THE CHROMOSOME CONSTITUTION OF GATES' 'NON-DISJUNCTION' (*v-o*) MICE[1]*

THEOPHILUS S. PAINTER
University of Texas, Austin, Texas

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INTRODUCTION

In a recent paper in this journal Professor W. H. GATES has presented the genetic evidence which led him to interpret the appearance of a waltzing mouse in the F_1 generation (in crosses between pure normal and pure Japanese waltzing mice) as being due to the loss (through non-disjunction) of the chromosome carrying the normal allelomorph of the waltzing gene. For the detailed history of this exceptional F_1 individual and the genetic evidence upon which this conclusion is founded the reader is referred to GATES (1927), "A Case of Non-Disjunction in the Mouse." Very briefly the situation is as follows: The normal gait is completely dominant to the waltzing factor and in crosses between pure normal and pure Japanese waltzing mice all of the offspring (of which there were several hundred) were normal except one female (No. 1913) which was a waltzer. In subsequent breeding tests this female showed the entire absence of the normal gene and GATES concluded either that the whole chromosome carrying the normal gene had been lost (non-disjunction), or that portion of the chromosome which carried the normal gene had been lost (loss of a part of a chromosome). Genetically the two types would be indistinguishable, in the absence of a linkage between the waltzing and any other known gene.

[1] Contribution No. 204 from the Department of Zoology, UNIVERSITY of TEXAS.

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Knowing that the cytology of the normal mouse was being studied at this institution, Professor GATES very kindly offered to send me a few extracted (*v-o*) individuals for study. I wish to make due acknowledgment and to express my thanks to Professor GATES for the opportunity of studying the *v-o* males.

MATERIAL AND METHODS

The testes of two *v-o* males have been examined.[2] Male No. 4717 was the offspring of the original "non-disjunction" female (No. 1913) with an extracted *V-o* male. The offspring of this cross consisted of five normal and two waltzers, one of the latter being male No. 4717. The second male (No. 5347) was obtained by mating an extracted *V-o* female with a pure waltzing (*v-v*) male.

The chromosome constitution of normal mice has been a subject of study at this laboratory for more than a year, and during this time a large number of normal mice have been carefully examined.

In addition to normal and *v-o* mice the testes of one pure Japanese waltzing mouse has been studied.

In all cases the technique employed was the same that I have used in my earlier mammalian chromosome studies (PAINTER 1925). The mouse testis lends itself to preservation with the methods employed and, as the figures will show, the testes of the *v-o* males were especially well preserved.

THE CHROMOSOMES OF NORMAL MALE MICE

Work done at this laboratory (Cox 1926) has shown that the normal male mouse has 40 chromosomes (figure 1). When these are carefully examined, especially in serial alinement (figure 29), we note that three chromosomes are somewhat smaller than the rest. Of these the two smallest (labelled "s") constitute a pair while the third which is a little larger is the Y sex chromosome. The remaining chromosomes form a fairly well graded series, the X sex chromosome being one of the medium sized elements. COX has shown that the chromosomes of one of the largest pairs often show a tendency to split early in metaphase plate stages so that they may appear unduly thick; this is not seen, however, in the cell illustrated in figure 29, but may be observed in figures 31 and 32.

[2] GATES represents normal and waltzing mice respectively by *VV*, and *v-v*. His original 'non-disjunction female' No. 1913 he designated by *v-o*, the *o* representing the absence of a gene. By appropriate matings he has been able to produce both *V-o* and *v-o* individuals, but such animals show a low vitality. He has never been able to produce *o-o* mice.

The haploid chromosome number is 20 including a typical mammalian X-Y sex chromosome complex. A side view of the latter components together with the s and a few associated tetrads is given in figure 2. Here again we note that the s chromosomes are a trifle smaller than the Y; this size relationship has proved to be very constant in all the mice I have studied, and it thus gives us a convenient standard of measure for the other chromosomes. Ordinarily the X and Y chromosomes do not segregate to opposite poles before the other chromosomes divide. In figure 33 a line-up of the haploid elements is given; this figure will be discussed in more detail later on.

