THE RELATION OF REPEATS TO POSITION EFFECT IN DROSOPHILA MELANOGASTER

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INTRODUCTION

IN *DROSOPHILA MELANOGASTER* it has been established that the action of at least some genes is affected by their position in the chromosomes. Critical evidence for this phenomenon of position effect has been given by STURTEVANT (1925), DUBININ and SIDOROW (1935), and PANSHIN (1935). Position effects have been detected only in cases in which the relative position of a gene with respect to its neighbors has been altered by a chromosomal rearrangement. It is conceivable that the effective position of a gene may also be changed as a result of a substitution in an adjacent gene of one allele by another. Such a possibility was put to experimental test by STURTEVANT (1928) for the dominant mutants, Delta (*Dl*) and Hairless (*H*), three units apart in the third chromosome; however, no difference could be demonstrated between *Dl H*/++ and *Dl*+/+*H*.

On the other hand when a similar type of comparison is made with mutants at the Star and asteroid loci, a position effect can be detected, as will be shown below. The purpose of this paper is to present the evidence for an asteroid locus as distinct from that of Star, to examine the conditions which may make possible the detection of a position effect in this instance, and to study "changes" at the Star and asteroid loci associated with certain chromosomal rearrangements.

THE STAR AND ASTEROID MUTANTS

Star (*S*) is a spontaneous dominant mutant at 1.3 in the second chromosome (BRIDGES and MORGAN 1919; STERN and BRIDGES 1926). *S*/+ flies have slightly reduced eyes with irregularly shaped facets and disarranged facet hairs. The *S* homozygote is lethal. In a line of *S*/+ flies which had been inbred for several generations in mass cultures, several individuals appeared which had smaller eyes than those of *S*/+. An analysis of one of these showed that it carried *S* and, in the opposite chromosome, a dominant intensifier of *S*. By itself this factor behaved as a recessive, rough-eyed mutant in the left end of the second chromosome. This mutant, formerly called Star-recessive (S^r), is known from evidence presented below to be close to the right of *S* and has been renamed asteroid (*ast*).

The compound *ast*/+ occasionally has a very slightly roughened eye, but it is usually inseparable from wild type. The eyes of flies homozygous for *ast* are smaller than those of *S*/+ and the longitudinal wing veins, L2, L3, L4, and L5, occasionally have terminal gaps. The compound, *S*+/+ *ast*, has an extremely small, diamond-shaped eye with many of the facets fused and the rest

of irregular size and distribution. The longitudinal veins, which are sometimes interrupted in *ast/ast*, usually have large terminal gaps in *S+/+ ast*.

Asteroid^2 (*ast^2*) is probably an allele of *ast*, although the possibility that it is a recessive allele of *S* has not been excluded. It appeared spontaneously in a single F_1 male from a mating of *ast* females to *al S ho/Cy* males (*al*, aristaless at 0.0; *ho*, heldout at 4.0). The fly had Curly (*Cy*) wings and rough eyes and was therefore readily distinguishable from the *Cy/ast* class. From an identical type of mating, conducted at a later time, another allele of *ast*, *ast^3*, arose in the first or second generation and was detected in the same way as was *ast^2*. In the case of both *ast^2* and *ast^3* it was shown that each had arisen in a Curly inversion chromosome. Although these mutants have not been separated from the inversion in the left arm of the second chromosome, they have been freed of the Curly mutant. Homozygous *ast^2* and homozygous *ast^3* types have good viability but the former is sterile in the female; in each case the eye effect is similar to that of homoyzgous *ast*, but the wing venation is normal. The compound, *S/ast^2* is lethal. The *S/ast^3* types are late-hatching, but those which emerge have good viability and fertility; they have normal venation and extremely small eyes which are similar to, but slightly larger than, those of *S/ast*.

In a search for spontaneous reverse mutations of *ast* to *ast$^+$* from homozygous *ast* females, a slight allele of *ast*, *ast^4*, appeared in a single F_1 male of composition, *al S ho/ast^4 ho*, from a cross of *al ast ho/ast* females to *al S ho/Cy* males. Here the calculated number of offspring in which *ast^4* could be detected was approximately 15,700, and the observed recombination value for the *al–ho* interval was 5.5 percent. The possibility that the observed association of mutation with crossing over in the *al-ho* region was due to chance is perhaps strengthened by the fact that an allele, *ast^5*, which closely resembled *ast^4*, arose in a noncrossover individual of composition, *al ast ho/al ast^5 ho*, from a mating (conducted at 30°C) of *al ast ho/ast* females to *al ast ho* males. *Ast^4/+* and *ast^4/ast^4* are wild type, although the latter may become as extreme as *ast/ast* under unfavorable culture conditions. The compound, *S/ast^4*, has a smaller eye than that of *S/+* and resembles rather closely *ast/ast* not only in having a similar eye effect but also in occasionally producing a similar pattern of gaps at the tips of the longitudinal veins.

The phenotypic effects of all possible combinations of +, *S*, *ast*, *ast^2*, *ast^3*, and *ast^4* are indicated in table 1 by means of a rough, arbitrary grading of the eye effect, based on inspection rather than measurement. Wild type is taken as Grade 1. Grade 2 has a very slight facet irregularity. Higher numbers indicate decreasing size and increasing roughness of the eye. The possibility that modifiers at other loci are a complicating factor in the phenotypic expressions of the various *ast* alleles has been excluded rather rigorously in the case of *ast*, and to some extent for *ast^2*, *ast^3*, and *ast^4*.

In summary, the alleles, *ast* and *ast*,^{4} are more nearly similar to each other than to *ast^2* and *ast^3* in having good viability when opposite *S*, a variable expression, and a tendency to interrupt the wing venation; whereas, *ast^2* and *ast^3* have peculiar lethal effects with *S* and, by themselves, a relatively constant expression and no perceptible effect on the wing venation.

TABLE 1

A rough classification of eye types of various combinations of S and ast alleles, including those with Enhancer of Star, E-S. The extent of variability is indicated, where necessary, in parenthesis. Asterisk (*) indicates that individuals of type in question may show gaps in the longitudinal wing veins.

	S^+ ast^+	S^+ ast^4	S^+ ast^3	S^+ ast	S^+ ast^2	S ast^+	S^+ ast^+ E-S
S^+ ast^+	1						
S^+ ast^4	1	1 (1–5)*					
S^+ ast^3	1	2 (2–3)	5				
S^+ ast	1 (1–2)	3 (1–5)*	4 (4–6)	5* (1–7)			
S^+ ast^2	1	4 (3–5)	5	6 (4–8)*	5 (♀, sterile)		
S ast^+	3 (2–3)	5* (4–6)*	7 (late hatching)	8 (6–8)*	lethal	lethal	
S^+ ast^+ E-S	1	2	2	4	3	5 (5–6)	2

In table 1 the interactions of *S* and the *ast* alleles with the dominant, Enhancer of Star (*E-S*, at 6 ± in the second chromosome) are also shown. The relative specificity of *E-S* in strongly enhancing $ast/+$ and $ast^2/+$ is shown by the fact that it had, when heterozygous, little, if any, such effect on other recessive rough-eyed mutants which were tested in the heterozygous condition. The mutants thus tested were: echinus, facet, split, roughex^2, uneven, morula, roughish, rolled, rubroad, scabrous, rough, and roughoid. Another interaction, not shown in table 1, is that with either homozygous net or homozygous plexus, each of which is partially suppressed by $S/+$ and tends to be completely suppressed by ast/ast and S/ast.

BRIDGES (1936) has reported that *S* is apparently normal in the salivary gland chromosomes. Since the *S* and *ast* loci are now known to lie in the region of the 21E1–2 doubtlet, particular attention has been paid to this region in a cytological study of *S*, S^2 and S^r (two alleles closely resembling S^1), *ast*, ast^2, ast^3, and ast^4. Each of these mutants has been found to be apparently normal in the salivary gland chromosomes.

THE EVIDENCE FOR AN ASTEROID LOCUS

Data summarized in table 2 represent part of the evidence that *ast* and *ast*^{4} are located close to the right of *S*, and they suggest that *ast*^{3} is located close to the right of *S*^{2}. The first indication that *ast* was not an allele of *S* was the appearance of a single+/*ast* fly among 3,235 offspring of a cross of *S*/*ast* females to *ast* males (Mating 1). Additional data were then collected from *al S ho*/*ast* females which were individually mated in separate experiments to *ast*, *al ast ho*, and *al S ho*/*Cy* males with the results shown in Matings 2a, 2b, and 2c, respectively. Mating 2a produced a normal fly which was shown by a cross to *al S ho*/*Cy* to have had the probable composition, *ast*/*ho*; seven other apparently normal offspring from Mating 2a were shown by the same test to have been instances in which homozygous *ast* had overlapped wild type. Mating 2b yielded no normal-eyed offspring. From Mating 2c, two non-*Cy* females with *ho* wings and eyes resembling those of *S*/+ were recovered in separate cultures. These females, which were non-virgin, were separately mated to *al S ho*/*Cy* males; in each case, some of the F_1 flies were phenotypically identical with the parental female. In one case a pair mating of these F_1 flies was made, and it produced an F_2 which had "*S*/+" eyes and normal eyes in the ratio, 2:1. Thus the females produced in Mating 2c had the probable composition, *al S ho*/*ho*.

In Mating 2 the "+" types, which may be interpreted as wild type crossovers between *S* and *ast*, occurred with a frequency of 0.009 per cent. Here the total number of offspring in which the "+" types could be detected is calculated, as shown in Column 3, from that class which had approximately the same viability as those types—namely, the "*ast*" offspring in Matings 2a and 2b, and the "*Cy*" class in 2 c.

A more satisfactory method of recovering the "+" crossovers was employed in Mating 3, table 2. Here the parental *S*/*ast* females were heterozygous for the mutants, *net* (net, at 0.0–), *dp* (dumpy, at 13.0) and *cl* (clot, at 16.5), as well as for *al* and *ho*. These mutants were arranged to give the approximately alternated composition, *net S dp cl*/*al ast ho*. In addition, the parental females were made heterozygous for inversions in chromosome arms other than 2*L*, since this procedure was known to increase the frequency of crossing over between *al* and *ho*. In order to obtain females of the required composition, *al ast ho In*(2*R*)*Cy*/*Cy*, *E*–*S* females were mated to *In*(1) *dl-49*, *cm*^{2}/*Y*; *net S dp cl*/*Cy*(2*L*) *dp*^{2} *b pr*; *In* (3*L*+3*R*)*P*/+ males. The F_1 *In* (1) *dl-49*, *cm*^{2}/+; *net S dp cl*/*al ast ho In* (2*R*) *Cy* females, of which one-half are expected to be heterozygous for the Payne inversions in both arms of chromosome 3, constituted the parental females employed in Mating 3. The parental males in this mating were homozygous for *In* (2*L*) *Cy*, *ast*^{2}. Since *S*/*ast*^{2} is lethal, the use of *ast*^{2} rather than *ast* served to reduce by one-half the total number of offspring to be examined for the "+" crossovers. At the same time, *ast*^{2} made easier the detection of such crossovers, since *ast*^{2}/*ast*, in contrast to *ast*/*ast*, had not been observed to overlap wild type.

