The Determination of Sex in Animal Development.

By

John Beard, D. Sc.,
University Lecturer in Comparative Embryology, Edinburgh.

With 1 plate and 3 text-figures.

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Abdruck

aus den

Zoologischen Jahrbüchern,

Abtheilung für Anatomie und Ontogenie der Thiere.

Herausgegeben von Prof. Dr. J. W. SPENGEL in Giessen.

Sechzehnter Band, 4. Heft, 1902.

Verlag von GUSTAV FISCHER in Jena.

"Das unaufhörliche Werden also, und nicht das unaufhörliche Bestehen, ist das Ziel im Haushalte der Natur." — Carl Ernst von Baer.

"For indeed, the further we go and the more closely we study, the more plainly is it brought home to us, that we merely are waifs, shipwrecked on the ocean of Nature; and ever and anon, from a sudden wave more transparent than others, there leaps forth a fact, that in an instant confounds all we imagined we knew." — Maurice Maeterlinck, The Life of the Bee.

Contents.

Preface.

In logical order the working out of the history of the germ-cells, and, as it has turned out, of the problem of the determination of sex, ought to have been the starting points for the course of research into the nature and mode of Metazoan development, entered upon by the writer some thirteen years ago. But, as every experienced embryologist knows, the starting point of a research often becomes the closing chapter of the narrative, while what should have been the commencement only reveals itself at the finish, if at all.

As demonstrated in the following pages, the prelude to every developmental history, usually played long before the actual story begins to unfold itself, is the determination of sex. The account to be here presented of the latter is of so simple and obvious a character, that it is difficult to understand why it was not found out long ago. The writer has no desire to give undue prominence to his own finds: indeed, he would suggest, that without them evidences of the actual existence of fourfold gametes, two sorts of eggs and two kinds of spermatozoa, sufficient to permit of the solution of the problem, have long been known. At any rate, their occurrence should have served to indicate the way.

Two sorts of eggs, sexually differentiated and of different sizes, have been known for many years. They were recorded for one species of *Rotifer* by J. Dalrymple in 1849, for other species by F. Leydig (1854) and, especially, by F. Cohn (1856), by Balbiani for *Phylloxera coccinea* (1873), and for *Dinophilus gyrociliatus* by E. Korschelt (1882). Cohn's services in this direction were great, for he realised more clearly than his predecessors the significance of his finds [1]).

Two kinds of spermatozoa were originally reported by von Siebold in 1836. For long after the discovery their occurrence found no admission into the Acta of the science; and, without examining the actual facts in the animal concerned, Kölliker disposed of them by denying the correctness of Siebold's observation! Later on Leydig was able to confirm the statements of the latter zoologist, but his work and the subsequent investigations of Duval, M. von Brunn, Auerbach, and F. Meves have hardly yet succeeded in finding a certain place in embryology for the worm-like spermatozoon of *Paludina*. The ordinary or hairlike and functional sperm is admitted: its remarkable brother is still either entirely ignored, or, if mentioned, then only as a curiosity.

And why? Because, forsooth, apart from the little understood hermaphrodites, there are but two kinds of individuals in the Metazoa, the males and the females. And, as the gametes, the eggs and sperms,

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1) in: Z. wiss. Zool., V. 7, 1856, p. 450.

were, and still are, believed to be produced from the tissues of these said individuals, there could be but two forms of gametes, eggs and spermatozoa.

It is purely an assumption, that only two forms of gametes exist in the Metazoa, an assumption, moreover, opposed to the actual facts. In the following pages the writer adduces proof of the occurrence of fourfold gametes, of which three are functional, in several instances. All current views are based upon the existence of but two sorts of gametes, and so sure have embryologists been of the truth of this, that they have never attempted to establish it in fact in any one case!

The existence of whole genera, in which functional hermaphroditism with the production of twofold functional gametes by one form of individual was the normal condition ought to have made it patent, that the Metazoan individual differentiated two sorts of gametes. The erroneous conception of the original or primitive nature of hermaphroditism however, stood, stubbornly in the way of any such recognition of the truth.

The conclusions here presented are portions only of a picture — the cycle of the development from one generation to the next: they are a small, though important, chapter in the history of the germ-cells. It may, therefore, help the reader, especially if he be not versed in the embryology of the higher animals, if a brief epitome of the writer's investigations and results concerning the course of the cycle from generation to generation, as well as a few other details of the story of the germ-cells, be given. A short account of the latter has recently been published elsewhere [1], and to this and nos. 5 to 8 of the list below the reader may be referred for fuller information.

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1) Beard, J., The morphological continuity of the germ-cells in Raja batis, in: Anat. Anz., V. 18, 1900, p. 465—485.

Other portions of the developmental cycle have been dealt with by the writer in the following memoirs:

1. On a supposed law of Metazoan development, in: Anat. Anz., 1892.
2. The history of a transient nervous apparatus in certain Ichthyopsida, Pt. 1, Raja batis, with 8 plates, in: Zool. Jahrb., V. 8, Anat., 1896.
3. On certain problems of Vertebrate embryology, Jena 1896.
4. The span of gestation and the cause of birth, Jena 1897.
5. Heredity and the epicycle of the germ-cells, in: Biol. Ctrbl., V. 22, 1902.

The question of the relationship, which may be supposed to subsist between the embryo — or for the matter of that, the mature organism — and the germ-cells within it, is one of the deepest and most important in the range of embryology. In the opinion of most embryologists the organism forms the germ-cells, and anon, when these are fertilised, they give rise directly to new organisms, which in their turns at the proper time produce from the tissues of their bodies new sets of germ-cells!

The organism neither forms the germ-cells, nor is it the chief, nor even the immediate, task of any one of these to give rise to a new organism! These statements cannot be proved here, nor is this the proper place for the production of the evidences, afforded by the study of the actual development. Briefly, after fertilisation the egg segments or cleaves itself a certain number of times. In the embryology of text-books the whole of the early cleavage products are regarded as the bricks, out of which the embryo is gradually built up. Thus, in an egg, which has divided five times, the number of segments will be at the most 2, 4, 8, 16 and 32, these 32 cells being generally considered to represent the beginnings of the future embryo. This period of 32 cells is taken for a special reason. In certain cases by the time the fifth cleavage is over, and 32 cells are present, there is no trace whatever of the future embryo. This statement may be made still stronger by the assertion, that at this period not even the commencement at the formation of an embryo has been set about. Of the 32 cells then present 31 are usually transitory or larval in nature, being destined to form the asexual organism, the larva or phorozoon, upon which the embryo arises. The remaining cell is, or becomes, the primitive germ-cell (= *U. K. Z*, the "Urkeimzelle" of German authors). In a way, which cannot be described here, this primitive germ-cell has a direct ancestry along a straight line from the fertilised egg. It now divides a certain number of times, this number varying in different species. Apparently, in the common frog it divides 3 times, in the lamprey 5 times, in the dogfish, *Scyllium canicula*, 7 times, in the development of the male skate 8 times, and in that of the female 9 times. In this way the number of the products of the primitive germ-cell comes to be one of the following: 2, 4, 8, 16, 32, 64, 128, 256,

6. The germ-cells of Pristiurus, in: Anat. Anz., 1902.
7. The numerical law of the germ-cells, ibid. 1902.
8. The Germ-Cells, Pt. I, in: Zool. Jahrb., V. 16, Anat., p. 615—702, 1902.

