline{XX}O$	$\underline{XX}Y$	69	—	—	—	—	—	—
		XO	—	—	—	—	—	26	21

† Additional $\underline{XX}O$ females.

grades (2 to 4). In the highest grades were two kinds of males not easily separable. The eyes of one kind were small, slightly lighter red, and less uniformly rough. These males were chosen as the male parents which produced the rougher line. A test of the high line derived from 58bi for a new factor for roughness not in the fourth chromosome gave a negative result. The other lines were not tested.

In some strains of *spa* which were obtained from exceptions among progeny of X-rayed males (MORGAN, REDFIELD, and MORGAN 1943) the eyes are smooth, in others the eyes are extreme *spa* both in males and in females. These phenotypes are recessive to wild type.

The distribution indices of normal genotypes (table 5) are widely different, varying from o percent to 100 percent for females and from o to 72 percent (in four experiments 79 to 98 percent) for males. Many of the higher indices were obtained from populations into which *spa* had just been introduced with

TABLE 2

F_1 *homozygous spa and samples of stock* [74], [a], *etc.* (*at* $18°\pm$, *at* $22\pm marked$,* *at* $25°$ *marked†*). *All parental fourth chromosomes spa unless denoted as* ci^D *or* ey^D. *58a, sc bb* [74]. *103a, sc bb* [*ext.*]. *59a, sc bb;* ci^D [a]. *121a, (bb?);* $ci^D\times(bb?)$. *121l, (bb?)*$\times$*(bb?).* *120a, (bb?);* $ey^D\times(bb?)$. *120l, (bb?)*$\times$*(bb?).* *58l, sc bb;* ci^D [a]. *58s, sc bb;* ci^D [a]. *23a, sc bb* [a]. *23t,† same. 58bi,* sc bb* [75]. *58y*, sc bb* [75^h]. *58z*, sc bb* [75^l]. *57l,* $\underline{XXY}$ $\underline{bb}\times sc\ bb$. *57v,* $\underline{XXY}$ $\underline{f}\times sc\ bb;$ ci^D.

NO.	F_1	o	1	2	3	4	5	6
58a	XX	4	6	51	63	16	—	—
	XY	72	23	6	1	—	—	—
103a	XX	—	—	24	29	19	9	—
	XY	26	38	10	11	3	—	—
59a	XX	—	—	—	9	74	8	—
	XY	—	27	58	38	3	—	—
121a	XX	—	—	3	11	32	20	—
	XY	1	13	19	6	9	2	1
121l	XX	—	—	—	—	32	163	—
	XY	—	16	47	67	29	2	1
120a	XX	—	—	—	3	30	5	—
	XY	3	23	23	6	—	—	—
120l	XX	—	—	—	9	55	95	—
	XY	4	38	36	26	17	—	—
58l	XX	—	—	—	—	—	23	—
	XY	1	2	4	16	12	—	—
58s*	XX	—	—	—	10	—	—	—
	XY	10	—	—	—	—	—	—
23a	XX	—	—	3	39	111	25	—
	XY	—	2	81	77	16	2	—
23t†	XX	2	31	106	37	3	—	—
	XY	162	29	1	—	—	—	—
58bi*	XX	—	1	4	12	3	8	14
	XY	16	3	—	—	9	15	3
58y*	XX	—	—	—	—	2	5	13
	XY	—	—	—	1	3	7	9
58z*	XX	1	5	4	5	5	—	—
	XY	4	9	3	2	1	—	—
57l	XY	—	69	67	7	—	—	—
57v	XY	4	58	78	18	—	—	—

some other chromosome, such as deficiency for chromosome 2 or 4, or ci^D and ey^D (eyeless dominant) in exps. 121a, 121l, 120a, 120l. In making the matings the more extreme *spa* flies were probably selected from the stocks from which they were chosen. Some of them may have represented a new extreme line rather than a sample from the higher grades of a uniform line.

EFFECT OF TEMPERATURE ON THE EXPRESSION OF *spa*

A high degree of *spa* had been noted whenever flies had been raised at low temperatures, but the wide range of modes of distribution of flies of normal constitution (cf. tables 2 and 5, right of table 3) makes it clear that the effect of a factor under investigation can be judged only by the most complete control. A sample of flies of stock (a) raised at about 22°C (table 2, exp. 58s) consisted of ten females of grade 3 and ten smooth males. A sample raised at the same time at about 18°C (exp. 58l) consisted of 23 females of grade 5 and 35 males of which 91 percent were above grade 3. About two years later a larger sample of the same line of flies was raised at about 19° (table 2, exp. 23a) and a control at 25° (exp. 23t) and gave similar results.

Two relations are evident. At both temperatures females show a higher degree of *spa* than do the males of the same populations, and at the lower temperature there is a shift toward more extreme *spa* in both sexes.

Exp. 23t shows an aggregation of 85 percent of the males in grade o; these are all the males below the highest two grades in which any males occur. In exp. 23a the males below the highest two grades, in this case 90 percent of males, are distributed among three classes. This must mean that beyond the threshold of what is called normal (that is, the grade which appears in the wild-type fly) there is, at 25°, variation in the degrees of *spa* which would have been visibly expressed if the flies had been raised at 19°. It would then follow that when a maximum frequency is found in grade o, the mode is beyond the threshold of normal and is not detectable. Accordingly, the grade of the mode cannot be determined (cf. tables 4 and 5). It may be that there are undetectable characteristics of the *spa* eye which are an expression of grades of *spa* beyond the normal threshold at 25°, just as at 19° differences are visible among the smoother grades.

