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CHROMOSOME NUMBER AND PAIRS
IN THE SOMATIC MITOSES OF
AMBYSTOMA TIGRINUM

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BY
CHARLES L. PARMENTER

A THESIS

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CHROMOSOME NUMBER AND PAIRS IN THE
SOMATIC MITOSES OF AMBYSTOMA¹
TIGRINUM

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THIRTY-SEVEN FIGURES (NINE PLATES)

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¹Also known as Amblystoma.

INTRODUCTION

It is believed (McClung, '17, pp. 536-38) that the chromatin of an organism is, for the most part at least, the idioplasm, and consists of a definite linearly arranged series of differentiated materials which is perpetuated from generation to generation. The chromosomes which are essentially constant in number in an individual are thought to constitute the visible mechanism for this perpetuation. This conception is known as the theory of the individuality of the chromosomes, which is quite generally accepted by all who have an intimate acquaintance with chromosome behavior. However, there are a few not so acquainted who strenuously oppose the theory.

Among these is Della Valle ('09, '11, '12), who presents some data and a large amount of discussion in an effort to disprove this theory upon the claim that the chromosome number in an individual is not constant, but is simply the quotient of the quantity of chromatin divided by the average size of the chromosomes. This removes from them any constancy of organization and contradicts the above theory. These observations have been cited by other opponents of the theory as cytological evidence in favor of their contentions. Della Valle's conclusions are based upon observations made upon dividing cells of the peritoneum and blood-cells of *Salamandra maculosa*, together with a large amount of data taken from the observations of others.

Meves ('11) and Della Valle ('12) further oppose the theory upon the basis of linear measurements made upon the spermatogonial and somatic chromosomes of *Salamandra maculosa* in denying Montgomery's ('01) and Sutton's ('02) claim that the chromosomes occur in pairs whose homologues are of equal length, and that approximately constant size relations among chromosomes are maintained from one cell generation to another.

In the spring of 1916 I was fortunate in obtaining peritoneal and other somatic tissues of *Ambystoma tigrinum*. This made it possible to repeat Della Valle's observations upon the somatic

cells of the same and other tissues of this closely related species and thus to determine whether such a variation as he claims is present in the somatic tissues of other Amphibians. Also the chromosomes of some cells in my material are sufficiently favorable for measurements to permit a reconsideration of their length relationships.

Since this paper is regrettably controversial, it is necessary to give careful attention to all the methods and conditions under which the preparations and the observations were made. Della Valle also lays much emphasis upon this point, and therefore considerable space is devoted to this problem.

For facilities in collecting and preparing this material I am indebted to the courtesy of the Department of Zoology of the University of Minnesota, and to Prof. C. P. Sigerfoos I owe the loan of several very excellent preparations. The work was done under the direction of Prof. C. E. McClung, of the University of Pennsylvania, toward whom I feel especially grateful for constant encouragement and valuable criticism, and for his characteristically generous and kindly interest at all times. I am also greatly indebted to other members of the department, especially to Dr. Eleanor Carothers and Dr. D. H. Wenrich, for helpful suggestions and very painstaking criticisms.

TECHNIQUE

The material used was obtained during the spring of 1916 from larvae of *Ambystoma tigrinum*, which were abundant in the ponds and lagoons near the College of Agriculture of the University of Minnesota. Mitotic figures in epithelial cells of the tail, gill plates, and lung, and of the endothelium from peritoneum and mesentery were studied.

Tail epithelium

Very excellent preparations of this tissue were kindly loaned me by Prof. Charles P. Sigerfoos, of the University of Minnesota. These were made from the tails of larvae $\frac{3}{4}$ to $1\frac{1}{2}$ inches in length obtained during the last of May and the first of June during several years.

The living larvae were thrown into Flemming's stronger solution. After about four hours of fixation, the tails were split dorsoventrally² into two thin plates of cells. These two plates of cells were then fixed twenty hours longer. After washing in running tap-water for twelve hours or more, the pieces were stained in toto in Heidenhain's haematoxylin, carefully dehydrated, and cleared in xylol and mounted in damar.

Gill-plate epithelium

The most successful gill-plate preparations were also obtained from larvae $\frac{3}{4}$ to $1\frac{1}{2}$ inches long. In each larva there are eight gill plates, one subtended from each gill arch and another behind each posterior gill cleft. These gill plates contain very numerous mitotic figures. They are composed of two epithelial lamellae with connective-tissue cells and capillaries lying between them. The two layers, unseparated, are so thin that they give very excellent preparations.

The material was fixed in situ by dropping the living larvae into the Flemming's stronger solution as soon as they were taken from the net. They were fixed in situ twenty-four hours and

² Haecker ('99) describes a very successful method of separating these two plates of cells. The posterior end of the larva is cut off after fixation just in front of the cloaca. With a sharp scalpel the thick cephalic end of the tail is split dorsoventrally through the middle of the vertebra to a depth of an eighth of an inch or more. By grasping with the forceps the ends thus made free, the two layers of epithelium can be pulled apart in a manner similar to separating two sheets of fly-paper with adhesive surfaces sticking together. Professor Sigerfoos advises separating the two layers after about four hours of fixation and then allowing to fix about twenty hours longer.

The numerous large mitotic figures in various stages with clear cell walls which can be studied without an immersion lens makes this material excellent for the class-room. The gill-plate preparations are equal or superior to those of the tail epithelium. Those of larger larvae are too thick when mounted in toto, but give very satisfactory preparations when separated. Peritoneal preparations of larger larvae contain fewer mitoses and the cell walls are indistinct. However, preparations can be made from the more rapidly growing shorter larvae from which the gill-plates were taken and would probably contain more divisions. Preparations of *Ambystoma punctatum* are less favorable than those of *A. tigrinum* because there are fewer figures, more pigment cells in the tail epithelium and the gill-plates are small and thicker.

washed in running tap-water. The larvae from which the gill-plates were taken were fixed for other purposes, and no special effort was made to insure good fixation of the plates. They were preserved in position in 5 per cent formalin, which was later gradually replaced with 80 per cent alcohol.

The fixed gill-plates were carefully removed from the larvae in 80 per cent alcohol and were left attached to the gill arches, which, in subsequent handling, were grasped by forceps to prevent injury of the plates. Hydrogen peroxide was added to the 80 per cent alcohol drop by drop through a fine glass capillary siphon until the solution amounted to equal parts of each. In this the plates were bleached for four to twelve hours and then transferred to the mordant by the above-mentioned drop-process and stained in iron haematoxylin. They were dehydrated by this drop method, cleared in cedar-wood oil followed by xylol, cut from gill arches, after being transferred to the slide, and then covered with damar and a thin cover-glass. While the damar was hardening they were kept for twenty-four hours or more under slight pressure to insure flat preparations.

The peritoneum, mesentery, and lungs

These preparations were made from larvae 3 to 4 inches long. All the tissues of a given individual were not only fixed together in the same fixative for the same length of time, but also received the same treatment in all subsequent processes. They were put into the fixatives within an estimated maximum of two minutes after the first incision. Two methods of procedure were used in preparing these tissues for fixation:

1. In order to avoid any possible unfavorable effect of captivity, the tissues were fixed in situ in the field as soon as the larvae were taken from the net. The animals were prepared for fixation as follows: With sharp scissors the body wall was cut open along the midventral line and also lateral incisions were made on each side at right angles to the first incision behind the pectoral girdle and in front of the pelvic girdle, so that the two halves of the body wall fell away from the viscera and opened

wide the body cavity. The folds of the viscera were pulled apart and the whole larva was plunged into the fixative. This secured immediate and uniform fixation. The operation requires less than a minute and the incisions are apparently painless, for the larva does not often struggle.

2. The body walls, lungs, and viscera were removed from the body of the larvae before fixing, either in the field or at the laboratory. The peritoneum was fixed in situ on the body walls. Only normally inflated lungs were used, and these were ligated anteriorly before removal from the body to prevent them from collapsing. After fixing one or two hours, they were cut into two or more flat longitudinal strips and returned to the fixative. The mesentery, attached to the intestine, was spread out flat on a piece of glass and the whole immersed in the fixative with the tissue beneath.

Fixatives

The fixatives used were: 1) Flemming's stronger solution, thirty hours; 2) Bouin's solution, forty-three hours; 3) Bouin's solution, to which was added $1\frac{1}{2}$ grams of chromic acid crystals per 100 cc., twenty to twenty-four hours; 4) Hermann's solution with two parts of osmic acid (Lee, '13, p. 38) twelve to eighteen hours; 5) a solution of saturated picric acid 75 cc., formalin 15 cc., glacial acetic acid 10 cc., urea crystals 2 grams, thirty to forty-three hours. The urea should be added gradually to the solution warmed to about 40°C., otherwise a precipitate is formed.

It is a difficult matter to decide which solution gave the best fixation. The prettiest cells were fixed in Hermann's and the chromic acid modification of Bouin's fluid. However, the peritoneum preparations of the osmic fixatives were a little thicker and less transparent than the others. If any fixative should be exclusively chosen, I believe it should be the chromic acid modification of Bouin's solution, because of its excellent fixation, convenience, and economy.

The peritoneum was removed as follows: The two sides of the body wall were detached by an incision along the back

close to the spine. Under the binocular lens, in water, the peritoneum was carefully loosened from the underlying tissue by scraping it with a sharp scalpel, first along the edge cut from the back. Sections of this loosened edge were then grasped by the forceps and relatively large sheets were easily pulled off from the underlying tissue. The peritoneum covering the dorsal and lateral portion of the body wall is deeply pigmented and to it adhere considerable muscle and connective tissue when the peritoneum is removed. This portion was grasped with the forceps in removing the peritoneum from the body wall, as well as in all subsequent handling. Consequently the cells in the ventral transparent region available for study have been undisturbed by instruments. However, there still remains a possibility that the strain of pulling the peritoneum loose might disturb some cells.

Peritoneum fixed in Flemming's and Hermann's solutions was stripped from the body wall after four hours of fixation and then fixed twenty hours longer. That treated with the various picric acid mixtures was stripped immediately after fixation. However, the peritoneum fixed in the chromic acid modification of Bouin's solution may be preserved in alcohol for as much as a year before stripping. That of *Ambystoma punctatum*, fixed in Flemming's stronger solution and preserved in 5 per cent formalin, can be stripped at least six months after fixation.

Material fixed in osmic acid fluids was washed five to fourteen hours in frequent changes of tap-water. Picric acid preparations were gradually transferred to 70 per cent alcohol, beginning with 10 per cent and progressing through successively stronger grades differing by 10 per cent. They remained in each grade five to ten minutes. The tissues remained in 70 per cent alcohol containing a few drops of saturated aqueous lithium carbonate solution until the picric stain was removed, and before staining they were returned to water by reversing the above process. All of the material was stained in Heidenhain's haematoxylin after mordanting in a $2\frac{1}{2}$ per cent solution of iron alum for four to six hours. No counterstains were used.

Dehydration was accomplished by passing the material through the above grades. The fluids were removed from, and added to, the containers without handling the material. Alcohols were followed by half xylol and half absolute alcohol, and finally by xylol.

The pieces of peritoneum were transferred from xylol to a slide where the above-mentioned pigmented area, with the attached muscle fibers, was removed quickly with a sharp scalpel just before mounting. After mounting in damar under a cover-glass, they were put under a light pressure for twenty-four hours or more while drying to insure as flat a preparation as possible.

OBSERVATIONS

It should be emphasized that the preparations upon which these observations were made are unsectioned surface membranes. This makes it possible to study the mitotic figures with the confidence that all of the chromosomes are present and that none have been cut and are being counted more than once. This is an important consideration in determining whether the number of chromosomes is constant.

A. The number of chromosomes

There are twenty-eight chromosomes in the somatic complexes of *Ambystoma tigrinum*. In forty-five unquestionable enumerations and in eighteen which contained either one or two chromosomes that might possibly be considered subject to interpretation, there are none which vary from twenty-eight. In three complexes, because of the alternative interpretations possible at one or more points, the number cannot be definitely determined and is interpreted to be either twenty-seven or twenty-eight. The fact that these numbers are so close to twenty-eight is strong evidence that these cells contain the usual number of chromosomes.