THE CHROMOSOMES OF JAPANESE WALTZING MICE

Although the desirability of studying the chromosomes of pure Japanese waltzers did not appear until after the *v-o* males had been examined, the main facts will be presented here. The one Japanese waltzer studied has 40 chromosomes (figs. 3 and 4). It will be noted that the s and Y chromosomes can be identified readily and that they have the same proportionate sizes as in the normal mouse. In the first maturation division it was observed that the sex chromosomes show a marked tendency to segregate early to the poles of the spindle when they are very conspicuous. In other respects no deviation from normal conditions was observed in the individual studied.

THE CHROMOSOMES OF *v-o* MALES

Spermatogonia

In my first preliminary counts on dividing spermatogonia I was able to find only 39 chromosomes, but more careful and extended study of a number of extremely favorable cells showed that 40 chromosomes is the true diploid number for both males (figures 5 to 8). On returning to the cells on which the first counts had been made in order to find out the source for the discrepancy in number, I found that when I counted 39 chromosomes I had failed to observe one very small chromosome, which is always present in cells with 40 chromosomes. Subsequently this small element was discovered in practically all of the cells which at first had shown only 39 chromosomes. When the individual chromosomes of spermatogonia are carefully examined, either in equatorial plate view (figures 5 to 8) or better in alinements in which each chromosome is drawn separately (figures 30 to 32), it becomes apparent that both s chromosomes and the Y sex chromosome are present in *v-o* males, and show normal size relations. The small chromosome, which I have labelled

LEGEND FOR PLATE 1

All figures on this and succeeding plates represent a magnification of approximately 3700 diameters as reproduced.

FIGURE 1.—The spermatogonial chromosomes of a normal mouse.

FIGURE 2.—A few chromosomes of a first maturation spindle of a normal mouse showing the X-Y sex chromosomes and the s complex.

FIGURES 3, 4.—Spermatogonial chromosomes of a pure Japanese waltzing mouse.

FIGURES 5, 6, 7, 8.—Spermatogonial chromosomes of two *v-o* males, showing 40 chromosomes including the fragment q_1.

FIGURES 9, 10.—First maturation spindles from *v-o* mice showing the position assumed by the sex chromosomes. Only a few other elements are shown.

FIGURES 11, 12.—Equatorial plate views of first maturation division taken from *v-o* mice showing 20 chromosomes.


Y
S
S
1
X
S
Y
S
2
S
S
Y
3
S
Y
S
4
Y
S
q_1
S
5
S
q_1
S
6
S
Y
S
q_1
7
Y
S
S
q_1
8
9
10
11
12

"q_1" for reasons given later, is typically smaller than either s chromosome, and there is no other chromosome of the same size to be found in the cell with it. The size of the q_1 chromosome varies somewhat depending on the degree to which differentiation of the stain has been carried. When the cell is heavily stained, as in figure 8, the q_1 approaches the s chromosome in size, but in less deeply stained cells (figures 5 to 7) it is markedly smaller than the s elements. Since the two s chromosomes are the smallest elements found in either normal (figures 1 and 29) or waltzing mice (figures 3 and 4), it is evident that the q_1 chromosome of *v-o* mice had no morphological homologue in either of these races. It must therefore be either a fragment of one of the chromosomes larger than the s and Y elements, or simply a supernumerary chromosome introduced by chance into this stock.

A mating up of the spermatogonial chromosomes, and their arrangement in a serial alinement shows (figures 30 to 32) that one of the smaller chromosomes, lying between the limits of the "p" and the "s" pairs (using Cox's terminology) lacks a mate of like size. Assuming that the q_1 chromosome is a fragment of a normal chromosome, its homologue must lie in this region. In the alinements I have placed the q_1 chromosome with the q element, my reason for doing this, however, is based more on primary spermatocyte than on spermatogonial evidence. It is clear that considering spermatogonia alone one would be unable to say whether the q_1 was a fragment of a normal chromosome or a supernumerary.

