Croonian Lecture: *The Bearing of Cytological Research on Heredity.**

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The privilege of speaking in this historic centre of learning was first accorded to me more than 30 years ago, through the extraordinary kindness of Prof. Huxley to a young and unknown student. I would like to think it more than a fancy that to the same source, possibly, I may trace the distinguished honour of having been invited, after the lapse of many years, to speak here once again on the subject of cytology in its bearing on heredity. Of all Huxley's wise and felicitous sayings none has more persistently lingered in my memory or appealed to my imagination than one which vividly pictured, 35 years ago, the basic phenomenon that the cytologist seeks to elucidate. Suggested, no doubt, by the researches of Hertwig, Strasburger, and Van Beneden, then but recently made known, this well-known passage is as follows:—

> "It is conceivable, and indeed probable, that every part of the adult contains molecules derived from both the male and the female parent; and that, regarded as a mass of molecules, the entire organism may be compared to a web of which the warp is derived from the female and the woof from the male. And each of these may constitute an individuality, in the same sense as the whole organism is an individual, although the matter of the organism has been constantly changing" (1878).

The advance of modern cytology has been in some important respects a development of the germ contained in these words. For the aim of cytology, in so far as it bears directly upon the problems of heredity, is to trace out in the individual life the history of maternal and paternal elements originally brought together in the fertilisation of the egg. And the drift of latter-day research, while it has not precisely confirmed Huxley's conception, has nevertheless been quite in harmony with the essential thought to which he gave such picturesque expression at a time when the labours of cytology were but just begun.

This thought has been most nearly realised through the study of the cell nucleus, and in particular of the bodies known as chromosomes. I ask attention especially to these bodies in connection with certain problems of

* It has been impracticable to reproduce here the original photographs and some of the other figures by which the lecture was illustrated.

genetics, not because the chromosomes are the only elements concerned in heredity, but because they offer the most available point of attack and have in fact yielded the most definite results. The limitations of time compel me to take a good deal for granted, and to pass over, for the most part, the historical and controversial aspects of the subject. I must be content, in the main, to state briefly what I believe to be established or indicated by the evidence. My task is much lightened by Prof. Farmer's earlier presentation of many important aspects of the subject in his Croonian Lecture of seven years ago. Permit me, nevertheless, for the sake of present clearness, to indicate briefly some of the essential facts determined prior to the re-discovery of Mendel's law in 1900.

(1) The work of cytology in its period of foundation laid a broad and substantial basis for our more general conceptions of heredity and its physical substratum. It demonstrated the basic fact that heredity is a consequence of the genetic continuity of cells by division, and that the germ-cells are the vehicle of transmission from one generation to another. It accumulated strong evidence that the cell-nucleus plays an important *rôle* in heredity. It made known the significant fact that in all the ordinary forms of cell-division the nucleus does not divide *en masse* but first resolves itself into a definite number of chromosomes; that these bodies, originally formed as long threads, split lengthwise so as to effect a meristic division of the entire nuclear substance. It proved that fertilisation of the egg everywhere involves the union or close association of two nuclei, one of maternal and one of paternal origin. It established the fact, sometimes designated as "Van Beneden's law" in honour of its discoverer, that these primary germ-nuclei give rise to similar groups of chromosomes, each containing half the number found in the body-cells. It demonstrated that when new germ-cells are formed each again receives only half the number characteristic of the body-cells. It steadily accumulated evidence, especially through the admirable studies of Boveri, that the chromosomes of successive generations of cells, though commonly lost to view in the resting nucleus, do not really lose their individuality, or that in some less obvious way they conform to the principle of genetic continuity. From these facts followed the far-reaching conclusion that the nuclei of the body-cells are diploid or duplex structures, descended equally from the original maternal and paternal chromosome-groups of the fertilised egg. Continually receiving confirmation by the labours of later years, this result gradually took a central place in cytology; and about it all more specific discoveries relating to the chromosomes naturally group themselves.

All this had been made known at a time when the experimental study of

heredity was not yet sufficiently advanced for a full appreciation of its significance; but some very interesting theoretical suggestions had been offered by Roux, Weismann, de Vries, and other writers. While most of these hardly admitted of actual verification, two nevertheless proved to be of especial importance to later research. One was the pregnant suggestion of Roux (1883), that the formation of chromosomes from long threads brings about an alignment in linear series of different materials or "qualities." By longitudinal splitting of the threads all the "qualities" are equally divided, or otherwise definitely distributed, between the daughter-nuclei. The other was Weismann's far-seeing prediction of the reduction division, that is to say, of a form of division involving the separation of undivided whole chromosomes instead of the division-products of single chromosomes. This fruitful suggestion (1887) pointed out a way that was destined to lead years afterwards to the probable explanation of Mendel's law of heredity.

(2) Such, in bird's-eye view, were the most essential conclusions of our science down to the close of the nineteenth century. A new era of discovery now opened. As soon as the Mendelian phenomena were made known it became evident that in broad outline they form a counterpart to those which cytology had already made known in respect to the chromosomes. Characters and chromosomes alike are singly represented (haploid or simplex) in the gametes, doubly represented (diploid or duplex) in the zygote and its products. In the formation of new germ-cells both alike are once more reduced from the diploid to the haploid condition. A parallelism so striking inevitably suggested a direct connection between the two orders of phenomena. And the hope was thus raised that the mechanism of heredity might be susceptible of a far more searching analysis than had yet been thought of.

It is a rather striking coincidence that almost at the moment of the re-discovery of Mendel's law, and apparently quite independently of it, microscopical studies were establishing the cytological facts upon which its explanation probably rests. Guyer's studies on hybrid pigeons led him, in 1900, to suspect a disjunction of maternal and paternal chromatin-elements in the reduction division, a conclusion which he developed further in 1902. But the real basis for an explanation of Mendel's law was laid by two conclusions announced in 1901 by Boveri and by Montgomery, independently of each other and apparently without knowledge of the Mendelian phenomena. Familiar as these conclusions are, I will dwell upon them for a moment, since they are fundamental to all that follows.

