THE MULTIPLE CHROMOSOMES OF HESPEROTETTIX AND MERMIRIA (ORTHOPTERA)

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EIGHT PLATES

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I. INTRODUCTION

Under the title "The chromosome complex of Orthopteran spermatocytes" ('05) I described certain unusual conditions in these cells, among them the union between the accessory chromosome and particular euchromosomes to form multiple chromosomes. At the time my material was limited and I was able to present only a partial account. Since then I have been accumulating a large series of specimens which have been studied both

cytologically and taxonomically. In the course of this study of the more extensive series of preparations a number of facts have been determined which were obscure in the early stages of the investigation. Although it is not yet possible to present a complete study of the two genera, owing to failure to secure cytological material from all the species, there are some facts known which should be presented in order to add what has definitely been determined and to correct errors in the earlier description.

Since the appearance of my former paper ('05) upon multiple chromosomes, a number of observations upon similar structures have been reported by different investigators. Some of these, such as the ones of Voinov ('14), are not clear in their nature, but recent papers by Robertson ('16) and his student, Woolsey, ('16) have made distinct contributions to our knowledge of chromosome relations which will certainly prove valuable. These are strongly confirmatory of the view expressed in my former papers ('05, '08) regarding the persistent organization of chromosomes even in the face of apparent numerical reductions. Very striking is the discovery of a structure, the octad, which I was not then able to find, but whose existence I anticipated so strongly that I gave it a name in advance of its actual observation. The occurrence of such a multiple chromosome I am now abundantly able to confirm, as well as the steps in its formation reported by Woolsey. The underlying principles of chromosome organization, permitting the anticipation of yet unobserved conditions, stands in as striking contrast to the conception of chance association of undifferentiated masses as does the periodic law of chemists to the vagaries of the alchemists.

In this reëxamination, advantage has been taken of the unusual opportunities for a study of the taxonomic characters of the two genera, especially *Mermiria*, offered by the splendid collections at the Philadelphia Academy of Natural Science under the charge of Messrs. Rehn and Hebard. Much good is sure to come from their active interest in all that concerns the group upon which they are specializing and from their cordial and generous coöperation with other students. By their very careful studies of large numbers of excellently preserved speci-

mens they reach conclusions regarding the relationships of individuals and groups which are soundly based upon well defined external characters and upon personal knowledge of the habits and distribution of the materials. Working quite independently of them I reach conclusions regarding the relationships of individuals and groups from the study of their germ cells and, in most cases, find that there is no difference in our estimate of these relationships. Since the discrimination between nearly related forms, upon cytological characters, sometimes reveals groupings that have been overlooked by earlier taxonomists, the feeling of confidence in the validity of the theories upon which such determinations are made is much strengthened. I feel confident that a full agreement between cytology and taxonomy depends only upon the quality of the criteria of differentiation and upon the accuracy of observation on the part of the followers of these two methods of gaining a knowledge of the organization of biological units of different degrees of complexity and extent.

II. CHROMOSOME CONDITIONS IN THE GENUS *HESPEROTETTIX*

1. *General observations*

When the observations on *Hesperotettix* were first announced I had only a few specimens, and, because of unfamiliarity with taxonomic characters, my assistants failed to distinguish the species and confused the sources of the material used for study. It was only when the germ cells were examined that it became apparent that all the specimens regarded as *H. viridis* did not belong to that species. Later and more careful collections enabled me to determine that *H. viridis* and *H. pratensis* were both represented. No difficulty is now experienced in discriminating between these species by germ cell characters. Together with *H. speciosus*, these represented the full extent of my acquaintance with the genus. Although the chromosome complex was, in its major features, consistently alike in the three species, I published no further observations, because I hoped to obtain preparations from the other North American forms in order to make the presentation complete. After waiting a number of

years I am now in a position to give a much fuller account of the chromosomal characters of the genus, although I have not yet seen all the species. Much to my surprise some of the new material shows marked departures from the uniformity prevalent in all my earlier slides. As a result, some of my generalizations are now rendered invalid, but it is hoped that fuller knowledge may make possible the formulation of principles having even wider application.

At present I have preparations from five different species, but in this article I shall discuss fully only the three of which earlier mention was made, for it is only in these that multiples have so far appeared. The number of specimens in each case, except *brevipennis*, is possibly sufficiently large to be representative.

2. *The complex of H. speciosus*

The observations recorded for *H. speciosus* are essentially correct and need only amplification here. It will be recalled that the accessory chromosome is united with one of the tetrads in the first spermatocyte to produce a hexad element. In the spermatogonium the union with one half of the tetrad also exists and is carried over into one of the second spermatocytes. This association was invariable in all the specimens studied, affecting always the same elements. So far as could be observed the unusual relations of the accessory chromosome did not modify its peculiar character and behavior in other respects. The striking appearance of a chromosome, one part of which is condensed and safraninophilous while the other portion is granular and tinged with the violet in Flemming's tricolor, is presented to our view in prophase and telophase of the first spermatocyte. As was pointed out at the time, an apparent reduction in chromosome number occurs without there being any real difference in this respect from other Acrididae. The diploid number of twenty-two becomes the normal twenty-three when it is noted that the accessory chromosome, instead of being free, forms one limb of a V-shaped element, easily distinguished among the

twenty-one ordinary rods. That there is here a true preservation of the physical identity of each member of the complex is evident from the continued characteristic behavior of the members which are apparently fused together. The conception of genetic continuity is therefore not merely formal, but expresses the actual morphological conditions of the species. Aside from the presence of the multiple chromosome the germ cells show no marked difference from most other Acrididae.

To complete my former account of the chromosomal characters of this species the following facts may be given: In the spermatogonium appear twenty-one rod-shaped chromosomes which are generally characteristic of the Acrididae. Besides these there is a V-shaped element with arms of unequal length (fig. 9, pl. 3). That this is not a simple euchromosome is indicated by the observation that one member becomes highly vesicular during the prophases. Such a condition is uniformly characteristic of the accessory chromosome under these circumstances and is sufficient to identify this arm of the V as the accessory chromosome. During the synapsis stage there is joined to this heterogeneous pair a third member, the homologue of the euchromosome portion. In the late prophase the accessory chromosome is precocious in condensation and appears as a dense, homogeneous rod, more or less bent, joined to a granular tetrad by endwise union. The contrast is most evident in preparations by the tri-color stain. In the first spermatocyte metaphase this hexad element goes on the spindle with its long axis parallel to that of the spindle and having fibers attached at the ends of the tetrad. This brings one fiber to the point of union between the accessory chromosome and the tetrad. There are visible, at this time, eleven separate chromosomes, of which ten are quite like the ordinary Acrididae type while the eleventh is the cane-shaped multiple (complex 6, pl. 1).

The anaphase groups resulting from this division each show eleven chromosomes, but in one there is a V which has no like mate in the sister group. This is constituted of the accessory chromosome and one half of the tetrad. In the second spermatocyte into which the V goes, the nature of this is again like that

of the spermatogonium—the member which was added during synapsis having been removed in the first spermatocyte mitosis.

The second spermatocytes are accordingly of the usual dimorphic character, one with the accessory chromosome—here permanently joined to a euchromosome—and the other without it. Since the V is made up of a dyad plus the accessory chromosome dyad, it is, in effect, a tetrad, but of unusual type since its parts are not homologous. It divides here just as do tetrads in the first spermatocyte, and the anaphase shows a V going to each pole along with the ten rods. The union of the accessory chromosome with one euchromosome is therefore not lost during all the changes of the maturation period, but persists into the spermatozoon and, by it, may be handed on to the female line of the next generation from which it passes into the male line upon the following fertilization.

3. *The complex of H. pratensis*

In this species, as in *H. speciosus*, there is uniformly present a multiple chromosome which differs from the *speciosus* form only in the proportion of its parts. Without some more definite criterion for the homology of the euchromosomes than we now possess it is not possible to say that it is the same member of the complex with which the accessory chromosome unites in the two species. If size only were used as a basis for judgment, then it would be necessary to say that different tetrads are involved, because the euchromosomes are much larger in *speciosus* than in *pratensis*. From the fact that the accessory chromosome itself may vary considerably in different species, it would appear that size is not alone a safe indication of homology. Leaving out of consideration the question of whether it is a particular one of the eleven tetrads with which the accessory is joined, we face the concrete fact that so far as our observations have gone, there is always such a multiple element present in the germ cells of the two species.

When we come to observe the character of the multiple element in the different generations of cells it is found to exhibit

throughout essentially the same features as mark its history in *speciosus*. The only observable difference is in the size of the parts. One individual, however, exhibits a variation in appearance of the multiple, which makes the conditions in *Anabrus*, already described ('05), directly comparable to those of *Hesperotettix* (fig. 23, pl. 4). Instead of the accessory being attached to the end of a rod-shaped tetrad it is joined to one extension of a ring. This relation appears in the prophase and is carried over directly to the metaphase, so that multiple chromosomes of very different shape from the ones commonly present are produced. Both forms appear, not only in the same animal, but also in a single cyst. It is probable on this account that the variation does not represent any fundamental difference, but only a divergence in some rate of movement of the parts.

The spermatogonial complex shows practically the same conditions which characterize the corresponding stage in *H. speciosus*. Minor differences of relative size of the elements are the only ones of note.

Figure 23, plate 4 exhibits the complex in the first spermatocyte. It is at once noticeable that the chromosomes are strongly of the *Hippiscus* type and in polar view appear as rings or V's lying in the equatorial plate. The only unusual feature is the tetrad, elongated in a plane of the spindle axis, with the accessory chromosome joined to one end at a more or less acute angle. The behavior of the chromosomes during division presents no unusual features and my general description of these types of chromosomes, given at length in a former paper ('14) may be held to apply here. The second spermatocyte mitosis likewise is typical for the euchromosomes, and the multiple chromosome behaves like the same structure in *speciosus*.

4. *The complex of H. brevipennis*

There is nothing in the specimens of this species, so far examined, to indicate the existence of any of the unusual conditions of chromosome association which occur in *speciosus*, *pratensis* and *viridis*. As may be seen by an examination of plate 3, figure

8, the complex is strictly of the *Hippiscus* type. There are neither multiple nor atelomitic¹ 1 chromosomes. It is possible that a more extensive series might show some variation, but this is doubtful. If multiple chromosomes are characteristic of the genus—which might fairly be said from their prevalence in the species so far studied—then *brevipennis* departs here from the type. Since this paper deals only with the question of multiple chromosomes, no further consideration will be given to species in which they are absent, except to note that the seriation in size is uniform.

5. *The complex of H. festivus*

This striking and characteristic species, of which numerous specimens have been studied, is like *brevipennis* and shows no trace of multiple chromosomes. The nature of the complex in the first spermatocyte may be observed in plate 1, complex 2. It seems therefore to have no immediate bearing upon the subject of the present discussion.

6. *The complex of H. viridis*

This species, like *speciosus* and *pratensis*, may possess a multiple chromosome, a hexad, constituted of a tetrad joined to the accessory chromosome dyad. The euchromosome portion is larger than those of the other species. This was the condition present in the first specimens which I studied and which served

¹ *H. viridis*. In a former paper I found it convenient to refer to various forms of chromosomes and to note that their forms depend upon the fiber attachment. Following de Sinety, the position of this attachment was designated as terminal, subterminal, or median. In the case of the annular chromosomes terminal attachment apparently results in rings whose plane in metaphase coincides with that of the equatorial plate, while with subterminal, or median, attachment the rings are so placed as to lie parallel with the spindle axis. For convenience these were spoken of as the *Hippiscus* type and the *Stenobothrus* type respectively. With the discovery of additional complexity of forms it has been found desirable to have general terms for the two conditions represented by the ring chromosome, and so it has been agreed in this laboratory to speak of chromosomes with terminal fiber attachment as 'telomitic,' while those with non-terminal attachment receive the name of 'atelomitic.' These terms may therefore be used to designate chromosome conditions similar to, but more general than, the ones involved in the two types of ring formation.

as the basis for my earlier discussion, although at the time they were not fully identified individuals. Now, with an extensive series secured for me by Miss Carothers, I find that the uniformity of association, so marked a feature of my early slides, has, for certain chromosomes, given way to an order characterized by multiples or non-multiples in equal proportions for the individuals studied. For any individual animal however there is no variation. But this species possesses added interest because of associations between euchromosomes to produce octad multiples in which the accessory chromosome does not occur. This is the first unquestionable instance of any such condition in the Acrididae, but its occurrence in a genus of the Locustidae has recently been reported by Miss Woolsey, working under the direction of Robertson. So clear and unequivocal is this condition in viridis that its interpretation can not be escaped, and that it should have failed to appear in my early material is most surprising. A careful restudy of my preparations makes it evident that no other multiples than those involving the accessory chromosome are to be found there. In some more recently prepared slides of earlier lots I was however somewhat chagrined to find individuals with the accessory chromosome free. These various modifications of the complex are most interesting and suggestive and merit extended consideration.

The following conditions of the first spermatocyte complex have been encountered so far (plates 1 and 2).

- Class (1)-12 separate chromosomes = 11 tetrads + the accessory dyad
- Class (2)-11 separate chromosomes = 10 tetrads + one hexad
- Class (3)-10 separate chromosomes = 8 tetrads + one octad + one hexad
- Class (4)- 9 separate chromosomes = 6 tetrads + two octads + one hexad
- Class (5)-10 separate chromosomes = 7 tetrads + two octads + one dyad
- Class (5a)-11 separate chromosomes = 7 tetrads + two octads + one dyad
and supernumerary
- Class (6)-11 separate chromosomes = 9 tetrads + one octad + one dyad

While the number of chromosomes varies from nine to twelve, the number of chromatids, morphologically recognizable, remain in all cases forty-six. The particular form of chromosome which I called an 'octad' in my former study ('05) presents itself very commonly in the new material.

A discussion of these various classes of complexes will serve to bring out most clearly the conditions prevailing in the species. Of these the first shows no unusual conditions whatever, and had specimens of this nature been the only representatives of the genus examined, they would have been regarded as coming from a group entirely typical of the family. There are the usual twelve chromosomes in the form of rings, V's, crosses and rods as found in *Hippiscus*, all of the telomitic type. The accessory chromosome is free and early passes undivided to one pole of the spindle. Of thirty-eight individuals studied, five had this chromosome constitution.

Seven of the thirty-eight belong to the second class and were the only kind of which I had preparations when my first study was made. Conditions here are as in class 1, with the striking exception that the accessory chromosome is joined permanently to one of the tetrads, forming a hexad element. The structure and behavior of this complex of eleven chromosomes instead of twelve, has been fully described and will not need further consideration here.

Very different is the aspect of the complex in the five individuals in class 3. Instead of twelve separate chromosomes as in class 1, or eleven like class 2 there are but ten. We recognize at once the hexad multiple of the second class and, in addition, eight telomitic tetrads of characteristic forms. The tenth element is much the largest and is otherwise strikingly different from its mates. In shape it is a much elongated ring of the *Stenobothrus* type, but, like the rings of *Chloealtis*, usually lacking any lateral extensions. Not infrequently there will be a separation on one side of the ring producing a C-shaped structure. The character of this element will be considered later in connection with other chromosome forms.

Members of class 4, of which I have seven individuals, show still further modifications. The number of chromosomes is reduced to nine, among which are distinguishable six tetrads of usual types, two large *Stenobothrus* rings and a hexad multiple. The two differential rings are of unequal size. The larger is directly comparable to the similar element of class 3, but the

smaller is always recognizable by its minor size and also by its variability of form. In this latter respect it may appear as a fully formed ring, or it may show varying degrees of separation at one of the polar angles, resulting in a large V-shaped structure. Any one individual animal however shows either a ring or a V and this does not suffer variation in any cells.