In addition to 16,068 "*ast*/*ast*^{2}" offspring, Mating 3 yielded 15 flies with normal eyes. Of the latter, one was sterile and another (with Minute bristles)

was lost. The remaining 13, composed of eight males and five females, were individually outcrossed to an *al S ho/Cy, E-S* stock. In each case, the F_1 non-*Cy* flies, except for those expected on the basis that the tested individual was a non-virgin female, were aristaless and had the "*S*/+" type of eye. Two types of *Cy* flies, those with normal eyes and those of the expected "ast²/*E-S*" type were obtained in the F_1 of each of these matings. An F_2 was raised from each

TABLE 2

The frequency of "+" crossovers between the S and ast loci. The inversions for which the parental females were homozyous are shown in column 2. Calculated total progenies (column 3) are based on total counts of the class shown in parenthesis. The number and composition of the tested crossover chromosomes are indicated in parenthesis in column 4.

	MATING	INVERSIONS	TOTAL	WILD TYPE CROSSOVERS	PER-CENTAGE
1	$\frac{S\ +}{+\ ast}$ ♀ × *ast* ♂♂	Unknown	2×1,728 ("*ast*")	1(?)	
2a	$\frac{al\ S\ +\ ho}{+\ +\ ast\ +}$ ♀ ± *ast* ♂♂	Unknown	2×6,933 ("*ast*")	1 (*ho*)	
b	(") ♀ × *al ast ho* ♂♂	Unknown	2×2,038 ("*ast*")	0	0.009
c	(") × $\frac{al\ S\ ho}{Cy}$ ♂♂	Unknown	15,753 (*Cy*)	2 (2, *ho*)	
3	$\frac{net + S + + dp\ cl}{+ al + ast\ ho + +}$ ♀ × *In*(2L)*Cy*, *ast*² ♂♂	*In*(1)*dl-49*, *cm*²; *In*(2R)*Cy*, *cn*²; and in about ½ ♀ ♀, *In*(3L+3R)*P*.	2×16,068 (*ast*/*ast*²)	15 (12, *al dp cl*)	0.047
4	$\frac{net\ S + + dp\ cl}{+ + ast^4\ ho + +}$ ♀ × *In*(2L)*Cy*, *ast*² ♂♂	*In*(1)*dl-49*, cm²; and in some, *In*(3L+3R)*P*, *ra*.	2×7,656 (*ast*⁴/*ast*²)	2 (2, *dp cl*)	0.01
5	$\frac{+\ S^2\ +\ In(2L)t}{al^2 + ast^3\ In(2L)Cy}$ ♀ × $\frac{al\ S\ ho}{Cy,\ E-S}$ ♂♂	*In*(1)*dl-49*, *cm*²; *In*(2R)*Cy*, *cn*²; and in about ½ ♀ ♀, *In* (3LR)*sep*, *ri p*ᵖ.	17,334 (*Cy*)	7 (4, *al*² *In*−*t*)	0.04

of 12 of the 13 progeny tests by mating the *Cy*, normal-eyed flies *inter se*. The F_2 non-*Cy* offspring had in each case normal eyes and were phenotypically *al*, *dp* and *cl*. Thus, at least 13 "+" crossovers between *S* and *ast* were recovered in Mating 3, of which 12 were shown to carry the marker *dp* and *cl*, as well as *al*. It will be noted that the composition of the crossover types is expected to remain intact in the above progeny tests since those types were constantly kept against the Curly inversion in the left arm of chromosome 2. Stocks of two separate occurrences of the "+" crossover types from Mating 3 were kept for further tests. These included matings to *ast*, *ast*³, and *ast*⁴ and to the *S* deficien-

cies of *Df-S1* and *Df-S2*, which will be described later. The results of each of these matings were consistent with the assumption that the "+" crossovers carried the normal alleles of *S* and *ast*. A cytological analysis of these two stocks showed no departure from normal in the critical *S* region.

The results of Mating 3 are consistent with those of Mating 2 in indicating that *ast* is close to the right of *S*. The frequency of normal-eyed flies in Mating 3 was 0.08 per cent. Since the S/ast^2 class is lethal, the calculated frequency of "+" crossover types is 0.04 per cent. Inspection of table 5 shows that significant increases in crossing over in the *al–ho* region are obtained by the method adopted in Mating 3 of introducing inversions into other chromosome arms. The increase in frequency of "+" crossovers in Mating 3, compared to Mating 2 can be best attributed to this procedure.

It was further found that $S+/+$ ast^4 females produced occasional "+" crossover types (Mating 4). The original ast^4 *ho* strain, kept by mass inbreeding was used as the source of ast^4. The "+" types were detected and tested by methods analogous to those used in Mating 3, although a less "efficient" inversion setup was employed. Only two normal-eyed flies were recovered in this experiment. Each was found to be a "+" crossover type similar to those obtained from $S+/+ast$ females. The lower frequency—namely, 0.01 per cent—of "+" crossovers from $S+/+ast^4$ females as compared with 0.04 per cent from $S+/+$ *ast* females is at least consistent with the use of fewer inversions in Mating 4 as compared to Mating 3. In their interactions with *ast*, ast^2, ast^3, *S* and *E–S*, the "+" crossovers from Mating 4 behaved as though they carried the normal alleles of *S* and *ast*. Likewise their cytological picture was apparently normal for the *S* region of the salivary gland chromosome.

Technical difficulties arising from the association of S^2 and asteroid^3 with *In(2L)t* and *In(2L)Cy*, respectively, prevented a study of their crossing over relationships with respect to either *ast*, ast^4, or *S*. However, since the sequences in these two inversions are nearly identical to each other (BRIDGES and LI 1936), the linkage relations of S^2 and ast^3 could be determined directly. For this purpose, *In(2L)Cy*, al^2 ast^3 *b pr* (homozygous) females were crossed to *In(1)dl-49*, cm^2/Y; *In(2L)t*, S^2*In(2R) Cy/Cy(2L)*dp^2 *b pr*; *In(3LR)sep*, *ri* p^p *sep* males. The F_1 "S^2/ast^3" females, one-half of which are expected to carry the third chromosome "separated" inversion of MULLER were individually mated to *al S ho/Cy*, *E-S* males with the results shown in Mating 5. Since S^2/S is lethal, only three general types of offspring were recovered. In addition, a total of seven non-Curly, aristaless flies resembling "$S/+$" were detected in the F_1.

It will be noted by reference to figure 1 that the left break points of *In(2L)t* and *In(2L)Cy* each lie in the region to the left of *ho* and to the right of *S*. Therefore, although no marker mutants close to the right of *ast* are employed in the parental females, that region is strategically marked by the inversions themselves. Of the seven "+" types, four were successfully studied cytologically. The procedure here was separately to cross the aristaless "$S/+$" flies to an *al S ho/Cy*, *E-S* stock. The F_1 Curly males were backcrossed to *al S ho/Cy*,

E-S females. In each of the four tests some of the F_2 larvae carried the overlapping inversion complex, *In(2L)t/In(2L)Cy*. At the same time it was determined that each of the "+" crossover types was apparently normal in the critical 21E region. The "+" crossovers were mated to *ast* and *ast*^{3} (as well as to *S* and *E-S*), and their behavior was that expected on the basis that they carried the normal alleles of *S* and *ast*. Thus the probable composition of each of the four crossover chromosomes was *al*^{2} *S*$^+$ *ast*$^+$ *In(2L)t*. The frequency of the "+" crossovers can best be computed on the basis of the Curly offspring which

TABLE 3

The frequency of "S ast" and "S ast^4*" crossovers. (See text and table 2 for description.)*

	MATING	INVERSIONS	TOTAL PROGENY	"*S as*" OR "*S ast*^{4}" CROSSOVERS	PERCENTAGE
6	$\frac{al\ S\ +\ ho}{+\ +\ ast\ +}$ ♀ × *al ho* ♂♂	Unknown	2,967	0	—
7a	$\frac{net\ +\ S\ +\ +\ dp\ cl}{+\ al\ +\ ast\ ho\ +\ +}$ ♀ × *al ho* ♂♂	*In* (1) *dl-49, cm*^{2}; *In* (2*R*)*Cy, cn*^{2}; *In*(3*L*)*P, Me* *In* (3*R*)*C, Sb e*	3,005	0	—
b	(") ♀ × *al Dp–S ho* ♂♂		6,222	0	—
8	$\frac{net\ S\ +\ +\ dp\ cl}{+\ +\ ast^4\ ho\ +\ +}$ ♀ × *al ho* ♂♂	*In* (1)*dl-49, cm*^{2}; and in some, *In* (3*L*+3*R*)*P, ra*	2,914	0	—
9	$\frac{+\ al\ (S\ ast)\ (+\ ast)\ ho\ +\ +}{net\ +\ +\ ast\ \ \ +\ dp\ cl}$ ♀ × *al go* ♂♂	*In*(1)*dl-49, cm*^{2}; *T*(2; 3)*Me, Me*	4,864	3 (2, *al S ast dp cl*)	0.06
		In(1)*dl-49, cm*^{2}; *T*(2; 3)*Me, Me*/ *In* (3*LR*)*CxD, D*	1,531	2 (1, *al S ast dp cl*)	0.1
10	$\frac{al\ (S\ ast)\ (+\ ast)\ ho\ +\ +}{+\ +\ ast^4\ \ \ +\ dp\ cl}$ ♀ × *net* ♂♂; *net ho* ♂♂; and *al ho* ♂♂	*In*(1)*do-49, cm*^{2}; *T*(2; 3)*Me, Me*/ *In* (3*LR*)*sep, p*^{p}.	6,667	3 (3, *al S ast*^{4} *dp cl*)	0.04

totaled 17,334. This gives an estimated frequency of 0.04 per cent (7/17,334). These results, when compared with those of Matings 2 and 3, indicate that *S*^{2} is probably an allele of *S* and not a dominant allele of *ast*; similarly, *ast*^{3} probably is an allele of *ast* rather than a recessive allele of *S*.

At first attempts to recover crossovers having *S* and *ast* or *S* and *ast*^{4} in the same chromosome failed. These experiments, shown in table 3, Matings 6, 7, and 8, were conducted on the assumption that *S ast*/++ or *S ast*^{4}/++ would resemble phenotypically *S*+/+ *ast* or *S*+/+*ast*^{4}, respectively, or that they would be at least more extreme than *S*+/++. In Mating 7b, use was made of a suppressor of *S*, *Dp-S*, in the parental males, on the basis that it might permit the survival and detection of *S ast*. However, no offspring with eyes more extreme than "*S*/+" (or wild type in the case of Mating 7b) were detected in any of these experiments among a total of 15,108 offspring.

An indirect way of obtaining the *S ast* and *S ast*^{4} combinations suggested itself as a result of independent studies of the tandem Star Duplication, *Dp-S* (LEWIS 1941). To understand the method employed, some of the properties of this duplication require review.