Boveri's masterly experiments on dispermic sea-urchin eggs gave the first conclusive proof that the chromosomes directly affect the process of

development, and that they are qualitatively different in respect to their individual influence. Eggs into which two spermatozoa are caused to enter develop into larvæ that are almost always pathological, deformed or monstrous. The first cleavage of such eggs is by a tripolar or quadripolar division, and the cytological examination proves that this involves initial and apparently irreversible aberrations in the distribution of the chromosomes to the embryonic cells. Boveri's analysis, carried out with characteristic sagacity and thoroughness, seems to leave no escape from the conclusion that the abnormal combinations of the chromosomes thus produced are the cause, and the only cause, of the abnormal forms of development. The chromosomes must therefore be qualitatively different. This conclusion has been confirmed and rendered more specific by many later researches. It was proved, for instance, that in certain animals one of the chromosomes, or a small corresponding group of chromosomes, stands in some special relation to the determination of sex and the heredity of sex-linked characters. The study of hybrid sea-urchin larvæ by Baltzer, Herbst, and others, gives strong reason to conclude that many of the aberrations which they show in respect to the combination of maternal and paternal characters result from corresponding aberrations in the distribution of maternal and paternal chromosomes. In the evening primroses, the researches of Lutz and of Gates have shown that the *gigas* type of mutant has arisen in association with a doubling of all the chromosomes; recently the same observers show that the *lata* type is characterised by, and probably has arisen through, the presence of a single extra chromosome. To the still more recent important results of Gregory on the Chinese primrose I will presently refer.

The conclusion of Montgomery was not less important, but failed at first to receive the consideration that it deserved. Among the suggestions that immediately followed upon Weismann's speculations concerning the reduction division, one of the most fruitful was that of Henking (1891), that the reduction of the number of chromosomes in the germ-cells is initiated by their conjugation two by two in pairs during synapsis, to be followed by their disjunction in the reduction division. Montgomery drew the bold conclusion that in this process each chromosome of paternal descent unites with a corresponding or homologous one of maternal descent; and he suggested that this process, though occurring at the very end of development, might be regarded as the final step in the fertilisation of the egg. This surprising conclusion was based on a comparative study of the size-relations of the chromosomes in the diploid and haploid nuclei. I well remember the scepticism with which I, like many others, first received it. The conjugation

of chromosomes, to say nothing of paternal and maternal homologues, has been obstinately contested; it must be admitted that the proof is still far from complete for the chromosomes generally. Nevertheless, in spite of all scepticism, the drift of later research has been, I think, steadily in its favour. Both in plants and in animals the diploid nature of the chromosome groups in the somatic cells is often clearly visible to the eye, owing to conspicuous size-differences among the chromosomes. In such cases, as was first urged especially by Montgomery and by Sutton, the chromosomes may be sorted out into pairs according to their size. In a few cases, of which the Diptera offer the most striking examples, the sorting out is performed by nature, all the chromosomes being actually grouped side by side in pairs according to their size (fig. 1).* The conclusion here becomes highly probable that each pair includes a maternal and a paternal member, and that these are destined to conjugate in synapsis. In the case of the sex-chromosomes, to which I shall return, the probability becomes a certainty.

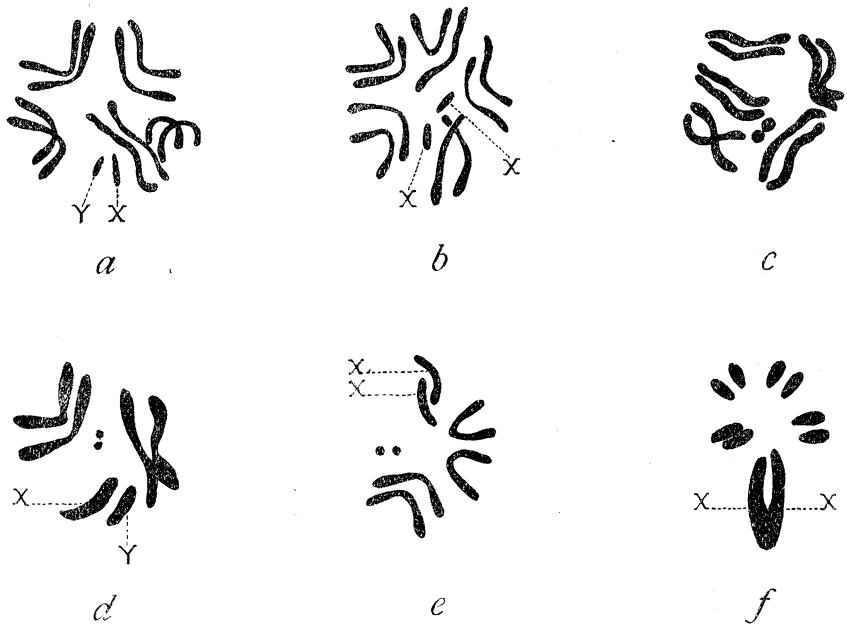

FIG. 1.—Exact drawings of the diploid chromosome groups in various Diptera, showing the chromosomes grouped in pairs; *a*, *b*, *c*, *e*, from Stevens; *d*, *f*, from Metz.

a, *Calliphora vomitaria*, ♂; *b*, the same, ♀; *c*, *Sarcophaga sarracina*, ♀: *d*, *Drosophila amœna*, ♂; *e*, *D. ampelophila*, ♀; *f*, *D. funebris*, ♀.

* These facts were illustrated by photographs of the chromosomes in Drosophila and Musca, from preparations by C. W. Metz, who has for some time been engaged with this problem in my laboratory.

The proof of the reduction division likewise remains incomplete for the chromosomes generally, and is fully demonstrative only in case of certain special kinds of chromosomes, in particular the sex-chromosomes and the "*m*-chromosomes" of the coreid Hemiptera. Strong confirmatory evidence of both conjugation and disjunction has, however, been afforded for the chromosomes generally by studies on the maturation-process in hybrids, especially in Drosera by Rosenberg, in Œnothera by Geerts, and in Lepidoptera by Federley and Doncaster.