In class 5, represented by seven individuals, we encounter again the number ten as in class 3, but it is constituted in a different manner. The absence of the hexad multiple is at once apparent, for the accessory chromosome is free and occupies a characteristic polar position. Its sometimes associated tetrad appears among the seven chromosomes of this type and there are in addition, two large rings as in cells of class 4. So far as can be seen, these two elements are as directly comparable with the corresponding two of the preceding class as are similar structures within either of these classes.

To the complexity resulting from combinations of separate elements into multiples, thus reducing the number of independent structures, there is added the opposite condition of a supernumerary chromosome in the animal found in class 5-a. This has a complex similar in its composition to the ones in class five to which is added a small, extra element with the usual characteristics of the supernumerary chromosomes. For the third time there appears the number eleven, but its make-up is different from the complexes of either class 2 or class 6.

The number eleven reappears in cells of the six individuals in class 6, but once again there is lacking the hexad multiple characteristic of classes 2, 3, and 4. Nine ordinary tetrads, a free accessory chromosome and one large *Stenobothrus* ring make up the complex in this group. In place of the smaller ring, or V, of classes 4 and 5 there are present two rod-shaped chromosomes of corresponding size. The large ring occurs in five of the six individuals and is directly comparable to the element falling in the same place in complexes of classes 3, 4 and 5. The sixth specimen of this group has, in place of the ring, a large V of the same construction as the smaller octad in class 4. This is the only individual in which the larger tetrads were not united at

both extremities and, until it appeared, I was inclined to believe that the unipolar union, rather common in the case of tetrads nine and ten, was lacking and that some modification of the association force existed. It is now evident that the conditions are essentially the same in both cases, except that the more frequent occurrence of the large ring and its complete character would suggest a longer history of multiple constitution.

III. CHROMOSOME CONDITIONS IN THE GENUS *MERMIRIA*

At the time of my first report ('05) upon *Mermiria* I had studied all the available Acrididae species and, in every individual, found only one type of chromosome—a telomitic rod in the spermatogonium, which, after synapsis with its mate, appeared in the form of a Hippiscus-type ring or in some modification of the type. Although familiar with the atelomitic form of chromosomes in other material, I had never seen it in the Orthoptera and had no reason to suspect its occasional appearance. Failure to consider the possibility of such chromosome forms was responsible for my misinterpretation of the multiple chromosomes in *Mermiria*, through which I made the serious error of reporting the segregation of whole tetrads. Although, with increased knowledge of the conditions in the group, it is now possible to determine with certainty the nature of the multiple chromosomes, at the time of my first acquaintance with the phenomenon of chromosome combinations the constancy of chromosome structure and behavior was so marked as to make quite unjustifiable any assumption of variation. Added to this is the fact, which seemed at the time very significant and which even yet is not satisfactorily explainable, that in certain individuals the multiple has a series of definite constrictions corresponding in number and position to what would exist in a deced element. Finally; actual separation in the first spermatocyte metaphase at the level of these constrictions in certain cells persuaded me of their value as an indication of chromosome boundaries. The theoretical difficulties involved in explaining the preservation of the complex on the assumption of tetrad segregation finally convinced me that I was probably mistaken, but

it was not until the discovery of the J-shaped chromosome in *Trimerotropis* by Miss Carothers that any explanation, consistent with the other known conditions in the group, was made possible. Meanwhile I had accumulated an extensive series of specimens and had studied the complex of the female and needed only the conception of the J-shaped tetrad to bring all the observations—with the exception just mentioned—into conformity.

In *Mermiria*, as in *Hesperotettix*, the fixity of combinations which seemed to mark the genus has failed to prevail throughout all its species. While such variation as is shown by *H. viridis* does not obtain in any species of *Mermiria* studied, multiples are lacking in *texana* and *neomexicana*. These results emphasize strongly the necessity of an extensive series of specimens in any investigation—a requisite I have always appreciated and sought to meet. Our recent work in the Orthoptera has shown that numbers of considerable magnitude are required for extensive generalizations.

Mermiria bivittata

It is not my purpose in the present paper to enter into a comparative history of the chromosomes in the various species of *Mermiria*. I wish merely to consider the multiple chromosome found in *bivittata*, with its modifications in certain groups of individuals which seem to have specific value. My former report on this species stated that the multiple chromosome consisted of two tetrads joined to the accessory chromosome. This is a mistake the occasion for which is discussed elsewhere. More extended study upon a large series of specimens has shown that in this genus, as in *Hesperotettix*, there is a hexad multiple instead of a decad. While there is a striking difference in configuration of the element in the two groups, it is due entirely to the form of tetrad involved. In *Hesperotettix* this is an ordinary extended rod with the accessory chromosome joined at approximately a right angle on one end. *Mermiria* has, on the contrary, a tetrad already of just this shape, to the straight end of which the accessory chromosome unites. Thus, while the composition is the same, the appearance is very unlike. How

this configuration is established, appears very definitely when the chromosome complex of the different cell generations is studied.

Conditions of the spermatogonial metaphase are presented in figures 44, 46, 47, 49, pl. 6. First it may be noted that the number of chromosomes present is twenty-two instead of the usual twenty-three, and that, of these, two differ from the remainder and from the usual spermatogonial rods in being atelomitic V's, with more or less unequal arms. Even a casual study of these differential elements reveals their divergence in both size and structure. One is distinctly larger, and, under favorable conditions, it may clearly be seen that its shorter arm is irregular in outline and more lightly staining. In these respects it conforms to the behavior of the accessory chromosome in many Orthoptera. So characteristic are these appearances that there can be no doubt whatever in the identification of this arm of the V chromosome as the accessory. The V chromosome, then, is a multiple, a tetrad, consisting of the two rod-shaped chromatids of the accessory chromosome joined to the two corresponding rods of a euchromosome, just as in *Hesperotettix*. At the angle of the V, where the fiber is attached and where the chromosomes join, there is, not infrequently, a clear break in outline. The compound nature of this V is clearly evident from these facts.

Inspection of the smaller V reveals no such indications of heterogeneity, and only in shape does it differ from the remaining elements of the complex. Its position in the metaphase plate is commonly near the multiple V. For convenience of comparison several of these pairs of V's in different stages are shown in figures 47 and 49. The number of chromosomes present in the complex is therefore found to be twenty free telomitic rods of various lengths, two rod chromosomes joined at their inner extremities forming a V and one other V-shaped chromosome—a total of twenty-three, the number characteristic of the family. The internal morphological evidence of the complex in this generation is sufficient proof to establish this conclusion. In addition however we have two confirmatory lines of evidence which are of great value, viz., the subsequent history of these structures in spermatogenesis and the conditions of the female

diploid complex. Before following out the later spermatogenetic history I will briefly indicate the conditions in the female cell.

Unfortunately it has not been possible to work out the history of the female germ cells, but the egg follicle cells show beautiful, clear chromosome groups, some of which are represented in figures 43, 45 and 48. It is at once observable that the resemblance to the male diploid group is very marked. Here again there are twenty-two chromosomes, among which are also two V's. A more careful study however reveals a significant sexual difference in the case of the two V's. Instead of being unlike in size they are of practically identical proportions. Owing to their somewhat sinuous course through the cell, with consequent foreshortening, it is difficult, if not impossible, to represent them accurately in drawings. Where they lie more nearly in the same plane, as in figure 43, pl. 6, their equivalence is clearer. Under the microscope there is no difficulty in appreciating the close resemblance existing. On comparing these two V's with the ones of the spermatogonium (figs. 47, 49, pl. 6) it is seen at once that in size and proportion they agree with the larger one of the male cells. In other words these are two multiples, the short arms of which are the accessory chromosomes. The count for the female complex, instead of the apparent twenty-two, is therefore twenty-four, corresponding to the conditions in other species. No differential behavior of the sex chromosomes was observed in these female somatic cells and but little evidence of separation at the point of fusion.

Other species of *Mermiria* show no multiple chromosomes in the first spermatocyte, and when the spermatogonial complex is observed there are found twenty-three rod shaped chromosomes of the usual type. Absence of a multiple chromosome in the spermatocyte is accompanied by the absence of V-shaped chromosomes in the spermatogonium. Animals of this type are not classified as *bivittata*, but, quite aside from their exact taxonomic disposition, it is plain that they must be very nearly related to *bivittata*. In such material, then, the direct relation between V chromosomes of the spermatogonium and multiple chromosomes of hexad nature in the first spermatocyte is

strongly suggested. Because of the constancy of chromosome organization, indicated among other ways by the constancy of fiber attachment, we would be justified in saying that the V's of the spermatogonium unite in synapsis to form the first spermatocyte multiple and that this should appear with two non-terminal fiber attachments. Such a condition is realized in the structure of the hexad in the first spermatocyte metaphase.

The conditions of the first spermatocyte complex in metaphase are readily determined, particularly if sections of sufficient thickness are used, and in smears are almost diagrammatic. Here there are clearly eleven chromosomes present, among which is a very large and distinctly different shaped one (figs. 55, 56, 57, pl. 7). This is characteristically in the form of a rod with the two ends of slightly different length bent sharply back in the same plane. One or both of the ends may exceed, or fail to reach, the common angle, producing some variety in form. Greater or less extension, preparatory to division, may result in considerable variation in length, but commensurate and opposite changes in diameter show that the volume remains very constant. Indications of internal composition are afforded by the contour of the element, although, as will be shown later, they are not entirely trustworthy. At each of the bends, where the fibers attach, there is a constriction, and nearer the shorter bent end, at about its length down the shaft, there is a pronounced fissure.

At the time of division, separation occurs at this point, producing two unequal V's in the anaphase. Although the chromosomes are much shorter and thicker than in the spermatogonium, relative proportions are preserved, and if these two parts of the long chromosome (figs. 47*a*, 50*a*, pl. 6) be compared with the two V's of the spermatogonium they will be found to correspond almost exactly. That is to say, two V-shaped chromosomes of certain proportions found in the spermatogonium reappear in the first spermatocyte (united by one limb) and are there separated at this point and segregated into different second spermatocytes. Since one limb of one V is the accessory chromosome, it remains undivided, as usual. Attached to the accessory chromosome is the rod portion of a J-shaped chromosome which

has separated from its V-homologue to which it was joined in synapsis. The only differences between *Mermiria* and most other Orthoptera are (a) the multiple chromosome V and (b) the euchromosome V. That the union of the accessory to a euchromosome is not a fundamental change of the nuclear state is indicated by the fact that certain species of *Mermiria* lack the association. No change in the distribution of the accessory results from its union, and, as we have seen in *Hesperotettix viridis*, the combination may be so weak as not to occur in some individuals.

Evidence that the euchromosome V is of like transitional character is not wanting in certain well marked members of this loosely constituted species. In these (figs. 58, 59, pl. 7) the multiple chromosome of the first spermatocyte metaphase is much like that of *Hesperotettix* except in proportions. There is a pronounced bend at one end at the point of fiber attachment, but the other extremity is almost straight, only a slight sub-terminal flexure indicating the place of the other fiber insertion. In some instances this point is almost at the end of the chromosome. Individuals with this peculiarity are clearly distinguishable by somatic characters and, I believe, constitute a distinct species. It is possible that, with fuller representation, forms similar in the constitution of their multiple chromosomes to those of *Hesperotettix* might appear.

This type of hexad caused me much difficulty and led to the conception of a decad chromosome. As will be noticed in figures 58 and 59, pl. 7, there are a number of constrictions along the length of the chromosome quite constant in position, and so placed as to indicate that there are five divisions or parts. It will also be observed that the chromosome may be divided at more than one of these levels. I can not now find any explanation for these separations at various levels on the chromosome, but that they are evidences of unions between various tetrads is not indicated by the full history of the chromosome complex. Again, in the anaphase and telophase of the first spermatocyte, the composite nature of the larger V becomes marked. As may be seen in figure 13 of a former paper ('05), one member of the

V consists of two granular rods of the same character as the remaining chromatin elements, while the other arm is composed of two dense and homogeneous rods of smooth contour. All these parts tend to diverge widely except at the level where the unlike portions join, which is the point of fiber attachment in the preceding cell generations and its site in the one to follow. This difference in constitution is the reverse of the one in the spermatogonium, but corresponds to the relative degree of concentration of the nuclear elements in the first spermatocyte prophase. The interval between the two spermatocyte mitoses is very brief and the chromosomes may be followed through their changes without any loss of identity.

The metaphases of all second spermatocytes show eleven separate chromosomes of which one is a V. On observing these V-chromosomes (figs. 63, 64, pl. 7), however, it is found that they are of two sizes which correspond in proportion to the two V's of the spermatogonium and to the two of the first spermatocyte anaphase. Upon division these are distributed to the spermatids which are accordingly of two classes, equal in number. It is clear from this very evident history of the two V-chromosomes that they go into different spermatozoa and so, upon fertilization, are contributed to different individuals. Of some theoretical interest is the fact that the rod-shaped homologue joined to the accessory chromosome has a criss-cross inheritance while its V-mate is confined to the male line. The presence of the accessory chromosome as a portion of one V means the addition of one chromosome to the count of eleven separate elements, and so conforms to the conditions in other Orthopteran species.

IV. GENERAL CONSIDERATIONS

The very extensive and detailed studies of chromosomes which have been in progress for many years would not be fully justified if the result were merely a record of interesting but non-significant protoplasmic manoeuvres. It is the belief that the substances of the chromosomes are specific materials which are intimately concerned with the development of a multitude of

dissimilar cells from a single cell that renders a knowledge of the finest details of their structure and behavior of the utmost importance. That this belief is well founded would seem to be clearly indicated by cumulative evidence from many sources. The fundamental concept involved is the Roux-Weismann hypothesis that the chromatin is the idioplasm, which is differentially organized and linearly arranged. As an indication of this differential organization and linear arrangement, the existence of definite aggregates of the chromatin substance into more or less thread-like chromosomes is regarded as most important.

Details of chromosome structure and behavior are significant, therefore, as indexes of the precision of organization in the material of which they are composed. Since it is the existence and perpetuation of this definite series of differentiated materials that is primarily required by the hypothesis, it is conceivable, and possible, that it may vary in the nature of its aggregates into definite masses (chromosomes) without affecting the manifestation of its various specific effects, except in their combinations. It is not the existence of a certain number of these aggregates that is of first importance, but the presence of the varied materials which enter into their composition. While this is true, it is to be expected that a somewhat exact correspondence should be maintained between the ultimate units of differentiated substances and the units of higher order into which they are assembled. But such a fundamental arrangement may be maintained in the presence of both lower and higher numbers of chromosomes through secondary combinations into units of still higher value in one case, or through duplications of the normal series in whole or in part, in the other. Numerical variation is not of itself *prima facie* evidence of altered organization—it must be shown that something of the complete series is lost, or new and unrepresented materials added, in order to demonstrate the existence of altered organization. The maintenance of the morphologically recognizable units of the original series in the face of changed conditions is indeed added proof of the exactness and stability of structural conditions in the chromatin substance. Evidence of a very important character in support of this position is fur-

nished by the facts disclosed in the study of *Hesperotettix* and *Mermiria*. For convenience these facts may be taken up under a number of different headings.