512 etc. The products of the primitive germ-cell are the primary germ-cells, on one of which falls the lot of developing into an embryo. The remaining primary germ-cells become the "sexual products" of this said embryo. They may be obliged, as in the skate, to wander into the embryo, or, owing to the mode of growth and evolution of the embryo, they may become enclosed by it.

The primary germ-cells are destined for future generations. After a long resting phase, during which the embryo gradually manifests itself, they begin to divide, and to form secondary germ-cells. It is with the division of the primary germ-cells into secondary ones, that, as revealed in the later pages of the present writing, the beginning of the determination of sex for the following generation is bound up. After a certain, usually limited, number of divisions these germ-cells become oocytes or spermatocytes, in which the final step in the determination of sex is taken by the numerical reduction of chromosomes. When this has been effected, there remains nothing more to complete the cycle of the germ-cells than that they should form gametes, and this they usually do by two divisions.

The greater portion of the present writing was completed — practically as now published — six months ago. In the interval it has undergone repeated examination and, where needed, revision. As it began to take form from the moment, when the existence of two sorts of eggs in the skate was ascertained, and as at that time the writer was far from suspecting the general, if not universal, occurrence of the forerunners of two forms of gametes in the male, or of two categories of gametes themselves, the manuscript has acquired a character, very different from that, which its present inditement would give it. Its gradual growth may account for, and, I hope, excuse, some apparent, though not real, contradictions between its earlier pages and the later ones. The question of re-writing it has often been considered, albeit rejected from anxiety, lest such a course should destroy the continuity of the argument. Many things, which now appear so obvious to the writer, might be mentioned without any evidence, were the manuscript to be re-cast.

The form, in which the work[1]) leaves the hands of its author, will at least enable the reader to follow the line of thought in the

1) And read, as now published, before the Royal Society, Edinburgh, on July 1st, 1901.

writer's mind during his attempts to unriddle the problem of the determination of sex.

Of the problems of sex three aspects stand contrasted: these are the determination, the regulation in nature, and the origin. The first two are considered in the following pages, of the third no absolutely certain knowledge is possible.

University of Edinburgh, June 1901.

Introduction.

In certain scenes of the cyclical drama of the germ-cells or sexual cells — in the course of the actual continuity of germ-cells from generation to generation, recently written of by the writer [1]) — what we term sexual individuals, males, females, or hermaphrodites, play certain rôles. It is to these and to the transformations, undergone by the germ-cells in their bodies, that the phenomena we term "sex" attach.

To finish off the main outlines of the cycle of the germ-cells from one generation to the next it has become necessary to inquire into the nature and determination of sex, and to attempt a solution from the morphological side. The writer was unaware, that the "sex-problem" was of necessity part and parcel of the great question of the developmental history of the germ-cells, until it forced itself into notice in a curious way. No attempt would have been made to solve the problem, had it not raised itself. For the past thirteen years the writer has been content, to follow out the track, revealed little by little by his researches into the nature and mode of Vertebrate development, without ever suggesting extraneous questions.

To go no further back than the last two centuries, these have witnessed, according to some authorities, upwards of five hundred theories of sex. More than half of these have already been termed "groundless hypotheses", and to the rest the same epithet may now be applied, for, so far as I have been able to find out, there is not one of them based in facts of morphology.

The account of sex to be here presented is a morphological one. It has nothing whatever to do with physiology; still less has it, like

1) Beard, J., The morphological continuity of the germ-cells in Raja batis, in: Anat. Anz., V. 18,, 1900 p. 465—485.

many theories of sex, leanings towards metaphysics — a subject, which is the very antithesis of morphology. In possessing a morphological basis the new features to be recorded concerning sex differ in toto from previous explanations. The present account merely points out the material basis of what we term "sex", and in itself it does not pretend to furnish indications how this can artificially be controlled or modified[1]). Anything of this nature is, of course, a question for physiology, and not for morphology.

Whilst I was engaged in putting together an account of my results, two publications, the one botanical, the other zoological, appeared. WEISMANN's writing treats of the determination of sex in the parthenogenetic development of the bee's egg, STRASBURGER's with the like question in dioecious plants. The results and conclusions of both observers will be referred to in the sequel.

Without attempting to give an account of previous inquiries and theories, one matter must be brought forward. The investigator owes a debt of gratitude and recognition to those, who have gone before him. His debt is all the greater, when the interval of time between their work and his own is a long one. And it still exists, when in any way — even in the enunciation of an idea — they may have anticipated something in his results.

Between the present writing and suggestions allied to the conclusions contained in it there lies an interval of nearly fifty years! In 1854 Dr. BERNHARD SCHULTZE[2]) from the consideration of the phenomena presented by double monsters and like-twins rightly drew certain conclusions.

His results (p. 527) include:

"All double monsters arise in one egg."

"In a single mammalian egg there invariably develops only the one sex, male or female."

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1) At the same time I would insist, in agreement with STRASBURGER (in: Biol. Ctrbl., V. 20, 1900, p. 785), that to all appearance any alteration of the relative numbers of the two sexes experimentally is an impossibility. In the present writing the experiments of YUNG, BORN, MAUPAS, Mrs. TREAT, and others have received no mention, because at the best their researches only prove what percentage of either sex will survive under given conditions.

2) SCHULTZE, B., Ueber anomale Duplicität der Axenorgane, in: Arch. path. Anat., V. 7, 1854, p. 479—531, 1 pl.

"Probably the conditions for the development of the one sex or the other are already laid down (gegeben) in the ovarian egg" [1]).

The like results are stated somewhat more fully on p. 522, and it is also recognised, that in the male element, the spermatozoon, the determination of sex (Bedingung des Geschlechts) does not lie.

Though quite on the lines of certain of the results of the present writing, one error underlies Prof. SCHULTZE's conclusions. It is that double monsters and like-twins must arise from a single egg, containing two germinal vesicles. This erroneous assumption was a very natural one to make: it has only now been proved to be unnecessary by my recent work [2]) on the germ-cells. If two primary germ-cells commence development within the same developmental foundation, the necessary larva or phorozoon, the conditions are given either for the formation of a double monster, or for the production of identical twins.

That SCHULTZE's work has in this long interval borne no fruit is proved by the nature of existing embryological opinion: that it was undoubtedly in the right direction may presently be demonstrated.

WEISMANN's recent contribution [3]) on the parthenogenesis of bees was published in the number of the Anatomischer Anzeiger, containing the resumé of my work [2]) on the germ-cells. Any connection between the two is apparently only a negative one; but it would have become positive, had I not kept back some of my conclusions.

The suspicion, which long amounted almost to a certainty, has at last been confirmed for the smooth skate, *Raja batis*. With the facts, relating to the primary germ-cells of both male and female skate before me, and with the resolution of the life-cycle of the germ-cells into one of unicellular organisms, that is, into correspondence with the life-cycles of many Protozoa, as indicated in my previous publication, it is at length possible to carry back the solution of the sex-question almost to its final limits, if not quite thereunto.

