THE SIDE-CHAIN THEORY OF THE STRUCTURE OF THE GENE[1]

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Received May 14, 1930

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INTRODUCTION

In 1923, after three years as research assistant to Professor CHARLES ZELENY during the time that the rates of mutation of the bar and ultrabar genes were being determined, it occurred to the writer that mutation of these genes may be explained as the gain or loss of certain discrete particles or units within the gene. This idea was developed and presented as a thesis in 1924 and a brief abstract published (THOMPSON 1925). During the past six years it has been possible to extend the theory by using data published by numerous geneticists.

[1] Contribution No. 396 from the Zoological Laboratories of the UNIVERSITY OF ILLINOIS, and the ILLINOIS STATE NATURAL HISTORY SURVEY.

This conception pictures the gene as consisting of a main particle firmly anchored in the chromosome with varying numbers of one or more kinds of other particles attached. The main particle is called the protosome and the attached particles the episomes. (In the article just referred to, these particles were called the "base" and the "modifier," terms which unfortunately are confusing since "modifier" is already used in genetics to mean accessory factor and "base" has also a wide range of technical meanings in the different branches of science.) Gene mutation is due most frequently to the loss of one or more episomes from the protosome and less frequently to the addition of episomes. When two or more episomes of the same kind are present in a gene, they are attached one to the other so as to form a side-chain. Different kinds of episomes do not enter into the same side-chain, but each kind has its own attachment with the protosome. Varying numbers of the same kind of episome produce "quantitative" series of multiple allelomorphs. Epistatic series and allelomorphs which are "qualitatively" different involve variations in the numbers of two or more kinds of episomes. Shifting of the position of an episome or chain of episomes on the protosome may account for changes in the rate of mutation or for different linkage relations. Episomes may be transferred occasionally, not only from one protosome to its homologue in the other chromosome, but also from one locus to another. The writer visualizes the genetic chromosome as the conventional string of beads used to demonstrate crossing over, except that almost every bead bristles with side-chains of episomes.

DIRECTION AND FREQUENCY OF MUTATIONS

There is a wide variety of types of eye in *Drosophila melanogaster* depending on genetic conditions at the bar locus in the X-chromosome. The eyes differ primarily in the number of facets, or ommatidia, which may be counted and accurate quantitative comparisons made. The individual ommatidium remains almost constant in size in the different types of eye under ordinarily good culture conditions, so that facet counts give an expression of area.

Origins and mutations of the genes of the bar series

All bar-eyed flies and all of the bar allelomorphs are directly descended from a single bar male found by TICE in 1913 in an experiment involving only normal wild-type eyes. Among the millions of Drosophila with wild-type eyes that have been examined since 1913, no recurrence of the bar mutation has been reported.

In 1917, MAY reported eleven occurrences of the reverse mutation, that

is, from bar to full, or round eye. His tests as well as those of a number of subsequent workers have failed to show that the full eye derived from bar by reverse mutation is in any way different from the wild-type eye.

In 1920, ZELENY described a new allelomorph, called ultrabar (double-bar of STURTEVANT), which was found in a homozygous bar stock. Whereas homozygous bar eyes ordinarily have about nine percent as many ommatidia as the wild-type eye, this new allelomorph has only about three percent as many facets. Females heterozygous for bar and full have an intermediate eye with about half as many facets as are found in the wild-type eye. On the other hand, heterozygotes of ultrabar and full, while still inter-

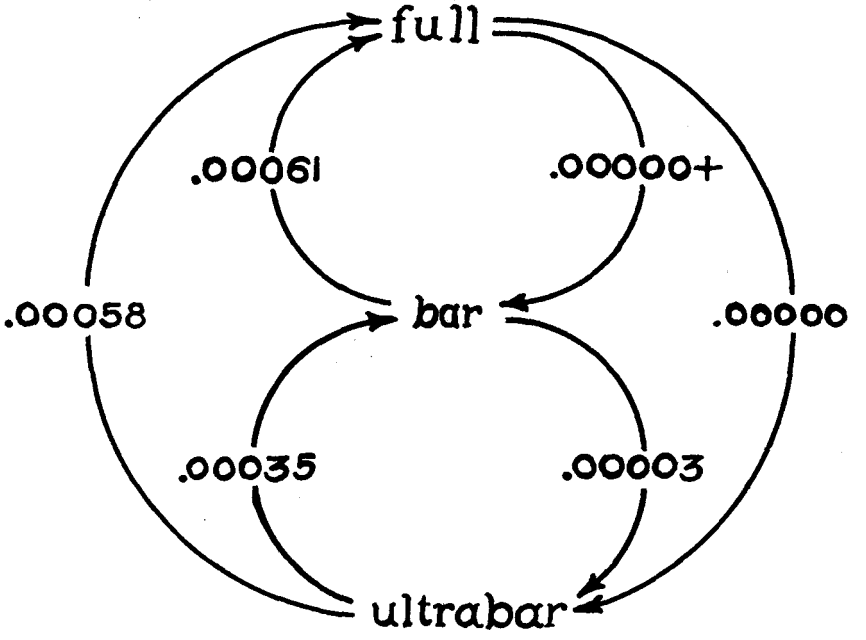

FIGURE 1.—Rates of mutation in the bar series of multiple allelomorphs. (After ZELENY 1921).

mediate, have only about six percent as many facets as full. Heterozygotes of ultrabar and bar are likewise intermediate, with about four percent as many facets as full. ZELENY'S tests indicated that ultrabar fulfills the requirements of allelomorphism with bar and full. There are no apparent "qualitative" differences between full, bar, and ultrabar which would prevent their consideration as a "quantitative" series of multiple allelomorphs in the order named. The ultrabar allelomorph not only mutates back to bar, from which it was derived, but also mutates directly to full without passing through the bar condition. The rates of mutation between these allelomorphs as determined by ZELENY (1921) are given in figure 1.

STURTEVANT (1925, 1928) explains the origin of ultrabar as due to unequal crossing over at the bar locus resulting in two bar genes lying next each other in the linear series of the genes. According to his explanation, reverted bar and presumably wild-type full, also, are not represented by an allelomorph at the bar locus; furthermore, homozygous bar should produce full and ultrabar mutants in approximately equal numbers. This has not been realized. ZELENY'S (1921) data show that among 85,008 bars examined the fulls outnumber the ultrabars 17:1. (During the course of this experiment, numbers of flies with low facet counts were tested and found not to be ultrabar.) As STURTEVANT has pointed out, by the inclusion of certain untested flies with unusually low facet numbers this ratio is lowered to 6.5:1. More recently (1928) he reports 8 full mutants and 2 ultrabar mutants from homozygous bar; a ratio of 4:1. While the true rate of mutation from bar to ultrabar may be considerably in excess of the 0.00003 given by ZELENY, it is difficult for me to believe that I overlooked numbers of ultrabars approximating the fifty or so full mutants which were found.