1. Chromosome numbers in the Acrididae

It is claimed by those who criticise the so-called chromosome theory of heredity that the maintenance of the specific number of chromosomes is required and that variations in number are direct disproof of the theory. There is an element of truth in this argument which has always been granted by students of cellular phenomena, but stated baldly and without reservation it may lead to entirely erroneous conclusions—indeed has done so in conspicuous instances. The fallacy in the argument lies in the circumstance that a primary organization may be maintained while having superimposed upon it secondary modifications. Instances of this are very common in organic structures. It is no argument against the reality of the pentadactyl type of limb that duplications or combinations or reductions occur in certain groups or individuals. Polydactyl or syndactyl individuals reproducing their modifications of the primitive type are not regarded as illustrations of the absence of a fundamental organization of limb bones. The diplopod condition is rightly considered as secondary to the usual arthropod arrangement—not a disproof of its existence. While it is true that these instances of organization are of a different order from those found in cellular structures they are true examples of meristic variation in individuals in the presence of a persistent type. All that is claimed by those who believe in the hereditary significance of nuclear structures is that the chromosomes are, in themselves, indications of structural organization in the materials of which they are composed. Provided the full complex of elements be preserved, the essentials of idioplasmic control of development exist, even in the presence of combinations or duplications. From this point of view, in the event of apparent variation in chromosome numbers in an individual or group, it is essential to discover whether any of the normal chromatic units are lost or others of different

nature added before it can be established that there is not maintained a specific organization.

The belief in such an organization as this has, for brevity, been called the theory of chromosome individuality. This has perversely been much misunderstood or misrepresented. According to some the theory demands an 'independent existence' of the chromosomes, although how this could seriously be maintained in the entire absence of any independently existing chromosome or claim for such, it is difficult to see. In the minds of others the theory demands that the chromosome shall 'make the cell'—whatever that may mean. Of course no such views are entertained by any cytologist and no fair interpretation of the theory of chromosome individuality would give occasion for such statements. Because it is desired to examine the nature of the evidence in favor of this theory, under conditions which might apparently controvert it, a statement of the facts, involved and an outline of the theory may be given to avoid useless discussion. They might take this form in the present state of our knowledge (1) The cell is a complex of organs having various functions; (2) of these the nucleus, is most concerned in reproducing the characters of the cellular organization; (3) of nuclear substances those involved in the structure of the chromatin are most important in carrying out this function; (4) these substances are likewise differentiated and have various rôles; (5) an indication of this differentiation is afforded by the aggregation of these substances into certain definite masses which are characterized by individual peculiarities of size, form and behavior; (6) these aggregates maintain their organization and reproduce themselves in each cell division; (7) their derivatives are characterized by similar attributes of size, form and behavior under given conditions; (8) such complexes therefore occur in all the cells of an individual, both germ and somatic; (9) because members of a species are related by descent their complexes are essentially the same; (10) the fact that a type of organization, which holds invariably for the individual and almost as constantly for the species, prevails with slight variations through genera and even a family is very strong evidence for exactness of organization:

(11) the behavior of the chromosome in maturation and fertilization is strictly in accord with the necessary mechanism for alternative inheritance; (12) the inner constitution of the chromosome is such as to afford an explanation for the linear arrangement of factors and for their variations; (13) in the instance of the most thorough analysis of characters in any one animal, *Drosophila*, the groups of linked characters and their magnitudes correspond to the number and size of the chromosome; (14) experimental disturbance of chromosome conditions is followed by the expected modifications of characters during development; (15) from the known conditions of nuclear organization in relation to character development predictions with regard to new characters and combinations of characters may be made.

As has been stated before, the number of diploid chromosomes in the various species of *Acrididae* is usually twenty-three in the male. Exceptions to this have been announced by Granata ('10) for *Pamphagus* and by various authors for *Chorthippus*, and by myself for *Hesperotettix* and *Mermiria*. Montgomery gave the number twenty for *Syrbula acuticornis*, but this is unquestionably an error, as Robertson has shown. In no case has a variation been reported for cells of the individual. The gametic complex therefore maintains itself, according to the reports of all investigators. Similar constancy prevails in the species, in almost all instances, except for the wide variation of eighteen to twenty-three here reported for *Hesperotettix viridis*. In the face of the admitted validity of numerical constancy as one test of the theory of chromosome individuality, how can it be upheld when actual variations of this magnitude exist? This would seem to be as severe a strain as it could be subjected to, and if it can be shown that the conditions in *H. viridis* are capable of explanation without invalidating the hypothesis, then other cases of apparent exceptions would be less weighty as evidence until their full character became known beyond question.

On first thought the conditions in this group would seem to be a particularly strong argument against the theory. Here is a species, beyond question intimately related to many others of a

group in which the number of chromosomes is almost constant, and yet, within the one species, there are wide departures from the normal number. If such changes may occur without producing any effect upon bodily characters that will serve to mark individuals by their variations, then surely it may be argued that there can be no direct relation between chromosomes and somatic structures. This would indeed be a vital objection to the theory if, under its terms, the existence of a fixed group of free chromosomes of unchanging behavior were postulated. Such however are not the conditions of the theory. If it can be shown that the smaller numbers present in some individual are not caused by the loss of any chromatic units, and further that all the conditions in the group are consistent with the maintenance of certain associations between chromosomes and their chance combinations in fertilization, then the conditions of the theory are not violated. In effect this position would extend the theory beyond the observed conditions of the chromosomes to their subdivisions. It would be most exact if it could be based upon constancy of chromomeres, the limit of our observational analysis, as has been done for certain chromosomes in *Phrynotettix* by Wenrich ('16).

The genus *Hesperotettix*, so far as our studies have gone, has two species, *brevipennis* and *festivus*, in which the normal haploid number in the male is twelve; two species, *speciosus* and *pratensis*, in which the number is eleven; and one, *viridis*, in which both of these numbers are represented and, in addition, nine, ten and thirteen. There can be little doubt that the occurrence of the typical family number in the genus is significant of conformity to type; the presence within one species of this number, and of variations from it, most strongly indicates that, whatever differences there are, they must not be due to any fundamental disturbance of the chromosome organization. This *a priori* argument, while in no sense conclusive, has its value and must be considered. Fortunately however there is strong objective evidence to support it.

First of this evidence is the case where the number is reduced, from twelve to eleven. This I have already considered at length

('02, '05), and the case is supported by observations of Sinety ('01) and others. In this instance an element, so well marked by peculiarities of form and behavior as to be unmistakable in every cell generation, is found to be united to one of the euchromosomes at all times so as permanently to reduce the haploid number to eleven. There is actually one less free and independent chromosome in cells of these animals than in those of other members of the group. This however is a far different thing from saying that there is one less chromosome present. In fact there is no difference at all in the number of chromosomes—the sole variation being the union of the accessory chromosome with one euchromosome. To put the case thus is to make a plain statement of fact and not to create an hypothesis. The apparently missing element is just as clearly present as in any other Orthopteran cells. Irrefutable proof is thus afforded of the process of the fusion of chromosomes without loss of their identity. As a principle of nuclear organization we have therefore to reckon with the ability of chromosomes to unite together while still retaining their morphological integrity.

It is also conceivable that where a tendency of this kind manifests itself between certain chromosomes in a species, it may also obtain between other members of the complex. Such combinations are more difficult to detect on internal structural evidence, because there is no such differential behavior as characterizes the accessory chromosome. Nevertheless there are valid criteria which may be employed and these make certain the existence of such multiple chromosomes. The conditions displayed in *H. viridis* are peculiarly fortunate and may now be considered in detail. From the study of thirty-eight individuals of *H. viridis* it is apparent that (a) the number of chromosomes in the first spermatocyte may vary from nine to thirteen and that (b) the same number may be present in two or three individuals and yet in each case be differently constituted. The necessity for a careful and detailed study of the complex before passing judgment upon the significance of numerical variation, is certainly apparent from these circumstances. In view of the fact that most explanations of changes in the number of chromosomes have concerned the smaller members of the series, it is of

much interest to find that in *Hesperotettix* this portion is relatively stable while the larger elements may be variously combined together. But however much difference there may be in numbers there is apparent no loss of any morphological structures in any of the classes. A comparison of homologous series of chromosomes, based on size, may be made on inspection of plate 2 where I have attempted to arrange the chromosomes of different classes in order so that homologous chromosomes are in vertical rows. In the case of multiple euchromosomes they are placed in an intermediate position between the columns where their members would properly come. The accessory chromosome, when in a multiple, is drawn as it is attached to its tetrad.

Even a casual inspection of this plate will show that, aside from the accessory chromosome, the seven smaller chromosomes, (nos. 1 to 8) constitute a graded series in which variation is not great. The eighth chromosome, however, may or may not have the accessory chromosome attached to form a multiple. In classes 4 and 5 there are above this chromosome only two other separate ones, but these are of a character to attract attention at once. Unlike most of the remaining elements of the complex, they lie extended, parallel with the spindle axis and are in the form of open rings, each half of which is greater than the whole of chromosome eight. There are a number of considerations which make it practically certain that these are multiples composed respectively of chromosomes 9 and 10, 11 and 12.

First, it is to be noted that if these are counted as octad multiples, the number of chromosomes becomes exactly twelve. In weighing the value of this piece of evidence it must be remembered that this number is characteristic of almost the entire family to which *Hesperotettix* belongs. This fact makes it certain that in this respect we deal with such a fundamental feature of organization as to require us to consider any variation, not as *prima facie* evidence of a lack of precision in organization, but rather as a modification of the form of it.² In the

² One of the most unjustifiable and unscientific attitudes imaginable is that which would regard the effort to interpret the form of organization in its various aspects only as an attempt to force a real divergence into a seeming agreement. Such a style of argument has been resorted to by a number of those whose belief

case before us the individual animals in class 1 have twelve chromosomes, those in class 4 have nine while the ones in class 5 have ten. The question we have to decide is, whether these conditions represent absolute differences in organization, or whether, within the nine and ten groups, the twelve elements of class 1 are morphologically present. On inspecting a complex of class 4 we find that the accessory chromosome is missing from its usual position in the series, but upon looking further it is discovered, with all its usual characteristics of structure and behavior, attached to one of the tetrads. As a structural element of the cell it is demonstrably present and must be counted, although it is not a free chromosome. In a complex which is of almost identical composition otherwise, (class 5) the accessory chromosome is a free element and would of course be counted. In both cases the entire history of the accessory chromosome in all cell generations is typical, whether free or attached. The remaining two chromosomes of classes 4 and 5 are strikingly different from all the others, and upon careful inspection are found to have morphological subdivisions, which if counted in the usual way, restore the apparently missing two. In other words, if the number of chromatids is counted the total is forty-six, just as it is in class 1. So far as the number of elements is concerned, therefore, we are dealing with the same series in each case. It is obvious that if the differences in number were the result of an unordered variation there would be every reason to expect numbers in excess of twelve, together with modifications of all the elements and not of precise changes in particular ones.

In a similar way, when the numbers in classes 2, 3 and 6 are considered, the same evidences of ordered change are seen. Only eleven separate chromosomes are found in class 2 but the largest of these shows very clearly the accessory chromosome as a con-

is that the chromosomes are unstable and indefinite structures of the cell. Back of such a position is the implication that evidence must be taken at its face value without interpretation. This I consider entirely wrong. The essence of observational work is interpretation, and in microscopical investigations it reaches its fullest development. The value of evidence submitted by a microscopist is in direct proportion to the quality of his interpretative ability.

stituent part and thus demonstrates the completeness of the series. In class 3 there are ten separate chromosomes, of which one is a multiple involving the accessory chromosome as in class 2, besides which there is a very large octad similar to the one in classes 4, 5, and 6. Class 6 differs from 3 only in respect to the non-union of the accessory chromosome with a tetrad. The one individual of class 5 a, of which I have only a smear preparation, presents the second instance of a supernumerary chromosome which has appeared in my material up to the present. Aside from this, the complex is like that of class 5. From all these considerations I think it may be said that the numerical variations within these specimens of *H. viridis*, instead of being an indication of lack of stability in organization of the chromosomes, are, in fact, very strong proof to the contrary. There is nothing whatever to indicate that the integrity of any element is lost, or, aside from the case of the supernumerary, that any additional members are added to the complex. Variation is not a question of loss or gain, but of relations.

2. Chromosome numbers in general

Variations in chromosome numbers, within the species and larger groups certainly exist, as is clearly manifest in the work of numerous investigators. The constancy of numbers is more definitely established in some groups of animals than in others. Thus the Acrididae have a common number for many of the genera, but in the Hemiptera, as Wilson and Montgomery have indicated, the families are much less uniform numerically. In using the conditions of the family which I have most studied as an indication of chromatin organization, I have recognized that the nature of this organization might be different in other groups, and I have not attempted to apply generally the immediate conclusions to which I inclined. Not only is this true but the specific statement to that effect appears in an early paper of mine ('08 a) in these words:

I am quite prepared to admit also that in one species even, there may be a variation in the integration of the chromatin material, resulting in some numerical variation of the chromosomes, without losing

my belief in the necessity for this definiteness in the grasshoppers. We do not yet know how much difference there may be in the organization of the various chromosomes of a complex nor how variable in importance they may be.

A more careful reading of my papers would have saved some of my critics much futile argument.

While I have thus avoided any consideration of the general topic of chromosome variations in other papers, I should like now to examine some of the data relating to this subject. It is important that this be done, because most of the attacks upon the theory of chromosome individuality have proceeded from investigators who either found, or think they found, variations in chromosome numbers within individuals or species. Such attacks have run the gamut, from assertions that chromosomes are merely physical aggregates without morphological value, down to questions regarding the definition of the term chromosome. In undertaking such a general discussion I shall base my opinion largely upon material with which I have personal familiarity. Much harm has come from attempts to homologize results from widely different materials by persons who have no first hand acquaintance with the conditions discussed. With our present knowledge of cellular phenomena as slight as it is, such lengthy critiques, involving the interpretation of other investigators' interpretations, can accomplish little good and may greatly retard progress by fixing attention upon relatively unimportant details. A striking instance of this has been the controversy regarding pre- or post-reduction, extended long past the time when it was definitely known that the question of the segregation division is one of the individual chromosome and not of a whole mitosis. The matter of chromosome organization is, however, fundamental to all our present conceptions of the cell in relation to the larger problems of biology, and the evidence is cumulative that the essential assumptions of our hypotheses are justified. On the other hand it seems clear that the nature of chromosome integration varies with different groups and that, for this reason, we must be cautious in carrying over the conclusions reached from a study of one population of organisms to

another. It is in full realization of this need for care in generalization that I wish to examine the conclusions of others regarding chromosome numbers in the light of my own and my students' studies.

In making such an examination I can profitably take up only the most general questions, because, in matters of detail, it becomes a case of interpretation of phenomena, and many times one is not justified in criticising the work of others without himself knowing the objective conditions. For this reason I should like to consider the work of Della Valle on the subject of chromosome numbers. I do not choose this author as representative, because of any inherent strength apparent in his papers, nor am I impressed by their number and size. On the contrary they appear to me weak, because of the small amount of observational basis and the large development of theory. For instance in the paper of 1909 of the hundred and seventy-seven pages, only forty pages are included under the heading "Data of observation" and, of these, twenty are concerned with matters of technique. Throughout all of his publications, indeed, there is apparent a painful lack of judgment regarding the distinction between fact and theory, and were he alone in his position he could fairly be dismissed with little attention.³

³ 'Objective analyses,' accomplished by the translation of morphological facts into terms of physical chemistry, carry their own indictment of lacking experience and judgment, and could safely be left without comment. Such an attitude toward chromosome organization is, however, but an extreme case of the position assumed by a number of biologists who would seek to discredit the hard won facts of cytology by an appeal to conditions or forces beyond observational control or by an arrogation of the whole problem to their own chosen field. It would seem that, in almost every case where such attitudes are assumed, there is some animus or prejudice in the mind of the writer which betrays itself in his injudicious or intemperate language. All those who fail to agree with his particular extreme views are depicted as banded together for the establishment of dogma and the suppression of truth. With much vigorous language and the plentiful use of exclamation marks all such are consigned to scientific oblivion with their obsolete methods and narrow views.