The promulgation of the conclusions of Boveri and Montgomery opened the modern period of cytological inquiry and, as has been said, provided a substantial basis for the cytological explanation of Mendel's law. This explanation follows in the most simple and natural manner from the observed facts. It assumes primarily that the Mendelian phenomena result from the shuffling (to employ the phrase of Farmer) of chromosomes that are concerned in the determination of the so-called unit characters. More specifically, the main assumption is that Mendelian allelomorphs are borne by corresponding pairs of chromosomes, each consisting of a maternal and a paternal member. By the conjugation of the homologous members of these pairs two by two, to form bivalents or gemini, as assumed by Montgomery, the maternal and paternal homologues assume such a grouping that they may be disjoined in the succeeding reduction division (in general accordance with Weismann's early prediction); and from this follows the disjunction or segregation of the Mendelian allelomorphs which these chromosomes bear. The independent distribution or assortment of different units is explained by the assumption (in favour of which definite evidence now exists) that the bivalents behave independently of one another.

The explanation as here outlined was first clearly and logically developed by Sutton in 1902–3, when a student in my laboratory. Naturally enough, however, several others came independently to more or less similar conclusions nearly at the same time—in particular Guyer, Correns, Boveri, Cannon, and de Vries. As will appear later, Sutton's elegant hypothesis was too simply framed to account for all the facts, and has had to undergo some modifications. In its main principle, however, it has received cumulative substantiation by later work in many directions. An important confirmation of the fundamental assumption is given by a discovery announced by R. P. Gregory before this Society only a few weeks ago. In certain plants of the Chinese primrose the usual number of chromosomes is doubled in both the gametes and the somatic cells. The genetic evidence obtained from such plants indicates that all the Mendelian units or factors thus far examined are correspondingly doubled. This result weighs strongly, I believe, in favour of

the view that these factors are borne by the chromosomes, and may open the way to its crucial experimental test.

The full force of the hypothesis only becomes apparent when we come to closer quarters with the facts. I shall attempt to illustrate this by considering certain phenomena which now stand in the foreground of interest and bring home to us the intimacy of the relation that has been established between cytology and genetics.

(3) I first ask attention to certain facts relating to the cytological basis of sex, a subject with which my own researches have been especially engaged during the past ten years. To the cytologist the interest of the phenomena extends far beyond the special problem of sex. Nature has here performed a series of experiments which gives a crucial test of many of our earlier conclusions, provides a secure basis for further advances, and at the same time brings vividly before us the connection of the chromosomes with heredity. I will here touch only upon the main facts, especially in their bearing upon the phenomena of linkage, to which, I believe, they give the cytological key.

That the chromosomes are involved in the determination of sex was first suggested, in 1902, by McClung, who argued on *a priori* grounds that the so-called "accessory chromosome," which enters but half the spermatozoa, is a sex-determinant. A substantial basis for this conclusion was provided in 1905, when the late Dr. N. M. Stevens and myself, working independently on Coleoptera and Hemiptera, discovered that in some of these insects the sexes differ in the composition of the diploid chromosome-groups. In the simplest type, first worked out in the Hemiptera, the "accessory"—or, as I have preferred to call it, the X-chromosome, or sex-chromosome—is unpaired in the male, but paired in the female. Since the sexes are identical in respect to the other chromosomes, the latter may be disregarded, the sexual formulas being written simply as XX for the female and X (or X0) for the male. All of the other chromosomes are paired; hence the male possesses an odd number of chromosomes, one less than that of the female. Thus is explained the fact, first discovered in 1891 by Henking in Pyrrhocoris, that in the reduction division of the male the X-chromosome passes undivided to one pole, so that two classes of spermatozoa are formed, one with X and one without. In the female, on the other hand, the two X-chromosomes conjugate to form a bivalent, as usual, and then disjoin in the reduction division, so that every egg receives one X. This fact, at first inferred from the other relations, was soon afterwards demonstrated by direct observation, first by Morrill in insects, on rather scanty evidence, later fully established by Boveri, Gulick, Mulsow, and Frolowa in nematodes. It thus became clear that fertilisation

of the egg by the X class of spermatozoa will produce the female combination XX, by the no-X class the male combination X (or X0) (fig. 2). From these relations, and those found in a second type, described below, Miss Stevens and I concluded that the determination of sex in these animals depends upon which class of spermatozoon enters the egg, and that the X-chromosome plays some special *rôle* in the process. In a general way this substantiated McClung's earlier suggestion, though he reversed the actual significance of the two classes of spermatozoa.

I discovered in my first researches on Hemiptera a second type, inde-

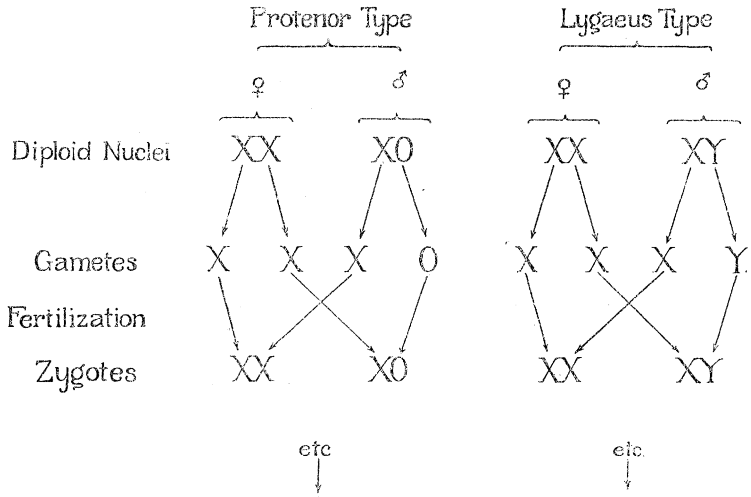

Fig. 2.—Diagram of the relations of the sex-chromosomes to sex-production, showing the two main types represented by the Hemiptera *Protenor* (Y-chromosome absent) and *Lygæus* (Y-chromosome present). In either case random union of the maternal and paternal gametes reproduces the original forms (males and females) in equal numbers.

