CYTOLOGIC STUDIES ON THE ABNORMAL DEVELOPMENT OF THE EGGS OF THE CLARET MUTANT TYPE OF DROSOPHILA SIMULANS

HELENA WALD

New York University, Washington Square College

Received November 30, 1935

INTRODUCTION

ALTHOUGH many instances of abnormal development of zygotes containing mutant genes have been described in the course of genetic studies on Drosophila, few attempts have been made to determine the mechanism of such development. Much of the material which has been studied has been poorly adapted to genetic analysis of this problem, since mutant genes usually produce developmental abnormalities so slight as to be difficult to identify, or so great as to produce complete inviability of the eggs containing such genes. The claret mutant gene of *Drosophila simulans* produces effects on the egg which are neither too great nor too small for study of this kind since some of these eggs develop into viable adults showing major abnormalities. For this reason genetic studies of the offspring of the claret mutant type have been completed, and have led to the suggestion of a probable mechanism for the production of the abnormalities found. A further study of this suggested mechanism may be made by the use of the cytologic method, basing such study on the genetic data at hand.

The writer wishes to express her thanks to Dr. ALFRED F. HUETTNER, under whose guidance the work was carried out, to Dr. RUTH B. HOWLAND for her assistance in the preparation of this report, and to Dr. ALFRED H. STURTEVANT for his kindness in reading and criticizing the manuscript.

REVIEW OF LITERATURE

In 1924, PLUNKETT found in a stock of *D. simulans* from Austin, Texas, a mutant fly with a claret eye color. Flies homozygous or heterozygous for this mutant gene were of good viability and normal in appearance, but homozygous females, on breeding, showed an additional abnormality. Although these females laid a normal number of eggs many of the eggs failed to develop, and those which did develop gave rise to adults only a few of which were normal. Females heterozygous for the mutant gene, and males both homozygous and heterozygous, produced the usual number of normal offspring. STURTEVANT and PLUNKETT, in 1926, located the claret mutant gene in the third linkage group, and found it to correspond to the claret mutant gene of *D. melanogaster*. The presence of the claret mutant

gene in *D. melanogaster* is not associated, however, with developmental abnormalities. STURTEVANT, in 1929, published the results of his genetic studies on the offspring of females homozygous for the claret gene. He described the kinds of abnormalities found among the viable offspring, and the frequency of occurrence of these abnormalities. He confirmed the observation of PLUNKETT that from the females it was possible to obtain only a very small number of viable eggs.

In addition to the high frequency of inviability, STURTEVANT found a second abnormality among the viable offspring of the claret females. This was the occurrence of flies whose sex-linked characters were unlike those expected from the matings made. Of the males produced by a cross, 50 percent were such exceptions, the sex-linked characters being apparently determined by the paternal rather than the maternal X chromosome. Of the females, 6 percent showed exceptional sex-linked characters. Such anomalous flies have been found in other stocks where they have been shown to be produced by failure of the X chromosomes to disjoin during the maturation of the egg. This process, however, would give rise to exceptional males and females in equal numbers rather than to such disproportionate numbers as STURTEVANT found. Similar disproportions of exceptional males to females had been observed earlier in *D. melanogaster* by BRIDGES (1916), SAFIR (1920), MAVOR (1924) and ANDERSON (1924) and in *D. simulans* by STURTEVANT (1921). These investigators suggested that the inequality between males and females might have been produced by occasional failure of the X chromosomes to reach either pole of the maturation figure, or by elimination of both maternal X chromosomes during the first cleavage. Either of these processes would produce only exceptional males while non-disjunction would produce both exceptional males and females. The fact that the inequality of males and females in the claret stock of *D. simulans* is greater than that found in other stocks was not explained by STURTEVANT.

A third type of abnormality found by STURTEVANT was the occurrence of flies having small bristles and frequent imperfections of the last section of the fourth vein of the wing. These "diminished" flies were shown by chromosome studies to be haplo-IV in constitution. Since no fourth chromosome mutant was available, it was not possible to determine whether the missing chromosome was that of the paternal or the maternal set.

In addition to these abnormal types, STURTEVANT found flies which gave evidence of similar abnormalities involving, however, only part of the tissues of the fly. This type of abnormality involved the X chromosome in 3 percent of the offspring, resulting in the production of gynandromorphs. The fourth chromosome was involved in 4 percent of the offspring, resulting in the production of "diminished mosaics" which were haplo-IV

in part of their tissues. The lack of a fourth chromosome mutant prevented further studies on the diminished mosaic flies.

From the complete studies of the sex-linked characters STURTEVANT found that two classes of gynandromorphs occurred among the offspring of the claret females. These classes may be described by a specific example. If a female, homozygous for the claret and yellow genes is crossed to a wild type male, the offspring are expected to be wild type females and yellow males. Exceptional yellow females and exceptional wild type males were also produced. The gynandromorphs produced by such a mating were of two classes. The first, including 90 percent of the gynandromorphs, consisted of wild type female tissue (phenotypically similar to the normal females produced by the mating) and wild type male tissue (similar to the exceptional males). The second class, including the remaining 10 percent, consisted of yellow female tissue (phenotypically similar to the exceptional females produced by the mating) and yellow male tissue, (similar to the normal males).

Additional observations which proved to be of importance in the formulation of a mechanical picture of the events causing the abnormalities of these eggs may be mentioned. In both the sex and fourth chromosome mosaics the abnormal tissues most often comprised $\frac{1}{2}$, $\frac{1}{4}$, or $\frac{1}{8}$ of the fly. More rarely fractions other than these were observed. Further, an abnormality of one linkage group in the whole or part of a fly was not necessarily accompanied by an abnormality of another linkage group. No major abnormality involving the second or third linkage groups was observed.