This is very unfortunate. It is rarely the case that scientific investigators are not honestly in search of the truth with regard to the subject of their enquiry, and to accuse them of conspiring for its suppression is most absurd. It is true that new views sometimes prevail but slowly, but it does not hasten their

The work of Della Valle is open to criticism from two sides. It may in the first place, well be questioned whether the knowledge of colloid chemistry, and of fluid crystals in particular, is sufficiently established to justify the extension of its principles into the operation of cellular phenomena. Certainly one is inclined to doubt the basis on which such application is made when the complicated phenomena of chromosome division are declared to be 'absolutely identical' with the cleavage of fluid crystals, or when the involved changes of the chromatin in the telophase are described as 'identical' with the solution phenomena of a gelatin cylinder in warm water. It is of course not to be doubted that in the activities of the chromosomes, and all parts of the cell indeed, chemical and physical laws are operative. Such a belief is however far removed from the one which conceives some particular manifestation of chemical or physical energy as 'identical' with the behavior of the chromosomes in mitosis. Much might properly be said with regard to this phase of Della Valle's work, but I desire rather to consider the nature of what he advances as direct evidence against the theory of chromosome individuality. Since this involves a thoroughgoing denial of all the facts and theories relating to the subject, such a discussion will touch upon most of the objections that have been raised by other critics and will avoid the necessity for repeating arguments involving only minor differences of material or opinion. A summary of his position with regard to matters relating to chromosome individuality follows:

He asserts that the number of chromosomes in a cell is due to the constancy in the amount of the chromatin and the median size of the single chromatin aggregates. This number suffers variation according to the law of fluctuating variations and is

acceptance to attack the motives of those who hold other opinions. A method does not commend itself as an instrument of value merely because it is termed an 'objective analysis' when it is obviously the application of a series of analogies. Neither does an observed fact cease to be such on being termed a 'hypothesis' or even a 'subhypothesis.' It is in fact not infrequently true that much of good in the work of certain biologists issues under a severe handicap because of the inherent evidence of poor judgment in their estimation of values in the work of others.

subject to conditions within the system of which the chromosomes are a part. The chromosomes are temporary and variable organizations of the chromatin, which form in the prophase and dissolve in the telophase. Variations in size of chromosomes are within the limits of observational error and are of the order of size variations in the droplets of an emulsion. Correspondence in size between chromosomes and nucleus finds its explanation in the physical condition of adsorption. The fact that chromosomes shorten proportionally to their original length shows they are homogeneous and indicates the identity of all the chromosomes in a mitosis. The previous history of the chromosomes in a line of cells can have no effect upon later generations because there is no continuity. Definite or specific organization is lacking and there is no perpetuation of a series through reproduction of individual chromosomes. All explanations of variations in number (called 'sub hypotheses') are declared to be untenable upon 'accurate examination' and certain determination of numbers in sections is stated to be impossible. As is customary with critics of this type, Della Valle, after decrying the spirit and purpose of those who do not agree with him, proceeds to attack their methods and declares that only his own technique yields infallible results. Sections can not give accurate enumerations of chromosomes, and only counts made upon stretched membranes are of value. Having thus put out of count the bulk of the work already done, he next makes a comparatively limited number of observations upon one type of material and arrives at the ultimate general conclusion that chromosomes are fluid crystals. With this solution of the problem accomplished, all minor questions are easily settled, because upon this major premise that chromosomes are fluid crystals and subject to all the laws known to pertain to such physical aggregates, certain variations of number, size, form and behavior must exist. I should like now to consider some of these assertions in the light of work known to me personally, first pointing out that all instances of order and system under varying conditions are direct evidence against his position.

The number of chromosomes within cells of an organism, he states, is variable, according to the law of fluctuating variations, because the number in any cell is due to the constancy in the amount of chromatin and the median size of the chromatin aggregates. Opposed to this is all the evidence, already given, regarding the high degree of regularity in the Acrididae where so many genera are alike in number, although the amount of chromatin varies widely. In this large group, the family, variation is infrequent, but in one species, *Hesperotettix viridis*, variation is common. Certainly under the terms of Della Valle's argument the greater variation should occur where there are the greater differences in amount of chromatin. But if there is any truth in the assumption, some variation would be expected in the cells of the individual and this does not occur in these Orthoptera. As reported for *Culex* by Whiting ('17) in the germ cells the diploid number is constantly six, Hance ('17) finds the same conditions generally true of the somatic cells, but Holt ('17), working upon the same material, discovers a range of variation extending from six to seventy-two in certain intestinal cells. It is very clear that the amount of chromatin is here not constant, while the median size of the chromosome remains practically unchanged. There is no correspondence, either, between the size of the nucleus, or of the cell, and the number of chromosomes. No evidences whatever of any balanced physical system, such as Della Valle advocates, appears in these cells of *Culex*.

Hertwig's outworn nuclear-plasma relation theory receives just as little support. In *H. viridis* the amount of chromatin is much the same in all the cells of a given generation and yet the number of chromosomes in the haploid condition ranges from nine to thirteen. Along with this fixity in the amount of the chromatin and variation in chromosome numbers, goes a constancy of size-series up to certain chromosomes, beyond which there is a sudden change. The phenomena relate to no means or averages, but concern definite morphological entities. In place of fluctuating variations there is definite and determinable order. What happens is explainable, not upon circumstances of

chance relations between masses of substance in different 'phases,' but through a knowledge of the history of certain individually recognizable chromosomes traced through a large group of animals. Worthy of note is also the fact that in such cases as *H. viridis* the range of variation is strictly defined and falls within the limits set by the organization established in the family, except in the occasional instance of supernumerary chromosomes—two instances in the thirty-eight individuals studied.⁴

It is only upon the basis of entire identity of all chromosomes in a complex and of their temporary character that the Italian author has any argument at all. Building upon this he seizes upon every reported instance of difference of chromosome numbers as a support for his thesis. Every descriptive fact is labeled a 'sub hypothesis,' and so many of these are secured in this way as to convince him that the main hypothesis of chromosome individuality has no standing. Curiously enough, he conceives a great importance for the fact that one explanation for all the reported cases of numerical variation is not sufficient. On the contrary, he says, there are so many ways of accounting for such variation that there can be no constancy and no individuality. Perhaps in no other way is the quality of his argument better indicated than here. The fact that an error in observation has been made, and even admitted, is only an indication of variation; when it is shown that a reported difference in number is due to the inclusion of more than one species in a study this is a subhypothesis weakening the main one; the production, by hybridization, of chromosome numbers different from those of the parents is accounted as an example of variation, as is the case of asymmetrical mitoses in neoplasms. The

⁴ If a chromosome is but a chromosome without character or distinction, there is in this case a violation of the rule stated. But if the nature of the element be significant, the rule holds, for the entire history of the supernumerary chromosome demonstrates that it is of a different order from the euchromosomes. It may in many ways depart from the history of typical chromosomes and finally end in complete elimination. Arguments, such as Della Valle's, require that such a structure be considered identical with all other bodies called chromosomes, without in any way regarding its individual history of ultimate extinction.

clear cut description by Wilson of the failure of certain chromosomes to enter into synapsis is not an explanatory fact but another 'subhypothesis.' Similarly, early separation of conjugants, delayed divisions of certain elements, the presence of supernumeraries are merely so many condemnatory 'subhypothesis.' The exact determination of the union of the characteristic and well marked accessory chromosome, with a tetrad to form a multiple is not a fact at all, not even a hypothesis, but only a 'subhypothesis.'

From this it is clear that Della Valle, and others like him, have set up in their minds the conception of an absolutely fixed and invariable number of chromosomes of constant and unchanging form as the hypothesis of chromosome individuality. This is a man of straw, fathered by no biologist of standing or character. His destruction in no way affects the existence of the real image of a constancy in chromosome organization consistent with our other knowledge of the living substance of which these structures are a part. Such misinterpretation merely condemns the judgment of the one who has no better discernment of the real problem. It seems incredible that a biologist should be capable of twisting the truth in such a way as to turn an investigator's meaning entirely around so as to make his results support a converse. Only when his basic conception of chromosome constitution is fully appreciated does an explanation of Della Valle's attitude appear. This is expressed by him in several places in some such terms as these: The chromosomes are temporary and variable aggregates forming in the prophase and disappearing in the telophase ('09). Between two mitoses the chromosomes are so completely lost as individuals that finally no trace of them can be found ('13). It is easy to see that, with such a view of chromosome organization, no constancy is possible and the quickest way to dispose of variable numbers is to consider them the result of chance.

But such a disposition of the case neglects certain objective facts which can not be disposed of by consignment to the class of 'subhypotheses.' It has long been known that the accessory chromosome, and other heterochromosomes, preserve their mor-

phological identity from one generation of cells to the other. All through the spermatogonial divisions each chromosome lies in its own vesicle and can be clearly recognized. Recently Wenrich ('16) has carefully traced certain differentiated chromosomes in *Phrynotettix* through much of the history of the maturing germ cells, without loss of their physical identity. Instead of appearing as "temporary and variable structures" they are just the opposite, being persistent and exact in organization to the highest degree. Not only are the chromosomes constant in number, but also, and more fundamentally, the chromomeres. Such conditions are probably unknown to Della Valle and are certainly unappreciated or he could not make such sweeping assertions regarding chromosome instability.

Perhaps one of the most striking and extensive instances of variation in chromosome numbers yet reported is the one described by Miss Holt ('17) for the intestinal cells of *Culex*. Here, in one individual, there may be a range from six to seventy-two. Without extending the analysis of the case beyond the simple determination of the numerical conditions, one would be justified in believing that no significance attaches to the mere number of the nuclear bodies. This would follow on the major premise that indefinite and fluctuating variations in numbers are direct evidence of lack of definiteness in organization. This might presumably be admitted for the somatic cells without entirely invalidating the theory of individuality so far as it applies to the germ cells, for it is possible that, among other evidences of differentiation in cells, correlative changes in the nature and integration of the chromatin substance might occur. Especially might this be the case in pupal *Culex* intestinal cells, because these are on their way to disintegration in preparation for the new epithelial lining of the enteron. Meanwhile the germ cells of the species preserve an unvarying constancy in number. But, as Miss Holt shows, the matter is not so simple as it appears. True, there is variation, but it is not indefinite and unordered. There are not always six chromosomes present, but, if more occur, the numbers are multiples of three and repeat in corresponding series the sizes of the primitive complex. Not

only this, but the grouping is maintained and the derivatives of each of the original series remains associated with its fellows and, with them, forms a common vesicle. So far as prophase and telophase conditions are involved it would not be observed that anything unusual in numerical relations obtains. Even in the matter of this multiplication the chromosomes exhibit individual differences, and one may have advanced in the process beyond the others and in this way produce a total number not strictly a multiple of three. But no matter how many or how few there may be, each one of the individual groups repeats the characters of the original member. How far removed from mere chance crystallization out of an indifferent matrix is the ordered and definite reproduction of distinct morphological entities which we see here. In the very face of what seems at first glance to be the grossest of variability there appears only another marked instance of exact organization. It is of interest to observe also that division of the chromosome may occur independently of mitosis, which suggests, in connection with the observation that the actual splitting of the chromosome is a prophase change, that mitosis may be more concerned with cell division than with chromosome separation.

3. *Chromosome sizes*

If the total amount of chromatin is constant for each cell and if the number of chromosomes is unchanged, it might reasonably be expected that the same series of sizes would always appear. These conditions are certainly realized in the individual, where the closest correspondence exists between the series and between individually recognizable elements. That like correspondence exists between individuals seems probable when the seriation in the complex is noted. An exact determination of chromosome sizes is however a very difficult undertaking, owing to the variety of forms they assume. One is forced to estimate relative volumes, and an exact determination between two nearly equal sizes of different form can not be made with certainty. Fortunately the difficulty of comparing sizes is less in the case of the

larger elements, owing to their volume and more uniform shape, and we may be sure of the series in this part of the complex. Since it is between these chromosomes that combinations occur, the members may easily be identified. The value of the evidence with regard to the constancy of chromosome organization as shown by size relation is best appreciated by a comparison of the different classes shown in plate 2.

In the one containing twelve chromosomes (1) there is a gradual increase in size from the smallest to the largest without any marked breaks. Class 2 shows the same condition, with the exception of the multiple chromosome, but if the presence of the accessory chromosome there be disregarded, the disproportion vanishes. Class 3 however presents a different aspect. There is the same gradation up to the largest element, which, in turn, is more than twice the size of the next one of the series. Similar conditions prevail in each of the other classes, with additional distinctness in classes 4 and 5 where the element succeeding number 8 is like the largest in class 3. In each of these cases there is an abrupt and disproportional increase in size, if these structures are regarded as simple units, which entirely disappears if their multiple constitution is recognized. Such jumps of size in the complex are lacking in any species of this family where the twelve chromosomes are present, and it is not reasonable to consider that they represent any great change in the organization of the chromosome such as would be involved if these huge chromosomes were in fact of the same value structurally as the lower ones of the series. However much difficulty there may be, for example, in locating chromosome 4 or 5 in the series, there can be no mistake made in judging the relative size of the largest element in classes 3, 4, 5 and 6. It is also easy to see that either half of this ring exceeds the dimensions of the next chromosome in size in somewhat the same degree as chromosome 11 does chromosome 10 of class 6. Similarly the members of the smaller ring in class 5 are related to chromosome 8 of class 6. The relations in size of the upper members of the series are demonstrated most conclusively by a comparison of them in classes 4, 5 and 6.

While these unequivocal conditions certainly obtain and justify the effort to homologise the chromosomes on the basis of size, it must be admitted that there appears to be some fluctuation in volume, both absolute and relative, between the same members of the series in different individuals. How much this may signify it is difficult to say. There are so many ways in which the size of a chromosome may be affected in the processes of microscopical technique that a just estimate of the significance of size variation is hard to reach. Thus it is known, for instance, that very marked differences in size may result from the method by which the animal is killed. If this be true, there are doubtless other ways in which these delicate structures may be similarly affected. Since however the animals themselves may vary greatly in dimensions without in any other way modifying their character, the same may be true of their chromosomes.

A very careful study needs to be made of chromosome dimensions, based upon material prepared with the utmost refinement of technique and studied with most careful mensuration. Under ordinary conditions there are many circumstances which would serve to obscure differences between nearly related members of a series and make their identification uncertain. While this is true, most of our observations would indicate that agencies affecting the size of chromosomes operate more or less uniformly on the entire complex, so that a proportionate change is found in each element. With the subject as difficult as it is there is no wonder that views as widely divergent as those of Meek and Della Valle exist. The former author thought at one time that he had detected a uniformity so great that only two diameters appeared in all the Metazoa, but a more extended experience did not confirm this opinion. It is true that there is much uniformity in the diameter of the chromosomes of a complex, and Meek's careful measurements disclosed this; but, on the other hand, there are extensive movements in the chromatin substances and the diameter varies inversely with the length. There is always the possibility of such movements and the extent of them at any given time is variable, so that we may expect to find differences in dimension although the volume remains constant.

Meek has done good service in pointing out the facts, but it is unfortunate that he should push his generalizations so far upon a limited experience. Very different are the methods and conclusions of Della Valle.