The difficulties of recognition of bar-ultrabar and full-ultrabar heterozygotes among an ultrabar population, as well as the preponderance of male mutants, make it seem likely that female mutants from ultrabar to bar were all overlooked and that part of those from ultrabar to full went unrecognized, so that the rates given in figure 1 (0.00035 and 0.00058) are probably too low.

STURTEVANT and MORGAN (1923) and STURTEVANT (1925, 1928), by a most ingenious and critical series of experiments, have shown that mutation at the bar locus is associated with crossing over in that region. A most important result of these experiments is that STURTEVANT by genetic manipulation apparently has conclusively demonstrated that the germinal difference at the bar locus resulting in a full eye on the one hand and a bar eye on the other is exactly equal to the germinal difference between a bar eye and an ultrabar (double-bar) eye.

Evidence from other sources shows that mutations at the bar locus may occur in the female at other stages in the life history than at the maturation divisions, and in the male as well as in the female. HANSON (1928a, 1928b) reported 9 reversions of bar to full among the offspring of irradiated bar males. It seems improbable that these mutations were associated with crossing over, since crossing over does not occur in the male in Drosophila. Other evidence of HANSON'S (1929) shows that irradiation has its effect on mature spermatozoa, which are haploid, thus making any possible association with crossing over quite out of the question.

PROFESSOR ZELENY has kindly permitted me to present here unpub-

lished data of his concerning an instance of coincidence of mutation from bar to full and from bar to ultrabar which occurred in 1922. A virgin female of an inbred white bar stock was mated to a brother and gave 105 offspring in 4 bottles. In bottle 3 of this mating there were 8 females and 12 males. Of these 8 females one appeared to be a heterozygote between bar and full, and another was as low in appearance as ultrabar, while the rest were typical bars. This full-bar heterozygote was not tested, but numerous others have always proved to be what they seemed, that is, heterozygotes for bar and full. The low female, when mated to a bar brother, gave only two offspring and a third pupa which did not hatch. One of the two offspring was a bar male with 83 facets and the other a male with 30 and 32 facets in the two eyes, respectively, at room temperature.

A similar coincidence of mutation happened in the spring of 1920 in the course of work with the character erect wing. A white bar erect female, which was homozygous for the white and bar factors and heterozygous for the sex-linked erect factor and for an autosomal factor which makes erect dominant (THOMPSON 1921), was mated to a wild-type male. Neglecting erect and its accessory factor, the expected classes were full-bar heterozygotes with red eyes and white bar males. The offspring consisted of 49 females and 47 males, respectively, for the expected classes and *one red full-eyed female* and *one red-eyed female classified as low bar.* Up to the time of the above-mentioned coincidence of mutation, this case could not be explained, but it now seems explicable in similar terms; that is, a bar female produced two mutant eggs, one of which bore a full and the other an ultrabar gene.

That the process which causes bar to mutate to full is associated with the process that produces ultrabar seems certain; for if they occurred independently, the probability of obtaining the two in a bottle of twenty flies would be one in several millions. While it has been shown by STURTEVANT (1925, 1928) that these two processes ordinarily are accompanied by forked-fused crossing over, these cases of coincidental mutation could not have involved crossing over; for in order to result in two mutants, the change in each case must have taken place one or more cell generations before the maturation divisions.

Another interesting case of coincidence of mutation came to light in November, 1923. An ultrabar forked female of a pure stock was mated to an ultrabar forked brother. The offspring consisted of 9 ultrabar forked females, 19 ultrabar forked males, *and one full forked female.* This full female could not have been the result of contamination because no forked full stock had been in the building for months, and no strays of this kind were in the

fly traps. Neither could she have resulted from the highly improbable fertilization of a mutant egg by a mutant sperm, since mutation in the male is not known to take place except by irradiation. It therefore seems probable that she had received both of her full genes from her mother. This egg, then, must have contained two X-chromosomes and was fertilized by a Y-sperm. In this case the full female should give exceptional offspring due to secondary non-disjunction. She was mated to an ultrabar forked brother and gave 47 forked daughters heterozygous for ultrabar and full, 37 full forked sons, and *one full forked daughter*. She was then mated to a full forked son and, after a few offspring by the previous male, gave all full forked offspring. This correlation of mutation with non-disjunction is all the more significant since non-disjunction is extremely rare in the stocks used. Recent experiments involving over 500 matings did not show a single exceptional offspring that could be due to non-disjunction.

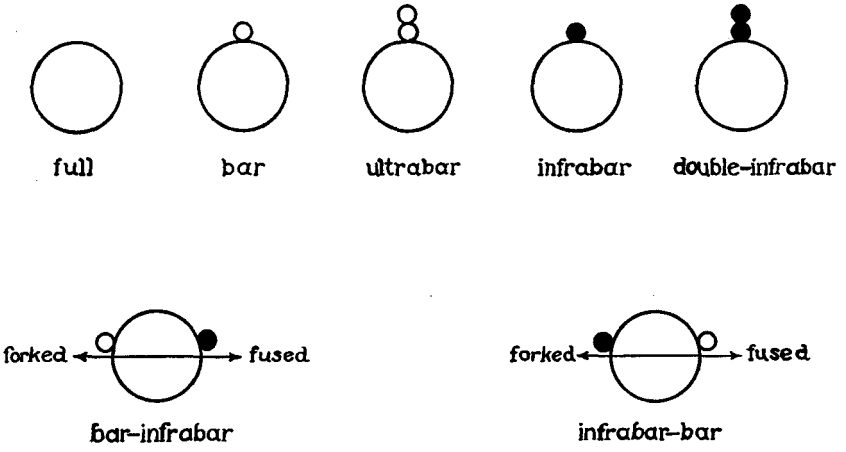

FIGURE 2.—Diagrams of the different genes known to occur at the bar locus.

The mutation phenomena of full, bar, and ultrabar thus far described may be interpreted briefly in terms of the structure of the gene as follows: The original bar mutation arose by the addition of a bar episome to a protosome at the bar locus in a full-eyed fly. This bar episome came from some other locus. The bar episome of the bar gene is frequently broken off, giving reverse mutations to full. When a bar episome is dislodged in a homozygous bar female, it may occasionally attach to the homologous bar gene to produce an ultrabar gene. Those episomes which are dislodged and do not become attached to the homologous gene may either be attached at some other locus or else be resorbed and lost to the germ-plasm. When a bar gene adds another bar episome to produce an ultrabar gene, we infer

that the second episome is attached to the first in a side-chain arrangement because the ultrabar gene loses both its episomes to produce a full mutant as readily as it loses one to produce bar.