THE EFFECTS OF DIFFERENT AMOUNTS OF HETEROCHROMATIN IN THE NUCLEUS

Sensitivity to amounts of heterochromatin in the nucleus which is shown by *spa* and by the variegation type of position effect (NOUJDIN 1939) was studied in different genotypes. The pronounced effect of low temperature on the expression of *spa* was utilized in experiments involving various amounts of heterochromatin. There was an advantage in raising the flies at 17° to 19° because *spa* would be visibly expressed in a greater number of flies and because rougher eyes are more easily classified than smoother.

It had been shown by the progeny of the first matings of sparkling flies which were also bobbed that the difference in the expression of *spa* in females and males was not, as in the case of *bb*, due to a normal allele in the Y chromosome; a differential of some kind associated with the character *spa* was found to be located in the fourth chromosome. The effect of Y might resemble the effect found in variegations which is correlated with the ratio of heterochromatin to euchromatin in the chromosome complement. Accordingly, the degrees of *spa* were measured when different amounts of heterochromatin and euchromatin were present in the sex chromosomes or in an autosome.

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TABLE 3

F_1 homozygous spa; left, modified genotypes, right, normal genotypes in the same population (at $18°\pm$ except 119k* and 90t* at $22°\pm$). All parental fourth chromosomes spa unless denoted as ci^D, Df 4 or 0. 130A, Dp(1; f) 101/y^2 cv v(bb?)/y^2 cv v(bb?); $ci^D \times y^2$ cv v(bb?); ci^D. 11a, same. 11m, y^2 cv v(bb?)$\times y^2$ cv v(bb?). 104k, y sc $bb^{Df\ G}$/bb; $ci^D \times bb$. 104n, same. 94a, same. 94k, bb; $ci^D \times bb$; ci^D. 119k, bb; $ci^D \times y$ sc bb^{Df}; ci^D.

NO.	P₁♀	F₁	0	1	2	3	4	5	6
130A	$X^{Dp}XX$	$X^{Dp}XX$	—	25	42	7	3	—	—
		$X^{Dp}XY$	19	(Sample)	—	1	—	1	—
11a	$X^{Dp}XX$	$X^{Dp}XX$	—	—	50	27	2	—	—
11m	XX	—							
104k	X⁻X	X⁻X	—	—	—	11	35	39	—
		X⁻Y	2	25	49	9	—	—	—
104n	X⁻X	X⁻Y	2	11	10	—	—	—	—
		—							
94a	X⁻X	X⁻X	—	—	—	—	16	38	29
		X⁻Y	—	49	55	—	—	—	—
94k	XX	—							
		—							
119k*	XX	X⁻X	—	—	1	5	15	7	—
90a	XX	XY^{bbl}	—	—	—	—	—	7	—
90t*	XX	XY^{bbl}	—	—	10	8	6	6	1
109	$2^-/2$	$XX2^-/2$	—	—	—	—	—	30	17
		$XY2^-/2$	—	—	—	1	11	24	10
131a	$2^-/2$	$XX2^-/2$	1	4	14	19	10	37	77
		$XY2^-/2$	4	8	4	8	11	25	89
131l	2/2	$XX2^-/2$	1	1	6	7	17	5	43
		$XY2^-/2$	2	6	6	4	1	15	25
125a	$4^-/4$	$XX4^-/4$	19	—	—	—	—	—	—
		$XY4^-/4$	20	—	—	—	—	—	—
128a	$4^-/4$	—							
128l	4/4	—							
114w	4/o	XX4/o	—	—	—	—	—	15	—
		XY4/o	—	—	26	30	11	3	—
114v	4/4	XX4/o	—	—	—	—	—	27	—
		XY4/o	—	1	22	43	23	3	—
129a	4/o	XX4/o	—	—	—	—	—	—	6
		XY4/o	—	—	—	—	1	5	—
129l	4/4	XX4/o	—	—	—	—	—	4	13
		XY4/o	—	—	—	4	30	15	1

Females carrying a Y and males without a Y chromosome were obtained in the same experiments (table 1, exps. 114a, j, k) among the progeny of attached-X females without a Y (XXO). Such females were daughters of attached-X homozygous *bb* females (XXY) mated to males in which nondisjunction was frequent; the character *bb* when visible in the females indicated the absence of the Y chromosome. Their XXY daughters were 100 percent normal, and the XO sons were of the highest grades of *spa*, 5 and 6 (some of the males were genetically *bb* and these showed exaggerated *bb*). The addition of Y to

TABLE 3

(Continued)

90a, sc bb×sc bb/Y^{bb1}. 90t, same. 109, Df(2) Ms10 ci^D. 131a, Df(2) Ms10×not-Df. 131l, not-Df ×Df(2) Ms10. 125a, Df(4) M-4×spa. 128a, same. 128l, spa×Df(4) M-4. 114w, (bb?); spa/0 ×(bb?). 114v, (bb?)×(bb?); spa/0. 129a, spa/0×spa. 129l, spa×spa/0.