First maturation period

During this period GATES' *v-o* mice show two unusual features not observed in normal mouse spermatogenesis, and for a considerable time I was misled with regard to the correct interpretation of the observed facts. A very careful study of diakinesis stages together with an examination of the waltzing mouse testes gave the necessary clues for clearing up the situation. For the sake of clarity and brevity these usual features will be discussed and explained before a detailed account of this period is given.

The very first tubule examined showed a first maturation spindle in which a large and small chromosome lay at opposite poles (figure 9). Near this was another cell in which, judging from size relations, the same elements were passing undivided to the same pole (figure 10). Numerous instances were noted in both males in which one of these two conditions obtained. There was no visible connection between these two aberrant chromosomes and since they did not divide and their distribution to the

same pole or to opposite poles appeared to be a matter of chance, it seemed obvious that we had at this period two chromosomes which behaved as univalent elements. And furthermore, since a small chromosome occurs in spermatogonia which had not been observed in normal mice, it looked as if the *v-o* males carried a supernumerary chromosome and a larger univalent chromosome which presumably had arisen through non-disjunction, as GATES had concluded. On the other hand, it was soon realized that the size relations of these two aberrant chromosomes was about the same as that of the X and Y sex chromosomes of normal mice and that while in spermatogonia the q_1 chromosome was smaller than the s elements in the first maturation stage, the small aberrant chromosome was a little larger than the s components. The upshot of the whole matter turned out to be this: That as a close study of the diakinesis stage showed, the connection or association between the X and Y sex chromosomes is often very weak, and as a result they may come into the spindle without any visible connection between each other. This lack of connection seems to retard their orientation in the spindle, and if they happen to lie on one side, as in figure 10, one gains the impression that they are passing together to one pole.

A second unusual feature of *v-o* males is the large number of degenerating first spermatocytes. GATES has emphasized the frail nature of these *v-o* mice, and as the testes were preserved a day or two after they had been received from a long railway journey, there can be no doubt that the hardships of the journey are responsible, in part at least, for the degeneration observed. Degenerating cells are characterized by a rounding up of the cell, and a darkening of the cytoplasm (with iron haematoxylin). At the same time the chromosomes seem to shrink or separate so that they are very favorable for counting. The exceptional feature which makes it necessary to eliminate such cells from consideration is that the chromatoid body, which in normal cells is extremely small and lightly staining, takes the stain deeply and may become quite large. In well differentiated material, the walls of the vacuole in which the chromatoid body appears to lie can be readily detected, but in deeply stained cells this chromatoid body looks like a small chromosome, and the writer mistook it for a time for the q_1 element.

Once the erratic behavior of the X and Y sex chromosomes was understood, and degenerating spermatocytes were eliminated from consideration, an analysis of observations on the first maturation period proved easy and very illuminating.

In the diakinesis stages, when the haploid chromosomes appear with ring-like and other characteristic shapes, we have the first opportunity of determining the make-up of the bivalent elements. If the small chromosome q_1 is a fragment of a normal chromosome, we should expect it to be associated with its homologue at this time, and to find among the tetrads an element, the two parts of which are very unequal. Needless to say, the X-Y chromosomes should also appear as a heteromorphic group, and must not be confused with the q_1 complex. In diakinesis stages I have found two heteromorphic groups, and it has proved possible to follow each of these through the first division in considerable detail as described below.

In diakinesis the X-Y complex (easily recognized because of its size relative to the s chromosome group) is found in two conditions. Usually the shorter Y is attached either to the end of the X or, as in figure 13, lying parallel to it on one side. More rarely, as in figure 15, the X and Y are entirely separated. The element which I have identified as the q_1 chromosome is associated with a chromosome some four times as large. Figure 14 is an early stage showing the relation between the larger q and the q_1 chromosome. Figure 15 is especially interesting because we have in the portion of the cell illustrated, not only the q_1 complex but the X-Y complex and the s chromosome pair. We are thus able to check up on the matter of relative sizes. The q_1 component is somewhat smaller than either s element, and in turn we note that the Y is a little larger than the s.