STURTEVANT and MORGAN'S demonstration that mutation at the bar locus is associated with forked-fused crossing over is interpreted to mean that crossing over, with its implied intimate contact at the point of breaking, is an effective agency for dislodging episomes or for transferring them to the homologous gene. That crossing over is not the only agency which can dislodge the bar episome is shown by HANSON'S production of mutations from bar to full by irradiating the spermatozoa of bar males. The two instances of coincidence of mutation at the bar locus occurred in the female during the oögonial divisions. Changes in cellular mechanics accompanying non-disjunction seem to have been responsible for the loss of the episomes from the two ultrabar genes of an XX egg.

The genes of the bar series of allelomorphs may be diagrammed as in figure 2.

Origins and mutations of the genes of the infrabar series

STURTEVANT in 1925 reported a new allelomorph of bar which he called infrabar. This mutation appeared as a single male in an experiment where all the fathers were bar and all the mothers had the two X-chromosomes attached, giving 100 percent non-disjunction. This mutation falls in a different category from those being considered in this paper, as it represents a change in the nature of the bar episome, that is, it involves episome mutation and not gene mutation. With this new kind of an episome STURTEVANT has duplicated most of the phenomena described earlier in this paper for the bar series of "quantitative" allelomorphs. Infrabar behaves like bar in that it reverts to full, but at a rate three to four times as great (STURTEVANT 1928). It also produces a new and more extreme allelomorph called double-infrabar, analogous to ultrabar. Just as homozygous bar produces ultrabar less frequently than it reverts to full, homozygous infrabar produces double-infrabar less frequently than it reverts to full. Double-infrabar likewise breaks down to give infrabar or reverts to full directly. Double-infrabar, infrabar, and full constitute a "quantitative" series of allelomorphs paralleling the ultrabar, bar, and full series.

This infrabar series differs qualitatively from the bar series. STURTEVANT (1925) says, ". . . homozygous infrabar is about like bar over round in facet number, but the two types can be separated by a peculiarity common to all the larger infrabar and double-infrabar types, namely, a roughened appearance of the eye, due to irregularities in the rows of facets. This

peculiarity is not present in bar eyes, and is almost completely recessive in bar over infrabar. In infrabar over round (which is not far from round in facet number) the roughness is variable in extent, and may be not at all evident, in which case the type can not be distinguished with certainty from homozygous round. In other stocks, where the modifiers are different, it often happens that infrabar over round is regularly conspicuously roughened and is easily distinguishable from round. This roughness of the eyes may be taken as evidence that the infrabar gene is qualitatively different from bar, rather than being merely a fraction of bar."

KRAFKA (1920) showed that wide differences in facet number result from differences in temperature at which bar or ultrabar larvae develop, approximating 10 percent decrease in facet number for each degree C increase in temperature. ROSELLE KARRER HERSH (1924) showed that full eye is affected in the same direction but to a less extent, being 2½ percent per degree. DRIVER (1926), in a very painstaking analysis of the temperature effect on bar and ultrabar, has shown that with increasing temperatures the rate of facet formation per unit of time during the facet-forming period is not much altered, but that the lower facet numbers result primarily from a shortening of the temperature-effective period.

Further evidence of the qualitative difference between infrabar and bar is found in LUCE'S (1926) discovery of the surprising fact that temperature has the opposite effect on infrabar and double-infrabar; that is, increasing temperatures give increasing facet numbers. His analysis shows that with increase in temperature the rate of facet formation during the temperature-effective period, instead of being almost constant as in bar and ultrabar, is increased enormously—so much so that it far more than counterbalances the opposite effect due to a shortening of the temperature-effective period.

The qualitative difference between the bar series and the infrabar series is represented in figure 2 by means of two different kinds of episomes, which, however, have the same structural relations to the protosome.

Composite genes of bar and infrabar

Evidence indicating some details in the relation of episomes and protosomes is found in the manner of origin of certain mutants. STURTEVANT (1925) has shown that when bar and infrabar genes are in the two X-chromosomes of a female a new allelomorph containing both may be produced in a manner analogous to the production of ultrabar or double-infrabar. This compound allelomorph breaks down to give either bar or infrabar mutants, but to date he has reported no mutations from it directly to full. These bar and infrabar mutants occur with a *special relation to*

crossing over. All the bar mutants were associated with crossing over between the forked locus and the bar locus, while all the infrabars were crossovers between the bar locus and fused. In another independently produced composite gene of bar and infrabar the relations were reversed, so that infrabar resulted from crossing over in the first region and bar from the second. He reports thirteen mutants consistent in this relation to crossing over, with no exceptions.

The facts just mentioned are interpreted to mean that the bar and infrabar episomes of these composite genes are not attached in side-chain arrangement but lie *near the left and right poles of the gene*, so that crossing over on the left side breaks off the one (or transfers it to the homologous bar allelomorph), while crossing over on the right side breaks off the other. The difference between bar-infrabar and infrabar-bar genes can be seen in figure 2. While, up to date, STURTEVANT has reported no direct mutations to full from these composite genes, an experiment showing whether or not they produce fulls at a rate approaching their rate of mutation to bar or infrabar would be a test for this postulated structure.

SOMATIC MANIFESTATIONS OF MUTATIONS

In the preceding discussion of the structure of the genes at the bar locus, only the most direct evidence available has been used, that is, evidence showing the origins of the different allelomorphs and the direction and frequency of mutation. Detailed discussion of facet number has been purposely avoided except as it was necessary to recognize the different mutations. All available data, however, do indicate that the gain or loss of one of these germinal units, or episomes, is accompanied by a definite jump in the facet number, and that these different units maintain their identity no matter what combinations they have entered into or what manipulations they have undergone in their past history. It is now proposed to discuss the somatic manifestation of these genes and to point out, in particular, how facet number is determined by the ratio of the number of episomes to the number of protosomes. Before undertaking the exact application of such a formula, however, it is necessary to consider two apparent exceptions, namely, the so-called position effect and sexual dimorphism.