NO.	P₁♀	F₁	0	1	2	3	4	5	6
130A	X^{Dp}XX	XX	—	—	11	45	31	3	—
		XY	36	1	2	1	(Sample)		(1 XO?)
11a	X^{Dp}XX	XX	—	—	13	46	16	2	—
11m	XX	XX	(1 XXY?)	—	16	90	35	11	—
104k	X$^-$X	XX	—	2	29	37	20	1	—
104n	X$^-$X	XY	78	1	—	—	—	—	(2 XO?)
94a	X$^-$X	XX	—	4	13	33	52	14	3
		XY	70	8	—	—	—	—	—
94k	XX	XX	—	—	—	8	33	18	2
		XY	40	17	1	—	—	—	—
119k*	XX	XY	n	—	—	—	1	—	(2 XO?)
90a	XX	XX	—	—	—	—	—	75	1
90t*	XX	XX	—	—	1	2	13	10	—
109	2$^-$/2	XX	—	1	7	19	8	1	—
		XY	9	11	5	—	—	(1 XO?)	—
131a	2$^-$/2	XX	17	41	33	20	33	25	6
		XY	2	59	47	15	8	11	6
131l	2/2	XX	17	14	18	16	19	6	—
		XY	36	9	9	1	2	—	—
125a	4$^-$/4	XX	—	—	2	16	20	2	—
		XY	6	19	15	—	—	—	—
128a	4$^-$/4	XX	—	—	6	18	42	54	3
		XY	13	34	53	27	11	6	—
128l	4/4	XX	2	3	8	22	31	39	4
		XY	23	29	36	17	7	6	2
114w	4/0	XX	—	—	35	17	—	—	—
		XY	42	1	—	—	—	—	—
114v	4/4	XX	—	5	20	10	4	—	—
		XY	29	10	—	—	—	—	—
129a	4/0	XX	—	—	—	27	19	1	—
		XY	6	9	23	—	—	—	—
129l	4/4	XX	1	3	34	36	30	9	—
		XY	19	20	5	—	—	—	—

XX in females suppressed *spa*, and in the XO males which lacked a Y the expression of *spa* was decidedly more extreme than in XY males.

It is to be noted that XXO females show a high degree of *spa*. This was observed in exps. 53A, 105p, 37l, and 37p (table 1, 37l at 22°) and in the stock which was examined at the same time. All of the XXO females were of grade 4 at least and usually even higher. The attached-X combination is deficient in heterochromatin due to loss of the right arms of the two X's and due perhaps also to uneven crossing over in the male in which the original attachment

of the X's arose. The lines used probably go back to the yellow attached-X which came from a yellow male (L. V. MORGAN 1938). However that may be, the Y chromosome suppresses a very high degree of *spa* for the $\underline{XX}$Y females in the same experiments were all smooth with the exception of a few rated as 1 in exp. 371 in which the ci^D controls were slightly granular.

The decided effect of the heterochromatic Y chromosome shown by the differences in the manifestation of *spa* in XO and XY, and in XX and $\underline{XX}$O as compared with XXY and $\underline{XX}$Y flies, led to experiments to show the grade of *spa* when only part of a Y chromosome is present.

The long arm of Y contains a mutant allele of *bb* and the short arm contains a normal allele. The loss of a region including the normal allele of *bb* may be detected when a deficient Y is used in combination with an X chromosome which carries *bb*.

A Y known as Y bobbed deficiency (Y^{bb-}) and described as deficient for *bb* lacks two-thirds of the short arm of Y; the wild-type allele of *bb* is included in the deficient region as shown by $\underline{XX}Y^{bb-}$ females that carry a mutant *bb* gene in each X and in the long arm of Y and are visibly *bb*. Such females when homozygous also for *spa* showed if any a very slight degree of *spa* at room temperature. At 19°C *spa* was more decided and was higher in the XY^{bb-} males than in the $\underline{XX}Y^{bb-}$ females.

Another loss of heterochromatin is recognized in so-called "Y sterile" (Y^{st}) which is a rod shaped chromosome cytologically resembling the long arm of Y in length and in having a similar constriction. Y^{st} carries a bobbed allele, X^{bb}; Y^{st} male is *bb* and sterile. Y^{st} is carried in stock of $w^e\ bb^l/w^ebb^l$; Y^{st} females and w^ebb^l; Y males. Bobbed females that survive are homozygous for bb^l and carry Y^{st} and are saved from the lethal effect of bobbed lethal (bb^l) by the allele of *bb* which is present in Y^{st}. After ci^D had been introduced into the stock, females were mated to *sc cv v f bb^l; spa* males and their spa/ci^D offspring were inbred. A few homozygous sparkling females of the F_2 and F_3 generations were obtained. They carried bb^l/bb^l; Y^{st} and were low grade *spa* at 25° and of higher grade at 19°. The heterochromatin of the long arm only of Y did not suppress *spa* when present with two X's carrying bb^l.

Males showing the character *bb* are found (rarely) in *bb* stocks. The manifestation of *bb* may be explained (as in the case of XY^{bb-} and XY^{st} where there is cytological evidence) as due to a deficiency, in the short arm of Y, of a region including the wild-type allele of *bb*.

Three such Y chromosomes have been introduced into *sc bb; spa/ci^D* flies. Y^{bb} was found by BRIDGES in 1926. Y^{bb36} and Y^{bbl} have been more recently found in two different *bb* stocks each in a single male showing *bb*.

XY^{bbl} males raised at 19° showed a very high degree of *spa* but so also did the XX females which can be used as controls for the effect of the Y (table 3, exps. 90a and t). The mode of distribution of males carrying a normal Y is usually well defined and is two or more grades below the mode for females. At 19° an effect lethal to males was present with Y^{bbl} (ratio of the total was 1:13) and there were but seven males in the sample; these were all of grade 5 though very slightly less *spa* than their sisters which were also within grade 5 except

one of grade 6. At 25° the males (in a 1:1 ratio) were more different from the females but again less different from their sisters than are XY males from their sisters.

XY^{bb1} males were directly compared at different temperatures and at different times with XY^{bb36} and XY^{bb}. The expression of *spa* was about the same in all of the males (a little more decided in the flies carrying Y^{bb36}). They all showed the same characteristic, the males were only slightly less *spa* than the XX females as is to be expected if the Y's (as the expression of *bb* in males indicates) were to some extent deficient.

