# THE ACCESSORY CHROMOSOME—SEX DETERMINANT?

C. E. MCCLUNG

*Laboratory of Zoölogy and Histology,*
*University of Kansas*
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## INTRODUCTION

That most organisms occur as two fundamental types—male and female—has been a commonplace observation since antiquity. However, the actual mechanism of sex determination was unknown until the early part of the 20th Century, when it was shown that sex of progeny was determined by the chromosomal makeup of the zygotes from which they developed.

In 1891, Henking noted that some insects (*Pyrrhocoris apterus*) showed sexual differences in karyotype and in meiosis. Females had 24 chromosomes that behaved as 12 pairs of homologs during meiosis, whereas males had 23 chromosomes that behaved as 11 pairs of homologs and one solitary chromosome. This lone chromosome became known as an ACCESSORY CHROMOSOME. (In one of his figures, Henking happened to label the accessory chromosome with the letter "X" and this ultimately led to the notion of an "X chromosome".)

With regard to the distribution of the accessory chromosome (which in his preliminary work he called a "nucleolus"), McClung quotes Henking as noting:

> Damit ergiebt sich aber die wichtige Thatsache, *dass wir zweierlei Spermatozoen erhalten: die einen besitzen einen Nucleolus, die anderen nicht.*

That is, spermatozoa were of two kinds: one with the nucleolus and one without. Similar findings were reported for other insect species and further observations on males carrying accessory chromosomes showed that they always produced two different kinds of sperm—one with an accessory chromosome and one without—in equal numbers.

In the present paper, McClung integrates these observations with the emerging belief in the importance of nuclear structures in determining heredity and offers the bold conjecture that the accessory chromosome might represent the long-sought mechanism of sex determination:

> A most significant fact … is that the [accessory chromosome] is apportioned to but one half of the spermatozoa. Assuming it to be true that the chromatin is the important part of the cell in the matter of heredity, then it follows that we have two kinds of spermatozoa that differ from each other in a vital matter. We expect, therefore, to find in the offspring two sorts of individuals in approximately equal numbers. ... [Since] nothing but sexual characters … divides the members of a species into two well-defined groups, ... we are

> logically forced to the conclusion that the [accessory] chromosome has some bearing upon this arrangement.

That is, McClung hypothesizes that a difference in chromosome number is the cause, not an effect, of sex determination. This paper represents the first effort to associate the determination of a particular trait with a particular chromosome.

Although McClung made some errors in the details of his proposal, his general suggestion of a CHROMOSOMAL MECHANISM OF SEX DETERMINATION has proven to be true for many different organisms. This demonstration of a link between chromosomes and a fundamental aspect of phenotype—an organism's sex—provided the first key support for the chromosome theory of inheritance. Now, of course, the multi-billion dollar, international human genome project is attempting to develop genetic maps that correlate all inherited traits with specific regions of chromosomes.

Robert J. Robbins
Seattle, Washington 2000

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# THE ACCESSORY CHROMOSOME—SEX DETERMINANT?

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## PART I. OBSERVATIONS AND COMPARISONS

The peculiar chromatic element discussed under this name in several recent papers is one that gives promise of throwing considerable light upon the nature of the chromosomes. So long as all chromosomes of the nucleus were observed to pass through a cycle of changes apparently identical in each case, there was little chance to gain an insight into their interrelations. With the discovery of the accessory chromosome and the recognition of its true chromosomic character, however, there has been offered an opportunity to draw comparisons and so to formulate conclusions which, in time, are certain to materially increase our knowledge of these most important nuclear structures.

In recognition of this fact, and with the hope of hastening such a desirable end, I have devoted some time to the study of the accessory chromosome and have also encouraged students in my laboratory to direct their attention to it. Much material has been collected, and is still

being accumulated, in order that as broad a view of the subject as possible could be obtained.

Since, however, the difficulties involved in securing and preparing material from widely different forms would unduly delay the attainment of any comparative results, I have confined my studies largely to the Orthoptera. This has been done in the belief that more substantial good can be derived from a thorough knowledge of a limited group than from a superficial acquaintance with a wider field. Once the basic principles underlying the cellular phenomena of one group are discovered, their recognition in other forms will be rendered much easier.

As a result of the studies so far pursued, it has been found that individual forms rarely present all the details of a problem equally well. Different species excel in the clearness with which certain points are brought out. A feature obscure in one species will appear distinct enough in another while for the elucidation of other structural peculiarities the relations might be reversed. By studying, therefore, an extensive collection of nearly related forms, it is possible to draw a composite outline of a process which will be found applicable in its main features to all the members of the group concerned.

This fact is taken advantage of in the series of studies upon insect spermatogenesis now being pursued by myself and students. Instead of taking one species and endeavoring immediately to make out the entire series of processes which characterize its spermatogenesis, we have considered restricted questions, and have chosen such species as would offer the best facilities for answering these. Thus, in the case of the accessory chromosome, it has been found advantageous to trace its course through the spermatogonial divisions in *Brachystola*, and through the spermatocyte changes in *Hippiscus*. Probably the spermatid transformations will demand another form for their best exemplification.

The danger involved in such a method is the liability which it offers toward accumulating a series of observations upon exceptional or strongly modified types. This has been guarded against, as far as possible, by verifying the appearances of one form by those manifested in others. While the work thus far done indicates considerable variations in the details of the different processes, it does not seem to suggest any wide deviations from general principles, so that the danger of error in the direction of exceptional instances would not appear to be great.

A recognition of the accessory chromosome as such is of very recent date and the literature upon it is therefore not very extensive. And while its chromosomic character has, in a number of instances,

been appreciated and even its participation in the supreme division act of the chromosomes in the metaphase noted, the mere resemblance in general features to the nucleoli has sufficed to caused its inclusion in the group of these questionable bodies. A majority of the references to it will accordingly be found in the literature devoted to a consideration of the nucleoli, and in only a few cases will it be found discussed as a chromosome.

On account of the scantiness of the literature, it has been thought advisable to give here all the probable references to the accessory chromosome in the language of the different authors. It may be possible thus to unify the conflicting opinions regarding the essential features and to leave the field clear for a discussion of the more involved points. Also, in furtherance of the plan previously mentioned of confining the question to a limited area, only the references to insects will be discussed in detail. By this restriction, however, very little material will be excluded since most of the work has been done upon insect testes.

In the following paragraphs will be found all the references—in the literature at my command—to structures which I regard as possibly identical with the accessory chromosome. These will be given chronologically and finally compared and commented upon.

1. The work of Platner (‘86) upon the Lepidoptera unfortunately sheds no light upon the nature of the accessory chromosome in that order of insects. Only casual mention is made of the nucleolus and the references do not enable us to gain much insight into its character. The following quotations embrace the principal references: “An letztern Punkten haben auch die Nucleolen ihre Lage. Diese erscheinen selten in der Einzahl, meist findet man deren zwei. Sie sind von ziemlicher Grösse, färben sich stärker und zeigen eine kugelige Form; doch geben meist die von allen Seiten an sie herantretenden Kernfäden ihnen ein unregelmässiges zackiges Ansehen.”

2. “Diese (Elemente) färben sich gleichmässig stark mit Safranin und zeigen nicht nur in ihren Dimensionen sondern auch hinsichtlich ihrer Zahl grosse Schwankungen. Unter ihnen befinden sich auch die Nucleoli.”

3. Henking (‘90) finds in the spermatogonia of Pyrrhocoris, in preparations fixed with picro-acetic acid, a nucleolus which is not apparent in those preserved with Flemming’s fluid. It appears rounded and of some size, and retains a yellow color while the chromatin stains dark red with carmine. It is observed occasionally to have divided.

4. Concerning the “nucleolus” of the first spermatocyte, Henking has this to say: “Vor allem auffällig ist es an diesem Zellen dass ein grosser Nucleolus zur Ausbildung gekommen ist. Derselbe bitt bei den verschiedensten Konservirungsmethoden stets sharf hervor.... Erscheint

der Nucleolus der jüngsten Hodenzellen bei der genannten Methode farblos, so nimmt er nun begierig Farbe auf, ein Verhalten, welches ganz regelmässig ist und vielfach von den beiderseitigen Nucleolus auf dem gleichen Schnitte beobachtet werden kann."

5. The position of the nucleolus is referred to as follows: "In einer Bucht an der Oberfläche der Chromatinkügelchen liegt der Nucleolus eingesenkt."

6. Regarding the constancy of appearance exhibited by this element, he says: "Allein der Nucleolus hat bei den Veränderungen von Kern und Zelle sein Aussehen nicht gewechselt. Er liegt noch wie zu Anfang als rundlicher Körper dem Rande des Kernes angenähert.... Der Nucleolus behalt seine Kugelgestalt unverändert bei, während die Chromosomen gewissermassen Pseudopodien aussenden und sich so zu einem Netz vereinigen. Der Nucleolus bietet seinerseits den Pseudopodien keine Ansatzfläschen und bleibt daher isolirt."