1) Recently the view of the ovarial determination of sex has been advocated by A. RAUBER (Das Geschlecht der Frucht bei Graviditas extrauterina, in: Anat. Anz., V. 17, 1900, p. 455—457). See also: RAUBER, A., Der Ueberschuss der Knabengeburten und seine biologische Bedeutung, p. 1—220, Leipzig 1900. From his experiments PFLÜGER also concluded, that sex must be decided in the ovary.

2) BEARD, J., The morphological continuity of the germ-cells in Raja batis, in: Anat. Anz., V. 18, 1900, p. 465—485.

3) WEISMANN, AUGUST, Ueber die Parthenogenese der Bienen, in: Anat. Anz., V. 18, 1900, p. 492—499.

In 1879 in his work [1]) on the *Daphnidae* WEISMANN wrote: "an explanation in the sense of a proof of the causes by which one egg develops into a male, another into a female, cannot be given; but it cannot at present be demanded. Only so much can be established, that fertilisation here has absolutely no influence on the determination of sex, etc."

Recently in dealing with a different case, the parthenogenesis of the bee, his conclusion is the very opposite, for he writes: "Therefore, I do not see how one can avoid the conclusion from the non-fertilisation of the eggs laid in drone-cells, that it is just the omission of fertilisation, which here conditions the development into the male sex, and conversely, that fertilisation of the egg at the same time determines the female nature of the embryo ('zugleich den Embryo zur Weiblichkeit bestimmt'). How this comes about we do not understand: but that there is a connection can no longer be denied." (Op. cit. p. 499.)

This conclusion cannot, I venture to think, commend itself to the embryologist. WEISMANN's standpoint of 1879 would seem to be more in accord with the facts. DZIERZON's theory [2]) of the determination of sex in the bee by the occurrence or not of fertilisation is a conclusion purely of the post hoc order. It is not a generalization applicable to all cases — even in the bee! — and it does not exclude other, and possibly more potent, determining factors. It would, however, carry me too far to discuss the matter at this juncture.

For reasons, to be presently briefly mentioned, its validity must be denied, and with this the correctness of the idea of there being any connection whatsoever between fertilisation and the sex of the resultant offspring, not only in this instance, but in any case.

1) WEISMANN, AUGUST, Beiträge zur Naturgeschichte der Daphnoiden, Leipzig 1876—1879, p. 457. Also in: Z. wiss. Zool., V. 27—33. The last paragraph is spaced in the original.

2) A sharp distinction must be drawn between two things; of these the one is, that the eggs of bees, which produce drones, are not fertilised, the other, that the absence of fertilisation is the cause of the development of a drone out of such an unfertilised egg. The latter is DZIERZON's theory of the determination of sex. To speak of the former as "DZIERZON's theory" is to lead to needless confusion, and, moreover, it is or is not a fact — not a theory — that as a rule the drone-eggs of the bee are not fertilised. From the supposed fact of the absence of fertilisation DZIERZON set up the theory, that omission of fertilisation in the bee determined the development to the male sex.

I. Theses concerning Sex, Hermaphroditism, and Parthenogenesis.

Here, I would lay down certain theses, concerning sex, hermaphroditism, and parthenogenesis.

1. The male gamete, the spermatozoon, has and can have absolutely no influence in determining the sex of the "offspring". Its rôle is simply to bring about the effects due to amphimixis.

2. After development has commenced, the nutrition of the developing germ, or of the mother, if development be in utero, cannot have the slightest effect on the sex.

3. Once the egg is fertilised, nothing whatever can influence the sex of its most obvious product, the embryo.

4. Nor is the sex determined in the moment of fertilisation: on the contrary, it is predetermined, in all probability from an epoch shortly antecedent to the formation of the egg-mother-cell or oocyte, which becomes an "egg" by giving off the polar bodies (Fig. 1, p. 750).

5. The sex is a function of the egg itself. With a given egg nothing whatever can alter the sex of the embryo, destined to arise from it.

Since in the human subject all the cells (oocytes), which later on become the actual ova, are already present long before birth, it seems to follow, that here the sex of each and everyone of the future offspring is already predetermined, even before the birth of the parent itself. Each oocyte in the ovary of a new-born child would, therefore, already at that early period have the stamp of sex impressed upon it.

6. There are two kinds of eggs: of these the one is destined to give rise to a male form, the other to a female one. For shortness and simplicity these may be spoken of respectively as "male-eggs" and "female-eggs".

7. The number of primary germ-cells is constant for the species, but it varies in different forms. It is always a member of the geometrical progression 2, 4, 8, 16, 32, 64, 128, 256 etc. It may be the like number in both sexes, thus in the dog-fish, *Scyllium canicula,* it is 128 for either sex; or, again, it may be unlike in the two sexes, and then the number of primary germ-cells in the female may be double that in the male, thus, in the smooth skate, *Raja batis*, it is 255 for the male and 512 for the female.

But in every instance the primary germ-cell, sacrificed to form the embryo, must be deducted; and, therefore, the number comes to be 2^n-1. Thus, it is 127 in the dogfish, *S. canicula*, 255 in the male, and 511 in the female smooth skate, *R. batis.* The possible existence of other numbers is not to be understood as here excluded.

8. From the facts concerning the number of primary germ-cells, and from certain other factors, to be afterwards referred to, it may be concluded, that sex is actually differentiated and decided during oogenesis. The facts point to the last division of the oogonia, and the formation of oocytes, as the particular epoch[1]), at which this happens. That is, the oocytes are differentiated into two categories, destined to become male- and female-eggs respectively.

9. In the male, as the researches of Meves[2]) and other factors render evident, a corresponding differentiation of the direct forerunners of two sorts of gametes may happen at the like period, at the final division of the spermatogonia into spermatocytes[1]) (Fig. B, page 750). But the second form of male gamete is not now of functional value. In other cases, as in the skate, the complete formation of a second form of gamete apparently does not come about.

10. Underlying the phenomena of sex, therefore, there are three sorts of functional gametes: in some, possibly in very many, instances a fourth sort of gamete is differentiated in the male, but in the latter never more than one kind of gamete is of functional import.

11. In parthenogenesis the sex of the "offspring" is not in any sense a consequence of the non-fertilisation of the egg. It would be more correct — at any rate in many cases, thus in the bee and plant-louse — to say, that the non-fertilisation of the egg was the final result of the production and maturation of either male- or female-eggs.

12. The phenomena of parthenogenesis depend upon the acquisition of the faculty of developing without fertilisation on the part of either male-eggs, or female-eggs, or of both male- and female-eggs.

13. Whenever in parthenogenesis long series of forms of the one sex appear from unfertilised eggs, the eggs, destined to form the other sex, must have been either delayed in their ripenings, or suppressed.

It must also be stated with great emphasis, that the degeneration and death of germ-cells, not only in pre-embryonic and post-embryonic life, but also in that of mature organisms, is a phenomenon constantly happening.