pendently found and fully worked out in the Coleoptera by Miss Stevens, in which the X-chromosome of the male is accompanied by a mate of different type, often much smaller than X, which I called the Y-chromosome. This was the first discovery of a heterogeneous chromosome-pair in any animal or plant. In this case X and Y conjugate and disjoin like any other chromosome pair—a fact here shown with incomparable clearness—so that half the spermatozoa receive X and half Y. The X-class are, as before, female-producing, while the Y-class are male-producing; and the sexual formulas become XX for the female and XY for the male (fig. 2). Owing to the small size of Y, these differences are in many species conspicuously visible in the diploid nuclei, despite the fact that the sexes here possess the same number of chromosomes. The two types are connected by a series of inter-

mediate conditions, varying with the species, and X may consist of two or more separate chromosomes. The Y-chromosome, on the other hand, is single in all cases thus far accurately known.*

At the time these conclusions were announced it seemed unlikely that the fertilisation of the egg by the two classes of spermatozoa could ever actually be followed out. This has, nevertheless, been accomplished recently by Mulsow in the case of a nematode, Ancyrocanthus, where the chromosomes remain distinct in the mature spermatozoa and can readily be counted, even in the living object. Both classes were here traced into the egg, and the sexual differences were clearly shown in the germ-nuclei at the time of their union.

I will not enter upon the many interesting modifications of detail which these phenomena exhibit. In principle, the facts are the same in many insects and nematodes, probably in the myriapods and arachnids, perhaps also in the mammals and in man, though the demonstration here still leaves much to be desired. An extremely interesting series of researches by Morgan, von Baehr, Schleip, Doncaster, and others have proved that the same principle applies also to the parthenogenetic forms, such as the aphids, bees, and ants. Hardly less interesting are the investigations, especially of Boveri, Schleip, Krüger, and Zarnik, which show that this principle may even be extended to certain types of hermaphrodites. The results of genetic experiments on Lepidoptera and on birds lead us to expect the existence in these forms of a different cytological type, in which the eggs, instead of the spermatozoa, are of two different classes; but the cytological facts have not yet become sufficiently clear to warrant any definite conclusion. In the case of birds, indeed, a conspicuous contradiction still appears between the cytological and the genetic results; but the cytological observations have not yet produced evidence that can compare in cogency with that available in case of the insects or the nematodes.

I turn to the broader significance of the cytological facts that have been made known in this field. They constitute a very definite advance upon Boveri's general demonstration of the qualitative differences of the chromosomes; for it is impossible to doubt that the X-chromosome stands in some special causal relation with sex-heredity. A powerful argument for this is given by the facts of sex-linked heredity, which I shall presently consider. The riddle which this form of linkage presents is solved by a cytological phenomenon to which I first drew attention in 1906. The Y-chromosome,

* In an extreme case, now under investigation by Mr. Goodrich in my laboratory, the X-element consists of not less than eight distinct chromosomes, opposed by a single Y. The females here show seven more chromosomes than the males (photographs).

when present, is confined to the male line, and hence always passes from father to son. The X-chromosome, on the other hand, always passes from father to daughter (because sperms of the X class produce females), while the sons receive their single X-chromosome from the mother (because the male-producing sperms are of the no-X or Y class) (fig. 2). I will show a little later that on this curious fact probably depends the "criss-cross" type of sex-linked heredity in which the sons are like their mothers, the daughters like their fathers.

The cytological phenomena of sex-production lend strong support to the theory of the genetic continuity of chromosomes. They give unquestionable proof, in case of a particular chromosome pair (XY), of the conjugation and subsequent disjunction of corresponding maternal and paternal chromosomes. They thus substantiate the conclusions of Henking and Montgomery and confirm Weismann's earlier conception of the reduction division. They are in full accord with genetic studies, which prove that one sex is homozygous, the other heterozygous, with respect to a sex-determining factor. They give the first direct evidence of a difference of nuclear constitution between the homozygous and the heterozygous conditions, and of corresponding gametic differences. And finally, in the case of a particular chromosome pair, they fully substantiate the general cytological explanation that has been offered of Mendel's law.

(4) The facts just considered now lead us to some of the most intricate and interesting of current problems. No phenomena appealed more strongly to the interest of earlier naturalists than those of correlation. A very interesting light is thrown upon this problem by the phenomenon now widely known as gametic coupling or linkage, and it is here, perhaps, that we may best appreciate to what an extent cytology and genetics reciprocally illuminate each other.

In the second of Sutton's original papers (1903) he pointed out what seemed to be an obstacle in the way of his own hypothesis, of which much has been made by later critics. The number of chromosomes is probably always much smaller than that of Mendelian units in any given case; hence each chromosome must bear many such units. From this it follows that if the composition of the chromosomes be fixed, or even fairly constant, the units should cohere in definite groups, equal in number to that of the chromosomes; but the earlier studies on heredity gave little definite evidence that such was the fact.* Sutton did not, I think, meet the difficulty, which he

* In a general way, of course, this fact was known to earlier observers, *e.g.*, "We appear, then, to be severally built up out of a host of minute particles, of whose nature we know nothing, any one of which may be derived from any one progenitor, but which are

himself had pointed out. It was perhaps the same difficulty that led Correns (1902) and De Vries (1903), in their attempts to explain Mendel's law, to treat the chromosomes as of quite secondary importance. Their explanations operated almost wholly with smaller elements, of which the chromosomes were supposed to consist. It is now clear, however, that only when the chromosomes are taken into account do all the facts fall into line. For the most recent studies in genetics have produced indubitable evidence that Mendelian units are often, in fact, more or less definitely linked together in groups, as they should be under the chromosome theory.