Reflecting the loose association of the diakinesis period we find that the X and Y chromosomes exhibit a considerable difference in their behavior in fully formed first maturation spindles. In a considerable majority of cases, these two components are attached end-to-end by a deeply staining chromatic bridge (figures 16 to 18).[3] Figures 19 to 21 are cells in which the X and Y are separate with no visible connections and represent perhaps, steps in the eventual arrangement and distribution of these elements. Figure 25 is a cell in which the sex chromosomes are entirely separated by the spindle. It seems altogether probable in the cells represented in figures 19 to 21 and 25, that there had been an entire separation of the X and Y in the diakinesis stage, similar to that shown in figure 15.

It has proved possible to follow the q_1 complex through this division often in the same cells showing the sex chromosomes. Figure 20 shows the q_1 element beginning to separate from its mate, and in figures 21 to 24

[3] The variations in size of the sex chromosomes and of other elements in the figures are due in part to differences in the degree of condensation, and in part to differences in staining. Differences in preservation may also play some part here.

LEGEND FOR PLATE 2

FIGURES 13, 14, 15.—Diakinesis stages taken from *v-o* mice to show the behavior of the sex chromosomes and of the q-q_1 complex. Not all of the elements are drawn in.

FIGURES 16,17,18.—Detailed drawings of sex chromosomes in *v-o* mice early in the first division.

FIGURE 19.—Side view of a first maturation spindle from a *v-o* mouse showing the sex chromosomes unconnected, but lying close together.

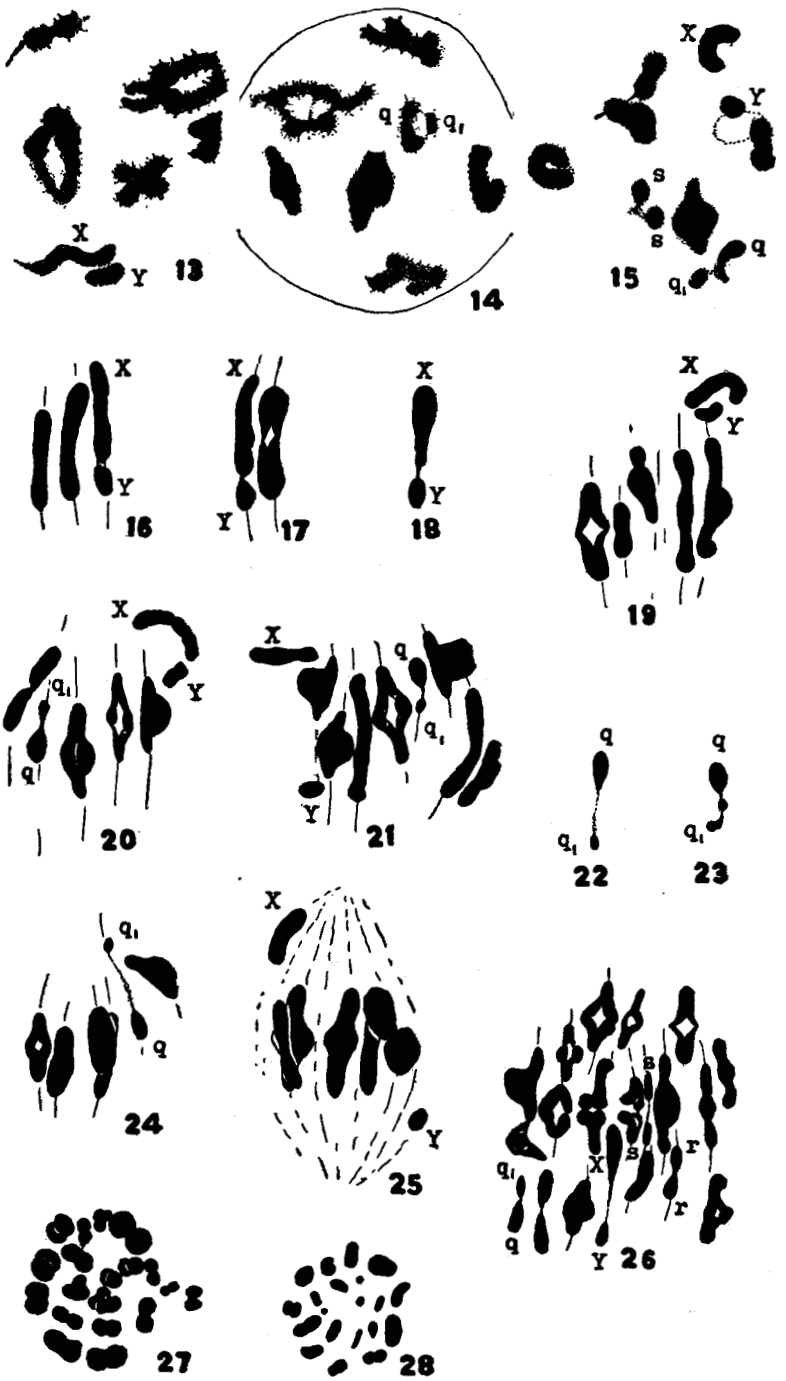
FIGURES 20, 21.—Side views of spindles from *v-o* mice showing both the sex chromosomes and the q-q_1 complex.