As one instance of this sort of elimination of germ-cells the following case from Haecker (in: Zool. Jahrb., V. 5, Anat., p. 219,

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1) Compare, however, Figs. A and B and Section VIII.

2) These are described in a later section (page 733).

220) may be cited. HAECKER found and described under the name of "Zwischenkerne" numerous germ-cells among the oocytes of *Cantho-camptus* in degeneration. It is especially of interest to note, that many of these obviously lie in the zone of sex-differentitaion, where the oogonia become oocytes.

14. Where the parthenogenetic development of eggs of the one sex, or of the other, has been initiated, there is an undoubted tendency for such eggs to become in the long run incapable of fertilisation. This would appear to be so as a rule in the drone-eggs of bees; but occasionally, as DZIERZON's experiments show[1]), and as WEISMANN has recorded (op. cit. p. 495) of one instance in 272, some drone-eggs may be fertilised[2]). From the experiments of the former it is clear, that such aberrant fertilisation is without influence on the sex of the offspring; but, if the two parents be of different races, it leads to "bastardism", i. e., crossing.

15. Where parthenogenesis becomes cyclical (WEISMANN), as in *Apus*, some *Daphnidae*, and Rotifera, and where thus the males tend to disappear, this must simply be due to the constant and regular suppression as such of the germ-cells, which ought to have produced male-eggs, and from these males. Perhaps one might also hold, that in those instances of the rare production of males, e. g., in *Artemia* (WEISMANN ISHIKAWA)[3]), these had come under the influence of panmixie. As WEISMANN has pointed out, they are useless organisms;

1) SIEBOLD, C. T. E. VON, Wahre Parthenogenesis bei Schmetterlingen und Bienen, Leipzig 1856, p. 96.

Compare section III, page 723.

2) In his remarkable researches on the *Daphnidae,* already cited, WEISMANN has discussed the possibility of the fertilisation of the summer-eggs (p. 324). "For certain species at least copulation of the virgin-females is impossible, because they lack the copulatory canal, which is present in the sexual females of the like species. This condition obtains in *Bythotrephes, Evadne* and *Podon.*"

These cases appear to represent a stage further than that, in which the fertilisation of the parthenogenetic egg has become impossible, for here fertilisation is superseded by impotence on the part of the female. The reader may be reminded, that the parthenogenetic females of *Aphis* possess no receptaculum seminis.

3) WEISMANN, A., u. ISHIKAWA, C., Weitere Untersuchungen zum Zahlengesetz der Richtungskörper, in: Zool. Jahrb., V. 3, Anat., 1888, p. 579.

and it is but a step further to the sisting of their production by the suppression of the male-eggs [1]).

16. Hermaphroditism is a lower and simpler state than parthenogenesis. As Fritz Müller, Brock, Yves Delage, Paul Pelseneer, Maupas, and the writer have maintained for certain cases, and as I would now generalize for all, functional hermaphroditism is a peculiarity of the female sex alone. That is, as a rule only the females of a species are capable of becoming hermaphrodite.

17. The possibility of hermaphroditism depends on the property possessed by the female Metazoon — and to all appearance by it only — of "producing" two kinds of functional gametes, the male-eggs and the female-eggs.

18. In hermaphroditism, instead of the production and maturation of two kinds of eggs, male and female in destination, we witness the maturation and fertilisation of one kind only, the female-egg. The germ-cells, which here should have become male-eggs, are converted into spermatogonia and from these spermatozoa are ultimately produced [2]) (Fig. C, page 750).

With the table (in: Biol. Ctrbl., V. 22, p. 326), showing the life-cycle of the skate, it is an easy matter, to see how this comes about. Following the history of a male-egg in the skate from its formation, through its fertilisation by a spermatozoon, to its cleavage, we have from this a certain number, 256, primary germ-cells arising. Of these 256 primary germ-cells one develops into an embryo, while the remaining 255 ultimately go to form the male "sexual products" of this embryo, its spermatozoa. In hermaphroditism, instead of the fertilisation of male-eggs, their cleavage, and the formation of primary germ-cells and embryo, we find the omission of these, and the production of spermatozoa some cell-generations earlier than would have been the case, had the said male-egg been fertilised, and had it developed. In my researches on the germ-cells the formation of an embryo has long been recognised to be a mere incident in a complicated life-cycle — an incident arising

1) Compare Section VIII, page 749.

2) A diagram, made up of the left half of Fig. 1 from *p.g.c* to the ripening of the female-egg and of the right half of Fig. 2 from *p.g.c* to the ripening of the ordinary or hairlike spermatozoa, gives a fair idea of what happens in hermaphroditism; this has been depicted in Fig. 3.

for a certain end — the creation of a harbour of refuge for a set of germ-cells. In hermaphroditism Nature dispenses with this incident in the cases of male-eggs, and they become converted into spermatozoa within a gametozoon or sexual generation, older than that, in which they ought to have arisen. From the new light, thrown by my researches upon hermaphroditism, the late FRITZ MUELLER [1]), and the writer [2]) were clearly in the right in disputing the validity of current views of the nature of hermaphroditism [3]). We denied, that hermaphroditism was the original condition, which prevailed prior to the evolution of separate sexes, and we regarded it as derivable from the dioecious state [4]). The opposite opinions were, and still are, maintained by leading zoological authorities.

19. A comparison of parthenogenesis and hermaphroditism demonstrates, that in the former there is what may be termed a precocious development of an embryo, male or female, in the latter the precocious development of male-eggs or their forerunners to form male gametes or spermatozoa without the intermediation of the otherwise necessary male embryo or sexual person. In hermaphroditism the males tend to disappear, because the male-eggs, which should have gone to form them, have been used in the development of spermatozoa. In parthenogenesis the males disappear for another reason, because their production may become unnecessary.

1) MÜLLER, FRITZ, Die Zwitterbildung im Thierreich, in: Kosmos, V. 2, 1885, p. 321—334.

2) BEARD, J., The life-history and development of the genus Myzostoma, in: Mitth. zool. Stat. Neapel, V. 5, 1884, p. 544—580, 2 pl.

3) Ordinary hermaphrodites might be defined as "Metazoan persons of one kind, each bearing germ-cells of two sorts". They would then be in contrast with dioecious forms, where there were two kinds of individuals, carrying germ-cells of four sorts, two for each description of individual. A definition of this character might have made clear the nature of the hermaphrodite person, for, where in hermaphroditism in animals a second kind of person is encountered, this is a male, the so-called 'complemental male'.

4) In 1884 the writer in an inaugural dissertation showed, that in *Myzostoma* and the Cirripedia hermaphroditism had been secondarily superimposed upon the females, and, independently in 1884 YVES DELAGE arrived at the like conclusion for *Sacculina*. This origin of hermaphrodites from what were really female Metazoa was extended to the Mollusca by PAUL PELSENEER in 1895. More recently still E. MAUPAS has found it also to hold good for certain Nematodes (vide: E. MAUPAS, Modes et formes de reproduction des Nematodes, in: Arch. Zool. expér., V. 8, 1900, p. 463—624, 11 pl., l. c. 582 et seq.).