From these results, STURTEVANT set up the following hypothesis to account for the abnormalities found. Elimination of chromosomes may occur during the maturation divisions of the egg or during the early cleavages. Elimination of an X chromosome during maturation produces a female pronucleus deficient in its X chromosome, and fusion of this with a male pronucleus produces an XO male or an inviable YO zygote. (Exceptional females may be produced by a second process, non-disjunction). Elimination of a fourth chromosome during maturation produces a fly of haplo-IV constitution. If an X or a fourth chromosome is eliminated from one of the nuclei formed during the first cleavage, a fly is produced which is deficient for this chromosome in approximately half of its tissue. Elimination during later stages of cleavage produces flies which are abnormal in varying amounts, depending on the time of elimination of the chromosome. Elimination of a second or a third chromosome during maturation or the cleavage divisions produces an inviable zygote.

To account for the peculiar classes of gynandromorphs found, a further assumption was necessary. As indicated above 91 percent of the gynandromorphs had normal female and exceptional male tissue. If, according to

STURTEVANT'S hypothesis, the exceptional male tissues are produced by the elimination of an X chromosome during cleavage, it must, in these cases, be the maternal X which is eliminated. This process produces a fly in which the male parts are XO in constitution, the remaining X chromosome being that of the paternal set. The assumption may also explain the second class of gynandromorphs, which may be produced by the elimination of a maternal X during cleavage, if such a chromosome is eliminated in an egg which began its development as an exceptional (XXY) female. The female tissue then remains exceptional while the male tissue, having suffered the loss of one of the X chromosomes, becomes similar genetically to the normal males expected from the same mating.

In other reported cases of the occurrence of gynandromorphs, no evidence of the selective elimination of maternal chromosomes has been found. Apparent evidence of such elimination was reported by BRIDGES (1925) in Minute-n *D. melanogaster*. But here elimination was of this type because the mutant gene caused elimination of the chromosome which carried it. If it had been carried by the paternal chromosome, that chromosome would have been eliminated (STERN, 1927).

In the cytologic study of the eggs of homozygous claret females STURTEVANT'S hypothesis has been a valuable working guide, indicating as it does, the probable stages in development when abnormalities are likely to occur. However, if an attempt is made to apply the hypothesis as a mechanical picture of the possible cytologic events, the explanation becomes less simple than it appears at first. To picture a mechanism which will permit the loss, during cleavage, of chromosomes of the maternal set, and rarely or never, those of the paternal set, is difficult. For, with the completion of the first cleavage, the two sets of chromosomes lie together in close association. To drop those of one set selectively would require a peculiar and complex mechanism.

A new hypothesis for the production of gynandromorphs has therefore been set up on the basis of the cytologic data. This hypothesis, to be presented below, fits the genetic data, and in addition lends itself to the postulation of a simple cytologic mechanism for producing the abnormalities. Further, an attempt will be made to establish the validity of STURTEVANT'S suggestions that chromosomes may be excluded from the female pronucleus as a result of the peculiarities of the first maturation division, and that this process, when it involves the second or third chromosomes results in failure of the egg to develop.

MATERIAL AND METHODS

To secure the eggs for study, a stock was used from which both normal and abnormal material could be selected. Since homozygous claret females

produce few viable eggs, the stock was maintained by mating heterozygous females with males homozygous for the mutant. For the study of the abnormal eggs, matings were made between homozygous claret females and heterozygous males. For control material, to show the normal course of development of *simulans* eggs, heterozygous females were mated to homozygous claret males. The stocks used were also homozygous for the third chromosome recessive, scarlet. This mutant gene had no appreciable effect on the viability of either the homozygous or heterozygous claret flies.

Flies were segregated as they emerged and fed on banana-agar mixture for five days before mating. Females were starved for several hours before collection of eggs was to begin, and permitted to mate. The wings of the females were cut off under ether to permit the posterior end of the abdomen to be observed during laying. For the collection of eggs such flies were placed in vials containing a glass slide on which was placed a piece of dark blotting paper moistened with fermented banana and yeast. During the period of laying these flies were kept at a temperature of 25.5 to 26.5° C.

To obtain abnormal and control eggs of comparable ages it was necessary to determine for each egg used the time interval between fertilization and fixation. In flies from which the wings have been removed, it is possible to see, after an egg has been laid, several protrusions of the uterus before the laying of the next egg. In the first few of these protrusions, the uterus appears yellow, translucent, and pointed at the posterior end. After several such protrusions the uterus, during one of them, becomes wider, and after another slight movement, becomes opaque, blunt at the end, and presents a shiny white tip. This tip is the posterior end of the egg, which has reached the end of the uterus. The anterior end of the egg is now in position to receive sperm. Fertilization was therefore assumed to occur when the white tip appeared at the end of the uterus, and timing of the eggs was begun at that moment. Slides made from control eggs timed in this manner showed similar stages of development when allowed to develop for similar lengths of time, justifying the timing procedure used.

The position of each egg on the blotting paper and the time of fertilization was recorded. After the desired time interval had elapsed, the slide was removed from the vial. The eggs were transferred to Kahle's formol-alcohol-acetic fixative and punctured at once with a sharpened steel needle (HUETTNER, 1923). To avoid distortion of the maturation figure which is found on the dorsal surface of the egg near the anterior end, eggs were punctured on the ventral surface, posteriorly. The puncture, when successful, permitted only a minute globule or a thin strand of oöplasm to escape.