FIGURES 22, 23, 24.—Detailed drawings of the q-q_1 complex from different cells of *v-o* mice.

FIGURE 25.—First maturation spindle from a *v-o* mouse showing the separation of the sex chromosomes.

FIGURE 26.—A "spindle dissection" of a first maturation spindle from a *v-o* mouse showing the morphology of the 20 haploid elements.

FIGURES 27, 28.—Second spermatocyte chromosomes showing 20 chromosomes from *v-o* mice.


X
Y
13
q
q1
14
X
Y
S
S
q
q1
15
X
Y
16
X
Y
17
X
Y
18
X
Y
19
X
q1
q
Y
20
X
q
q1
Y
21
q
q1
22
q
q1
23
q1
q
24
X
Y
25
q1
S
X
S
r
r
q
Y
26
27
28

other views and stages of separation are shown. As the two components draw apart it is not unusual to see a distinct knot in the chromatic bridge (figure 23) and a little later (figure 24) the chromatic bridge is much heavier than before suggesting that this knot has contributed to the bridge. I have gained the impression that as the q and q_1 chromosomes go apart in this division the heavy bridge goes along with the q_1 element.

Figure 26 is a "spindle dissection"[4] of one of those rare cases in which all the elements of the first maturation spindle can be recognized in one cell and in figure 34 these are lined up in the approximate order of their size. From the standpoint of the present paper the most interesting elements are the sex chromosomes, the q_1 and the s complexes. Certain phases of this figure will be taken up below.

Primary spermatocytes have 20 chromosomes. In many cells the sex chromosomes lie well outside of the general circle of autosomes, as in figures 11 and 12. If the X and Y happen to be separated the count may run to 21, or if the X and Y have already segregated to opposite poles, we may find only 19 elements. The higher and lower counts are obviously correlated with the erratic behavior of the sex chromosomes.

All the evidence of the first maturation division points to the segregation of the q and q_1 elements to opposite poles of the cell, just as in the case of the sex chromosomes.

Second maturation division

It has proved difficult to reach any definite conclusion regarding the behavior of the q_1 chromosome during this division. Twenty chromosomes are found in second spermatocyte cells (figures 27 to 28), but the small size of all the chromosomes makes it difficult to identify the q_1 element. In side views of spindles I have seen no evidence which would indicate that this was other than an equation division for all of the chromosomes.

DISCUSSION

In interpreting his genetic results, as I have stated above, GATES concluded either that a whole chromosome had been lost, or that a part of a chromosome was absent. In the former event we should expect *v-o* mice to show 39 chromosomes, but if it were a case of a loss of a part of a chromosome, there should be 40 chromosomes, and the detection of the

[4] In "spindle dissections," as I have used the term in various papers, the individual chromosomes are drawn more or less out of place so that their form may be clearly seen. In reality they lie in a normal spindle with much overlapping, but by focusing up and down on the semi-transparent elements the shape of the lower lying members can be accurately determined.

loss cytologically would be dependent upon how large a piece of the chromosome was gone. The non-viable character of *v-o* mice and GATES' failure to obtain *o-o* individuals both suggest that the loss may be extensive in character.