Linkage was first clearly recognised in sex-limited or sex-linked heredity, to which I have already referred. A form of linkage having no relation to sex was brought to light a little later by Correns and by Bateson and Punnett in certain plants, and is now known to be of rather wide occurrence. I will confine my attention mainly to the case in which both these forms of linkage are now most accurately known, that of the fruit-fly, *Drosophila ampelophila*. In this species a very extended experimental analysis of the genetic phenomena has been carried on during the past four years in the laboratory of Columbia University by my colleague, Prof. T. H. Morgan, and his pupils and co-workers, Sturtevant, Bridges, and many others, from the investigations of whom the following results are reported. Drosophila (to paraphrase the words of Lacaze Duthiers) seems made for the experimental study of genetics. It passes through a complete generation from egg to egg in about twelve days. A single female not infrequently produces upwards of a thousand eggs. These fortunate circumstances have made it possible, in the course of four years of continuous study, to accumulate a prodigious mass of data, far surpassing in extent any others thus far made known. During this period these flies have given rise to more than a hundred definite mutations which are inherited in accordance with Mendel's law. They are of many different kinds, affecting the colour, shape, and structure of the body and of the eyes, the structure of the wings, legs, antennæ, and so on. Up to the present time 72 of these characters have been more or less completely tested as to their behaviour when crossed with the normal or "wild" form, and with one another. The all-important fact which these tests have established is that the characters fall into four definite linkage-groups, of which the first now includes 31 characters, the second 23, the third 17, the fourth, so far, but a single one. These numbers represent of course only a beginning. They steadily increase as observation continues.

As the elaborate experimental analysis has proceeded, carried on by a

usually transmitted in aggregates, considerable groups being derived from the same progenitor" (Francis Galton, 'Natural Inheritance,' 1889).

number of specially trained co-operating observers, it has been more and more conclusively demonstrated that the units of each group are more or less firmly linked together in heredity, while those belonging to different groups are quite independent. This at once suggests that the units of each group (or corresponding things on which they depend) are borne by a particular chromosome which constitutes their common vehicle of transmission, and that to this fact is due their cohesion or linkage in heredity. Conversely, the several groups are independent of one another, because of the independence of the chromosomes which bear them. This hypothesis would have been a plausible one even were the number of chromosomes in Drosophila unknown. In point of fact, however, the gametic number of chromosomes in this species (or of chromosome-pairs in the diploid groups) is actually the same as that of the linkage-groups, namely, four (fig. 1, *d*, *e*). It is at least an odd coincidence that one of these chromosomes, like one of the linkage-groups, is extremely small. One is tempted to guess that this may explain why for a long time but three linkage-groups could be identified, and that the fourth thus far contains but a single character, recently discovered by Muller.

Thus far, admittedly, the hypothesis presents a somewhat speculative aspect; but fortunately there is a means of testing it specifically, for the cytological evidence demonstrates that one of the four chromosomes is definitely connected with the determination of sex. One of the four groups of units, therefore, should likewise exhibit some special relation to sex. And this is in accordance with the facts, for every one of the 31 characters of the first group exhibits sex-linked heredity, of the same type as that which appears in colour-blindness or hæmophilia in man. It was pointed out, in 1910–11, by Morgan, Gulick, and myself that the heredity of sex-linked characters of this type exactly follows the course of the X-chromosome; that is to say, that the history of such characters is precisely such as it should be if they were dependent upon factors borne by this chromosome. Like the latter, the sex-linked units are always simplex or haploid (hence heterozygous) in the male; and they zigzag between the sexes in exactly the same way. No other group shows this relation.

Without entering far into the detail, let me illustrate these phenomena by a single example, that of the so-called "criss-cross" heredity. The normal Drosophila possesses red eyes; a common mutant has white eyes, recessive to red. If a pure-bred white-eyed female be paired with a normal red-eyed male, all the resulting sons are white-eyed, like their mother; all the daughters red-eyed, like their father. Exactly analogous results appear when, instead of white eyes, any other units of the first group, such as yellow body colour or miniature wings, are similarly tested. The results at once lose their

apparently bizarre character if we assume that the production of each sex-linked character depends upon something (which we may call a "factor" or a "gen") that is borne by the X-chromosome. I have already emphasised the fact that the sons derive this chromosome from their mother. In the cross just considered the sons therefore inherit with this chromosome the white eyes of their mother; the daughters, on the other hand, are red-eyed like their normal father, because they receive from him in every case a normal X-chromosome bearing the factor for the dominant red colour (fig. 3, A).

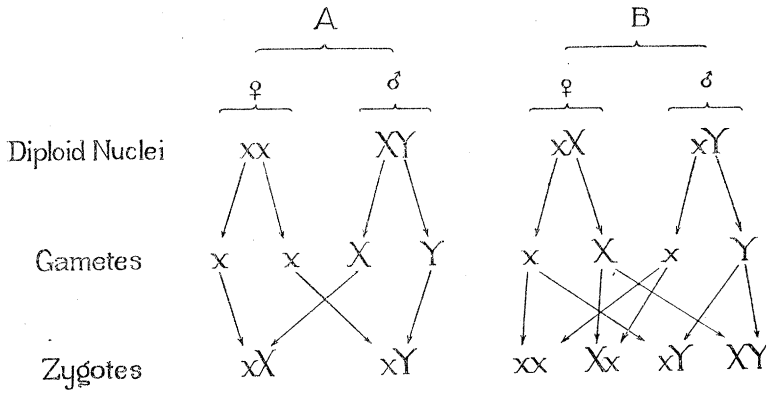

FIG. 3.—Diagram of the relations of the sex-chromosomes to sex-linked heredity. Any normal (dominant) sex-linked character (*e.g.*, red eyes) is assumed to depend on the presence of a particular factor contained in the X-chromosome. Loss or modification of this factor produces a corresponding recessive (*e.g.*, white eyes), X now becoming *x*.

A. Criss-cross heredity, when the double recessive, white-eyed female (*xx*) is paired with the normal, red-eyed male (XY). The heterozygous daughters (*x*X) are red-eyed, with white-eye recessive; the sons (zygotes *x*Y) white-eyed.