The so-called position effect

STURTEVANT (1925, 1928) has pointed out what he calls the position effect of genes on facet number. According to his unequal crossing over explanation of mutation at the bar locus, it is to be expected that homozygous bar females, with a bar gene in each X-chromosome, would have the

same facet count as full-by-ultrabar heterozygotes, with two bar genes in one X-chromosome and none in the other. When these two are compared he finds that the heterozygote always has fewer facets than the homozygote, the number varying in different stocks from $^2/_3$ to $^4/_5$ as many. The same relation holds when infrabar homozygotes are compared with heterozygotes of full and double-infrabar and when bar-by-infrabar heterozygotes are compared with heterozygotes between full and the composite bar-infrabar allelomorph. Such comparisons, at first, appear to present the same difficulty of explanation on the basis of side-chain structure of the gene as on the basis of unequal crossing over, since the same structural elements are present in both; but there is an essential difference. I am indebted to Professor ZELENY for the suggestion that the difference in facet number shows that the two bar factors (episomes) in an ultrabar gene do not exist as separate entities but are bound up with a third and different member (the protosome) to form a single gene which, with a normal allelomorph (protosome alone) in the homologous chromosome, may be expected to give a different result from that produced by a bar episome on each of two protosomes. If two episomes always gave the same result no matter how they were distributed, there would be no obvious need of postulating the existence of the protosome. Furthermore, if the same facet number always resulted, it would indicate that two allelomorphic genes, although located in two separate chromosomes, could together constitute a genetic unit of a more fundamental nature than a single gene—a situation without parallel among the units of the physico-chemical world. Finally, the absence of "position effect" on bristle number has been shown by STURTEVANT (1928) in a case in which the genes unquestionably exist as separate entities.

Sexual dimorphism

Males of the different allelomorphs at the bar locus have consistently higher facet numbers than the corresponding homozygous females. The sex coefficients in the order of decreasing facet number, and the authority quoted for each, are as follows: full 0.984 (ROSELLE KARRER HERSH 1924); infrabar 0.729 (STURTEVANT 1925); bar 0.791 (KRAFKA 1920); double-infrabar 0.831 (STURTEVANT 1925); bar-infrabar 0.899 (STURTEVANT 1925); ultrabar 0.953 (ZELENY 1920). Since the stocks on which these dimorphism coefficients are based seem to have been more or less closely inbred, these observed differences are brought about by the male having a single dose of the facet-determining factors in the X-chromosome while the female has a double dose of these factors. These differences, however, are

not necessarily due to single and double doses at the bar locus alone; for A. H. HERSH (1929) has demonstrated the existence of plus and minus modifiers of facet number in other parts of the X-chromosome. His findings bear out ZELENY'S (1921) explanation that the marked decrease in sexual dimorphism following inbreeding results from the elimination of differences due to X-chromosome factors affecting facet number.

Sexual dimorphism is greatest in the middle range of facet number, where a more labile condition is also indicated by temperature coefficients and by coefficients of variability. The so-called position effect also falls in this labile range.

The facet numbers determined by genes at the bar locus

Facet number is more closely correlated with the ratio of the number of episomes to the number of protosomes than with the total number of each or with other mathematical relations of these structural elements. If the different facet numbers are determined by quantitative changes in the gene, there should be some mathematical relation between the number of bar episomes and facet number when the number of protosomes is held constant. The males of the bar series, having only three members, cannot be expected to show the relationship as readily as the female series with its six members. The facet counts at 25°C for full eye by ROSELLE KARRER HERSH (1924) and for the other members of the series by A. H. HERSH (1922) have been used because all of them were derived by recent mutation from one stock and should have about the same combination of accessory factors. Since homozygous bar females and full-ultrabar heterozygotes both have two bar episomes, their facet numbers will be averaged, leaving five facet numbers in the female series as follows:

Kind of female		*Number of episomes*	*Facet number*
Ultrabar homozygotes		4	24.5
Bar-by-ultrabar heterozygotes		3	28.1
Bar homozygotes Full-by-ultrabar heterozygotes	average	2	49.0
Full-by-bar heterozygotes		1	325.0
Full homozygotes		0	740.0

The interval increases rapidly for the first four members in this facet-number series but is not much larger for the last member than for the one before it. This again involves the consideration of qualitative differences. For instance, if A and B are different kinds of things, A is qualitatively different from the combination of A+B. On the other hand, A+B is quantitatively different from A+2B; and A+2B from A+3B; and so on. Since

the last interval in the above tabulation represents a qualitative difference in the composition of the gene, the full facet count should be excluded from the quantitative series. Analogous results are to be found in a consideration of the physical properties of series of organic chain-compounds, in which the zero, or end-member, of the series does not lie on the curve determined by the other members.

ZELENY (1920b) found an exponential relation to exist in the variations of the facet numbers of the different members of the bar series. KRAFKA, the HERSHES, and DRIVER found the same kind of relation in the facet counts of flies reared at successive intervals of temperature. It is then desirable to express the facet number in this female series in terms of the number of episomes, preferably by a generalized exponential equation, as follows:

$$\log_{10} f(n) = a + bc^n$$

Here the number of episomes is represented by n, and the facet number by the right-hand member of the equation, in which the constants a, b, and c were determined by the different combinations of the four remaining facet numbers taken three at a time. The average values thus obtained were:

$$a = 1.363$$
$$b = 4.209$$
$$c = 0.276$$

By substituting these constants and the number of episomes, we may calculate the different facet numbers and extrapolate the facet numbers of hypothetical allelomorphs and their heterozygotes which have more than four episomes on the two protosomes of the female. The observed facet numbers and the calculated results are as follows:

Number of episomes in females	*Calculated facet number*	*Observed facet number*
0	373,250	740
1	335	325
2	48.2	49.0
3	28.3	28.1
4	24.4	24.5
5	23.4	——
6	23.2	——
7	23.1	——
8	23.1	——
∞	23.1	——

The calculated facet numbers fit the observed facet numbers very closely, except for full, the zero condition, which has already been pointed out as qualitatively different. This seems to substantiate the conclusion

reached earlier by the rates of mutation, namely, that the full gene consists of a protosome with no episomes.

The main point of this discussion of facet number is to bring out mathematically what seems evident from an inspection of the facet numbers, that is, that facet number in the bar series approaches a limit. This calculated limit is 23.1 facets. This formula has been applied to the corresponding facet numbers given by STURTEVANT (1925), also at 25°C. While his facet numbers run somewhat higher than those used here, the limit approached is almost identical, being 23.05 facets. Females homozygous for these hypothetical allelomorphs with three or more episomes on each protosome would not have a facet number appreciably different from homozygous ultrabar.