A cytological study of *v-o* mice has shown that they carry the full normal diploid number of 40 chromosomes. At first this would seem to eliminate the possibility of the loss of a whole chromosome, but this would not be the case if the *v-o* stock should happen to carry a supernumerary chromosome. As a matter of fact, in my preliminary study when I confused the deeply staining chromatoid body of degenerating cells with a true chromosome, I suspected the presence of a supernumerary body, but later observations gave no valid evidence for this.

With the elimination of the loss of an entire chromosome we must now consider if we have critical evidence for the loss of a part of a chromosome. The answer is clearly in the affirmative. Both *v-o* males show a very small chromosome in their spermatogonia which is not present in either normal or waltzing mice, and in the first maturation division this fragment is actually observed paired up with a chromosome four or more times as large. This is just the sort of behavior we should expect to find in case the q_1 chromosome was a fragment of one of the chromosomes of the normal mouse complex, and is inexplicable should we try to interpret the q_1 as a supernumerary. It is clear that a loss of part of a chromosome would fully explain GATES' exceptional mouse as well as non-disjunction. We only need to assume that the normal chromosome q carries the factor for waltzing and that the fragment q_1 lacks this gene (and many others no doubt).

The cytological study of *v-o* mice has thus made a distinct contribution to our knowledge of the genetic consequences of aberrant chromosome behavior. For it is the only case on record in which the absence of a part of one chromosome (cytologically determined) is accompanied by an absence of a gene (genetically determined) although the fundamental principle involved is familiar to us in non-disjunction and is suggested in cases of so-called "chromosome deficiency." Cytologists have known for a long time of animals in which one autosome was markedly larger than its synaptic mate (see WILSON, 1925, p. 931), but such cases have not been linked-up with the absence of genetic characters. On the genetic side, cases of "chromosome deficiency" have been reported which were not accompanied by any visible loss of chromatin. In *v-o* mice both cytological and genetical conditions are fulfilled. At first sight, one would describe this as a case of "chromosome deficiency," which literally

it is of course, but unfortunately this term has been used by cytologists and geneticists to mean slightly different things. In cytology it has meant the absence of a part of a chromosome, but BRIDGES who first employed the term in genetics applied it to cases where a gene or a group of genes were genetically absent, but it was thought that this might be due to an inactivation of genes, since no morphological deficiency could be observed in the chromosome involved. For this reason the writer proposes the term "chromomere deletion" to cover cases such as GATES' *v-o* mice, meaning what this term literally implies, the loss of a segment (large or small) of a chromosome from the cell. It may well be that cases of "deficiency" in the genetic sense are conditioned, in most instances, on chromomere deletion.

From a cytological point of view the behavior of the chromosome fragment in the mouse is like that of similar structures in Orthoptera. And it is interesting to note that in the mouse we have evidence of a random segregation of the chromosome fragment, using the sex chromosomes as a criterion, just as CAROTHERS (1913) so clearly described in the grasshopper Brachystola. In figures 20 and 26, the fragment q_1 is passing with the X chromosome to one pole, while in figure 21 it is going to the same pole as the Y chromosome.

From a theoretical standpoint, perhaps the most interesting feature of *v-o* mice is that they afford us the first opportunity of locating a definite gene in a definite chromosome in mammals. Because of this theoretical interest a great deal of study has been given to the exact identification of the snyaptic mate of the chromosome fragment q_1. The first attempt at this was made in spermatogonial cells in which the individual chromosomes of the cell were copied separately and then matched up according to relative size and to a slight extent shape. This mating was checked under the microscope so as to eliminate, as far as possible, any error due to foreshortening. The results of these alinements are given in figures 30 to 32, and allow us to locate the mate of q_1 among some one of the smaller chromosomes. In most cases it appears to fit in best with the q chromosome, but the method due to unavoidable sources of error is not sufficiently exact to allow us to more than approximate the location of the q_1 element on spermatogonial evidence.

