# A GENETIC AND CYTOLOGICAL ANALYSIS OF A TANDEM DUPLICATION AND ITS INCLUDED LOCI IN DROSOPHILA MELANOGASTER

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# TABLE OF CONTENTS

## THE STAR AND ASTEROID LOCI



## THE STAR DUPLICATION

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## I. The Star and Asteroid Loci.

### Introduction

In general the action of a gene appears to be either unaffected by a substitution in a neighboring gene in the same chromosome of one allele by another, or, at least, not to be affected in a way other than would occur if the replacement were made in the opposite chromosome. On the other hand, if a gene is brought into a new genetic environment as a result of a chromosomal rearrangement occurring near it, then it sometimes happens that the effect of that gene is altered, without, and this has been proved in some instances, a change in the gene itself.

The former statement can perhaps be reconciled with the latter as a result of studies of the two closely linked loci, Star and asteroid, in the second chromosome of D. melanogaster. For, as will be shown, the results constitute an important exception to the former statement; they show that a mutant at one of these loci acts differently according to whether the replacement of an allele at the other locus is made in the same or in the opposite chromosome, other conditions being constant. The existence of such a relationship suggests that position effects, associated with certain types of chromosomal rearrangements, may be phenomena restricted to regions which show a similar relationship.

# I. The Star and Asteroid Loci.

## The Star Locus

Star is a dominant mutant at 1.3 in the second chromosome. Some twelve spontaneous occurrences have been recorded (Drosophila Information Service: 9). In what follows, the symbol, S, refers to Star-1, which was found by Bridges and analyzed by Bridges and Morgan (1919), and Stern and Bridges (1926). S / + has a slightly reduced eye with irregularly shaped facets and disarranged facet hairs; infrequently, it may overlap wild-type. A photograph of a type nearly if not indistinguishable from S / + is shown in Fig. 2. S / S is lethal. By itself, S is probably without a detectable effect on the wing venation; however, in combination with net or plexus, it partially suppresses the extra venation characteristic for those mutants, while in combination with Hairless, it enhances the interruption in certain wing veins characteristic for that mutant.

Bridges (1936) has reported that S is apparently normal in the salivary gland chromosomes. Since the locus of S is now known to lie in the region of the 21 E 1-2 doublet, S has been reexamined, as have also two other alleles, $S^2$ and $S^R$, paying particular attention to this region. No visible disturbance was found associated with any of these alleles.

## The Asteroid Locus

At least four recessive, rough-eyed mutants have been

found in the left end of the second chromosome, which, opposite S, either further reduce the size of the eye and increase its roughened appearance or produce lethality. Evidence to be presented shows that at least three of these are at a separate locus very close to the right of S. This new locus has been given the name, asteroid (ast), because of its similarities with the S locus. When used specifically, the symbol, ast, refers to asteroid-1. Two alleles, ast and $ast^4$, have been studied in most detail. The symbol, $ast^2$, refers to a possible allele of this series, but it has not been excluded that it is a recessive allele of S. $Ast^2$ and $ast^3$ both arose in, and have not been separated from, the Curly inversion chromosome.

A rough, arbitrary system of grading has been adopted to represent the eye effect in the various possible combinations of the ast alleles and S. The results are shown in Table 1. Wild-type is taken as Grade 1, and increasing numbers indicate decreasing size and increasing roughness of the eye. Grade 2 has a very slight roughening. Grade 3 typifies S / + . An example of Grade 5 is shown in Fig. 5; Grade 6 is based on a type similar to that shown in Fig. 7; and Grade 8 is shown in Fig. 3. Grades shown in parenthesis in Table 1., indicate the approximate extent of variability around the chosen grade.

It is also important to consider qualitative differences which distinguish members of the ast series of alleles.

Table 1. A Rough Grading of Eyes of Combinations of +, S, and ast alleles. (see text for description.)

* gaps at the tips of longitudinal veins.

** extensive losses in longitudinal veins.

| | + + | + ast$^4$ | + ast | + ast$^3$ | + ast$^2$ | S + |
|---|---|---|---|---|---|---|
| + + | 1 | | | | | |
| + ast$^4$ | 1 | 1 (1-5)* | | | | |
| + ast | 1 (1-2) | 3 (1-5)* | 5* (1-7) | | | |
| + ast$^3$ | 1 | 2 (2-3) | 5 (5-6) | 5 (5-6) | | |
| + ast$^2$ | 1 | 3 (3-5) | 6 (6-7) | 5 (5-6) | 5 (5-6) ♀-sterile | |
| S + | 3 (2-3) | 5 * (3-6) | 8 ** (6-8) | 7 (7-8) viability (=) 40% | lethal | lethal |

Ast and $ast^4$, in the combinations with themselves or with S, have an effect on the wing venation. S + / + $ast^4$ and ast / ast frequently show a loss of the tips of any or all of veins, L2 - L5, similar to that shown in Fig. 7. S + / + ast shows extensive interruption of these veins as can be seen in Fig. 3. All combinations involving ast or $ast^4$ have, in general, good viability and fertility; although S + / + ast usually hatches with wild-type, its chances for survival are greatly reduced under unfavorable culture conditions. $Ast^2$ and $ast^3$ are more similar to each other than to ast or $ast^4$ in their general effect; they do not affect the wing venation, they are more constant in expression, and they show peculiar lethal effects with S. S + / + $ast^2$ is always lethal although $ast^2$ / $ast^2$ is viable (but sterile in the female). S + / + $ast^3$ has a viability of around 40% as compared with wild-type, but after emergence it has a better survival than S + / + ast, presumably due to the absence of a wing effect.

The possibility that modifiers at other loci are a complicating factor in the phenotypic expressions discussed above has been excluded rather rigorously for ast and to some extent for $ast^2$, $ast^3$, and $ast^4$.

Ast arose either from + or S but the latter is improbable. $Ast^2$ and $ast^3$ probably arose in a normal Curly inversion chromosome. $Ast^4$ appeared as a single $ast^4$ ho / al S ho male among approximately 15,700 (Cy)

offspring of al ast ho / ast females mated to al S ho / Cy males ( al = aristaless, 0.0 ; ho = heldout, 4.0). A similar experiment conducted at 30° C. produced an allele, closely resembling $ast^4$, but whose origin was not associated with crossing over in the al - ho region. Here the total number of offspring in which $ast^4$ could be detected was only 1,513. Unfortunately, this possible reoccurrence of $ast^4$ was lost before fully tested. It is unlikely that ast mutates to $ast^4$ with an appreciable frequency, for many cases which resembled such a mutation have been found in other experiments and have proved, on testing, to be the result of the normal variability of ast. It should also be added that homozygous ast females have produced no reversions of ast to wild-type in over 80,000 offspring in which reversion could be detected had it occurred.

The salivary gland chromosomes show no evidence for chromosomal rearrangement accompanying ast, $ast^2$, $ast^3$ or $ast^4$. They have usually been examined opposite a normal chromosome as well as homozygous; ast has also been examined carefully in unpaired chromosomes. Throughout this analysis, as with the S alleles, special attention was paid to the region of the 21 E 1-2 doublet which probably includes the ast locus, as well as the S locus.

## The Evidence for an Asteroid Locus

Preliminary linkage data placed ast near if not at the S locus. Extensive data summarized in Table 2., represent part

| | Mating | Inversions Heterozygous in Parental ♀ | Total* | "Wild-type" Crossovers** | Frequency |
|---|---|---|---|---|---|
| 1. | $\frac{al\ S\ +\ ho}{+\ +\ ast\ +}$ ♀ x al ast ho ♂ | | | | |
| | " ♀ x $\frac{al\ S\ ho}{Cy}$ ♂ | unknown | 31,106 | 4 (3, ho) | 0.01% |
| 2. | $\frac{net\ +\ S\ +\ +\ dp\ cl}{+\ al\ +\ ast\ ho\ +\ +}$ ♀ x In(2L)Cy, $ast^2$ ♂ | In(1)dl-49, In(2R)Cy, and in ca ½ ♀♀ In(3L+3R)P. | 2 x 15,367 | 15 (12, al dp cl) | 0.046% |
| 3. | $\frac{net\ +\ S\ +\ +\ dp\ cl}{+\ +\ ast^4\ ho\ +\ +}$ ♀ x In(2L)Cy, $ast^2$ ♂ | In(1)dl-49, and in ca ½ ♀♀ In(3L+3R)P. | 2 x 7,654 | 2 (dp cl) | 0.01% |
| 4. | $\frac{al\ S\ +\ ho\ +\ +}{+\ +\ ast^4\ +\ dp\ cl}$ ♀ x al ast ho ♂ | In(1)dl-49, In(3LR)sep. | ---- | 1 (ho) | --- |
| 5. | $\frac{+\ S^2\ +\ In(2L)t}{al^2\ +\ ast^3\ In(2L)Cy}$ ♀ x $\frac{al\ S\ ho}{Cy,\ E\text{-}S}$ ♂ | In(1)dl-49, In(2R)Cy, and in ca ½ ♀ ♀ In(3LR)sep. | 17,334 | 7 (4, $al^2$In(2L)t | 0.04% |

* The total number of offspring, based on the most viable class, in which $S^+$ $ast^+$ could be detected.

**The number and constitution of those fully tested is indicated in parenthesis.

Table 2 . The frequency of "wild-type" crossovers between Star and asteroid.

of the evidence that the ast locus is close to the right of S. Fifteen established "wild-type" crossovers between S and ast were recovered from S + / + ast females, in Matings 1. and 2., Table 2. Three such crossovers were obtained from S + / + $ast^4$ females in Matings 3. and 4. Four were recovered from $S^2$ + / + $ast^3$ females in Mating 5. A consideration of the strategic marker genes used in these matings and the composition of the "+" crossovers with respect to those markers shows that in every case the results are consistent with the assumption that the ast locus is to the right of S. The region to the left of S (1.3) was marked by al (0.0) and / or net (net, 0.0-). The closest mutant to the right of ast was ho (4.0); in Mating 5., the closest "marker" to the right of ast was the left break point of In(2L)Cy which is closer to ast than ho, as can be seen in Fig. 8, from cytological evidence. The mutants, dp (dumpy, 13.0) and cl (clot, 16.5), were used in some cases. The alternated use of many marker genes close to the left and right of S and ast served to minimize the possibility that any of the "+" crossovers were due to contamination or that they represented reversions which were associated by chance alone with crossing over.

Although testcrosses were not employed in Table 2., the genetic composition of most of the "+" types was determined by obtaining them in the homozygous condition or by testing them to the marker mutants. In addition, some of the "+" types

were tested against all of the ast alleles, to S, and to deficiencies for S. There was no evidence from those tests which contradicts the assumption that the "+" crossovers carry a normal allele of S and a normal allele of ast.

The frequency of "+" crossovers obtained in Table 2. varied between 0.01 - 0.046%. The higher frequencies were undoubtedly due mainly to the use of females which were heterozygous for inversions in some or all of the chromosome arms other than 2L; namely, X, 2R, 3L, and 3R. It is sufficient to note here that such a procedure is known to give a significant increase in crossing over in the al - ast region, and, in general, that the increase is probably related directly to the number of inversions used and to their effectiveness in reducing crossing over in other arms. Where known, the exact inversion set-up in any one experiment has been specified in Table 2. and other similar tables.

A salivary gland chromosome analysis of two of the "+" types from Mating 2., as well as the two cases from Mating 3., failed to show any departure from normal. In the case of Mating 5., four "+" types were apparently normal cytologically with respect to the S region and carried In(2L)t, as expected on the basis that they represent crossovers between $S^2$ and $ast^3$.

Technical difficulties arising from the association of $S^2$ and $ast^3$ with In(2L)t and In(2L)Cy, respectively, prevented a study of their crossing over relationships with respect to

ast, $ast^4$, or S. However, the results of Mating 5. would indicate that $S^2$ is probably an allele of S and not a dominant allele of ast; similarly, is probably an allele of S and not a dominant allele of ast; similarly, $ast^3$ is an allele of ast rather than a recessive allele of S. It should be noted that in Mating 5. there is a complication due to the presence in the parental female of the two closely overlapping inversions, In(2L)t and In(2L)Cy; however, there was little or no decrease in the al - ast recombination value, which was 2.6%, or actually higher than the standard value, 1.3%, presumably due to inversions in other arms.

At first, attempts to recover a crossover complementary to "+" types having S and ast, or S and $ast^4$, in the same chromosome failed. These experiments presented in Table 3., Matings 6. and 7., were conducted on the assumption that S ast / + + and S $ast^4$ / + + would resemble phenotypically S + / + ast and S + / + $ast^4$, respectively, or that they would be at least more extreme than S + / + + . In Mating 8., use was made of a suppressor of S, Dp-S (see Part II.), in the parental male on the basis that it might permit the survival and detection of any types more extreme than S $ast^+$. No complementary types were detected, however, in these experiments among a total of 11,141 offspring.