The effect of reduction of heterochromatic content of the X chromosome was also studied; *spa* was rated in normal flies and in sibs which carried X chromosomes known to be deficient for *bb* and for neighboring heterochromatic regions. Such an X is GERSHENSON's bobbed deficiency (X$^{bb\ DfG}$ or X^{-}) in which the locus of *bb* and heterochromatin to the right and left of *bb* are missing. The deficient chromosome is marked by the mutant yellow; and when used with a chromosome which carries *bb*, all classes are distinguishable.

In exp. 94a (table 3) the expression of *spa* in X^{-}X and X^{-}Y offspring of X^{-}X females was markedly higher than in the XX and XY offspring respectively, and the same relation held as between the two classes of females in exp. 104k and as between males in exp. 104n. Reduction of heterochromatin in X was correlated with more extreme *spa*.

Spa was observed also in flies carrying the deficient X found by DOBZHANSKY. Both DOBZHANSKY's and GERSHENSON's chromosomes are deficient for the locus of *bb* and both are visibly shorter in heterozygotes than the normal X.

DOBZHANSKY's deficient chromosome (X^{DfD}) measured two-thirds the length of the normal X in metaphase plates of larval ganglia of females. SCHULTZ (unpublished) observed in salivary gland preparations that breaks of an inversion found by STURTEVANT and BEADLE were at bands 4D1 and 19F and that the deficiency was for the section 20C–D (BRIDGES' salivary chromosome map, 1935). GERSHENSON's deficient X was approximately three-fourths as long as the normal X in oögonial metaphase plates and SCHULTZ (unpublished) observed in salivary chromosomes a deficiency from before 19F to right of 20C. Accordingly, the sections missing from the two chromosomes are not coextensive, and it appears that their effects on the expression of *spa* are also probably different.

The distribution of grades of *spa* when Df^{D} was present was not noted, but the eyes were directly compared at various times with those of flies deficient for Df^{G} and the latter appeared to be rougher in corresponding classes of flies. More definite data might show whether there may be an effect on *spa* of heterochromatin to the left of the locus of *bb*, as this region is absent in GERSHENSON's deficiency but present in DOBZHANSKY's.

A third kind of X which may also be deficient for the locus of *bb* is known as X bobbed lethal (*bb^l*). The extent of a possible deficiency is not known. The chromosome has the same kind of effect on expression of *spa* in heterozygous females and in males as have the two known deficiencies but to a lesser degree than X^{DfG}. This was seen in direct comparisons of *spa* stocks into which each

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TABLE 4

Summary of data relating to homozygous spa, modified genotypes.
(At 18° ±, at 22° ± marked.*)

NO.	P_1♀	F_1	TOTAL	X % ABOVE GRADE Y		GRADE OF MODE	F_1	TOTAL	X % ABOVE GRADE Y		GRADE OF MODE
				X	Y				X	Y	
130A	$X^{Dp}XX$	$X^{Dp}XX$	77	4	3	2	$X^{Dp}XY$	19	0	0	
11a	$X^{Dp}XX$	$X^{Dp}XX$	79	2.6	3	2	—				
104k	X^-X	X^-X	85	82	3	4–5	X^-Y	85	68	1	2
104n	X^-X	—					X^-Y	23	44	1	1–2
94a	X^-X	X^-X	83	100	3	5	X^-Y	104	53	1	1–2
119k*	XX	X^-X	28	78	3	4					
119m	X^-X^-Y	X^-X^-Y	92	0	2	1–2	X^-Y	97	64	2	3
							X^-YY	64	0	0	
87B	X^-X^-Y	X^-X^-Y	47	0	1	1	X^-Y	103	100	2	3–4
							X^-YY	66	0	0	
53B	X^-X^-Y	—					X^-Y	44	55	2	2–3
							X^-YY	42	0	0	
53A	XXY	XXY	n	0	0		X^-Y	60	100	2	3–4
		XXO	29	100	4	5–6					
105p	XXY	XXY	55	0	0		X^-Y	43	95	2	4
		XXO	18	89	4	5	X^-YY	5	0	0	
37l*	XXY	XXY	40	0	1		X^-Y	91	72	2	3
		XXO	21	28	4	4	X^-YY	16	0	0	
37P	XXY	XXY	62	0	0		X^-Y	74	95	2	4
		XXO	14	79	4	5	X^-YY	3	0	0	
90a	XX						XY^{bb1}	7	100	4	5
90t*	XX						XY^{bb1}	31	100	1	2–3
114	XXO	XXY	123	0	0		XO	99	100	4	5–6
109	$2^-/2$	$2^-/2$	47	100	4	5	$2^-/2$	46	98	3	4
131a	$2^-/2$	$2^-/2$	162	70	3	3, 6	$2^-/2$	149	84	3	1, 6
131l	2/2	$2^-/2$	80	60	3	4, 6	$2^-/2$	59	70	3	2, 5
114w	4/0	4/0	15	100	4	5	4/0	70	20	3	2–3
114v	4/4	4/0	27	100	4	5	4/0	92	28	3	3
129a	4/0	4/0	6	100	5	6	4/0	6	100	3	5
129l	4/4	4/0	17	100	4	6	4/0	50	92	3	4

of the three deficiencies in question had been separately introduced.

An effect of heterochromatic deficiency in two X's is visible in X^-X^-Y females with either deficiency G or D. XXY females and the XXY females that have been examined have smooth eyes, but in the eyes of $X^{DfG}X^{DfG}Y$ females the range of *spa* is from grade o to 2 (table 1). They overlap normal, but they show that with the loss of heterochromatin in the two X's the Y does not entirely suppress the character *spa*. X^-XY females, however, carrying either of the deficiencies in one of the X's are smooth at room temperature.

The effect of an extra Y appears in half of the males of the $X^{DfG}X^{DfG}Y$ line. The males fall into two large classes (table 1, exps. 119m, 53B, and 87B), one which comprises X^-Y males, all above grade 2, and a second class of smooth eyed males which have received a Y from each parent and are X^-YY males.