A combination of genetic and cytological studies now indicates that this duplication includes two known loci, *S* and *ast*. Cytologically, this aberration is a tandem repeat in direct order for the four bands: 21D3,4 and the 21E1–2 doublet structure or "capsule" (see fig. 1). As originally obtained, this duplication, which arose from an *ast* female, had the probable composition, (+*ast*)(+*ast*), formerly written (S^r)(S^r). This notation is to be taken as indicating that the regions in parenthesis are in adjacent, direct order and that each carries the normal allele of *S* and the recessive *ast*. Many variations in the genetic composition of *Dp-S* have been obtained, but for present purposes only the derivative, (*S ast*)(+*ast*), formerly written (*S*)(S^r), need be considered. This derivative was obtained among the offspring of a mating in which (+*ast*)(+*ast*) *ho/net S dp cl* females were individually mated to *In(2L)Cy*, *ast*^{2} *b pr* males. The great majority of the F_1 had normal eyes—that is, the (+*ast*)(+*ast*)/*ast*^{2} class; in addition, there were 206 offspring with eyes resembling "*ast/ast*^{2}." Four possibilities seemed likely for the origin of these types. Thus, by unequal crossing over, *ast* could have been extracted from the duplication in two ways, either as (1) *ast dp cl*, or as (2) *net ast ho*. *S* could likewise be inserted into the left section of the duplication in two ways, either as (3) *net* (*S ast*)(+*ast*) *ho*, or as (4) *net* (*S*+)(+*ast*) *ho*. Two other duplication types possible here—namely, (+*ast*)(++) and (+*ast*)(*S*+)—were known to be wild type opposite *ast*^{2} and therefore could not be detected in this experiment. Of the 206 cases, 192 were successfully tested to a *net ast ho* stock. In each of these progeny tests some of the F_1 flies were phenotypically *net ast ho* (except for two which were *ast ho*). Thus the first possibility, the extraction of *ast* from the left section of the duplication, was not realized. A salivary gland chromosome analysis was then made of 83 of the "*ast*" crossover types. Only two of these proved to carry *Dp-S*. These were indistinguishable phenotypically from the 81 *net ast ho* types which did not carry the duplication. Since type (4) is complementary to type (1), which did not occur in this or related experiments, it can be assumed that the composition of these two duplication types was (*S ast*)(+*ast*). This genetic structure, at least with respect to the *S* locus, was confirmed by extracting from the left (distal) section of the repeat the newly introduced *S*. This was done in three ways. The results of two of these methods are shown in Matings 9 and 10 of table 3. Females of composition, *al* (*S ast*)(+*ast*) *ho/net ast dp cl* (Mating 9) or *al* (*S ast*)(+*ast*) *ho/ast*^{4} *dp cl* (Mating 10) when mated to *al ho* males produced for the most part only normal-eyed flies. However, each type of female produced several aristaless offspring with eyes resembling those of "*S*/+" flies. These rare types were also produced when *al*(*S ast*)(+*ast*)/*dp cl* females were mated to *al ho* males. No types with eyes more extreme than "*S*/+" were detected in any of these experiments.

In the next step, three separate occurrences of what may be called the "*S*" crossovers from Mating 9, and two such cases from Mating 10 were tested for the presence of *ast* or *ast*^{4}. The results of these five tests are grouped and summarized in Matings 11, 12, 13, and 14 of table 4. Matings 11 and 12 were tests of "*S*" crossovers derived from Mating 9 and in each of these three tests one (*net*) *ast dp cl* crossover was recovered. Likewise, at least one *net ast*^{4} *dp cl* crossover was detected from each of the two tests of "*S*" crossovers from Mating 10.

These linkage studies indicated that the probable composition of the "*S*"

TABLE 4

The frequency with which ast and ast^4 *were recovered from S ast /++ and S ast*^{4}*/++ females, respectively. (See text and table 2 for description.)*

	MATING	INVERSIONS	TOTAL	"*ast*" OR "*ast*^{4}" CROSSOVERS	PERCENTAGE
11	$\frac{al\ \ S\ ast\ \ dp\ \ cl}{+\ +\ +\ +\ +}$ ♀ × *In(2L)Cy, al*^{2} *ast*^{3} ♂♂	*T(2;3)Me, Me*	2×2,304	1 (*ast dp cl*)	0.02
12	$\frac{+\ al\ \ S\ ast\ +\ dp\ \ cl}{net\ +\ +\ +\ ho\ +\ +}$ ♀ × *al ast ho* ♂♂ and *In(2L)Cy, al*^{2} *ast*^{3} ♂♂	*In(1)dl-49, cm*^{2}; *Dp(2)T(2;3)Me, Me*	2×1,594	2 (2, *net ast dp cl*)	0.06
13	$\frac{+\ al\ \ S\ \ ast^4\ +\ dp\ \ cl}{net\ +\ +\ +\ ho\ +\ +}$ ♀ × *In(2L)Cy, al*^{2} *ast*^{3} ♂♂	*In (1) dl-49, cm*^{2}; *Dp(2)T(2;3)-Me, Me*	2×2,754	4 (3, *net ast*^{4} *dp cl*; 1, *net ast*^{4})	0.07
14	$\frac{net\ +\ \ S\ ast\ \ \ \ \ \ +\ dp\ \ cl}{+\ al\ \ (+\ ast)(+ast)\ ho\ +\ +}$ ♀ × *In(2L)Cy, al*^{2} *ast*^{3} ♂♂	*In(1)dl-49, cm*^{2}/ *In(1)AM, ptg*^{4}; *T(2;3)Me, Me*	2×996	1 (*al ast dp cl*)	0.05

crossovers fromMatings 9 and 10 were *al S ast dp cl* and *al S ast*^{4} *dp cl*, respectively. In these studies the *ast* and *ast*^{4} crossovers were derived from *S ast/++* and *S ast*^{4}*/++* females, respectively. Still another *S ast* crossover type from Mating 9 was tested in Mating 14 by a different procedure, but one analogous to that by which the *S ast* type was first recovered. In this case *net S ast dp cl/al (+ast)(+ast) ho* females were mated to *In(2L)Cy, al*^{2} *ast*^{3} *b pr* males. Here, too, it was possible to recover an individual whose composition was shown to be *al ast dp cl/In(2L)Cy, al*^{2} *ast*^{3} *b pr*. The evidence that the rare crossover types obtained in the matings shown in table 3 carried *ast* or *ast*^{4} was based on phenotypic studies which included obtaining homozygotes for the crossover chromosome as well as testing to *S+*, *S ast*, and *S ast*^{4}.

A cytological study was made of five of the *S ast* types from Mating 9, two *S ast*^{4} types from Mating 10, and several of the *ast* and *ast*^{4} crossovers from Matings 11–13. None of these carried *Dp-S*, and, with the exception of one *S ast* type, all were apparently normal in the critical *S* region of the salivary gland chromosomes. The exception here showed a slight disturbance

near the 21E1–2 doublet. The exact nature of this irregularity was not determined but it appeared to involve a duplication or inversion of a single faint band. This aberrant *S ast* type was not included in the crossover studies shown in table 4; however, a cytologically normal *ast* crossover was extracted from it by the same procedure as that used in the other cases.

In their phenotypic reactions, *S ast* and *S ast*^{4} were indistinguishable from

TABLE 5

A comparison of the standard recombination values (together with their standard deviations) for the al-ho region, with those obtained when the parental females were heterozygous for various inversion (or translocation) complexes.

NUMBER OF MATING	SEQUENCE IN MAJOR CHROMOSOME ARMS OF PARENTAL ♀♀					PERCENTAGE RECOMBINATION	
	X	2L	2R	3L	3R	*al-S*	*al-ho*
6	(standard)					1.2±0.2 (36/2,967)	4.7±0.4 (140/2,967)
7a, b	*dl-49* / +	+ / +	*Cy* / +	*P* / +	*C* / +	4.3±0.4 (128/3,005)	10.9±0.3 (1,011/9,227)
12, 13	*dl-49* / +	+ / +	+ / +	*Dp(2)T(2;3)Me* / +		4.2±0.3 (167/4,008)	12.1±2.7 (18/149)
—	+ / +	*Dp-S* / +	+ / +	+ / +	+ / +	—	4.3±0.2 (343/8,000)
9	*dl-49* / +	*Dp-S* / +	*T(2;3)Me* / +; +			—	13.8±0.6 (455/3,297)
9	*dl-49* / +	*Dp-S* / +	*T(2;3)Me* / +; *In(3LR)CxD*			—	17.3±1.7 (85/490)

S ast$^+$ in tests to +, *E-S*, *ast*^{2} and *ast*^{3}. All combinations in which *S* was homozygous were lethal regardless of the *ast* composition. However, opposite *ast*, and similarly opposite *ast*^{4}, *S ast*^{4} produced a larger eye than *S ast* which in turn produced a larger eye than *S ast*$^+$. Thus, the following sequence, arranged in order of decreasing eye size, could be established: *S ast*^{4}/+*ast*^{4}, *Sa st*/+*ast*^{4}, *S*+/+*ast*^{4}, *S ast*^{4}/+*ast*, *S ast*/+*ast*, and *S*+/+*ast*. The largest eye produced here is still somewhat smaller than that typical of *S*/+.

It was felt that the difference in eye size between *S ast*/+*ast* and *S*+/+*ast* (or between *S ast*/+*ast*^{4} and *S*+/+*ast*^{4}) was not sufficiently distinct owing to

the inherent variability of these types to make practicable the detection of an *S ast* crossover from a mating of *S+/+ast* females to *ast* (or ast^4) males. However, it was possible to recover *S* ast^4 from *S+/+*ast^4 females on the basis of the more distinct difference existing between *S* ast^4*/+ast* and *S+/+ast*. In this experiment, mating 4a, not shown in the tables, *In(1)dl-49*, cm^2*/+*; *al S ho/*ast^4 *dp cl; In(3LR)sep/+* females were mated to *al ast ho* males. Total counts were not made but among a large number of offspring several aristaless flies were obtained which had eyes intermediate between *S+/+ast* and *ast* /ast^4. Three of these upon further testing were found to carry the desired *S* ast^4 crossover, on the basis that it behaved phenotypically exactly like the *S* ast^4 types obtained from Mating 10. The remaining cases were found to be instances in which *S/ast* had overlapped wild type.

In addition, a stock of one of the complementary *ho* crossovers which occurred in Mating 15 was established. It was then possible to make up flies having the composition *al S* ast^4 *dp cl/ho* whose second chromosomes were the two complementary crossovers derived from mating 4a. The phenotype in this case was again indistinguishable from *S* ast^+*/++*.

In summary, some 39 crossovers were obtained between the Star and asteroid loci. Twenty-two of these were "+" crossovers derived from *S+/+ast*, *S+/+*ast^4, or S^2*+/+*ast^3 females. Their frequency varied between 0.009 per cent in the absence of inversions in the parental females to 0.047 per cent when the parental females were heterozygous for *In(1)dl-49*, *In(2R)Cy*, and, in some, *In(3L+3R)P*. Six *S ast* or *S* ast^4 crossovers were detected in the progeny of (*S ast*)(*+ast*)*/ast* and (*S ast*)(*+ast*)/ast^4 females, respectively. The frequency varied between 0.04 and 0.1 per cent, depending on the particular inversions present.