From independent experiments on the Star Duplication, Dp-S, described in detail in Part II.), a stock was available whose composition, with respect to the duplication was: ( S ast)( + ast) ; this notation indicates that the regions

| Mating | Inversions (see Table 2.) | Total Progeny | S ast/+ +, or S ast$^4$/+ +* | Freq. |
|---|---|---|---|---|
| 6. net + S + + dp cl / + al + ast ho + + ♀ x al ho ♂ | In(1)dl-49, In(2R)Cy, In(3L)P, In(3R)C. | 3,005 | 0 | --- |
| 7. net S + + dp cl / + + ast$^4$ ho + + ♀ x al ho ♂ | In(1) dl -49, & in some In(3L+3R)P. | 2,914 | 0 | --- |
| 8. net + S + + dp cl / + al + ast ho + + ♀ x al Dp-S ho ♂ | as in 6. | 6,222 | 0 | --- |
| 9. + al (S ast)(+ ast) ho + + / net + + ast + dp cl ♀ x al ho ♂ | In(1)dl-49, T(2;3)Me. | 4,864 | 3 (2, al S ast dp cl) | 0.06% |
| | In(1)dl-49, T(2;3)Me, In(3LR)CxD. | 1,531 | 2 (1, al S ast dp cl) | 0.1% |
| 10. al (S ast)(+ ast) ho + + / + + ast$^4$ + dp cl ♀ x al ho ♀♂, net ♂, & net ho ♂ | In(1)dl-49, T(2;3)Me, In(3LR)sep. | 6,667 | 3 (al S ast$^4$ dp cl) | 0.05% |
| 11. al (S ast)(+ ast) ho + + / + + + + dp cl ♀ x al ho ♂, net ♂, & net ho ♂ | In(1)dl-49, T(2;3)Me, & in ca ½ ♀♀ In(3LR)sep. | 8,390 | 2 (1, al S + dp cl) | --- |

*Although these types may have occurred in Matings 6, 7, and 8, they were only detected in Matings 9 and 10.

Table 3. The recovery of S ast and S ast$^4$.

in parentheses are duplicated in tandem, direct order in the same chromosome and that the left region contains S and ast while the right section contains $S^+$ and ast. In as much as ( S ast)( + ast) / + is "wild-type," this duplication suggested an indirect way of obtaining S ast and S $ast^4$. By mating females of composition, (S ast)( + ast) / ast and (S ast)( + ast) / $ast^4$, to $S^+$ $ast^+$ males, Matings 9. and 10. Table 3., a few "S / +" phenotypes were recovered among otherwise normal-eyed offspring.

Upon further testing of some of these " S / +" types, they proved to be S ast / + + in the case of Mating 9., and S $ast^4$ / + + for those from Mating 10. Their origin may be visualized in the following manner, which assumes that unequal crossing involving the left region of the duplication has occured between the S and ast loci, or possibly between ast and the break point of the duplication for the origin of S ast:

$$\left(\frac{S\ ast}{+\ ast}\right)\left(\frac{+\ ast}{}\right) \quad \text{or} \quad \left(\frac{S\ ast}{+\ ast^4}\right)\left(\frac{+\ ast}{}\right)$$

As a "control" one substantiated case of S $ast^+$ was recovered from (S ast)( + ast) / + females, Mating 11. The frequency here is omitted because of classification difficulties arising from the presence of the third chromosome dominant, Hairless, which was used in this experiment only and which slightly suppresses the " S / +" type of eye. Therefore some of the desired types may have been missed.

Although S ast / + + and S ast$^4$ / + + are phenotypically indistinguishable from S + / + + , their true composition was revealed by extracting from S ast / + + or S ast$^4$ / + + females as a result of crossing over, an unchanged ast and an unchanged ast$^4$, respectively. This was done for each of three separate occurrences of S ast derived from Mating 9., and for each of two separate occurrences of S ast$^4$ derived from Mating 10. The results are summarized in Table 4.

It is also interesting to note that an unchanged ast was recovered from a S ast type, derived from Mating 9., by a procedure shown in Mating 15., Table 4., analogous to the method by which S ast was originally recovered. Its origin may be visualized as follows:

$$\frac{(\ +\ \text{ast}\ )(\ +\ \text{ast}\ )}{\text{S ast}}$$

Here the derived product, S$^+$ ast, carries the normal allele of S which was originally present in the left section of Dp-S. The results of the latter experiment indicate again that no unexpected complications have arisen in using Dp-S to obtain the S ast and S ast$^4$ crossovers. Nevertheless, it is desirable to obtain those crossovers without the use of the duplication, if possible.

A practical solution to this problem was reached by making use of the fact that S ast$^4$ / + ast has a sufficiently larger eye (Fig. 7) than S + / + ast (Fig. 3) to make feasible an attempt to recover S ast$^4$ in the $F_1$ of a

| Mating | Inversions (see Table 2.) | Total Progeny* | "ast" or "ast$^4$" types | Freq. | al - ast Recombination* |
|---|---|---|---|---|---|
| 12. $\frac{al\ S\ ast\ dp\ cl}{+\ +\ +\ +\ +}$ ♀ x In(2L)Cy, $al^2\ ast^3$ ♂ | T(2;3)Me. | 2 x 2,304 | 1 (ast dp cl) | 0.02% | --- |
| 13. $\frac{+\ al\ S\ ast\ +\ dp\ cl}{net\ +\ +\ +\ ho\ +\ +}$ ♀ x al ast ho ♂ & In(2L)Cy, $al^2\ ast^3$ ♂ | In(1)dl-49, Dp(2;3) - T(2;3)Me. | 2 x 1,254 | 1 (net ast dp cl) | 0.03% | 3.9% (48/1,254) |
| 14. $\frac{+\ al\ S\ ast\ +\ dp\ cl}{net\ +\ +\ +\ ho\ +\ +}$ ♀ x In(2L)Cy, $al^2\ ast^3$ ♂ | as in 13. | 2 x 340 | 1 (net ast dp cl) | 0.1% | --- |
| 15. $\frac{net\ +\ S\ ast\ \ \ dp\ cl}{+\ al(+\ ast)(+\ ast)ho}$ ♀ x In(2L)Cy, $al^2\ ast^3$ ♂ | In(1)dl-49, In(1)AM, T(2;3)Me. | 2 x 996 | 1 (al ast dp cl) | 0.05% | --- |
| 16. $\frac{+\ a\ l\ S\ ast^4\ +}{net\ +\ +\ +\ ho}$ ♀ x In(2L)Cy, $al^2\ ast^3$ ♂ | as in 13. | 2 x 1,844 | 2 (net $ast^4$, 1) | 0.05% | 3.7% (68/1,846) |
| 17. $\frac{+\ al\ S\ ast^4\ +\ dp\ cl}{net\ +\ +\ +\ ho\ +\ +}$ ♀ x In(2L)Cy, $al^2\ ast^3$ ♂ | as in 13. | 2 x 910 | 2 (net $ast^4$ dpcl) | 0.1% | 6.0% (56/912) |
| * Calculated from most viable class, $ast^3/+$. | | 13,304 | 7 | 0.05% | 4.3 ± 0.3 % |

Table 4. The Recovery of ast and $ast^4$ from S ast and S $ast^4$, respectively.

cross of S + / + $ast^4$ females to ast males. The experiment was carried out by mating al S ho / $ast^4$ dp cl females to al ast ho males. This experiment is identical with that shown in Mating 4., Table 2., where it was desirable to include only the "+" crossovers. Total counts were not made and no attempt was made to recover all of the "+" types that may have occurred, but one case of the latter was obtained which had the composition: $S^+$ $ast^+$ ho. Three complementary crossovers having S and $ast^4$ in the same chromosome were detected and had the expected composition: al S $ast^4$ $ho^+$ (not tested for dp and cl). They proved to be indistinguishable from the S $ast^4$ types recovered indirectly by the use of Dp-S; i.e., further testing showed that opposite ast each of the three cases gave a significantly larger eye than S + / + ast. It should also be noted that several cases were found which resembled S $ast^4$/ + ast but which proved on testing to have been the result of the normal variability of S + / + ast.

The frequency of S $ast^4$ crossovers from Mating 4. can be roughly estimated from the fact that, although total counts were not made, 83 al ast crossovers were recorded. The proportion of the total crossovers between S and $ast^4$ to the total crossovers between al and $ast^4$ may be estimated as 3% ( 3 / 86 ). As will be seen in the next section this result agrees well with calculations of a similar proportion from other data.

From Mating 4., the single "+" crossover was tested against one of the complementary, S $ast^4$, crossovers obtained from the same experiment. In this crucial test the "+" crossover behaved exactly like $S^+$ $ast^+$ in the sense that over S $ast^4$ the eye was indistinuishable from S $ast^+$ / + +. This result also held for similar tests of "+" crossovers obtained from S + / + ast (Mating 2.) or S + / + $ast^4$ females (Mating 3.) when either of such crossovers was tested opposite the S ast and S $ast^4$ crossovers recovered by the use of Dp-S.

The behavior of S ast in all combinations tried is not sufficiently distinct from S $ast^+$ to make practicable an attempt to recover it directly from S + / + ast females. Moreover, there is little necessity for such a direct recovery since S $ast^4$ has been obtained by an analogous procedure.

No attempt was made to recover $S^2$ $ast^3$ from $S^2$ + / + $ast^3$. Technical difficulties also prevented the possible recovery of S $ast^3$ by the Dp-S method.

It is assumed without proof that ast, $ast^4$, and $ast^3$ are allelic to each other. This is consistent with the origin of $ast^4$ from ast, the linkage relations of ast and $ast^4$ to S and the linkage relations of $ast^3$ to $S^2$.

All matings in Tables 2., 3., and 4. with the exception of Mating 5, employed only one parental female per culture. An analysis of the data shows that there is no significant evidence for grouping of either type of crossover, which permits

the conclusion that crossing over between S and ast occurs at the same time as normal crossing over.

The Map Distance Between Star and Asteroid

Inspection of Tables 2., 3., and 4. shows that a total of 38 crossovers between the Star and asteroid loci have been established. Much of the data was obtained from females heterozygous for inversions ( or translocation) in some or all of the other chromosome arms, since such females show an increase in the frequency of crossovers between S and ast. On the assumption that this increase is roughly proportional to a concomitant increase in the al - ast region the proportion of crossovers between al and ast which occur between S and ast has been calculated for those cases where data is available.

A summary of Matings 13., 16., and 17., Table 4. gives a value of 3% ( 5 / 172) for this proportion. This is consistent, considering the small numbers involved, with a value of 1% ( 2x0.01 / 1.8 ) obtained from Mating 1., Table 2., where the al - ast recombination was 1.8% (112 / 6,202 ) or slightly higher than the standard al - S of 1.3%. As already described a value of 3% for this same proportion was found in Mating 4. A rough estimate therefore of the map distance from S to ast is 1-3% of 1.3% or 0.01 - 0.04 standard map units. The lower value would be more nearly correct if the presence of inversions in other arms results

in a relatively greater increase in the S - ast distance than in the al - ast region as a whole.

Matings 9., 10., 11., and 15. are more difficult to analyze for frequency data since they are complicated by the presence of Dp-S. On the other hand the results are valuable for an analysis of unequal crossing over in Dp-S heterozygotes, which is presented in Part II.

No systematic study has been made of the effect of single, or combinations of, inversions, heterozygous in other chromosome arms, on crossing over in the al -ast region. The aim has simply been to introduce into each mating as many inversions as was practicable at the time. They were chosen for their probable effectiveness in reducing crossing over in the arms involved, and, for the most part, only those were chosen which could be easily followed genetically with the use of markers; in no case did these markers interfere with critical classifications.

An indication of the effectiveness of the inversion method is given from a summary of Matings 13., 16., and 17., Table 4., which used identical inversions in other arms. Here the al - ast recombination was 4.3%, with a standard deviation of 0.3%; this is an increase of three times the standard value. In those same matings, the total frequency of crossing over between S and ast was 0.1% or between three to ten times what was estimated as the standard value.

The particular inversion conditions in those matings, which may be assumed responsible for the observed increase, were the presence, heterozygous, of In(1)dl-49, $cm^2$; and Dp(2;3) T(2;3)Moire. The effectiveness of In(1)dl-49 in decreasing crossing over in the X was shown by Sturtevant and Beadle (1936) who estimated that the frequency of non-crossover X-tetrads in XXY females, heterozygous for the inversion, was 70%. The effectiveness of T(2;3)Me, or its duplication derivative, may be best judged by Whittinghill's analysis (Drosophila Information Service: 9) of the salivary gland chromosomes, which showed an inversion in 2R, and superimposed on In(3L)Payne five more breaks throughout the third chromosome whose distribution and reunion accomplished a rather thorough shuffling of the contents of 3; the tip of 2R was carried in an undetermined way in heterochromatin of 3. Dp(2;3)T(2;3)Me has a normal second chromosome and is therefore essentially a multiple inversion complex in the third; the duplication for the tip of 2R is fertile and practically wild-type.