7. Zur Zeit der zusammenballung der Chromatinmassen ist er durch seine beträchtlicher Grösse immer noch leicht zu sehn und seine Kugelform macht ihn kenntlich wenn die Chromosomen durch Zusammenfliessen des Chromatins an Volumen ihn zu überragen beginnen. Wenn dann aber die Auflockerung des centralen Haufens anhebt, tritt eine Verkürtzung der Chromosomen ein, wodurch dieselben ihm immer ähnlicher werden. Schliesslich ist er nicht mehr mit Sicherheit heranzufinden. Dafür das er gänzlich rückgebildet wird habe ich gar keine Andeutung erhalten. Er ist so lange in voller Ausbildung deutlich zu erkennen, als ihn seine characteristiche Gestalt vor einer Verwechselung mit anderen Gebildung schützt. Allerdings müssen wir annehmen das er späterhin eine Einschnürung erfahrt da auf einem definitiven Stadium alle Chromosomen eine gleiche Form besitzen.

8. Having thus traced his "Nucleolus" up to its final disappearance among the group of chromosomes in the first spermatocyte, Henking leaves it and goes on to a consideration of the division of these elements. He notes here the peculiar character and behavior of one "pair" of chromosomes which is noticeable on account of its deep staining power and inertia during division. This latter is so strong as to cause it to remain undivided in the second spermatocyte mitosis, and so to pass into only one of the two resulting spermatids. It is, therefore, as the "Doppelelement *x*." instead of the "Nucleolus *n.*" that we trace its further history.

9. After an extended discussion of the subject, embracing an account of the delayed division of the unusual chromosome of the first spermatocyte mitosis and its later behavior, Henking sums up his conclusions in the following words: "Demnach glaube ich sagen zu

dürfen: Bei der letzen Theilung der Spermatocyten wird das Chromatin ungleich getheilt, derart, das die eine Spermatide nur 11 Chromosomen erhält, die andere dagegen ausser den 11 Schwesterchromosomen noch ein ungetheilt bleibendes Chromatinelement."

10. With respect to the further behavior of this latter element, Henking observes that "Nur das isolirt Körperchen hält sich davon entfernt, tritt nicht in so einige Berührung mit den 11 Chromosomen."

11. Under the treatment of the spermatid transformations, Henking discusses the participation of his "isolirt Einzelelement" in the following language: "Von der allgemeinen Vertheilung hält sich nur das isolirt Einzelelement zuruck, welches bei der letzten Halbirung der Spermatocyten ungetheilt in die eine Tochterzellen übergegangen war. Es sind somit die, wenn wir so wollen, bevorzugten Tochterzellen auch jetzt immer noch zu erkennen.

12. His final opinion of the element is expressed in the following quotations: "Ich glaube, ein jeder unbefangene Beobachter wird mit mir diesen runden von dem übrigen Chromatin scharf unterschiedenen Körper für das Kernkörperchen ansehen. Damit ergiebt sich aber die wichtige Thatsache, *dass wir zweierlei Spermatozoen erhalten: die einen besitzen einen Nucleolus, die anderen nicht."*

13. In the summary of his results, he expresses the same thoughts in slightly different language. He says: "Es sind zwei verschiedenwerthige Arten von normalen Samenfäden vorhanden. Die einen enthalten nur 11 chromatische elemente, die andern ausser 11 chromatischen Elementen auch noch ein einzelnes zuletzt ungetheilt gebliebenes Chromatinelement, welches wahrscheinlich als Nucleolus anzusehn ist."

14. The investigations of Toyama ('94) upon *Bombyx* and several other Lepidoptera, like those of Platner, do not afford us any very definite idea of the accessory chromosome in this order. Several references to nucleoli are made, however, and these I will quote:

15. "The nucleolus (first spermatocyte), lying either in the chromatin mass or outside of it, persists, as is unusual in skein stages of other animals, till to the end of the skein stage shortly to be described.... The nucleolus, however, shows no change from the first resting stage till the present stage, and always consists of small chromatic granules imbedded in a less stainable matrix.

16. "In a still later stage the chromatin granules again commence to separate from one another, and the nucleus again presents the appearance shown in Figs. 31 and 32. In most cases two nucleoli are found in the nucleus of this stage. These gradually migrate toward the periphery of the nucleus facing the center of the cyste (rarely, facing the wall of the cyste) and are finally pushed out into the cytoplasm one

after the other through the nuclear wall at this point. Placed in the cytoplasm the nucleoli seem to change their quality, since they now stain differently from what they did when they were in the nucleus. This is shown by the use of Hermann's triple staining, by which the nucleolus in the cytoplasm takes a brownish color, while it colors deep red so long as it is within the nucleus. The further fate of the nucleoli in the cytoplasm is not known.

17. "In this stage I have not found any nucleolus in the 'Kernplatte,' while Henking observed it in a spermatocyte of *Pyrrhocoris apterus*."

18. Wilcox ('95) in his studies upon *Caloptenus femur-rubrum* and *Cicada tibicen* finds peculiar nucleolar structures which will be found described in the following excerpts:

19. "Cytoplasm and achromatic nuclear parts were stained green, the chromosomes, nucleolus, and centrosomes red (safranin and victoria-green).... In *some* stages the chromosomes were stained green, indicating that a chemical change takes place in the chromatic substance. But even in such cases the nucleolus was bright red.... By this method (Henneguy's) the chromosomes and nucleoli are stained bright red, the individual chromosomes being sharply outlined.... During the stages shown in Figs. 49, 51, 52 (spermatocytes of *Cicada),* there appears to be a chemical change in the constitution of the chromosomes. By the safranin and victoria-green method the chromosomes stain red, though not so deeply as the nucleoli. At later stages the chromosomes assume a green color, while the nucleoli continue to stain red. In still later stages the chromosomes again take the red.

20. "One or often two nucleoli are to be seen (spermatogonia of *Cicada).*... The cells of *b* (a spermatocyte cyst) each contain one or two bodies which I consider nucleoli, since they react to the stains quite differently from the chromosomes.... The nucleolus then moves to the periphery of the nucleus, and appears meantime to have divided into two portions, one of which passes into the cytoplasm, while the other remains in the nucleus; later, both parts appear outside the nucleus and on diametrically opposite sides of it."

21. With this incomplete account, the nucleolus is left by Wilcox. But in a subsequent paper ('96) he takes up the later history of the element and carries it into the spermatozoon. Two paragraphs will give his conclusions.

22. "The body which appears in the vacuole of the nucleus is rather problematical, both as to its origin and its fate. It appears usually as a rod of deeply staining substance, whose longest axis is in the long axis of the vacuole; but the rod may have the form of a crescent.

23. "The tentative conclusion to which I have come with regard to this body is, that it represents the nucleolar substance of the nucleus of the spermatid, and that it subsequently passes into the mass of chromatin, with which it becomes homogeneously mingled. My evidence for this is as follows: Very soon after the second division of the spermatocytes a body is seen in the nucleus. It *(cres.)* lies at first among the chromatic granules of the nucleus, but is distinguishable from any of the latter by its greater size and deeper color. Then it comes to lie in the vacuole of the nucleus. At length, what I consider its remains are found for some time faintly discernible in the chromatic mass of the head of the immature spermatozoon. In later stages this body is not to be distinguished from the rest of the chromatic mass. I was at first inclined to believe that this body allied itself with the centrosome to help in forming the neck-body, but was soon convinced that this is not true, because I observed that the two parts of the centrosome and this problematical body exist at the same time in the same spermatid. In Figs. 90, 92, and 106 the body in question is seen in contact with the chromatic mass, and in Figs. 103, 104, it is nearly included in the chromatic crescent. Later, as already indicated, it becomes indistinguishable from the rest of the head of the spermatozoon. Accordingly, I am unable to determine whether or not it forms any definitely limited portion of the head."

24. While Henking was the first to discover the unusual behavior of a "nucleolus" in the spermatocyte which, in all of its manifestations but one, corresponds to an ordinary chromosome, Montgomery ('98) deserves the credit for observing that this body is merely a chromosome of the spermatogonia that pursues a somewhat different course from the others. Unfortunately, however, his interest in nucleolar structure led him to denominate it, with Henking a nucleolus and it is accordingly under the name "chromatin nucleolus" that we shall trace its history through the spermatocytes of *Euchistus.*

25. The persistence of a color reaction in this element that is characteristic of chromosomes in the metaphase first attracts Montgomery's attention. He states in regard to this that "in each nucleus, from the commencement of the anaphase on, one of the chromosomes still retains the red stain characteristic of all of them in the immediately preceding period, and this particular element is destined to become the chromatin-nucleolus, the metamorphosis of which will be described later."

26. Subsequently he discusses the chromatin nucleolus at some length and from this part of his paper I will quote passages bearing upon its name, staining reaction, form, behavior, final disposition, and function. He says:

27. "To return to the chromatin nucleolus. I give this name in order to express its genetic origin, and to distinguish it from the true nucleolus; it differs also from the "karyosomes" found in many cells, which are nothing more than temporarily thickened portions of the chromatin reticulum.... But when the chromosomes have become more or less elongated, all of them stain violet (with increasing intensity of color), except one, which remains red (saffranine), and by strong light may be easily distinguished from the other chromosomes. This one is the chromatin nucleolus, characteristic for the spermatocytes. At least one whole chromosome becomes thus metamorphosed; and it is very probable, judging from my observations, that only one becomes thus changed. This chromatin nucleolus retains in all stages up to the formation of the spermatids its red coloration after the use of Hermann's double stain, and so can be easily distinguished from the true nucleolus as well as from the chromatin of the rest stage and anaphase.