B. History of the following generation, produced by crossing *x*X and *x*Y. The offspring of both sexes are now indifferently white-eyed (*xx*, *x*Y), or red-eyed (X*x*, XY).

This has been tested in many ways, with results always in accordance with the hypothesis. Very convincing evidence in its favour has recently been obtained by Bridges through the study of a particular race of Drosophila, which regularly shows about 10 per cent. of exceptions to the "criss-cross" type of heredity. This holds true for all the sex-linked characters thus far tested. Bridges' analysis—too intricate to be entered upon here—led to the conclusion that these exceptions are due to a failure of the two X-chromosomes to undergo disjunction in the reduction division of the female, so that the mature egg sometimes receives both X-chromosomes, sometimes neither. Eggs of the XX type might be expected to produce females even if fertilised by the no-X type of sperm, the XX combination (characteristic of the female)

being supplied solely by the egg. Sex-linked characters shown by such females must be derived from the mother. On the other hand, eggs of the no-X class fertilised by the X class of sperm (normally female-producing) should produce males, and these should show only sex-linked characters derived from the father. This hypothesis was first tested, very ingeniously and thoroughly, by combining different sets of sex-linked characters derived respectively from the mother and the father. The results uniformly sustain the hypothesis. Very recently Bridges has tested his assumption cytologically. The expectation is that eggs of the XX type fertilised by sperm of the X class should produce females with three X-chromosomes, while if fertilised by sperms of the Y class the females should possess two X's and a Y. The cytological examination has demonstrated that certain females of this race actually possess three of these chromosomes.

Taken as a whole, the foregoing evidence gives almost crucial proof in favour of the conclusion that both the sex-determining factor and the sex-linked ones are borne by the X-chromosome. Sex-linked heredity of the type seen in birds or in Lepidoptera requires an explanation somewhat different in detail but similar in principle (Spillman, Castle). In the facts of sex-linkage generally the chromosome hypothesis finds, I think, its strongest support, for the linkage of sex-linked factors with one another is of quite the same type as that which appears in other groups that are independent of sex, and the conclusion can hardly be avoided that in both cases linkage is due to the same cause.

We now take a final step in order to consider a seeming difficulty which introduces us to the most recent inquiries in this field. If our hypothesis is correct, how does it come to pass that linkage is not complete? How can we explain the variations in the so-called strength of linkage? Let me again illustrate by a single example taken from Drosophila, showing the heredity of two pairs of sex-linked characters of the first group. One pair comprises the normal grey body colour (G) and its recessive mutation yellow (Y), the other the normal red eye-colour (R) and its recessive mutation white (W). Let the pure-bred dominant female RG be crossed with the pure-bred recessive male WY and the hybrid offspring be inbred. Were linkage complete we should expect to find in the grandchildren the same combinations as those which entered the hybrid, and these alone—that is to say, RG or WY. In point of fact, this expectation is nearly always realised, but about one individual in eighty shows one or the other of the new combinations RY or WG. Genetically this means that R and G, or W and Y, are strongly linked, yet now and then may dissolve their union and recombine. Cytologically it means that the original X-chromosomes, bearing in one case

RG, in the other WY, usually maintain their original constitution, yet occasionally may undergo an exchange of units so as to produce the new combinations observed. How is this possible?

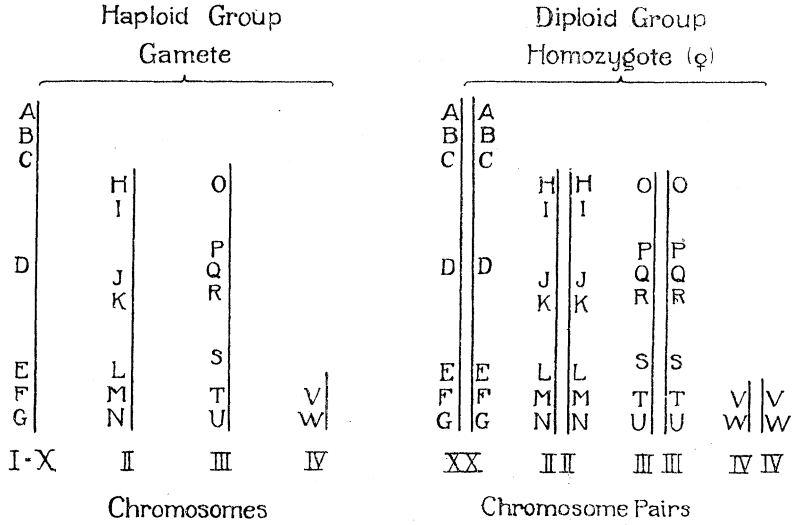

FIG. 4.—Diagram of chromosomes and linkage-groups, based on the relations observed in *Drosophila ampelophila*. Heavy vertical lines represent chromosomes (or the chromatin-threads from which they arise), letters different factors or gens, assumed to be aligned in linear series in the threads. In the diploid groups corresponding chromosomes are paired side by side in the position assumed by them during conjugation or synapsis.

Each of the four series A–G, H–N, O–U, V–W, forms a definite linkage-group in which the factors tend to cohere, while independent of all other factors.

In the male diploid groups one X-chromosome is missing, its place being taken by a Y-chromosome (*Lygæus* type). The nature of the latter is still unknown.