TABLE 5

Summary of data relating to homozygous spa, normal genotypes.
*(At 18° ±, at 22° ± marked *, at 25° marked †.)*

NO.	P_1 ♀	TOTAL NUMBER OF F_1 XX	% ABOVE GRADE 3	GRADE OF MODE	TOTAL NUMBER OF F_1 XY	% ABOVE GRADE 1	GRADE OF MODE
114w	4/0	52	0	2	43	0	
114v	4/4	39	10	2	39	0	
131a	$2^-/2$	175	37	1, 4	148	59	1, 5
131l	2/2	90	23	2, 4	57	21	
58a	(74)	140	1.4	3	102	5	
119k*	XX	—			n	0	
104k	X^-X	89	25	3	—		
104n	X^-X	—			79	0	
94a	X^-X	119	58	3^-4	78	0	
94k	XX	61	87	4	58	2	
103a	(ext)	81	35	3	88	17	1
130A	$X^{Dp}XX$	90	38	3^-4	40, n	7.5	
11a	$X^{Dp}XX$	77	23	3	—		
11m	XX	152	30	3	—		
109	$2^-/2$	36	25	3	25	20	1
125a	$4^-/4$	40	55	4	40	37	1
128a	$4^-/4$	123	81	4	44	67	2
128l	4/4	109	68	5	120	57	2
129a	4/0	47	43	3^-4	38	61	2
129l	4/4	113	35	3	44	11	1
57l	XXY	—			143	52	1^-2
57v	XXY	—			158	61	1^-2
121a	ci^D	66	79	4	50	72	2
121l	not-ci^D	195	100	5	161	90	3
120a	ey^D	38	92	4	55	53	1^-2
120l	not-ey^D	159	94	5	121	65	2
59a	(a)	91	90	4	127	79	2
58l	(a)	23	100	5	35	91	3
58s*	(a)	10	0	3	10	0	
23a	(a)	178	76	4	178	98	2^-3
23t†	(a)	179	17	2	192	0.2	
58bi*	(75)	42		3, 6	46		0, 5
58y*	(75^h)	20	100	6	20	100	5^-6
58z*	(75^l)	20	50	2^-5	19	32	2
90a	XX	76	100	5	—		
90t*	XX	26	89	4^-5	—		

This again is an example of the suppression of the character *spa* in the presence of an extra Y.

The same kind of effect is found also when a duplication for heterochromatin of the X chromosome is present in the nucleus. Dp. (1)101 was chosen as suitable for the experiment because both males and females carrying the duplicating fragment are viable and the fragment consists almost entirely of heterochro-

matin; the euchromatic portion of the chromosome from 2A2 to between 19F4–5 and 20A is missing. The females that carried the duplication (tables 3, 4, 5, exps. 130A, 11a, 11m) showed a lower degree of *spa* than did the controls in the same populations. The modes of distribution were at grade 2 in duplication females and at 3–4 in normal females (exp. 130A). The actual difference due to the duplicating fragment may be greater than the apparent difference because a slight roughness of the eye sometimes occurs in the presence of a duplicating fragment.

If the combinations of sex chromosomes of flies which were rated for *spa* are arranged in sequence according to the modes of frequency of distribution at 18° $\pm$ the order is: XO, $\underline{XX}$O, XY^{bbl}, X⁻X, [X⁻Y(F₁ of $\underline{XX}$Y)], [X⁻Y(F₁ of X⁻X⁻Y)], XX, X^{Dp}XX, X⁻Y(F₁ of X⁻X), [XY(F₁ of $\underline{XX}$Y)], XY, X⁻X⁻Y, (X⁻XY, XXY at 22° $\pm$), $\underline{XX}$Y, X⁻YY(F₁ of $\underline{XX}$Y), X⁻YY(F₁ of X⁻X⁻Y), X^{Dp}XY. The combinations in square brackets are those for which there has been no direct control.

Other combinations that have been observed but have not been rated for *spa*, namely, flies carrying Y^{bb}, Y^{bb36}, Y^{bb-}, or X^{DfD}, X^{bbl}, have been found by comparison with measured combinations to fit according to their degrees of expression of *spa*, in an orderly way into the series.

Within the series are subseries the terms of which differ only by the addition of one chromosome element. When the terms in the subseries are arranged in the order of progressive suppression, they form the same sequence as that based on the modes of distribution. The symbol "$>$" means "is more *spa* than"; the types cited have all been measured by the scale of grades except $\underline{XX}$Y^{bb-} and XY^{bb-}; these can be used because from direct comparisons it is known that XO$>$XY$^{bb-}>$$\underline{XX}$Y^{bb-}, and $\underline{XX}$Y^{bb-} is slightly *spa* and therefore ranks between $\underline{XX}$O (of very high grade) and $\underline{XX}$Y which is always smooth.

The subseries may be arranged in four groups that show: 1) The effect of addition of a part or whole of a Y to an X or to a male or female complement of chromosomes; XO$>$XY^{bb-}, XO$>$XY$^{bbl}>$XY, X⁻Y$>$X⁻YY, and $\underline{XX}$O $>$$\underline{XX}Y^{bb-}>$$\underline{XX}$Y; 2) The effect of the addition of an X to an X or to a male complement; XO$>$XX, X⁻Y$>$X⁻XY, XY$^{bb-}>$$\underline{XX}$Y^{bb-}, and XY$>$$\underline{XX}$Y; 3) The effect of the addition of a fragment of X composed for the most part of heterochromatin; XX$>$X^{Dp}XX, XY$>$X^{Dp}XY; 4) The effect of the addition of an X deficient for a large part of the heterochromatin; XO$>$X⁻X, X⁻Y $>$X⁻X⁻Y, and XY$>$X⁻XY.