Variations in size, according to Della Valle, fall within the limits of observational error and are of the order of size variations of droplets in an emulsion. Correspondence in size between chromosome and the nucleus finds its explanation in the physical condition of adsorption. As in the case of numbers, the appeal is from an explanation based on order of determinable character to one in which only the chance circumstances of physical relations obtain. Opposed to this conception stand all the careful investigations in which definite size relations are shown to be preserved, not only in a single series but in a duplicate one. Within this occur occasional inequalities, which, in turn, are of definite character, pertaining to certain chromosomes and so recurring as to be accounted for most readily through genetic continuity and chance distribution and recombination. Upon this point again the circumstances in *H. viridis* offer important evidence. The smaller members of the complex form an ascending series which, in class 1, is continued without breaks through the larger members. But in the remaining classes there are sharp breaks in seriation of exactly the same character in all the cells of the individual, entirely beyond the range of observational error and past the possibility of chance variation. The conditions are altogether of the character of order and system common to living structures and foreign to purely physical phenomena. The material upon which the Italian author worked is not nearly so favorable for size determinations as are many other kinds, because of the great length and irregular contour of the elements, but even here much more accurate results could have been obtained by the exercise of such care as was employed by Meek. From some studies being pursued in this laboratory by Mr. Parmenter I feel certain that the Amphibia are no less exact in their size relations than are other groups of animals.

4. *Chromosome forms*

In a previous paper ('14) I have considered at length the subject of chromosome forms in the Orthoptera and do not here desire to go into the subject beyond what is necessary to an understanding of the multiples. A glance at plate 2 will show how strongly these elements differ from the tetrads in metaphase. The typical Orthopteran chromosome is telomitic and, in polar views of the spermatogonium and second spermatocyte metaphase, it appears as an approximately straight rod which, in the anaphase, becomes divided into daughter rods of similar nature. In the first spermatocyte these rods, joined in pairs to form tetrads, show a great variety of shapes in metaphase, but in the anaphase again become reduced to the form of two rods joined at one end to form a simple V, the enclosed space of which lies between two chromatids of the former tetrad. Upon division of the hexad in the first spermatocyte of *Hesperotettix* this condition is again realized for the rod-portion, but opposite this in the other anaphase group stands a V, the arms of which are not simple rods but double ones, and the enclosed space of this lies, not between chromatids, but between whole chromosomes. The nature of this double-V I have considered fully in former papers. Its peculiarity of form is due to the fact that fiber attachment is non-terminal—a condition resulting from the union of non-homologous chromosomes and not from a real shift of the fiber. All this is very clear in *Hesperotettix*, and the character of the double V is beyond question and easy of determination. From this one would be inclined to generalize and to say that where fiber attachment is shifted from the terminal position, producing double V's in the first spermatocyte anaphase, it is due to the union of non-homologous chromosomes. Of course to make this a valid argument there would have to be a corresponding reduction in the group number of free elements. This condition is fully met in *Hesperotettix* and is confirmed by the differential morphological characters of the elements involved. In the absence of such criteria based upon structure, the sole test regarding the character of the double V's would be that of numerical conformity.

Upon turning to *Mermiria bivittata* we encounter these conditions: a spermatogonial complex of twenty-two free elements among which are two atelomitic V's. If it were assumed that the latter are always multiples, then the male diploid complex would be twenty-four. There are two arguments against this conclusion however. First there is the circumstance that the genus—a well marked one—has species with the normal twenty-three free chromosomes. The value of such evidence has already been considered. A second piece of evidence is the actual morphological structure of the V's. One of them shows by all its history that it is directly comparable to the similar structure in *Hesperotettix*—it is a multiple composed of the accessory chromosome and a rod-shaped euchromosome. The other V is found in the first spermatocyte attached to this multiple element. Unless this combination be of greater valence than a hexad, the second V can be only a dyad. Strong evidence that this V is indeed only a simple dyad appears from a study of certain individuals which I have called the 'green' *bivittata*. In these specimens the multiple chromosome is almost the shape of the one found in *Hesperotettix*, the fiber attaching near the free end instead of toward the middle (figs. 58, 59, 60, pl. 7). The valence of the part is very clearly indicated in this way. Owing to mistaken enumeration in the spermatogonium, because of insufficient material, I regarded the multiple as a decad instead of a hexad in my earlier work. In this opinion I believed myself confirmed by the persistent type of chromosomes in the family. It was only when new material revealed to Miss Carothers that, in a full complex of twenty-three chromosomes, there might be numerous double V's, and that one such an element might be united to a rod, producing a J-shaped tetrad, that I found it possible to account for the form of the *Mermiria* multiple. With plenty of favorable material I was also able to determine accurately the diploid number of chromosomes in the male to be twenty-two. Having all these facts it was not difficult to solve the problem.

It is apparent, therefore, that form alone is not sufficient to establish the nature of a chromosome. The double V may or

may not be a multiple. In *Mermiria*, in the same chromosome of the first spermatocyte, we have two such V's, one of which is multiple, the other not. In *Trimerotropis* double V's are numerous although the complex is not reduced in number. But the form of the chromosome, taken in connection with the number in the complex, the relative sizes of the elements, their structure and behavior, is important. With these limitations on the value of chromosome form in mind, I wish to consider the evidence they afford regarding the nature of the *Stenobothrus* type of rings and V's in *H. viridis*.

In such a study the occurrence of the incomplete ring is very helpful because it constitutes an intermediate form between the two rod condition and the ring. An examination of these elements in classes 4, 5 and 6 (plate 2) will demonstrate these relations. In the last class, chromosomes 9 and 10 are rods arranged on the spindle parallel with its axis, and there divide at the middle constrictions. Similar structural conditions prevail in class 4, with the exception that, in place of chromosomes 9 and 10 as free elements, their position in the size series is occupied by a single chromosome in the form of a V placed in the same relation to the spindle. Upon division, the elements of this unusual chromosome separate at their middle constrictions just as did free chromosomes 9 and 10, and in one of the anaphase groups exactly the same conditions obtain as in cells of class 6 (fig. 28, pl. 5). The other group has, in place of two rods, a double V corresponding in the number and size of its subdivisions to the sum of those in the two rods from which it separated. All the circumstances of the case indicate that the only unusual condition here is the union of chromosomes 9 and 10 at one end, producing, in the diploid condition, a V, whose arms are unequal in size and otherwise non-homologous. The origin of such a combination has not yet been determined, but it is not a temporary condition during the maturation period, for it is found in all generations of the cells and is undoubtedly present in the somatic cells. Since it is present in half of the sperm it may as readily perpetuate itself as the two rod condition. If like relations exist during the maturation of the egg, then a means

for producing all the chromosomal arrangements found in *H. viridis* is at hand. Thus a two rod spermatozoon and a two rod egg would produce a complex having chromosomes 9 and 10 free as in classes 1 and 6. A double-V spermatozoon and a two rod egg would bring about the condition shown in class 4 where these chromosomes are joined at one end, while such a spermatozoon, united with an egg of similar composition, would result in the complete ring of class 5. It seems evident therefore that the forms of chromosomes found in the male germ cells may be accounted for on the basis of persistent fusions, segregations and recombinations.

Similarly, if chromosomes 11 and 12 are compared, we find the two rod, incomplete ring, and ring, and the argument for their relation is the same as for the preceding case. There are however two notable differences between the series. Of the six classes, only two show multiples of chromosomes 9 and 10, while four classes have combinations between 11 and 12. Again, the incomplete ring is as frequent as the complete in classes 4 and 5, while only one individual out of twenty-six belonging to classes 3 to 6 failed to have a union of the two tetrads at both ends. Thus we learn that the tendency of the large elements to unite is more pronounced and complete. In this connection it may be noted that in class 2 the largest element, although failing to form a union with a euchromosome, is united to the accessory chromosome. Only four individuals of the thirty-seven show this element not joined to another chromosome. Of similar import is the combination between the accessory with the largest free euchromosome in class 4. It is possible that the largest free tetrad is involved in class 3, although I judged it to be next to the largest when the drawing was made. Another thing to be noted is that only tetrads of nearly equal size unite together. The accessory chromosome is never joined to a chromosome of lesser size.

The form of the multiples, as represented on the tabular series of plate 2, is the one best adapted to serve as a means of comparison between them and the free chromosomes of other individuals. This relatively simple configuration is, however, not

the only one assumed, and some of the figures would be difficult of conception in the absence of acquaintance with the possible range of chromosome movements. For a number of reasons these variable forms are of importance and require consideration. It is to be noted that this extended ring may be the final shape assumed by other metaphase forms just previous to the anaphase, or it may represent the status attained by chromosomal movements antecedent to the metaphase. Probably all that is involved throughout is a difference in relative movements of the chromatids. An examination of figures 14b, 16c, plate 3 will reveal a chromosome, one part of which is extended over the spindle as are the members of the flattened ring already described, while, embraced between its ends, lies another ring within the plane of the equatorial plate. By comparing this chromosome with others shown in photomicrographs Q-V, plate 8 it is seen that this second ring may be progressively reduced until it corresponds in form and position to the extended element to whose ends it is attached—the sole remnant of the equatorial ring being an enlargement at the middle of the curved rod. It is apparent that the enclosed spaces in these two rings, lying at right angles to each other, can not be the same morphologically, unless the chromatids in synapsis have shifted. One must represent an interchromosomal space, the other the longitudinal split of the chromosomes. In this respect the element is similar to the double ring figures in certain Orthopteran tetrads.

Another form of not infrequent occurrence is similar in outline to the hexad of *Mermiria* and consists of a curved rod applied conformably to the spindle, with the fibers attached at the ends. From these ends also there spring at various angles more or less straight chromatin rods equal in length, approximately, to half the curved rod to which they join. Superficially such an element is almost the counterpart of the *Mermiria* hexad, but is nothing more than the large extended ring whose shorter half has divided at the equatorial constriction in advance of the longer (fig. 24). A more puzzling case is that of the curved rod extending from one pole of the spindle to the other and almost equalling the combined length of the two halves of the ring or V (fig. 19, pl.

4 and photomicrographs P., Y., pl. 8). Such a structure would result if the V were unfolded on the spindle with fibers attaching at each end, but in this case the parts on either side of the equatorial constriction would be unequal and division at this place would mean separation of whole tetrads. Because of the relatively slight difference in size of the joined tetrads, I have not yet been able to satisfy myself of the exact relation of parts in this elongated chromosome, but I suspect that separation of chromatids has occurred in a plane at right angles to that which obtains in the V. A full study of the prophase history should clear up this matter.

Emphasis needs to be placed upon the statement already made that the mere form of the chromosome is not conclusive evidence, either of its valence or its method of division. Valence can be determined only by a study of the element through the various generations of cells and in numerous individuals of the species. Planes of division are certainly identified only by some morphological character which marks one of the synapsed elements. Instances of the exactness in configuration of chromosomes of tetrad, hexad and octad valence have already been given, but it would seem desirable to consider the ring chromosome a little more at length. In former papers I have called attention to the importance of such elements as indicators of the planes of division, and in one ('14) I pointed out the distinction between the *Hippiscus* and *Stenobothrus* types and indicated how failure to recognize such distinctions has led to much confusion. At the same time it was recognized that, in the presence of *parasynapsis*, the opening in the ring might represent either the space between homologous chromosomes or their longitudinal division. Since there was, so far, nothing to suggest two types of ring formation, it was considered that only one exists and the available evidence would indicate that the annular space lies between homologous chromosomes. In the presence of one type of ring there must necessarily be two forms of first spermatocyte division, for the *Hippiscus* ring divides along the length of the ring while the *Stenobothrus* type separates transversely. Owing to the fact that, up to that time, all rings of the *Stenobothrus* type

has been found in complexes of less than the full twenty-three numbers, some uncertainty existed regarding the connection between these two circumstances. That this connection was not necessarily causal was indicated by the case of the unidentified 'Stenobothrus-like' species with a complex of twenty-one and numerous rings of this species-type. But not until the work of Carothers on *Trimerotropis* was it certain that there is no necessary relation between the *Stenobothrus* ring and lesser chromosome numbers. It is now clear that this form of chromosome is not in itself evidence of multiple constitution above the tetrad. Structurally all that is involved, apparently, is an atelomitic fiber attachment.

But if it is not true that such rings and the double-V's which unite to form them, and which reappear upon their division, are criteria for octads, it is very suggestive of such composition to find them so frequently in cases of reduced numbers. Where it can be shown that progressive steps in such unions occur within a species, as in *H. viridis*, and that morphologically recognizable divisions of these elements which, if counted as units, exactly restore the reduced number to that characteristic of the species and family, then the form of the chromosome is of value in determining composition. Especially is this true if by such means always the exact number characteristic of the group is restored, and if, in groups of different chromosome numbers, the distinctive numerical relations are preserved. All of these desiderata are realized in *Hesperotettix* of the twenty-three chromosome family and in *Jamaicana* of the thirty-three and thirty-five group as reported by Woolsey. The parallel between the two cases seems to be very exact, with the exception of a more marked tendency toward combinations in *Hesperotettix*.

The circumstances appear unequivocal in the cases of *Hesperotettix* and *Jamaicana* where multiples may or may not exist within one species and where intermediate steps indicate the method of combination. The question which next presents itself is with regard to the application of these criteria to cases, such as *Chorthippus*, where there appears to be a permanent and fixed reduction of number, not only for the species, but also for the

genus and related genera. In a recent paper Robertson ('16) has considered this matter with great care and has advanced strong arguments in support of the position that the family number may be maintained in the face of apparent reduction. His argument may be thus summarized. (1) The number of chromosomes for the family is twenty-three in the male; (2) the form of the chromosomes, when uncombined, is that of a rod with terminal fiber attachment; (3) size relations are constant and valid indications of homologies; (4) in the presence of less than the typical number of chromosomes certain ones of the complex are V's with non-terminal fiber attachment; (5) if the limbs of the V's are counted as units, the number twenty-three is restored; (6) the behavior of the limbs of the V's in maturation is parallel to that of corresponding free elements in other species of the family; (7) the morphological composition of the V is indicated by a non-chromatic bridge at the angle where the fiber attaches.

I believe that in the species studied by Robertson the criteria are largely applicable and the conclusions essentially justified. The evidence is, to a considerable extent parallel to that of *H. viridis*, with the exception of intermediate steps in linkage.

There is clearly a permanent reduction in the number of free chromosomes—it then becomes a question as to whether there is a definite loss of morphological entities in the chromosome complex, or whether all the twenty-three elements are potentially present, even though in combinations. It must be recognized, I think, that, however fundamentally alike are the cases of *Hesperotettix* and *Chorthippus*, they stand on a somewhat different basis morphologically. In the former, independence of certain chromosomes is strongly evidenced by their free condition in some individuals and their partial union in others; in the latter the number of free elements is constant, not only for this genus but for others nearly related, and evidence of combinations must be more indirect. Considering the criteria which I have employed in *Hesperotettix* and *Mermiria* I would regard the following as probably applying in the case of *Chorthippus*: (1) The number of chromosomes, so strongly indicated in the

family, is made exactly normal by counting the limbs of V's as units, (2) the graded size series, sharply broken if the V's are considered as simple units, is restored if they are compound elements, (3) the form of the univalent chromosomes is so generally a rod in the family that the occurrence of V's, in connection with the circumstances in (1) and (2), is strongly suggestive of multiple constitution. To these should be added the conformatory evidence of cases like *H. viridis* which establish on direct observation the principle of chromosome combinations.