As I have elsewhere already insisted (in: Mitth. zool. Stat. Neapel, V. 13, 1898, p. 304) hermaphroditism and parthenogenesis are mutually exclusive. This may now be obvious. Similarly in the animal kingdom males, females, and hermaphrodites do not and cannot obtain within the limits of a species.

II. The Gametes of the Metazoa. — The four Categories of Gametes, and the three Kinds of functional ones — Pseudo-Hermaphroditism.

The above are in brief form further conclusions, drawn from my researches on the germ-cells. In part they are based upon certain facts, disclosed in the course of the work; and in part they would seem to be legitimate inferences from known facts, from the resolution of the life-cycle into that of a unicellular organism, and from the table of the life-cycle of the skate, published in the paper on „Heredity and the Epicycle of the Germ-cells."

From enumerations of the primary germ-cells of *Raja batis* it presently became evident, that the total always approximated more or less closely to one or other of two numbers. It was either 256, or double this, 512. These numbers of primary germ-cells cannot be derived from germinal areas of like sizes. In the smooth skate there must be "macro-eggs" and "micro-eggs" — exactly as in *Dinophilus apatris*, *Phylloxera*, and *Hydatina senta* — for, whether there be 256 germ-cells or 512, these cells are practically of the like average size, 0,02 mm. This was found to be the case: the germinal discs of segmenting eggs of the smooth skate are of two sizes [1]).

1) At present it cannot be stated whether or not this applies to the yolk-mass also. Possibly it does not, for in both males and females the embryo remains within the egg-capsule for the like period. But I have long been struck by the fact, that in certain periods of the development of *R. batis* there are obviously two sizes of embryos, thus in embryos of 8—10 mm some embryos of the former size exhibit the same degree of development as others of the latter dimensions, even under the identical preservation. It does seem possible, that the marked sexual dimorphism, so often obtaining among animals, e. g., *Lepidosteus osseus*, *Cyclopterus lumpus*, *Bonellia*, and *Sacculina*, may be in association with, and a consequence of, a considerable difference in the number of primary germ-cells in the two sexes.

From a fairly large number of exact measurements of large *Raja batis*, kindly made by my friend, Mr. P. Jamieson, in the fish-market at Aberdeen, a small but constant difference in size between male and

From the start there was a natural suspicion, that the larger number of primary germ-cells related to the female embryos. I had no certain knowledge of the relative numbers of the two sexes in *Raja batis*; and, moreover, the number of such germinal areas in my possession was not great enough for statistical purposes [1]). I was thus obliged to determine the relative numbers of the primary germ-cells in embryos, in which the sex, though not yet announced, was betrayed by one or other of the well-known characters, a knowledge of which we owe to SEMPER. The result of this further census demonstrates, that in embryos potentially female the number of primary germ-cells is double that in embryos potentially male.

As such embryos are relatively in embryological sense "late or older embryos", it cannot be expected, that the total number of primary germ-cells will always be found to be 255 or 511 — the degeneration of some or many of them may already have taken place. In potentially male embryos the number of primary germ-cells is, however, always such as to be derivable from 256, in potential females from 512, and from these two numbers respectively and only [2]).

Lest too much stress should be laid upon this fact, let another one be added to it. I had, perhaps not unnaturally, expected to find a similar sexual difference in other Elasmobranchs, thus in *Scyllium canicula*; and to this end the primary germ-cells of 7 embryos of this fish were counted. The variety of this species at my disposal was obtained in Wales. I believe it to be in some respects distinct from, for instance, the Neapolitan form. Therefore, it by no means follows, that the like conditions will be found in the latter variety. In 4 potentially female embryos and in 3 potentially male ones of *S. canicula* the number of primary germ-cells was found to approximate to 128. From this it may be concluded, that here the number is alike in both sexes.

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female skate was verified. As was anticipated, the males are smaller from end to end, and also across the broadest part of the pectoral fins.

1) So far as they went, the study of germinal areas of the smooth skate seemed to point to an equality in numbers of the two sexes. From statistics, kindly furnished by Mr. P. JAMIESON, of the staff of the Scottish Fishery Board, this conclusion finds its warrant in fact. Among 1486 smooth skate he found 735 females and 751 males.

2) The actual figures and tables will be published in Pt. 3 of the memoir on the germ-cells.

Notwithstanding the expenditure of labour for an apparently negative result, I am very glad to have carried out the count here. At any rate it has prohibited any erroneous generalization, that the number of primary germ-cells of the female is always double that of the male. Moreover, the find has served to help to clear up certain cases of twofold gametes, simulating hermaphroditism, in the males of certain species.

Of these there may be here mentioned 1) the presence of apparently true eggs in the testis of the lamprey, *P. planeri*, and of the common toad, *Bufo vulgaris*, and 2) the two kinds of spermatozoa — the wormlike and the hairlike or ordinary forms — in certain snails, *Paludina vivipara*, *Ampullaria*, *Murex brandaris*, and in a moth, *Pygaera bucephala*, .etc. These, and possibly very many other cases, may be interpreted as either abortive, or futile, attempts on the part of the male to differentiate two sorts of gametes. But it must be insisted, that nothing goes to show both ever to be of functional import.

As to the "eggs" of the testis, of course, these are not "eggs". Like the instances of apparent eggs in the testis of *Rana esculenta*, or in its fatty bodies, of both of which the writer has seen several cases, they are merely spermatogonia, or spermatocytes of the first generation. Being such, they are the true "homologues" [1]) of ovarian eggs. I regard these and other instances of the usual or the sporadic production of "eggs" in the testis as abortive or useless gametes, or the forerunners of such, of a second form [2]). In the toad, and prob-

1) The writer feels unable to maintain the existing embryological opinion of the complete homology of the sexual products of the male with those of the female.

2) The reader may be reminded, that abortive and useless gametes are not treated of here for the first time: embryologists have long recognised their general occurrence in the conjugation of the Protozoa, and in the formation of the "polar bodies", separated by every oocyte in the Metazoa before it becomes an egg, capable of fertilisation.

Apart from degeneration of germ-cells at various periods of the cycle — of which, while it is by no means uncommon (e. g., *Ascaris*, *Salamandra*), no estimate has yet been made in any single instance — it must now be recognised, that in all cases of spermatogenesis with twofold spermatozoa, fully differentiated, there is always one abortive gamete more than obtains in the formation of an egg from an oocyte, i. e., in the shape of the three polar bodies. But while, for example, in the "ripening" of a male- and of a female-egg in *Paludina* there may be six abortive gametes, in the corresponding period in spermatoably in other cases, this second form of gamete seldom gets beyond the stage of being a spermatocyte — hence its superficial resemblance in some instances to an egg — and in the toad it very often degenerates in this stage of development. Supposed hermaphrodite male frogs — for the forms are usually at the basis functionally male — are continually cropping up, and getting themselves described as remarkable instances of hermaphroditism in the Vertebrata: the like is true of cod-fish, *Gadus morrhua.* In the Anat. Anz., V. 11, p. 104—112, my friend, F. J. Cole, has brought together in tabular form the described cases of frogs. According to him, of 13 recorded only 3 are "hermaphrodite" females, while 10 are males. To the latter and to Cole's frog one or two more in my own possession may be added.