The first maturation division has given much more satisfactory evidence regarding the synaptic mate of q_1. In figure 34 the bivalent chromosomes of a complete first maturation spindle are shown individually. Beginning at the end with the small chromosomes, we note the smallest or s chromosome pair. A trifle larger is the r pair, and next to it in size is the q chromo-

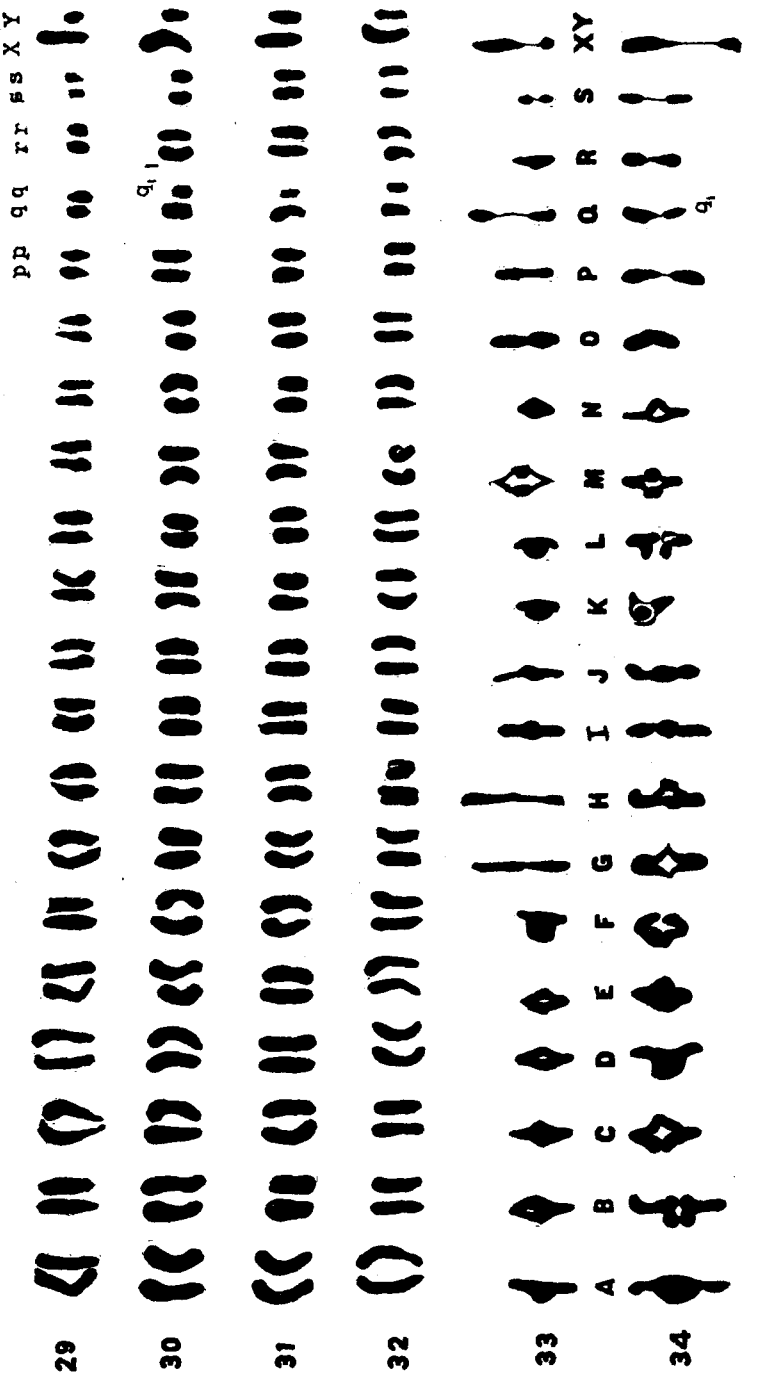
LEGEND FOR PLATE 3

FIGURE 29.—A chromosome alinement of the spermatogonial chromosomes of a normal mouse taken from figure 1.