A SURVEY OF THE EXTREME LEFT END OF CHROMOSOME 2

Early in the genetic analysis of *S* and *ast*, an attempt was made to correlate those loci with the salivary gland chromosome structure in the left end of chromosome 2. This correlation was particularly necessary as a basis for a cytological analysis of the *S* and *ast* mutants. Genetic and cytological correspondence were obtained not only for *S* and *ast* but also for most of the other mutant loci in their immediate vicinity. A genetic map of this region, which includes approximately the first four map units of the left end of chromosome 2, is shown as the top line in figure 1. This section includes at least ten known loci. Descriptions and linkage data for the mutants, aristaless (*al*), expanded (*ex*), and dachsous (*ds*), as well as for Star, have been given by STERN and BRIDGES (1926). The mutant, telegraph (*tg*, at 0.0±), also described by these authors, has been lost and is omitted from the linkage map shown here. An account of net (*net*), shrunken (*shr*), heldout (*ho*), and lethal giant larvae (*l-gl*), together with BRIDGES' revised location of *l-gl* at 0.0±, is given by BRIDGES and BREHME (1944).

The drawing, in figure 1, of the first two sections of chromosome 2L is

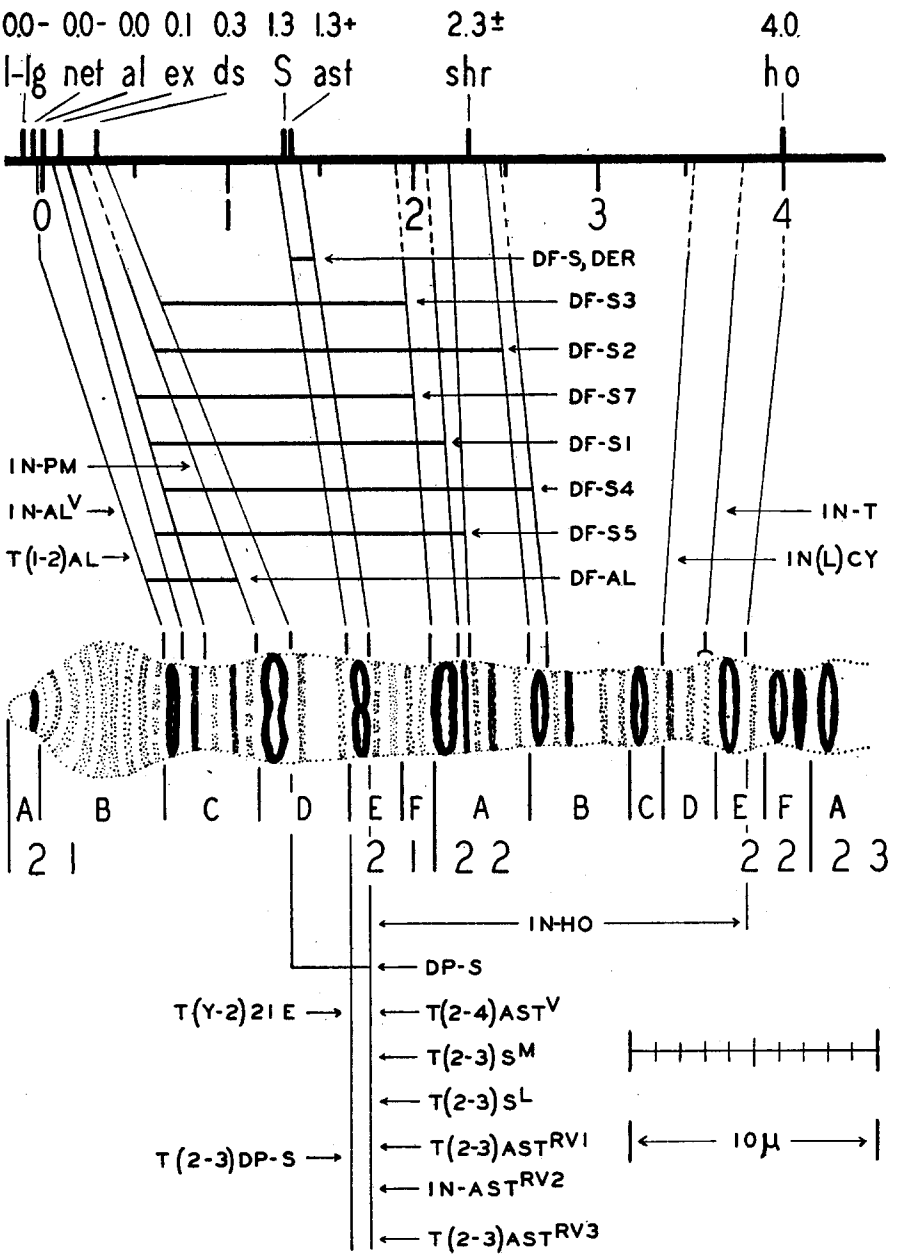

FIGURE 1.—A correlation of the linkage map of the extreme left end of the second chromosome with the salivary gland chromosome structure.

semi-diagrammatic and composite, based on moderately stretched chromosomes which were stained with aceto-orcein according to the method of LA COUR. The chromosome is labeled to correspond with BRIDGES' (1935) map.

Reference to the latter map shows that the section extending from 21A to just after 23A1–2 corresponds, as calculated by BRIDGES, to about 4.4 map units on the genetic map. In figure 1, this value has been used as the basis for the correlation of the genetic and cytological maps.

Much of the material which has made possible the genetic and cytological correspondences shown in figure 1 was obtained from an experiment in which adult wild type (Canton-S) males were X-rayed (3,000 r units) and mated to *al ast ho* females. The results of this experiment are summarized in table 6. Total counts were not made; however, a random sample of 16 out of 196 cultures, in which the parental males were allowed to remain for the first five days, averaged 82 flies per culture. A total of 103 transfer cultures produced an additional 3,398 flies. Therefore the combined total number of offspring

TABLE 6

Results from X-rayed (3,000 r units) wild-type ♂ × al ast ho ♀. Total offspring: approximately 19,000.
(Df = Deficiency, In = Inversion, T = Translocation.)

CHANGES RESEMBLING:	NUMBER NOT ANALYZED	NUMBER ANALYZED				TOTAL
		ASSOCIATED WITH ABERRATIONS			"POINT MUTATIONS"	
		Df	*In*	*T*		
aristaless	5	1	1	0	0	7
Star	5	5	0	1	1?	12
asteroid	4	0	0	1	1?	6
heldout	9	0	1	0	0	10
Notch	13	—	—	—	—	13

was roughly 19,500. F_1 aberrant individuals, in which the eyes were smaller and rougher than in *S*/+ flies, or whose phenotypes resembled *al* or *ho*, were separately crossed to an *al S ho/Cy, E-S* stock. A preliminary examination was made of the salivary gland chromosomes of F_1 larvae from each of the fertile progeny tests. This was followed up in most cases by a more detailed cytological analysis after a balanced culture had been established.

No substantial case of "point mutation" to either *al* or *ho* was detected in this experiment. A possible change of this type to a *S* and to an *ast* allele, simultaneously, will be discussed later. The following is a description of those analyzed changes shown in table 6 which were found to be accompanied by a chromosomal aberration; an additional *S* deficiency (*Df-S7*) obtained from a related X-radiation experiment is also included:

Df-al-Deficiency (2) *aristaless.*—LEWIS 1940. *Df*/+ is an extreme Minute with rough eyes and slight *ex*-and *ds*-like effects. Deficient for *al, ex* and *ds;* but not for *l-gl, net* or *S. Df-al/net* has a weak net effect. The loss extends from just before the 21C1–2 doublet to just before the 21D1–2 doublet.

Df-S1-Deficiency (2) *Star.*—LEWIS 1940, *Df*/+ has a slightly smaller and rougher eye than "+" and is less extreme and more variable than "*S*/+"; viability and fertility are good. *Df/ast* is somewhat less extreme than *S*+ /+*ast*, and more like *S ast*/+*ast;* otherwise the *Df* acts exactly like *S*. Deficient for *ds*, *S*, and *ast;* but not for *l-gl*, *net*, *al*, *ex*, *shr*, or *ho*. The breaks follow the medium 21C3 band and just precede the heavy 22A3 band.

Df-S2-Deficiency (2) *Star-2.*—LEWIS 1940. Phenotypic effects are like those of *Df-S1*. Deficient for *ds*, *S*, *ast*, and *shr;* but not for *l-gl*, *net*, *al*, *ex*, or *ho*. The loss appears to extend from just to the left of 21D1–2 doublet to just preceding the 22B1–2 doublet. It is also possible that the breaks occur in the middle of these two doublets.

Df-S3-Deficiency (2) *Star-3.*—LEWIS 1940. Phenotypic effects are like those of *Df-S1*. Deficient for *S* and *ast;* but not for *l-gl*, *net*, *al*, *ex*, *ds*, *shr*, or *ho*. The loss extends from just to the right of the 21D1–2 doublet to just before the 22A1–2 doublet.

Df-S4-Deficiency (2) *Star-4.*—LEWIS 1940. Phenotypic effects are like those of *Df-S1*. Deficient for *ds*, *S*, *ast*, and *shr;* but not for *l-gl*, *net*, *al*, *ex*, or *ho*. The breaks follow the medium 21C3 band and the 22B1–2 doublet.

Df-S5-Deficiency (2) *Star-5.*—LEWIS 1940. Phenotypic effects are like those of *Df-S1* except that *Df-S5*/+ has a slight *ex*-like effect. Deficient for *ex*, *ds*, *S* and *ast;* but not for *l-gl*, *net*, *al*, *shr*, or *ho*. The breaks occur just after the 21C1–2 doublet and after the heavy 22A3 band.

In(2LR)al^V—Inversion (*2LR*) *aristaless-variegated.*—LEWIS 1940. *al^V/al* is similar to homozygous *al;* however, in the presence of an extra *Y* chromosome, *al^V/al* is wild type. The homozygote is lethal. *Df-al/al^V* is viable and variegated in that sometimes only one instead of both of the aristae is missing. The euchromatic break appears just to precede the 21C1–2 doublet; the right break is in the heterochromatin of chromosome 2R.

T(2;3)S^L—Translocation (*2;3*) *Star.*—LEWIS 1940. *S^L*/+ resembles *S*/+; *S^L* also resembles *S ast$^+$* opposite *ast*, *ast^2*, *ast^3*, *ast^4*, and *E-S*. This is a complex rearrangement involving at least three breaks: one follows the 21E1–2 doublet of chromosome 2L; chromosome 3R has a break which apparently just precedes 88D8 and another in the basal heterochromatin. The tip of chromosome 2L up to and including 21E1–2 is translocated to heterochromatin of chromosome 3, but it has not been possible to determine from a salivary gland chromosome analysis whether the section from the base of 3R to 88D is inverted or whether there is an insertion of heterochromatin (without inversion) between 88D and 21E. The tip of 3R to 88D is exchanged for the tip of 2L.

In-ho—Inversion (2) *heldout.*—LEWIS 1940. *In/ho* is phenotypically like homozygous *ho*. Opposite *Df-S3*, *S* or *ast*, the inversion acts as though it carries the normal alleles of *S* and *ast*. The homozygote has reduced eyes with anterior indentation; the wings are reduced to tiny stubs (*In/vg* is wild type); the female is fertile, but the male lacks genitalia and the anal apparatus. The break points of this short inversion occur just to the left of the 21E1–2 doublet and just to the right of the 22E1–2 doublet.