The counts as indicated in the accompanying table have been obtained from twenty-three different individuals varying in age

approximately from six to ten weeks. The preparations of tail epithelium loaned by Professor Sigerfoos were taken from a collection which he has been accumulating for a number of years. It is probable, therefore, that the counts in these preparations represent the chromosome number present during a series of years and that the number is constant from year to year.

Table showing for each tissue studied, the number of different individuals represented and the number of complexes with their distribution into classes as described on page 178. The total number of different individuals represented is twenty-three

TISSUE	NUMBER OF INDI- VIDUALS	CLASSES			TOTAL
		I	II	III	
Peritoneum.....	7	14	6	1	21
Mesentery.....	1	1	0	0	1
Lung.....	4	7	2	1	10
Tail epithelium.....	5	9	4	0	13
Gill plates.....	8	14	6	1	21
Totals.....		45	18	3	66

a. Method of determining number. Since one of the chief purposes of this study is to determine accurately whether there is any variation in the number of chromosomes, considerable care has been taken to eliminate from the evidence every possible source of error. An important part of the presentation of this evidence is, then, a concise description of the exact procedure employed in obtaining it.

1. Procedure. In order to avoid overlooking any mitotic figures, the entire surface of every piece of tissue was completely surveyed systematically before beginning to count any of the chromosomes in any of the complexes. The survey was accomplished with a 4-mm. objective and an 8× ocular supplemented by a mechanical stage.

In determining the number of chromosomes in each complex, a camera lucida sketch of it was first made at a magnification of 2633 diameters. This sketch was carefully compared with the cell in order to make certain that no errors had been made in

sketching it. The chromosomes were then numbered consecutively, the number being placed on both ends of each chromosome. This method avoided any possibility of overlooking any chromosome or of counting any chromosome twice.

2. Clearness and classification of the complexes. All the complexes counted were polar views of late prophases and of metaphases and have been divided into three classes on the basis of their clearness. The first class consists of forty-five complexes in which every chromosome was so clearly separated from adjacent chromosomes that it could be optically traced continuously over its entire length, without losing sight of it at any point. Only the counts from complexes of this group are submitted as data which are unquestionably free from objection and uncertainty.

In the second class of cells there are eighteen complexes in which the chromosomes are all exactly as clear as those of the first class, with the exception that either one or two chromosomes cannot be clearly traced over their entire length as they could be in class I and therefore might possibly be hypercritically considered to necessitate interpretation.

The three cells of the third class differ from those of the second class in that they each contain places in which the number of chromosomes cannot be determined with confidence and consequently are actually subjects for interpretation.

Complexes of the first class. Complexes of this class are represented by figures 1 to 8 which have been made in carbon and are attempts to represent the actual appearance of the chromosomes and their relative positions in the complexes. Representative cells from each of the tissues studied, except the mesentery and lung, have been so drawn. Other complexes of this group have been outlined in ink, figures 9 to 20, to give a further assurance of the nature of the complexes constituting this class of conditions.

Since it is impossible to represent chromosomes in a drawing as clearly as they are seen in a cell, it is necessary to consider briefly this situation in order to prevent misunderstanding, and incorrect impressions concerning the clearness of the cells and

the faithfulness of the description of the conditions under which the number of chromosomes was determined. The difficulty lies in the necessity of representing on a plane surface chromosomes which in the cell occupy several levels. The effect can be produced by shading, but at the same time at points where chromosomes cross or overlap each other for various distances they might create the impression in the drawing that they cannot be "optically traced continuously over their entire length." There are such cases in every drawing. This is especially true of the late metaphases of the tail epithelial complexes (e.g., figs. 7, 8) where every chromosome in the cell can be clearly and faithfully traced as described above.

There is also the condition in which parts of the same chromosome are so related to one another that their appearance in the drawings might create a doubt as to their clearness in the cell. Examples of this are represented in figures 6 and 8, chromosome 'a,' in which the two arms of the same chromosome turn abruptly upon one another and the appearance might be subject to the criticism that there are two different chromosomes involved—a portion of one lying exactly upon another with their ends terminating at the same point. Such cases were carefully examined and the two arms can clearly be seen to follow into each other.

In four of this first class of cells there is another condition that needs mention. These cells contain one or two chromosomes which appear to be broken into two parts (e.g., figs. 19 and 20, *f*). The parts in each case are separated by very short spaces and are exactly in line with each other. Della Valle ('09, fig. 11) shows two cases of this sort as one chromosome, but discusses them (p. 116) as uncertain. That there is a single chromosome concerned in each of these cases is further evidenced by the fact that there are twenty-one similar cases in other cells of this class (e.g., figs. 5, 7, 14 and 15, *f*) and thirty-five cases in cells of class II in which the parts are connected by various amounts of chromatin. In some instances the connection is seen as faintly staining chromatin, in others as a single or double darkly stained thread.

Complexes of the second class. In fourteen cells of this class there is one point in one chromosome and in four cells there is one point in each of two chromosomes which, to persons hypercritically inclined, might possibly appear uncertain. To one acquainted with the material, each of these points is entirely clear, and even when accepted as subject to interpretation it is very plain how the interpretation should be made—so plain that I am certain that the count of twenty-eight chromosomes is accurate and dependable. But for the sake of unquestionable fairness I have placed these cells in a separate group. As to the exact nature of the interpretations in these eighteen complexes, four of them have some small portion of only one chromosome so covered by others that it cannot be traced over its entire length without losing sight of it as stated above (p. 178). Two other cells had two chromosomes of this nature. Five complexes have a single chromosome lying in such a relation to another chromosome that it might possibly be interpreted as a part of the other chromosome (e.g., fig. 23, chromosome *i*), and in three more cells there were two such chromosomes. In the remaining five complexes a single chromosome was so situated or otherwise involved, that it might be interpreted that there were two chromosomes present (e.g., fig. 21, *i*).

In considering all the interpretation possible in each of these eighteen cells the minimum number in any one of them would be twenty-seven and the maximum number thirty. Even granting this much variation, it is far removed from that expected in a series of chance variants as Della Valle claims them to be.

The points in question were sketched as described above before the chromosomes were counted, so that the determination of the number of chromosomes was not influenced, either consciously or unconsciously, by a knowledge of how many chromosomes were present or by how they should be sketched in order to produce the expected number. This procedure and the fact that the number counted always agreed with the number present in the forty-five cells of class I make it practically certain that the enumeration is correct. It should be emphasized again that these cases are only subject to question when hypercritically

considered and would otherwise constitute a part of class I. In fact, an experienced cytologist of this laboratory, in examining these, without a knowledge of the number of chromosomes present, could see no reason for considering them as subjects for interpretation, and it seems almost absurd to place them in a separate class.

Complexes of the third class. There was a very large number of cells which were beautifully clear everywhere except in regard to one or two chromosomes. However, only three of these were sketched, because the number of clear counts was so large that an increased number of these uncertain counts is of little value.

Each of the three cells drawn contains two points of uncertainty as to whether there are one or two chromosomes present. The number is interpreted as either twenty-seven or twenty-eight. The minimum number of chromosomes possible of interpretation in one cell is twenty-six, the maximum is twenty-eight; in the other two cells the minimum is twenty-seven, the maximum is twenty-nine.

These three cases were interpreted while the sketch was being made and before it was known how many chromosomes were present. It is not true, therefore, that the interpretations were prejudiced nor that any cases which did not agree with the expected numbers were cast aside and consequently ignored. On the contrary, they are here included as part of the evidence in forming the conclusions drawn from this study.

Rationally considered, then, of the cells sketched there are sixty-three in which the enumeration of chromosomes is accurate and dependable and three in which there are unavoidable interpretations necessary. These sixty-six complexes constitute very strong evidence that the number of chromosomes in *Ambystoma tigrinum* is constant.

b. Possible variation in number in uncounted complexes. As to whether or not there was any variation in chromosome number in this species can be judged from the results obtained from the sixty-six cells which were studied. If as few as 2 per cent of the total complexes studied varied from the usual number, at least one of these should have made its appearance. Furthermore,

Della Valle ('09, p. 117) claims that variation of chromosome number is probably a general law and (p. 120) that his counts strikingly bear out the expectation expressed by Newton's theoretical binomial curve. Were this the condition in *Ambystoma tigrinum*, a good proportion of the sixty-six complexes should have shown variation in number. Since no variation was found, it is safe to conclude that there is none in the cells that could not be counted.

c. Abnormal complexes. Seven apparent variations from the usual number were found. These were groups of chromosomes in which the number was clearly other than twenty-eight (figs. 22, 24, 25, A.B., 26, A.B.). But when these groups are thoroughly analyzed it is certain that they are nothing else than cases of a very unusual behavior of four cells and do not constitute a variation from the usual number of chromosomes.

Figure 22 shows a peritoneal cell which has lost a part of the chromosomes. Chromosome *a* is but part of a chromosome, showing very unmistakable evidence that a portion of it has been broken off and there is a conspicuous depression in the tissue from which it is evident that the remainder of the chromosomes of this cell have been lost. The cell lies close to a tear in the peritoneum. It is a bare possibility that the tear and the loss of the chromosomes is due to the same cause.

The second case is a very early metaphase from the peritoneum. It consists, as represented in figure 24, of one group of twelve chromosomes and another group of sixteen immediately adjacent to it. These two groups and figures 2 and 20 are very similar to Della Valle's dicentric cell ('09, fig. 6) and Flemming's ('91) figures 31 to 39, table 40. A study of these two groups makes it practically certain that they are separated parts of one and the same cell. This is evidenced by the following facts: 1) these two groups together constitute the normal number twenty-eight. 2) The chromosomes of both groups of cells are in the same stage of mitosis. 3) Both groups represent a half circle and indicate strongly that they are separated parts of one cell which have rotated a total of 180° to their present positions. 4) When these chromosomes are arranged side by side linearly they

form a series (fig. 31) like that (figs. 27 to 30) made by a similar arrangement of the chromosomes of normal cells (figures 1 and 3). The length relationships of these chromosomes as shown graphically in figure 37 are practically identical with those of normal cells (figs. 33 to 36). Unfortunately, the cell walls are not visible. 5) Both of the homologues of chromosome pairs, as determined by measurements and indicated in figure 24 by a duplicate series of numbers, in some cases are found in the same separated part of the cell and in other cases one homologue is found in part *a* and the other in part *b*.

The third case is a compact metaphase in the epithelium of the lung (figs. 26 A and B) and is similar to the second case. These two groups are somewhat more separated than those of figure 24. Figure 26 B represents the chromosome number and characteristics and figure 26 A shows the relative positions of the two groups omitting some of the chromosomes in *a*.

That these two groups of chromosomes are parts of the same cell which have become separated is made highly probable by the following facts: 1) As in the second case (fig. 24), the two groups are near together, one containing eight and the other twenty chromosomes—a total of twenty-eight. 2) The chromosomes are in the same stage of mitosis, the chromatids of those of *b*, however, being separated a little more than those of the twenty chromosomes in *a* which may be due to a less crowded condition. 3) The two groups are practically of the same diameter and of the same shape. An outline of *a* on transparent paper can be perfectly fitted to *b*. 4) These chromosomes also form a linear series of lengths (fig. 32) similar to those of normal cells. 5) Group *a* is not a complete cell because the cytoplasm can be seen only below the chromosomes, while above the chromosomes are bare. The boundaries of the cytoplasm of *b* cannot be seen. 6) The homologues of the chromosome pairs are numbered and distributed in the two groups like those of figure 24.

The fourth case is the peritoneum of another individual. It evidently is a cell which has been divided into two parts like those of cases 2 and 3. Figure 25 A is a camera-lucida drawing

representing the relative positions of the two groups and figure 25 B shows the chromosomes enlarged and numbered consecutively. As in case 3, the two groups are not immediately adjacent, but are separated by the longer diameter of a resting nucleus.