28. "The chromatin nucleolus appears to undergo the same changes of form as do the other chromosomes, up to about the synapsis. Then it ceases to elongate, and in the post synapsis gradually commences to assume a spherical form, which is characteristic for it during the telophase and the rest. When it may first be distinguished in the early anaphase, and also during the synapsis, it lies within the nuclear cavity, not in contact with the nuclear membrane; but at the end of the synapsis it gradually takes up a more peripheral position, so that usually during the synapsis, and always in the telophase and rest, it is closely apposed to the nuclear membrane.... But in most cells in the synapsis it occurs in the nuclear cavity apart from the chromosomes. In such cases it is found to be usually rod-shaped, often more or less curved, occasionally even lobular; but so great is its irregularity in form, that in no two cases does it have exactly the same shape.... Afterwards it either gradually shortens up into the ultimate spherical form, or first becomes constricted at one or more points on its surface, showing then a more or less beaded appearance, and then, by division at these points, breaks into a number of unequal fragments, each of the latter subsequently rounding off.... But it is most probable that at first only a single one is present, *i.e.,* that only one chromosome becomes changed into a chromatin nucleolus: for in the synapsis, when it may best be distinguished from the chromosomes, I have never seen more than one long chromatin nucleolus. From the synapsis on, the surface of the chromatin nucleolus gradually becomes smooth, so that its process of rounding off may be regarded as a mode of concentration of its substance. It finally becomes homogeneous, quite different from the microsomal chromosomes.

29. "These phenomena show us the remarkable process of the casting off of an entire chromosome, which is itself possibly a mode of chromatin reduction; and in the two spermatocytic divisions we shall find that the chromatin nucleolus does not again become a chromosome.... There are only two other thinkable modes of origin of the chromatin nucleolus: 1) that it be extranuclear in origin, or 2) that it be a secretion of the chromatin.

30. "In the spermatocytes of *Harpalus* a chromatin nucleolus has been seen by me, besides the true nucleolus; and judging from the observations of authors on various objects it would seem that such a structure is generally characteristic of spermatocytes. Thus Moore ('95) found in Elasmobranchs about the beginning of the synapsis, 'a curious secondary nucleolus surrounded by a vacuole, which, so far as I can ascertain, is in these fishes characteristic of this change.'

31. "Thus the true nucleolus passes from the periphery toward the center of the nucleus, the chromatin nucleolus in the reverse direction.

32. "The chromatin nucleolus lies now in contact with the nuclear membrane, and is rounded with the exception of that side flattened against the nuclear membrane.

33. "In the early prophases the chromatin nucleolus becomes rounded, but at first retains its central clearer globule. At the loose spireme stage it commences to grow smaller, at the same time losing the central globule.... The decrease in size continues until the end of the loose spirem, when a dimension is attained which is approximately uniform for the chromatin nucleoli of all cells; one or more of the smaller bodies, which arose as fragments of the original chromatin nucleolus, may still be seen in the nucleus, and often up to the monaster stage. At the time when the chromosomes have attained their definitive form, it usually becomes likewise elongated and dumb-bell-shaped; in the majority of cases it appears to assume this form before the nuclear membrane disappears. Thus it looks like a diminutive chromosome among larger ones. As the true chromosomes now stain with saffranine it likewise resembles them in coloration. This peculiar structure acted like a nucleolus in the rest stage, but in the monaster is destined to lie in the equator among the chromosomes, where it also becomes divided in metakinesis, and so terminates in acting like a chromosome, as at the commencement it has been formed from one.

34. "In a few cases, so few that they must be considered abnormal, a whole undivided chromosome passes into a second spermatocyte, but I have met with only two or three such cases. Henking found in *Pyrrhocoris* and later in some other cases, that the second spermatocytes receive an unequal number of chromosomes, *i.e.*, that one of them may frequently if not usually receive a whole undivided

chromosome; either *Pyrrhocoris* shows a marked peculiarity in this respect, or else Henking had mistaken either a yolk globule or a chromatin nucleolus for an undivided chromosome.... Each second spermatocyte appears as a rule, if not always, to receive a half of the original chromatin nucleolus.

35. "The 7 chromosomes and the chromatin nucleolus gradually become arranged in the equator of the spindle, their axis parallel to the latter, and the plane of their constrictions perpendicular to it. Then follows the metakinesis, with a consequent transverse (reduction) division of all the chromosomes, and apparently in most cases of the chromatin nucleolus, with the result that each daughter cell (spermatid) receives 7 daughter chromosomes and 1 daughter nucleolus."

36. Paulmier ('99) records in his observations that "These interesting bodies (the small chromosomes) were first recognized in the equatorial plate of the spermatogone divisions in the form of two chromatin masses very much smaller than the chromosomes and connected with them by chromatin bands. In the resting spermatogones they appear as two rather indefinite bodies staining with the chromatin stains, and apparently not breaking down to the same extent as the rest of the chromatin. During the period of spermatocyte growth they come to view again in the synapsis stage, as a single body. This body has at first an irregular shape, then it elongates, splits longitudinally, and again transversely, thus forming a tetrad in essentially the normal manner, though passing over many of the stages which the other tetrads go through. During these stages it shows a decided difference in its staining reactions, taking at all times a deep black stain with hæmatoxylin, while the rest of the chromatin is scattered irregularly and stains gray. It always lies close against the nuclear membrane. In the first spermatocyte division it lies in the center of the ring of chromosomes, and divides somewhat before the others. The two parts are connected with each other by two threads precisely as are the normal tetrads.

37. "In the second spermatocyte division it goes bodily over into one of the two daughter-nuclei without showing any traces of division, beyond a slight elongation due to the pull of opposing spindle fibers. In a slightly later stage it again shows its difference from the other chromosomes by retreating as far as possible from them. Soon the disintegrating force overtakes it and it becomes indistinguishable from the others.

38. "I think that we may say without hesitation that this body is not a true nucleolus, a possibility precluded by its different staining reaction, the constancy of its occurrence, and by its division. We find

also in addition to it a perfectly normal, true nucleolus in both the resting spermatogones and spermatocytes.

39. "I agree with Henking that it is chromatin, and that the nuclear substance is thus divided unequally. This body is absent in one-half of the spermatozoa which nevertheless, as far as we know, produce normal descendants. I would make the suggestion that it is degenerating chromatin; in other words, that these small chromosomes, or idants (to adopt for the moment Weismann's terminology), contain "ids" which represent somatic characters which belonged to the species in former times, but which characters are disappearing. The ''ids" which represented these characters are much slower in disappearing than the characters themselves, and persist as the two small chromosomes of the spermatogones. These then undergo a pseudo-reduction and form a tetrad which is unable to complete the second spermatocyte division."

40. My own early views concerning the accessory chromosome, as manifested in the Locustidae, may be found expressed in the following quotations from my first paper ('99) upon the subject:

41. "As it first appears in the spermatogonia of *Xiphidium fasciatum,* there would be no hesitation in calling it a nucleolus except for its unusual situation on the surface of the nuclear vesicle. It is a small, irregularly rounded body, and lies immediately under the nuclear membrane. Before the division figure is established, however, it takes on the form of a thread which becomes 'U'-shaped. Still further contraction ensues, and by the time of the metaphase the thread has become very short and thick and is bent in the middle with an obtuse angle so as to resemble a boomerang. At this time, it may be observed lying at one side of the circle of chromosomes arranged in the equatorial plate, and plainly distinguishable from them by reason of its greater length. From the pole the chromatin appears as a broad, fenestrated plate, and the accessory chromosome is indistinguishable from the ordinary ones. Because of the rapidity of the division none of the anaphases are to be seen, but in the telophases the ordinary chromosomes of the cell may be seen grouped in the typical manner at the two ends of the spindle, while extending down towards the equatorial plate from each mass is a half of the boomerang-shaped body which has been divided longitudinally in the same manner as the ordinary chromosomes.

42. "In the resting stage of the spermatocyte that succeeds the appearance just described, the accessory chromosome again appears as it did in the resting stage of the spermatogonia, and would easily be taken for an ordinary nucleolus. Soon, however, it commences to assume a thread-like form which finally results in the production of a long 'U'-shaped body, a form that is retained during the greater part of

the spireme stage. In this condition it lies at the surface of the vesicle and stains in its usual intense manner. Concurrently with the formation of the 'rings' from the spireme thread, it commences to shorten and grows into the form of a horseshoe, and is finally to be distinguished from the chromatic rings only by its deeper staining quality and by the smoothness of its outline. In the formation of the mitotic figure of the first spermatocyte division, it assumes its position on the outside of the group of chromosomes as it did in the spermatogonial division, and again has the boomerang shape that marked its appearance in the early figures. When the chromatin separates and moves to the two poles, the accessory chromosome divides longitudinally and presents the appearance of two horseshoes with their rounded ends in contact. In the second spermatocyte division apparently the same process is followed.