T(2;4) ast^V—Translocation (2;4) asteroid-variegated.—LEWIS 1940. *ast^V/+* is wild type; *ast^V/ast* and *ast^V/S* resemble but are more variable than *ast/ast* and *S+/+ast*, respectively. *ast^V/Df-S3* is lethal. *ast^V/ci* gives a cubitus interruptus (*ci*) effect. The variegated *ast*- like effect is completely suppressed in the XXY female. Duplication and deficiency derivatives are viable. *Df-ast^V/+* resembles *Df-al/+* except that the former has a much more roughened eye; the venation of *Df-ast^V/net* resembles homogygous net; *Df-ast^V/l-gl* is lethal. *Dp-ast^V/l-gl/l-gl* is viable. One break just follows the 21E1–2 doublet and the other is in the heterochromatin of chromosome 4.

Df-S7-Deficiency (2) Star-7.—LEWIS 1940. From X-rayed *net ho/net Dp-S sp cl* male. Associated with *net* and *ho*. Loss extends from just after the medium 21C3 band to just preceding the heavy 22A1–2 doublet. Lost.

With this material it was possible to locate with varying degrees of precision most of the known mutants at the extreme left end of the second chromosome. The results are diagrammed in figure 1. The locations of *al*, *ex*, and *shr* are based directly on tests of these mutants to the *S* deficiencies and *Df-al*. It is likely that the locus of *ho* is in the neighborhood of section 22E on the basis of genetic and cytological studies of *In(2)ho*. The location of *l-gl* follows from studies of the duplication and deficiency derivatives of *T(2;4) ast^V* which "cover" this locus in contrast to the *S* deficiencies and *Df-al*.

Net (0.0–)

The process of locating the *net* locus was complicated by the enhancement of *net* by Minute deficiencies of the *Df-al* and *Df(2)ast^V* type, and by the possible complete suppression of the *net* phenotype by the *S* effect of the *S* deficiencies. The latter possibility was excluded by the fact that *net Df-S7/net* flies had only a very slightly suppressed *net* venation. Critical evidence for the location of *net* was obtained by the use of a duplication for the left end of chromosome 2 derived from *T(2;4)b* of DOBZHANSKY. Females of composition *T(2;4)b, net/net* were crossed in separate experiments to *Df-al/Cy* and *Df-S5/Cy* males. The non-Curly duplication offspring having the composition *Dp(2)b, net/net/Df-al* and *Dp(2)b, net/net/Df-S5*, respectively, had normal venation. On the other hand, when females of the above type were mated to *T(2;4)ast^V/Cy* males, some of the duplication offspring had typical *net* venation and therefore presumably had the genetic make-up *Dp(2)b, net/net/Df(2)ast^V*. These results are consistent with the fact that *Df(2)ast^V/net* flies show a more extreme *net* venation than *Df-al/net* flies. Hence the locus of *net* must lie to the left of the 21C1–2 doublet.

The locus of *net* (originally 0.3± on the genetic map) was reexamined, but the results were negative with respect to whether *net* is to the left or to the right of *al*. Thus, on the basis that it is to the right of *al*, no crossovers were obtained between the locus of *al* and *net* among 90 tested crossovers between *al* and *S*. Again, on the basis that it is to the left of *al*, there were no crossovers between *net* and *al* among ten tested crossovers between *net* and *S*. From the cytological evidence, however, it is likely that the *net* locus is to the left of

al. It should be noted here that the map order of *net* (0.0—) and *l-gl* (0.0—) is unknown.

As has already been mentioned, (+*as*^{l})(+*ast*) *ho/net S dp cl* females produced a very rare type having the composition, *ast ho*. A total of all matings in which females of this type were used produced three *ast ho* to 247 *net ast ho* crossovers, the latter occurring with a frequency of 0.35 per cent. The *ast ho* types, which were normal cytologically, were first interpreted as indicating that the locus of *net* was included in the region duplicated in *Dp-S* (LEWIS 1941). This tentative conclusion is now no longer consistent with the deficiency evidence for the location of *net*. The *ast ho* types may have resulted from double crossing over, which is very improbable for a region only one to two map units in length, or from unequal crossing over involving sister chromatids of the duplication chromosome.

Dachsous (0.3)

The deficiencies, *Df-al*, *Df-S2*, and *Df-S6* (identical with *Df-S2*, but independent in origin) appear to be deficient for the *ds* locus as determined by tests of these deficiencies not only to the mutant *ds*^{1}, but also to *ds*^{W}, *ds*^{k}, and *ds*^{38k}. Yet, cytologically the above *S* deficiencies do not appear to overlap *Df-al* (fig. 1). In the case of both *Df-S2* and *Df-S6* it is possible that the loss involves only the right half of the 21D1–2 doublet; in any case, they are separated from *Df-al* by at least one disc of the chromosome. Perhaps the simplest explanation of these ambiguous results is that either *Df-al* or *Df-S2* (and *Df-S6*) is a true deficiency for the *ds* locus while the other has a position effect at, or an interaction with, the *ds* locus resembling deficiency for that locus.

Star (1.3)

Purely genetic evidence has shown that the *S* locus is included in the repeated section of *Dp-S*. That evidence has included not only the "extraction" of the normal allele of *S* from each section of (+*ast*)(+*ast*) but also the "insertion" of *S* itself into each section. Hence, the *S* locus must lie in the region from 21D3 to 21E1–2, inclusive—that is, the region present twice in *Df-S*. This shows that the deficiencies, *Df-S1* to *Df-S7*, inclusive, which have in common a loss of that section and which closely resemble *S*, phenotypically, are indeed deletions for the *S* locus.

A more precise location of *S* can be made with the use of *T(2;4)ast*^{V} and *T(Y;2)21E*. The latter translocation was kindly supplied by DR. SCHULTZ, who found (unpublished) that the second chromosome was broken just to the left of the 21E1–2 doublet and translocated with the Y chromosome. *T(Y;2)21E* over *S* or *ast*, acts as though it carries the normal alleles of *S* and *ast*. The deficiency for the tip of chromosome 2 derived from this translocation closely resembles *Df-al* in its effects. Whereas *Df(2)ast*^{V}/*S* is lethal, *Df(2)21E/S* is very similar to *Df(2)ast*^{V}/+. This suggested that the loss of the 21E1–2 doublet is associated with an *S* effect. To eliminate as far as possible the effects

of deficiencies for euchromatic regions other than the 21E1–2 doublet, these two translocations were combined to produce a derivative having the tip of chromosome 2L up to and including 21D4, derived from *T(Y;2)21E*, and the remainder of the second chromosome, from 21E3 on derived from *T(2;4)ast^V*. For this purpose *y/Y* (attached-X): *T(Y;2)21E/Cy, E-S* females were mated to *T(2;4)ast^V/Cy, (2L)dp^2 b pr* males. F_1 Curly flies with eyes resembling those of *S/E-S* were mated *inter se* to establish a balanced stock of this newly derived deficiency. An examination of the salivary gland chromosomes of this stock showed that the expected combination of the two translocations was realized. This synthesized deficiency has been called Deficiency (2) Star-derived, *Df-S-der*. As kept in stock, *Df-S-der* has a deficiency for the 21E1–2 doublet, a duplication for part of the Y chromosome, and a deficiency in the heterochromatin of chromosome 4. Phenotypically, *Df-S-der* was indistinguishable from the other *S* deficiencies in its effects opposite +, *S*, *ast*, *ast^3*, and *ast^4*. Thus, the *S* locus is closely confined to the region of the 21E1–2 doublet structure of the salivary gland chromosomes.

Asteroid (1.3+)

It is well recognized that the determination of genetic and cytological correspondences by deficiency evidence alone may sometimes be misleading, owing to the possibility of position effects accompanying these deficiencies. As has already been described, such difficulties were encountered in attempting to ascribe the *ds* locus to some precise section of the chromosome. In view of the position effect known to exist at the *S* and *ast* loci, it is apparent that these loci present a special problem. In the case of *S*, its location by means of deficiencies could be independently checked by the use of a tandem duplication for that locus—namely, *Dp-S*. Similar methods have been used in the location of *ast*.

It can be safely assumed that *Df-S4*, which involves a loss of the shrunken locus, is a deficiency for the *ast* as well as the *S* locus. Moreover, it more closely resembles *S ast* than *S ast$^+$* in its effects opposite *ast*. Inasmuch as *Df-S-der* is phenotypically indistinguishable from *Df-S4*, it is likely that it too involves a loss of both the *S* and *ast* loci. It is important to note here explicitly that *Df-S-der* is derived from *T(Y;2)21E*, which has no detectable departure from normal when opposite *S* or *ast;* and from *T(2;4)ast^V* which likewise behaves like wild type when the variegation is suppressed by an extra Y chromosome. Now it has been determined that the presence of an extra Y chromosome does not change the phenotypic effects of *Df-S-der*. It may therefore be assumed that those effects are attributable mainly to the deficiency for the 21E1–2 doublet.

Considerable evidence is available for supposing that the *ast* locus, like the *S* locus, is included in *Dp-S*. This evidence will be described in a later section and comes from a further study of the position effect at the *S* and *ast* loci by means of chromosomal aberrations and by means of various derivatives of

Dp-S. Formal genetic proof that the *ast* locus is included in *Dp-S* is lacking, however, since the occurrence of the crossover between an asteroid locus in the left section of the duplication and the break point of the duplication has not been definitely detected.

Suppressor of Star

The dominant Suppressor of Star, *Su-S*, found by CURRY, is mentioned here since it has been reported to be close to, if not an allele of *S*. A reinvestigation of *Su-S* has shown that it is probably not the result of a point mutation but rather that its effect is attributable to the double deficiency involved in *In(2L)Cyt*, with which it was associated at the time its effect was first detected. This was shown by deriving, anew, *In(2L)Cyt*, from a mating of *In(2L)Cy*, $dp^2/In(2L)t$ females to *S dp/Cy* males. *In(2L)Cyt* was detected in the progeny of this cross by virtue of its suppressing effect on *S*. The complementary or double duplication derivative was also detected in this experiment by virtue of its slight enhancement of *S*. A cytological analysis of *In(2L)Cyt*, made by BRIDGES and LI (1936), showed the presence of a deficiency for 22D3 and all of section 34A. There is some evidence of a preliminary nature for supposing that it is the deficiency in 22D which is responsible for the suppression of *S* by *In(2L)Cyt*. Whether or not the location of Enhancer of Star, *E-S*, (locus, 2- 6±) coincides with that of *Su-S* is not known. Existing stocks of *E-S* that have been examined cytologically show the presence of the Curly inversion and not the double duplication derivative complementary to *In(2L)Cyt*. The enhancement of *S* by this duplication type is by no means as extreme as that by *E-S*.

THE PHENOTYPES OF DP-S DERIVATIVES

Unequal crossing over provides a mechanism whereby different *S* and *ast* alleles can be introduced into *Dp-S*. It seemed likely that a study of the derivatives, so obtained, should shed further light on the nature of position effect at these loci. Using only the following six combinations of *S* and *ast* alleles: ++, $+ast^4$, +*ast*, *S*+, $S\ ast^4$, and *S ast;* there are 36 possible ways of varying the composition of *Dp-S*. Actually, it was not feasible to vary the composition of the *ast* locus in the left section of the duplication. This leaves twelve combinations, of which all but two, $(+ast)(+ast^4)$ and $(S\ ast)(+ast^4)$, have been synthesized. The derivatives may be divided into the following two groups:

Group I	Group II
1. (+*ast*)(++)	6. (*S ast*)(++)
2. (+*ast*)(+*ast*)	7. (*S ast*)(+*ast*)
3. (+*ast*)(*S*+)	8. (*S ast*)(*S*+)
4. (+*ast*)(*S ast*)	9. (*S ast*)(*S ast*)
5. $(+ast)(S\ ast^4)$	10. $(S\ ast)(S\ ast^4)$

In each of these ten types the presence of the duplication was confirmed by an examination of the salivary gland chromosomes. Only a brief description

will be given of the methods by which these types were originally obtained.