I observe with pleasure, that Bonnet[1]) takes up very much the same attitude towards many supposed cases of hermaphroditism in various individuals among vertebrates. He demands, that as a proof of their true hermaphroditism the ripening of both eggs and sperms shall be demonstrated. With emphasis he states, that this proof has never yet been furnished. I quite agree with him, and would add, that the proof can never be offered; for were the apparent eggs in the testis of a frog or toad to ripen, the result would be a second form of spermatozoon, comparable to the wormlike one of *Paludina.* This follows from observations by von La Valette St. George[2]), who found in *Bufo calamita* occasionally ("kaum unter Hunderten") large spermatozoa of double the usual size. In *Bufo,* as my own observations prove, they are rare, because many or most of them degenerate before reaching the spermatozoon-stage. While such giant-spermatozoa were also rare in *Hyla*, von La Valette St. George

genesis of the four hairlike and the four wormlike spermatozoa formed from two spermatocytes, there are only four abortive gametes, the wormlike spermatozoa. Therefore, in spite of the non-functional nature of the second form of spermatozoon, a greater percentage of abortive gametes may still obtain in oögenesis than in spermatogenesis. Thus, whereas 50 per cent of the spermatozoa may be useless gametes, this number is always much exceeded in oogenesis, where invariably, except in parthenogenesis with a percentage of 50, the corresponding number is 75 out of every 100.

1) Bonnet, R., Giebt es bei Wirbeltieren Parthenogenesis? in: Ergebn. Anat. Entw., V. 9 for 1899, 1900, p. 865.

2) von La Valette St. George, Spermatologische Beiträge, III, in: Arch. mikrosk. Anat., V. 27, p. 393—395.

stated, that they were abundant ("recht häufig") in *Rana esculenta*. Such large spermatozoa have, therefore, been described in the testis of the frog and toad, whereas ripe eggs, or eggs anywhere near ripening, have never been seen there. From a partial search of the literature of spermatogenesis it is curious and very significant to note how relatively often a second form of spermatozoon has been mentioned: the text-books naturally hardly ever refer to it, or, if they do so, their notice is of the briefest description. Probably it has hitherto appeared to be too mysterious an object for general mention!

Without citing more recent cases, for to do so would serve no useful object, one further example of an hermaphrodite male frog may be noticed. It is the paper, rather than the frog, which is of interest. The author, F. FRIEDMANN [1]), comments upon the "höchst seltsame Behauptung" of KNAPPE [2]), that the so-called eggs of the testis of *Bufo,* if they develop further, yield spermatocytes and spermatozoa. According to the former, in face of our present knowledge this is "unhaltbar"; for eggs, or oocytes, cannot yield spermatozoa, (notwithstanding the current opinion, that eggs and sperms are completely homologous). A theory may be made untenable by a fact, or by facts: a fact is either true or false, and it cannot be influenced in any way by a theory. Facts are sometimes awkward things — for theories. To my mind there is nothing at all remarkable or improbable in KNAPPE's statement: it would be a wonder — a miracle even — were the supposed eggs in the testis to become actual functional eggs. They may develop into spermatozoa, and they often do so, for they are the forerunners of a second sort of spermatozoon. On the other hand, there is nothing to prevent the occurrence of eggs and sperms in the ovary. Without doubt the female can on occasion form spermatozoa from the forerunners of male-eggs: the male can never differentiate any sort of egg. Moreover, we know any number of instances of the parthenogenetic development of eggs in the Metazoa, but not one of that of spermatozoa. The female, therefore, "produces" two kinds of gametes, either of which on occasion being capable of developing without the aid of another gamete or spermatozoon. On the other hand, no instance of the parthenogenetic development of a spermatozoon is known, or likely to be. The male only „produces" spermatozoa, often

1) FRIEDMANN, F., Rudimentäre Eier im Hoden von Rana viridis, in: Arch. mikrosk. Anat., V. 52, 1898, p. 248—262.

2) KNAPPE, E., Das BIDDER'sche Organ, etc., in: Morph. Jahrb., V. 11, 1886, p. 538—542.

of two kinds, and, therefore, the male is never capable of becoming hermaphrodite.

A tabulation and discussion of several instances of supposed hermaphrodite cod-fish have been given by Prof. G. B. HOWES [1]). From his finds and from various considerations HOWES comes to the conclusion, that the original Vertebrate stock was hermaphrodite. It is, perhaps, needless to remark, that this is, and must be, erroneous.

The conversion of hermaphrodites into dioecious forms, and of males of dioecious animals into hermaphrodites are things believed in, but hitherto without evidences of a reliable kind. The idea of the original or primary nature of hermaphroditism was never founded at all in facts, and with the lapse of time and the accumulation of the results of research it has not emerged unscathed. In those instances, where the relations of the hermaphrodite and dioecious states to each other have been looked into, it has, as a rule, turned out, that the former had been derived from the latter, and that in all cases it was upon the females, that the hermaphroditism had been superimposed. This has now with greater or less certainty been established in Cirripedia, certain Teleostei (BROCK), *Sacculina* (YVES DELAGE), Mollusca (PAUL PELSENEER), *Myzostoma* (the writer), *Myxine* (J. T. CUNNINGHAM), and, lastly, various Nematoda (E. MAUPAS).

Because of these facts and the now obvious correctness of the conclusion, that only the female Metazoon can become functionally hermaphrodite, and because of the further consequence, that all hermaphrodites must be altered females, the writer has not deemed the inclusion of a special chapter upon hermaphroditism in this writing needful.

In connection with the question the reader's attention may be directed to the two following publications: 1) E. MAUPAS, Modes et formes de reproduction des Nematodes (in: Arch. Zool. expér., V. 8, 1900, p. 463—624), and 2) Prof. PERRIER's report upon MAUPAS' researches in the competition for the "Grand Prix des Sciences physiques" (in: CR. Acad. Sc. Paris, No. 25, 16 Déc. 1901, p. 1089 to 1096).

In his extensive researches upon Nematodes MAUPAS confirms for still another group the writer's conclusion of 1884 that "hermaphroditism, probably all hermaphroditism, had its origin in a unisexual

1) HOWES, G. B., On some hermaphrodite genitalia of the Codfish, in: Journ. Linn. Soc. London, Zool., V. 23, 1891, p. 539—558.

condition" (in: Mitth. zool. Stat. Neapel, V. 5, 1884, p. 578). This conclusion, and subsequent attempts on the part of the writer to establish it more firmly, have, except upon the part of the late FRITZ MÜLLER, who fully endorsed it, attracted more discredit, and even abuse, than anything else. MAUPAS cites the researches of various authors [1]), including FRITZ MÜLLER and W. M. WHEELER, but for some inscrutable reason my work upon *Myzostoma* is nowhere referred to by him, although it furnished in part the pioneer-basis of his results, which confirm, while naturally extending, my conclusions. The citation of WHEELER's work with the neglect of mine is curious, for unlike my own the conclusions of the former are diametrically opposed to MAUPAS' results.