With standard sequence presumably present in all chromosomes the effect of high temperature (30 C.) on crossing over in the al - ho region was negligible; the observed frequency was 3.6 ± 0.5% as compared with a map distance of 4.0%. The effect of temperature in combination with inversions was not studied.

## Position Effect Without Demonstrable Aberration

With the use of the S ast and S ast$^4$ combinations, obtained from Matings 4., 9., and 10., certain phenotypic comparisons which are equal on a quantitative genetic basis have been made; namely, (1) S ast / + + vs. S + / + ast; (2) S ast$^4$ / + + vs. S + / + ast$^4$ ; and (3) S ast / + ast$^4$ vs. S ast$^4$ / + ast . Most striking is the difference between S ast / + + (Fig. 2.) and S + / + ast (Fig. 3.). Whereas, the former has normal wings and a rough eye slightly smaller than normal, the latter has large sections of the longitudinal veins missing and an extremely small eye with many of the facets fused. Less striking, but equally significant, is the difference between S ast$^4$ / + + (Fig. 4.) and S + / + ast$^4$ (Fig. 5.). Here S ast$^4$ / + + , like S ast / + + , is phenotypically indistinguishable from S ast$^+$ / + + ; while S + / + ast$^4$ usually has a smaller eye and occasionally interruptions at the tips of the longitudinal veins; the latter effect is not shown in Fig. 5.

The examples shown in Fig. 2 - 7. were raised at the same time under similar conditions, namely, at room temperature and in uncrowded cultures. A typical female of each type was chosen from the first few days' hatch from each type of culture. An important qualitative difference in comparisons (1) and (2), not apparent from Fig. 2 - 5., makes unnecessary a rigid control of environmental conditions to establish the existence of a position effect in these two cases. Thus, when S and ast or ast$^4$ are on the same chromosome with normal

Fig. 1. Wild-type female.

Fig. 2. S ast / + + female.

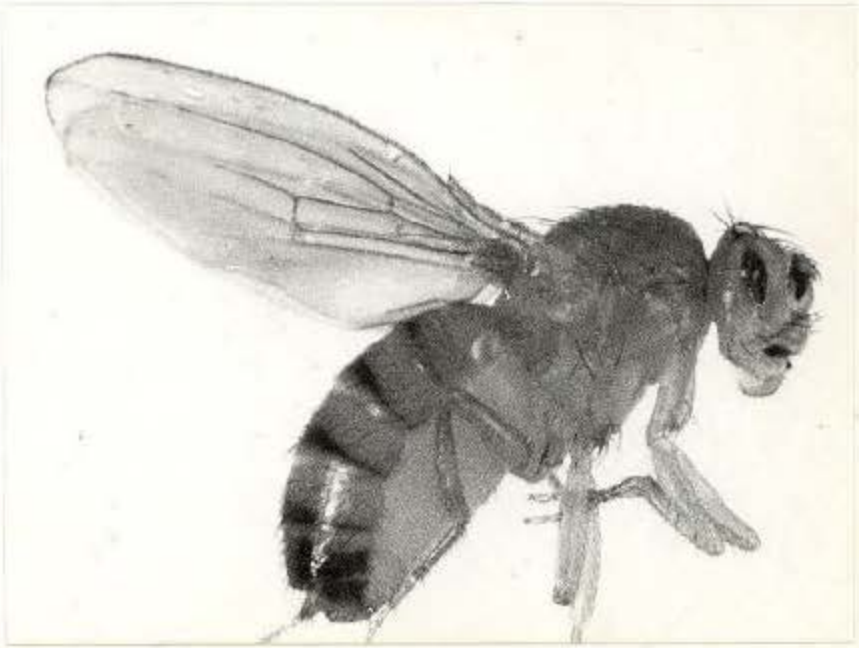

Fig. 3. S + / + ast female.

Fig. 4. S $ast^4$ / + + female.

Fig. 5. S + / + $ast^4$ female.

Fig. 6. S ast / + $ast^4$ female.

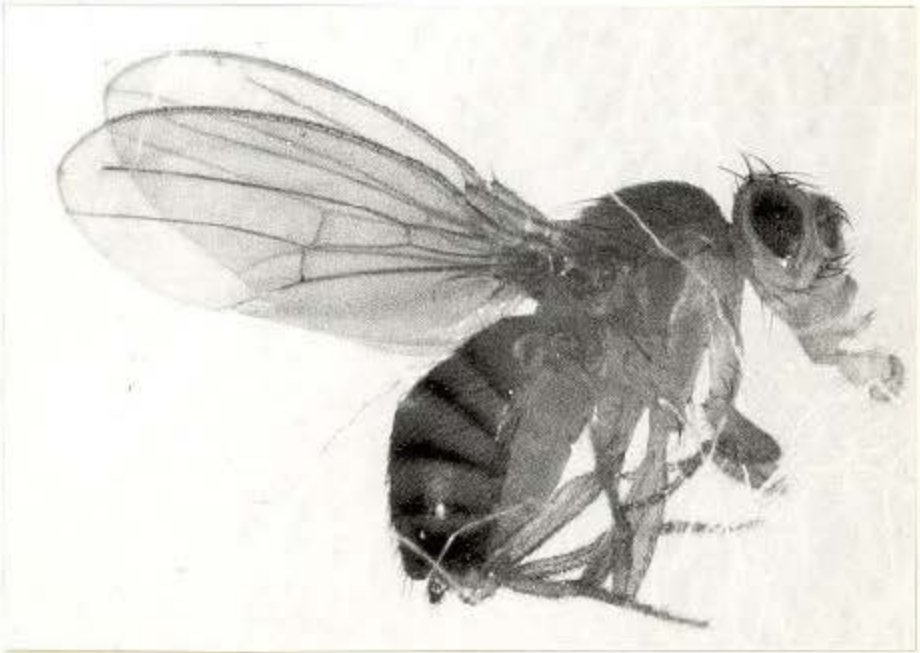

Fig. 7. S $ast^4$ / + a st female.

alleles in the other, the phenotype is quite constant, as it is in S ast$^{+}$ / + +, and has never been observed to overlap the smaller-eyed types. On the other hand, when S and ast or ast$^{4}$ are on opposite chromosomes, the phenotype is variable, depending, as was pointed out before, chiefly on environmental conditions. The extent of that variability in terms of arbitrary grades is shown in Table 1.

One comparison (3) remains: S ast / + ast$^{4}$ (Fig.6.) probably has significantly larger eyes, in general, than S ast$^{4}$ / + ast (Fig.7.). Also, the wing veins show much less tendency to be broken in the former than in the latter. Since both types are variable, however, it would be desirable here to have measurements, and more rigid control of conditions than was used.

The above differences observed in comparisons (1) and (2) hold whether or not the normal chromosome there used was one of the wild-type crossovers between S and ast or between S and ast$^{4}$, or was one obtained from unrelated stocks wild-type with respect to these two loci. Material close to the left and to the right of S ast and S ast$^{4}$, as well as other regions, has been replaced by unrelated material without altering the characteristics of S ast or S ast$^{4}$ in any way. Moreover, comparison (2) was also made with the use of S ast$^{4}$ and its "+" complement, each freshly derived from one experiment (Mating 4.) in which they were recovered directly from S + / + ast$^{4}$ females. In this

critical case, the existence of a position effect exactly similar to that described above for comparison (2) was established.

Beyond doubt, the observed differences in comparisons (1), (2), and probably in (3) are due to the way in which S and ast or S and $ast^4$ are distributed with respect to each other in the two chromosomes. Moreover, as has already been pointed out, an analysis of the salivary gland chromosomes has shown no evidence for a visible disturbance associated with S, ast, $ast^4$, S ast, or S $ast^4$. The existence of a position effect without the complication of chromosomal rearrangement should prove an important simplification for the study of the mechanism of position effect.

Another way of expressing the observed position effects at the S and ast loci is that S and ast act phenotypically as alleles when they are in opposite chromosomes; when they are in the same chromosome the intensifying of S by ast, or vice versa, disappears. In practice, this has been the basis of the difficulty in deriving the S ast and S $ast^4$ combinations.

A Genetic and Cytological Survey of the Tip of Chromosome 2L

Before inquiring as to the location of S and asteroid in the salivary gland chromosomes it will be useful to survey what is known about the general region in which these loci lie. The first four map units of the left end of the second chromosome are known to include nine mutant characters of which all but one, telegraph, are still in existence. The linkage relationships and phenotypes of aristaless, dachsous, expanded, Star and telegraph have been given by Stern and Bridges (1926).

Agenetic map of the region under consideration is shown in the top line of Fig. 8. The following is a brief description (after Bridges) of some of the characters; omitting Star and asteroid, which have already been described, and Suppressor of Star, which will be given special treatment elsewhere:

al - aristaless. 2- 0.0. Aristae greatly reduced; posterior scutellars erect and divergent. RK1.

ds - dachsous. 2- 0.3. Wings blunt and broad with crossveins closer; legs and abdomen chunky; slight dominance of crossvein effect. RK1

ex - expanded. 2- 0.1. Wings wide and broad; eye somewhat reduced and roughish; body size large. RK2

ho - heldout. 2- 4.0. Wings held at right angles to body. RK1

net - net. 2- 0.0-. Wings have a heavy network of extra veins; semi-dominant extra vein parallel to L5 near crossvein;

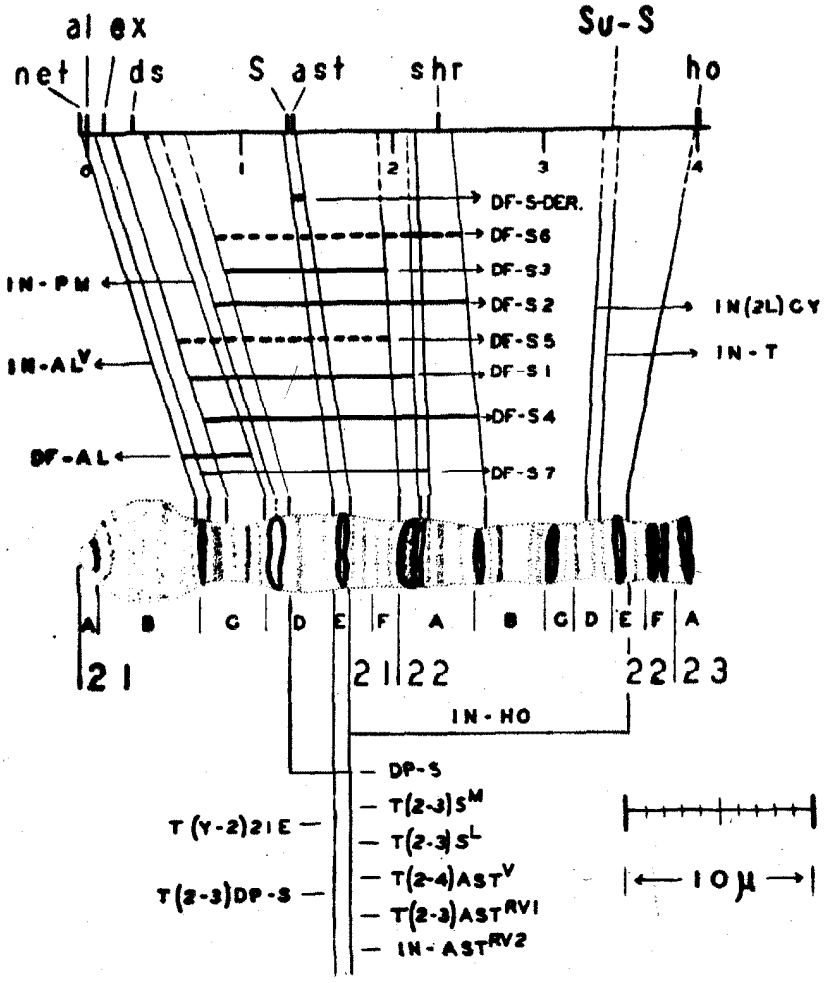


Fig. 8. A correlation of the linkage map with the salivary gland chromosome structure for the extreme left end of the second chromosome.

partially suppressed by Star; usually completely suppressed by homozygous ast and S / ast.

shr - shrunken. 2- 2.3±. Body smaller and narrower, dark. Overlaps wild-type.

Chromosomal aberrations which have enabled the correlation of some of these characters with salivary gland structure were obtained by X-radiation of wild-type males with a dosage of 3,000 r-units; these males were mated to al ast ho females with the results shown in Table 5.