All members of Group I were obtained from (+*ast*)(+*ast*). The unequal crossover, *net* (+*ast*)(++) *ho*, was detected among the progeny of *net* (+*ast*)(+*ast*) *dp cl/al ho* females as a complete suppressor of S^2 *E-S* (*Cy*); whereas, (+*ast*)(+*ast*) only partially suppresses S^2 *E-S*. The derivatives 3, 4, and 5 could be obtained with relative ease from (+*ast*)(+*ast*)/*S*, (+*ast*)(+*ast*) /*S ast*, (+*ast*) (+*ast*)/(*S* ast^4) females, respectively, when mated to *S/Cy* males. The marker genes are omitted here for simplicity. The new combinations, 3, 4, and 5, when opposite *S*, are found to be inseparable in appearance from *S*/+; while (+*ast*)(+*ast*)/*S* has a normal or only very slightly roughened eye.

A brief description has already been given of the method of deriving (*S ast*) (+*ast*) from (+*ast*)(+*ast*). Because of the difficulty of introducing *S* into the

TABLE 7

Phenotypic effects of certain Dp-S heterozygotes. The composition of the duplication chromosome is shown at the left while that of the normal chromosome is shown at top. The entries in the table indicate standard diploid types to which the appropriate Dp-S heterozygotes are very closely comparable.

		+	*ast*	*S* ast^4	*S ast*	*S*	*Df-S3*	*Df-S-der*
1	(+ *ast*)(+ +)	"+"	"+"	"+"	"+"	"+"	"+"	"+"
2	(+ *ast*)(+ *ast*)	"+"	"+"	"*ast*/+"	"*ast*/+"	"*ast*/+"	"*ast*/+"	"*ast*/+"
3	(+ *ast*)(*S* ast^4)	"+"	"*ast*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"
4	(+ *ast*)(*S ast*)	"+"	"*ast*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"
5	(+ *ast*)(*S* +)	"+"	"*ast*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"
6	(*S ast*)(+ +)	"+"	"*ast*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"	"*S*/+"
7	(*S ast*)(+ *ast*)	"*ast*/+"	"*ast*"	"*S* ast^4/*ast*"	"*S ast*/*ast*"	"*S*/*ast*"	"*S ast*/*ast*"	"*S ast*/*ast*"
8	(*S ast*)(*S* ast^4)	"*S*/+"	"*S* ast^4/*ast*"	lethal	lethal	lethal	lethal	lethal
9	(*S ast*)(*S ast*)	"*S*/+"	"*S ast*/*ast*"	lethal	lethal	lethal	lethal	lethal
10	(*S ast*)(*S* +)	"*S*/+"	"*S*/*ast*"	lethal	lethal	lethal	lethal	lethal

left section of *Dp-S*, and of detecting the product when it is produced (*S ast*)(+*ast*) was used as the source of the remaining members of Group II. This, then, was accomplished by methods similar to those by which members of Group I were derived from (+*ast*)(+*ast*) except that the detection of the Group II derivatives was on a different basis. One example will be given to illustrate this difference. Females of composition *al* (*S ast*)(+*ast*)/*net S dp cl*, were mated to *al ho* males. F_1 *al* "*S*/+" offspring were then outcrossed to an *al ho* stock, and the F_1 larvae from this latter mating were then analyzed cytologically for the presence of *Dp-S*. It was found in this way that roughly one-half of the *al* "*S*/+" crossovers had carried the duplication; in other words, they were of the expected type, *al* (*S ast*)(*S*+). Similar methods had to be used for each of the remaining members of Group II.

The phenotypic effects of ten *Dp-S* derivatives when opposite +, *ast*, *S* ast^4, *S ast*, *S*, *Df-S3*, or *Df-S-der*, are shown in table 7. Those types which have a normal eye or occasionally a very slightly roughened one are compared with *ast*/+, to which they are perhaps most similar. Although a deficiency for, and only for, the region present twice in *Dp-S* is not available, an approximation can be realized by comparing the effects opposite *Df-S-der*, and *Df-S3*, whose

deficiencies are respectively smaller and larger than that region. From table 7, it is apparent that these deficiencies act alike in all combinations shown. Two important combinations of these deficiencies are those with (+*ast*)(*S*+) and (*S ast*)(++). In each case the effect is more nearly similar to that of *S*/+ than to *Df-S*/+. The effects of these two deficiencies when opposite (*S ast*)(+*ast*) are compared with those of *S ast*/*ast*; they might equally well be compared with *ast*/*Df-S*.

It has already been reported that types analogous to double-Bar and quadruple-Bar have been obtained from *Dp-S* (LEWIS 1941). The triple form, since it arose from (+*ast*)(+*ast*) females by unequal crossing over, has the composition (+*ast*)$_3$. The subscript, 3, here is used to indicate that the section present in parenthesis occurs three times in tandem repetition. Using a similar notation, the quintuplication, which was derived from homozygous (+*ast*)$_3$ females, has the composition (+*ast*)$_5$. A comparison of (+*ast*)$_2$/(+*ast*)$_2$ and (+*ast*)$_3$/*ast* shows no detectable difference. That is, the eye looks normal in each case, and slight extra veins are occasionally present in each. No (detectable) position effect was found in a comparison of (+*ast*)$_3$/(+*ast*)$_3$ with (+*ast*)$_5$/*ast*. Each of these latter types has an eye slightly larger than normal with bulging and somewhat disrupted facets; extra veins are also present in each to apparently the same extent.

Experimental evidence for the nature of the position effect in (+*ast*)(+*ast*) has been derived from a study of a translocation, *T*(*2;3*) *Dp-S*, obtained from X-radiation of (+*ast*)(+*ast*) males. A salivary gland chromosome analysis showed the presence of a reciprocal translocation, having one of its breaks within the duplication, just to the left of the 21E1–2 doublet of the right section of *Dp-S*, and the other break in heterochromatin of 3R. In the presence of a normal heterochromatin balance, the phenotypic effects of *T*(*2;3*)*Dp-S* are such as might be expected if it contained two doses of *ast*, just as the notation (+*ast*)(+*ast*), would indicate; thus, *T*(*2;3*)*Dp-S*/*S* is similar to, although more variable than, homozygous *ast*. However, when studied in the XXY female, this latter combination has a normal eye, or occasionally a slightly roughened one, typical of that of (+*ast*)(+*ast*)/*S*. In other words, the effects of (+*ast*)(+*ast*) appear to be the same whether the *S ast* regions are relatively close, as in *Dp-S*, or are widely separated, as in *T*(*2;3*)*Dp-S*. It is important to note here explicitly that the phenotypic effects of (+*ast*)(+*ast*), itself, are not changed by the addition of a Y chromosome.

X-ray induced changes at the S and ast loci

An analysis of X-ray-induced changes at the *S* and *ast* loci offers another line of attack on the nature of the position effect at these loci. Such a study must be limited by the practical difficulty of obtaining a sample of such changes without employing their phenotypic effects as a basis for their detection. In the experiment shown in table 6 it was possible to detect changes from wild type which closely resembled either *S* or *ast*. Neglecting deficiencies, a change of each type was detected—namely, *T*(*2;3*)*S*^{L} and *T*(*2;4*)*ast*^{V}. To these aberrations should

be added the *S* translocation of MULLER (MULLER and PAINTER 1929), *T(2;3)S*^{M}, which likewise arose from wild type. The latter *S*, however, was detected by its dominant *S*-like effect opposite wild type. In spite of the differences in the bases for detection of *S*^{L} and *S*^{M}, the phenotypic effects of these changes, as far as could be determined by tests to various *S* and *ast* alleles, were identical with that of *S* itself. The addition of an extra Y chromosome did not alter the phenotype of either *S*^{L} or *S*^{M}. The recessive change, *ast*^{V}, was more similar to *ast*^{1} than the other *ast* mutants in its effects on the venation and eyes.

Cytologically, *S*^{M}, *S*^{L}, and *ast*^{V} have in common a break immediately following the 21E1–2 doublet, as indicated in figure 1. In this connection, one other aberration obtained from wild type, *In(2)ho*, also has a break at this point. As already noted, *In(2)ho* does not appear to be associated with a change at the *S* or *ast* loci. Similarly, *T(Y;2)21E* has no detectable effect at these loci. Its break in the second chromosome, however, is immediately to the left of the 21E1–2 doublet.

Only one change at the *S* and *ast* loci not associated with a demonstrable rearrangement in the salivary gland chromosomes occurred in the experiment shown in table 6. This change has been given the tentative symbol, *S*^{X} *ast*^{X}, since, as shown below, it behaves genetically as though it involves a simultaneous change at each locus. *S*^{X} *ast*^{X}/+ flies show an occasional gap near the tip of the second longitudinal vein and have slightly smaller eyes than those of S/+ flies. It was found that if these slight differences from *S* are due to modifiers, those modifiers must be very closely linked to *S*^{X} *ast*^{X}. Opposite the *ast* alleles, *ast*, *ast*^{2}, *ast*^{3}, amd *ast*^{4}, *S*^{X} *ast*^{X} closely resembled *S ast*$^{+}$.

On the assumption that *S*^{X} *ast*^{X} represented merely a new dominant allel of *S* a search was made for "wild type" crossovers from *S*^{X} *ast*^{X}/*ast*^{4} females similar to those obtained from *S*/*ast*^{4} females. In this experiment females of composition *In(1)dl-49*, *cm*^{2}/+; *S*^{X} *ast*^{X}/*ast*^{4} *ho*; *In(3LR)sep*/+, were individually mated to *al S ho*/*Cy*, *E-S* males. This mating produced, among 3,505 non-Curly offspring, a single female resembling the expected wild type class. The latter fly had normal (non-*ho*) wings, but the eyes were slightly smaller than those of *S*/+. Upon further testing, the crossover chromosome appeared to carry a very slight *ast* allele, *ast*^{X}. It is of course possible that *ast*^{X} arose in the above experiment as a mutation of *ast*^{4}. The following properties of *ast*^{X} were determined: *ast*^{X}/+ and *ast*^{X}/*ast*^{X} are wild type; *S*/*ast*^{X} closely resembles *S*^{X} *ast*^{X}/+ except that the former frequently, if not always, has normal venation; *S*^{X} *ast*^{X}/*ast*^{X} has a somewhat smaller eye size than that of *S*^{X} *ast*^{X}/+. A salivary gland chromosome analysis of *ast*^{X}, as well as of *S*^{X} *ast*^{X}, failed to show any disturbance in the left end of the second chromosome. The possible significance of the *S*^{X} *ast*^{X} change will be taken up in the discussion of this paper.