Eggs were stained with erythrosin in 85 percent alcohol to facilitate orientation in paraffin under the binocular microscope. Sections were cut sagittally, 7μ in thickness and stained with Heidenhain's iron haematoxylin.

DESCRIPTION OF CYTOLOGIC PREPARATIONS

1. Control stock—development of normal eggs of D. simulans

The development of the normal control eggs follows closely that described by HUETTNER (1924) for *D. melanogaster*. With one exception the chromosomes of the two species are similar. The Y chromosome of *D. melanogaster* consists of one long and one short arm. The short arm is absent in the Y chromosome of *D. simulans*. The earliest fertilized eggs obtained were in the anaphase of the first maturation division. This figure lies, as in *D. melanogaster*, at the periphery of the egg, in a small, yolk free, cytoplasmic island on the dorsal surface near the bases of the filaments of the chorion. In eggs fixed 5 minutes after fertilization the chromosomes lie at the poles of the first maturation spindle (figure 1). No reorganized nuclei are seen at the completion of this period, the chromosomes lining up immediately on the spindle for the second maturation division. Stages of this division are found in eggs fixed between 5 and 10 minutes after fertilization (figure 2). Eggs which have developed for 11 minutes (figure 4), show the four reorganizing polar nuclei, three of which, as is true in *D. melanogaster*, remain in the cytoplasm at the periphery of the egg. One of the four nuclei lies nearer the center of the egg, where the sperm, not shown in this figure, is undergoing reorganization.

Preparations of eggs fixed during the next few minutes do not permit of identification of the reorganized egg nuclei which at this time stain poorly, and are again visible as clear vesicles only in preparations of eggs which have been permitted to develop for 15 minutes. Eggs prepared during the next 5 minutes show these four nuclei, each containing a haploid set of chromosomes. One of these vesicles, the female pronucleus, lies near the center of the egg, in the protoplasmic island containing the male pronucleus, wherein the haploid set of chromosomes is also visible. Material fixed from 20 to 23 minutes after fertilization shows the polar chromosomes lying free in the peripheral cytoplasm in three haploid groups, the nuclear membranes having disappeared. At this stage, two spindles are seen in association with the chromosomes of the male and female pronuclei. Figure 4 shows a portion of such a preparation, in which the three haploid groups of polar chromosomes may be seen. The cleavage figure present near the center of the egg is not shown. During later cleavages the two spindles characteristic of the first cleavage are no longer to be seen, the

maternal and paternal chromosomes being closely associated on a single spindle. Such late cleavages further indicate the persistence of the polar chromosomes or nuclei in the peripheral cytoplasm.

2. Homozygous claret stock—development of abnormal eggs of D. simulans

a. First maturation division. As in the control preparations, the abnormal eggs immediately after fertilization are in the anaphase of the first maturation division. The figures at this stage are distinctly abnormal, showing one general type of abnormality; namely, separation of the chromosomes at either end of an abnormally wide maturation spindle. Figure 5 shows such an abnormal figure. The chromosomes are greatly separated at one pole, less so at the other. Other preparations show modifications of this type of abnormality, the extreme being a separation of the chromosomes into several widely scattered groups as seen in figure 12. The spindles in these figures are correspondingly distorted.

b. Second maturation division. The abnormal eggs again differ from the control eggs at the time of the second maturation division. In the control stock, stages of the second maturation division are found in eggs prepared from 6 to 10 minutes after fertilization. Those of the abnormal stock of comparable age seldom show a second maturation division in progress, the chromosomes lying as they did at the end of the first maturation division. In some preparations the double nature of these chromosomes is already evident. During the next 5 minutes of development preparations show no evidence of the polar chromosomes, which lose their staining capacity as do those of the control preparations at the conclusion of the period of the second maturation division.

Fixation 15 minutes after fertilization discloses the presence of the vesicular nuclei formed by the reorganization of the chromosomes at the end of the maturation divisions. In contrast to similar preparations of control eggs, where four such nuclei are seen, the abnormal egg shows the presence of four to twelve reorganized nuclei.

c. Cleavage. In material fixed during the following period (20 to 30 minutes after fertilization) the chromosomes within the polar nuclei and pronuclei are visible. The chromosomes are long and thin, twisting about each other, thus making identification of the fourth chromosomes, the smallest of the set, impossible. The other chromosomes, however, are readily recognized. Where more than four of these egg nuclei are present, each contains less than the haploid number of chromosomes. The extreme of this condition is seen in those preparations where twelve nuclei are present, each containing but a single chromosome (figure 6).

The pronuclei in preparations of this age again show differences from those of the control preparations, where each of the pronuclei, lying side by side, showed the presence of its own spindle. In the abnormal stock

the male pronucleus occupies its normal position and contains a normal spindle. The female pronucleus may be abnormal in position and in spindle formation. One or several of the nuclei produced by the maturation divisions may lie in association with the male pronucleus, but in many cases all these egg nuclei lie at the periphery of the egg so that no one of them may be identified as a female pronucleus. Spindles are rarely seen in association with any of these egg nuclei. When the total number of such nuclei is twelve, no spindle is ever seen in association with the female pronucleus, even when this is normal in position. Many preparations with fewer nuclei also lack the spindle of the female pronucleus. Figures 7 and 8 show two unusual preparations in which spindles are present in both pronuclei. In each of these, the female pronucleus contains both the second and third chromosomes, figure 7 indicating further a lack of an X chromosome and figure 8 the presence of an extra X chromosome. In no preparation was the female spindle present when the female pronucleus lacked a second or a third chromosome.