The breaks points of these aberrations where they occur in the left end of the second chromosome, are indicated in Fig. 8. The drawing of this part of the chromosome is a composite one designed to show all bands that have been clearly seen in well stretched preparations of this region. It is labeled to correspond with Bridges' 1935 map.

The following is a description of those changes shown in Table 5. which were analyzed and found to be accompanied by an aberration:

Df(2)al - Deficiency (2) aristaless. Df / + is an extreme Minute with rough eyes, slight ex- and ds- like effects and normal aristae. Deficient for al, ex and ds; but not for net or S. Df / al has aristae completely missing. Salivary analysis shows loss from just before 21 C 1-2 doublet to just before 21 D 1-2 doublet.

Df(2)Sl - Deficiency (2) Star-l. Df / + has slightly smaller

Table 5.

Results from X-rayed (3,000 r-units) wild-type ♂ x al ast ho ♀.

Total offspring: approx. 19,000.

| Changes Resembling | Unanalyzed* | Analyzed Associated with Aberration** | | | "Point Mutations" | Total |
|---|---|---|---|---|---|---|
| | | Df | In | T | | |
| Aristaless | 5 | 1 | 1 | 0 | 0 | 7 |
| Star | 5 | 5 | 1 | 0 | 1 | 13 |
| Asteroid | 4 | 0 | 0 | 1 | 1? | 5-6 |
| Heldout | 9 | 0 | 1 | 0 | 0 | 10 |
| Delta | 3 | - | - | - | - | 3 |
| Notch | 13 | - | - | - | - | 13 |
| Plum | 2 | - | - | - | - | 2 |

* Sterile, died or discarded

** In= inversion; Df=deficiences; T=translocation.

See text for description.

slightly rough eye, usually less extreme than S / + ; bristles normal; viability and fertility good. Df / ast is somewhat less extreme than S + / + ast, and more like S ast / + +. Deficient for ds, S and ast; but not for net, al, ex, shr, ho. Salivary analysis shows break follows medium 21 C 3 and just preceding heavy 22 A 3.

Df(2)S2 - Deficiency (2)Star-2. Phenotypic effects like Df-S1. Deficient for S, ast, shr; but not for net, al, ex, ds or ho. Salivary analysis shows breaks following s1 D 1-2 doublet and following 22 B 1-2 doublet.

Df(2)S3 - Deficiency (2)Star-3. Phenotypic effects like Df(2)S1. Defieient for S and ast; but not for net, al, ex, ds, shr or ho. Salivary analysis shows loss probably extending from just to the right of 21D1-2 doublet to just before the 22 A 1-2 doublet.

Df(2)S7 - Deficiency (2)Star-7. Phenotypic effects like Df(2)S1 / +, except Df / + is slight "ex" type. Deficient for ex, ds, S, and ast; but not for net, al, shr or ho. Salivary analysis shows breaks just after 21 C 1-2 doublet and after heavy 22 A 3.

Df(2) S4 - Deficiency (2)Star-4. Df / + resembles Df(2)S1 / +. Deficient for ds, S, ast, and shr; but not for net, al, ex or ho. Salivary analysis shows breaks following medium 21 C 3 and following 22 B 1-2 doublet.

In(2LR)al$^{V}$ - Inversion (2LR) aristaless-variegated. al$^{V}$ / al is similar to homozygous al but "+" in XXY ♀. al$^{V}$ / Df(2)al

is viable, with aristae frequently but not always absent. $al^V$ / $al^V$ is lethal. Salivary analysis shows left break probably just precedes 21 C 1-2 doublet, right break in heterochromatin of 2R therefore probably an inversion across the spindle fiber.

In(2) ho-Inversion (2) heldout. In / ho is exactly like homozygous ho. In acts phenotypically like wild-type opposite Df(2)S5, S or ast. In / In has smaller eye with anterior indentation; wings reduced to tiny stubs ( In/vg is "+"). Fertile in ♀ but ♂ lacks genitalia and anal apparatus; testes are oval (unattached?), and contain mature sperm.

T (2;3) $S^L$ Translocation (2;3) Star - Lewis. $S^L$ / + resembles S / + ; $S^L$ / ast is like S + / + ast; $S^L$ / S, $S^L$ / Df(2)S2, and $S^L$ / $S^L$ are lethal. Salivary analysis shows three breaks: following 21 E1-2 doublet; 3 heterochromatin; and before 88 E 1-2 doublet. The new arrangement is presumably: tip of 2L to 22E; 3 het. to 88D; 3 het. through to 3L tip. 3R tip to 88D; 21 E through to spindle attachment of 2 and normal 2R.

T(2;4) $ast^V$ - Translocation (2;4) asteroid - variegated. $ast^V$ / + is "+"; $ast^V$ / ast and $ast^V$ / S resemble but are more variable than ast / ast and S + / + ast, respectively. Df(2)S3 / $ast^V$ is lethal. $ast^V$ / ci = ci. The variegated asteroid-like effect is completely suppressed in the XXY ♀. Duplication and deficiency

types are viable. Salivary analysis shows break just following the 21 E 1-2 doublet and a break in heterochromatin of chromosome 4.

In addition to these aberrations, use was also made of a translocation, T(Y;2)21E, kindly supplied by Dr. Schultz. This translocation proved particularly valuable in the cytological location of Star. It involves, as shown by Schultz (unpublished), a reciprocal exchange between the Y chromosome and the tip of the second chromosome, which is broken just to the left of the 21 E 1-2 doublet (see Fig. 8.)

With the above material it is now possible to locate with some degree of precision some of the mutants at the extreme left end of the second chromosome. The results are diagrammed in Fig. 8 and a description of them follows:

-- aristaless and expanded --

The probable location of al (0.0) is the 21 C 1-2 doublet region, based, in particular, on a comparison of Df-al with Df-S4. It is also likely that the extreme Minute effect of Df-al is due to its deficiency for this doublet since Df-S4 does not show this effect. The locus of ex (0.1) is very close to aristaless in the region of the medium staining band, 21 C 3.

-- dachsous --

Although the data from Df-al, Df-S2, and Df-S3 would seem to indicate that ds is located to the right of 21 C 3 and to the left of the heavy 21 D 1-2 doublet, the results

are somewhat ambiguous in view of preliminary studies of a S deficiency, Df-S6, which was obtained in another X-radiation experiment and which apparently has a loss extending from the right of the 21 D 1-2 doublet to just after the 22 B 1-2 doublet (Fig. 8.). Yet unlike Df-S2 and Df-S3, Df-S6 acts as though it were a deficiency for ds. This discrepancy if it exists, may have its explanation in a position effect or mutation at ds in Df-S6. It has not been excluded, therefore, that the locus of ds is not included in the 21 D 1-2 doublet.

-- shrunken and heldout --

The locus of shr (2.3±) lies in the region from the right of 22 A 3 to the left of 22 B 4 . It is likely that the locus of ho (4.0) is in the neighborhood of 22 E on the basis of a probable position effect in that region in In(2)ho.

-- net --

The location of net presented difficulties at first. Df-al / net had a slight net effect while all of the S deficiencies were normal opposite net. In order to avoid the known complication of enhancement of net / + by Minute deficiencies of the Df-al type, and the possible suppression of net by the S effect of the S deficiencies, a duplication for the left end of the second chromosome was used. It was derived from T(2;4)b of Dobzhansky and extends from the tip of the chromosome to after the dumpy locus (13.0). As used it carried net, and will be referred to as Dp, net. Flies of composition: Dp, net / Df-al / net; and Dp, net / Df-S4 / net; had normal venation. On the other hand when deficiencies

from T(2;4) ast$^{V}$ and T(Y;2)21E, were used, flies of composition: Dp, net / Df(2;4)T(2;4)ast$^{V}$ / net; and dp, net / Df(Y;2)T(Y;2)21E/ net showed typical net venation. Since the latter deficiencies involve a loss of the entire tip extending to section 21 E, the above results show that the locus of net is to the left of 21 C 1-2 and, therefore, probably to the left of aristaless.

From unpublished data of Bridges it is known that net is to the left of S but its locus was only roughly determined as 0.3$^{+}$ . A reexamination of the locus of net was made 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 net is to the left of al there were no crossovers between net and al among 10 tested crossovers between net and S. However, when these results are combined with deficiency evidence they may be taken as indicating that net is probably close to the left of aristaless.

-- Star --

The smallest S deficiency that has been obtained directly is Df-S3. The locus of S therefore lies to the right of the heavy 21 D 1-2 doublet and to the left of the 22 A 1-2 doublet. This region is also missing in the other S deficiencies as can be seen in Fig. 8.

From experiments which are described in detail in Part II., it is known that the S locus is included in Dp-S,

whose break points occur just after 21 D 1-2 and just after 21 E 1-2 as shown in Fig. 8. Hence the S locus lies in the region bounded by those break points. This fact justifies the assumption that all of the deficiencies which have been described above and which are known to resemble S rather closely are, indeed, deficiencies for S.

A much more precise location can be made with the use of T(Y;2)21E and T(2;4) $ast^V$, which have already been described. It can be seen in Fig. 8. that the former has its break point in the second chromosome just to the left of the 21 E 1-2 doublet while the latter has its break point just to the right of that doublet. Df(Y;2)T(Y;2)21E / + is a strong Minute with slightly rough eyes and resembles Df-al / + . Df(Y;2)T(Y;2)21E / S has a much rougher eye which is smaller than S / + but which exactly resembles the eye of Df(2;4)T(2;4)$ast^V$ / + . The latter deficiency over S is lethal; apparently it includes the S locus while the former does not. That this was true was shown in a more refined way by combining the two translocations to give: Df(2;4)T(2;4)$ast^V$; Dp(Y;2)T(Y;2)21E., or Df-S-der. This derivative has a deficiency for the 21 E 1-2 doublet, a duplication for part of the Y chromosome and a deficiency for heterochromatin of the fourth chromosome. Df-S-der./ + has slightly roughened, slightly smaller eyes than + ; Df-S-der. is lethal opposite S. Again, as with the other S deficiencies, Df-S-der. / $ast^{-}$ has a slightly larger eye

than S + / + ast. In other less diagnostic combinations, such as opposite ast$^3$ or E-S, it behaves exactly like S and other S deficiencies.

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 acts normal when the variegation is suppressed by an extra Y chromosome. Now it has been determined that the presence of an extra Y does not change the phenotypic characteristics of Df-S-der.; thus it may be assumed that those charactertistics are attributable mainly to deficiency and not to position effects. S is therefore located in the 21 E 1-2 doublet region of the salivary gland chromosomes.

-- asteroid --

Since the larger S deficiencies are deficient for shr they almost certainly are deficient for ast as well. Moreover, all of the S deficiencies obtained, including Df-S-der., act essentially alike, when tested to ast; i. e., they resemble S ast more than they do S ast$^+$ or S ast$^4$. This suggests that even the smallest of them, Df-S-der., may include the ast locus. It also should be pointed out, though, that opposite a normal chromosome all of the deficiencies are more variable in their expression than are either S ast$^+$, S ast, or S ast$^4$.

More satisfactory evidence that ast is included within the confines of the 21 E 1-2 doublet has come from a study of

Dp-S. Phenotypic studies (Part II.) show that Dp-S, as originally obtained, acts as though it carries $ast^+$ in the left section and ast in the right section. Assuming from this that the section present twice in Dp-S includes the locus of ast, then, since ast is to the right of S and since the duplicated section does not extend beyond 21 E 1-2, the ast locus must be included with S in the 21 E 1-2 doublet.

A check on this location of ast can be derived from other evidence, to be presented, which can be taken as excluding the presence of an ast locus to the right of the 21 E 1-2 doublet.

-- Suppressor of Star --

Suppressor of Star, Su-S, found by Curry, acts as a dominant suppressor of S and as a dominant partial suppressor of homozygous $ast^3$. A reinvestigation of Su-S has shown that it is not the result of a point mutation but rather that its effect is probably attributable to the double deficiency derivative, 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 mated to S dp / Cy males. It was detected by its suppressing effect on S. The complementary or double duplication derivative was also detected by its dominant enhancement of S. A cytological analysis of In(2L)Cyt, made by Bridges and Li (1936), showed the presence of a defiency

for 22 D 3 and all of section 34 A. There is some evidence, of a preliminary nature, that the suppression of S in Su-S is directly attributable to the deficiency for the 22 D. Thus, two translocations have been obtained, from X-radiation of ast males, in each of which the 22 D region is brought close to heterochromatin of the third chromosome and in each case the translocation over S shows a partial suppression of the S + / + ast effect. Presumably, therefore, the 22 D region may show changes resembling Su-S.

Whether or not the mutant, Enhancer of Star (E-S), locus, 2- 6± , found by Bridges (Drosophila Information Service: 9), is also due to a change in the 22 D 3 region 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, which has a slight dominant enhancing effect on S as noted above but which is by no means as extreme as that of Enhancer of Star.