In group *a* there are eleven chromosomes. In the other group there are apparently seventeen, but unfortunately in this second group the chromosomes are so overlapped at one point that they cannot be counted with confidence. There are, however, thirteen chromosomes which can be clearly delineated and the interpretation that there are four chromosomes in the group (14 to 17) which so badly overlap is likely correct. The total number of chromosomes in the two groups is then probably twenty-eight.

The chromosomes are so much foreshortened, and at the above-mentioned point so crowded, that I have not attempted to measure and arrange them in a series as was done for the chromosomes of figures 24 and 26. However, a glance at figure 25 B shows that such a series might be arranged.

The shape and size of both groups of chromosomes, and of the cytoplasm about them, are such that one can be fitted upon the other. Although these two relations are not positive evidence they indicate that one of these groups, possibly the smaller, has been separated from the other.

To summarize, it may be said that in the first case considered (fig. 22) it is certain that the smaller number of chromosomes is due to a loss of a part of the chromosome complex from the cell. Although the facts stated for cases 2, 3, and 4 may not be considered absolute proof, they do constitute a very strong probability, which closely approximates a proof, that in each of these cases a cell has been separated into two parts.

B. Somatic chromosome pairs

a. Introductory statement. Since Van Beneden's ('83) hypothesis that one-half of the chromosomes of an individual are of paternal origin and that one-half are of maternal origin there

have been many confirmatory observations and some that oppose it.

Montgomery ('01, p. 220) advanced evidence that for each of the chromosomes of maternal origin there is a homologous mate among the chromosomes of paternal origin, and that these homologues unite during synapsis. He also maintained that these pairs³ can be recognized in the spermatogonia. This view has been supported by many authors. Among these are Sutton ('02), who compared numerous camera-lucida drawings of spermatogonial complexes of *Brachystola magna*; Meek ('12 a), who measured the lengths of spermatogonial chromosomes of a somewhat wide range of animals, and Hance ('17 b, '18 a), who made linear measurements on the germinal and somatic chromosomes of the primrose, *Oenothera scintillans* and the pig. On the other hand, Meves ('11), on the basis of measurements made upon the spermatogonial and somatic complexes of *Salamandra maculosa*, fails to confirm the claim for the former and denies it (p. 282) for the latter. Della Valle ('12), who measured the chromosomes of peritoneal cells of the same form, also denies the existence of pairs.

Some of the somatic cells studied in *Ambystoma tigrinum* are quite favorable for a linear measurement of chromosomes, and these complexes have been used to obtain further data upon the query as to whether the chromosomes of the somatic cells form a duplicate series (based upon length and form) as is shown by their progenitors in the germinal line during the maturation period.

b. Mensuration. Since the possibilities of error in measurements are so great, it is necessary to consider the conditions

³ The two mates constituting a pair are usually of equal length, so that homologues may be recognized by such equality. In some cases, for example, in the Diptera, Stevens ('08, '11), Metz ('14, '16 a and b), Holt ('17), Whiting ('17), Hance ('17), the two members lie near each other or even closely approximated in the spermatogonia and somatic cells, while in many other cases, for example, in Orthoptera and Amphibia, the homologues may be widely separated in these cells. In the present paper the term 'pairs' will refer to the two chromosomes which are homologues as determined by length and form regardless of the relative position in the cell.

under which these measurements were made in order to judge their value correctly.

1. Type of cells. Only cells were used in which every chromosome was perfectly clear and, except as noted (p. 189), lay exactly level in the equatorial plane throughout their entire length. Only three cells (figs. 1, 3, and 9) of this quality were available, and these were polar views of early metaphase stages in cells of the peritoneum and lung. The chromosomes of one other cell (fig. 10) approximated this condition and were also measured. The care with which these cells have been chosen may be judged from the fact that they were the only suitable cells in material from over one hundred larvae containing large numbers of division figures. In material with chromosomes so long and so numerous it is not surprising that so few cells were perfect enough for measurement.

2. Method. In addition to choosing cells with chromosomes of the above character, three different camera-lucida sketches of each chromosome were made on different days with extreme care at a magnification of 2633 diameters. Each of these sketches was measured three or more times along the median line with an Ott compensating planimeter modified for this purpose, or with an opisometer. These nine determinations obtained for each chromosome were averaged to represent its length. This method is important because the extremes of these nine measurements in about one-fifth of the cases may differ 1 mm. from the average (and occasionally more). This demonstrates that one measurement upon a single drawing might give rise to an erroneous difference in the lengths of the homologues of some pairs ranging from 1 to 2 mm., the actual amount depending upon the respective errors in each homologue. Averages largely eliminate this error.

3. Sources of error. The various sources of error may be classified in three groups: 1) instrumental errors, 2) personal errors, 3) errors inherent in the condition of the material.

In the first place, it should be emphasized that no attempt has been made to determine the actual length of any chromosome. These measurements have all been made on the drawings

described above and have to do only with relative lengths. This fact eliminates at once a number of errors which would otherwise be very serious.

The instrumental errors. The possible instrumental errors are *a*) failure to maintain a critical illumination, *b*) failure to maintain a constant wave length of illumination, and *c*) errors inherent in the planimeter and opisometer.

It so happened that the strongest light was obtained a little above the point of critical illumination, and might therefore cause an error in measurement. However, several chromosomes were drawn a number of times under both conditions and no perceptible difference was observed. Any slight error overlooked would be equal in the homologous chromosomes and would not interfere with a relative measurement.

Farmer and Digby ('14) state that errors can arise from the use of varying wave lengths of light. The same optical equipment and illumination were maintained in all of my operations so that relative values were unaffected.

In making measurements with the planimeter the polar arm was held rigidly stationary in two grooved blocks so that the tracing point moved around in a circle having a diameter of 33 cm. The sharp tracing point was kept upon the median line of the chromosome figure by moving the drawing around (without slipping) into line with the path of this tracing point, which was used as a pivot for orienting the drawing. A constant, representing the value of each of the divisions of the vernier, was determined by measuring a series of known distances on a straight line.

The accuracy with which this instrument was operated is indicated by the fact that the average difference between the extremes of measurements made upon each of several drawings is 0.3 mm., the standard deviation, computed from the combined measurements of several drawings, is 0.17 mm. The measurements, obtained more quickly with the opisometer, are slightly less accurate, the average of the above extremes and the standard deviations being 0.4 mm. and 0.26 mm., respectively. Finally, the average of the nine measurements made upon the three drawings of each chromosome reduces this instrumental error

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to approximately zero. This error is, of course, inherently included as a part of the personal error discussed below.

Personal errors. Probably the greatest personal error was due to inaccuracies in making camera-lucida drawings. To reduce this error to a minimum, each chromosome, as stated above (p. 186), was drawn three times with extreme care. These sketches were made at the same point on the drawing-board so that any error due to different drawing distances and consequent differences in magnification was eliminated. The estimated median line of the sketch, upon which the measurement was made, was indicated with a lead pencil. The average deviation from the mean of the nine measurements is 0.6 mm. and the standard deviation, computed from combined measurements of several drawings, is 0.37 mm., which indicates that the instrumental and personal errors in the average of these measurements are practically zero for relative purposes.

Errors due to conditions inherent in the material. This class of errors is much more important than the preceding. The errors of this kind are an unequal shortening of the whole chromosome and a foreshortening of parts or all of the chromosome. Measurements made without very careful attention to foreshortening are of questionable value, for small amounts can give rise to large errors, especially in short chromosomes. Shortening is caused by a twisting of the chromatids about one another (figs. 1 to 8, 27 to 30). The amount of shortening in each twist of the chromatids, as determined by computation,⁴ at the mag-

⁴ The amount of shortening in each twist of a chromosome was determined by adding together the separately computed amounts of shortening due to the lateral deviation of the chromatids and that due to the vertical deviation of the chromatids. The shortening in each twist due to the lateral deviation was computed by averaging the lengths of the two chromatids of a chromosome and subtracting the length measured upon the median line of the whole chromosome. This total difference divided by the number of twists is 0.2 mm., which is approximately the amount of shortening due to the lateral deviation in each twist. In determining the amount of shortening due to the vertical deviation of the chromatids, the width or thickness of the chromatid, as determined with an ocular micrometer was assumed to be the amount of vertical sag of the chromatid in each twist. This thickness multiplied by the magnification amounted to 1 mm. This was used as the altitude of a right triangle, the base of which represented half of the measured longitudinal length of the shortened part, and the hypotenuse of which then represents very closely one-half of the true length

nification of the drawings (2633 diameters), amounts very closely to 0.4 mm. However, the effect of this condition is either completely or largely neutralized by equal or nearly equal amounts of twisting in the homologues of each pair. The maximum amount of error due to this cause may be judged by an examination of figure 28 which contains the most twisting. In this cell there are seven pairs in which the amount of twisting is equal and the error completely neutralized, two pairs containing an error of 0.4 mm., two pairs with 0.8 mm. error, one with 1.2 mm., and two pairs in which it is uncertain. Since these errors that occur at critical points will be considered individually later, no corrections for them are included in the measurements.

Foreshortening occurs only in certain chromosomes as indicated in figures 27 to 30 and 33 to 36. This is, however, in the cells represented by figures 27, 28, 33, and 34 so slight that the whole chromosome can be seen at one focus, the foreshortened part appearing only slightly hazy. In the cells represented by figures 29, 30, 35, and 36 it is a little more. Measurements with the fine-adjustment graduated wheel, made more accurate with a sharper pointer made of a pin, indicated this sagging to be not more than 2.5μ (one division of the fine adjustment wheel) in any case. Corrections⁵ made for this foreshortening are

of the shortened portion. Double the length of this hypotenuse minus the original measurement of the shortened portion is 0.2 mm., which is the maximum amount of shortening due to the vertical sag of any twisted portion. This amount added to that caused by the lateral deviation made the total shortening in one twist amount to 0.4 mm. Although this determination cannot be considered entirely accurate, it is a close approximation.

⁵ The correction was made as follows. The amount of vertical deviation was read from the fine-adjustment wheel when the objective was focused as nearly as could be judged upon the middle of the lowest and highest points of the foreshortened portions. All measurements for a given complex were made with the same part of the fine-adjustment screw, thus avoiding different pitches in the thread. The reading (2.5μ for each division) gave the actual differences of vertical positions. This multiplied by the magnification and divided by 1000 converted the figure into millimeters, the units made use of in the drawings. By using this distance as the altitude of a right triangle and the measured longitudinal extent of the foreshortened portion as the base of the triangle, the hypotenuse (which represented approximately the correct length of the foreshortened part) was determined. This was substituted for the original measurement of the foreshortened portion.

only approximate, because it is very difficult to determine accurately the amount of vertical deviation, and its longitudinal extent, as well as its exact course. In figures 27 to 30 and 33 to 36 corrected figures are used, and the amount included in each measurement for foreshortening is indicated.

There are two other conditions which do not give rise to actual errors in measurement, but do interfere with precision of results and may well be considered here. 1) A possible unequal contraction of chromosomes. Wenrich ('16) observes that chromosomes A and B condense before the other chromosomes in the spermatogonia and tetrads of *Phrynotettix*, and ('17) he shows that one homologue of chromosome 4, cell E, plate 2, contracts more rapidly than the other. 2) Since so many of the chromosomes of *Ambystoma* are so long and composed of two intertwined chromatids, there is considerable possibility of a stretching due to bending and other stresses still present in the complexes nearing the metaphase. As Meves ('11, p. 247) points out, under these conditions two chromosomes could be of different length and of equal volume. Even an imperceptible difference in diameter of parts or all of two chromosomes of equal volume might cause considerable difference in their lengths. This difference would of course be proportionally greater in the longer chromosomes so that measurements of the shorter chromosomes of a cell might strongly indicate the presence of pairs while the homologues of the longer pairs would show quite wide differences in length. A case of very perceptible stretching is to be seen in chromosomes 's,' figures 9 and 12.

Effects of technique. The differences which may arise in the chromosomes of different cells of even the same tissue due to different effects of fixatives, and all other effects of technique, do not affect relative measurements of chromosomes in the same cell. For it is extremely improbable that the lengths of chromosomes of the same cell which are so equally close to the surface of these membranes would not be similarly affected by the action of these various reagents and processes. It is also improbable that inherent differences among the homologues would cause a differential change of length under these conditions.