In attempting to answer this, it is necessary to bear in mind that the recombinations with which we are dealing affect units that are usually linked together, and hence belong to the same group—in the example just given to the sex-linked or X-group. The recombination or exchange of units must accordingly take place between the corresponding or homologous chromosomes of a pair (here the X-pair). It therefore becomes probable that the exchange is effected during the intimate association of these chromosomes in conjugation or synapsis. It was long since suggested by Boveri that an exchange of particular elements might take place between conjugating chromosomes as between conjugating Protozoa, but this suggestion is too vague for our present purpose. The basis for a more specific explanation was offered by Janssens in 1909 in his theory of the "chiasmatype," more recently

elaborated in a remarkable manner by Morgan and by Sturtevant. This explanation is as follows:—

It has long been known that subsequent to the process of conjugation—sometimes, as now seems probable, during this process—the two chromosomes of each pair, while still in the form of long threads, often twist about each other, thus producing the so-called "strepsinema" stage. Janssens concluded from a careful study of the facts in the Amphibia that the double spirals thus formed may in some cases come into contact and fuse at certain points where the threads cross, and then may be separated again by a straight longitudinal split through the points of fusion. Such a process would lead to an exchange of certain regions between the two threads. Janssens named this the "chiasmatype," and briefly called attention to the possible bearing of such a process on the Mendelian phenomena. Morgan afterwards very ingeniously developed this thought as follows: The "determining factors" or "gens" of unit-character are assumed to be aligned, as Roux long since suggested, in linear series in the chromatin-threads and in a definite order. In the process of conjugation corresponding or allelomorphic gens (large and small letters in the diagrams) are assumed to lie opposite one another in the two threads, as is shown in diagram in figs. 4 and 5. If in a certain proportion of cases

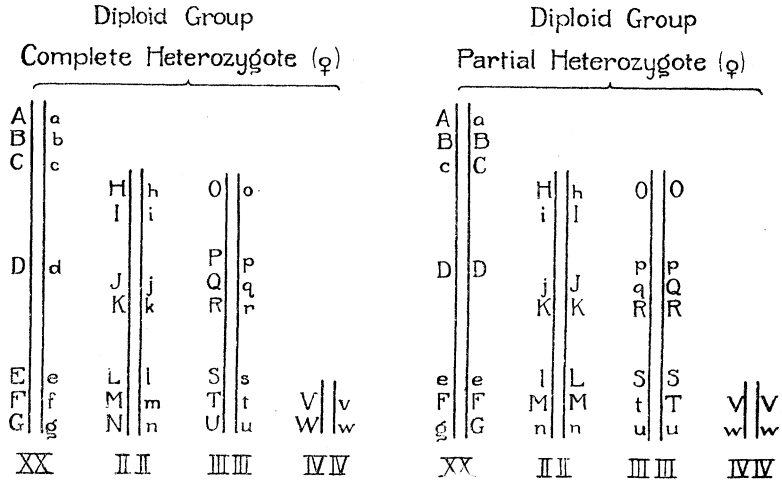

FIG. 5.—Diagram illustrating heterozygous conditions. Homologous or allelomorphic factors (*e.g.*, C and *c*) occupy corresponding loci or levels in homologous chromosomes. The condition at the left is heterozygous for all factors; at the right, heterozygous for some (A*a*, *g*G, etc.), and homozygous for others (BB, *ee*, etc.).

the two threads (linear series of gens) twist together, unite, and separate in the manner described by Janssens, the result will be an exchange between

them of certain gens, as shown in fig. 6. A simple and elegant solution of the problem of recombination or of "crossing over" is thus given.

It should be pointed out that Janssens' actual observations on the chiasmatype still lack adequate confirmation, that the strepsinema has been observed in the longitudinally split single chromosomes of the somatic divisions, and that nearly all cytologists have hitherto believed the twisted threads to untwist again before actual separation takes place. I have not yet been able fully to satisfy myself concerning the facts; but there is no doubt that in the Amphibia many of the appearances seem to be in favour of Janssens' conclusions.

The most ingenious part of the explanation relates to the varying strength of linkage. It is obvious that if the twisting be not too close, the likelihood of a chiasma taking place in the interval between any two units (and hence of their separation or dissolution of linkage) increases with the distance between them in the thread. Conversely, the nearer together two units lie the greater

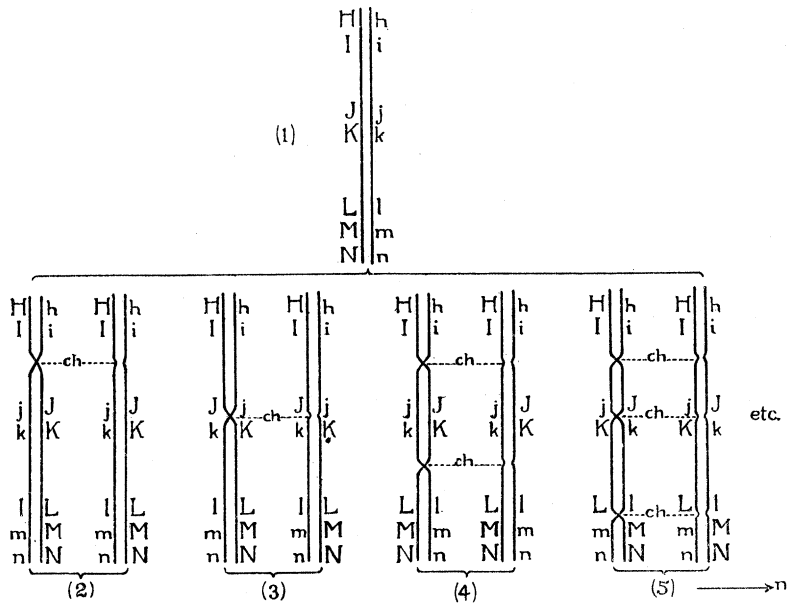

Fig. 6.—Diagram of the exchange of factors (dissolution of linkage) as explained by the chiasmatype hypothesis. The second chromosome-pair of fig. 5 is here employed as an example. The original condition is assumed to be that shown above (1). The lower figures (2–5) show a few of the many possibilities of exchange or "crossing over" between the two homologous linkage-groups or chromosomes, H–N and *h–n*. In each case the point of crossing, fusion, and subsequent splitting is shown at the left, with the result at the right. The position of the chiasma indicated in each case by *ch*. In (2) and (3) but one chiasma is present, in (4) two, and in (5) three. A very large number of recombinations is possible, even with so small a number of factors as is here represented.

the probability of their remaining in association; in other words, the greater the "strength of linkage." Hence the astounding possibility which this suggests of using the "strength of linkage" as an index of the serial order of the units in the threads and the relative distances between them. This, it seems to me, is the most remarkable result to which these researches have led, for it opens the possibility of a detailed experimental analysis of the nuclear organisation—almost, we might say, of the topographical anatomy of the germ-plasm.