Several conclusions can be drawn; Y suppresses *spa;* a deficient Y has the same kind of effect but to a lesser degree. From 3) it can be concluded that a heterochromatic fragment of X has the same kind of effect as has the heterochromatin of Y.

From 2) it appears that addition of a whole X also has the same kind of effect; this might be expected if it is the ratio of heterochromatin to euchromatin and not the actual amount of heterochromatin which is effective. The ratio of heterochromatin to euchromatin in a whole X is higher than the ratio in the whole normal male or female complement of chromosomes so that the addition of an X, although it contains actually more euchromatin than heterochroma-

tin, increases the ratio of total heterochromatin to total euchromatin. The subseries 4 shows that even the deficient X^- which retains some proximal heterochromatin but is composed almost entirely of euchromatin, likewise acts in the same direction as Y, that is, as a suppressor.

The difference between X and X^{Dp} is a difference in the amount of euchromatin. X^{Dp} contains all of the heterochromatin of X and very little of the euchromatin; therefore if the proportion of heterochromatin to euchromatin is the factor involved in the additions of heterochromatin, the addition of X to the nucleus should be less suppressive than the addition of X^{Dp} and the addition of X^- (largely euchromatic) should have still less effect than has X. The members of the series, X^-XY, XXY, and $X^{Dp}XY$, in which the three elements are present in addition to the combination XY are beyond the threshold of wild type and all three cannot be compared. However, the effects of X and X^- have been measured and with increase in the proportion of euchromatin to heterochromatin in the X, there is less effect of suppression; X^- suppresses *spa* less than does X (cf. X^-Y and XY, X^-X and XX, Table 3, Exps. 94a and 94k).

In no genotype used with *spa* has the ratio of euchromatin to heterochromatin been high enough to enhance *spa*. SCHULTZ (1936) reporting results with variegations mentions "duplications for active regions, some of which annul, others of which stimulate, the effects of the Y chromosome."

The effect of increasing amounts of heterochromatin in the nucleus has been shown to be progressive regardless of sex.

There are besides the subseries showing the effect of addition of one chromosome element, the contrasting pairs of combinations which show a fairly constant relation between females and males, that is between chromosome complements differing in the presence of an X or a Y; XX > XY at 18° ± and at 22° ±. $X^-X > X^-Y$, $X^{Dp}XX > X^{Dp}XY$, to which may be added in anticipation of the next section, XX Df(2) > XY Df(2) and XX haplo-4 > XY haplo-4. If the X or the Y is altered in one sex, the difference between the degrees of *spa* in the two sexes is correspondingly changed, e.g., XX and XY^{bbl} are more alike than are XX and XY. Other changes in the sex chromosome complements are, as noted above, accompanied by corresponding changes in the relative degrees of *spa* in females and males, sometimes shifting the direction of the difference; XY > $\underline{XX}$Y and $XY^{bb-} > \underline{XX}Y^{bb-}$ are examples in which *spa* is more extreme in males than in females.

There is a deficiency for heterochromatin in one of the large autosomes which was tested for an effect upon the expression of *spa*. Df(2)Ms10 which lacks a block of heterochromatin in the proximal region of 2R produces a Minute effect and is lethal when homozygous. When present in flies which were homozygous *spa* (exp. 109), all of the Minute females and 74 percent of the Minute males were of grades 5 and 6 although the distribution indices of the not-deficient controls were average. Two other experiments, 131a and 131l, (tables 3, 4, and 5) were complicated by what appears to have been a mixture of two separate lines. This gave a bimodal effect to the scattered distributions of both classes (Minute and not-Minute) of females and males (apparently

semi-lethal in the higher grade not-*M* males). The combined effect of the two lines and the deficiency for 2R resulted in exceptionally high grades while the distribution index of control females was noticeably low. It is evident (especially from exp. 109 in which there is no complicating factor) that in the presence of the normal amount of heterochromatin of 2R in the control flies the expression of *spa* was decidedly decreased as compared with expression in the deficient flies. This is in agreement with effects of the tested heterochromatic regions of the sex chromosomes.

Df(4)M-4 in salivary chromosomes shows a loss of about ten bands. The locus of *spa* is not included in the missing region, for the eyes of Minute flies heterozygous for *spa* are smooth (table 3) showing the presence of the normal allele in the deficient (Df 4) chromosome. The effect on *spa* of the deficiency cannot be studied unless *spa* can be introduced into the deficient chromosome.

Haplo-4 flies carrying only one dose of *spa* show the character to a high degree; the mode is at 5 or 6 in females and 3 to 4 in males (tables 3 and 4) while at the same time the indices of diplo-4 controls (table 5) are above average. The absence of one fourth chromosome from the genotype is largely a loss of heterochromatin and the enhancing effect might be regarded as due to this loss were it not that the absence of chromosome 4 involves the loss of one dose of *spa* in the diploid fly which introduces another situation. The high degree of *spa* in Dp(2; 4)b described earlier is another example of exaggeration effect. In the latter case the balance between the heterochromatin and euchromatin in the fragment 2L-4 (tip of 2L and the proximal heterochromatic region of 4) might be a factor producing a discernible effect. The effects of different doses of *spa* have not been studied.

It has been stated that the eyes of *spa* do not show a mosaic pattern. A suggestion of what may be mosaicism was sometimes observed in the lowest grades, making classification difficult. When the eye is turned to receive illumination from different directions, a slight roughness sometimes may be seen in an otherwise smooth eye, usually at the edge. When the posterior rim is involved the pattern resembles that of *Cat*. In one male the left eye was of this kind; the rough edge was like the right eye (grade 2). The surface of eyes of some males of derived high lines appeared uneven.