E-S / S has a small eye similar to that shown in Fig. 7; E-S / $ast^3$ and E-S / $ast^4$ have only slightly roughened eyes; but E-S / ast and E-S / $ast^2$ have eyes which are slightly smaller and rougher than S/ + . The relative specificity of E-S, in enhancing strongly S, ast, and $ast^2$, was shown by the fact that it had little or no such effect on other rough-eyed mutants which were tested in the heterozygous condition.

Those mutants tested in that fashion included: echinus, facet, split, roughex$^{2}$, and uneven, in the X chromosome; morula, roughish, rolled, rubroad, and scabrous, in 2; and rough and roughoid, in 3.

X-ray Induced Changes at the Star and Asteroid Loci

A description has already been given of a translocation derived from X-radiation of "+" males and having an effect like S ast$^{+}$. This translocation, T(2;3)S$^{L}$, is closely similar, from the standpoint of origin and phenotypic effects, to the Star translocation of Muller (1929), analyzed in the salivary gland chromosomes by Bridges and Li (1935). In both instances, one of the breaks occurs just to the right of the 21 E 1-2 doublet. T(2;3)ast$^{V}$ also arose from "+" as a result of X-radiation and has one of its breaks just following the 21 E doublet; it, however behaves as a variegated, asteroid-like change, the variegation presumably being induced in the doublet region by heterochromatin of chromosome 4. That not all rearrangements having a break just following this doublet need give an effect on the S and ast loci, is shown by the case of In(1)ho, which acts exactly like S$^{+}$ ast$^{+}$ in several combinations already noted.

From X-rayed al ast ho/ net ast dp cl males mated to S / Cy, E-S females, two instances of reverse "mutation" of ast to ast$^{+}$ were detected; both of these however were found to associated with a rearrangement which, like those above, had

one of its breaks just following the 21 E doublet which in each case was translocated to euchromatin of other regions. These two aberrations are also indicated in Fig. 8., as In(2L)$ast^{rv2}$ and T(2;3)$ast^{rv1}$. Another aberration, Dp-S, is known to have a break just following the 21 E doublet and although it arose spontaneously from ast its effects, as shown in Part II., indicate that the left section of the duplication acts as though it carries a reversion of ast to $ast^{+}$. The fact that no spontaneous instances of reversion of this type were found in over 80,000 offspring in which it might have been detected, and the fact that no reversions were obtained,from X-radiation of ast,which were not associated with an aberration, strongly suggests that the reverted effect in T(2;3)$ast^{rv1}$, In(2L)$ast^{rv2}$, and Dp-S is due to a position effect rather than to mutation. On this basis, it can be seen that the ast locus must lie in the 21 E doublet (or to the left of it) since it is this doublet and not the material to the right of it which has undergone rearrangement in all three instances.

Two breaks are known to occur just to the left of the 21 E 1-2 doublet in the aberrations, T(Y;2)21 E and T(2;3)Dp-S, As has been noted, the former has no detectable effect on the originally present $S^{+}$ $ast^{+}$, and the latter appears also, from studies presented in Part II., to have no effect on the S and ast loci in the right doublet of Dp-S but rather an effect on those loci in the left section of the duplication.

## Discussion

A plausible interpretation of many of the observed phenomena at the Star and asteroid loci can be made in terms of a naturally occurring repeat in the chromosome. The starting point of this interpretation rests on the finding that the two loci are probably included in the 21 E 1-2 doublet structure of the salivary gland chromosomes. The possibility that such structures might represent instances of duplication of a single band was first pointed out by Bridges (1935) from purely cytological considerations. It is interesting to note that Bridges chose the 21 E 1-2 doublet or "capsule" as a characteristic example of the two-band type of repeat.

That such doublet structures probably involve two discrete bands was shown experimentally by Bridges (1936) in an analysis of the spontaneous second chromosome deficiency, Notopleural. 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 two halves of a doublet. Similarly, Metz (1937) has analyzed a series of small deficiencies in the salivary gland chromosomes of Sciara ocellaris, some of which involve a loss of only one band of a doublet structure. Metz (1938) also holds to the view that doublets have arisen by duplication of single bands and that some of the single band deficiencies,

which occur commonly in Sciara stocks, are either the original unduplicated band condition or are the result of secondary loss from a duplication which has already become established in nature.

The two discs, present in capsules of the 21 E type, are homologous to the extent that they pair tightly with one another in the same chromosome, giving the appearance of a shell of chromatic material enclosing an achromatic center. The 21 E doublet may, therefore, be considered as divisible into two separate, and homologous discs, and as such it provides a cytological basis for predicting the existence, genetically, of two similar and adjacent loci.

An analysis of mutants which are included in the 21 E capsule has shown that this sort of genetic situation does exist. Thus, ast has been shown to be at a separate locus very close to the right of S by an estimated distance of 0.01 - 0.04 map unit. Moreover, the essential similarity between the S and ast mutant types is reflected in a number of ways. The property of reducing the size of the eye and of causing, at the same time, facet disturbances which result in a roughened appearance, is shared by mutants at each locus. A tendency to cause an interruption in the wing veins can be detected in S as well as in $ast^{1}$ and $ast^{4}$. A high degree of similarity in action for S and $ast^{1}$ is suggested by the strong

intensification of each by Enhancer of Star, whose effect was shown to be relatively specific for these loci. Again, when S is in one chromosome and any of the ast mutants is in the other, there is a strong reduction in the size of the eye as compared to S $ast^{+}$ / + + / Ast is not behaving here like a typical non-allelic intensifier of Star, such as Enhancer of Star; for, when S and ast or S and $ast^{4}$ are in the same chromosome with normal alleles in the other, the result is indistinguishable from S $ast^{+}$ / + + . Moreover, this position effect was not, as far as could be determined from an analysis of the salivary gland chromosomes, the result of a cytological alteration of the chromosome.

Now the possibility that a position effect of the above type might exist was tested by Sturtevant (1928) with the use of the dominant mutants, Delta and Hairless, three units apart in the third chromosome. No position effect, however, was found, and at that time the only position effect that had been demonstrated remained the Bar case, although still other possibilities were tested. Sturtevant concluded "that the association of the two elements in double-bar is much more intimate than the association between identical loci in homologous chromosomes, and that the 'position effect' perhaps rests on something more than mere closeness together." It would now appear that the "something more" is the essential similarity in those elements.

As a result of the cytological finding of Muller, et al., (1936) and Bridges (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 in one homolog and the fourth in the other. The position effect, here, would appear to extend over a distance as great as the length of the triplicated region, measured from one point in the most distal section to the same point in the most proximal section, or a distance, perhaps of 13-14 bands. This is remarkable in view of the fact that other rearrangements, involving exchanges occurring between euchromatic segments of the chromosomes only, and which seem to have position effects on known loci, do not show this spreading effect but rather a position effect within a band or so of the break point.

The impression is gained that the spreading effect observed in the Bar case is somehow related to the close juxtaposition of identical regions. On this basis, there is a striking analogy with the position effect observed at the S and ast loci, since these, too, appear to be homologous to a certain extent. To go further, the probable position effects at the S and ast loci observed in a study of rearrangements, one of having their breaks just to the right of these two loci, can be considered as a secondary effect on a

position effect which exists normally in the chromosomes. From a broader standpoint, position effect may be confined only to repeat regions of the chromosomes, the most frequent type of which are the doublet structures or two-band repeats. In general it may be predicted that such regions will act, in a physiological sense, as units, but, in a genetic sense, as though they were made up of discrete loci which show position effects with one another.

Recently, Muller (1941) and also Schultz, unpublished, have suggested that position effect may somehow be related to the phenomenon of somatic pairing. Muller notes that the forces of somatic pairing "acting between unlike genes, should tend to deform them, and this deformation might well affect the nature and the quantity of the gene products which they form." Its seems more plausible from the above considerations that it is the similar, or "repeat" genes which may be affected in that way rather than the unlike ones. This would permit a more precise statement of the possible significance for position effect of somatic pairing; namely, that the synaptic forces, by governing the degree of association of the repeat genes, are capable of modifying the extent to which the gene products of each member of the repeat interact with one another.

# II. The Star Duplication

## Introduction

Changes at the Bar "locus" in the X-chromosome occur with a measurable frequency among the progeny of females homozygous for the dominant small-eyed type, Bar. Two types of changes were noted by Zeleny (1921); namely, a reversion of Bar to normal and a change to a form with more extreme eye reduction than Bar, called ultra-Bar, now referred to as Double-Bar. Unequal crossing over was shown by Sturtevant (1925) to be the mechanism responsible for these changes; reverted Bar, on this basis, could be thought of as a deficiency for the Bar gene while the complementary, Double-Bar type was considered as a duplication for the Bar gene.

The salivary gland chromosomes of Bar were investigated by Muller, et al., (1936) and Bridges (1936), who showed, independently, that Bar was a tandem duplication in direct order for a short section of the X-chromosome, composed, according to Bridges' analysis, of about 6 bands. Double-Bar, Bridges found, had this section thrice repeated, whereas, the reverted type had the banding of a normal chromosome.

Another instance of a tandem duplication being associated with a dominant change is that of Hairwy Wing, Hw, which was analyzed, cytologically, by Demerec (1939) and Rapoport (1940).

They found a duplication for three discs near the tip of the X-chromosome; Rapoport noted that Hw was possibly an example of a reverse, rather than a direct, repeat. Rapoport was unable to demonstrate unequal crossing over in homozygous Hw females, in over 33,000 offspring examined.

A third case of spontaneous tandem duplication was reported in a preliminary note (Lewis, 1941). This duplication includes two known loci, Star and asteroid, and is referred to as the Star Duplication, Dp-S. Changes, due to unequal crossing over and analogous to those occurring in the Bar duplication, could be derived from homozygous Dp-S females. Moreover, unequal crossing over could be detected in females heterozygous for Dp-S. The analysis has been greatly facilitated by the use of markers at the Star and asteroid loci and a relatively high frequency of unequal crossing over could be obtained by the procedure, noted in Part I., of introducing into the female many inversions in other chromosome arms.

More recently, Schultz (Morgan, Schultz, and Curry, 1941) has shown that the dominant, Confluens, change is a tandem duplication for the Notch region of the X-chromosome. In this case, also, it was possible to vary the genetic composition of the duplication as a result of unequal crossing over occurring in females heterozygous for Confluens.

## The Origin and Nature of Dp-S

In a search for reversions of ast, 48,945 offspring were raised from a mating of al ast ho / ast females to al S ho/ Cy, E-S males. Actually the total number of offspring in which reversion could be detected is higher due to the reduced viability of the non-Curly class (63% as compared to the Curly class); a corrected estimate gives an approximate total of 78,000. The total frequency of crossing-over between al and ho was 5.2% (34716,674). No substantiated case of reversion of ast to wild-type was recovered in this experiment. However, it did yield a single normal-eyed, heldout individual, whose genetic composition was shown to be Dp-S ho / al S ho. The origin of Dp-S was therefore associated with crossing over in the al - ho region.

An analysis of the salivary gland chromosomes shows that Dp-S is a tandem duplication in direct order for a section of four bands extending from 21 D 3 to just after the 21 E 1-2 doublet. A more complete description of the cytological picture will be deferred to a later section.

The phenotypic effects of Dp-S are "flexible" in the sense that by varying its genetic composition with respect to the Star and asteroid loci, the resultant phenotype is changed. It will therefore be convenient to anticipate the evidence for its genetic composition, which has still to be presented, and assign to Dp-S, as originally obtained, the more restrictive notation: ( $S^+$ ast )( $S^+$ ast ), or simply

$( + ast)_2$, which indicates the presence of two sections in tandem duplication having identical composition at least with respect to the Star and asteroid loci. The symbol, Dp-S, is reserved for indicating the presence of the duplication without regard to its genetic make-up.

When $( + ast)_2$ is opposite ast, $ast^2$, $ast^3$, or $ast^4$, the combination is "wild-type." When it is opposite S, $S^2$, or deficiencies for S, it acts as a suppressor of the rough eye effect although occasionally the eye is very slightly rough and possibly slightly smaller. The best diagnostic combination is $( + ast)_2$ / $S^2$ E-S; here the eye is larger than in + / $S^2$ E-S but suppression is not complete, the eye being rougher than S / + and closely similar to ast / + E-S. This result is consistent with other evidence to be presented that $( + ast)_2$ acts as though it carried one dose of ast and a normal allele of asteroid instead of two doses of ast as indicated and as its origin from homozygous ast would suggest. In this connection it would be desirable to know the effect of $( + ast)_2$ over a deficiency for, and only for, the region present twice in the duplication. An approximation to this ideal situation can be realized by a comparison of two of the Star deficiencies, which were described in Part I; namely, Df-S3 which involves such a loss but in addition a loss of four or five bands to the right of the 21 E 1-2 doublet; and Df-S-der. on the other hand which is essentially a loss of the 21 E1-2 doublet only. When either of these deficiencies is opposite ( + ast) the result is the same; the eye is normal or

only slightly roughened. Again the result might be compared with + / ast which is usually normal but which occasionally shows a slight roughening of the eye. The eye of homozygous $( + ast)_2$ is normal; the wings may be normal or they may occasionally show a slight extra vein parallel to the fifth longitudinal vein near the posterior cross vein. It is difficult to exclude the presence of modifiers which may cause or contribute to this wing effect, but there is good reason to believe that the tendency to show extra veins is a property of the duplication itself; e. g., in the presence of some of the Minute bristle types, $( + ast )_2$ / + may show the extra vein effect as well as branches at the tips of the longitudinal veins.