By the application of this method to an immense body of experimental data, Morgan and his co-workers, Sturtevant in particular, have actually plotted the location of most of the units in each chromosome, and constantly use the diagrams thus obtained as working models for further analysis. The order and relative distances of the units in each linear series, once determined, are found to be remarkably constant when tested by varied experiments designed to this end. The practical value of the hypothesis is attested by the fact that when the distance (strength of linkage) between any two units, A and B, is known, and also that between B and a third unit, C, the relation between A and C may be *predicted* with considerable accuracy. This fact gives reality to the assumption that each unit has a definite locus in the linear series (chromatin-thread), and that allelomorphic units occupy corresponding loci in homologous chromosomes. Further corroboration is found in the interesting phenomena presented by multiple allelomorphs, of which an example is given by the four eye-colours: white, eosin, cherry, and the normal or red colour, all of which belong to the first or sex-linked group in Drosophila. Any two of these are allelomorphic to each other, and the important fact is that all exhibit the same strength of linkage with all other sex-linked units. The inference is that each of the units in question must occupy the same locus in the sex-chromosome. Since no two can occupy the same locus at the same time, it follows that not more than two of them can co-exist in any particular female, and not more than one can be present in the male. And this corresponds with the facts as actually observed.

To those not actually engaged in such investigations this hypothesis will, perhaps, seem of highly speculative character. But is it more so than many working hypotheses of experimental physics or organic chemistry that have proved themselves fruitful in the past? I will not pretend to answer. There is no doubt that it provides us with a simple, easily intelligible and effective means of handling enormous masses of intricate data, of devising new experiments, of predicting results. Such an hypothesis, venturesome though it may seem, is something more than a speculation.

I have endeavoured to show how the chromosome-theory, first outlined in very general form, has been more and more specifically developed until it has become an important instrument for the detailed analysis of intricate genetic phenomena. I am well aware that some eminent students of genetics are still reluctant to accept this theory, at least in its more detailed applications. I am not disposed to reproach them for such scepticism. The cytologist suffers under the disadvantage of working in so unfamiliar a field that some of his conclusions, even among those most certainly established and most readily verifiable, are apt to give a certain impression of unreality, even to his fellow naturalists. It is undeniable, too, that in this subject, for better or for worse, hypothesis and speculation have continually run far in advance of observation and experiment. It is quite possible that some of my hearers may consider some of the views I have touched upon as a fresh illustration of this fact. If so, I beg them to bear in mind that no conclusion which I have considered has been reached as a merely logical or imaginative construction. I have endeavoured to limit myself to matters of observed fact, and to conclusions that are either demonstrated by facts or directly and naturally suggested by them.

To those who have had opportunity to come into intimate touch with both cytological and genetic research the conclusion has become irresistible that the chromosomes are the bearers of the "factors" or "gens," with the investigation of which genetics is now so largely occupied. What are these gens? How do they operate? We do not know what they are. We assume only that a gen is *something* that is necessary to the development of a particular character. We do not know how they operate; for, despite all that experimental cytology and embryology have taught us concerning development, we are still without adequate understanding of its mechanism. We may nevertheless guess that gens play their several *rôles* by virtue of their specific chemical nature, and that the study of chemical physiology as applied to development is destined to take an important part in the future investigation of this problem. In the meantime it would be well to drop the term "determiner" or "determining factor" from the vocabulary of both cytology and genetics. What we really mean to say is "differential" or "differential factor," for it has become entirely clear that every so-called unit character is produced by the co-operation of a multitude of determining causes. Embryologists long since demonstrated by direct experiment that the cell-protoplasm as well as the nucleus is concerned in the determination of development. Our whole study of the cell leads us to the conclusion that it is an organic system, in the operation of which no single element can be wholly dissociated from the rest. When, therefore, we speak of nuclei or

chromosomes as the "bearers of heredity" we are employing a figure of speech. They are such just to the extent that they are necessary to development and heredity; but how far this conclusion carries we are as yet unable to say. Genetic experiment has already given some ground for the conclusion that definite types of hereditary distribution may be immediately dependent upon elements contained in the protoplasm. Recent advances in our knowledge of the "chondriosomes" or "plastosomes" provide this conclusion with at least a possible cytological basis.

Our conceptions of cell-organisation, like those of development and heredity, are still in the making. The time has not yet come when we can safely attempt to give them very definite outlines. It is our fortune to live in a day when the business of observation and experiment leaves little time or inclination for *a priori* speculations concerning the architecture of the germplasm or of the cell. Nevertheless it is impossible not to be struck with the fact that recent advances in cytology and genetics are in certain important respects in line with theoretical views put forward nearly 30 years ago by Roux, Weismann, and de Vries. These views were, it is true, almost purely imaginative or logical constructions. Some of them, especially as applied to the mechanism of embryological development, have been experimentally disproved, others are incapable of verification, and hence have fallen into disrepute. We have become chary of theories which assume all parts of the cell to be built up of ultimate, self-propagating, vital units, such as "gemmules," "pangens," or "biophores." The working hypothesis that has here been considered must not be identified with those far-reaching speculations; it is at once more limited in scope and more flexible in form. And yet, as far as the cell-nucleus is concerned, those visions of a bygone speculative era are now beginning to seem more real than would have been thought possible by some of us ten or even five years ago. We read in the latest productions of cytology and genetics of the division and genetic continuity of factors or gens, of their linear alignment in the chromatin threads, of their conjugation and disjunction, of their linkage or independent distribution, in heredity. We find such conceptions no longer treated as belonging to an age of cytological romance, but employed every day in the most matter-of-fact way as practical instruments of laboratory experiment, analysis, and prediction. We are bound to no speculative systems or extravagances of an earlier day if we recognise in this, let the outcome be what it may, a triumph for the men who first endeavoured to bring cytology and the experimental study of heredity into organic relation.