MAUPAS, at any rate, has no belief in the existence of the miraculous conversion of males into hermaphrodites, which under WHEELER's views would daily happen.

Something of the well-deserved honour, paid to MAUPAS' researches upon the hermaphroditism of Nematodes by the Academy of Sciences, Paris, is thereby reflected upon my earlier pioneer-investigations into the nature of hermaphroditism. And, ignoring the discredit, and even the personal abuse, the latter have called forth, I venture to deduce from the recognition given to MAUPAS' work a compliment to my own. The high source of this only makes it all the greater and more valuable.

III. The supposed Influence of Fertilisation upon Sex.

In other parts of the present writing some of the evidences, pointing to the absence of any influence of fertilisation upon the sex of the subsequent offspring, are briefly referred to, and it is denied, that the sex of an egg, destined to develop either after fertilisation or parthenogenetically, can be influenced or altered by anything subsequent to the period of the formation of the oöcyte, which becomes "the egg" by giving off the rudimentary sister-gametes, the polar bodies.

1) The priority of the discovery, that "l'hermaphroditisme est d'origine secondaire et la dioïcité l'état antérieur" is assigned by MAUPAS to YVES DELAGE and PELSENEER (l. c. p. 594). PELSENEER's researches were not published until 1895, while DELAGE's conclusion and my own identical one appeared independently in 1884. My words were "hermaphroditism, probably all hermaphroditism, had its origin in a unisexual condition".

The belief in the actual existence of this influence is so widespread, it is advocated by zoological investigators of such high standing, that its detailed consideration may be called for. From the results, to which his studies have led him, and from the consideration of the known facts of other cases, the writer does not for himself regard the question as now any longer worthy of serious discussion. The facts, concerning the twofold gametes of the male, and those of the female, as well as the non-functional nature of a second form of spermatozoon, seem to be decisive against any influence whatever of fertilisation upon sex.

Were the female organism the one to differentiate one kind of functional gamete only, in the shape of an egg, and were there two categories of functional spermatozoa, the matter would stand in another aspect. Then, but then only, the sex of the "egg", that is to say, of the zygote, might be determined by the union with either the "A" sperm or the "B" sperm. This condition is, however, non-existent in nature as we know it, and it is with existing things and factors we have to deal, not with imaginary ones.

Many recent writers accept as more or less firmly established what may be termed DZIERZON's view of the determination of sex (in the bee) by fertilisation, for instance, WEISMANN, STRASBURGER, and RAUBER. In a recent contribution [1]), already referred to on an earlier page, WEISMANN writes: "Therefore, I do not see how one can escape from the conclusion from the non-fertilisation of the eggs laid in drone-cells, that it is just the absence of fertilisation, which here conditions the development to the male sex, and conversely, that fertilisation of the egg of the bee at the same time determines the female nature of the embryo. How the connection obtains we do not understand, but that there is a connection can no longer be denied."

The reader is probably aware of the main facts of the parthenogenesis of the bee, as established by DZIERZON and VON SIEBOLD. They maintained, that fertilised eggs here gave rise to female young, because they were fertilised, non-fertilised eggs to male forms or drones, because they were not fertilised. At the basis of this supposed determination of sex in the bee by the occurrence or omission of fertilisation is the idea or belief, that the queen-bee can at will fertilise an egg, or omit to do this. This belief assumes the existence

1) WEISMANN, A., Ueber die Parthenogenese der Bienen, in: Anat. Anz., V. 18, 1900, p. 499. The above translation is not strictly literal.

of too great an intelligent power in the bee, and one elsewhere denied, even unto the highest animals. How can the queen-bee know whether or not a certain egg should be fertilised? If she can exercise this power at will, her embryological knowledge is truly remarkable, and probably for thousands of years it has been far in advance of that of mankind!

The two statements given above may be put in rather different ways. 1) The eggs, which produce females, are fertilised. 2) The eggs, which give rise to males, are as a rule not fertilised.

While it is generally true, that the eggs of drone-cells are not fertilised, it is not universally so. The eggs of drone-cells, the male eggs, are sometimes capable of fertilisation, and they are not infrequently fertilised. This, however, does not influence their sex. From the zoological literature dealing with the bee, quite apart from apicultural writings not at the writer's disposal, there is abundant evidence of the truth of this. WEISMANN records (p. 495), that in 272 drone-eggs, sectioned and studied by himself and PETRUNKEWITSCH, there was one fertilised[1]). Regarding this instance he says: "with this egg the queen must have 'erred', and by mistake fertilised an egg, although it was laid in a drone-cell. Such an error has indeed long been recognised by apiarists as possible and as happening."

In the latter sentence he doubtless refers to the well-known fact, that sometimes a queen-bee may make no distinctions as to the cells in which she lays, whole combs of drone-cells may produce workers instead of drones.

As the experiments of BESSELS[2]) prove, the like results can under proper conditions be produced experimentally, that is to say, a queen-bee can be induced to lay drone-eggs, which invariably give rise to males, in worker- and even in queen-cells, or worker-eggs (female-eggs) in drone-cells without alteration of their prospective destinies. In BESSELS' experiments the queen-bee, even when provided with a spermatheca filled with sperms, never "erred" by neglecting to fertilise the eggs, which by the conditions of the experiments she was compelled

1) He also mentions (p. 494), that in the earlier observations of PAULCKE in 3 cases of freshly laid drone-eggs it could not be decided whether certain dark bodies should not be identified as sperm-nuclei.

2) BESSELS, EMIL, Die LANDOIS'sche Theorie widerlegt durch das Experiment, in: Z. wiss. Zool., V. 18, 1867, p. 124—141.

to lay in drone-cells, for in all the cases of this kind described the resulting young from the drone-cells were workers, not drones! The converse experiment with queens laying in worker-cells was, of course, carried out with females, which had been prevented from copulation, or which were known to contain no sperms.

These facts[1]), however, while significant for the question of the existence of two categories of eggs in the bee, have little bearings upon that recorded by WEISMANN. Because sometimes whole batches of eggs, which become females, are laid in drone-cells, the assumption that in the one instance observed by WEISMANN the egg would have given rise to a worker, instead of to a drone, had it developed further, is not warranted.

Additional and very strong evidence of the possibility of fertilisation of the eggs in drone-cells in the bee without influence upon their sex is afforded by the experiments of DZIERZON, PEREZ, MULOT, DICKEL and others in crossing German or French and Italian races.

These experiments of DZIERZON's will be found recorded in VON SIEBOLD's well-known work[2]). According to DICKEL[3]), they have since been repeated by himself, MULOT, and others named by him, in the converse order, but with the like result. It had recently become the practice to "bastardise" hives of bees by the introduction of Italian queens into colonies of German workers and drones, or of

1) BESSELS' experiment no. 5 (l. c. p. 131—132) places the still current belief, that the queen-bee can influence the sex of her eggs by fertilising them or not, in another light. The queen here in question had her spermatheca filled with sperms, as a later examination proved. She was compelled to lay her eggs in a comb of drone-cells. This she finally did unwillingly. BESSELS writes "the result of this experiment could naturally already a priori be foreseen", that is, under normal circumstances the eggs laid would have given rise to workers. This turned out to be the case, although they were all laid in drone-cells! If she possesses the power, why did she not omit to fertilise these eggs? The true reason is clear. In the first place, the sexes of the eggs in question had already been decided in the ovary, and in the second, as a result of this they were capable of fertilisation, and she could not prevent it.