The majority of the preparations at hand show no cleavage figure. Eggs fixed during the next few minutes may still show the presence of the sperm spindle, but this is lost in older preparations, the male pronucleus appearing again as an interkinetic, lightly staining vesicle. Still later preparations, where second and third cleavages figures are expected, show instead the picture of nuclear degeneration, the pronuclei and polar nuclei being irregular in outline and lightly stained. An increased number of polar nuclei is visible at this time, the number of nuclei approximately doubling as the period for each unrealized cleavage passes. Spindles are not seen in association with this nuclear increase.

Those preparations which show evidence of normal cleavages following the first, still show the presence of polar chromosomes whose nuclear membranes have disappeared. These polar chromosomes lie scattered throughout the cytoplasm between the periphery of the egg and the centrally located protoplasmic islands containing the cleavage nuclei (figure 9). As in the more normal preparations, the number of these chromosomes increases at each cleavage.

d. Unfertilized eggs. It is important to determine the time of development at which the first sign of abnormality appears in the eggs of the claret females. Preparations were therefore made of eggs dissected from females before fertilization or laying. Figure 10 shows the earliest of these, in which the prophase of the first maturation division is seen. Figure 11 shows a later prophase figure. Both these preparations show no sign of the abnormality to follow. Figure 12 shows the first visible sign of the distortion of the maturation figure, groups of chromosomes being widely separated in the cytoplasm rather than in the single compact group characteristic of the control preparations at this stage of development.

THEORETICAL DISCUSSION

In the discussion to follow, consideration of the fourth chromosome will be omitted. This chromosome is small and is identified in normal eggs by its position in close association with the other chromosomes. In the abnormal preparations where the chromosomes are scattered, this relation is lost, and it becomes impossible to differentiate the small chromosome from nearby yolk granules of the same size.

The first abnormality which appears in the development of the eggs of the homozygous claret females is the distortion of the first maturation spindle and the separation of the chromosomes at its poles. The subsequent abnormalites of development may be explained as results of this initial abnormality. To carry out this explanation each of the abnormalites will be reviewed in the light of those which took place in the preceding stages of development.

The picture presented at the time of the second maturation division may be considered to consist of two distinct abnormalities. The first of these is the failure of the second maturation to occur in orderly fashion in most eggs. Spindles for the separation of the chromosomes at this time are rarely seen. During this period the chromosomes lie as they did at the conclusion of the first maturation division, three long chromosomes being visible at each pole of the spindle. The small fourth chromosome is difficult to identify. These six chromosomes disappear during the next few minutes, and on their reappearance are found to have increased to twelve. Thus the chromosomes have doubled during the period of the second maturation division, but this division was an abnormal one, unaccompanied by any spindle.

The second abnormality of this period becomes evident at the conclusion of the second maturation period, and concerns the formation of an abnormal number of polar nuclei, each containing fewer chromosomes than the haploid set. The chromosomes at one end or at both ends of the distorted first maturation spindle are often widely separated from each other. No immediate effect of this is visible since no nuclear reorganization follows this division. However, when nuclear reorganization does occur, at the completion of the period of the second maturation division, it is apparent that the widely separated chromosomes have not been able to enter in normal fashion into the organization of these nuclei. For, instead of forming a compact nucleus containing all the chromosomes at one pole of the spindle, these chromosomes, scattered widely from each other, form instead, separate nuclei, each containing only a single chromosome. If each of the four chromosomes in each of four haploid sets found at the end of the two maturation divisions were thus to form its own nucleus, sixteen nuclei would be found. Such is not the case, however, the largest number at this time being twelve. The small fourth chromosome does not form

its own nucleus, either remaining with one of the others or, if separated, failing to form a membrane about itself. This point could not be determined because of the difficulty of following the fate of the fourth chromosome.

The phenomenon of the formation of nuclei by single chromosomes has been called "idiomerie" by HAECKER and other workers, and occurs in both normal and experimentally treated tissues. POLITZER (1934) has reviewed the early work in this field. STRASBURGER (1880) and HAECKER (1895) described the formation, during normal mitosis of independent vesicles formed by each independent chromosome, these blending to form the typical single interkinetic nucleus. SCHILLER (1909) and TOBIAS (1914) were able to accentuate this slight degree of normal idiomerie by treatment of the eggs of Cyclops at high temperature. In all these cases the several vesicles fused finally to produce a normal interkinetic nucleus. ALBERTI and POLITZER (1934) so accentuated idiomerie by treatment with X-rays that the independent vesicles failed to fuse. It is this type of idiomerie that is found to occur in the untreated eggs of the claret mutant females under consideration.

The varying number of polar nuclei found in the abnormal eggs ready for first cleavage is the result of varying degrees of abnormality of the first maturation division. Complete separation of all the chromosomes at this time, with the exception of the fourth chromosome which apparently does not act independently, would produce twelve nuclei. Other variations in the number of these nuclei may be produced by partial scattering of the chromosomes of the first maturation division, separating two chromosomes from a third, or separating only those at one pole as in figure 5.

The next abnormality to be seen is the failure of cleavage in the greater number of eggs studied at this stage. Cleavage fails in several types of eggs. Such eggs include those in which twelve nuclei are present after the maturation divisions, none of these nuclei ever showing the formation of a maternal cleavage spindle. Also, eggs in which these nuclei are more nearly normal in number often fail to cleave. In the rare cases in which cleavage does occur, the female pronucleus is always found to contain at least the second and third chromosomes, in accordance with the genetic data presented by STURTEVANT.