Summary. The combined instrumental and personal errors are reduced to practically zero by averaging several measurements made upon different drawings. The errors due to twisting of chromatids about one another are largely neutralized and are considered individually later. Therefore, except for possible unequal contraction and stretching of homologues, only the measurements of those chromosomes which are foreshortened contain appreciable errors. It is thought that these errors, after corrections have been made, probably do not in any case exceed 1 mm. The presence and amount of error due to unequal contraction and stretching in any particular chromosome is an uncertainty, but if existing would probably be greater in the longer chromosomes.

c. Results of measurements. 1. Criteria for determining pairs. Before considering the results of the measurements, it seems desirable to state what the criteria are that will demonstrate whether pairs (p. 185) are present among the chromosomes at these particular stages of mitosis. In the absence of definite minute morphological characteristics, such as a repeated occurrence of marked granules, constant in position and size, which Wenrich ('16) describes for certain Orthopteran chromosomes, the next most exact criterion for determining the presence of pairs in diploid cells would be a duplicate series of chromosomes of equal volume. But in these chromosomes trustworthy volumetric determinations cannot be obtained, for the above-mentioned intertwining of the chromatids and the stretching of the chromosomes would cause variations in diameter which could not be measured accurately, and these errors would be cubed in the volume. Consequently linear measurements, supported by form, have been chosen as giving more trustworthy data.

Upon this basis, in order to constitute undeniable evidence that the chromosomes form a duplex series, there are two conditions which should be met. First, when the chromosome lengths are plotted in a graph (e.g., figs. 33 to 37), they should definitely associate themselves in twos of equal lengths. Second, the differences in length between successive pairs, as indicated

by the first condition, should exceed the errors of measurement by a good margin. Unless the above conditions are met, the errors of measurement make it possible to contend that the chromosomes are arranged in a series of successively increasing lengths which bear no relation to one another and therefore do not represent pairs.

In addition to the above, the form of the chromosome, which is probably determined in large part by the position of the spindle fiber attachment, may be used as an aid in determining which chromosomes are homologues. McClung ('14, p. 674) pointed out that, although the spindle fiber attachment may be different on different chromosomes, nevertheless, for each chromosome "it is most precise and constant" in the individual. Carothers ('17, p. 470) has shown that for certain tetrads (e.g., figs. 32 and 63) the point of spindle fiber attachment on one homologue is different from that on the other. But she also finds that the point of fiber attachment is constant on a given homologue for each individual. She shows (figs. 32, 32a and 63, 63a) that the point of spindle fiber attachment on these homologues in the spermatogonia is preserved in the tetrads. However, an exception to constancy of fiber attachment in the individual has been noted by Wenrich ('16). He found in a rod-shaped tetrad of another genus that the fiber attachment might shift from one end of the chromosome to the other in certain individuals. Therefore, according to the theory of the individuality, in the somatic chromosomes the homologues of certain pairs of chromosomes may be expected to be unlike in form. However, individuals showing such conditions are very few and should be considered exceptions rather than the rule. There is the possibility that these heteromorphic homologues may not be confined to the Orthoptera, and certain cases in *Ambystoma* make this appear to be so.

Finally, it would be remarkable if any material satisfied the above criterion in all points. The small difference in length between some chromosomes makes it impossible to demonstrate beyond doubt the presence of pairs among them. Again, the possibility that the chromosomes may not condense at equal

rates, and that unequal stretching, especially between the longer chromosomes, may occur during mitosis, increases the difficulty in obtaining accurate metric comparisons, and may interfere with perfect certainty in the interpretation of the results. Furthermore, some variation is characteristic of living material and hence slight differences in relative length and form in different cells would be expected rather than absolute uniformity. McClung ('17, p. 567) finds in certain Orthoptera that the accessory chromosome, although unmistakably distinguished from the euchromosomes, is not always of the same relative length in different individuals of a given species, for it sometimes occupies the fourth and sometimes the fifth position in the series of lengths. However, it must be remembered that this is the sex chromosome which at the metaphase (the stage of greatest condensation of the euchromosomes) is already becoming diffuse. Differences in the degree of condensation might therefore be involved in the differences of relative lengths. And again, since individuals vary in their morphological characteristics, why should it be expected that the chromosomes of two different individuals should be of exactly the same relative lengths at the same stage of mitosis? In view of universal variability, homologous chromosomes, which are derived from different individuals and which may be expected to maintain their individuality, should not invariably be of exactly the same length. As discussed (p. 217), the observations on different Orthoptera by several authors indicates this to be so.

On account of these interfering factors it cannot be expected that homologous chromosomes will always be of exactly the same length at any particular stage of mitosis. Therefore, length and form, considered in a limited number of cells, from different individuals, cannot be regarded as conclusive evidence for or against the presence of chromosome pairs. Much more conclusive evidence would be had in a comparison of several somatic complexes from a single individual and with those of other individuals, a comparison of these with the diploid and haploid chromosomes of the germinal line, and a comparison of the complexes of parents and progeny.

However, although the measurements may not meet the above criteria in all chromosomes, there are certain cases which do meet them definitely, and strongly evidence the existence of pairs. This fact, together with the above consideration, makes it possible that all the chromosomes are in pairs.

Furthermore, it may be mentioned here that conditions which do not meet the above criteria fall far short of proving that pairs do not exist. The possibility still remains that two or more pairs may be of equal or nearly equal length. Such a condition is known to exist in certain Orthopteran chromosome pairs (Carothers, '17, pl. 1, tetrads 7 and 8) where the chromosomes are unquestionably known to be paired.

2. Evidence for the existence of pairs. On plate 9, figures 33 to 37, are five rows of vertical lines representing the relative lengths of the chromosomes of as many cells. The differences in the lengths of these lines and also the space between adjacent lines represent relative differences in chromosome length. For convenience the lines are made twice the length of the chromosomes as drawn and the width of the spaces are made eight times the difference in length. The lengths of the chromosomes, the amounts included for foreshortening, and the form of chromosomes for each of these cells are also shown, respectively, in figures 27 to 31 and 33 to 37.

A part of the evidence which these graphs present for the existence of pairs is three outstanding characteristics common to all of them. First, there is a graded series of chromosome lengths from the shortest to the longest; second, there is a marked sameness in the relative chromosome lengths of these cells which appears in the approximately constant presence of groups containing the same chromosome pairs, and, third, a similarity of form between homologues.

The pairs, in accordance with the above criteria, were determined primarily on the basis of chromosome length, supported by a comparison of form. The graphs and figures of each complex measured show that certain chromosomes are very probably homologues. In other cases a number of chromosomes are so nearly of the same length that, according to the criteria, the

homologues of the pairs cannot be determined with certainty, but they doubtless exist. Since the measurements of figures 33 and 34 are very nearly accurate and present the most reliable evidence, these will be considered separately from the others. For convenience the pairs may be considered in two groups, the first including those which differ greatly in length from their neighbors (pairs 1, 2, and 8) and the second including the remainder in which the pairs are not so clearly distinguished.

It is all but certain that the chromosomes represented in each of pairs 1, 2, and 8 in both of these figures are homologues, for they almost completely satisfy the criteria outlined above. In these pairs there is foreshortening in only one chromosome and in pair 8 the error of 0.8 mm. in both complexes due to twisting of the chromatids is negligible. The homologues are of approximately equal length, and the difference between each pair and the adjacent pairs is so much greater than the error of measurement that it is improbable that the condition represented by these three pairs in both complexes is merely a matter of chance. Furthermore, there is a close resemblance of form between these homologues. A comparison of other cells of the same individual, if available, would be expected to show that this condition is constant in all the cells as is shown by comparable cases of constancy in the germ cells of individuals of certain Orthoptera (p. 219). It can, therefore, be maintained with considerable confidence that these particular chromosomes of equal length and sameness of form actually constitute pairs. The measurements of the chromosomes of these pairs in other cells as discussed below give similar although less conclusive evidence.

Among the chromosomes of the second group in these two cells there is strong evidence for the existence of pairs, but the small difference in length and the errors due to twisting and possible stretching make it inconclusive. In figure 33 the homologues as shown in each of pairs 3 to 7 are so nearly of the same length and form (fig. 27) that one may believe that they constitute pairs as represented. Pair 3, in addition, is well separated from those adjacent. Although pairs 4 to 7 appear to be actual pairs, the chromosomes of this series differ so little in length that

the criteria adopted are not entirely satisfied. There is a chance of doubt, therefore, of the validity of the pairs as indicated.

As mentioned above, it will be noted that the groups into which the chromosomes of this cell are associated are repeated in the other cells. The similarity of grouping is very marked, especially in the formation of two large and distinctly separated groups, one containing pairs 3 to 7 and the other pairs 9 to 14. In pairs 3 to 7 of figure 34 the condition present in figure 33 is duplicated, except that pair 3 is not so well separated from pair 4, due to the fact that both homologues of pair 4 are relatively shorter in the former complex. Concerning the homologues of pair 6 there is some doubt. I have interpreted the end of chromosome 30 + (fig. 28) as bending back upon the main portion of the chromosome, and have estimated the length of this portion.

Of the remaining six pairs (9 to 14) in both cells several are quite clear, but on the whole the possibilities of error and the differences in length between successive pairs is too small to satisfy the second criterion fully. In figure 33, on account of the practical absence of twisting in pair 12 and adjacent pairs, the condition for determining pairs is very closely satisfied. The chromosomes of pair 9 were considered homologues through a process of elimination. They differ 12.4 mm. in length, but agree in form (fig. 27). This condition will be discussed later (p. 217).

In figure 34, pairs 9, 11, and 13, although not sufficiently separated to constitute an unquestionable demonstration of pairs, are fairly well separated and the homologues of each pair, after allowance is made for errors due to twisting, are of nearly equal length. One homologue of pair 10 (fig. 28) is imperfect. Pair 14 is well separated in the graph from pair 13. Approximate corrections made for the kink in the shorter member and for the slight foreshortening at that point make its length approximately the same as that of the longer member. The homologues of all these pairs agree very well in form (fig. 28), in spite of the fact that some may not yet have assumed their final shape.

The measurements represented in figure 35 are nearly as reliable as those of the preceding cells. The amounts included for very slight foreshortenings are indicated in figure 29. The relative chromosome lengths form approximately the same groups as those of the other cells, and the evidence for the existence of pairs is strong. As contrasted with figures 33 and 34, the chromosomes of pairs 1 and 2, when approximately corrected for foreshortening, do not entirely meet the conditions of the criteria, but their lengths and form strongly support the probability that they are homologues. The chromosomes of pairs 7, 8, and 10 differ somewhat in length. They do not appear foreshortened, and although possibly present there is no perceptible stretching in them. These differences may be due to an unequalness of homologues as discussed later (p. 217). On the other hand, the chromosomes of pairs 3 to 6, 9 and 11 to 13 are, except as noted in figure 29, unforeshortened and of equal length, they agree in form and present strong evidence for the existence of pairs. The greater part of the difference in length between the homologues of pair 14 is due to stretching.

The measurement of the chromosomes of the cell represented in figures 30 and 36 are somewhat less favorable for measurement than those of the preceding cells, because in addition to a moderate amount of twisting there is slight foreshortening in many of them. Although the lengths have been approximately corrected for this foreshortening (figs. 30 and 36) the measurements cannot be considered so accurate and reliable as those of figures 33 and 34. The relative lengths of the pairs closely parallels that of the preceding figures which results in a similar distribution in the series. As contrasted with figures 33 and 34, the chromosomes of pairs 1 and 2 fail to satisfy the criteria, and this is apparently not due to errors in measurement. The large difference of 5.6 mm. between the homologues of pair 7 recalls a similar difference in pair 9 of cell 33. Pairs 8 and 9 clearly satisfy the conditions of the criteria and the remainder of the pairs duplicate the conditions in figures 33 and 34.