The genes of the infrabar series and facet number

The facet numbers given by STURTEVANT (1925) for the homozygotes and heterozygotes of the full, infrabar, and double-infrabar genes have been substituted in the same formula, and the constants calculated, as was done for the bar series. By using the four facet numbers determined by 1, 2, 3, and 4 infrabar episomes on the two protosomes of the female, and by extrapolating, it is evident that facet number in this series does not approach any positive number. The trend of the curve indicates that a female homozygous for an allelomorph with three infrabar episomes on each protosome would be almost, if not quite, without facets. If this were true, the threshold ratio of episomes to protosomes in this infrabar series would be 3:1. Assuming, as before, that flies with the same episome:protosome ratio have similar facet numbers, an allelomorph with six or more infrabar episomes would be completely dominant over full, and the flies would be without facets.

With all this discussion of hypothetical allelomorphs with more than two episomes, the reader may well wonder why they have not been found. STURTEVANT (1925), and the writer as well, have tried to find such allelomorphs without success. One may surmise either that the decrease in viability which accompanies decrease in facet number in these series may prevent the appearance of these more extreme forms, or else that the protosome at the bar locus is "saturated" by two episomes.

Other quantitative series with a completely dominant allelomorph

The bar series of allelomorphs, without having realized a completely dominant member, can be brought into harmony with other quantitative series, such as the albino allelomorphs in guinea pigs (WRIGHT 1925) or the white eye series in *Drosophila melanogaster* (MULLER 1920), each of which has a

completely dominant allelomorph. It is to be expected that in the bar series all allelomorphs with four or more episomes on each protosome would be *completely dominant* over the three known allelomorphs. The facet formula given above shows that there is a *threshold ratio of episomes to protosomes*, which is 2:1. Accordingly, there can be no change in facet number until this ratio goes below the threshold value. For example, mutation from a gene with four episomes to five episomes would go undetected either by a count of the facets of the homozygotes or of their heterozygotes with the known allelomorphs. On the other hand, two allelomorphs, one with three episomes, and another with four episomes, while indistinguishable in the homozygous condition, should be capable of separation by their heterozygotes with full because the episome:protosome ratio of the first is below the threshold of manifestation and the other is not.

The postulation of apparently identical end-members of a series, which differ only by their complete or incomplete dominance over the allelomorph at the other extreme of the series, has been realized in the vestigial series in *Drosophila melanogaster* reported by BRIDGES and MORGAN (1919). Here the four members allelomorphic to wild-type, named in the order of increasing wing reduction, are nick, antlered, strap, and vestigial. The nick homozygotes are indistinguishable from the wild-type homozygotes on the basis of somatic appearance; however, they can be separated by their dominance over vestigial, since the normal allelomorph is completely dominant while nick gives an intermediate compound with vestigial very much like the sex-linked character "notch."

The dominance of bar over infrabar

Using STURTEVANT'S (1925) facet counts of the different combinations of genes at the bar locus, WRIGHT (1929) pointed out ". . . that infrabar is virtually indistinguishable in its effects from bar in all combinations, as long as there is at least one true bar gene present, that is, is almost or quite completely recessive to bar, . . ." This dominance relation, as well as other evidence already brought out, shows that bar and infrabar are qualitatively different and throws some light in such other allelomorphic series as the eye and ocelli color series in the wasp, *Habrobracon juglandis* (A. R. WHITING 1926), in which there are three genes allelomorphic to the completely dominant wild-type, with its jet black eyes and ocelli. These mutant allelomorphs are: light ocelli, in which the eyes are black and the ocelli somewhat lighter; orange eyes and ocelli, which vary ". . . from very light cream to a deep red and from yellowish orange to pink"; and ivory eyes and ocelli, which are greenish white. These allelomorphs not only cannot be arranged

in any quantitative series but show further heterogeneity in that each is completely dominant over the ones following in the order named. This epistatic behavior is similar to that of bar and infrabar and makes it likely that these three mutations represent the loss (or, less probably, the gain) of three different kinds of episomes.

Other manifestations of mutation at the bar locus

As a test of the structure of the gene pictured in this paper, a study was made of other quantitative character changes accompanying mutation at the bar locus. On the basis of facet number, we arrange full, bar, and ultrabar eyes in a quantitative series in the order named. If other quantitative character changes caused by the same mutations gave reason for arranging these three in a different order, it would, obviously, be a serious criticism of the theory since it would indicate different structures for identical genes. An example of such other manifestations of these mutations is the increase in vigor accompanying a full mutation in a bar stock, where the full-eyed flies tend to crowd out the bar flies in a few generations. Likewise, bar mutants appearing in ultrabar stock crowd out the latter, which in turn are also crowded out if a full mutation occurs. Conversely, ultrabar flies never crowd out bar flies. This crowding out effect is obviously due to a variety of physiological differences affecting such things as number of eggs, length of immature period, and vigor. In a series of experiments designed to determine the quantitative relations of these other manifestations, flies of all possible combinations of the bar series were produced under uniform conditions at 25°C. The stocks used were forked ultrabar, forked bar, and forked full derived from the forked ultrabar by reverse mutation. The results are summarized in the following paragraphs.

While facet number may be regarded as a combined expression of the length and breadth of the eye, the width of the head gives an expression of the depth of the eye. The average widths of the heads of one hundred each of the six kinds of females and the three kinds of males are : Females—full homozygotes, 873 microns; full-bar heterozygotes, 760 microns; bar homozygotes, 681 microns; full-ultrabar heterozygotes, 658 microns; bar-ultrabar heterozygotes, 640 microns; ultrabar homozygotes, 637 microns. Males —full, 814 microns; bar, 636 microns; ultrabar, 601 microns.

It was noticed that ultrabar flies usually lack the median ocellus while the two lateral ocelli are unaffected. The median ocellus was measured by estimating the ratio of its diameter to the diameter of the lateral ocelli. These ratios were thrown into five classes ranging from that for equal-sized median ocelli down to zero, representing the absence of median ocelli. Flies

of the intermediate types occasionally had a double-appearing median ocellus, which recalls the double origin of this ocellus in development. The average of the two diameters of such ocelli were used. The average ratio of one hundred each of the six kinds of females and the three kinds of males are: Females—full homozygotes, 0.985; full-bar heterozygotes, 0.920; bar homozygotes, 0.623; full-ultrabar heterozygotes, 0.522; bar-ultrabar heterozygotes, 0.360; ultrabar homozygotes, 0.048. Males—full, 0.960; bar, 0.598; ultrabar, 0.042.