It will be recalled that the spontaneous *S* duplication, *Dp-S*, has a break immediately to the right of the 21E1–2 doublet (of the left section of the duplication), similar to that found in *S*^{L}, *S*^{M}, and *ast*^{V}. Phenotypic studies of *Dp-S*

suggested that the *ast* mutant originally present in the left section of the repeat acts as though it had reverted to wild type. With this result particularly in mind, *al ast ho/net ast dp cl* males were X-radiated (6,500 r units) and mated to *S/Cy, E-S* females. "Reversions" of *ast* were looked for among the F_1 flies. The following three changes closely resembling reversions of *ast* to *ast+* were found, all of which were fertile:

T(2;3) ast^{rv1}—Translocation (2;3) asteroid reverted-1.—LEWIS 1942. The translocation carries the mutants *al* and *ho*. Phenotypically, *ast^{rv1}* is inseparable from wild type opposite +, *ast*, *ast^3*, *ast^4*, *S ast$^+$*, *S ast*, *S ast^4*, *E-S*, and *ast^V*. The homozygote is lethal. However, *ast^{rv1}/Df-S4* is viable and resembles *S/+* rather than *Df-S4/+*. The eye is slightly less rough in either *ast^{rv1}/S^M* or *ast^{rv1}/S^L* than it is in *S^M/+* or *S^L/+*. At least three breaks are involved in this complex rearrangement. Chromosome 2L is broken just to the right of 21E1–2; chromosome 3L has a break just after the heavy dotted 68C2 band and 3R has a break which probably just precedes 88D9 (BRIDGES' 1935 map). The new rearrangement is in the form of cyclical exchange of tips. Thus, 2L tip replaces the tip of 3R, which in turn replaces the 3L tip, which in turn replaces the tip of 2L.

In(2L)ast^{rv2}—Inversion (2L) asteroid-reverted-2.—LEWIS 1942. The inverted chromosome also carries the mutants, *al* and *ho*. Phenotypically, *ast^{rv2}* is identical with *ast^{rv1}*, including its effects opposite *Df-S4* and *S^L*. *S^M/ast^{rv2}* overlaps wild type. *ast^{rv1}/ast^{rv2}* is wild type. A preliminary salivary gland chromosome analysis showed a break immediately following the 21E1–2 doublet and one in section 31, the section between then being inverted.

T(2;3) ast^{rv3}—Translocation (2;3 ast^{rv3}.—LEWIS 1942. The translocation was associated at the time of its origin with the mutants, *net*, *dp*, and *cl*. The homozygote and *ast^{rv3}/Df-S4* are lethal. Incomplete phenotypic studies indicate that opposite *S*, *S ast*, *S ast^4*, and *S^L*, *ast^{rv3}* produces a slightly rougher eye than that characteristic of *S/+*. *ast^{rv3}/ast^3* and *ast^{rv3}/E-S* are wild type. The rearrangement involves a reciprocal exchange of the extreme tips of chromosomes 2L and 3L, with breaks just to the right of 21E1–2 and to the left or right of 61C1. In its new location this latter region of chromosome 3L assumes a heterochromatic character.

Again, as in *Dp-S*, apparent reverse mutation of *ast* was associated with a chromosomal rearrangement in which the 21E1–2 doublet was transferred to a new position. As yet, no spontaneous or induced reverse change of *ast* to *ast$^+$* unaccompanied by a chromosomal rearrangement has been detected.

The above results further support the conclusion that *ast* as well as *S* is included within the confines of the 21E1–2 doublet, for it is this doublet and not the material to the right of it which has undergone rearrangement in both *Dp-S* and the X-ray induced asteroid-reverted changes. That is, in *Dp-S* the material to the right of 21E1–2, since it not present twice, is in its normal position following the 21E1–2 doublet of the right section of the repeat.

From the same X-radiation experiment which produced the asteroid-re-

verted changes, a complete suppressor of *S* was detected as a single (sterile) male with normal eyes and wings. Several changes resembling partial reversion of *ast* were also detected. Among those of the latter which were analyzed, two were found to be due to permanent changes. They were complex rearrangements in which the 21E region was normal, but each had in common a break in or close to section 22D and had that section translocated to heterochromatin of chromosome 3. It is possible that the partial suppressing effect on *S/ast* of these aberrations is related to that shown by the double deficiency *In(2L)Cyt* or *Su-S*, which has one of its deficiencies in 22D.

DISCUSSION

A plausible interpretation of the *S* and *ast* loci can be developed by assuming that they have resulted from duplication of an ancestral locus, that duplication now being established in the species. This notion is chiefly based on the finding that *S* and, very probably, *ast* are included in the 21E1–2 doublet structure of the salivary gland chromosomes. The possibility that such structures might represent instances of duplication of a single band, or short section, was first pointed out by BRIDGES (1936). It is interesting to note that BRIDGES chose the 21E1–2 doublet as a characteristic example of this type of duplication.

That the doublets involve two discrete bands was shown experimentally by BRIDGES et al. (1936) in an analysis of the Notopleural deficiency. In this case the break points of the deficiency occurred between the halves of two doublets. BRIDGES (Drosophila Information Service **9**) has reported other instances, chiefly spontaneous, where breaks have separated the halves of a doublet. Similarly, METZ (1937) has found that some of the small deficiencies which occur in stocks of *Sciara ocellaris* involve the loss of only one band of a doublet structure. METZ (1938) also holds to the view that doublets have arisen by a process of duplication and, on this basis, that these "deficiencies" are either the original unduplicated condition or are secondary losses from duplications already established in the species.

Small, intrachromosomal duplications have been given the convenient name "repeat" by BRIDGES. The doublet structures fall in the category of "tandem repeats" in which the duplicated section immediately follows (or precedes) the original section. The tandem repeat may be either "direct" or "reverse." Thus, if the section present twice in the repeat is represented by the letters, CD, then a direct tandem repeat can be written as CDCD, and a reverse tandem repeat as CDDC or DCCD. The direct repeats such as Bar, or the Star Duplication, are unstable in the sense that they can give rise by unequal crossing over to the unduplicated condition, CD, or to a complementary triplicated form, CDCDCD. Reverse repeats, however, are expected to be stable in the sense that unequal crossing over (if it occurs at all) should result only in a dicentric chromatid, which will be lost to the polar bodies, and an acentric fragment.

The problem arises as to which of these two categories of repeats the 21E1–2 doublet may be assumed to belong. This cannot be decided from cytological

evidence alone, since only the duplication of one disc is involved. *A priori*, only the stable or reverse type would be expected to become established in the species. This is supported by the fact that all of the tandem repeats, which have been described for the normal salivary gland chromosomes of *Drosophila melanogaster* and in which the sequence can be determined, are of the reverse type. Moreover, if the 21E1–2 doublet were a direct repeat, then it might be anticipated that some of the changes observed at either the *S* or *ast* loci would represent either the unduplicated or the triplicated forms. However, no cytological changes whatsoever in the 21E1–2 doublet were apparent in any of the mutants at these loci or in any of the analyzed crossovers between these loci.

Assuming from the above considerations that the 21E1–2 doublet is a tandem reverse repeat, we can draw the genetic analogy that *S* and *ast* are reversely repeated loci. There are at least two methods by which reverse repeats may offer a basis for producing changes which resemble mutation but which are due to crossing over in the repeat. Firstly, genetic differences in the duplicated section of a naturally occurring repeat may arise within a stock or between different stocks. Such differences need not be detectable, phenotypically, since the repeated section is present in four doses. Applied to the *S* and *ast* loci, this mechanism suggests that such differences as are observed between $S+/+ast$ and $S\ ast/++$, or between $S+/+ast^4$ and $S\ ast^4/++$, may be due, not to position effect, but to unknown genetic differences in what were thought to be normal alleles. However, this is very unlikely in that the "wild type" crossovers derived from either $S+/+ast$ or $S+/+ast^4$ females are identical not only with each other but also with normal chromosomes from unrelated stocks, even in the critical test opposite $S\ ast$ or $S\ ast^4$. In particular, the critical test held in an experiment in which the "wild type" crossover and $S\ ast^4$ were each recovered in the same experiment from $S+/+ast^4$ females.

Secondly, consider a tandem repeat in which the two halves are no longer exactly identical, genetically, and assume that an inversion of the repeat has been produced. The original repeat may be written CDD′C′, where the primes indicate genetic differences between the repeated regions. For simplicity, the inversion may be assumed to involve only the repeat itself and may be represented as C′D′DC. It is apparent that this simple case has a highly important consequence—namely, that the inversion cannot be detected cytologically. Crossing over in CDD′C′/C′D′DC heterozygotes will give rise to genetically different products. For example, if crossing over occurs between D and D′, then these products are CDDC and C′D′D′C′. This mechanism is suggested as a possible basis for the behavior of the X-ray induced *S*-like change, $S^X\ ast^X$, which from linkage studies appeared to involve a change at the *ast* locus as well as the *S* locus. Here it is necessary to assume that the *X*-rays induced an inversion of just the repeated region, since $S^X\ ast^X$ was normal, cytologically. The new sequence may be written $ast^+\ S^+$, and the *S*-like effect may then be attributed to a position effect rather than to a point mutation. On this basis, the "mutant" ast^X, recovered from $S^X\ ast^X/++$ females may be interpreted as a duplication for the *ast* locus and a deficiency for the *S* locus—that is, its

composition may be written ast^+ ast^+. This mechanism does not seem applicable to the spontaneous *S* and *ast* mutants, since the crossover corresponding to ast^+ ast^+ does not differ detectably from S^+ ast^+; furthermore, S^+ could not be here assumed to be identical with ast^+, for then the inversion itself would accomplish nothing new.

The concept of *S* and *ast* being repeated loci is also useful in that it relates, by analogy, the position effect observed at these loci to that occurring at a known repeat region—namely, the Bar Duplication. As a result of the cytological finding of BRIDGES (1936) and MULLER, et al. (1936) that Bar is a duplication, the position effect demonstrated by STURTEVANT (1925) may now be stated as depending on the way in which four, rather than two, homologous sections are distributed between the two homologs, Thus, equal distribution of the four doses of the Bar region results in a larger eye than when three doses are present in one homolog and the fourth in the other. The position effect, here, would appear to extend over a distance at least as great as the length of the duplication, or perhaps as great as the length of triplicated region. This is remarkable in view of the fact that other types of rearrangements which involve only euchromatic portions of the chromosomes and which seem to be accompanied by position effects do not show this spreading effect.

It is suggested that this spreading effect is due not alone to the closeness of the two elements in Bar, or the three elements in *BB*, but likewise to the homology of those elements. This possible role of repeats in producing pronounced position effects will be discussed more fully in connection with position effects at the *S* and *ast* loci.

Several methods of attack were used in studying position effect at these loci. Firstly, their effective position could be altered by allelic substitutions alone. Thus, no demonstrable chromosomal aberrations appeared to accompany *S*, *ast*, ast^4, *S* ast^4, or *S ast;* yet, a striking difference exists (1) between *S/ast* and *S ast/+*, and (2) between *S/*ast^4 and *S* ast^4*/+*. Similarly, *S ast/*ast^4 is genetically equivalent to *S* ast^4*/ast;* yet the former has a larger eye and less interrupted wing venation than has the latter. It may be emphasized that in comparisons (1) and (2) there is an important qualitative difference in that neither *S ast/+* nor *S* ast^4*/+* has ever been observed to overlap the small, roughened eye types characteristic of either *S/ast* or *S/*ast^4; whereas, *S ast/*ast^4 and *S* ast^4*/ast* may occasionally resemble one another.