The chromosomes of the cell represented by figures 24, 31, and 37 are foreshortened in nearly every case and were measured

only in order to learn whether they constitute a series which would indicate that they belong to one cell. Only one set of measurements was made. Consequently, the figures are not so accurate as those of the other cells. Pairs 1 and 2 are readily recognized because they are well separated from each other and adjacent pairs. Pair 8 which stood out clearly in figures 33, 34, and 36, occupies a similar position here, but its homologues according to these less correct measurements differ about 4 mm. in length. The relative positions of the pairs practically duplicate those of the other cells. I have not attempted to make corrections for foreshortenings, but as nearly as I can judge, the chromosomes of pair 1, if corrected for foreshortening, would differ in length a little more, homologues of pair 2 would differ less in length, and the homologues of pair 8 are foreshortened about equally. Approximately the same condition exists in the other pairs, so that the matching as indicated would not be disturbed sufficiently to alter the grouping of the pairs. In this cell chromosomes of pair 12 differ by approximately 10 mm., which recalls a second time the condition in pair 9 of figure 33.

Further consideration of the form of the chromosomes in all these figures furnishes additional strong evidence that the chromatin is definitely organized. As indicated in anaphases, the general form of the chromosomes in the metaphase of these somatic mitoses is determined by the point of spindle fiber attachment. The complexes represented in figures 27 to 32 are early metaphases, and the final form which the chromosomes will take is quite apparent, although in some cases (e.g., pair 8, fig. 27) it is not entirely clear.

In all of these figures, as previously mentioned, the form of the homologues of each pair is practically the same, even in cases where the final form has not been reached. Only three pairs (5, fig. 29; 7, fig. 30; 5, fig. 31) are exceptions, and this may be expected as indicated by a like condition in the heteromorphic pairs of certain Orthoptera (Carothers, '17). A comparison of several complexes from the same individual would probably show this condition constant for the individual as in the Orthoptera. A further comparison of each pair in any figure with

the corresponding pairs of all the other figures also shows a striking correspondence of form in these chromosomes. The homologues of the unusual cells represented by figures 31 and 32 may not be correctly determined, as previously mentioned.

Such agreement and constancy of form between homologues and between corresponding pairs of different individuals cannot well be considered as due to chance and indicates a definite organization of the chromatin.

3. Summary. The strongest evidence for the existence of pairs is the fact that the chromosomes indicated as pairs 1, 2, and 8 in figures 33 and 34 completely satisfy the criteria. Although these pairs fail to do so in figures 35 and 36, they are recognizable. Among those chromosomes composing the two large groups in which the chromosomes differ so little in length (pairs 3 to 7 and 9 to 14) the evidence presented by the lengths of the chromosomes does not conclusively demonstrate nor deny the existence of pairs because of the various factors inherent in the nature of the material. However, as represented in the graphs and figures, the lengths and forms of these chromosomes strongly indicate such a duplexity. The cases in which the chromosomes of a pair differ somewhat in length do not constitute contrary evidence since homologues are not always of equal length as explained on page 217. Furthermore, the constancy of chromosome number, the presence in all the cells of certain groups composed of the same number of chromosomes with approximately the same relative lengths is further strong evidence of a constancy of chromatin organization and that the lengths of the chromosomes are not due merely to chance.

It seems to me to be a very difficult task to demonstrate conclusively by means of measurements the existence of pairs in these and similar somatic complexes. To furnish anything more than strong supporting evidence is almost impossible because of the various difficulties inherent in the nature of the material and because of the fact that homologues, as shown in exceptional cases, are not always of equal length, a fact which has been actually observed in the germ cells (tetrads) of certain Orthoptera by several authors. The same conditions make it

just as difficult to demonstrate the absence of pairs. It seems to me that the evidence in the Dipteran somatic complexes, where the members of a pair lie parallel and adjacent to one another, together with the already large and well-supported evidence of pairs in the various generations of the germ cells throw the balance greatly in favor of the presence of homologues.

DISCUSSION

A. Introductory statement

The foregoing observations upon the constancy of chromosome number and the existence of pairs in the somatic chromosome complexes have their chief importance in their relation to the Roux-Weismann hypothesis that the chromatin is the idioplasm, which is differentially organized and linearly arranged, and that this organization is perpetuated. This hypothesis received important support from the theory of the individuality of the chromosomes as set forth by Van Beneden ('83) and strongly maintained by Rabl ('85), Boveri ('88, '02), and numerous other more recent investigators. The morphological evidence accepted as supporting this proposition is an essential constancy of number, size, form, and behavior.

Since McClung ('17) has so recently thoroughly considered the theory of individuality, this discussion is confined to the particular phases of the supporting evidence which are directly related to the observations made upon this material. These phases are essential constancy of number, of size, and of form.

B. Constancy of chromosome number

Della Valle has strongly attacked this theory on the basis of inconstancy of chromosome number. He arrives at the conclusion ('09, p. 120 ff.) that the number of the chromosomes is the quotient of the quantity of the chromatin divided by the average size of the chromosomes; that their size is variable according to the nature of the elements and the conditions in which they are found, and ('11, p. 188) that the size and number

of the chromatic elements are directly comparable to the size and number of the fluid crystals which are formed in a solution under different conditions.

These contentions he supports with the claim ('09) of a variation of nineteen to twenty-seven chromosomes in forty mitoses of the peritoneum, and ('11) by a very large variation in the blood cells of *Salamandra maculosa*. These observations he supplements with a long list of citations of chromosome numbers which he interprets as supporting his contention.

But, following an apparently frank and critical discussion of the accuracy of his observations in the peritoneal cells, he says ('09, p. 116) that he is only sure of his enumeration in twenty-five of the forty cells discussed. These twenty-five complexes he describes as being very clear. The range of variation in these cells is as follows:

Number of chromosomes....	19	21	22	23	24	25	26	27	
Number of mitoses.....	1	1	1	6	16	12	2	1	total 40
Number of mitoses.....		1	1	3	10	8	2		total 25

An examination of his descriptions and figures may indicate to some extent the reliability of these counts.

His count of 22 chromosomes was made upon a polar view of a very late anaphase (fig. 2) in which he states the smaller chromosomes in the center of the complex were beginning to go to pieces and becoming indistinct, and his only doubt is whether the chromosome numbered 18 is one or two chromosomes. But, judging from similar stages in my material, it seems to me that where the chromosomes are beginning to go to pieces in as crowded a condition as this must be, such a complex is not a safe object for an exact chromosome count.

Instances of this kind make it seem possible that conditions which he considers clear for an exact count might be much less conclusively clear to others, and that his drawings do not represent the actual conditions in his complexes.

Since his citations of chromosome number variations found in the literature have been discussed by Montgomery ('10), Wilson ('10), and by McClung ('17, p. 548 ff.), it is not necessary to

review them extensively. But Della Valle's attitude and his conception of what constitutes clearness and certainty may be better understood in the light of some of these citations of chromosome variations, especially in the Amphibia, which he presents as valid evidence of variation in chromosome number. He quotes ('09, p. 35) Flemming ('81, ['82] p. 51) and Rabl ('84, ['85] p. 248 to 250) as reporting variations of from seventeen to twenty-four in the gill epithelium of *Salamandra maculosa*. Flemming explicitly states (pp. 51 and 52) that in the three cells which admit an exact count there are twenty-four chromosomes, that in about twenty other cells he counted from seventeen to twenty-four, but was not certain of the number, and assumed that there were twenty-four. Rabl says ('85, p. 248) that up to that time only eleven unquestionable counts had been made and each of them showed twenty-four chromosomes. In no exact counts in any cell had a different number been found. Della Valle seems to think that Török's ('88) figures of erythrocytes of *Salamandra maculosa* show a variation. This work was not concerned with chromosome number and the figures were not intended to show the number of chromosomes in the cell. His citations of the work of Carnoy and Lebrun ('00) on *Rana temporaria*, and of Lebrun ('02) on *Diemyctylus* and *Bombinator* may be criticised because the authors were primarily concerned with other considerations and only gave approximate number determinations. Winiwarter ('00, p. 699), as cited by Della Valle, reports a variation of chromosome number in the rabbit; but he states that he is uncertain of his counts. The variations reported by Barratt ('07, p. 376), in proliferating epithelium of the rabbit are in pathological tissue, and, moreover, his counts are uncertain. Montgomery ('10) has shown that many other such citations are misinterpreted.

The above cases represent Della Valle's inexact and uncritical attitude in relation to data that seem to serve his purpose, and this creates the suspicion that his attitude interfered with the accuracy of his observations when he counted the chromosomes in his own material. This suspicion approaches a probability in view of the fact that numerous competent investigators who

have made extended and careful studies of the germinal and somatic mitoses of the same species mention no variation in chromosome number. Furthermore, Meves ('11), who attacks the theory of individuality, fails to substantiate Della Valle's observations, and it is not at all likely that he would have failed to mention any variation observed. He appears to believe (p. 296) that the number is constant. However, Heidenhain ('07, p. 176, figs. 80 and 81) shows a polar view of a late prophase and a lateral view of a metaphase with twenty-six and twenty-two chromosomes, respectively, and states that such irregularities *occasionally* occur. An occasional variation is not surprising, but variations as numerous as Della Valle claims to be present are unusual. Flemming ('90, p. 78) states that he observes in the lungs of *Salamandra maculosa* numerous atypical mitoses with very short chromosomes. He gives no further discussion and no figures to indicate what kind of cells they are nor whether they are normal. In the ten cells of the lung of *Ambystoma tigrinum* (table, p. 177) there were no variations in chromosome number, and with the exception shown in figure 26 I observed no abnormalities. Della Valle's ('11) figures of blood cells in *Salamandra maculosa*, which he claims show an extreme variation in chromosome number, appear very much like cells undergoing disintegration.

To the above evidence of the questionableness of Della Valle's results may be added the results of the sixty-six counts in *Ambystoma tigrinum* showing no variation in number. The important fact that these counts were made with extreme care (p. 177) in the somatic cells of the same and other tissues of a closely related species, and made in uncut membranes (which Della Valle emphasizes as important for accurate counts), further strengthens the already strong probability that his number determinations are incorrect.

There are certain characteristics in his figures that also indicate that his drawings are none too accurate. He notices that the chromosomes are twisted, but he does not show what constitutes the twist. That the peritoneal chromosomes of *Salamandra maculosa* are each composed of two separate chromatids twisted

about one another is plainly evident in Meves' ('11) figures 11 to 15 which show each chromosome to be of variable width. These figures are exactly comparable to my figures 1 to 8, and 9 to 23 which demonstrate that this variation in width is due to the twisting of the chromatids. Della Valle represents each chromosome to be of uniform width excepting an occasional split in the end of some chromosomes. If he does not see chromatids in any of the chromosomes which he has drawn, either his observations, his technique, or both are faulty. Furthermore, the above evidence together with his attitude make it uncertain whether his preparations were as clear or the chromosomes as distinctly separated from one another as his drawings indicate.

Finally, Della Valle's above demonstrated attitude, the absence of confirmatory evidence for his contentions, his questionable ability as an observer as indicated by his drawings, and the results of critical counts in *Ambystoma tigrinum*, all support the view that his observations and conclusions are incorrect.

But upon the assumption that they may be partially correct, there are some possible explanations for the presence of variation in the peritoneum of *Salamandra maculosa*. 1) One or more chromosomes of a complex easily could have been disturbed, as is evident from my figures 22 and 24 to 26. This could account for number deficiencies and perhaps also for excesses. 2) Champi ('13, p. 181) claims that chromosome number can vary by fragmentation under the influence of certain external stimuli. Della Valle ('09, p. 86) says the number of mitoses can be increased by keeping the larvae covered with a blue glass. If Della Valle did this, and if such a stimulus could produce fragmentation, a bare possibility is offered for a disturbance of chromosome number. 3) There is also a slight possibility that the larvae had been kept in captivity and might in consequence have been sufficiently pathological to produce abnormal mitoses. 4) In an investigation on certain Orthoptera now in progress in this laboratory, Mr. Carroll observes that in three individuals some of the few dividing spermatogonial cells contain, in addition to the normal number of twenty-three, one, and some two

extra chromosomes (dyads). This is a small variation in chromosome number in the individual of from twenty-three to twenty-five. In each of four individuals, including the above three, he finds that in from one to three of the primary spermatocytes observed in division, one of the eleven tetrads normally present is replaced by two separate dyads. Both of these dyads in the division of the cell may pass undivided to either daughter cell with or without the accessory chromosome. One of the secondary spermatocytes resulting from the division of the cell in which these two dyads accompany the accessory chromosomes receives twelve dyads plus the accessory and the other receives ten dyads. The other spermatocyte in which two dyads do not accompany the accessory chromosome gives rise to one secondary spermatocyte with ten dyads plus the accessory, and another with twelve dyads. This would make possible four classes of spermatozoa containing ten to thirteen chromosomes.