In connection with this work on the median ocellus it was noticed that the ultrabars often had 10 or more ocellar bristles while the fulls usually had 7 or 8. The average ocellar bristle numbers of one hundred each of the above-mentioned material are: Females—full homozygotes, 7.50; full-bar heterozygotes, 7.56; bar homozygotes, 8.13; full-ultrabar heterozygotes, 7.92; bar-ultrabar heterozygotes, 8.53; ultrabar homozygotes, 8.74. Males —full, 7.17; bar, 7.41; ultrabar, 9.11.

The percentage of sterile females also varies in the allelomorphs of the bar locus. Data on hand from large numbers of single pair matings and uniform culture methods indicate that about 10 percent of the full females, 30 percent of the bar females, and 90 percent of the ultrabar females are completely sterile.

It appeared in a number of experiments that the ultrabars were hatching somewhat later than other kinds. An experiment was carried out to find the differences in the time of emergence of the different combinations of full, bar, and ultrabar in females. An excellent control of this determination was obtained by mating each of the three kinds of heterozygotes to the three kinds of males. Each of these nine different matings gave two kinds of females and two kinds of males which developed in the same bottle from the same parents. The flies were cultured in 8-dram vials containing 9 cc of banana agar food at 25°C. Eight pairs of parents were used in each mating and transferred at three-hour intervals until they had laid eggs in a series of thirty bottles. The time of emergence was recorded at three-hour intervals throughout the hatching period. The average time of emergence of the full females was 218 hours after egg-laying. The average differences in hatching time as determined by this method, and their probable errors, are given in the following tabulation based on 2,013 females. Each number represents the mean difference in hours between the time of emergence of the female listed at the top of each column as compared with the one listed to the left when the two were produced in the same culture bottle and from the same parents at 25°C.

	full / bar	bar / bar	full / ultrabar	bar / ultrabar	ultrabar / ultrabar
full / full	+0.30±1.50		+4.08±1.04		
full / bar		−0.34±1.43	+2.02±1.39	+1.81±1.21	
bar / bar				+2.64±1.37	
full / ultrabar				+4.54±1.01	+10.10±2.57

These other somatic manifestations of mutation at the bar locus agree with facet number in that the values for bar are intermediate between those for full and ultrabar. The similarity of the full-ultrabar heterozygote to homozygous bar is consistent, and unless other experiments prove it untrustworthy, it provides a useful tool in working out the structure of the gene in other quantitative series of multiple allelomorphs. A comparison of the effects of these genes on these somatic characteristics shows nothing inconsistent with the view that they may accomplish their effects through a single intermediate agent, perhaps an enzyme. More critical evidence on this point should be furnished by a comparison of the effects of temperature on these different manifestations.

Dominance

At this point in the discussion it seems obvious that dominance, *per se*, is a property of the somatic effects of genes but not of their structure, since it not only varies from one manifestation to another but also depends upon the point of view with which we approach a single manifestation. Not only are the threshold ratios of episome number to protosome number different for these different manifestations, but some manifestations occur *above* certain episome:protosome ratios while others occur *below* certain other ratios. For example, variations in the median ocellus, which are parallel to variations in facet number, occur where the threshold of manifestation is below a 2:1 ratio; while variations in the number of ocellar bristles, which have an inverse relation to facet number, occur above a 0:1 ratio.

TWO DIFFERENT BAR DEFICIENCIES

A comparison of two cases of mutational phenomena involving a change at the bar locus substantiates the conclusion reached earlier in this paper

that the wild-type eye, as well as reverted bar, is represented by a gene at the bar locus. In the first of these cases (BRIDGES 1917) the bar locus of the deficient X-chromosome behaves like a full gene, indicating the presence of a protosome. In the other case (MORGAN 1927) the bar locus of the deficient X-chromosome does not behave like an allelomorph; that is, no protosome is present, and females are haploid for the bar locus. Tests showed that the latter case did not involve adjoining loci in the X-chromosome. From the evidence presented, no other interpretation of this case seems possible but that it was a "real" deficiency and that both the protosome and the bar episome were lost. In this case, the deficiency and a bar gene gave females with bar eyes; in BRIDGES' case, the deficiency and a bar gene gave females with eyes typical of full-by-bar heterozygotes.

The deficiency reported by BRIDGES (1917) occurred in a germ cell of a white bar male. It also produced changes at the forked and bar loci. In effect, it changed a bar gene to a full gene, and the normal gene for the forked locus to a forked gene. Forked mutations and similar allelomorphs of forked (MOHR 1922) have repeatedly arisen from the wild-type gene, with no reverse mutations reported. Differential rates of mutation in the two directions are interpreted, by methods discussed in connection with the bar series, to mean that the wild-type gene at the forked locus consists of a protosome with episomes attached in side-chain arrangement. Forked mutations and forked allelomorphs arise by the loss of all, or a part, of these side-chains. Applying this same interpretation to BRIDGES' forked-bar deficiency, we find that both the forked and bar loci lost episomes very much as if the genetic chromosome had been shaved of its side-chains for a short distance.

The same explanation may be used for other regional mutations such as the notch-8 deficiency described by MOHR (1923) which involved the loci for white, notch, and abnormal abdomen.

THE MUTABLE GENES OF *DROSOPHILA VIRILIS*

The migration of the episome on the chromosome

DEMEREC (1926, 1926a, 1927, 1928, 1929, 1929a) has found genes at three loci in the X-chromosome of *Drosophila virilis* which exhibit a higher rate of mutation than is known elsewhere in the animal kingdom. These genes arose by mutation from the wild-type, probably as single occurrences, and they frequently revert to the wild-type gene, depending on certain genetic factors in other chromosomes, crossing over, developmental stages, and age of the female. Under certain conditions 100 percent mutation is

realized. Each of these mutable genes has produced non-mutating genes which are indistinguishable in other respects from the parent genes. In all these mutable genes, there are differential rates of mutation in the two directions, with the high rate toward the wild-type indicating that the few original mutants resulted from additions of an episome and that their reversion represents its loss. Mutable reddish (body color) was the first to be found (DEMEREC 1928). In an experiment involving reddish, mutable miniature (wing character) was found (DEMEREC 1926a). The origin of mutable magenta (eye color) is not certain, as it may have gone unnoticed for several generations (DEMEREC 1927). These three mutable genes have so much in common in their mutational behavior that they may be regarded as resulting from the *same episome* acting at three loci on the X-chromosome.

The greater mutability of newly formed genes

From the evidence of chromosome maps, as well as the rates of mutation of genes under so-called natural conditions, it is evident that most genes are stable. Furthermore, the very numerous lethal mutations and the several cases of multiple allelomorphs wherein the greater rate of mutation is away from the wild-type indicate that most genes have side-chains of episomes. These old established genes seem to have achieved a degree of stability and a nicety of balance not found in genes formed by the recent additions of episomes. This is well borne out by the mutable genes of *Drosophila virilis* and by those at the bar locus in *D. melanogaster*, all of which represent additions to the germ-plasm.