43. "The recently formed spermatids possess a nucleus in which the ordinary chromatin is extremely scant and very weak in staining power, while the accessory chromosome shows as prominently as ever and stains in the same uniform manner. It is not easy to trace out the part that the different elements of the nucleus take in the formation of the spermatozoon, but in the light of present knowledge it appears as if the accessory chromosome was prominently concerned in the formation of the head.

44. ''In seeking to point out the features that characterize this peculiar nuclear element, perhaps the most striking thing to be noticed, is the almost uniform staining power exhibited.

45. "Thus there seems to be no reason to suppose that the accessory chromosome of *Xiphidium* arises by the direct transformation of one of the ordinary ones, although such a change may be possible. This does not argue against the chromatic origin of the body, however, for it is almost certainly modified chromatin, but in *Xiphidium* it arises during the resting stage and may represent derivative substance from one or all the chromosomes."

46. A later, more detailed, study of various members of the Acrididae ('00) gave me a much better insight into the nature of the accessory chromosome and the result of my observations upon its behavior during the spermatocyte divisions are summarized in the following words:

47. "As a result of the last spermatogonial division, the much reduced daughter cells are each provided with the somatic number of chromosomes. All but one of these rapidly disintegrate and from their substance produce the spireme of the first spermatocyte. One persists in its original form and, assuming a peripheral position, continues to stain as does a chromosome of the metaphase. During metakinesis it is divided like other chromosomes. This is the accessory chromosome.

48. "The accessory chromosome is a constant and important element of the germ cell. It arises, in the Acrididae, from a spermatogonial chromosome, and from that time forward maintains a separate and distinct existence. During the prophases, when occur the profound changes that result in the production of a nucleus with only half the ordinary number of chromosomes, this structure stands aloof and self-contained. With the establishment of the mitotic figure of the first spermatocyte, however, it takes its place with the other chromatic elements and becomes indistinguishable from them henceforth until the spermatids are formed. Here it again becomes distinct and conspicuous."

49. Up to the present time, we have learned nothing of the real origin of the accessory chromosome. That it is one of the spermatogonial chromosomes that passes over into the spermatocytes without taking part in the formation of the spireme is pretty well established; but as to the manner of its appearance in the spermatogonia, we have no knowledge. Sutton ('00) is the only one who has traced the history of the element at all fully in the spermatogonia. Concerning its first appearance and its subsequent history in this generation of sex cells he says, "In *Brachystola*, the accessory chromosome appears probably in the first, and certainly in the third, secondary spermatogonial division, and goes through precisely the same changes in each cycle up to the last.

50. "It may occasionally be distinguished from the other chromosomes in the metaphase and anaphases by its granularity and greater length, though it always divides like the others, and in the actual process of division, as a rule, is indistinguishable from them. In the telophase it constructs its own membrane just as the others do, but soon becomes sharply contrasted with them by the deposition of its chromatin in a diffused condition upon the inner surface of its vesicle (vesicular chromosome), and also by the fact that from this point to the following metaphase the cavity of its vesicle remains distinct from that formed by all the others. In these stages the vesicle of the accessory chromosome may lie on any portion of the nuclear membrane proper; in some cases occupying a position between the 'fingers' or sacculations of it (Fig. 34). This vesicular stage is of comparatively long duration, and is followed by a receding of the chromomeres from the membrane to form a chromatic rod, first loose, rough, and granular, but gradually growing more slender, and compact, and often becoming twisted (Fig. 16). It also betrays a longitudinal split at a stage later than that at which a similar occurrence is observable in the ordinary chromosomes.

51. "From the middle prophases to the telophases, its conduct is so similar to that of the other chromosomes that it would hardly be an error to speak of the cells of these stages as having two nuclei, one having a single chromosome and the other a large number, the small nucleus always lagging slightly behind the large one. This lagging of the accessory chromosome is nicely shown in Fig. 21, already alluded to, where in a stage just before the metaphase the nuclear membrane is seen to be dissolved while that of the body in question is still intact.

52. "In the last or transformation division of the secondary spermatogonia, some differences are noticeable in the behavior of the element under consideration. The vesicular stage seems to be of slightly longer duration, and while, after its close, the same condensation of the chromatin takes place, no longitudinal split appears until the late prophases or 'ring stage' of the spermatocyte; and in the course of the earlier prophases of the growth period its vesicle gradually becomes fused with the nuclear membrane, its outer half completing the smooth contour of the latter, while its inner portion projects into the nuclear cavity. In this stage it has the appearance of an irregular vesicle filled with a homogeneous, darkly staining liquid or semi-liquid body, suspended within the membrane of the nucleus. Later it again becomes granular, and in the first spermatocyte division divides as it did in the spermatogonia.

53. "The resting stage of this element, as shown by its staining violet with Flemming's three-color stain, is what I have called the vesicular stage, and this only, since at all other times it stains a bright red. The absence of the formation of a spireme at any stage in the development of this element is paralleled by Henking's description of the normal process in all the chromosomes in the spermatogonia of *Pyrrhocoris.*

54. "Perhaps the most important thing to be gained at present from the knowledge of the behavior of the accessory chromosome in *Brachystola* is the light which it throws upon the question of the individuality of the chromosomes. In the first place, the fact that it is a true chromosome, though different from the others, is shown by its staining reactions and by the parallelism between its development in the spermatogonia and that of its more generally recognized fellows. Although it shows a tendency to lag behind the other chromatic bodies, the only radical difference between the two is the absence of the loose spireme in the accessory, and this is paralleled, as shown above, by the normal process of all the chromosomes of *Pyrrhocoris,* according to the statement of Henking. The apparent radical difference in the case of the vesicular stage is, in reality, only a matter of degree, for it frequently happens that the ordinary chromosomes, in going into the diffused

condition, leave a very appreciable hollow in their centers (Fig. 30). Apparently there is, for some reason, a necessity that the chromatic granules of the accessory come into closer relation with the cytoplasm than those of its mates, and the result is their deposition upon the vesicle itself—this vesiculation being really a substitute for the loose spireme so conspicuously lacking.

55. "Now, if it be admitted that the body *is* a chromosome, inspection quickly shows us that it maintains throughout the spermatogonial divisions, as well as in those that follow, an indubitable independence, being enclosed, in all stages except those of actual division, in its own individual membrane. Having, then, one of the chromosomes which preserves its individuality in this way, and seeing the other chromosomes enclosed for a part of their development in similar individual vesicles, which only become intercommunicating by absorption of a part of their walls, have we not a right to suppose that at one time they too enjoyed the same independence as their more exclusive mate? In other words, have we not a right to suppose that their phylogeny is paralleled by their ontogeny? If this be granted, then we have at least more ground for belief in the individuality of the chromosomes than if we had never known of a time when they were of necessity independent."

56. Of interest in connection with the question of the general distribution of the accessory chromosome are the observations of Miss Wallace ('00) upon the male germ cells of the spiders. The general facts of the case are found in the following quotations from her preliminary paper: "In this spider the peculiar chromosome is conspicuous in the late spermatogonic stages, and in the prophase of the first spermatocyte, its peripheral position in the nucleus making it easy to observe. Its origin in the spermatogonia has not yet been traced, but a gradual change of form has been made out in the early stages and, suffice it to say here, that in all of them it appears to be double.

57. "In the monaster of the first spermatocyte the accessory chromosomes are easily distinguished from the others by their sharpness of outline, slightly greater affinity for staining reagents and above all by their eccentricity of position. They are always found on the periphery of the spindle and often near the periphery of the cell. It is a curious fact that in the majority of cases both of these elements are found nearer one pole than the other."

58. Regarding the unequal distribution of the accessory chromosome to the spermatozoa, she is unable to speak definitely. Her observations on this point are stated in the following words: "In the spider the position of the two chromosomes nearer one pole gives the impression that this unequal distribution occurs in the first

spermatocyte division. One thing, however, opposes this interpretation and that is that in the monaster of the second spermatocyte division two elements are again found in eccentric position but of half the size of those found in the preceding cell-division. The position nearer one pole might mean merely delayed distribution but there is not yet at hand sufficient data from which to draw a conclusion."

59. In the latest edition of his work upon the cell, Wilson ('00) takes cognizance of the investigations being pursued by insect spermatologists and practically adopts Paulmier's views concerning the accessory chromosome. We find in his summary that "A comparison of the foregoing results indicates that the small tetrad (dyad) corresponds to the extra chromosome observed by Henking in *Pyrrhocoris*, and perhaps also to the 'accessory chromosome' of *Xiphidium.* Whether it corresponds to the 'chromatin nucleolus' of *Pentatoma is* not yet clear. The most remarkable of these strange phenomena is the formation of the small tetrad, which seems to be a non-essential element, since it does not contribute to all the spermatozoa. Paulmier is inclined to ascribe to it a vestigial significance, regarding it as a degenerating chromosomes which has lost its functional value, though still undergoing in some measure its original morphological transformation; in this connection it should be pointed out that the spermatocyte nucleolus, from which it seems to be derived, is represented in the spermatogonia by *two* such nucleoli, just as the single small tetrad is represented by two small chromosomes in the spermatogonia mitoses. The real meaning of the phenomena is, however, wholly conjectural."