If, similarly, in *Salamandra maculosa* one of the twelve tetrads normally present in the spermatocytes and in the oocytes should be replaced by two dyads, there would be produced gametes with eleven, twelve, and thirteen chromosomes. These gametes would produce zygotes (individuals) having twenty-two and twenty-six chromosomes, respectively, a variation comparable to that claimed by Della Valle. Further, if extra chromosomes can thus appear in the germ cells of an occasional individual, the same might also occur in the somatic cells. But this variation should be expected in but few of the total individuals, making the proportion of cells containing the normal number greatly predominating. In Della Valle's counts about one-half contain the normal number which is far too small a portion unless such variation is much more common than is at present known.

Although the above is a clear case of a small variation in chromosome number *in the individual*, it must be clearly understood that these cases are exceptional and do not represent the normal condition. The chromosome number may vary in the species, but it is usually constant for the individual, as has been especially pointed out by Wilson ('09, '10), Carothers ('17),

McClung ('05, '07, and '17), and others. But it is very important to note that these irregular chromosomes arise and perpetuate themselves in a manner entirely consistent with a definitely organized chromatin and furnishes no support whatever for Della Valle's contention that the chromosomes are comparable to crystallizations of a salt solution and that their number in any cell is dependent upon the law of chance.

C. Variations in other Urodeles

Snook and Long ('14) find in the spermatogonial cells of *Aneides lugubris* nine containing clearly the usual number of twenty-eight chromosomes, and one cell with twenty-three. There are no other authentic reports of variable chromosome number in individuals of the Urodeles. The counts of Kölliker ('89), Fick ('93), and Jenkinson ('04) in the cleavage stages of *Axolotl* (*Ambystoma tigrinum*) were made for other purposes, and were not presented as accurate number determinations. Likewise, the counts of about eighteen to twenty-four, Champi ('13, p. 124) in several other Salamanders are only approximate.

D. Variations in other forms

Since a somewhat extensive tabulation of comparative germinal and somatic counts has been made and discussed by Hoy ('16), Harvey ('16), and briefly reviewed by Hance ('17 b), a repetition of this discussion is of little value. However, in the review of reported cases of variation a few general considerations have impressed me as worthy of mention. These may seem commonplace, but are evidently not altogether realized by some who are none too critical in their discussion of the significance of these enumerations. 1) As Montgomery ('01) long ago suggested, it is important to distinguish between variation in the chromosomes in the germinal line and those of differentiating somatic cells. In the germ cells I believe it can be stated safely that there are no certainly demonstrated variations in number which do not conform to a definite organization of chromatin. From time to time cases of apparent variation have appeared

and again disappeared when thoroughly understood (e.g., Metapodius Wilson, '10, and the sex group of *Ascaris lumbricoides* Edwards, '10, and multiple chromosomes of certain Orthoptera Woolsey, '15, Robertson, '16, and McClung, '05, and '17). 2) In considering the significance of variations, it should be remembered that there are normal and abnormal conditions (p. 202). 3) Metz ('16), Hance ('17 a, b), and Whiting ('17) have called attention to the necessity of proper technique. This is not always an easy matter to judge, especially in absence of material for comparison. 4) In determining the presence or absence of variation in any material, a very rigid line should be drawn between accurate enumerations and those involving varying degrees of interpretation, e.g., Winiwarter's ('00) cited variations in the amnion and omentum of rabbit embryos were uncertain and interpreted. 5) Finally, observers should maintain an exacting standard in distinguishing between that which is considered 'certain' and that which is interpreted. This is especially true in counting small chromosomes.

E. Fragmentation

Hance ('17 b, '18 a) found the spermatogonial number in the pig, and the diploid pollen-mother cells in *Oenothera scintillans* to be constantly forty and fifteen, respectively. But the somatic chromosomes vary from forty to fifty-seven in the pig and from fifteen to twenty-one in *Oenothera scintillans*. He presented metrical evidence that this variation is due to a fragmentation, probably of the longer chromosomes. He maintains that these fragments divide normally with the other chromosomes, and that therefore this fragmentation does not oppose the theory of the individuality of the chromosomes.

However, the probability that this variation in *Oenothera* is much less, and that most if not all of these fragmentations are invisibly connected with the main part of the chromosome is strongly supported by the conditions in *Ambystoma* material and by Hance's ('18 b) later observations upon additional *Oenothera* material from the same source. Both he ('17 b, p. 90)

and Hoy ('16, p. 356) review other cases of fragmentation in *Ascaris megalocephala* (Boveri, '99, '04), *Angiostomum* (Schleip, '11) and *Fragmatobia* (Seiler, '13).

In *Ambystoma tigrinum* (p. 179) and in *Salamandra maculosa* (Della Valle, '09, fig. 11), and as stated above, in *Oenothera*, the fragmented portions are directly in line with the main portion of the chromosome. This may be due to the absence of chromatin on the linin or failure of the chromatin to stain at that point. The fact that in several cases (e.g., *f*, figs. 5, 6 and 7) the space between the fragment and the main portion of the chromosome was uniformly faintly stained lends support to the suggestion. Other cases exhibited connections consisting of various amounts of strongly stained chromatin (e.g., *chr.f.*, figs. 5, 14, 15, and 21). All mitoses in the gill plates (the most in prophases, fig. 5) showed the largest number of instances of this condition; the peritoneum contained scarcely any. This might be explained as an effect of inferior fixation (p. 173) were it not for the fact that a considerable amount of apparent fragmentation is present, even in the metaphases of the tail epithelium which are fixed under the most favorable conditions. The reason for this is not clear.

F. The existence of pairs

The question whether the chromosomes exist in a duplicate series is significant in two respects: 1) in its relation to the mechanism of heredity as suggested by Janssen's chiasmatype theory and by the brilliant work of Morgan and his co-workers; 2) as a further index of the constancy of the organization of the chromatin. This constancy is vitally related to the theory of the individuality of chromosomes.

It will be convenient to consider separately the evidence of the existence of pairs in the germ cells and in the somatic cells.

a. Pairs in germ cells. Van Beneden's ('83) hypothesis, that one-half of the chromosomes of an individual are of maternal origin and that the other half are of paternal origin, has been verified in many cases. That this double set of chromosomes

exists in pairs in the germinal line is evidenced by their behavior during the maturation period.

Montgomery ('01) presented evidence for the recognition of pairs in the spermatogonia, basing his argument upon the significance of the chromosome number in *Euschistus*. Sutton ('02) showed by means of a comparison of many camera-lucida drawings of spermatogonial complexes of *Brachystola magna* that these chromosomes form a duplicate series of lengths, and by means of measurements with a pair of dividers that the chromosomes of the early primary spermatocyte prophases are graded into the same series of relative sizes. Meves ('11) interpreted his measurements upon spermatogonia of *Salamandra maculosa* as failing to demonstrate pairs. Meek ('12) has made linear measurements upon the spermatogonia and secondary spermatocytes of a number of animals, interpreting his results as confirming the claim of the existence of pairs. Robertson ('15, '16) also supports this view with metrical data in certain Orthoptera, and Hance ('17 b; '18 a) confirmatively interprets his measurements in the germinal and somatic cells of *Oenothera* and the pig. In unmeasured spermatogonial chromosomes of the Diptera, Stevens ('08, '10, '11), Metz ('14, '16), and Whiting ('17) show very convincing evidence of pairs for the homologues are associated side by side. Wilson's ('06, p. 11) figures of *Anasa* and Hoy's ('16, p. 336; '18) figures of *Anasa*, *Epilachna*, and *Diabrotica* also support this conclusion. The majority of other authors as a result of their general observations have expressed the belief that the chromosomes exist as a duplicate series.

Furthermore, the existence of pairs in the spermatogonia is practically proved by parasynapsis where the chromosomes of the last spermatogonial division unite side by side and remain so until separated by the reduction division. This statement is made possible by Wenrich ('16) who, besides confirming the already numerous and all but conclusive evidences of parasynapsis by Janssen ('05, '09), A. and K. E. Schreiner ('06, a and b; '08, a and b), Wilson ('12), and many others, carries the demonstration a step further by actually tracing a well-marked chromosome pair A (p. 76) continuously through every stage of

spermatogenesis from the early spermatogonia to the spermatids. He thus demonstrates that the conjugating elements are chromosomes and are morphologically identical with the spermatogonial chromosomes. That one of the homologues of each conjugated pair is maternal and the other paternal is very probable, as has been shown by the observations of Van Beneden ('83) and numerous later authors, especially Mulsow ('12).

It remains to be seen whether the pairs of maternal and paternal homologues present in the germ cells during the maturation period maintain their identity in the germinal line between the time of fertilization and the first observations upon the spermatogonia. This has been accomplished in part. Mulsow ('12) has followed the actual chromosomes of the living spermatozoon of a parasitic trematode, *Ancyracanthus*, into the egg, and has found the expected number of chromosomes in the two pronuclei and cleavage stages. He also observes that the chromosomes of the cleavage nuclei show in many cases a tendency to lie parallel to one another, and suggests that this is an approximation of maternal and paternal chromosomes. Boveri ('87, '92) traced the chromosomes of the primordial germ cell of *Ascaris univalens* through the cleavages from the two-celled stage, and Moenkhaus ('04), Morris ('14), Richards ('17) have traced the persistence of individual chromosomes through several cleavages of hybrid eggs of *Fundulus*. If this persistence of the chromosomes is permanently maintained, the observations of the above authors make it probable that the maternal and paternal chromosomes form a duplicate series throughout the germinal line.

b. Pairs in somatic cells. Since the existence of chromosome pairs can be considered to be all but proved throughout the germinal line, it remains to be seen whether or not the chromosomes of the somatic cells, which are really descendants of those of the germinal line, still retain this duplicate series and thus give evidence of maintaining their individuality.

The earliest observations bearing upon this question were made on *Salamandra maculosa* by Flemming ('82) and Rabl ('85), who observed that the chromosome segments were not of

equal lengths in the early spireme and metaphase stages. The general observations of many later authors, especially those studying Dipteran somatic cells (Metz, '14, '16 a, b; Hance, '17; Holt, '17; Whiting, '17) indicate these chromosomes to be paired. Observations of pairs in a large number of animals and plants have been extensively reviewed by Metz ('16, p. 245).

But no cases are recorded in which an attempt was made to determine accurately by measurement what relation the lengths of somatic chromosomes bore to one another until the work of Meves ('11). As a result of the discussion centering around the observations of Montgomery ('01) and Sutton ('02), he was led to attempt measurements in an effort to obtain more definite data, as suggested by Della Valle ('09, p. 109). He measured both spermatogonial and somatic chromosomes of various tissues in *Salamandra maculosa*. Della Valle ('12) made further measurements upon the same form, and agrees with Meves that their results do not confirm the observations of Montgomery and Sutton. Hance ('17 and '18 a) interprets his measurements upon the somatic chromosomes of *Oenothera scintillans* and the pig as confirmatory.

1. Meves' results. Since Meves has (p. 282) failed to confirm the results of other authors, it is desirable to reconsider his data in comparison with linear measurements upon cells of the same nature in the same kind of preparations and of the same tissues of another salamander, *Ambystoma tigrinum*, in an effort to form a judgment of the validity of his conclusions. For this purpose it is necessary to recall what criteria are required (p. 191) definitely to affirm or to deny the existence of pairs and under what conditions these criteria were satisfied.