The migration of the episome on the protosome

DEMEREC (1928) has supplied pertinent evidence on the process by which a rapidly mutating gene becomes stabilized. Reddish-alpha, immediately after its origin, was crossed repeatedly to yellow (a stable allelomorph) and gave a decreasing yield of reversions to wild-type. The reversion in the second generation was 12.4 percent; in the third generation, 1.6 percent; fourth, 0.6 percent; fifth, 0.32 percent; sixth, 0.043 percent; and in the seventh generation there were no reversions. On the basis of our side-chain explanation of gene mutation, such evidence is interpreted to mean that there are certain positions on a protosome where an episome will give a more stable gene than other positions. If it be supposed that the "equatorial" position is most unstable and that an episome varies somewhat in its position from one cell generation to another, then episomes at the "equator" would be eliminated most rapidly and those nearer the

"poles" of the protosome would be in a more "protected" position. When DEMEREC saw that the mutation rate was dropping rapidly, he attempted to bring back the original high rate by selecting for mutability. In this he was partly successful, being able to maintain an average level of reversion amounting to 2.9 percent. It is understandable that he should not attain the original rate of reversion (12.4 percent), if we suppose that the "pressure" of mutability soon reaches an equilibrium with the "pressure" of selection when such high actual rates of mutation are involved.

The explanation of decreasing mutation rate by migration of the episome toward one, or both, of the poles of the protosome is further clarified by DEMEREC'S (1928) data. He found that almost half of the reddish-alpha reversions are crossovers between yellow (stable allelomorph of reddish-alpha) and scute, a gene 0.6 unit away from the reddish locus. No reversions were crossovers between yellow and sepia, which is 2.2 units on the other side of reddish. The remainder of the reversions were about equally distributed between non-crossovers and crossovers in other parts of the X-chromosome. It seems clear that the episome migrated toward the scute pole of the protosome but not toward the sepia pole.

DISCUSSION

Attraction and repulsion phenomena

Since homologous chromosomes lie side by side during synapsis, each gene attracting its homologue in the other chromosome, and since most genes are identical with their homologues in paired chromosomes, we may conclude that like genes attract each other. In "quantitative" series of multiple allelomorphs which are interpreted to be due to varying numbers of one kind of episome, the mutation rates indicate that like episomes unite to form side-chains. In the composite bar-infrabar and infarbar-bar genes, the two different kinds of episomes seem to repel each other and they take up positions near the opposite poles of the protosome as shown in figure 2. The unusually high mutability of the reddish-alpha gene in heterozygotes and not in homozygotes also indicates attraction and repulsion.

The symmetry of genes

Since the data assembled here indicate that the gene is a three-dimensionally differentiated entity, there are certain corollaries which cannot be altogether neglected. For example, the ordinary assumption that genes "split" during cell division seems rather too naïve. General considerations of growth and symmetry phenomena among living things make it more rational to regard a gene, in its rôle of autocatalyst, as "regenerating" a

homologue by its side. In the same way this "regenerated" gene may also be expected to be a mirror image of its progenitor. A number of interesting analogies may be drawn between such "dextro" and "laevo" genes and optically active organic substances, but their genetic applications seem too problematical to discuss further.

WILLSTÄTTER'S structure of enzymes

I am indebted to Doctor A. H. HERSH for pointing out that the structure of the gene developed in this paper resembles in several points the structure of enzymes as given by WILLSTÄTTER (1926), who says: "The 'molecule' of an enzyme appears to consist of a colloidal carrier and a purely chemically active group. The specificity of the enzyme is vested wholly in this active group, which can apparently be transferred from one colloidal carrier to another. It has not yet been possible to separate the enzyme molecule proper from the colloidal carrier without loss of activity." Genes probably produce their effects through, and are the parent substances of, enzymes, so that it is fitting that they should be similar in structure.

Other structural explanations of gene mutation

When MAY (1917) reported the occurrence of reverse mutations of bar to full, he postulated the following explanation:

"If the normal wild fly carries a limiting factor with respect to the facet number then it is possible by partial non-disjunction for the factor to pass from one chromosome of a pair to the other, giving one chromosome without a limiting factor and the other with a double limiting factor. The bar-eyed race of Drosophila may be derived from such a chromosome with two limiting factors or factor groups, the mate of the chromosome having been lost in the maturation of the egg. If then in the bar-eyed race a second non-disjunction again separates the two factors the result should be one chromosome with triple factors and one heterozygous female. If the former passes into the egg it should give rise to a further reduction in the facet number, but it is possible that a fly with such a chromosome does not live. It is possible also that the male with 34 facets contained a chromosome with a triple reducing factor."

According to the mathematical relations among the members of the bar series developed in the present paper, ultrabar fulfills the above prediction, but the evidence from mutation rates and other data indicates that bar does not represent the doubling of an inhibiting factor already present in the full gene but rather the addition of a new factor (episome).

CORRENS (1919), in explaining variegation in certain plants, postulated

a structure in which he conceived of the gene as a large molecule with varying numbers of the same chemical radical attached, thus producing varying amounts of green and white in variegated foliage. The gene for intermediate variegation was held to have an intermediate number of radicals, to which new ones might be added or old ones sloughed off to produce plants with larger or smaller green or white areas. The addition of the greatest possible number of these chemical radicals or the loss of all of them, he inferred, would give a self-green plant or a self-white plant, one or both of which should be constant. As long as the number of radicals was intermediate, selection would be effective in either direction until one or the other extreme was reached.

The large and complicated series of multiple allelomorphs in the cob and pericarp colors of maize has been investigated by many workers. In a study of the mutational phenomena accompanying variegation, EYSTER (1924, 1925) has concluded that these genes consist of varying numbers of pigment-producing and non-pigment-producing *gene elements.* In an intermediate color, such as orange pericarp, he conceives that mitosis may divide the two elements of the gene unequally between the two daughter genes, so as to produce adjacent areas of lighter and darker color. The side-chain explanation can equally well account for the same facts by the gain or loss of episomes between the two daughter genes.

A rather superficial examination of the data and material suggests an explanation that is alternative to both CORRENS' and EYSTER'S, namely, that there are varying numbers of one kind of episome determining the total amount of pigment present, and that another kind of episome causes the pigment present to be evenly distributed or, if it be lost, to be concentrated in certain areas. This latter phenomenon would not involve the unequal assortment of gene elements, but rather the critical concentrations of chromogenic substances unequally distributed during the ontogeny, very much as CONKLIN found among the organ-forming stuffs of Crepidula.