Because of the greater prominence of the element in the spermatocytes, observations upon its occurrence and changes have been more numerous and accurate than in the case of the spermatogonia. During the prophase of the first spermatocyte, particularly, the appearance of the element is so striking as to render its oversight impossible. Regarding the main features distinguishing it, there is a convincing agreement in all the published reports and these speak further for the morphological exclusiveness so plainly manifested by the element in the spermatogonia. There are some slight discrepancies in the accounts of its very early appearance but concerning the later stages there appears to be no confusion.

From the Hemiptera (¶ 27) and the Orthoptera (¶ 47), we learn that the chromosomes of the last secondary spermatogonia, with the exception of one, break down rapidly into their constituent chromomeres and that these enter at once into the formation of a spireme—at first thin and fine but later coarse and granular. The exception to this process is the accessory chromosome. It is, in the beginning, somewhat irregular in outline but quickly condenses its

substance and becomes homogeneous, transparent, and sharply outlined. It early assumes a position upon the periphery of the forming nuclear vesicle and maintains this during all the prophase. Throughout this period, and the remaining stages of its existence, it persistently stains according to the reaction exhibited by chromosomes of the metaphase. It is thus, in both its chemical and physical properties, strikingly different from the remainder of the chromatin. It may be said, therefore, that it is a chromosome of the previous generation (*i.e.*, one formed from the spireme of that generation) which exists as such while its fellows pass through the prophase of another mitosis.

So far as the first spermatocytes are concerned, this is the only point at which the accessory chromosome differs from the others. When metakinesis occurs, all the chromatin elements divide at the same time and in the same way. The participation of the accessory chromosome in this act is generally conceded (¶ 33, 36, 47), but the exact process has not yet been observed because no form has been studied where the element stands out conspicuously enough to be noted. From the nature of the element, however, the only reasonable thing to expect would be that it should divide as it has done in all previous mitoses, *i.e.*, longitudinally.

With regard to the action of the accessory chromosome in the second spermatocyte mitosis, there are two opinions. One is that it takes part with the ordinary chromosome in the act of division (¶ 34), the other that it passes undivided into only one of the two resulting cells (¶ 9, 12, 13, 37). Examination of the literature shows that the weight of evidence, so far as observations are concerned, supports the latter view. In addition to this, the fact that it differs from the other chromosomes must be taken into account. When it divides in the first spermatocyte it has finished the entire act of separation begun for it in the prophase where it was formed. It has no need, therefore, to divide in the second mitosis where the other chromosomes complete the separation of the chromatids formed in the prophase of the first spermatocyte. Thus, when the origin of the element is taken into consideration, a phenomenon at first apparently inexplicable according to the usual laws governing cellular activities is seen to be in strict accord with them. The variation from normal conditions is, accordingly, manifested by those chromosomes which emerge from the spireme of the first spermatocyte instead of by the one which fails to enter into it.

At the completion of the second spermatocyte mitosis the apportionment of the chromatin to the germinal elements is accomplished and the further changes in the spermatid would seem to indicate that there is no longer the necessity for the chromosomes to

maintain their separate identities. This, because the chromatin first becomes diffuse and later condensed so as to form the head of the spermatozoon where there is no distinction of parts. In the spermatid where the accessory chromosome is present, there is no apparent difference in the behavior of the nuclear elements. The accessory chromosome runs the course of the ordinary chromosome and sooner or later becomes indistinguishable in the homogeneous mass of the spermatozoon head.

It is, therefore, impossible to trace the location of the accessory chromosome to any portion of this nuclear mass. The fact is apparent enough, however, that it does remain a part of the nuclear contribution to the mature element (¶ 12, 13, 23, 37, 43) and does not go to form a part of the archoplasmic derivations as Wilcox was at first inclined to believe (¶ 23). The merging of the chromatic elements into one mass makes it impossible to go further in the investigation of the accessory chromosome at this point. We can hope to know more about it only by learning its part in fertilization of the egg. As a result of the action of the accessory chromosome in one maturation mitosis, this fact, at any rate must be apparent, viz: that there are two kinds of spermatozoa; those *with* the accessory chromosome and those *without*. Beyond this is speculation only, but with accumulated observations on many forms it may not be long until we are able to reach a definite conclusion regarding the exact function of this well defined element.

From the different observations, I hope (1) to bring out the essential features which characterize the accessory chromosome, (2) to show the extreme probability of its universal occurrence among insects, (3) to outline its history in the different cell generations of the testis, and (4) to suggest a theory in explanation of its function.

Confirmation of the statement that most references to the accessory chromosome would be found under discussions of nucleolar structures has just been given in the quotations from various papers. When a reason is sought for the classification of such a purely chromosomic element with this heterogeneous group of bodies it is difficult to find any that is sufficient. Interest in other problems has, perhaps, induced investigators to concentrate their attention elsewhere and as a result the accessory chromosome has been assigned relationships entirely foreign to its true nature.

I have, in previous papers (’99, ‘00), given my reasons for regarding it as a chromosome, so that I shall not have to go much into detail on this point. It would seem sufficient to show that the element *is* a chromosome of the spermatogonia (¶ 27, 41, 47, 49–53) and that it divides in a subsequent spermatocyte mitosis as a chromosome (¶ 33, 36, 42, 47, 48) in order to insure its classification as such. Yet in full

recognition of these conditions Montgomery (¶ 27) calls it a nucleolus and insists (¶ 29) that it never again (*i.e.*, in the spermatocytes) becomes a chromosome. While Henking fails to detect the origin of his "nucleolus" from a chromosome of the spermatogonia he recognizes its chromatic character and its participation in the act of metakinesis and even occasionally calls it a "chromatinelement" (¶ 13).

Wilcox (¶ 19) is very careful to note the staining reaction of the nucleolus and that of the chromosomes. This he finds to be identical except in certain stages, and in these, it is the chromosomes proper that weaken in their affinity for the basic anilines while the "nucleolus" consistently reacts to them with the true chromatin reaction. But in considering the "nucleoli" of the spermatocytes (¶ 20) he regards them as such because "they react to the stains quite differently from the chromosomes" Just in what respect this difference lies is not quite manifest from the text, but it is apparently in being uniform in staining instead of variable.

In his early paper Wilcox ('95) does not trace the final history of the element so carefully as he does in a later one ('96). Here, even after more extended investigation, he is uncertain "both as to its origin and its fate" but inclines to the belief that it is "nucleolar substance" despite the fact that it does become "homogeneously mingled" with the chromatin. As will be noted (¶ 23), he was at first inclined to consider it related in some way to the centrosome, but as a result of more careful study decided that this was a mistake.

In addition to noting the obvious staining reaction and peripheral position of the element in the prophase of the spermatocyte, Paulmier also observed its behavior in the spermatogonial divisions (¶ 36) and subsequently in the different phases of the spermatocytes (¶ 37). As a result of a recognition of its very apparent chromosomic character he agrees with me in calling it a chromosome, but prefers to speak of it as the "small chromosome." I have already pointed out in a previous paper ('00)[1] my reasons for regarding this as a misnomer on account of its usually being larger than the other chromosomes so need not again refer to the subject. Thus, we have as a result of the latest and apparently most accurate work upon the Hemiptera, the history of a small chromosome that puts it in almost complete agreement with the behavior of the accessory chromosome in the Orthoptera.

From the preceding statements of different investigators, it will be apparent, I think, that there is in the spermatocytes of all insects so far

[1] ¶ 3, p. S5; latter part of ¶ 3, p. S6; and ¶ 5, p. S9; were attached to the proof as footnotes but were included by the printer in the body of the article. It thus happens that on page S9 a reference to Paulmier's last paper ('99) appears before a criticism of his earlier one ('98).

studied an unusual nuclear element which is characterized (1) by a remarkable uniformity in staining power, similar to that exhibited by chromosomes in the metaphase; (2) by a continuous peripheral position during the spireme stage, at least; (3) by an isolation from the chromatin reticulum and nonparticipation in its changes; and (4) by fission during metakinesis after the manner of chromosomes. In addition to these features which have generally been recognized, there are others which material of exceptionally good character has rendered apparent to several observers. Of these I wish to speak later but desire here only to show the great probability of the general distribution of the structure among insects.

The published reports of almost all investigators certainly speak very strongly in favor of such a supposition, but in order to assure myself by personal observation that views of such a character were correct, I examined representatives of the different families of the Orthoptera and became convinced that I was right so far. Later, material from the Hemiptera, Neuroptera, Coleoptera, and Lepidoptera was examined only to confirm the opinion that the element in question is a constant character of the insect testicular cells. The recent work of Miss Wallace upon the spider (‘00) would seem to indicate that there is no doubt of its presence in the Arachnids, and with its determination in this class the probability of its general occurrence in the Arthropods is largely increased. I may also say in passing that some hurried examinations of vertebrate spermatocytes lead me to believe that the accessory chromosome is likewise present here.

If it be conceded that we are dealing with a common element of the sperm-forming cells, it must also be admitted that there exist extensive variations in its appearance and manifestations. To do this, however, is no more than to concede what is known to be true of all other chromosomes so that such an admission can in no way impair the standing of the accessory chromosome as a constant and important nuclear element. It will therefore be understood that the outline history of its behavior in the different cell generations, which I intend to give, will apply only in general particulars. Its purpose is more to show the importance of the element than to postulate a type.