The following conditions under which Meves' measurements were made allow the introduction of such a varying amount of error that the conclusions drawn from his results are of questionable value.

Instrumental and personal errors. Meves made his measurements evidently upon a single drawing, probably somewhat carefully executed, which means, according to a series of tests in my own attempts to be accurate, that he has a minimum instru-

mental and personal error of about 0.6 mm. at his magnification. This, of course, is negligible in comparison with the large errors arising from foreshortening in numerous chromosomes.

Concerning the favorableness of the spermatogonial chromosomes for measurement, Meves says (p. 274) that by no means do all of the chromosomes lie in the equatorial plane; without exception the bend lies in the plane while the ends lie outside; in the drawings such chromosomes seem shortened and therefore the measurements upon these chromosomes would give only an approximate value. Judging from these statements and from the magnitude of error due to the slight foreshortenings in my material, his measurements very likely contain errors which amount to as much as 4 or 5 mm.

For measurements of somatic chromosomes he chose (p. 280) polar views of the transformation stages between the prophase and metaphase stages in the epithelium of the gill plates (figs. 16 to 18) and extraordinarily well-flattened polar views of pro-phases (figs. 11 to 13) and metaphases (figs. 14 to 15) in the peritoneum. In the three prophases of the peritoneum the chromosomes lay nearly or entirely parallel with the upper surface of the cell. According to this description, it is evident that these three prophases are the most favorable cells, and even in these the chromosomes are not entirely free from foreshortening. The chromosomes of the other cells probably were more foreshortened. Therefore, judging from results in *Ambystoma*, his measurements contain errors due to foreshortening which probably vary from 2 to 5 mm.

The amount of the errors which are due to the twisting of the chromatids of these chromosomes is uncertain. Such twisting is evidently present, as indicated by the irregular contour of his chromosome drawings which are similar to those of my own. The errors due to this twisting may largely neutralize each other as explained above (p. 189). He mentions also the possibility of different rates of contraction of the chromosomes, especially in the earlier stages. Having before us the conditions under which Meves made his measurements, we are in a position to judge their value more or less correctly.

As Meves states (p. 276), the differences in length between the spermatogonial chromosomes are too small, and the possible errors too great to affirm that these chromosomes are present in pairs of equal length. On the other hand, it should be added that these conditions offer no evidence against this claim. Furthermore, he shows a practically constant difference in length of 6 half-millimeters between chromosomes 8 and 9 in his table of measurements. An examination of these figures shows unmistakable evidence that there is foreshortening in chromosome 9 in three of these cells, and a probability that chromosome 8 is much less, if any, foreshortened than chromosome 9. There results, therefore, in all of these cells a relatively uniform distribution of the chromosomes into two constant groups, a point which supports the claim that the relative lengths of these chromosomes remain constant.

Concerning the somatic measurements, Meves concludes (p. 282) "Die Fragen, ob bei gewöhnlichen Gewebszellen des Salamandra Chromosomenpaaren unterschieden werden können, ist bereits von C. Rabl ('06, S. 72) verneint worden, ich muss mich ihm auf Grund der mitgeteilten Zahlen anschliessen" and "Die erhebliche Längendifferenz zwischen chromosomen VIII und IX, welche wir bei den Spermatogonien festgestellt haben, besteht bei den gezeichneten somatische Zellen nur in der Hälfte der Fälle." This means, of course, that he believes that the chromosomes are not distributed into two constant groups in each cell and that therefore the evidence of the constant organization of the chromatin is lacking in this respect.

As already stated, the possibilities of error are so great that nothing is conclusively affirmed or denied by his measurements. However, an examination of the drawings of the chromosomes in his figures indicates certain probabilities.

1. In three of the four cells (his figs. 15 to 17) in which the marked difference between chromosome 8 and 9 is not present, chromosome 9 is conspicuously foreshortened, and is in reality longer than his measurements indicate. Furthermore, chromosome 8 in these cells may or may not be foreshortened, at any rate it is probably much less foreshortened than chromosome 9.

A conservative estimate of foreshortening upon chromosome 9 in these cells, based upon computation of foreshortening in my material, amounts to a minimum of 2 mm., which restores in these cells the difference in length between chromosomes 8 and 9 found in the spermatogonial and the other somatic cells. These same chromosomes in the remaining cell (fig. 13) which lack this difference do not appear foreshortened, but owing to the various sources of error pointed out in the preceding pages, it is entirely possible that such a difference may also be present in this cell. These considerations make it probable that the difference between chromosomes 8 and 9 is present in eleven and possibly in all of the twelve somatic cells which Meves measured. It is beyond expectation that this difference should be exactly the same in every cell whether of the same or of different stages of mitosis.

2. Meves also observes a difference of 3 to 4 mm. between other chromosomes which theoretically should be homologues. In figure 11 between chromosomes 17 and 18 and between chromosomes 23 and 24; in figure 12, between chromosomes 11 and 12; in figure 13, between chromosomes 7 and 8, 15 and 16, 17 and 18, 23 and 24.

Attempts to apply corrections for the foreshortening evident in these cells, estimated upon the basis of his drawings as compared with similar cells in *Ambystoma* (figs. 5, 13, and 14), leave the situation about as it was. This is not surprising, considering the difficulty of judging the amount of foreshortening that is conspicuously present in some chromosomes and the uncertainty of its presence, or absence, in others. Meves' statement quoted above concerning the type of cells used for measurements makes it quite possible that a great many of these chromosomes are foreshortened in amounts varying from 2 to 5 mm. This opinion is strengthened by his generalized and unprecise statement concerning foreshortening and by comparisons with similar cells in *Ambystoma*.

The conspicuous foreshortening in some chromosomes, and the probability of it in others, seriously weakens the validity of his measurements. This is especially true of figures 11, 12,

and 13 showing late prophases in which there is usually much foreshortening and considerable inequality of homologues, as stated above. In five other cells (figs. 14 to 18) Meves' figures show but one case of a difference of 3 mm. and only five cases of a difference of as much as 2.5 mm. between homologues, as they are indicated by his measurements. But the various sources of error already mentioned make it uncertain as to what the actual lengths are.

To summarize the examination of the results of Meves' measurements, it may be said, 1) that the chromosomes of the spermatogonial cells fall into two groups, one containing chromosomes 1 to 8, the other chromosomes 9 to 24. 2) In the somatic cells, when corrections are made for evident foreshortenings, it is probable that in eleven of the twelve cells, and possibly also in the twelfth, the same grouping is present. This indicates a constancy of organization of the chromatin. 3) It is impossible either to demonstrate conclusively or to deny that these chromosomes are paired because, *a*) of the various sources of error present and, *b*) the small differences in length in the majority of cases between adjacent chromosomes.

2. Della Valle's measurements. Della Valle ('12) measured the lengths of chromosomes in the peritoneal cells of *Salamandra maculosa* shown in his ('09) figures 1 to 3, 8 to 9, and 12. The length of each chromosome was obtained by averaging two measurements made with a curvimeter upon a single camera-lucida drawing. He also attempts to determine the degree of concordance between the measured lengths of each of these chromosomes and the dimensions which would exist if the lengths of these chromosomes were determined by the laws of fluctuating variation. These latter figures he obtains by calculation from a table of figures compiled by Sheppard and published by Galton ('07). He interprets his data as demonstrating, 1) that the chromosomes of *Salamandra maculosa* do not exist in pairs; 2) that there is no constant grouping of chromosomes, such as is evident in the measurements of Meves and myself, and, 3) that the chromosomes are a series of variants subject to the laws of fluctuating variation as shown by the comparison,

given in his tables and curves, between the measured lengths of the chromosomes and the computed lengths that would be expected if the chromosomes were such a series of variants.

I believe Della Valle's conclusions are incorrect for the following reasons: 1) He fails to demonstrate the presence of chromosome pairs because, *a*) as discussed on page 201, his chromosome enumerations are probably incorrect and therefore his measurements do not represent the actual conditions; *b*) his measurements probably contain numerous errors of varying magnitude due to foreshortening (as well as to errors arising from measurements upon single drawings) even though he chose for measurements strongly flattened cells (p. 126); *c*) the differences in the lengths of these chromosomes are so small and the errors so great that it is impossible either to demonstrate or to deny a presence of pairs. 2) Failure to find a constant grouping among the chromosomes would result from the causes given in (*a*) and (*b*). 3) His interpretation that the chromosome lengths are controlled by the law of fluctuating variations is untenable because, even if his measurements were reliable and whether pairs do or do not exist, the differences in length between the chromosomes of *Salamandra maculosa* are so small that the degree of correspondence between their measured lengths and the calculated lengths of a series of variants, corresponding respectively to each of these chromosomes, would be fully as close as those which he presents in his tables and curves containing numerous and large differences.

3. Results in *Ambystoma tigrinum*. The chromosomes of *Ambystoma tigrinum*, fortunately, are more favorable subjects for measurements than those of *Salamandra maculosa*, because the relative differences in length between many pairs is so large that certain pairs and certain groups of pairs stand out conspicuously. The evidence presented in figures 33 and 34 is free from all errors of measurement except those due to twisting of the chromatids and to minute foreshortenings at non-critical points in the series. These errors have been approximately eliminated (p. 188) and do not seriously disturb the critical evidence of the chromosome pairs which are much shorter or longer

than adjacent pairs. The evidence in the other figures is but little inferior to that of figures 33 and 34. However, measurements of the chromosomes of so few cells are insufficient to furnish more than strongly supporting evidence of the existence of pairs, and of an approximate constancy of size relations between pairs in different individuals.

It may appear that these measurements support equally well Della Valle's claim that there are no chromosome pairs, but that the chromosomes form a series of variants. However, the consistent evidence of the presence of pairs among the shorter chromosomes, the possibility of unequal stretching of the longer homologues together with the known condition in Orthoptera that homologues of tetrads may be of unequal lengths lends greater support to the probability of the existence of homologues.

The following points need further consideration.

Large differences in length between homologues. The difference in length of 12.4 mm. between the homologues of pair 9 in figure 33 and the similar difference of 9.5 mm. between the homologues of pair 12 in figure 37, the smaller difference in pairs 7 and 8, figure 35, pair 7, figure 36, and pair 8, figure 37, may possibly be explained as follows:

1. Unequal homologues have been reported in Orthoptera by Baumgartner ('11), Hartmann ('13), and more thoroughly studied by Carothers ('13), Robertson ('15), and Wenrich ('16). The latter's observations are particularly significant. He found different conditions of inequality in two of the small tetrads of *Phrynotettix*. He designated these two tetrads as 'B' and 'C' and traced their history from the pachytene stages through the first maturation division. The homologues of tetrad 'B' were unequal in eleven of the thirteen individuals studied and were equal in the other two. Tetrad 'C' was found in three forms, designated as 'C₁, C₂, C₃.' 'C₁' is composed of very unequal elements, the larger of which possesses a relatively large terminal knob or granule which is not present on the other two. 'C₂' is a pair with equal members, each of which appears to be homologous to the smaller member of 'C₁.' 'C₃' is a pair of unequal elements, neither member of which appears to be

exactly homologous to the components of 'C₁' and 'C₂.' The smaller member resembles those of 'C₂' and may be homologous with them. It is important to note that the three last-mentioned authors find that each particular condition is constant for the individual in which it is found.

Although tetrads with unequal homologues among the longer chromosomes have not been observed in the Orthoptera, they might possibly exist in other animals. The above observations, especially those of Wenrich, offer a possible explanation for the inequalities between homologues observed in *Ambystoma* as well as in *Salamandra maculosa*. Furthermore, the condition found in tetrad 'C₂' may offer a parallel explanation for the different relative lengths shown in some cases between corresponding pairs in complexes of different individuals (e.g., pr. 4 and pr. 9). Of course much further data from both the somatic and germinal chromosomes is necessary before the above can amount to anything more than a suggestion.