This position effect at the *S* and *ast* loci may be stated more analytically. The viable, diploid combinations of *S*, ast^4, *ast*, *S* ast^4, *S ast*, and wild type can be arranged in the following series (the symbol, >, means " has a larger and less roughened eye than," while the symbol, =, means " is phenotypically approximately equivalent to"): $+/+ = ast^4/+ > ast/+ > ast^4/ast^4 > S\ ast^4/+ = S\ ast/+ = S/+ > ast/ast^4 > S\ ast^4/ast^4 > S\ ast/ast^4 > S/ast^4 = ast/ast > S\ ast^4/ast > S\ ast/ast > S/ast$. From this seriation it may seen that if (n) represents any one of the six combinations of these alleles, then the order may consistently be written: $+/n > ast^4/n > ast/n > S\ ast^4/n \geqq S\ ast/n \geqq S/n$. Here, the order, *S* ast^4*/*n>*S ast/*n>*S/*n, is determinate only when (n) is *ast* or ast^4.

The above analysis shows that the order of effectiveness of the *ast* alleles is different, depending on whether they are adjacent to S^+ on the one hand or to *S* on the other. Thus, when these alleles are adjacent to S^+, their effective order may be written: $+ > ast^4 > ast$; while adjacent to *S*, it becomes: $ast^4 > ast > +$. The difference between *S* ast^4/ast and *S* ast/ast^4 leads to the conclusion that more than just a change in the order of effectiveness of the *ast* alleles is involved; however, the above change of order is another way of stating the pronounced position effects detected in comparisons (1) and (2). The problem arises as to which, if any, of these six haploid combinations of *S* and *ast* alleles does not exhibit a (detectable) position effect. (In this discussion the term, position effect, when applied to the haploid chromosome, is meant to imply an interaction between two, or more, neighboring genes or their products, which leads to a different phenotype than would arise if the gene s were widely separated from one another.) As yet, no solution to this fundamental problem is available, since it has not been possible to study the effects of one locus independently of the other. Although none of the available aberrations which have breaks in the vicinity of *S* and *ast* appear to separate these loci, or to involve a loss of one locus and not the other, the effects of these aberrations do have a bearing on the above problem. The study of these aberrations constitutes a second attack on position effect at these loci.

Exclusive of the *S* deficiencies, the available rearrangements involving the *S* region exhibit two significant features. First, only two of these, *T(Y;2)21E* and *T(2;3)Dp-S*, have a break just preceding the 21E1–2 doublet. It is likely that in the latter as well as the former translocation, there is no detectable effect on the immediately adjacent *S* and *ast* alleles. On the other hand, the remaining aberrations have a break immediately following the 21E1–2 doublet, and all of these, except for *In(2)ho*, are associated with a pronounced effect at these loci. This result parallels that found with certain other loci. Thus, among those rearrangements, which have breaks on either side of the scute locus and which do not involve heterochromatin, only those having a break just to the right of the locus are accompanied by a scute-like change. (For a discussion of this case and its bearing on the evidence for position effect accompanying euchromatic exchanges, see MULLER 1941.) The Bar "locus" is another example. Although little material is as yet available, the wholly euchromatic exchanges having a "Bar" effect, including Bar itself, consistently appear to have a break just preceding the 16A1–2 doublet, which structure is known, particularly from studies of GRIFFEN (1941) and SUTTON (1943) to be associated with the Bar effect.

The second important feature is revealed by a study of the non-variegated rearrangements involving the *S* and *ast* regions—namely, all of the available aberrations except for *T(2;4)ast*^{V} and *T(2;3)Dp-S*, whose effects are changed by the addition of a Y chromosome. The former types are associated either with no change whatsoever at the *S* and *ast* loci, as in the case of *In(2)ho* and *T(Y;2)21E*, or they are acccompanied by an identical type of change, the type itself depending on whether the rearrangement occurred in wild type or in

ast. Thus, the dominant *S*-like changes, S^M and S^L, are the only non-variegated aberrations, originating from wild type, associated with a change at the *S* and *ast* loci. In spite of their completely independent origins, and the different bases on which they were detected, these two changes are indistinguishable from each other in all combinations studied. Similarly, the rearrangements which arose from *ast* and which are accompanied by a change at this locus act like complete reversions of *ast* to wild type. That is, partial reversions, although detectable in this case, were not recovered. In the above category belong the X-ray induced changes, ast^{rv1}, ast^{rv2}, and ast^{rv3}. To these may be added the spontaneous *Dp-S*, which appears to have a change to wild type in the action of the *ast* allele in the left section of the repeat.

The above *S*-like and reverted-like *ast* changes can be related on the basis of a possible identical association of the 21E1–2 doublet region in S^L and ast^{rv1}. Thus, these translocations exhibit a remarkable coincidence in that they apparently have identical breaks in 21E and 68C. Whether the 21E1–2 doublet is intimately associated with 68C in S^L, as well as in ast^{rv1}, is not determinate, owing to the complication of an additional break in heterochromatin in the S^L rearrangement. However, it is likely that there is a causal rather than a chance basis for this coincidence of breaks.

From these considerations, it may be surmised that the same rearrangement which gave rise to an *S* change from wild type, would, if the original constitution had been *ast*, have given rise to a reversion to wild type. The behavior, if the original composition had been *S*, is conjectural. In this connection, however, extensive experiments on the X-radiation of *S* (unpublished) have failed to give reversions of *S* to wild type. Unfortunately, *Dp-S*, which promised to be the most suitable material for attacking this problem, has so far failed to give crossing over between the *ast* locus of the left section and the break point of the duplication. The effects of *Dp-S* combinations in which the left section carries *S ast*, instead of *ast*, tend to indicate that the effects of that section are similar to those of *S ast* (or *S*).

The third method of attack on the *S-ast* position effect makes use of *Dp-S* to obtain combinations equal on a quantitative genetic basis, but involving differences in the distribution of the *S* and *ast* alleles between the two homologs or between the two sections of the repeat. Inspection of table 7 shows that a great many combinations of this type are lethal; such as, (*S ast*)(*S ast*)/*S* and (*S ast*)(*S* +)/*S ast*. In many cases, no difference can be detected between the members—that is, $(+ast)^4(S+)$ cannot be distinguished from (*S ast*)(++) in a great many combinations studied. In certain instances, very sharp differences exist, as in each of the seven comparisons involving the equivalent types, (+ *ast*)(*S ast*) and (*S ast*)(+ *ast*). Superficially, such differences appear to indicate an interaction between the two sections of the repeat—that is, a position effect extending across the repeated regions. A much more likely and consistent assumption is that in those duplication derivatives having S^+ *ast* in their left section, the rearrangement has induced a position effect on these loci causing them to act like S^+ ast^+. There are three reasons for making this

assumption. Firstly, it satisfactorily explains the effects observed for each of the *Dp-S* derivatives which carry *ast* in their left section, without introducing the additional assumption of position effects extending from the *S* and *ast* loci in one section to their duplicates in the other section. Secondly, three other aberrations, ast^{rv1}, ast^{rv2}, and ast^{rv3}, have just such a reverse type of change in the originally present *ast* mutant, and, as in *Dp-S*, this change appears to be a position effect arising from the new association of the 21E1–2 doublet. Finally, the study of *T(2;3)Dp-S* indicated that, at least in the case of $(+ast)_2$, the effects of these two sections are the same whether in close juxtaposition, as in the duplication itself, or are widely separated, as in the translocation.

These conclusions do not necessarily imply that the behavior of *Dp-S* and the *B* duplication are at variance. For it now seems clear that the *B* effect is induced primarily by the new association of the 16A1–2 doublet of the right section of the repeat, rather than to mere duplication of that region, or solely to a position effect between the two sections of the duplication. The difference between *B/B* and *BB/+* indicates that the latter type of position effect does occur; however, it is not clear as to which combination, *B* or *BB*, has the stronger effect in bringing about that difference. Unfortunately, the comparison of $(+ ast)_2/(+ ast)_2$ with $(+ ast)_3/ast$, in which no difference was detected, is not critical, for in neither of these cases does the increase in dosage of the *S-ast* region result in an appreciable change of the eye from normal.

STURTEVANT (1925) and MULLER (1941) have suggested that position effect may be related to the phenomenon of somatic pairing found in the Diptera. This suggestion appears particularly plausible when applied to tandem repeats, since the forces of somatic pairing bring about a very intimate pairing of the duplicated sections within a homolog, and, by so doing, should increase the possibility of the genes in those regions, or the genic products, interacting with one another or competing in a gene reaction. From these considerations, the pronounced position effect existing between the *S* and *ast* loci has been interpreted as a function of their repeat nature and the very short distance between them, both genetically and cytologically speaking. A mutant at one of these loci is viewed as a change in the gene which alters the forces of somatic pairing normally existing between those loci. This possibility is of interest in that it is not necessary to assume that the immediate gene product is altered. The rearrangements which are associated with changes at these loci are likewise considered as upsetting the normal somatic pairing relations between them. It appears that certain relatively non-specific changes in the material just to the right of these loci can cause a considerable change in the position effect normally existing between them.

In general, it may well be found that rearrangements, at least of the wholly euchromatic type, which are associated with position effects, either have a naturally occurring repeat at the basis of those effects or are themselves repeats. *Dp-S* and *B* may be considered as complex types, since they involve a duplication of a doublet structure. The doublets, 21E1–2 and 16A1–2, appear to be responsible for the strong position effects associated with *Dp-S* and *B*, respec-

tively. Superimposed on this position effect is another one which presumably arises from the close juxtaposition of additional doublet regions—a phenomenon as yet detectable only in the case of *B* and/or *BB*.

A repeat interpretation was suggested by OLIVER (1940) for the behavior of two "alleles" of lozenge, lz^r and lz^{gl}, in *Drosophila melanogaster*. This case appears at present to be in many ways similar to that reported here at the *S* and *ast* loci. The alleles, yellow and reddish-alpha, in *Drosophila virilis*, analyzed by DEMEREC (1928), may also be interpreted as duplicate loci, but it is also clear that this alone will not account for the remarkable behavior of the reddish-alpha character. If doublet structures are repeats, as the evidence thus far indicates, then, judging from their widespread occurrence in the salivary gland chromosomes of Drosophila, it is likely that other multiple allelic series may be resolved into duplicate loci which act, by reason of a position effect, as a developmental unit.

SUMMARY

Two loci, Star and asteroid, in the second chromosome of *Drosophila melanogaster* were found to be extremely closely linked, the normal distance between them being estimated as 0.02 map unit.

These loci exhibit a position effect which can be detected solely by varying their genetic composition.

A correlation of genetic and cytological analyses gave evidence that *S* and *ast* are included in the 21E1–2 doublet structure of the salivary gland chromosomes. It also gave pertinent data on the location of other mutants in the vicinity of these loci.

Position effect at the *S* and *ast* loci was also studied by means of chromosomal rearrangements which had breaks in the neighborhood of these loci. A special case was the study of a spontaneous tandem duplication of the Bar type—namely, the Star Duplication, *Dp-S*.

From certain cytological considerations, *S* and *ast* may be interpreted as repeated loci which have become established in the species. The possibility is discussed that repeats have special potentialities for showing pronounced position effects.

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