That these circumstances justify the generalizations which Robertson draws regarding the multiple nature of V-chromosomes in general seems much less certain. Because of the great importance which attaches to the exact determination of chromosomal conditions in the germ cells it is essential that our cytological evidence be most carefully considered. I should like therefore briefly to indicate where it appears that limits should be placed upon the extension of the principles indicated by conditions in *Hesperotettix*, *Mermiria*, *Anabrus* and *Jamaicana*. Since Robertson has made the widest application of these by his conception of the multiple constitution of V-shaped chromosomes, an examination of his presentation will illustrate the nature of the difficulties attending such generalizations, into some of which I was myself led. It is fortunate in this connection that there is no question regarding the accuracy of the observations. His work is very carefully done and the drawings are clear and definite.

Agreement has already been indicated with the first two premises of his argument, i.e., that the number twenty-three is typical for the family, and that the form of the univalent chromosome is a rod with terminal fiber attachment. Like most other biological generalizations, however, these are not without exceptions in the Acrididae. *Pamphagus*, with telomitic elements, has only nineteen chromosomes and *Trimerotropis* with numerous and variable V's has a full complex of twenty-three. My unidentified Acridian reported in a former paper ('14) (which may be a species of *Circotettix*) has nine atelomitic chromosomes in twenty-one chromosomes, while *Circotettix*, as

reported by Miss Carothers, has similar conditions of less than the typical family number and more than enough V's to compensate. Despite these exceptions the value of the evidence for constancy of number and form is strong because of the few departures from type.

With regard to size as a test for homology there is much less certainty. No chromosome is more definitely to be determined than the accessory chromosome and this falls in no constant size position, either for the family, genus or species. Robertson reports it varying in size from number 1 to number 5 in the *Tettiginae* and from number 8 to number 10 in the subfamily *Oedipodinae*. It seems to vary from number 3 to number 5 in the genus *Hesperotettix* and from number 4 to number 5 in the species *viridis*. Variation in size of the individual chromosomes, recognizable by other morphological characters, has been reported by Wenrich ('16) after a most careful study of *Phrynotettix*. In this latter case variation appears to be the result of actual loss of certain parts of the chromosome, but in general it is due to the extent of condensation and possibly to the amount of more fluid substance held within the chromosome. All our observations indicate the existence of a fairly definite series of sizes throughout the group, and the extreme members of the complex can not be confused, but it is clear that no certain identification of neighboring elements can be made by size alone. For this reason, even if the achromatic bridges did mark the limits of joined chromosomes, it would not be certain that specific numbers united as is indicated by Robertson. Here it may be noted that in *H. viridis* chromosomes of nearly equal size, and the largest in the series, are joined together. Practically the same conditions are reported by Woolsey in different species of *Jamaicana*. Judging from her figures I should be inclined to say that it is not improbable that neighboring chromosomes, and those of the largest size, are united here. Measurement of the chromosomes as drawn is not a very accurate way of determining their size, since the amount of foreshortening can not be known, but it is so frequently the case that the element marked '14' in her figures of the multiple, is clearly larger than the free number '15,' that the possibility of confusion exists.

The significance of the form of V-chromosomes, as an indication of multiple constitution, is bound up intimately with the question of numbers. Only when the limbs of the V, counted as units, with the rod chromosomes, total the number characteristic of the family can there be such evidence of multiple constitution, according to the terms of Robertson's argument. If it appears that such elements exist under other circumstances, then it is necessary to assume that there may be more than one type of V. This Robertson does and uses as a criterion for the V, indicative of multiple composition, the presence of a distinct achromatic bridge at the apex of an acute angled structure. The final test of the validity of his generalization becomes therefore a structural one. There is no doubt of the accuracy of his determination of such a condition and he has with great care traced it through all the stages of maturation. It is unfortunately true, however, as Carothers and Wenrich have shown, that equally clear non-staining bridges occur in atelomitic chromosomes of *Trimerotropis* where the full complex of twenty-three is present. Conversely, in cases where undoubted combinations exist, as in *H. viridis*, no distinct achromatic bridges at the point of fiber attachment appear. This seems to be true of *Jamaicana* also, judging by the figures of Woolsey and of Robertson himself. It seems certain therefore that the V-form of chromosomes, is, in itself, no indication of a multiple condition. If this be true for the *Acrididae* it is quite useless to consider its value in other groups where the chromosome constitution is less well known.

The form of certain first spermatocyte chromosomes and the behavior of their parts in this mitosis is regarded by Robertson as further confirmation of his belief in their multiple character. He draws a close parallel between them and free elements of other species through homologies of size and form. Since the limits of these supposed elements are fixed by the achromatic bridges at the point of fiber attachment, their validity as tests for chromosome boundaries is questioned for the same reasons as were given above. I should however like to speak more at length regarding this form of chromosome—the double rings.

These are represented very exactly in Robertson's ('16) figures 174, 179, 180 and 182, and the achromatic break is considered the point of union between tetrads. I believe that the weight of evidence supports this interpretation: that these are indeed potentially multiple chromosomes, but at the same time I feel that there is no justification for the belief that this form of chromosome is the result of the union of two tetrads of such shapes that this configuration results. My evidence for this is that just such shapes occur in full complexes of twenty-three chromosomes, of both telomitic and atelomitic type. They are found in many species and have been figured by Sutton, Granata, and myself. As I have pointed out, the two rings lie in planes perpendicular to each other and their origin is easily conceived if the chromosome is constituted of four parallel rods. A similar conception appears in the interpretations of the other two authors cited. The same difference between double rings of the telomitic and atelomitic types holds as in the case of simpler annular chromosomes of the two types, for the fiber attachment is persistent and determines the position of the chromosome on the spindle. Therefore such structures are very much alike in *Trimerotropis* and *Stenobothrus* and are so placed on the first spermatocyte spindle that the ring to which the fiber attaches lies parallel with its axis, while in forms with telomitic chromosomes only, such as *Hippiscus*, *Tropidolopnus*, *Brachystola* and others, the ring to whose 'lugs' the fibers attach lies in the equatorial plate. Such differences are however entirely independent of simple or multiple composition of the diploid chromosomes.

Finally I consider them valueless as criteria of multiple constitution, because in *Chloealtis*, a genus nearly related to *Chorthippus*, the same numerical and size relations obtain, and there are no double rings to suggest preservation of chromosome forms such as are found in more widely removed genera. So far as the form of the chromosomes is concerned, the conditions in the *Tryxaline*, *Chloealtis*, are much more nearly like those in *H. viridis*, of the *Acridiinae*, than they are in *Chorthippus*, a very nearly related genus.

At this point it may not be out of place to speak of the condition of cross segmentation as an index of chromosome boundaries, since this is a matter that has led many, including myself, into mistakes. These non-chromatic segments are doubtless definite indications of structure in the chromosome and probably are always of the nature of the spaces lying between the chromomeres, as in the 'selected' chromosomes described by Wenrich ('16). Such regions are more pronounced in appearance and more persistently free of chromatin where the fiber attaches than elsewhere in the chromosomes, but probably do not differ otherwise. Even this distinction may be lacking, as in *Mermiria* (figs. 58, 59, pl. 7) where the chromosomes seem not infrequently to separate at different levels. It was this strong cross segmentation at definite levels, with subsequent division, that led me to believe in the decad constitution of the multiple in *Mermiria*.

Another thing which has contributed to Robertson's misinterpretation of the V-chromosome is his belief that only in their presence do pachytene loops appear in the nucleus. No one feature is more characteristic of the prophase condition in all *Acrididae* (from which the *Tettiginae* should be removed, as I suggested in 1908 and as Robertson believes) than the bending of the chromosome so that both ends lie at one side of the nucleus. Even the univalent accessory chromosome takes this position, as was shown in an earlier paper ('02, fig. 12). Wenrich finds that the looped condition is not confined to the large elements, but is characteristic of the small chromosomes of which he made careful, detailed study. It is of interest to note that, in this respect, the members of the octad in *H. viridis* behave as though they were independent, each forming its own loop. This is a very strong indication, indeed, of their multiple nature. The conditions at this time in the *Tettiginae* are unique so far as my experience goes. Here, as Robertson states, the chromosomes are extended in a more or less straight line, and since they are much longer than the nuclear diameter, the whole nucleus is drawn out, producing very irregular shaped structures. It

was this circumstance, of which he had detailed knowledge, that led Robertson to believe that loops are unusual.

The form of the chromosome is conceived by Della Valle to be uniformly that of a rod with rounded ends. Such is frequently the shape of metaphase chromosomes, particularly in the diploid condition, but no one who has studied the complicated changes through which the members of the haploid series pass at the synaptic period, could entertain the simple conception of a homogeneous rod spontaneously dividing along its length, advocated by this author. The most remarkable and striking phenomena of biology are entirely ignored by this explanation. Precision of organization, shown by Wenrich to extend to the limit of our observational powers, is entirely disregarded by the gross conception of a formed and homogeneous rod cleaving like an unorganized colloid mass. The extensive and significant changes of the prophase, when the division actually occurs, receive no recognition in this crude explanation of chromosome division. The form of the chromosome is a much more involved question than would appear from the statement of the case by Della Valle.

5. Chromosome behavior

Of very great importance for the theory of chromosome individuality is the behavior of the elements united into multiples. If the chromosomes are merely colloid masses—products of the cell—it would not be expected that they should exhibit any differential characters. The very fact that such differences of behavior exist is a disproof of all theories which postulate complete loss of identity and reformation of chromosomes at each mitosis. Unless there is continuity of substance there can not be continuity of organization, because we know of no organization independent of material basis. Besides, such persistence of structure and substance is observable. As indicating the high degree of this specificity of chromosome characters, the multiples are of much value, especially when they involve the accessory chromosome. If all the elements of a mitosis are

'identical' and 'homogeneous,' as Della Valle asserts, there would not be the marked difference in behavior of the euchromosome and the accessory chromosome which we always witness in the Orthoptera. Most especially would this be true where the two types constitute a single mitotic element. What a contrast the actual facts regarding exactness and differential character of the chromosomes present to the crude and vague generalizations of those who conceive the cell to be only a microscopic laboratory for the play of the chemical forces involved in colloidal crystallizations. It is however fortunate that there are minds not content with a half way denial of the facts relating to nuclear constitution and the substitution of indefinite generalization about the activity of the cell as a whole or of specific enzymes, etc., but which push the denial of precision of organization to an unconscious *reductio ad absurdum* in some field of chemistry. For through this service we are brought to see the contrast between clearly defined facts, gained through researches of the most exacting and painstaking character and the uncertain and contradictory theories springing from impatient and prejudiced minds.

The facts regarding the differential behavior of the chromosomes, together with those relating to numbers, sizes and forms, are, many of them, now commonplaces of observation and are regularly determined by beginning students in microscopic anatomy. Others, such as those exhibited by the euchromosome multiples described in this paper, are no less striking and significant. They all speak clearly and unequivocally in favor of an order, in terms of morphology, and against one of a more remote and molecular character. But no single feature of chromosome behavior possesses the significance and fundamental importance which attaches to the unquestioned reproduction of each individual chromosome during mitosis. Why, unless there be something in the organization to be preserved, should the elements so carefully reproduce themselves down to the most minute structural peculiarities? It is inconceivable that the complexity and exactness of this process should exist unless there is some correspondingly important function to be carried out.

If the chromosomes are homogeneous and identical, as Della Valle asserts, their substance could most easily be divided while they were in 'solution.' The complication of the mitotic process is all out of proportion to the simple end to be served under such an assumption.

6. Chromosome distribution

In most mitoses it would seem that every step of the process is directed toward securing the exact distribution of the chromatin elements. The outstanding exception to this rule has been the independent movement of the accessory chromosome in one of the maturation mitoses, where it goes into one cell entire without division. This is clearly a differential mitosis, featured by an asymmetrical distribution of the chromosomes. One of the elements possesses a distinctive character not shared by the others—it has an individual and more or less independent movement which takes place at only one time in all the history of the organism. Up to this one point it is distributed in mitosis like the other chromosomes, but just here it betrays the inherent difference of its nature. So much of the chromatin substance acting as a unit in mitosis, possesses distinctive characters. Are these the consequence of this separate unity, or is there some specific nature of the material? The history of the hexad multiple chromosome answers this question definitely in favor of the latter alternative, for, although joined to another element, the same characteristic features of behavior and distribution mark the accessory chromosome as when it is free. It is incomprehensible that there should be this definiteness of action on the basis of mere chance—such a conclusion is foreign to all our experience with living structures. Taken in connection with the parallelism between the development of sex and sex linked characters, and its distribution during maturation, all the history of the accessory chromosome speaks for specific organization and self perpetuation. The fact that it violates the practically universal rule of a complete division of all the members of the complex in each mitosis in order to accomplish its necessary distribution and self perpetuation is an unanswerable argument

for its specific individuality. That this distribution is properly maintained, although the element is no longer free in the multiples, is only another confirmation of the conclusions otherwise determined.

Another evidence of specific organization through acts of distribution comes from the studies, upon unequal and heteromorphic chromosomes by Miss Carothers ('17). Organization, expressed in size or form, is perpetuated by a distribution and a conceivable recombination according to chance movements. There is no indication that any indefinite variation exists. While it is true that no explanation of the origin of the heteromorphic condition is strongly suggested by the facts so far observed, it may be noted that Wenrich's work affords objective evidence regarding inequalities in size. Always a means for the maintenance of given conditions appears in the mechanism of mitotic distribution.

The same precision in distribution which marks the accessory chromosome is true also of the elements which unite to form the euchromosome multiples. No difference exists between the distribution of these members of the complex, whether free or combined. It might be thought that the intimate and apparently permanent association of two chromosomes, resulting in the formation of a new mitotic unit of different value, would materially change the activities of the components. So far as I can see this is not the case, and even the details of movements in the prophase are preserved in the face of the multiple condition.

The problem of accounting for the occurrence of a given variable series of heteromorphic chromosome combinations in Trimerotropis has been considered by Miss Carothers and she properly arrives at the conclusion that segregation and chance recombination of components offer a competent explanation of the observed phenomena. At the same time there is not excluded the possibility of a readjustment of relations between the chromosome complex and the new cytosome at the time of fertilization, which, within a limited range of possibilities, would result in a similar variable series. The conditions within the

species of *H. viridis* would not be fully met by the explanation through segregation and recombination, without assuming a heterogeneous character of the material or a lack of survival of certain classes. While both of these possibilities exist they are remote, and the phenomena, taken in their entirety, are much more suggestive of a reconstitution of relations in the new individual, probably at its origin on fertilization. This is a problem which concerns the most fundamental activities of the chromatin substances, and about which we lack information on all the stages of fertilization and maturation of the egg. Breeding experiments should serve to decide between the possibilities suggested, and these will be carried out, but the nature of the processes will not be clear until the complete cycle of the chromosome complex has been studied. This fact has been realized since the beginning of the work on the Orthoptera, but the technical difficulties involved are great and so far have not been overcome.

While, therefore, it is not possible to draw any definite conclusions from our present knowledge, it may be desirable to direct attention to the conditions which confront us. Reasons are given elsewhere for considering the individuality of the chromosomes as well established by the observed uniformity of number, size, form, behavior, etc., but in the case of the multiple chromosomes we face a series of variations. As has been pointed out, variability is a universal property of living matter and is a subject for investigation, not an evidence of lost individuality. Taking into consideration, first, the accessory chromosome, whose individuality is marked so definitely as to be unquestionable, we find, that, within the species *H. viridis*, it may possibly vary slightly in size although this may be only apparent. From this we would draw the conclusion that, for the species, size is a fairly safe criterion of homology, although not absolutely exact. Using size to identify homologues, then, we discover that the accessory chromosome is joined to the largest chromosome in class 2 (plate II), to the third or fourth from the largest in class 3, and to the fifth from the largest in class 4. With persistent union, segregation and chance com-

bination it is apparent that there could be no union between a spermatozoon of class 2 and an egg of classes 3 or 4, for instance, without synapsis of non-homologous chromosomes. Still using size as a test for homology, we find no such indication of non-homologous chromosome unions in this species.