There is a class of mosaics which may be explained, by somatic elimination of a chromosome, as composed of two different genotypes each including the region of an eye. Three males had each one eye of grade 6 and the other eye of a lower grade, one each of 0, 1, 3; these may be explained by elimination of Y from a cell which developed into XO tissue including the grade 6 eye. Two other males had each one eye of grade 0 and the other of grade 5, which is the mode of haplo-4, and the eye of high grade may have been included in haplo-4 tissue resulting from somatic elimination of chromosome 4. The grades are not defined enough to exclude either interpretation of any one of the five mosaics. Two mosaics (incompletely described) had each one eye of grade 4 and the other of grade 6 (the grade of haplo-4 females, while the grade of XO males is 5–6). One mosaic had shorter wing and abdomen on the side of the extreme *spa* eye and the abdomen was extreme *bb*. The fly may have been a gynan-

dromorph by elimination of an X. The abdomen of the other mosaic was shorter on the side of extreme *spa* but the bristles were uniform and the fly was perhaps a female mosaic for haplo-4.

MATERNAL EFFECT

It is known that in some variegations there is a maternal effect of a Y chromosome on the expression of variegation in flies that developed from X eggs of an XXY female. The effect is in the same direction as that of an extra Y in the individual which carries the Y. That is, if a maternal effect is present the expression of variegation is less extreme in flies developed from X eggs of an XXY female than in the progeny of XX sisters of those females.

Experiments were not specifically planned to study the effect of different proportions of heterochromatin in the variants used as P_1 females, but in making experiments for observing the direct effect of altered chromosomes variant and normal females were sometimes used separately as P_1. No indication of a maternal effect was found in these experiments which were not refined enough to detect small effects. The results are given in tables 2, 5 where the contrasted matings are numbered alike but have different letters.

The character ey^D is associated with a heavily banded (euchromatic) homozygous duplication (exps. 120a, 120l). The expression of *spa* was the same in the female progeny of ey^D and not-ey^D females. It was slightly more extreme in males whose mothers had been not-ey^D, an effect opposite to the maternal effect found in variegations.

Other modifications tested for maternal effect were Df(2)Ms10, Df(4)M-4, haplo-4, X^{Dp}; with none of these was there any difference in grades in F_1 when the mothers were different (tables 3, 4, 5).

Homozygous *bb; spa* XX females which had developed from X eggs of XXY and daughters of XX females have been compared but not measured. There was a slight difference but the slightly more extreme *spa* appeared in the daughters of XXY, an effect opposite to the effect seen in variegations due to the presence of Y in the mother.

The XX and XY progeny of X⁻X and XX females from the same mass culture may be compared in exps. 94a and 94k, table 3. The XX daughters of XX mothers (exp. 94k) showed significantly more extreme *spa* than did the XX daughters of X⁻X mothers (exp. 94a). Again the difference is opposite to that to be expected if lack of heterochromatin in the mother enhances the expression of *spa* in her XX progeny. The sons showed no difference.

An unexplained difference appeared between X⁻Y sons of X⁻X females and X⁻Y sons of X⁻X⁻Y or of XXY females, the last two from matings for which there is no control. X⁻X females rate about 90 percent above grade 3 (table 4), while a higher proportion of heterochromatin is indicated both in the X⁻X⁻Y flies (which overlap normal and are never above grade 2, table 1) and in XXY females which are normal (except a few doubtful cases of grade 1). The X⁻Y sons of X⁻X are about 55 percent above grade 1, but the X⁻Y sons of X⁻X⁻Y are about 73 percent grade 2, and X⁻Y sons of XXY nearly 100 percent above grade 2 (72 percent in one experiment at about 22°). There was a marked

difference in expression of *spa* in the sons of X⁻X as compared with the sons of the other two kinds of females. The differences among the three sets of males indicated a series in which the expression of *spa* varied not inversely but directly with the proportion of heterochromatin in the mother. But since the three lines of flies were not related, it must be borne in mind that uncontrolled modifiers may have been responsible for the more extreme expression of *spa* found in the progeny of the two kinds of mothers which were most heterochromatic.

<div align="center">DISCUSSION</div>

The genetic study of *spa* has shown that it manifests two characteristics of variegations, one often accompanying mutation, the other so far found to be peculiar to the type of position effect called variegation; *spa* phenotype is variable, and it is affected by the proportion of heterochromatin to euchromatin in the nucleus. Because of these resemblances the properties of variegations are of special interest.

A gene mutation may occur in a rearrangement of chromosomes whether the new chromosomal material which comes to be adjacent to the mutating gene is euchromatic or heterochromatic. In either case the mutation is specific for the locus or region of the gene next to the new contact. But there are differences in the phenotypic effect depending upon whether the material in the new environment within the chromosome is heterochromatic or euchromatic (DEMEREC 1941).

When the new contact is euchromatic only the immediately adjacent locus is affected and the mutant gene is stable. But when the new material adjacent to the mutating gene is heterochromatic, there is a variable phenotypic effect resulting in mosaics of what appear, in white variegations, to be alleles of the mutating gene. Loci beyond the one immediately adjacent to the heterochromatin may also be affected. This may be seen in white variegations because there are other loci in the region which may be marked. The mosaicism is exhibited in somatic tissue as in *Pm*, *rst*,[3] the white series and many other variegations. MULLER (1930) "found that at least two distinct genetic strains (of white mottled) could immediately be obtained from one original mottled line—a lighter and a darker."

Factors outside of the rearranged chromosome which affect the extent of mosaicism are factors of the environment both of the mutated gene and of the adjacent heterochromatin. Such a factor is the proportion of heterochromatin to euchromatin in the whole chromosome complement.