In the spermatogonia, our knowledge of the accessory chromosome is due almost entirely to Paulmier and to Sutton. The former author notes in a general way (¶ 36) that in the metaphase of the early cell generations there are present two smaller chromatin bodies which, during the breaking down of the spermatogonial chromosomes to form the spireme, do not suffer any very extensive dissolution. Later these appear as a single body in the prophase of the spermatocyte. Montgomery makes no mention of their behavior earlier than the

telophase of the last spermatogonial division but agrees with Paulmier's view concerning their later changes.

Sutton, on the contrary, has devoted his entire attention to the spermatogonial divisions and has given us a fairly complete account of the changes taking place in *Brachystola* (¶ 49–55).

It is probable that this object presents an extreme view of the accessory chromosome. From an inspection of Sutton's preparations and a comparison with other forms, however, I am strongly inclined to the behalf that it is merely a very marked example of a normal process. We may, therefore, take the course followed by the accessory chromosome in the spermatogonia of *Brachystola* as representative since it is all that we have, at present, to base our knowledge upon.

Even with this we have no hint, as to the real origin of this problematical body—a point of great importance. It is hoped that more extensive collections of material will render it possible to learn something of this, but the problem is one of considerable difficulty owing to the compact nature of the early cells and their apparent irregularity in arrangement. A favorable object may be found, however, in which the structure will stand out clearly enough to be accurately studied, and in this event we may discover the conditions determining the setting apart of this one chromosome from all the others.

Sutton was first able to distinguish the accessory chromosome in cysts of eight or sixteen cells. Here, as in the spermatocytes, it seems to be removed as far as possible from the influence of the ordinary chromatin. This is accomplished by an enclosure in a separate vesicle which, as Sutton observes, may almost be regarded as a separate nucleus (¶ 51). A distinct existence is maintained during all the stages when a possible exchange of material between the accessory chromosome and the other chromosomes might be accomplished. Only after the chromosomes are definitely established as independent bodies are the barriers removed and then only long enough to permit the act of metakinesis to take place.

During the period intervening between the acts of division, the conduct of the accessory chromosome parallels that of the nucleus containing the remainder of the chromatin (¶ 50). A vesicle is formed, the chromatic substance is deposited upon its wall in intimate relation with the cytoplasm, a concentration ensues and a definite chromosome is produced.

These changes, it seems to me, are easily explainable if we regard the conditions under which the processes operate. The divisions of the spermatogonia are rapid and continuous and every factor concerned is subordinated to filling the follicles with spermatogonia as quickly as possible. This, of course, requires a rapid increase in amount of

chromatin including that of the accessory chromosome, and so the anabolic processes are facilitated by bringing the chromatin into a position where it can best derive its nourishment from the cytoplasm. The vesiculation of the accessory chromosome is merely an incident, the isolation of the element being the end sought.

Throughout the divisions of the secondary spermatogonia this process continues until the accessory chromosome of, let us say, the primary spermatogonium has been apportioned to each of the many cells that are now ready to transform into spermatocytes, and during this time it has practically been as independent as if it were the chromatin of a separate nucleus.

## PART II. THEORETICAL CONSIDERATIONS

In seeking an explanation for the unusual phenomena connected with the history of the accessory chromosome in the male germ cells, it is most natural to surmise the existence of a phylogenetic significance. In the spermatagonia what amounts to practically two nuclei in each cell is strongly suggestive, in mere general features, of the appearances manifested in the Protozoa where both macro- and micronuclei are present. The accessory chromosome might be homologized with the micronucleus which serves as a medium of exchange between the organisms during the act of fertilization, but it would be extremely difficult to trace any parallelism between the macronucleus and the real chromosomic vesicle of the spermatogonia. I do not, therefore, believe that we can look in this direction for an explanation of the peculiar character exhibited by the accessory chromosome.

Nor do I believe that there is the least basis for Paulmier's theory that the structure is a degenerating chromosome. There are many facts which argue against it. Of these I should like to speak in some detail, since the theory they controvert is the only one yet advanced upon the proper basis that the element is a chromosome and not a mere nucleolus.

Paulmier considers the element a chromosome in the process of disappearing from the species and reaches this conclusion after observing that in the last spermatocyte mitosis it fails to divide and is thus unequally apportioned to the resulting spermatozoa. Before considering a theory based upon so unusual a phenomenon as this, it would be well to make certain that it is an actual and common occurrence. That it is must be granted, I think, after the work of Henking, Paulmier, and myself, upon so many different forms, has shown it to be of such wide distribution. Granting this, then, there

remains to be examined the validity of the assumption that the act presages the final extinction of an element.

In combating the suggestion of Paulmier, I shall make use of the evidence offered in the different cell generations, commencing with the spermatogonia. Every instance, according to my interpretation, shows facts strongly incompatible with this author's view. I would suggest in this connection that the extreme importance of the structure is unmistakably manifested by its course in the spermatogonial divisions. What could more strongly emphasize the special importance of an element than to have it set apart in a separate vesicle while its fellows are provided with one common investment? Morphologically, this is simply raising the accessory chromosome to the rank of a nucleus coordinate with the one commonly present in a cell. Its careful and uniform division during the mitoses of all the spermatogonia suggests anything but an unimportant structure. Had we no further refutation of the degeneration theory than that afforded by the spermatogonia, it would, I think, be sufficient. The evidence of the other cell generations, however, strengthens this position and is well deserving of attention.

From the spermatogonia, each spermatocyte receives one chromosome (the accessory) which through all the subsequent stages exists as a chromosome and never suffers extensive disturbance of its chromomeres either for the purpose of metabolic activities or for the possible exchange of mutual influence with the other chromosomes. Montgomery assigns the origin of the accessory chromosome to a single spermatogonial chromosome, in the Hemiptera, and I have clearly traced it to the same source in the Orthoptera. It must be regarded, therefore, as *a single* element and as the possessor of *two* chromatids, after its longitudinal division, and so differs from the other elements which are constituted of *four* chromatids. This is a matter of considerable importance as will appear when we consider the division of the spermatocytes.

The period which witnesses a breaking down of the spermatogonial chromosomes and the construction of a thin chromatin thread from their chromomeres is marked by changes just the reverse on the part of the accessory chromosome. It enters the prophase as a definite body with a staining reaction that is constant and marked. These characters it maintains until it becomes indistinguishable in the spermatid.

Meanwhile the other chromosomes of the spermatogonia are lost in the substance of the spireme, in which condition their chromomeres exist in relations far removed from those prevailing in the component individual chromosome. This spireme stage is one of extreme importance to the structures it involves. In many cases, as has

frequently been pointed out, it is a phase occupying relatively, and sometimes actually, a long period of time. During its continuance, profound changes take place in the nucleus as a result of which the chromatin emerges in the form of chromosomes the like of which we are unable to find in any other cells of the body. Instead of having two chromatids at the time of the metaphase, each of these has four. Instead of being as numerous as those of the spermatogonia, there are but half as many. Throughout all the time involved in the production of these fundamental differences, the accessory chromosome has existed quite apart from the field of mutual influence in which the other chromosomes operate. It is thus apparent that it has its characters fixed, not in the generation which witnesses its division but in the previous one. In other words it is a spermatogonial chromosome which divides in the spermatocyte mitosis.

From this it will be apparent enough why there is an undivided chromatin element in one spermatocyte mitosis. It is but a single chromatin and so cannot be separated into halves. If, therefore, Paulmier bases his theory upon this phenomenon (as it appears he does) it would seem that he has a very insufficient foundation for it. True it is that he considers the element a tetrad and so would be more justified in his conception, but the evidence he brings forward in the proof of this is not convincing. Montgomery clearly recognizes its unit character in other members of the Hemiptera, Sutton traces it through the spermatogonia of the Orthoptera as a single chromosome, and my own observations are positive as to its valence. The weight of evidence is therefore strongly against Paulmier on this point—the essential one in his theory. Moreover, I might point out that the division of the elements has already been accomplished in the prophase of the first spermatocyte and only their separation remains for a succeeding metaphase. The active agents here are the archoplasmic fibers, so that failure to act in unison with the other cell structures would reflect upon their vigor rather than upon that of the chromosomes.

Again, the great regularity of the divisions by means of which exactly one half of the spermatozoa are unprovided with the element would seriously weaken any assumption of degeneration. If the usual course of degenerating structures were followed, it would demand great irregularity and uncertainty in the occurrence of the unequal division, whereby varying numbers of the spermatozoa would be marked by the presence of the undivided element. In none of the forms studied is there any suggestion of an indeterminate and indefinite division, and in the absence of this, Paulmier's theory loses its strongest support.

It is also pertinent to ask whether the dropping of specific characters would take place by the elimination of an *entire*

*chromosome,* if so whether this would occur in the germ cells, and, if in the germ cells, why in the last generation?

In view of all the objections advanced, I believe it would be impossible for Paulmier's hypothesis to maintain its ground without the support of numerous others equally difficult to base upon observed facts. It will be necessary on this account to look elsewhere for an explanation of the various phenomena involved in the problem. I shall therefore venture to advance a theory which has been suggested to me by a careful study of the structure in various species in the hope that an early elimination of the improbable factors of the question will bring us closer to the true explanation.