We may further compare those chromosome groupings in the female pronucleus which permit of normal cleavage with others which do not. Figures 7 and 8 show two eggs in which the first cleavage is in progress, each of these eggs containing the diploid set of second and third chromosomes. Figure 7 indicates further a lack of an X chromosome in the female pronucleus and figure 8 the presence of an extra X chromosome, lying in its vesicle just outside the female pronucleus. Despite the X chromosome irregularities, cleavage is in progress. Figure 6, on the other hand,

shows an egg which has failed to cleave. Surrounding the normal male pronucleus are three of the twelve nuclei produced by the maturation divisions, these containing one of the second, third and X chromosomes. These three chromosomes, when associated with the male pronucleus in other eggs, were sufficient to permit of normal cleavage. Here, where the necessary chromosomes lie in separate nuclei, cleavage has not taken place. It is thus apparent that in order for normal cleavage to occur, the second and third chromosomes must reach the male pronucleus and that they must do so enclosed within a single nucleus. If they do not lie in a single nuclear membrane, no spindle is formed for their division, and cleavage fails. Figure 8 illustrates this further. Here a single female pronucleus containing the second, third and X chromosomes is functioning in cleavage. But an extra nucleus (one formed by the maturation divisions), lying above the female pronucleus and containing a single X chromosome, has formed no spindle and has been unable to enter the cleavage figure up to this time.

The first abnormality observed, that of the first maturation division, is thus again responsible for the abnormality found at this time. Moreover, this early abnormality has at last achieved the result of stopping any further development of the egg, by causing, in most cases, the separation of the second and third chromosomes. Cleavage could take place only when these chromosomes remained sufficiently close at the end of the maturation division to become incorporated into a single nucleus. Cleavage could include the other chromosomes only when these remained sufficiently close to the second and third to be included with them in a single nucleus. Further, cleavage failure in these cases is seen to be characterized by the failure of spindle formation in the female pronucleus, for at this stage no spindle is ever formed by a female pronucleus deficient in the second or third chromosomes.

This observation lends itself to orderly interpretation if we assume that an interaction between the second and third chromosomes, or portions of them, is necessary in order that a spindle be produced. Re-examination of the abnormal maturation figures shows the accordance of these with the assumption made. For here, at the first maturation division, the second and third chromosomes are often separated from each other, relatively few eggs developing normally at this time. These abnormal eggs, if the assumption is correct, should now fail to form a spindle for the second maturation division. This is found to be the case.

The assumption that no spindle is ever formed unless the second and third chromosomes are in close proximity leads to the corollary that the presence of a spindle at any time during development must have been preceded immediately by a close proximity of these chromosomes. How-

ever distorted the spindle may be, then, the preceding prophase must have been normal, containing in a single nucleus all the chromosomes to appear on the spindle at the succeeding metaphase. This test of the validity of the assumption made must be applied to the first maturation division, the last appearance of spindle formation in the eggs that are completely abnormal. This spindle, if the assumption is valid, must be preceded by a prophase in which all the chromosomes lie in a single nucleus, surrounded by a nuclear membrane. This is found to be true. No preparations have been found in which the nucleus during the prophase of the first maturation division was abnormal. In every case the germinal vesicle was single, containing within it all the chromosomes. Figure 10 shows the single germinal vesicle of an egg before laying. Figure 11 shows the nucleus of a later stage of development of an unlaid egg, in which the nuclear membrane has just broken down. Again the chromosomes are found in a single compact group. Figure 12 shows the anaphase of the first maturation division. The spindle is completely formed, and for the first time the chromosomes are seen to be widely separated from each other in the cytoplasm. In such an egg, where the second and third chromosomes no longer lie together, no orderly mitotic figures can again be formed and the egg can never develop into an adult. Thus at no stage of development have figures been found which will invalidate the assumption that the second and third chromosomes must be in close proximity for spindle formation to take place.

STURTEVANT'S hypothesis for the production of gynandromorphs and fourth chromosome mosaics suggests that a chromosome may be dropped from one pole of the cleavage figure. To determine whether this process ever occurs or not is difficult, since the formation of gynandromorphs is rare. The fact that no evidence of the elimination of chromosomes has been found cytologically is thus no proof that this process never occurs.

The cytologic preparations studied, however, indicate that gynandromorphs may be formed by a process simpler than that which STURTEVANT suggests; namely, by the addition of a chromosome to a deficient nucleus. This new hypothesis accounts as well as does STURTEVANT'S for the two types of gynandromorphs which he found. Figure 7 shows the possibility of formation of the first class of gynandromorph, that having exceptional male and normal female tissue. The figure shows a preparation in which first cleavage is in progress. The diploid set of chromosomes lacks a single X chromosome, which was excluded from the female pronucleus by the separation of the chromosomes at the first maturation division. Near the upper pole of the figure, beyond the centriole, a single X chromosome is seen, lying free in the cytoplasm. This chromosome must be one that had been eliminated during a previous division, since it does not lie in a posi-

tion indicating its elimination during the division in progress. Since this is the first cleavage the extra chromosome can only have been eliminated during the maturation divisions of the egg. It is therefore derived from one of the abnormal polar nuclei formed at that time. Further, this chromosome lies in what may become the path of the upper set of cleavage chromosomes when the division progresses. If, during this or later cleavages, the chromosomes at one pole of the cleavage figure lie close to the region occupied by the extra chromosome, the latter may become incorporated within that nearby cleavage nucleus. That one cleavage nucleus would then be enriched by this chromosome to contain the diploid set of X chromosomes, the remaining nuclei still being deficient for this chromosome and containing only the single X chromosome present before cleavage began. The deficient nucleus would thus lack a maternal X chromosome, missing since the formation of the female pronucleus. A gynandromorph would result in which the female parts, derived from the enriched nucleus are normal, and the male parts carrying a single X chromosome derived from the paternal set, are exceptional. This is the type of gynandromorph which was found by STURTEVANT to make up over 90 percent of those studied.