The maternal effect observed in variegations in comparing XX progeny of XXY females with those of XX females is associated with a difference in the cytoplasmic environments of the maturing nuclei depending on the presence or absence of a Y chromosome.

Lastly variegations are affected by temperature, a developmental factor in the physical environment.

It is not yet known to which, if any, category of known mutations *spa* may

belong. It is inherited in the manner of a recessive gene, point mutation or recessive accompanying rearrangement.

The expression of the gene *spa* is in some sense variable and in this it resembles variegations. It does not show detectable mosaics as do, for example, variegations of the white series and *rst³*, but as with them there is variation among sibs, which with *spa* and with the 4th chromosome character *ci* (STERN 1943) is measurable.

If *spa* eyes are in fact mosaics in which the pattern cannot be distinguished, it would be expected that the two eyes of a pair would often show over-all different grades of expression; but there is high correlation between the two eyes as is true also of the wings of *ci* flies. Two eyes of an individual have not been observed that differ by as much as one grade of the scale chosen for measurement. Both eyes were not always examined, but very large numbers have been compared. On the other hand differences in extent of mosaicism are found, among variegated individuals, and it may be that the differences between individual *spa* flies correspond to those differences.

The proportion of heterochromatin to euchromatin in the chromosomal environment is negatively correlated with the extent of the expression of *spa* which is in agreement with a similar effect in variegations. One example from the graded series showing progressive suppression of *spa* with greater proportions of the heterochromatin of the sex-chromosomes is the difference in expression in XX and XY. It is not a difference between females and males as such, because an XO male is more *spa* than an XX female, XX is more *spa* than an XY male, but the XY male is more *spa* than an XXY female. The expression of *rst³* is also different in males and females, but with *rst³* the mutant character is more extreme in XY males than in XX females.

The temperature at which development takes place affects the expression of *spa* and of *rst³*; both are more extreme at lower temperatures. The relative degrees of expression in XX and XY are maintained at the temperatures observed. The difference is also maintained with *spa* when there is a shift in distribution connected with loss of heterochromatin of the sex-chromosomes or of 2R and in haplo-4.

Another character, cubitus interruptus (*ci*), in chromosome 4 (STERN, 1943) is like *spa* in being variable and the degrees of expression of *ci* and of *spa* are shifted in the same direction in response to temperature. The two mutants differ however in that *ci* is more extreme in males than in females.

Experiments which might have indicated a maternal effect gave inconclusive results.

In summary it may be said that factors responsible for the variability observed in *spa* have not been determined, but that the variability resembles in some sort that seen in variegations. In both instances the variability is affected by the proportion of heterochromatin to euchromatin in the nucleus and also by the temperature at which development takes place, but a maternal effect depending on an effect of Y upon cell cytoplasm of the developing egg has not been observed in *spa*.

The phenotypic expression of the mutant *spa* resembles that of the 4th chromosome mutant *ci* in being variable, enhanced by low temperature during development and also in the constant relative degree of expression between XY males and XX females, though in the reverse direction.

The gene *spa* is in some way related to the dominant *Cat;* the heterozygote shows roughness in the part of the eye which in *Cat* heterozygous for wild type is smooth. LEWIS (1945) has shown that in an eye heterozygous for recessive asteroid (which is also variable) and dominant Star there is an effect of *ast* not seen in *S* when heterozygous for wild type. This phenotypic test of allelism is always inconclusive when applied to the relation between a dominant and a recessive.

The genetic study of *spa* shows the need of further investigation of old lines of *spa* and of new lines that have been derived from them and of salivary gland chromosomes; also there is need of a study of dosage effects and of exceptions that have been obtained from X-rayed flies.

SUMMARY

The phenotype sparkling (*spa*) is associated with a gene in the fourth chromosome of *Drosophila melanogaster*. The gene *spa* is tentatively located by evidence from crosses with T(2; 4)b and from a few salivary preparations in the distal range of region 102C.

Spa is inherited in the manner of a recessive gene, but shows characteristics not common to recessive genes. *Spa* bears some relation to dominant *cat*; the eye of the heterozygote shows a low grade of *spa* anteriorly where the eye of *Cat* is smoother.

Distributions of frequencies of grades of the phenotype *spa* show a single mode. The frequencies in one line which had shown monomodal distribution later showed a bimodal distribution. The line was separated into two which after about 25 generations (with almost no selection) bred true.

Among exceptions from X-rayed males smooth lines and extremely rough lines have been derived.

The degree of expression of *spa* varies in the progeny of pairs of flies. Individual flies differ, but definite mosaicism was not detected in single eyes.

Mosaics in which the two eyes of an individual were different have been seen though rarely. They can be explained by somatic elimination of an X, a Y, or a fourth chromosome.

Spa is more extreme at lower temperatures, $17°-19°$, than at $22°-25°$. A constant relative difference was found between XY males and XX females at all observed temperatures.

Distribution of males in a controlled temperature experiment indicates that beyond the normal threshold (grade of wild type) there are differences which are not detectable in the phenotype.

The expression of *spa* is less extreme with addition of heterochromatin to the nucleus. Addition of increasing amounts of heterochromatin of the sex chromosomes gives a corresponding graded series of decrease in the degree of

expression of *spa*. The expression is lessened also with increase in the proportion of heterochromatin to euchromatin; X which contains more heterochromatin in proportion to euchromatin than does deficient X⁻ has the greater effect in suppression of *spa*. The constant difference between XX and XY falls consistently into line with progressive differences correlated with increase in proportion of heterochromatin to euchromatin.

In the absence of normal proximal heterochromatin of 2R *spa* is enhanced.

Spa is of high grade in haplo-4 flies and with Dp(2; 4)b.

Negative results were obtained in experiments which might have indicated a maternal effect.

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