This difficulty entirely disappears, however, if we assume that the formation of multiples takes place at fertilization. Such a view is in strict accord with our knowledge of the importance of this step in the production of differences between individuals and of constancy of character exhibited by each of these variants throughout its subsequent history. Also there is offered a ready explanation of the formation of multiples, which would, at the same time, explain the variety of elements sometimes involved, as in this case; for if there be a period at which chromosomes are brought into intimate relations in a linear series, multiples would be merely persistent cases of such associations. The contrary might also be true, the multiples showing a tendency to overcome forces keeping the chromosomes apart, but, in view of the variety of elements involved in *H. viridis*, this would seem less reasonable than the first explanation. There are also other considerations, into which I have not the time to enter here, which incline me to believe that multiples indicate the operation of a common principle of chromosome association. The alternative for this explanation of the conditions in *H. viridis*, by reason of associations established at the time of the union of the spermatozoon and egg complexes, is the assumption that, at some time, each of the classes represented in plate 2 became established in some way and has since maintained itself by the segregation of its elements and their chance recombinations, with the elimination of all the unrepresented conditions. By this we are still left without an explanation of the first instance of each class and must confront a selective mortality of considerable magnitude, for which there is no evidence.

Such an explanation, as that to which I incline, for the conditions of the multiple chromosome in *H. viridis* does not make impossible another for the case of *Trimerotropis*, and it may

well be that in the latter instance, which concerns questions of fiber insertion or other variation between homologous chromosomes, the simple method of segregation and chance recombination obtains, while in *H. viridis*, where non-homologous chromosomes are involved, another mechanism is operative. Genetical experiments will shed some light on these difficulties and it is hoped that these may be completed before long.

7. Chromosome individuality

As has been stated elsewhere in this paper, the circumstances relating to the multiple chromosomes are strong proofs of the theory of chromosome individuality, as indeed are all facts indicating order and definiteness in form and behavior. So far as I can see there is no half way ground between the assumption that the chromosomes are definite, self-perpetuating organic structures and the other which presents them as mere incidental products of cellular action. According to one view individual chromosomes are descendants of like elements and possess certain qualities and behavior because of their material descent, the visible mechanism for which is the process of mitosis: according to the other any similarities that may exist in the complexes are the result of chance aggregations of non-specific materials. It is a choice between organization and non-organization in the last analysis, at least in terms of cellular structures. To attempt the substitution of a conception of molecular organization, which is beyond the experience of the biologist and which exceeds the present powers of the chemist to analyse, is to cast aside all hope of solving the problem of cellular action, because it is necessary to understand, not only the physical and chemical phenomena involved, but also their different forms in the various parts of the cell. It is readily admitted that these are not the same in the nucleus and in the cytosome, but some hesitate to recognize differences between parts of the nucleus, and more are disinclined to grant specific activities to the chromosomes.

Since it is not possible to observe directly the action of the chromosome we are obliged to make use of indirect evidence,

seeking parallels between elements of structure and action in the chromosomes, and the mass effect of cellular action as exhibited in the so-called body characters. Such a method is justified by all our other experience in tracing relations between structure and function in organisms, and while it apparently resolves the individual into parts of greater or less independence, has given us our best conceptions of it as a whole. Homologies have been much more significant of relationship than analogies when concerned with organic parts, and I do not doubt that conclusions drawn from structural relations will greatly exceed in value the remote and equivocal analogies between nuclear structure and colloidal phenomena. On the basis of our experience we anticipate a commensurate relation between structure and function, but this does not mean a fixed and exclusive correspondence—it does not signify that a function necessarily entirely lapses in the absence of a certain structure. All our observations indicate that the relatively few functions of the living substance—irritability, contractility, metabolism and reproduction—are shared by all its parts in varying degrees, and when we speak of the function of any somatic organ or cell part we mean its outstanding and preponderant activity.

With this understanding of the relation between morphology and physiology, we speak of the chromosomes as having to do with the process of reproduction, and conceive of them as non-homogeneous within themselves and individually unlike. In this sense they bear factors, or have parts, which are most concerned with certain peculiarities which, through cumulative action in cell reproduction, come to fullest expression in regions of the complete soma. Through what manner a factor operates to produce a cell structure which, in a given cell aggregate, summates in the form a somatic character, we do not know, and that is not the question immediately at issue in the individuality hypothesis. What is postulated there is that the chromosomes are self-perpetuating entities with individual peculiarities of form and function to identify them. Characteristics of form and behavior we see; certain very definite parallels between these and the manifestations of somatic characters exist

beyond question; provision for the perpetuation of the organic unity of the individual chromosomes is found in the process of meiosis; the actual direct result of its operation appears in the uniform conditions of the complex in the individual animal; the extension of this beyond the organism to the group and the means for it in the phenomena of maturation and fertilization are easily established by observation; the age old existence of all these circumstances is revealed by the near approach to uniformity in the chromosome complex of the multitude of species of unnumbered individuals constituting a family. And yet, in the face of this overwhelming mass of evidence indicative of order, system and specific chromosome organization, some conceive only the action of ordinary chemical forces, or the chance association of indifferent substances, while others, over impressed with the thought of a general coordinating force in the organism, deny significance to the orderly play of its cellular parts.

While opponents of the individuality hypothesis differ thus widely in what they would substitute for it, they almost invariably seize upon one supposed condition of the chromosome as a basis for discounting the remaining positive evidence which is presented for the hypothesis. Even some well informed cytologists who accept the implications of the facts, regard this circumstance as a severe weakness in the chain of evidence. That the chromosomes do not maintain a compact and easily recognizable form in the interval between mitoses is accepted by many such biologists as proof that they no longer exist as entities. All the other manifold indications of character and continuity do not weigh against this apparent loss of identity. Doubtless it would be more satisfying if we could at all times perceive the chromosomes in unchanging form in all stages of cellular activity, but why we should demand this condition as a test for individuality in the chromosomes when we unhesitatingly admit the unity of an organism in all the varied changes of its development from a single cell, through such complexities of change and metamorphosis as to give rise to doubts of even the phyletic position of some stages, it is difficult to see. Being organic, the chromosomes must change their form, they must

suffer division of their substance and they are obliged to restore this loss through metabolic changes. Since these changes of substance take place at surface contacts there is an obvious advantage in increased superficies and, in common with other, larger structural elements, the chromosomes become extended and their substances are diffused. In this state their boundaries may not be well defined and this circumstance has been seized upon as a disproof of their continuity.

For myself, even were the visible limits of the chromosome completely lost, this would not appear as a convincing disproof of persistent individuality in the face of the large number of facts pointing in the opposite direction. Of such facts those relating to the history of the multiple chromosomes, outlined in this paper, are of great importance. Their bearing upon the various aspects of numbers, sizes, forms and behavior, has been mentioned under these different headings. At this point however I wish to summarize this evidence upon the general topic of individuality. But before doing so I should like to repeat statements previously made that the accessory chromosome in the germ cells of the male presents us with the history of a particular chromosome, at all times distinct and well delimited, whose physical identity suffers no eclipse during the various metabolic changes of growth and division. It is readily distinguished where it occurs, not alone in a given cell, but in all the germ cells of the species, genus, family and order. The same element, always recognizable by reason of its structural character, is identified in different phyla and is shown to have a definite relation to the development of sex characters. It would seem impossible to conceive any more definite marks of individuality than is possessed by this chromosome, in whose history there is no confusion of character or break of continuity, and yet it does not receive even mention in some attacks upon the theory of individuality.

Again, its position may be granted, but with the reservation that it is unique and no criterion for other chromosomes. Upon this point it is now merely necessary to say that the accessory chromosome differs only in degree from the euchro-

mosomes, and general conclusions regarding the nature of chromosome organization may properly be drawn from its history. That its condition is indeed not unique is shown by various approaches to it on the part of certain chromosomes in *Phrynotettix*, as demonstrated by Wenrich ('16). The great similarity to the euchromosomes is also indicated by the union with the accessory chromosome in multiples, and by its entirely typical nature in the female when paired.

The mere academic question of individuality is not here important, the practical matter before us is to decide whether the metaphase chromosomes of two cells are individually identical organic members of a series because they were produced by the observed reproduction of a similar series of the parent cell, or whether the resemblance is independent of this genetic relation and due to chance association of indifferent materials, or to a reconstituting action of the cell as a whole. It is my belief that the observed act of reproduction, by which the organization of the chromosomes is materially transmitted in each mitosis, together with all facts indicating extensive distribution of given conditions, definiteness of organization, uniformity of behavior and consistency of deviation from the normal, are so many clear indications of the individual character of the chromosomes. Transmutation of form, even to an extreme degree, can not be held as a valid argument against a persistent individuality. A consideration of the criteria applied to larger organic aggregates well supports this view. Such objects are said to possess individuality when they exhibit a more or less definite unity which is persistent and characterized by peculiarities of form and function. Most clearly defined is this individuality when it may be perpetuated through some form of reproduction to find expression in new units of similar character. The term does not connote unchangeability, and there may be fusions with more or less loss of physical delimitations, followed by separation, even after exchange of substances. The test of individuality is material continuity, but it does not necessarily involve complete or entirely persistent contiguity. An organism may bud off new individuals similar to itself, the substance of its body

differs from time to time, movements of parts take place, fragmentation occurs, extreme attenuation or extension of substance is found, even separation and recombination of parts may happen and yet the individual maintains itself. What it may have been in the past, what its possibilities of future development are, what potentialities of multiplied individuality it suppresses do not affect the reality of its individuality. It is, as Huxley says, "a single thing of a given kind." If one such thing divides into two, there are two individuals; if two unite into one indistinguishably there is a single individual; if a fusion of two things occurs in part, without loss of physical configuration, there are still two individuals in existence. Only when the substance of one thing disappears or becomes incorporated integrally into the organization of another does its individuality depart.

If all these variations of physical state may occur in the history of an organism without sacrifice of individuality, there can be no reason for urging them against a conception of the individuality of the self perpetuating chromosomes. Especially is this true in face of the facts recorded here and in other papers showing the high degree of chromosome constancy, for any given period, in all the attributes by which we usually judge individuality. The conditions in *Hesperotetix* and *Mermiria* at first seem to be a contradiction to this generalization, but, when examined with care, show, not an instance of fundamental change, but merely of modified detail. From these results it is very clear that a chromosome number less than normal for the group is not a necessary evidence of lost elements. In a similar manner the studies of Miss Holt on the intestinal cells of *Culex* indicate definitely that numbers in excess of the normal do not signify the addition of anything not previously represented. Loss or gain in chromosome numbers is a condition to be investigated, not implicit evidence of an altered organization with loss of organic individualities. That the chromosomes are not the ultimate structural units is, however, indicated in many ways, but it remained for Wenrich to show the nature of this more minute portion of the chromosome architecture. From

his results it would appear that all chromosomes are, in a sense, multiples, and it is conceivable that these parts might be variously associated, producing even extensive numerical variation, still without loss or gain of essential structural elements.

The peculiar case of *Ascaris megalocephala*, where evident multiple chromosomes are retained intact in the germ cells while they suffer fragmentation in the somatic cells, is an extreme instance of numerical variation which occurs in one individual. How common such relations may be we do not know, since so little has been done in the study of the complete history of a single organism; but it is obvious that the Orthoptera stand at the opposite extreme from *Ascaris* in the persistence of chromosome organization, for here all the cells of an individual seem to be invariably the same, and the individual is representative of the group. But even in this stable assemblage we encounter the conditions which I have described for *H. viridis*. This case, with its diminution in number, and that of *Culex*, with its great increase, are alike in the fact that the measures of difference are entire chromosomes and not parts of such, as seems to be the case in *Ascaris*, unless indeed these chromosomes be multiples of a high order of complexity.

If it were possible for chromosomes to reproduce themselves and still preserve their physical configuration unchanged, there would probably be little question of their continuity and individuality—the demonstration would be self evident. But it happens that the necessities of the case require that each newly produced chromosome should take part in the formation of a new nucleus, through whose activities the cell as a whole and each chromosome, individually, is enabled to restore the volume diminished by the act of division. During this process the outlines of the chromosomes become materially changed and in their extreme diffusion can no longer be traced in many cases. Because of our limitations in observational power they appear to be lost as separate individuals and we are thus deprived of the simple test of observed continuity. Later, in the same cell, there reappears a series of chromosomes severally like those which seemed to disappear during the period of metabolic activity.

We confront two alternative explanations for this re-integration of the chromosomes; either they actually persist as discrete units of extremely variable form, or they are entirely lost as individual entities and are reconstituted by some extrinsic agency. There is no other possible explanation and we must weigh the evidence for one or the other of the alternatives.

All facts which indicate order and system in chromosome features speak for the former, those which demonstrate variability and indefiniteness, for the latter. The case for discontinuity is strongest in the absence of any chromosome order, and becomes progressively weaker with the establishment of definiteness and precision in form and behavior. Evidence has already been given in other papers on the Orthoptera to show the exactness in organization of chromosomes, their persistent continuity between mitoses in certain cases, the uniformity in number and behavior through large groups, and the parallel behavior of chromosomes on one hand, and of somatic characters on the other. In this paper are discussed what appear to be the widest departures from normal conditions so far found in the group. Accepting as most important, next to actual observed continuity, the evidence afforded by constancy in number, size, form and behavior of the chromosome, I have been able to show that these criteria are here applicable and that variation is only apparent and not real. It seems to me that the facts disclosed in *Hesperotettix* and *Mermiria* not only fail to weaken the case against individuality, as tested by constancy in number, size, etc., of the chromosomes, but, on the contrary, greatly strengthen it, for the reason that all of the distinguishing features of the individual chromosomes are maintained, even in the presence of unions into multiples of higher order. The fact that, although the number of independent units is reduced, nothing is lost from the complex, is most important and significant.

Finally, the main feature of chromosome individuality must not be lost in a discussion of whether it is the chromatin or the linin that persists with less change. However great in importance this distinction may be, it does not alter the aspect of the problem of individuality in its present phase. One or both of

these substances may be responsible for the integration of the chromosomes, but it is the fact that there is a definite structure to be identified which concerns us most. The substances of the chromosome divide into new units of similar relative sizes, and these structures, now of half the original content, take up unlike materials and replace the missing portions. The complexes of the daughter cells are now equivalent to that of the mother unit. It is obvious that, since the chromosomes, as such, divide, all of their substances must be involved. There may be some question whether one may be lost as a morphological entity while another persists—although this is much open to question—but something maintains the organization which we recognize in the chromosome and it is this organization which we study. In view of the fact that our microchemical tests are so far from specific in their action, it is not the part of wisdom to build very extensive theories upon their evidence. It is quite possible that one substance, in different phases of its activity, may present alternately the aspects of chromatin and achromatin, and some conditions of the staining reaction suggest this, so that it would be unwise to involve the whole question of chromosome organization in a dispute regarding the nature of their substance, based merely upon uncertain staining reactions.