In offering a theory to account for the function of the accessory chromosome, I do so with considerable reluctance, for I realize how little real general knowledge we have of this structure. It seems to me, however, that something is necessary to concentrate the interest of spermatologists upon the fundamental character of this most suggestive chromatin element, and I know no better way of aiding in this than by publishing the working hypothesis with which I have attacked the problem.

This has led me into the field of theories concerning sex and its determination, but I have tried to avoid any more extensive discussion than is necessary to outline, in a preliminary way, the opinion I hold concerning the meaning of the accessory chromosome. Even with this reservation I have nevertheless been obliged to go further afield than I should desire with our present knowledge as a guide. I can only hope that my excursions may accomplish a measure of the purpose for which they were undertaken.

Briefly stated, then, my conception of the function exercised by the accessory chromosome is that it is the bearer of those qualities which pertain to the male organism, primary among which is the faculty of producing sex cells that have the form of spermatozoa. I have been led to this belief by the favorable response which the element makes to the theoretical requirements conceivably inherent in any structure which might function as a sex determinant.

These requirements, I should consider, are that: (*a*) The element should be chromosomic in character and subject to the laws governing the action of such structures. (*b*) Since it is to determine whether the germ cells are to grow into the passive, yolk-laden ova or into the minute motile spermatozoa, it should be present in all the forming cells until they are definitely established in the cycle of their development. (*c*) As the sexes exist normally in about equal proportions, it should be present in half the mature germ cells of the sex that bears it. (*d*) Such disposition of the element in the two forms of germ cells, paternal and

maternal, should be made as to admit of the readiest response to the demands of environment regarding the proportion of the sexes. (*e*) It should show variations in structure in accordance with the variations of sex potentiality observable in different species. (*f*) In parthenogenesis its function would be assumed by the elements of a certain polar body. It is conceivable, in this regard, that another form of polar body might function as the non-determinant bearing germ cell.

(*a*) If we accept the theory that the chromatin is the bearer of hereditary qualities, there could be little doubt regarding the necessary chromosomic character of a sex determinant. Sex being an elementary characteristic of protoplasm, it would be firmly established in the hereditary basis along with metabolic activity, irritability, etc., and if any argument were needed at all it would be a general one, not concerned immediately with the question under discussion, but with the broader one suggested. It will therefore be assumed that the chromatin is this basis. This being true, it will only be necessary to point out that the work of a majority of investigators definitely proves that the accessory chromosome *is* a chromosome, and its standing in this respect is established.

(*b*) With regard to what would theoretically be required of a chromosome whose function should be the determination of sex, it is probable that almost every investigator would hold an opinion differing in some respects from those entertained by others. What I can suggest in this connection will therefore be merely tentative and an expression of my own views. One thing, however, would seem to be necessary; *i.e.*, that the determinant should exist in the cells until they are definitely established as elements of either an ovary or of a testis.

If it be that the production of male elements is a sign of katabolic conditions, or, in other words, of those that make a greater demand of energy expenditure upon the developing cell, then it would seem most natural that the determinant should be for the purpose of carrying the transformation beyond the production of ova to spermatozoa. It would therefore be a necessary content of the cells until they had passed through the stages of development beyond that at which they might pause and become laden with yolk or, in other ways, postpone the period of maturation. It is conceivable that the production of four functional cells from one spermatogonium would call for the employment of more energy than would the formation of one functional egg from an oögonium, especially since many cells contribute their substance or support in the upbuilding of the egg.

Accordingly, it would be most reasonable to expect the presence of the determinant in the latest possible stage consistent with its equal distribution to half the spermatozoa. This we find to be the case with

the accessory chromosome which regularly occurs in all the cell generations up to the last and is only withheld, finally, from half of the spermatids. By its consistent course in this respect, the accessory chromosome plainly manifests its intimate influence upon the germ cells of which it is a part, and most strongly suggests a relation to sex determination. It may further be pointed out in reference to this relation, that during the multiplied spermatogonial divisions, the accessory chromosome exhibits a somewhat distant attitude toward the remainder of the chromatin, and it is only at the time of the definitive spermatocyte divisions that it comes to be an intimate member of the cell nucleus. In what manner it is borne from the fertilized egg to the testis of the embryo we do not know, and, lacking this knowledge, are placed at a considerable disadvantage for a proper appreciation of its real character.

(*c*) A most significant fact, and one upon which almost all investigators are united in opinion, is that the element is apportioned to but one half of the spermatozoa. Assuming it to be true that the chromatin is the important part of the cell in the matter of heredity, then it follows that we have two kinds of spermatozoa that differ from each other in a vital matter. We expect, therefore, to find in the offspring two sorts of individuals in approximately equal numbers, under normal conditions, that exhibit marked differences in structure. A careful consideration will suggest that nothing but sexual characters thus divides the members of a species into two well-defined groups, and we are logically forced to the conclusion that the peculiar chromosome has some bearing upon this arrangement.

I must here also point out a fact that does not seem to have the recognition it deserves; viz, that if there is a cross division of the chromosomes in the maturation mitoses there must be two kinds of spermatozoa regardless of the presence of the accessory chromosome. It is thus possible that even in the absence of any specialized element a preponderant maleness would attach to one half the spermatozoa, due to the "qualitative" division of the tetrads.[1]

*(d)* As I elsewhere suggest, it is most appropriate that the sex determinant should have its locus in the spermatozoa. These elements are most commonly freed from any close relation to the parent organism at maturity, and thus lose the opportunity to receive from it any bias toward the production of an unusual proportion of the one sex or the other as environmental conditions might require. It is otherwise

[1] It is suggestive that in all those cases where there appears to be no cross division of the chromosomes in maturation, nothing like the accessory chromosome has been noted. This would seem to be some indication that there might be two types of division.

with the ova. They are usually retained by the maternal organism in such intimate relation to it that surrounding conditions might easily imprint their demands upon them. Even up to the time of fertilization the female elements are so placed as to react readily to stimuli from the mother. Here they are approached by the wandering male elements from which they may choose—if we may use such a term for what is probably chemical attraction—either the spermatozoa containing the accessory chromosome or those from which it is absent. In the female element, therefore, as in the female organism, resides the power to select that which is for the best interest of the species.

(*e*) The strength with which sex is established in different species of animals is variable. Moreover it is a fact of common observation that all cell elements vary widely in different animals. We should not be surprised to find, then, that a determinant would exhibit marked varieties of form which might even be carried to the extreme of its entire suppression as a definite element. Incomplete as are the observations upon the behavior of the accessory chromosome in various species, enough evidence is forthcoming to show wide departures from anything that might be considered a typical form. And here it is that it may be possible to secure more or less definite information with regard to the meaning of the accessory chromosome. If a large number of observations show variations that parallel well-marked instances of unusual sex characters, then greatly increased probability will attach to the theory I have advanced.

(*f*) Concerning the bearing of parthenogenesis upon the problem of sex determination we know little. In eggs, no structure comparable to the accessory chromosome has yet been observed and the presence of any such element is extremely improbable. But it is known that different sexes come from parthenogenetic eggs, and in the familiar example of the aphides, these are produced in strict response to environmental demands.

Parthenogenesis, however, is regarded as a degenerate method of sexual reproduction in which polar bodies perform the function of the spermatozoa. Sex might, therefore, be determined by the particular polar body that restored the needed amount of chromatin to the egg, for these, like the spermatozoa, would be of two kinds where a reduction division took place in the process of maturation. These facts would indicate an element of truth in Minot's view regarding the meaning of the polar bodies. In respect to this matter, however, we have only theory to guide us and must wait for more thorough study of the question.

The suggested hypothesis affords a reasonable basis for a number of theories that have been advanced and supported upon empirical data.

Among these are Thury's and Düsing's on the time of fertilization; the ones relating to the nutrition of the parents and embryo; and possibly others in which age or "comparative vigor" is assigned as the influential factor.

In general, I would point out, my theory confirms these by showing that the condition of the ovum determines which sort of spermatozoon shall be allowed entrance into the egg substance. In this we see an extension, to its ultimate limit, of the well-known *rôle* of selection on the part of the female organism. The ovum is thus placed in a delicate adjustment with regard to surrounding conditions and reacts in such a way as to best subserve the interest of the species. To it come the two forms of spermatozoa from which selection is made in response to environmental necessities. Adverse conditions demand a preponderance of males, unusually favorable circumstances induce an excess of females, while normal environments apportion an approximately equal representation to each of the sexes.

Those theories regarding sex determination which contain any element of truth within them will be found dependent upon this principle. It is expressed by Geddes and Thompson in these words: "But the general conclusion is tolerably secure—that in the determination of the sex, influences inducing katabolism tend to result in production of males, as those favoring anabolism similarly increase the probability of females" The authors just cited clearly recognize that we must consider the sexual elements in the light of their elemental structure and function when the final explanation of sex is sought. They say: "That the final physiological explanation is, and must be, in terms of protoplasmic metabolism, we must again, however, remind the reader."