## The Right Section of Dp-S

The genetic composition of Dp-S has been deduced from a study of unequal crossing over in females heterozygous for the duplication. From $( + ast )_2$ / S + females a total of 258 tested "ast" types were recovered in Matings 18., 19., and 20., Table 6. The great majority of these are to be interpreted as the extraction of $S^+$ ast from the right section of $( + ast )_2$ as the result of crossing over occuring in the region between the break point of the duplication and the locus of S. Using a more extended and arbitrary notation this result may be visulized as follows:

$$\frac{(\cdots\cdot+\cdot\cdot ast\cdot\cdot)(\cdots\cdot+\cdot\cdot ast\cdot\cdot)}{(\cdots\cdot S\cdot\cdot + \cdot\cdot)}$$

| Mating | Inversions Heterozygous in Parental ♀ | Total Progeny* | "ast" Types** | Freq. |
|---|---|---|---|---|
| 18. $\frac{+ \text{ al } (+\text{ ast})(+\text{ ast}) \text{ ho } + +}{\text{net } + \quad S \quad + \quad + \text{ dp cl}}$ ♀ x al S ho/Cy, E-S ♂ | In(1)dl-49, & in some In(3L+3R)P | 2 x 976 | 8 (ast ho) | 0.4% |
| 19. $\frac{+ \quad (+\text{ ast})(+\text{ ast}) \text{ ho } + +}{\text{net} \quad S \quad + \quad + \text{ dp cl}}$ ♀ x In(2L)Cy, $\text{ast}^2$ ♂ | as in 18. | 2 x 9,156 | 64 (57, net ast ho) (1, ast ho) | 0.35% |
| 20. as in 19. | as in 18. | ---- | 190 (net ast ho) 2 (ast ho) | --- |
| 21. $\frac{\text{al } + \quad (+\text{ ast})(+\text{ ast}) \text{ ho } + +}{+ \quad \text{ds} \quad + \quad + \quad + \text{ dp cl}}$ ♀ x al ast ho ♂ | Unknown | 8,000 | 13 (ast ho) (2,ds ast ho) 1 (al ast ho) | 0.16% |
| | | | **278** (272) | 0.16 - 0.4% |

*Calculated number of offspring in which ast could be detected.
**Genetic composition in parenthesis and in most cases determined by progeny tests.

Table 6. The Extraction of Asteroid from the Right Section of Dp-S.

The "new" type was detected on the basis that it acts exactly like ast. The frequency of this class was 0.36% (72 / 2x10,132) in Matings 18. and 19., where the females carried inversions in some of the other chromosone arms.

That the duplication was no longer present in the great majority of the "ast" types was shown by a cytological analysis of 83 of them recovered from Mating 20. In Mating 18. it was also possible to detect a complementary crossover having S ast$^+$ inserted into the right section of the duplication; over S the resultant phenotype was inseparable from S / + but a cytological analysis demonstrated the presence of Dp(2)S. This type occurred with a frequency of 0.3% (6 / 2x974 ) and its composition may be written ( + ast)( S + ).

Results similar to those shown above were obtained when unequal crossing over was followed in ( + ast)$_2$ / + females, Mating 21.,Table 6. Here ast was recovered from the right section of the duplication in 13 cases, or with a frequency of 0.16%. This reduced frequency is probably due in part to the fact that few if any of the females in Mating 21. were heterozygous for inversions in other chromosome arms. Although it was not possible to detect a complementary crossover to ast in the $F_1$ of this mating, one case of ( + ast)( + + ) was found among 13 $F_1$ (al) crossovers between al and ho which were tested against $S^2$ E-S; unlike ( + ast)$_2$, ( + ast)( + +) results in complete suppression of the $S^2$ E-S effect.

To summarize, the right section of Dp-S$_2$ was shown to

carry a normal allele of S and the recessive, ast. This is consistent with the origin of Dp-S from homozygous ast. In Dp-S / + females the frequency of unequal crossing over where the right section of the duplication and the normal chromosome were involved varied between 0.3 - 0.72% for the region from the break point of the duplication to the locus of S. The lower value may be taken as the standard value. The higher value represents an almost two-fold increase, attrituable probably to the inversion set-up used; but as will be seen later an even greater increase was obtained.

The Left Section of Dp-S

To turn to a study of the left section of Dp-S it has already been shown in PartI., Table 4., Mating 15. (more complete details are given in Table 7.), that $S^+$ could be extracted from the left section as the result of crossing over, occurring in $( + ast)_2$ / S ast females, between the S locus in the left section and the break point of the duplication; diagrammatically:

$$\frac{(\cdots\cdot+\cdot\cdot ast\cdot\cdot)(\cdots\cdot+\cdot\cdot ast\cdot\cdot)}{\cdots\cdot S\cdot\cdot ast\cdot\cdot}$$

In this case the detected product was $S^+$ ast and a consideration of the marker genes used shows that the left and not the right section of Dp-S was involved in crossing over. Whether or not the accompanying ast was derived from the left section of the duplication or from the normal chromosome obviously

cannot be determined in this experiment.

A crossover complementary to that described above would result in the insertion of S into the left section of the duplication. It was early recognized that ( S ast)( + ast) might for practicable purposes be indistinguishable in its action from ast, itself. For this reason 83 of the "ast" types obtained in Mating 22. were examined cytologically for the presence of Dp-S; only two were found which showed the presence of Dp-S, the remainder were normal as expected on the basis that they arose from crossing over in the right section of the duplication. Evidently the two rare cases had the composition desired: ( S ast)( + ast) or ( S + )( + ast), whose origin, in this experiment, may be indicated as follows:

$$\frac{(\cdots\!+\!\cdot ast\cdot\cdot)(\cdots\!+\!\cdot ast\cdot\cdot)}{\cdots S\cdot\!+\!\cdot\cdot}$$

Again, whether or not the crossover occurred between S and ast or to the right of ast is difficult to tell from this experiment alone but there are other reasons to be presented which indicate that the more probable constitution is ( S ast )( + ast ) and not ( S + )( + ast ). An estimate of the frequency of crossing over between the S locus in the left section of the duplication and the break point gives a value of 0.02% ( 2x2 / 83 x 0.36%) which is of the order of magnitude of the distance between S and ast. Actually the new type, ( S ast)( + ast), did prove to be nearly if not inseparable, phenotypically, from ast. That it actually contained S in the left section was shown by extracting it from the duplication

in three different experiments which have already been reported in Part I., Table 3., Matings 9., 10., and 11. It is useful here to summarize the results as follows (The number which were fully tested for the indicated composition is shown in parenthesis.):

Mating 11.

$$\frac{(....S..ast..)(....+..ast..)}{....+..+..} \quad \text{S ast+ (1)}$$

Mating 9.

$$\frac{(....S..ast..)(....+..ast..)}{....+..ast..} \quad \text{S ast (3)}$$

Mating 10.

$$\frac{(....S..ast..)(....+..ast..)}{....+..ast^{4}..} \quad \text{S } ast^{4} \text{ (3)}$$

It will now be recalled that this was the method by which the combinations, S ast and S $ast^{4}$, were first obtained. Mating 11., served here as a control in showing that a typical S $ast^{+}$ type could also be extracted.

On the other hand the extraction of ast from the left section of the duplication or the insertion of $ast^{+}$ into that section has not been detected, at least, in an unequivocal way. It is important therefore to examine what the chances were for the recovery of ast from the left section. The recovery of ast had it occurred could have been detected among the tested sample of 271 "ast" types which arose among the offspring from Matings 18., 19., 20., and 21., Table 6; that

is, with respect to the marker genes used they would have appeared as "complementary" to the only type recovered. Now it was determined cytologically, as already mentioned, that two out of 83 "ast" types tested in one of these experiments represented crossovers between the locus of S in the left section and the break point of the duplication. One may guess therefore that roughly six or seven ( 2x271 / 83 ) such crossovers in the latter region were recovered, part of which, at least, were presumably crossovers between S and ast. Hence the number of flies examined has been too small to make it very probable that ast would have been recovered from the left section even if the frequency of crossing over between ast and the break point was of the order of magnitude of the frequency of crossing over between S and ast. It is likely from Matings 10., and 11., that the former frequency is lower than the latter since four tested crossover between S and the break point all occurred between S and ast, and not to the right of ast.

Because of this negative result, it might be assumed that the failure to detect the extraction of the ast locus from the left section of Dp-S in some of the experiments given above was not due to its close proximity to the break point but rather to the representation of that locus by an allele which was being extracted with an appreciable frequency but which could not be detected in those experiments. This would, of course, require an additional assumption that at the time of origin of Dp-S a mutation at the ast locus in

the left section had occurred. That the first assumption is unnecessary is shown by reference again to Matings 9.,10., and 11., where the value and importance of the results lay in their being independent of just such an assumption. Those results indicate clearly that in every case where S was recovered from the left section of the duplication the crossover could consistently be interpreted as having occurred between the S and ast loci. It may,however,be useful to point out that there is some support from phenotypic considerations for supposing that the left section contains $ast^+$ and not ast. but just as $ast^+$ was not recovered in Matings 9., and 10., it was not recovered in an experiment shown in Table 7., Mating 24.[3], which was especially designed to detect it in the $F_1$ had it occurred. In this case, ( + $ast^{+?}$)( + ast ) / net ast dp cl females were mated to net S dp cl / Cy males and a search was made for S / + types in the $F_1$. Total counts were not made, but 198 "net" crossovers between the net locus and the locus of ast in the left section of Dp-S were recorded; their frequency was 1.4% (63/4,442). Since no S / + types were recovered the value of postulating a mutation from ast to $ast^+$ ceases to exist for all practical purposes. In other words, there is no genetic data which contradicts the assumption that ast and not a mutation thereof is present in the left section of Dp-S.

It has been tacitly assumed in the above discussion that the ast locus is actually included in Dp-S but the evidence for this has remained indirect and is based chiefly on

cytological evidence, and phenotypic considerations which have yet to be taken up in detail.

To summarize, the left section of Dp-S, as originally obtained, was shown to contain a normal allele of S which again is consistent with an origin from homozygous ast, and with the notation ( + ast )$_2$. But it was not possible to determine whether the left section carried the recessive, ast; presumably, the locus of ast is too close to the break point for it to be extracted with an appreciable frequency. Into the left section of ( + ast )$_2$, S could be inserted and subsequently could be extracted unchanged. Since the derivative, ( S ast)( + ast), resembled ast, itself, and could only be detected cytologically, it was included among the "ast" crossovers obtained in Matings 18., 19., and 20., Table 6., which were interpreted as having involved the right section of Dp-S. However, the frequency with which ( S ast)( + ast) arose in those matings was so low, estimated at 0.01% that it does not seriously affect the frequency of crossovers in the right section, nor the main conclusions, from those matings.

Unequal Crossing over in Dp-S Heterozygotes

It would would be desirable to compare in a quantitative way the left and right sections of Dp-S with respect to the frequency of unequal crossing over within a region of those sections which could be specified with known loci. From deficiency data presented in Part I., Fig. 8.,it is

known that the loci of net, al, ex, and probably ds lie outside the duplicated region of Dp-S; that is, to the left of the 21 E 3 band. Apparently, only the S and ast loci are included in Dp-S. They are so close together, however, that it would be impracticable to obtain sufficient data for a comparison such as that indicated above.

On the other hand, some indication of the effect of Dp-S, when heterozygous, on crossing over in the al - S region can be obtained from Matings 18., 22., and 15., shown in Table 7. Here it was possible to measure, simultaneously in each experiment, the frequency of crossing over between the al locus and the S locus in the left section of Dp-S as well as the the frequency in the region from S in the left section to S in the right section of the duplication. It will be convenient to refer to these as the al - $S_1$ and the $S_1$ - $S_2$ regions, respectively. How then does the sum of these two regions, or the al - $S_2$ region, measured in terms of crossover frequency, compare with the map distance between al and S under similar inversion conditions but in the absence of Dp-S. The answer to this can be obtained from a comparison of the results of Mating 15., Table 7., with the average of the results from Matings 13., 16., and 17., Table 4., Part II.