The egg pictured in figure 8 shows the possibility of formation of the second class of gynandromorphs. Again first cleavage is in progress but in this case the female pronucleus is complete. Above the complete maternal group of chromosomes is a vesicle containing an extra X chromosome which is not at this time entering into the cleavage. During cleavage the nuclear membrane surrounding this chromosome breaks down, setting the chromosome free in the cytoplasm as are those shown in figure 7. The extra chromosome may then pass to one pole of the cleavage figure, there to enrich one of the cleavage nuclei. If the egg pictured began its development as a normal male, the enriched nucleus now becomes XXY in chromosomal constitution, the other nucleus remaining XY as before the cleavage. A gynandromorph is thus produced in which the female tissues, derived from the enriched nucleus are exceptional female in constitution, and male tissues, derived from the unenriched XY nucleus are normal male. Genetically the result is the same as that obtained by STURTEVANT'S method, in which he suggests the formation of this type of gynandromorph by the elimination of an X chromosome from an egg which began its development as an exceptional female.

That chromosomes may be picked up during later cleavages is indicated by figure 9, in which, during the third cleavage, polar chromosomes are still seen to persist in the cytoplasm. Gradual degeneration of these chromosomes accounts for the fact that few gynandromorphs are found in which very small mosaic patches occur.

Such chromosome additions at later cleavages must be discussed more

fully than the simpler first cleavage additions which produce gynandromorphs that are half male and half female. The addition of chromosomes at later cleavages produces gynandromorphs that are more than half female or less than half female. Those that are more than half female are produced when a chromosome is added at a later cleavage to an egg that has already undergone addition at first cleavage. Those that are less than half female are produced by additions of chromosomes at later cleavages to eggs that have undergone no previous additions. STURTEVANT (1929) found that 45 percent of all the gynandromorphs are more than half female while only 15 percent are less than half female. To fit the addition scheme to these facts it is necessary to show that chromosome additions occur so frequently at first cleavage, and so frequently again at later cleavages, that 45 percent of the eggs are likely to suffer at least two successive additions. Estimates of the number of eggs affected by abnormality at each cleavage, if the elimination hypothesis is used as the basis for such estimates, do not lend themselves to the support of the addition scheme, as indicated by Dr. STURTEVANT in a personal communication to the writer. However, estimates based on the elimination hypothesis may not be applied directly to the addition scheme.

The error in such a direct application may be illustrated as follows. Cleavage abnormality, if that abnormality is elimination, produces male tissue. The extent of male tissue in the adult indicates, therefore, the developmental stage at which this specific abnormality occurred. Each gynandromorph which is one half or more than one half male has thus developed from an egg in which elimination occurred at first cleavage, or successively at first and later cleavages. This group of gynandromorphs includes 55 percent of the total, and indicates the frequency with which first cleavage elimination occurs. Addition of chromosomes, on the other hand, produces female tissue, and the extent of female tissue in the adult indicates the developmental stage at which this particular abnormality occurred. Here, each gynandromorph that is one half or more than one half female has developed from an egg in which addition of chromosomes occurred at first cleavage, or successively at first and at later cleavages. This group of gynandromorphs includes 85 percent of the total, and indicates the frequency with which first cleavage addition occurs. Such estimates can thus be made only when a specific mechanism is under consideration, and the estimates based on one mechanism do not apply when the other mechanism is considered.

Similarly, second cleavage elimination is characterized by gynandromorphs that are one quarter, three quarters or more than three quarters male. These total 35 percent. Second cleavage addition, however, is characterized by gynandromorphs that are one quarter, three quarters or more than three quarters female. These total approximately 53 percent.

Thus the elimination scheme implies that 55 percent of the eggs become abnormal at first cleavage and 35 percent at second cleavage. On this basis it is to be expected that 19 percent (55 percent×35 percent) of the gynandromorphs will bear evidence of successive elimination of chromosomes at both first and second cleavage. STURTEVANT'S counts of such gynandromorphs, characterized by the elimination scheme as being more than one half male, number 15 percent of the total. The addition scheme, however, leads to the expectation of 45 percent (85 percent×53 percent) of gynandromorphs bearing evidence of successive addition of chromosomes at first and second cleavage. Counts of such gynandromorphs, characterized by the addition scheme as being more than one half female, number 45 percent of the total. The actual percentages here are not significant, because of the necessarily rough estimates of each class of gynandromorph made by STURTEVANT. They indicate, however, that either scheme may be used to explain the relatively large amount of female tissue found in the gynandromorphs.