2. Certain inequalities might be explained as due to the presence of a multiple chromosome similar to that which has been described by McClung ('05, '17), in Orthoptera, by Boveri ('09), Edwards ('10, '11), and Frolova ('12), for *Ascaris megalocephala*, Boveri ('11) and Edwards ('11) for *Ascaris felis*, Stevens ('11) in *Anopheles*, and by King ('12) for *Necturus*. In these cases the sex chromosome has been interpreted as being attached to one of the euchromosomes, and thus there is present in the male an unequal pair of chromosomes which may parallel the condition in pair 9 of figure 33 and pair 12 of figure 37. In certain Orthoptera McClung ('17) finds that the accessory may be attached to different chromosomes in different individuals, which lends support to the possibility that this is the condition in *Ambystoma*. The presence of an X and a Y chromosome would also produce unequal homologues.

If either of these explanations be valid, such an unequal pair should appear in all the diploid complexes of approximately one-half of a somewhat large number of individuals, and similar conditions should be found in the maturation period. Unfortunately, the difficulty of obtaining a sufficient number of suitable

cells prevents extensive investigation of this point in the somatic cells for the present and makes this explanation only suggestive.

Constancy in the individual. It should be emphasized that the observations in the Orthoptera concerning the unequal tetrads (p. 217) and other heteromorphic tetrads (p. 192) strikingly demonstrate a constancy in the individual for the particular characteristic of each homologue concerned. In Ambystoma it has been impossible to obtain sufficient material to verify this point.

Whether the members of these Orthopteran unequal and other heteromorphic tetrads maintain their organization from one generation of animals to the next is yet to be demonstrated by breeding experiments now in progress in this laboratory. The expectation is that they do, since Wenrich ('16) and Carothers ('17) find every possible combination which would arise from the segregation and recombination of the members of these various types of tetrads.

The presence in the Orthoptera of unequal tetrads does not indicate a lack of individuality. On the contrary, the persistence of this condition throughout the individual, and perhaps from generation to generation, is strong evidence to the contrary. Of course a change has taken place at some time (if it be correct to assume that the homologues were all alike at some earlier period), but this is to be expected if these chromosomes are to parallel genetic behavior.

Bridges ('17, p. 445-6) presents parallel genetical data in connection with the chromosomes of *Drosophila*. He finds in certain cases that the genes for 'bar' eye and 'forked' bristles, whose loci are located near one end of the sex-chromosome, have been lost and that the region between these two loci has also been affected. He suggests that this deficiency may be due to a physical loss of this portion of the chromosome. He also reports ('19, p. 357) a case in which "a section of the X-chromosome, including the loci for vermilion and sable, became detached from its normal location in the middle of the X-chromosome and became joined on to the 'zero' end (spindle fiber) of its mate." In other instances the locus for sable alone, as

far as known, has been lost from one homologue and joined to the end of its mate. Another case is the transposition of a piece of the II chromosome to the middle of the III chromosome. He has exhibited definite cytological evidence (unpublished) supporting a part of the above. This condition produces homologues of unequal length which parallels the observations in the germ cells of the Orthoptera and the apparent similar condition in certain somatic homologues of *Ambystoma*.

c. Constant relative size relations. In addition to verifying Montgomery's ('01) and Sutton's ('02) observations of paired homologous chromosomes of equal length in the germ cells, Meek ('12), Robertson ('16), and Hance ('17 b, '18 a) confirm Sutton's ('02) observation (based upon comparisons of camera-lucida drawings of many spermatogonial cells and upon measurements of early prophase tetrads) that the proportional difference in size between any two pairs in one nucleus is practically the same as that between the corresponding pairs in any other nucleus. In *Ambystoma tigrinum*, as is seen in figures 33 to 37 and the table of percentages accompanying them, while the relative lengths are not exactly the same in every cell, there is in general a marked constancy of relative lengths. Were Meves' and Della Valle's measurements correct, the same would probably appear there.

d. Summary of measurements. The data here presented in connection with measurements upon the chromosomes of *Ambystoma tigrinum* and *Salamandra maculosa* cannot well be interpreted as a confirmation of Meves' and Della Valle's contention that pairing of the chromosomes and a constant organization of the chromosomes do not exist because: 1) Their data, on account of errors inherent in the material, are too unreliable to command confidence; 2) the differences in the lengths of the chromosomes of *Salamandra maculosa* are too small to permit one to deny or to affirm the existence of pairs, and, 3) it has been shown that because of obvious foreshortenings, individually mentioned above, which are unaccounted for in Meves' measurements, there probably is a somewhat constant difference between chromosomes 8 and 9 in eleven out of the twelve cells which he

measured. This confirms the contention of a constancy of chromatin organization so far as is possible in material having chromosomes differing so little in length as those of *Salamandra maculosa*. 4) The measurements of chromosomes in the somatic cells of *Ambystoma tigrinum* show duplication of sizes, especially pairs 1, 2, and 8 in figures 33 and 34, pairs 8, 9, and 10 in figure 36 and indications of the same in the other chromosomes of all the cells which differ too little in length to constitute reliable evidence. Explanations are offered for cases in which the homologues differ in length. 5) The chromosome lengths show approximately constant relative sizes in all of the cells measured.

Based upon the above considerations and upon the unequal and other heteromorphic tetrads in Orthoptera, my expectations are that the pairs and their relative lengths in the somatic cells of *Ambystoma* are constant for the individual, and although not exactly the same, they are approximately the same in different individuals. However, as stated above (p. 199), the measurements cannot be considered to demonstrate conclusively the presence of a duplicate series of chromosomes.

SUMMARY OF CONCLUSIONS

1. No variation is found in the somatic chromosome number of twenty-eight in *Ambystoma tigrinum*.
2. Della Valle's contention that variation in chromosome number is the rule is unconfirmed.
3. The chromosomes form approximately a duplicate series of sizes and forms, supporting the contention that they consist of pairs of maternal and paternal homologues.
4. An approximate constancy of size relations between pairs in the complexes of different individuals is also maintained.
5. Della Valle's claim that the chromosome lengths are a series of variants is not substantiated.
6. There is evidence of unequal homologues in these cells.
7. There is also a suggestion that there is a sex chromosome attached to a euchromosome.

8. There is apparently but little complete fragmentation of the chromosomes.

9. The above observations support the theory of the individuality of the chromosomes.

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PLATES

EXPLANATION OF PLATES

The drawings were made with the aid of a camera lucida, using a Zeiss 2-mm. apochromatic immersion objective, N. A. 1.30, and a Spencer compensating ocular $20\times$ which produced a magnification of 2633. The illumination consisted of light from a 100-watt frosted-globe Mazda concentrated-filament lamp passed through a daylight glass or a common cobalt-blue glass filter, and an Abbe condenser. The observer was shaded from the light in front of him and from the sides by a black-cloth screen.

In reproduction plates 1 to 8 have been reduced one-third, and plate 9 three-fourths, giving a final magnification of 1755 and 1316, respectively.

Figures 1 to 20 represent complexes of class I; figures 21 and 23, complexes of class II.

PLATE 1

EXPLANATION OF FIGURES

1 to 3 Very late peritoneal prophases.

4 A peritoneal metaphase.

The numbered pairs of chromosomes correspond to pairs bearing, respectively, the same numbers in figures 27 and 28, and in the legend of figures 33 and 34.

PLATE 2

EXPLANATION OF FIGURES

- 5 and 6 A gill-plate prophase and metaphase.
7 and 8 Metaphases of the tail epithelium.

5

6

7

8

PLATE 3

EXPLANATION OF FIGURES

9 to 12 Late prophase complexes of the lung epithelium.

The pair numbers correspond, respectively, to those in figures 29 and 30, and in the legend of figures 35 and 36.

PLATE 4

EXPLANATION OF FIGURES

13 to 16 Gill-plate complexes. The chromatids are shown only in figure 13.

13

14

15

16

PLATE 5

EXPLANATION OF FIGURES

- 17 to 19 Two prophases and one early metaphase of gill-plate epithelium.
20 A peritoneal metaphase.

PLATE 6

EXPLANATION OF FIGURES

21 and 23 Gill-plate prophases of class II. Chromosomes '*f*,' figure 21, interpreted as one; '*i*,' figure 23, as two (p. 12).

22 A peritoneal complex with part of the chromosomes missing.

24 A peritoneal complex separated into two parts. The pair numbers are duplicated in figure 31 and in the legend of figure 37.

23

24

PLATE 7

EXPLANATION OF FIGURES

25A Another peritoneal complex separated into two parts which are drawn in their relative positions. $\times 866$.

25B The chromosomes of figure 25A. $\times 1755$.

Chromosomes 14 to 17 are interpreted; note that the chromatids of these four chromosomes are well separated.

26A A lung epithelial cell separated into two parts which are drawn in their relative positions. $\times 866$.

26B Chromosomes of 26A. $\times 1755$. Pair numbers duplicated in figure 32.

25A

25 B

26A

26 B

PLATE 8

EXPLANATION OF FIGURES

27 to 32 Chromosomes of figures 1, 3, 9, 10, 24, and 26, respectively, arranged in pairs with pair numbers and figures indicating their lengths in millimeters at $\times 2633$. The amount included in any figure for foreshortening is indicated above that figure. The pair numbers are duplicated in the above figures and in the legend of figures 33 to 37, respectively.

PLATE 9

EXPLANATION OF FIGURES

33 to 37 Representing the lengths of the chromosomes in the cells indicated in the table below at a magnification of 1316. The differences in the lengths of the lines and also the spaces between the lines represent relative differences in chromosome lengths. For convenience the width of the spaces between the lines are made eight times the differences in lengths. The lengths of the chromosomes and their percentage of the average length in the cell is shown in the table below. The amount of foreshortening in any chromosome is indicated in plate 8.

PAIRS	FIGURES									
	33 (1, 27)		34 (3, 28)		35 (9, 29)		36 (10, 30)		37 (24, 31)	
	Milli- metres	Per cent	Milli- metres	Per cent	Milli- metres	Per cent	Milli- metres	Per cent	Milli- metres	Per cent
1	12.5	37	14.0	35	18.0	44	14.5	34	8.5	27
	13.0	38	14.5	36	18.5	45	17.5	41	10.5	33
2	15.1	44	17.8	45	19.0	46	18.0	42	14.0	44
	16.2	47	17.9	45	19.5	47	20.0	47	16.5	52
3	22.5	66	25.5	64	26.7	65	24.7	58	20.0	63
	23.3	68	26.9	68	27.5	68	25.0	59	20.0	63
4	26.5	77	27.8	70	29.0	71	27.0	63	22.0	70
	26.8	78	28.6	72	29.5	72	29.0	68	22.0	70
5	27.2	80	31.6	79	31.0	76	31.0	73	23.5	75
	27.2	80	32.1	80	31.6	77	33.0	78	23.5	75
6	27.6	81	30+	—	33.5	82	35.4	84	24.0	76
	27.6	81	33.0	83	35.5	87	35.5	84	24.0	76
7	28.4	83	33.6	84	36.5	89	36.5	86	25.0	79
	28.9	84	33.6	84	39.5	97	42.0	99	26.0	81
8	36.0	105	42.0	105	40.4	99	44.8	103	29.0	91
	36.0	105	42.0	105	46.0	112	44.8	103	33.5	106
9	40.4	118	49.7	125	47.5	116	49.0	116	39.0	124
	52.8	154	49.7	125	48.0	117	49.5	117	41.0	130
10	43.0	126	50.7	127	50.0	122	54.0	127	41.0	130
	44.0	129	52.3	132	54.0	132	55.0	130	41.0	130
11	44.4	130	53.8	135	55.5	136	57.3	135	43.0	136
	45.3	132	53.8	135	55.5	136	58.8	138	44.5	141
12	45.7	134	55.4	139	56.5	138	60.0	142	44.5	141
	45.8	134	56.8	143	56.5	138	61.3	145	54.0	171
13	48.9	143	58.7	148	58.5	143	62.2	147	47.0	149
	49.3	144	58.9	148	58.5	143	64.7	153	47.5	150
14	51.0	149	62.2	156	60.0	147	66.3	156	48.0	152
	52.0	152	64.0	161	62.0	151	69.3	163	49.5	157

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