FIGURES 30, 31, 32.—Chromosome alinements from *v-o* mice based on figures 5 to 7 respectively.

FIGURE 33.—The haploid elements of a normal mouse.

FIGURE 34.—The haploid elements of a *v-o* mouse, based on figure 26.


pp qq rr ss X Y
q_1
A B C D E F G H I J K L M N O P Q R S XY
q_1
29
30
31
32
33
34

some to which the q_1 is attached. Using the s chromosome as a standard of measure, I have checked up repeatedly this identification of the mate of q_1 and I am convinced that the q element is its homologue.

There is one additional reason for identifying the chromosome fragment as belonging to the q pair. In normal mice as Cox (l.c.) has shown, the q chromosome typically divides much earlier than the rest of the elements. In *v-o* males I have not observed the presence of this precocious pair, except in so far as the q and q_1 elements tend to separate early (figures 20 to 24). Furthermore, the erratic behavior of the q elements in normal mouse spermatogenesis would seem to make them particularly liable to an accident during maturation. All of the foregoing considerations have led me to the conclusion that the initial loss of chromatin occurred in a q chromosome.

There is one additional feature of the present study which deserves mention, and that is the unusual behavior of the sex chromosomes. Why should the sex chromosomes exhibit, in some cells, such a loose association? Is it not possible that this would lead to an irregular distribution of the sex chromosomes, with the attendant production of sexually abnormal individuals? These two questions can be answered only in a provisional way.

Since normal mice only occasionally show the early separation of the sex chromosomes, while in the one Japanese waltzer examined this is the rule, it seems probable that the unusual behavior of the sex chromosomes has been brought into the *v-o* mice from the waltzing strain. In this connection it should be mentioned that recently GATES (1926) has shown that the Japanese waltzing mice are probably derived from a species different from the common fancy mouse. This might account for the loose association between the sex elements in crosses. With regard to the second question, it seems inevitable that, if the sex chromosomes separate entirely before they enter the first maturation spindle, then there is a chance that the X and Y would pass together to one pole, and thus give the basis for sexual abnormalities. However, my observations indicate that spermatocytes showing this behavior are not a large class numerically, although they are very conspicuous, and hence the chance of a sperm carrying both the X and Y sex chromosomes fertilizing an egg is not very great.

SUMMARY

1. A cytological examination of two of GATES' *v-o* mice shows that each has the full diploid number of 40 chromosomes.

2. A close examination of the individual spermatogonial chromosomes reveals that in *v-o* mice there is a small chromosome fragment which is smaller than any element in either normal or waltzing mice.

3. Evidence from the first maturation division indicates that the chromosome fragment is a part of the q chromosome with which it unites in synapsis.

4. In the first maturation division the fragment segregates from its homologue so that mature spermatozoa will carry either the normal q element or the fragment q_1.

5. GATES' original waltzing female (*v-o*) is to be explained, therefore, on the basis of an absence of a part of a chromosome rather than on the basis of the absence of an entire chromosome.

6. Since the term "chromosome deficiency" has come to have two slightly different meanings in cytology and genetics, the writer suggests the term "chromomere deletion" to cover cases such as GATES' *v-o* mice where there is a visible absence of a part of a chromosome.

7. The *v-o* mice allow us for the first time in any mammal to locate a definite gene in a definite chromosome.

8. The erratic behavior of the X-Y sex chromosome complex during the first maturation division has been described.

LITERATURE CITED

CAROTHERS, E. E., 1913 The Mendelian ratio in relation to certain orthopteran chromosomes. Jour. Morph. **24**: 487–512.

COX, E. K., 1926 The chromosomes of the mouse. Jour. Morph. **43**: 1–14.

GATES, W. H., 1925 The Japanese waltzing mouse. Carnegie Publ. No. **337**: 83–138.
1927 A case of non-disjunction in the mouse. Genetics **12**: 295-306.

PAINTER, T. S., 1924 A technique for the study of mammalian chromosomes. Anat. Rec. **27**: 77–86.

WILSON, E. B., 1925 The cell in development and heredity. 1232 pp. 3rd Ed. New York. MacMillan Company.