The addition scheme may thus explain the formation of gynandromorphs and account for two peculiarities shown by them, namely, the absence of the maternal chromosome in over 90 percent of the gynandromorphs, and the fact that generally half or less than half of tissues of each of these gynandromorphs lack this chromosome. While the addition scheme presents no advantage over the elimination scheme in explaining the genetic data, it presents the advantage of a mechanical basis for the fact that it is always a maternal chromosome, derived from the egg, that is lacking in the deficient tissue. As the direct result of the abnormality of the first maturation division, this chromosome failed to be included in the female pronucleus and was thus lacking before cleavage began. When no additional chromosome is picked up by one of the cleavage nuclei the entire fly is deficient for this chromosome. When one of the nuclei produced by the first cleavage is enriched, only half of the tissue remains deficient for the maternal chromosome. A similar process during later cleavages will produce varying amounts of male and female tissue. The gradual degeneration of the extra polar chromosomes will reduce the frequency of this process in older eggs, making less likely the formation of gynandromorphs with small patches of mosaic tissue.

SUMMARY

1. Cytologic preparations of the eggs of the claret mutant type of *Drosophila simulans* are described.

2. The initial abnormality in these eggs is the separation of the chromosomes at each pole of the distorted spindle of the first maturation division.

3. The second abnormality is the failure of the second maturation

division in most eggs, and the reorganization of the chromosome groups at the end of this period, into an abnormally large number of nuclei, each containing an abnormally small number of chromosomes.

4. The third abnormality is the failure of cleavage in most eggs. This is correlated with the failure of a maternal spindle to appear in the absence of the second or third chromosomes from the female pronucleus.

5. These events are explained in the light of the abnormality of the first maturation division.

6. A new hypothesis is presented to account for the formation of gynandromorphs in this stock. The hypothesis is based on the cytologic data presented.

LITERATURE CITED

ALBERTI, W. and G. POLITZER, 1924 Über den Einfluss der Roentgenstrahlen auf die Zellteilung. II Mitteilung. Arch. Mikr. Anat. **103**: 284–307.

ANDERSON, E. G., 1924 X-rays and the frequency of non-disjunction in Drosophila. Pap. Mich. Acad. Sci. **4**: 523–526.

BRIDGES, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics **1**: 1–52, 107–163.

1925 Elimination of chromosomes due to a mutant (Minute-n) in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **11**: 701–706.

HAECKER, V., 1895 Über die Sebständigkeit der väterlichen und mütterlichen Kernbestandteile während der Embryonalentwicklung von Cyclops. Arch. Mikr. Anat. **46**: 579–618.

HUETTNER, A. F., 1923 The Origin of germ cells in *Drosophila melanogaster*. J. Morph. **37**: 385–423.

1924 Maturation and fertilization in *Drosophila melanogaster*. J. Morph. **39**: 249–265.

MAVOR, J. W., 1924 The production of non-disjunction by X-rays. J. Exp. Zool. **39**: 381–432.

MORGAN, T. H. and C. B. BRIDGES, 1919 The origin of gynandromorphs. Contributions to the genetics of *Drosophila melanogaster*. Pub. Carnegie Instn. **278**: 1–122.

POLITZER, G., 1934 Pathologie der Mitose. Protoplasma Monographien, Bd. VII. Berlin, Gebrüder Borntraeger.

SAFIR, S. R., 1920 Genetic and cytological examination of the phenomena of primary non-disjunction in *Drosophila melanogaster*. Genetics **5**: 459–487.

SCHILLER, I., 1909 Über künstliche Erzeugung "primitiver" Kernteilungsformen bei Cyclops. Arch. Entw. Mech. Org. **27**: 560–609.

STERN, C., 1927 Über Chromosomenelimination bei der Taufliege. Naturwiss. **15**: 742–748.

STRASBURGER, E., 1880 Zellbildung und Zellteilung. Jena, G. Fischer.

STURTEVANT, A. H., 1921 Genetic studies on *Drosophila simulans*, II. Sex-linked group of genes. Genetics **6**: 43–64.

1929 The claret mutant type of *Drosophila simulans*. Z. wiss. Zool. **135**: 323–356.

STURTEVANT, A. H., and C. R. PLUNKETT, 1926 Sequence of corresponding third-chromosome genes in *Drosophila melanogaster* and *Drosophila simulans*. Biol. Bull. **50**: 56–60.

TOBIAS, A., 1914 Über den Einfluss erhöhter Temperatur auf den Kernteilungsmodus von Cyclops. Arch. mikr. Anat. **84**: 369–429.

EXPLANATION OF FIGURES

The figures were drawn with a Leitz 10× ocular and a Leitz oil immersion objective, N A 1.30, at table level with the aid of an Abbe camera lucida. A magnification of about 2540× was obtained. The figures have been reduced to one half in reproduction.

FIGURE 1.—Control stock. First maturation division. Five minutes after fertilization.

FIGURE 2.—Control stock. Second maturation division. The chromosomes at the extreme left will form the female pronucleus. Ten minutes after fertilization.

FIGURE 3.—Control stock. Reorganization of the four egg nuclei following the second maturation division. The nucleus at the left will form the female pronucleus. Eleven minutes after fertilization.

FIGURE 4.—Control stock. Three polar nuclei remaining at the periphery of the egg during cleavage. Twenty minutes after fertilization.

FIGURE 5.—Abnormal stock. First maturation division. The spindle is distorted and the chromosomes scattered at its poles. Five minutes after fertilization.

FIGURE 6.—Abnormal stock. Period of first cleavage. Twenty minutes after fertilization.

(a) Nine reorganized egg nuclei remaining at the periphery. Each contains a single chromosome. In the preparation these appeared in seven consecutive microscopic sections.