ACKNOWLEDGMENTS

This explanation of gene mutation was developed during 1923 and 1924 in the Zoological Laboratories of the UNIVERSITY OF ILLINOIS under the direction of Professor CHARLES ZELENY. Any contribution it may make to the literature of genetics is a reflection of the intimate association I have enjoyed with him during the past eleven years. I wish to thank him particularly for his stimulating criticism and for the repeated admonition that a hypothesis is valuable only in so far as it is capable of being tested.

Whatever clarity may attend the presentation of this paper is largely a result of the untiring efforts of my friend, Mr. H. CARL OESTERLING, editor for the ILLINOIS NATURAL HISTORY SURVEY.

I wish to thank Doctor C. N. WALL, of NORTH CENTRAL COLLEGE, and Doctor H. W. BAILEY, of the Department of Mathematics, UNIVERSITY OF ILLINOIS, for the calculation of the constants and aid in the application of the facet formula.

LITERATURE CITED

BRIDGES, CALVIN B., 1917 Deficiency. Genetics **2**: 445–465.

BRIDGES, C. B., and MORGAN, T. H., 1919 II. The second-chromosome group of mutant characters. Pub. Carnegie Instn. 278.

CORRENS, C., 1919 Vererbungsversuche mit buntblättrigen Sippen. I. *Capsella Bursa pastoris* albovariabilis und chlorina. Sitzungsberichte der Akademie der Wissenschaften zu Berlin. **33**: 585–610.

DEMEREC, M., 1926a Reddish—A frequently "mutating" character in *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **12**: 11–16.

1926b Minature—A second frequently mutating character in *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **12**: 687–690.

1927 Magenta-alpha—A third frequently mutating character in *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **13**: 249–253

1928 Mutable characters of *Drosophila virilis*. I. Reddish-alpha body character. Genetics **13**: 359–388.

1929a Genetic factors stimulating mutability of the miniature-gamma wing character of *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **15**: 834–839.

1929b Changes in the rate of mutability of the mutable miniature gene of *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **15**: 870–876.

DRIVER, ERNEST C., 1926 The temperature-effective period—the key to facet number in Drosophila. J. Exp. Zool. **46**: 317–332.

EYSTER, WILLIAM H., 1924 A genetic analysis of variegation. Genetics **9**: 372–404.

1925 Mosaic pericarp in maize. Genetics **10**: 179–196.

HANSON, FRANK BLAIR, 1928a The effect of X-rays in producing return gene mutations. Science **67**: 562–563.

1928b The effects of X-rays on productivity and the sex ratio in *Drosopila melanogaster*. Amer. Nat. **62**: 352–361.

1929 Duration of effects of X-rays on male germ cells in *Drosophila melanogaster*. Amer. Nat. **63**: 511–516.

HERSH, A. H., 1922 The effect of temperature upon the heterozygotes in the bar series of Drosophila. Thesis, Univ. Illinois Grad. School (not published).

1924 The effect of temperature upon the heterozygotes in the bar series of Drosophila. J. Exp. Zool. **39**: 55–71.

1929 The effect of different sections of the X-chromosome upon bar eye in *Drosophila melanogaster*. Amer. Nat. **63**: 378–382.

HERSH, ROSELLE KARRER, 1924, The effect of temperature upon the full-eyed race of Drosophila. J. Exp. Zool. **39**: 43–53.

KRAFKA, JOSEPH, JR., 1920 The effect of temperature upon facet number in the bar-eyed mutant of Drosophila. J. Gen. Physiol. **2**: 445–464.

LUCE, WILBUR M., 1926 The effect of temperature upon infrabar, an allelomorph of bar eye in Drosophila. J. Exp. Zool. **46**: 301–316.

MAY, H. G., 1917 Selection for higher and lower facet numbers in the bar-eyed race of Drosophila and the appearance of reverse mutations. Biol. Bull. **33**: 361–395.

MOHR, O. L., 1922 Cases of mimic mutations and secondary mutations in the X-chromosome of *Drosophila melanogaster*. Z. indukt. Abstamm.- u. VererbLehre., **28**: 1–22.

1923 A genetic and cytological analysis of a section deficiency involving four units of the X-chromosome in *Drosophila melanogaster*. Z. indukt. Abstamm.- u. VererbLehre. **32**: 108–233.

MORGAN, T. H., 1927 Exceptional classes of individuals in an experiment involving the bar locus of Drosophila. Hereditas **9**: (Festskrift för W. Johannsen) 1–9.

MULLER, H. J., 1920 Further changes in the white-eye series of Drosophila and their bearing on the manner of occurrence of mutation. J. Exp. Zool. **31**: 443–473.

STURTEVANT, A. H., 1925 The effects of unequal crossing over at the bar locus in Drosophila. Genetics **10**: 118–147.

1928 A further study of the so-called mutation at the bar locus of Drosophila. Genetics **13**: 401–409.

STURTEVANT, A. H., and MORGAN, T. H., 1923 Reverse mutation of the bar gene correlated with crossing over. Science **57**: 746–747.

THOMPSON, DAVID H., 1921 A new type of sex-linked lethal in Drosophila. Anat. Rec. **20**: 215.

1925 Evidence of structure in the gene. Amer. Nat. **59**: 91–94.

TICE, SABRA COLBY, 1914 A new sex-linked character in Drosophila. Biol. Bull. **26**: 221–230.

WHITING, ANNA R., and BURTON, RAYMOND H., 1926 Quadruple allelomorphs affecting eye-color in Habrobracon. Amer. Nat. **60**: 285–290

WILLSTÄTTER, R., 1926. Chemical progress. Chem. Soc. of London, Rep. Prog. Chem. 232–234.

WRIGHT, SEWALL, 1925 The factors of the albino series of guinea pigs and their effects on black and yellow pigmentation. Genetics **10**: 223–260.

1929 The dominance of bar over infra-bar in Drosophila. Amer. Nat. **63**: 479–480.

ZELENY, CHARLES, 1920a A change in the bar gene of *Drosophila melanogaster* involving further decrease in facet number and increase in dominance. J. Exp. Zool. **30**: 293–324.

1920b The tabulation of factorial values. Amer. Nat. **54**: 358–362.

1921 The direction and frequency of mutation in the bar-eye series of multiple allelomorphs of Drosophila. J. Exp. Zool. **34**: 203–233.

1922 The effect of selection for eye facet number in the white bar-eye race of *Drosophila melanogaster*. Genetics **7**: 1–115.