In Mating 15., the origin of one of the crossover types in the al - $S_1$ region may be indicated as follows:

| Mating | Inversions | Total Progeny* | Crossover Region** al-$S_1$ | Crossover Region** $S_1$-$S_2$ | % al-$S_1$ | % $S_1$-$S_2$ | % al-$S_2$ |
|---|---|---|---|---|---|---|---|
| 15. $\frac{al\ (+\ ast)(+\ ast)}{+\qquad S\ ast}$ ♀ x In(2L)Cy, $al^2\ ast^3$ ♂ | In(1)dl-49, In(1)AM, & T(2;3)Me. | 2x995 | 22, $al(+ast)_2$. | 22, ast. (1, al ast dp cl) | 2.2 | 2.2 | 4.4 |
| 18. $\frac{al\ (+\ ast)(+\ ast)ho}{+\qquad S\ +\qquad +}$ ♀ x al S ho/Cy,E-S ♂ | In(1)dl-49, & in some In(3L+3R)P= | 2x976 | 17, $(+\ ast)_2$. | 8, ast. 6, (+ast)(S+). | 1.7 | 0.7 | 2.5 |
| 22. $\frac{+\ (+\ ast)(+\ +\ )\ +}{al\quad S\ +\qquad ho}$ ♀ x al S ho/Cy, E-S ♂ | In(1)dl-49, In(3LR)CxD. | 2x1,057 | 20, $al(+ast)_2$. | 6, $al\ S^+\ ast^+$. 11, (+ast)(S+). | 1.9 | 0.8 | 2.7 |
| 23. $\frac{+\ (+ast)(+ast)\ +\ +}{net\quad ast\quad dp\ cl}$ ♀ x net ast dp cl ♂ | In(1)dl-49. | 2x4,442 | 63, $net(+ast)_2$. | ----- | 1.4 | --- | --- |

*In Matings 15 and 23., the calculated total is twice the $(+\ ast)_2$/ ast class; in Matings 18 and 22, it is twice the non-Cy class.

**See text for description. In Mating 23, the net - $ast_1$ region may be considered as identical with the al - $S_1$ region.

Table 7. The frequency of crossing over in the al - S region in Dp-S heterozygotes.

al (....+..ast.)(....+..ast.) ho ——— ( + ast )$_2$ ho
+ ....S..ast.

The frequency of this type was 1.2% (22/ 2x995); the complementary crossover, al S ast, was not recorded since it fell into the poorly viable S ast / + ast[3] class of offspring. The best estimate of the total frequency of crossing over in the al - $S_1$ region is therefore 2.3% (22/ 995). In the same experiment it was also possible to detect a crossover between the S locus in the left section and the break point of the duplication. This had the composition, al ast, and occurred with a frequency of 0.05% (1/ 2x996); it was convenient to note the occurrence of this type in studies on the left section of Dp-S where it had special significance. The complementary crossover to the latter is ( S ast)( + ast) ho, but if it occurred in the offspring of Mating 23., it must have been included in the phenotypically indistinguishable ast ho class, which resulted from crossing over in the region between the break point and the S locus in the right section of Dp-S. Now the frequency of the "ast" ho types in Mating 15., was 1.2% (22/ 2x995) and it follows that the total frequency of crossing over in the $S_1$ - $S_2$ region is 2.3% (22/ 995), or the same as that observed in the same experiment for the al - $S_1$ region. Thus, the total frequency in the al - $S_2$ region is 4.4% under the particular conditions used in Mating 15. This high value is presumably due to the presence in that mating of In(1)dl-49, In(1)AM, and T(2;3)Me, which were heterozygous in the parental female.

The above result may be compared with the average of the results from Matings 13., 16., and 17., in which the parental females did not carry Dp-S and were heterozygous for In(1)dl-49 and $\overset{\wedge}{T}{}^{Dp(2;3)}(2;3)$Me; it is likely that the inversion conditions here are only slightly less efficient than they are in Mating 15. In the former matings the average total frequency of crossing over between al and S was 4.2% (167/4,022) which is in good agreement with 4.4% for the al - $S_2$ region as measured in the latter mating. Apparently, then, the heterozygous presence of Dp-S does not appreciably affect the total amount of crossing over in the al - S region of a normal chromosome, at least under the particular inversion conditions used.

The problem of how the crossovers between al and $S_2$, in Dp-S heterozygotes, are distributed with respect to the left and right section of Dp-S can be attacked indirectly by calculating the ratio of the al - $S_1$ region to the $S_1$ - $S_2$ region in terms of genetic length. A summary of Matings 15., 18., and 22., Table 7., gives a value of 59 : 22 + $\frac{1}{2}$x31 (in terms of absolute numbers of crossovers in each region) or 1.6 : 1 for that ratio. Considering the fact that the al - $S_1$ region is known to be longer cytologically than the $S_1$ - $S_2$ region, it seems safe to conclude that the frequency of unequal crossing over involving the left section of Dp-S is the same as that for the right section in females which are heterozygous for the duplication. It is important to note, though, that this conclusion is based on an assumption, which

may not be justified, that differences in inversion conditions in Matings 15., 18., and 22., have had a negligible effect on the calculation of the above ratio. Again, whether or not the observed ratio would obtain in the absence of inversions in other arms is not known. If it be assumed that the ds locus is close, genetically, to the left end of the duplicated section, as it appears to be cytologically, then the theoretical al - $S_1$ : $S_1$ - $S_2$ ratio may be estimated as 1.3 : 1.3 - 0.3 or 1.3 : 1, which is in good agreement with the observed value.

## The Possibility of Sister Strand Crossing over in Dp-S Heterozygotes

The apparent removal of the ast locus from the right section of Dp-S without association with crossing over in the al - ho region has been observed in a total of 5 cases from heterozygous Dp-S females, distributed among Matings 19., 20., and 21., Table 6., and Mating 22, Table 7. Each case occurred in a single culture; four out of five of the cultures each had over 100 offspring which were derived from a single parental female. The fifth culture was from Mating 22., which had two parental females and which produced only 72 flies in that culture. It is quite possible that the net $S^+$ $ast^+$ dp cl type in Mating 22., and the al ast ho case in Mating 21 were the result of contamination; this explanation probably does not apply to the three cases obtained from Matings 19., and 20. Here it was established that each of the three cases arose as

net$^{+}$ ast ho dp$^{+}$ cl$^{+}$ / In(2L)Cy, ast$^{2}$ individuals and in each case it was shown that the ast ho type was apparently normal in the salivary gland chromosomes. The frequency of these types may be estimated as 1.6% ( 5/ 309 ) of the total number of cases of removal of ast from the right section of Dp-S. This frequency is about what would be expected if these types represent double crossovers within the region from net (or al) to S and if there is no interference in that region. But a coincidence close to one in an interval which in these experiments was probably not over 3 units is so unlikely that the possibility is open that the rare types are the result of unequal crossing over involving sister strands of Dp-S.

An experiment was designed to detect the possible occurrence of sister strand crossing in females having Dp-S in one homolog and one of the large S deficiencies in the other. Females of composition, In(1)dl-49 / In(1)AM ; al ( + ast )$_2$ ho T(2;3)Me / Df(2)S4 ; In(3LR)CxD, were mated to In(2L)Cy, al$^{2}$ ast$^{3}$ males and a search was made for ast / ast$^{3}$ types in the $F_1$; none were found however in over 2300 offspring (based on the ( + ast)$_2$ / ast$^{3}$ class ). The absence, in the homologous chromosome, of the section present twice in Dp-S does not appear to induce sister strand crossing over in Dp-S with an appreciable frequency.

## The Phenotypes of Dp-S Derivatives

In unequal crossing over studies of Dp-S heterozygotes, it was shown that the genetic composition of ( + ast )$_2$ could be

varied by introducing S into the left or right section of the duplication, or by replacing ast in the right section with $ast^+$. Many other substitutions are theoretically possible. Thus, using the 6 combinations, $S^+$ $ast^+$, S $ast^+$ , S ast, S $ast^4$, + ast, and + $ast^4$, there are 36 possible ways of varying the composition of Dp-S, making use of this material only. Actually, however, it has not been feasible to vary the composition of the ast locus in the left section so that the possible ways of varying that section are reduced to 2; this leaves only 12 combinations which can be obtained relatively easily. All but two of these, (+ ast)(+ $ast^4$) and (S ast)(+ $ast^4$), have been synthesized. The combinations may be divided into Group I., all members of which were derived originally from $(+ ast)_2$ by substitutions in the right section; and Group II., all members of which were originally derived from (S ast)(+ ast). The members of these groups are as follows:

| 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 $ast^4$) |

As has already been noted, (+ ast)( + + ) / $S^2$ E-S may be compared with $S^+$ $ast^+$/ + + E-S; while (+ ast)(+ ast) / $S^2$ E-S is similar in effect on the eye to $S^+$ $ast^+$/ + ast E-S ; similarly, (+ ast)(S + ) / $S^2$ E-S acts like $S^+$ $ast^+$/ S + E-S.

Members 3., 4., and 5., of Group I. have been found to be not only indistinguishable from each other, phenotypically, but also from $S^+$ $ast^+$ in all combinations which have been tried, including tests to S $ast^+$, S ast, S $ast^4$, and deficiencies for the region present twice in Dp-S. All members of group I. look normal in the homozygous condition, at least with respect to the eye; 1. and 2. occasionally show the extra vein effect as already described. The effects of every member of Group I. can consistently be interpreted as indicating that the left section acts as though it carries $S^+ast^+$ instead of $S^+$ ast, that the right section acts in each case according to the indicated composition, and that the two sections act independently of each other.

The effects of each member of Group II. are such as would be expected if each carried only the composition of the right section; thus, 6. acts like $S^+$ $ast^+$; 7., like $S^+$ ast; 8., like S $ast^+$; 9., like S ast; and 10., like S $ast^4$. These conclusions are based on tests of each member of Group II. to +, S, ast, $ast^4$, S ast, S $ast^4$ and E-S. Tests to S deficiencies were not made. No apparent position effects could be demonstrated for any of the following comparisons: (S ast)(S + )/ ast vs. (S ast)(+ ast)/S; (S ast)(S ast) / ast vs. (S ast)(+ ast)/ S ast ; and (S ast)(S $ast^4$) / ast vs. (S ast)(+ ast) / S $ast^4$. However, the possibility that slight differences may exist in some or all of these comparisons is still open, since eye measurements were not made and possible genetic variability was

not adequately controlled.

A comparison of Group I. with Group II. shows that members 3. and 6. are identical with respect to genetic material; as far as can be determined they are also identical phenotypically; e. g., (+ ast)(S +) / S and (S ast)(+ +) / S, each have a typical S / + type of eye. On the other hand, there is a striking difference between the derivatives, 4. and 7; for, whereas (+ ast)(S ast) / S + looks like S / +, (S ast)(+ ast) / S + is nearly if not inseparable phenotypically from S + / + ast. Superficially, the results of the latter comparison might be interpreted as an instance of position effect extending from the S and ast loci in one section of Dp-S to those loci in the other section. The results may also be explained on the simple assumption that there is a "primary" position effect on the ast locus in the left section of (+ ast)(S ast), causing it to act like $ast^{+}$, by virtue of the rearrangement in Dp-S, which just follows the 21 E 1-2 doublet of that section. The latter interpretation is more likely for three reasons. Firstly, it satisfactorily explains all of the effects observed for members of Group I. Secondly, two other aberrations, T(2;3) $ast^{rv1}$ and In(2L)$ast^{rv2}$, have been found which have one of their breaks just following the 21 E 1-2 doublet, as in the case of Dp-S, and which act as though the originally present ast had reverted to $ast^{+}$. Finally, it has been demonstrated, as shown below, that in the case of $(+\ ast)_2$ there is no reason to

suppose an additional "secondary" position effect, extending from the S and ast loci in one section to those loci in the other section.

Experimental evidence for the nature of the position effect in $(+\ ast)_2$ has been derived from a study of a translocation, T(2;3)Dp-S, obtained from X-radiation of Dp-S males, whose composition with respect to the duplication was $(+\ ast)_2$. A salivary gland chromosome analysis showed the presence of a reciprocal translocation, having one of its breaks within the duplication and just to the left of the 21 E 1-2 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 $S^+$ ast, just as the notation, $(+\ ast)_2$, 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, T(2;3)Dp-S / S has a nearly normal eye which may be compared with that of $(+\ ast)_2$ / S. In other words, the effects of $(+\ ast)_2$ are 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 likely that the "primary" position effect occurring at the S - ast region of the left section of $(+\ ast)_2$ has been changed in T(2;3)Dp-S as a result of the rearrangement, and that the change in that region is to a variegated ast-like effect, which, however, can be suppressed by an extra Y chromosome. It is important to note here that the phenotypic effects of $(+\ ast)_2$ are not changed by the addition of a Y chromosome.