(b) Male pronucleus and three reorganized egg nuclei, that at the left containing the X chromosome, those at the right the second and third chromosomes. Figure reconstructed from two consecutive microscopic sections, one containing the male pronucleus and the autosome at the lower right, the other containing the X chromosome and the autosome at the upper right.

(c) Diagrammatic reconstruction of the above. The group of nuclei at the periphery at the left are the nine nuclei shown in (a), the central group the pronuclei shown in (b).

FIGURE 7.—Abnormal stock. First cleavage. Above the upper centriole is an extra X chromosome lying free in the cytoplasm. Twenty minutes after fertilization.

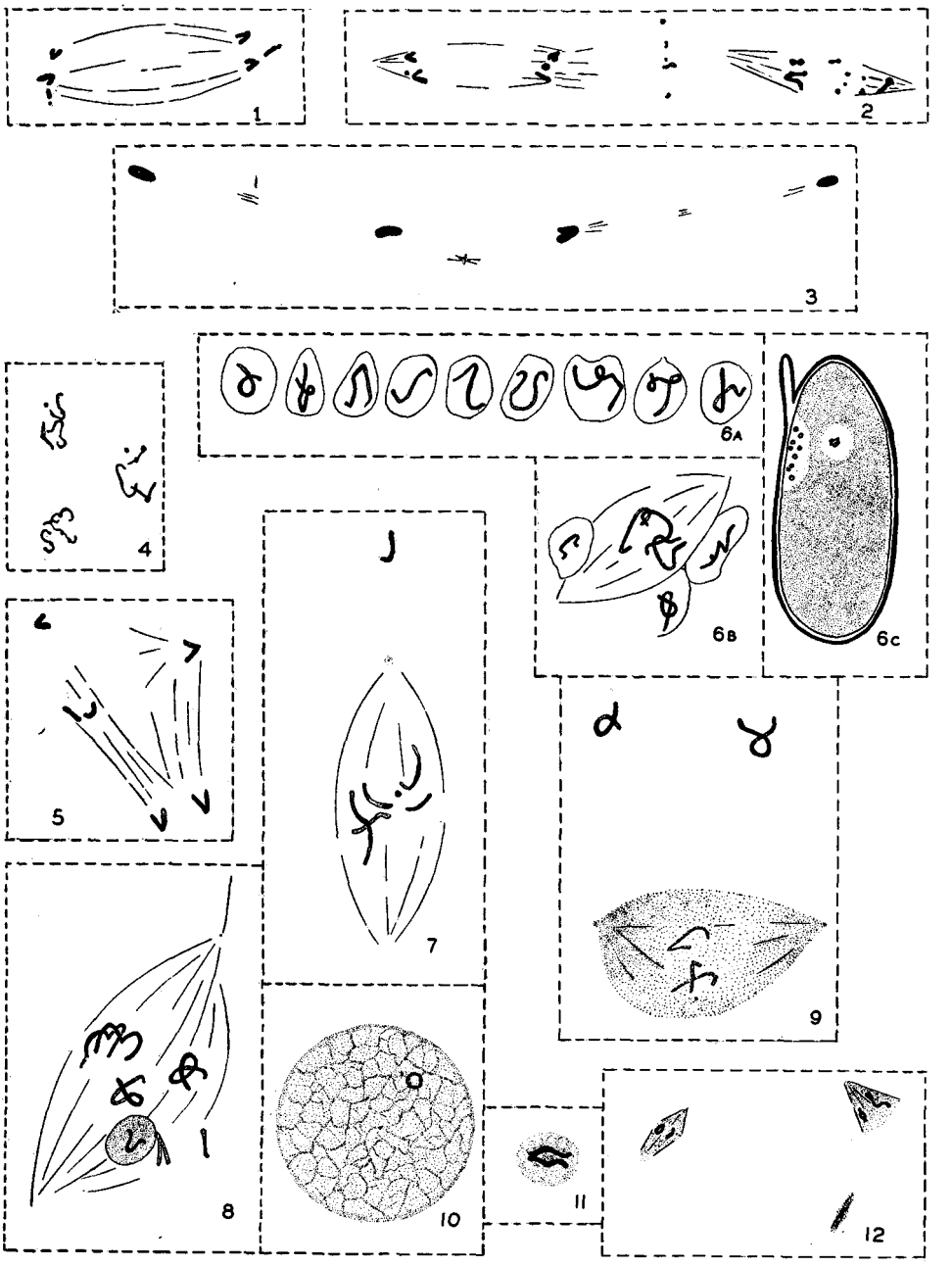
FIGURE 8.—Abnormal stock. First cleavage. The figure shows the paternal chromosomes at the left and the maternal at the right, these constituting a full haploid set. An extra vesicle containing a single chromosome lies above the maternal group. It has failed to form a spindle. Twenty-three minutes after fertilization.

FIGURE 9.—Abnormal stock. One of four nuclei of the prophase of the third cleavage. Two polar chromosomes are seen near it, lying free in the cytoplasm. Thirty-six minutes after fertilization.

FIGURE 10.—Abnormal stock. Prophase of the first maturation division. Single germinal vesicle. Unlaid, unfertilized egg.

FIGURE 11.—Abnormal stock. Shows a later stage than the previous figure. The nuclear membrane has broken down, the nucleus now lying at the dorsal surface of the egg. Unlaid, unfertilized egg.

FIGURE 12.—Abnormal stock. Shows a later stage than the preceding figure. The spindle is distorted, the chromosomes scattered into three widely separated groups. Unlaid, unfertilized egg.


1
2
3
4
6A
6B
6C
5
7
8
9
10
11
12