The *rôle* that I have suggested for the accessory chromosome in no way changes the ordinary conception of the part played in sex determination by the various observed factors, but it does offer some tangible means by which to correlate these and to fix the nature of their participation.

The conception of two forms of sexual elements which would be operative in the determination of sex is not new. It has been assumed on purely theoretical grounds that there are two kinds of ova, one of which, in the event of fertilization develops into a male organism while the other under similar conditions gives rise to a female. This theory is dismissed by Geddes and Thompson on the ground that the two forms of ova have never been observed and for the further reason that later influences might possibly change the earlier tendency.

The latter objection would prove fatal to any theory which located the determination of sex in a structural difference of the germinal

elements. I do not consider this position well taken for reasons that I will give later. The more serious objection lies in the fact that, so far as observation has gone, all eggs of a species are practically alike. It is also to be depreciated because of the fact that it reverses the ordinary relations of the elements and removes the power of choice from the female.

We have in the case of the spermatozoa, however, the observed fact that there are two essentially different forms and that they are present in equal proportions. No other feature save sex, separates the resulting offspring into two approximately equal groups. By exclusion then, it would seem that the determination of this difference is reposed in the male element.

There are, I am aware, certain observations upon the determination of sex with which my hypothesis does not seem to agree. Some of these I should like to mention in order to suggest possible explanations or reasons for regarding them within the limit of error set by our present knowledge of the subject. These objections may be suggested by the following questions:

Is sex potentiality—by which I mean the tendency of the species to perpetuate itself in individuals of two sexes of approximately equal numbers—a constant and uniform factor prevailing throughout all classes of animals? Is sex determined at the time of fertilization; if so, is such determination absolute, or may it be changed by varying conditions? Under the unusual circumstance of parthenogenesis, will it be possible to reconcile a theory which postulates the presence of a determinant in the male element with the fact of the entire absence of this element in unisexual reproduction?

An answer to the first question is not difficult. It is a matter of common observation that all animals are not alike in their methods of reproduction. In the insects, for instance, it is known that certain forms invariably produce young after the sexual method and that parthenogenesis never occurs; in others, parthenogenesis is the common method and sexual union of male and female only an infrequent occurrence; while in yet others, one sex is produced by fertilized eggs and the other sex from those unfertilized.

The logical conclusion to be drawn from these facts is that sex, *per se*, is not an unchangeable attribute of organisms but is an adaptation of the species to secure the most favorable conditions for its perpetuation. Given favorable conditions of environment and aphides will reproduce indefinitely with only one sex as a representative of the species. Adverse conditions, on the contrary, cause the appearance of the male form which then shares with the female the representation of its kind

Again, some species are influenced by favorable external conditions to such an extent as to cause, not the entire suppression of the male form, but only its subordination in numbers. Finally, there are forms where the numerical proportion of the sexes is preserved with only slight variation even under great extremes in environment. The first case we would consider as an example of a weak, the second as one of moderate, and the last as one of strong, sex potentiality.

Let it be granted, then, that the demand for sexual representation is not equally strong in all species. It follows that we may expect to find corresponding variations in the method by which sex is determined. Such forms as exhibit a ready response to environmental conditions will certainly be more easily influenced even at a late stage of development than would the more stable forms at the beginning.

On this account, an answer to the second question could not be a simple one. It is very probable that, in certain species, sex is determined at the time of fertilization and can not be altered by any later influences. Conversely, it has been experimentally proved that the proportion of sexes may be materially altered by changed nutritive conditions operating upon larval forms, or may possibly be changed several times in the same individuals. But because Yung raised the proportion of females from a normal one of about 56% to the unusual one of 92% in the sexually unstable tadpoles of the frog, it does not follow that in all forms sex is such a variable factor. It is simply an evidence that sex is not a fixed attribute of organisms and that in this particular case it is extremely unsettled. By no means can it be taken as an argument that sex may not be established in the act of fertilization.

In refuting this view, moreover, we are not forced to rely entirely upon negative inferences. In the case of the honey-bee, it has long been known that sex depends solely upon the matter of fertilization. From the impregnated eggs come the females, queens or workers as circumstances dictate; from the unimpregnated eggs always males. This fact Dzierzon demonstrated by observation in 1853, and the absence of spermatozoa from the eggs which develop into drones has very recently been proven in the laboratory of Weismann by the use of modern cytological methods.

A further proof, although inferential, is that afforded by “true” twins, in which case it appears that the sex of the two individuals is always the same. If sex were established at the time of fertilization of the ovum, then sex would be shared along with the other qualities possessed by the normal individual that would have developed from the ovum under ordinary conditions. In case sex were not established at the time of impregnation, it would be natural to expect the two sexes to be

occasionally represented in one birth because of the inequality of nutrition in the embryos or for other reasons.

Sex, then, *is* determined sometimes by the act of fertilization and can not be subsequently altered. But between this extreme and the other of marked instability there may be found all degrees of response to environment. It must accordingly be granted that there is no hard-and-fast rule about the determination of sex, but that specific conditions have to be taken into account in each case. The objection that Geddes and Thompson raise against the possibility of two forms of eggs, viz., that it is a useless adaptation on account of the fact that subsequent conditions may determine sex in some cases, is not a valid one in general. Such *may* be the case in some instances, but such *is* not the case in others.

Finally, with respect to the evidence to be derived from parthenogenesis, it should be remembered that we are here dealing with a practical suppression of sexuality and it is to be expected that extensive modifications of the ordinary process will follow. If the egg takes upon itself all the functions commonly exercised by it in conjunction with the spermatozoön, it must be that the determination of sex is included. This, in some instances, is a final choice on the part of the ovum and ever afterward one sex only is produced by it; again, however, it maintains a responsive attitude toward environments and gives rise to the sex most needed by the species. It is to be hoped that the very promising field opened up by the work upon artificial parthenogenesis will throw much light upon these vexed problems.

## SUPPLEMENT

During the period of a year and a half that has elapsed since the completion of the foregoing article, a number of important changes in, and additions to, our knowledge of the accessory chromosome have been made. These are noted in another paper, "The Spermatocyte Divisions of the Locustidae," soon to be published, so that extended reference to them will not be given here. For the sake of completeness, however, I deem it proper to make brief mention of such as affect the main points of this contribution.

First, I may observe that the exact character of the unequal division of the accessory chromosome in the spermatocytes of the Orthoptera has been established. As may be noted in ¶ 42, I could not determine the behavior of this element in *Xiphidium* with certainty. From its absence in large numbers of spermatids, though, I was inclined to support Henking's view that it remained undivided in one of the

spermatocytes. In other genera of Locustidae, *Orchesticus, Anabrus, Microcentrum,* and *Scudderia,* I have since been able to demonstrate with certainty that the accessory chromosome divides but once in the spermatocytes. Here, unlike *Pyrrhocoris,* the second spermatocyte mitosis witnesses the separation of the chromatids of the accessory chromosome. The end result is, nevertheless, the same in each case.

Further confirmation of the fact may be found in the work of R. de Sinéty upon other species of Orthoptera, in which he records exactly similar processes. I feel safe in stating it as established therefore, that in the Orthoptera—and in the Hemiptera—the accessory chromosome is normally present in exactly one half the spermatozoa.

With regard to the general distribution of the accessory chromosome, I may state that a student in this laboratory, Mr. M. W. Blackman, has been able to demonstrate its presence in the Myriapoda where it evinces, in connection with all the other cell elements, a tendency to depart from typical appearances. Montgomery has investigated the spermatogenesis of *Peripatus* and declares that the "chromatin nucleolus" is not present in that form. His evidence, however, I regard as not convincing in this respect.

Credit is assigned Montgomery (¶ 24) for the discovery that the accessory chromosome is merely a spermatogonial chromosome that comes over unchanged into the spermatocytes. This must now be withdrawn, for, at present, he accepts the views of Paulmier that it is formed by synapsis from differentiated elements of the spermatogonia. That it *is,* on the contrary, the same in both cell generations, at least in the Orthoptera, is shown by the work of Sutton (¶ ¶ 49, 52).

Perhaps the most important advance in our knowledge of the accessory chromosome pertains to its relation to the other chromosomes of the cell. During the early investigations upon it, the tendency was to consider it widely removed from the type chromosome, but we are now beginning to perceive that practically its only divergence consists in its isolation. This feature is most pronounced in the prophase of the first spermatocyte where the element bears some little resemblance to a nucleolus—enough, in fact, to have induced several investigators to so call it. But, as has already been shown, this is merely superficial, and later researches upon the Locustid cells have brought to light the fact that the accessory chromosome forms a close spireme of its own, and so parallels the activities of the ordinary chromosomes at the point where it seemed most to diverge. We may therefore regard the accessory chromosome as practically normal in its behavior throughout the different cell generations of the testis up to the point where it is thrown into prominence by the unusual action of the remaining chromosomes

during the pseudo-reduction. It may well be, as previously suggested, that the distinction bestowed upon the accessory chromosome at this time is due to its fidelity to the type form of division which, at this point, is abandoned by its fellows.

Regarding the theory of its function advanced in this paper, I can say only that it has, if anything, been strengthened by later researches, and more nearly explains the phenomena involved than any other that has been conceived.

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