## Discussion

It is perhaps significant that the origin of Dp-S was associated with crossing over. It suggests that the mechanism of the origin of tandem repeats, in which the sequence is in direct order, may be either one of non-homologous crossing over or a type of unequal crossing over occurring within a repeat already established in the species. Thus, assuming that there is a small, not strictly tandem, repeat with a sequence,..J K L M N O P L M Q R., then as a result of pairing in an unequal manner, diagrammatically:

.. J K L M N O P L M Q R S ..
..J K L M N O P L M Q R..

the occurrence of crossing over in the L M region would give rise to a tandem, direct repeat with the sequence, .. J K L M N O P L M N O P L M Q R .. , and a complementary deficiency type. There is however no obvious indication for the existence of the original type of repeat, here postulated, in the normal region of the salivary gland chromosomes in which Dp-S lies. There is occasional evidence from studies of Dp-S, itself, for an association of the doublets, 21 D 1-2 and 21 E 1-2, which might be taken as indicating that they provide the necessary repeat region; on the other hand, it is probably more likely that the association is not real but the result of the confusion in pairing shown by the two sections of Dp-S.

The study of unequal crossing over in females, heterozygous for Dp-S, gave no indication for a disturbance in the total amount of crossing over in the al - S region; furthermore, the distal and proximal sections appear to undergo crossing over with the common homologous region with about the same frequency. This suggests that, at meiosis, pairing of those two sections is a t random. The fact that there is little or no reduction in crossing over in the al - S region in Dp-S is in agreement with cytological evidence derived from a study of the salivary gland chromosomes of heterozygous Dp-S which shows that failure of pairing in the S region is very infrequent. Indeed, so complete is this somatic pairing between the repeat section of Dp-S that in some instances it appears as though it had the normal banding. This lateral pairing, if it existed at the time of crossing over might permit a type of sister strand crossing over although involving, perhaps, four strands at one level. But if this phenomenon occurs, and there is some evidence which suggests that it may, it may be difficult to determine whether crossing over between sister strands has occurred in the four strand level, i. e., pairing of the two homologous sections in the sister strand stage; or in the two strand condition.

# SUMMARY


## I.

Two loci, Star and asteroid, in the second chromosome of D. melanogaster were found to be extremely closely linked with an estimated standard map distance of 0.01 to 0.04 unit. It was demonstrated that these two loci are included in the 21 E 1-2 doublet structure of the salivary gland chromosomes. Not only do the Star and asteroid mutants affect the eye in a similar way, but they also show position effects depending on how they are distributed between the two chromosomes. An interpretation of the observed phenomena is made in terms of a naturally occurring repeat in the chromosomes.

## II.

The Star Duplication is a tandem repeat in direct order for the four bands, 21 D 3, 4 and 21 E 1-2. It includes the Star and asteroid loci and with the use of mutants at these loci as markers, it has been possible to study unequal crossing over in females heterozygous as well as homozygous for the duplication. The genetic composition of the Star Duplication has been varied in ten distinct ways; the results indicate that there is probably a position effect exerted on the asteroid locus in the left section of the duplication causing the originally present asteroid change to act like a reversion to normal. It is likely that there is little or no position effect exerted by the Star and asteroid loci in one section on those loci in the other section.

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# *ANOTHER CASE OF UNEQUAL CROSSING-OVER IN DROSOPHILA MELANOGASTER*

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Females homozygous for the sex-linked dominant, Bar, occasionally give rise to wild-type reversions and to forms with more extreme eye reduction than Bar. This behavior was shown by Sturtevant[1] to result from unequal crossing-over. The Bar-reverted type was considered to be a deficiency for the Bar gene; while the extreme form, called Ultra-Bar or Double-Bar, was interpreted as a duplication for that gene. Later, Wright[2] suggested that Bar itself had something additional present which when lost by unequal crossing-over would give back a normal chromosome (Bar-reverted).

The cytological nature of Bar was cleared up independently by Bridges[3] and Muller, *et al.*,[4] who investigated the salivary gland chromosomes. They found Bar to be a tandem duplication, in normal order, for an *X*-chromosome section composed, according to Bridges' detailed analysis, of six bands. Bridges further demonstrated that Bar-reverted had the identical banding of a normal chromosome, whereas Double-Bar had a serial triplication for the region present twice in Bar and once in Bar-reverted.

The second case of a tandem duplication being responsible for a dominant "mutation" is that of the sex-linked Hairy wing, which Demerec[5] has shown is a repetition for a single heavy band near the tip of the *X*-chromosome. However, its location in a region of extremely low crossing-over prevented a study of unequal crossing-over.

This paper is a preliminary report on an autosomal tandem repeat which was detected as a suppressor of the dominant mutant, Star (*S*, 2-1.3).

An analysis of the salivary gland chromosomes of this suppressing factor, when homozygous, when closely paired with a normal chromosome and when present as an unpaired haploid strand, consistently showed the presence of a tandem duplication in direct order near the left end of the second chromosome. The section present twice appears to include the two faint bands, 21 *D* 4—5, and the heavy, frequently capsulated doublet, 21 *E* 1—2; i.e., a section of at least four bands.

*Dp*(2)*S* appeared in a study of changes at the Star locus, a consideration of which is essential before discussing the properties of this repeat. *S*/+ has roughened, slightly reduced eyes; *S*/*S* is always lethal. Star-reces-

sive ($S^r$) is the name tentatively given to a recessive mutant near if not at the Star locus. $S^r/S^r$ has smaller, rougher eyes than $S/+$, and may have gaps at the tips of the wing veins. The compound, $S/S^r$, is much more extreme, having a narrow diamond-shaped eye and extensively interrupted venation.

Bridges has reported that the salivary gland chromosomes of Star are apparently normal. The same appears to be true for Star-recessive and also for the dominant Suppressor of Star (*Su—S*, 2—1.3±) found by Curry.

The allelic relation between $S$ and $S^r$ is, as yet, ambiguous. From *al S ho*/$S^r$ females (*al* = aristaless, 2—0.0; *ho* = heldout, 2—4.0), wild-type "reversions," which are always heldout crossovers and which are cytologically normal, occur with a frequency of 0.01% (4 : 31, 106); by using females heterozygous for inversions in all of the other chromosome arms, their frequency has been stepped up to 0.046% (12:26, 370). Yet, a crossover complementary to the reversions has not been detected. The situation may be similar to a case, recently reported by Oliver,[6] of reversions, associated with crossing-over in one direction, arising from females carrying two alleles of the lozenge eye mutation. For the sake of simplicity, $S$ and $S^r$ are considered as alleles in this paper.

*Dp*(2)*S* arose spontaneously as a single individual among approximately 49,000 offspring of *al* $S^r$ *ho*/$S^r$ females individually mated to *al S ho*/*Cy*, *E*—*S* males. The fly had normal aristae, nearly wild-type (Star suppressed) eyes and heldout wings. Tests showed that the mother had contributed $al^+$, *ho* and *Dp*(2)*S*, whose origin was therefore associated with crossing-over. *Dp*(2)*S*/+ and *Dp*(2)*S*/$S^r$ look wild-type. *Dp*(2)*S*/*Dp*(2)*S* is also normal except for an occasional slight extra vein near the fifth longitudinal vein. This wild-type action is in striking contrast to the pronounced phenotypic effects of Bar and Hairy wing.

A study of unequal crossing-over in the heterozygous duplication has shown that the Star locus is included in the repeated sections; i.e., it has been possible to recover from *al Dp*(2)*S ho*/*S* females unequal crossover products which have $S$ inserted into the left (distal) section of the repeat, and others with $S$ introduced into the right (proximal) region. The latter occur with a frequency of 0.3% (6:1948) or roughly thirty times as frequently as the former (0.01% or 2:*ca* 23,000). In terms of genetic length this indicates that the $S$ locus is included in the extreme right portion of each of the two regions present in duplicate. The original duplication, since it arose from homozygous $S^r$, might be expected to have a $S^r$ gene in each of these positions. Using parentheses to bound the repeated regions, its composition may be written: *Dp*(2)*S* = (...$S^r$.)(...$S^r$.). That $S^r$ is present in the proximal section has been demonstrated by its recovery from

$$\frac{al\ (\ldots S^r.)(\ldots S^r.)\quad ho}{(\ldots S.)}$$

females as $S^r$ *ho* cross-overs, whose cytological picture is normal; their frequency is 0.35% (57:16,568) or approximately that of the complementary *al* $(\ldots S^r.)(\ldots S.)$ class mentioned above. In the two cases where $S$ was inserted into the left section, the product may be written: $(\ldots S.)$-$(\ldots S^r.)$ *ho*, and its origin visualized as the result of the following pairing:

$$\frac{al\ (\ldots S^r.)(\ldots S^r.)\quad ho}{(\ldots S.)},$$

accompanied by a cross-over between the $S$ locus and the break point of the duplication. The complementary crossover is expected in this case to be *al* $S^r$, or the removal of $S^r$ from the left section. Yet, although a total of 243 $S^r$ *ho* types have been detected, no cases of *al* $S^r$ have occurred. This may mean that $S^r$ is slightly to the right of $S$, as was suggested, in part, by evidence given above. On this basis, either $S^r$ is just outside the duplication or it is so close to the break point that crossing-over has failed so far to remove it from the left section.

Although the phenotypic effects of the original duplication are consistent with the assumption that one $S^r$ and a normal allele of $S^r$ are acting, the origin of $Dp(2)S$ from homozygous $S^r$ would seem to indicate that this action is more likely a position effect. A preferable notation, for the present, would be $Dp(2)S = (\ldots S^r?.)(\ldots S^r.)$.

From *al* $(\ldots S^r?.)(\ldots S^r.)$ *ho*/+ females, normal $S^r$ *ho* chromosomes and *al* $(\ldots S^r?.)(\ldots S^{r+}.)$ occur with approximately equal frequencies as expected.

There is genetic evidence, not of a crucial character, that the locus of net (2—0.3±) is also included in $Dp(2)S$ at the extreme left end of each section. If this is the case then the total frequency of crossing-over between the loci of net and Star in heterozygous $Dp(2)S$ is greater when the distal section is involved (1.4%) than when the proximal one takes part (0.7%). As in the experiments previously given, these data are obtained from females heterozygous for inversions in some of the other chromosome arms with the result that the normal *net*–$S$ distance of 1% is increased to 2% or more.

From females homozygous for the original duplication whose repeated sections may be supposed occasionally to pair in an unequal manner, diagrammatically,

$$\frac{al\ (\ldots S^r?.)(\ldots S^r.)\qquad ho}{(\ldots S^r?.)(\ldots S^r.)},$$

two types of unequal cross-over products have been obtained which, apart from phenotype, are analogous to the derivatives produced by homozygous Bar females. The normal chromosome products, corresponding to Bar-reverted, are detected on the basis that they carry $S^r$; their frequency is 0.25% (9 *al* $S^r$ + 5 $S^r$ *ho*: 5594). New chromosomes with three sections in tandem repetition, as is the case with Double-Bar, occur with approximately the same frequency as the normal types, namely, 0.18% (8 *al* + 3 *ho*, triplications: 6000); their action is to suppress, completely, *S E–S* (*E–S* = Enhancer of Star, 2- 6.±), whereas *Dp*(2)*S* (...$S^r$?.) (...$S^r$.) only partially suppresses the small rough eye effect seen in *S E—S*/+. The unequal crossover types have been examined in the salivary gland chromosomes and the analogy with the Bar derivatives has been found to hold. The homozygous triplication, symbol, *Tr*(2)*S*, has slightly bulging eyes with large facets; in addition to occasional slight extra veins, described for the homozygous *Dp*(2)*S*, there is often a branching of the second longitudinal vein. The wing effects are perhaps to be ascribed to the locus of net.

Homozygous *Tr*(2)*S* females have produced $S^r$ chromosomes with only one section present, and also a new "dominant" unequal crossover product, which over $S^r$ (or $S^{r+}$) has eyes resembling those of homozygous *Tr*-(2)*S*. A cytological analysis supports the conclusion that the "dominant" is a repeat of five sections. When homozygous this quintuplication, symbol, *Qn*(2)*S*, is still quite viable and fertile, and has the same effects, but much more intensified, as homozygous *Tr*(2)*S*. *Qn*(2)*S* would correspond to Quadruple-Bar obtained by Rapoport[7] from attached-*X* females homozygous for Double-Bar.

*Summary.*—1. An autosomal tandem duplication is described, whose origin was associated with crossing-over.

2. Genetic evidence indicates that the locus of Star and possibly the net locus are included in each of the duplicate sections.

3. The homozygous duplication gives unequal crossover products analogous to Bar-reverted and Double-Bar. A repeat of five sections has been derived from the homozygous triplication.

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