8. Chromosome specificity

That chromosomes might be genetically continuous and still be of the same nature, without differential character, is, of course, conceivable. In fact it is more than probable that, in a sense, they are all alike, for they probably share the general properties or functions of protoplasm. But that they carry out these activities in the same manner is a conclusion quite foreign to the evidence. Just what the nature of this differential action may be has not yet been discovered, but the action of the sex determining chromosome and the groupings of characters in *Drosophila* are suggestive. Male and female possess the same series of parts, and the difference between them is one of relative development. Nothing unique for either sex exists and such a thing

as an 'exclusively male' or exclusively female character is not to be found. The evidence here would indicate that the 'factors' for maleness, are such controls of the developmental processes as will eventuate in a certain degree of differentiation of each cell of the body. The sum of all these, in any somatic region, produces a condition which we call a male character, and the total complex of these constitutes an individual which is a male. In another individual the same series of elements obtains, but each cell and each part is slightly different and the sum total of the characters produces an assemblage which we recognize as a female. Should the conditions be varied, even by the internal secretion of the gonad, in some cases, these may be altered to resemblance of the opposite sex.

However much the entire complex is involved in the production of characters which are called sexual, it is apparent from the history of the accessory chromosome in the Orthoptera that it is a differential agent. All other things apparently being equal, the presence of one accessory chromosome so shapes the developmental processes that a male results, while if two are involved a female is produced. In some way, not now apparent, the action of this particular one of the chromosomes differs from all the others in producing an effect of which they are not capable—that is, it has a specific action. Apparently it is unlike them in being concerned with the entire body, including the germ cells, which are differentiated into eggs or spermatozoa. A further indication of the specific nature of this particular chromosome is afforded by sex-linked characters whose development is conditioned by exactly the same circumstances of distribution as those which mark the alternatives of maleness or femaleness. The facts relating to the accessory chromosome are the strongest evidence we have in support of chromosome specificity, because the history of this element is so clear, its continuity so unbroken and its relations to certain characters so definite. But any evidence for specificity of one chromosome is, at the same time, support for the general conception of the differential nature of chromosomes, and for this reason the facts concerning the accessory chromosome have additional value.

The only direct evidence for any such a differential nature of the other chromosomes is afforded by the work on *Drosophila*. In this case a whole group of characters follows the rule of distribution governing sex. This is the only element that can certainly be identified, but the groups of observed characters, not sex linked, correspond to the three remaining pairs of chromosomes, and the numbers of characters in each group are in proportion to the size of the chromosomes. Of these one pair is very small and there is a corresponding small group of characters. As is well known, the analysis of the conditions in *Drosophila* has gone so far that the relative loci for the different factors have been calculated. It would seem from these results that there is every reason to regard the chromosomes of this fly as qualitatively different. While there is no correlation of this sort known for the Orthoptera, the actual history of the chromosomes which their germ cells exhibit affords a mechanism adequate for the facts of alternative inheritance and for the segregation and chance recombinations of characters. While the multiple chromosomes do not afford any direct evidence for specificity of function, it would seem, because of all the facts, that the purpose for which these studies on the Orthoptera were begun—a correlation between germ cell structure and somatic characters—is much more feasible of accomplishment than appeared at the beginning.

V. SUMMARY OF RESULTS

1. Chromosomes are definitely organized chromatic bodies acting as units in mitosis.
2. These units are of unlike morphological value in the different generations of germ cells.
3. While any one cell generation is marked by one general type of chromosome organization, individual chromosomes may differ from the type in higher or lower degree, by definite steps or intervals.
4. In a given species the integration of the chromosome complex may vary from individual to individual.
5. For any one individual this integration is fixed.

6. Despite this individual variation in the composition of the mitotic units, there is no loss in the total morphological elements of the specific complex, or departure from the usual habits of synapsis and segregation of homologous elements.

7. The maintenance of the original complex in the presence of a variable number of mitotic units results from associations of these to produce others of higher valence.

8. Where variation between individuals exists for any one cell generation, it is the result of differences in association between certain definite mitotic units.

9. Such variations are not indefinite, but occur in a fixed order between certain units.

10. The occurrence of associations between units of lower order into those of a higher results from the operation of a common integration principle.

11. The union of homologous chromosomes to produce tetrads is temporary and is terminated in one of the following maturation divisions; multiples of non-homologous chromosomes are permanent for the individual and the union does not end in either maturation mitosis.

12. Associations in the first spermatocyte of a higher order than tetrads are the result of lower associations which are persistent throughout all cells of the individual.

13. Associations into tetrads in the first spermatocyte occur between homologous chromosomes.

14. Higher associations than tetrads in the first spermatocyte are necessarily non-homologous.

15. Associations may be complete and persistent, or only approximations.

16. Associations may be complete in parts of chromosomes and incomplete in other regions.

17. The point of union between associated units in metaphase is at the ends of the chromosomes.

18. In telomitic chromosomes this is at the point of fiber attachment.

19. Atelomitic chromosomes of unit value have not yet been observed in multiples, except in that part of the tetrad of *Mer-miria* not joined to the accessory chromosomes.

20. Multiple chromosomes are of two types (a) those between euchromosomes and heterochromosomes and (b) those between euchromosomes alone.

VI. BIBLIOGRAPHY

A. Additions to my former list ('14) of papers on Orthopteran spermatogenesis

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1916 A study of the germ cells of *Gryllotalpa borealis* and *Gryllotalpa vulgaris*. Jour. Morph., vol. 28.
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B. Articles cited on other than Orthopteran material

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1911 La continuita della forme di divisione nucleare ed il valore morfologico dei chromosomi. Archivio Zoologico, vol. 5.

- DELLA VALLE. PAOLA 1912 La morfologia della cromatina dal punto di vista fisico. Archivio Zoologico Italiano, vol. 6.
 1913 Die Morphologie des Zellkerns und die Physik der Kolloide. Zeitschrift für Chemie und Industrie des Kolloide, Bd. 12.
- HANCE, R. T. 1917 The somatic mitoses in the mosquito, *Culex pipiens*. Jour. Morph., vol. 29.
- HOLT, CAROLINE 1917 Multiple complexes in the alimentary tract of the mosquito. Jour. Morph., vol. 29.
- WHITING, P. W. 1917 The chromosomes of the common house mosquito, *Culex pipiens*, Linn. Jour. Morph., vol. 28.

DESCRIPTION OF PLATES

All figures are drawn at an initial magnification of 2860 diameters under the camera lucida, and appear in the reproduction at a magnification of 1800. The photomicrographs were obtained with a Zeiss 2 mm. objective of 1.40 N. A., projection ocular 4 and a Watson 'Holoscopic' oil immersion condenser. The original magnification of 1000 diameters is here reduced to 666, except fig. IX.

PLATE 1

EXPLANATION OF FIGURES

Horizontal rows represent complete first spermatocyte complexes, usually in lateral view, numbered 1 to 6. These are arranged so that chromosomes of homologous size are brought into vertical rows, numbered from 1 to 12. Blank spaces in vertical rows represent the normal position of the accessory chromosome which is joined to a tetrad.

- 1 A complex of *Hesperotettix brevipennis*. All twelve chromosomes are free.
- 2 *Hesperotettix festivus*, polar view.
- 3 *Hesperotettix viridis*, class 1—twelve chromosomes plus a supernumerary.
- 4 *H. viridis*, class 2.
- 5 *Hesperotettix pratensis*.
- 6 *Hesperotettix speciosus*.

PLATE 2

EXPLANATION OF FIGURES

Complexes, arranged as in plate 1, of representatives of the various classes of first spermatocytes in *Hesperotettix viridis*. The classes are indicated by the figures at the left, 1 to 6. Euchromosome multiples are arranged between the rows to which their tetrads belong. Not all conditions are represented here but may be found in other figures. Thus the smaller octad of class 4 may also be a ring in some individuals; the smaller ring of class 5 may be a V; and the ring octad in one individual of class 6 is a V.

MULTIPLE CHROMOSOMES

PLATE 2

CLARENCE E. MCCLUNG

PLATE 3

EXPLANATION OF FIGURES

1 to 5, 7 to 9 Polar views of spermatogonial metaphase complexes. 1 to 7 are of *Hesperotettix viridis*, 8 of *H. brevipennis* and 9 of *H. speciosus*.

1, is of class 2, showing twenty-two chromosomes, of which one is a multiple. The accessory chromosome portion of this is marked 'X.'

2 An example of class 3. The members of the large first spermatocyte euchromosome multiple ring are the larger V-shaped elements, while the smaller V includes the accessory portion of the hexad. Twenty separate chromosomes.

3 A complex of class 4, in which the largest two V's are parts of the first spermatocyte ring, the next largest one of the octad V, and the smallest of the hexad. Nineteen separate chromosomes.

4 In this group, representing class 5, there are three V's belonging to two octads of the first spermatocyte, the smaller of which is a V. The accessory chromosome is free. Twenty separate chromosomes.

5 From an individual of class 6 with a free accessory chromosome and one octad ring in the first spermatocyte. Twenty-one chromosomes.

6 An anaphase group of class 4 showing the 4 V's (fig. 3 above).

7 A complex of class 6 in which the octad of the first spermatocyte is a V instead of a ring. The one V in this spermatogonial group is involved in the formation of this multiple. Twenty-two free chromosomes.

8 From *H. brevipennis* in which all specimens studied showed twenty-three rod shaped chromosomes.

9 The one V in this complex of twenty-two chromosomes will constitute a part of the hexad in the first spermatocyte. The accessory chromosome forms one limb.

10 An octad ring of *H. viridis* in the late prophase. It will commence to separate at the apex.

11 Late prophase condition of such a chromosome as shown in figure 10.

12 Shows metaphase condition of such octad rings. These are from different cells.

13 Two metaphase octad rings of *H. viridis* from the same cell. Class 5.

14 Similar elements from another cell of the same individual as shown in figure 13. The large octad *b* has one tetrad forming a ring lying in the equatorial plate at right angles to the other member. The small octad *a* shown edgewise.

15 Five examples of the V-shaped octad of class 4. The inequality of the constituent tetrads shows clearly.

16 Multiples of class 4; *a* the large octad and the hexad; *c* the two octads, the larger like figure 14 *b*.

11

PLATE 4

EXPLANATION OF FIGURES

17 Examples of octad rings in late prophase showing various aspects during the process of opening out. These are arranged somewhat in the order of progress from *a* to *k*.

18 Lateral view of first spermatocyte metaphase, class 6. One octad ring, and accessory chromosome at one pole.

19 Three examples of extended V's, such as shown in Photo Y.

20 Lateral view of first spermatocyte metaphase of class 5 with the V portion of the smaller octad going to same pole as the accessory chromosome. Ten free chromosomes.

21 A complex of a first spermatocyte of class 6 in which the one octad is a V.

22 Two first spermatocyte octads from one cell.

23 A complex of *H. pratensis* in which the hexad is similar in shape to that of *Anabrus*.

24 A complete complex of *Hesperotettix viridis*, class 3, in which the octad has opened at one side, simulating the appearance of the hexad in *Mermiria*.

25 The three multiples from a complex of class 4.

26 An entire complex of class 3, from the same animal as in figure 24.

27 A representative complex of class 5 with one V.

PLATE 5

EXPLANATION OF FIGURES

28 First spermatocyte anaphase, class 4 with one V, showing the distribution of the rod and V parts, in black. The entire complex, including the other multiples is shown.

29 to 42 Various second spermatocyte metaphase complexes (except fig. 33) in polar view, of *H. viridis*.

29 Class 1 with accessory chromosome.

30 Class 2 without accessory chromosome.

31 Class 2 with accessory chromosome in multiple.

32 Class 4, (one ring, one V and hexad in first spermatocyte) no accessory chromosome, two rods of V, and half of ring.

33 First spermatocyte anaphase of class 4, no accessory chromosome.

34 Class 6 (in first spermatocyte one ring, free accessory chromosome) no accessory chromosome.

35 Class 6 with accessory chromosome.

36 Same condition as figure 35, same animal.

37 Same condition as figure 35, another animal.

38 to 42 From one animal of class 6 with the octad in the form of a V, accessory chromosome free.

38 With V and no accessory chromosome—ten chromosomes.

39 With V and with accessory chromosome—eleven chromosomes.

40 No V and no accessory chromosome—eleven chromosomes.

41 As in figure 40.

42 No V, but with accessory chromosome—twelve chromosomes.

PLATE 6

EXPLANATION OF FIGURES

43 to 54 From *Mermiria bivittata*.

43 Somatic complex of the female with twenty-two free chromosomes and two similar multiples, each containing an accessory chromosome. The euchromosomes are all telomitic.

44 A spermatogonial complex of twenty-two free chromosomes with two unlike V's, in one of which the accessory chromosome betrays its presence by its characteristic granular condition. The other V is smaller and is confined to the male line by always going into the opposite cell from the accessory chromosome in its segregation division.

45 Another female somatic complex.

46 Spermatogonial complex.

47 Pairs of spermatogonial V's in different stages showing disproportion in size.

48 A female somatic complex—one V foreshortened.

49 Spermatogonium, V-chromosomes. *a* and *c* associated pairs.

50 *a* The multiple in first spermatocyte anaphase, lateral view; *b*, *en face*; *c*, as these elements would appear in second spermatocyte metaphase.

51 Multiples in late first spermatocyte prophase showing the marked difference in appearance between the smooth, homogeneous, condensed accessory chromosome and the yet granular tetrad to which it is joined.

52 First spermatocyte multiples, *b* almost fully condensed and showing non-chromatic clefts. These are about in the relative positions of the fiber attachments and the plane of division.

53 As in figure 51.

54 Like 52 *b*.

PLATE 7

EXPLANATION OF FIGURES

55 to 65 From *Mermiria bivittata*.

55 First spermatocyte metaphase showing the multiple and the five smaller chromosomes divided.

56 Three multiples of first spermatocyte metaphase.

57 Like figure 56.

58 Three multiples of the 'green' variety of *M. bivittata* in which fiber attachment is almost at the end of the bent portion. At *a* complete separation, in *b* and *c* beginning stages.

59 Similar to figure 58. Note the various constrictions, simulating a decad constitution, emphasized at *a* by separation at two levels.

60 Complete separation of bent rod opposite accessory chromosome.

61 Polar view of second spermatocyte metaphase.

62 Lateral view of same stage.

63 to 65 Polar view of second spermatocyte metaphase. All have eleven chromosomes, one of which is a V, but in one case, figure 63, this is a simple chromosome and in the other, figure 64, it is a multiple involving the accessory chromosome.

PLATE 8

EXPLANATION OF FIGURES

A to Z and II to III are photo micrographs of *Hesperotettix viridis* cells and IV to IX of *Mermiria bivittata*. A to D and IV to VI smears, the remainder sections.

A, B, C show the appearance of multiple rings with the longitudinal split and numerous clefts, D is a lateral view of a first spermatocyte metaphase showing the multiple ring in profile and the accessory chromosome at one pole.

E Polar views of spermatogonial metaphase with telomitic and atelomitic chromosomes.

F, G, H Profile views of hexad multiple.

I to N Lateral views of V-shaped multiples showing characteristics of form and movement.

O Ring and V. multiples.

P, A V-multiple with one limb out of position.

Q to T Ring multiples in which one tetrad has the plane of its ring perpendicular to the other.

U, V Late stages in the movement of such rings as appear in Q to T.

W, X Late metaphase stage of ring multiples.

Y An extended V similar to that of P.

Z A ring multiple about to divide.

II Anaphase condition of a V-multiple (cf. fig. 28).

III Late anaphase condition of a divided ring. The resulting V's are double.

IV Prophase condition of a hexad showing the accessory chromosome condensed and the tetrad thin and granular.

V, VI Metaphase hexads showing constrictions.

VII, VIII Profile views of hexads.

IX Lateral views of mitoses $\times 400$.