2) VON SIEBOLD, C. T. E., Wahre Parthenogenesis bei Schmetterlingen und Bienen, Leipzig 1856, p. 96.

3) in: Anat. Anz., V. 19, p. 110—111.

German queens into Italians swarms[1]). The ultimate result of the procedure is the same, whether it relate to an Italian (Ligurian) or to a German queen; but, as the drone-eggs are not as a rule fertilised, in the first instance the drones are not of mixed blood, that is, they exhibit all the characters of the maternal line.

Dzierzon had such "bastard-hives". In one of two such, where the queen was a pure German bee, there suddenly appeared among the drones some few ("einige wenige") of a brilliant golden hue. These were, of course, "bastards" or crosses. This unexpected and surprising result naturally led Dzierzon to gravely doubt the truth of his own theory, while von Siebold, Baron von Berlepsch and others thought, that it could be explained away. They supposed, that the queen in question was not of pure German blood: a curious, even lame, apology considering the practical knowledge of bees possessed by Dzierzon, and not forgetting the fact, that the practice[2]) was only of recent origin[3]). The fertilised drone-egg examined by Weismann furnishes the complement to Dzierzon's results, which, as already mentioned, have been since obtained by Dickel, Mulot, and others, experimenting in the converse order. If drone-eggs be not as a rule fertilised in bees, because they be now generally incapable of fertilisation, Dzierzon's case is readily explicable. There is not the smallest chance of escaping from the conclusion, that these few eggs had been fertilised. In all probability they had undergone fertilisation, because smaller sperms had been able to enter them through the micropylar apparatus. The result, so far as concerned the sex, was not affected by this: the offspring were still drones, because the products of male-eggs; but they were "hybrids" or crosses, because the progeny of an Italian father and of a German mother.

Two interesting articles relating to the Dzierzon theory are: J. Perez, Memoire sur la ponte de l'Abeille reine et la theorie de Dzierzon, and A. Sanson, Note sur la parthenogenese chez les Abeilles, both in: Ann. Sc. nat., V. 7, Zool. 1878.

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1) This was done, apparently, because of the more brilliant golden colouring of the Italian or Ligurian bees.

2) Only several years later were Italian (Ligurian) bees first introduced into England.

3) As will presently appear, a similar far-fetched explanation is offered by von Siebold for like unwelcome and awkward results in the case of *Nematus ventricosus*.

Perez imported from Italy in the spring of 1877 a swarm of pure Ligurian bees, in order to "italianise" three swarms of the black French ones, "by substituting for their queens young females, daughters of the one fertilised in and imported from Italy".

The general results of the experiments were: 1) that all the progeny of the original Italian queen were pure Ligurian forms, and 2) that a considerable number of the males produced by its daughters, which had been fertilised by French drones, were of mixed blood. Of 300 of the latter half exhibited characters of the French drone to a greater or less degree!

Sanson endeavours to explain away these finds by supposing the result to be due to "atavism" or "reversion" (!), but he makes no attempt to show why this feature was entirely lacking in all the drones produced by the original Ligurian queen. The explanation is far-fetched and totally inadequate, besides transgressing the rule "entia non sunt multiplicanda", but it is, perhaps interesting as exemplifying the subterfuges, to which the human mind can resort, when placed in a dilemma by a novel fact, at variance with accepted but unproved dogmas. The human imagination is, indeed, boundless for the invention of non-existent things: the mind is strictly limited, when the simple hard facts of Nature are offered to it for its acceptance and comprehension.

As already insisted, it is inconceivable, that with but one form of functional male gamete the sex of the egg should ever be influenced by fertilisation. In the bee this influence is supposed to lead to the production of females. Apply the supposition to other cases, and curious results will be obtained. Thus, in *Aphis*, *Apus*, and *Artemia* the females produce at certain periods parthenogenetically young of both sexes, males and females. The eggs of these latter, when fertilised by the males, give rise to females, reproducing parthenogenetically. Hence here under the assumption the logical result would be, that, while fertilisation determined the production of female young, in its absence the young for many generations might be female, and finally — without the intervention of fertilisation — male and female. Which goes to show, that, as in *Daphnia* according to researches of Weismann, fertilisation has no influence at all upon the sex of the offspring. Pointing to the like result are the well-known facts as to the conditions in various species of gall-flies.

An interesting case is that of *Nematus ventricosus*, as described by von Siebold [1]), who from a long series of experiments thought he had proved, that as in the bee non-fertilisation of the eggs induced the development to the male sex. In four of his experiments with the sexes of the eggs produced by virgin-females he unexpectedly obtained a few females among the male young produced in each case. The results are as follows:

No. of experiment	male cocoons	female cocoons
13	265	2
14	493	2
15	374	8
16	168	1

That is to say, in not fewer than four out of nine experiments one or more females appeared among the parthenogenetic young. von Siebold accounts for this by supposing, that in these cases young laid in nature by fertilised females had by accident been introduced with the food into his cultivations.

The acceptation of this explanation would vitiate the experiments, for it would be clear, that proper precautions had not been taken. Quite apart from the unnecessary multiplication of causes — "entia non sunt multiplicanda praeter necessitatem" [2]) — the zoologist, who has read, and admired, von Siebold's record of patient observations, and who can realise the significance of the new facts and factors of the present writing, will probably attach far greater weight to the care and precautions of the observer, than to any such needless hypothesis.

The case of the bee is the only one, in which a supposed influence of fertilisation upon sex, has ever appeared at all plausible. In face of the actual facts concerning it, of the glaring contradictions afforded by the observations of Dzierzon, Perez, Mulot, Dickel, Bessels, and others, of the conditions in other insects such as the gall-flies, of von Siebold's observations upon *Nematus ventricosus*, of the known existence of sexually differentiated eggs in certain examples from the Rotifera, Insecta, and Vertebrata, of the existence, so far as we know and can judge, of but one form of functional gamete in the male, it is not logical or reasonable to generalize from it, that fertilisation influences the sex of the offspring, or that this holds even for the bee.

1) von Siebold, C. T. E., Beiträge zur Parthenogenesis der Arthropoden, 1871, p. 106—130.

1) William of Occam's Razor. Vide Karl Pearson's Grammar of Science, 2. edit., London 1900, p. 537.

Indeed, granting the right to explain away the awkward facts of certain of the above cases — and it would not be according to the methods of strict scientific investigation to do this — there exist far more instances, where it is clear as the light of day, that sex is not determined or influenced by fertilisation, where it is beyond any possible doubt an attribute, a function, of the egg itself.

Were it possible to furnish proof of a convincing character of the determination of sex in any particular direction, male or female, by the spermatozoon in any single instance, the facts concerning the twofold sexually differentiated gametes, the eggs, of certain females, and those relating to the two categories of spermatozoa [1]), would be meaningless and useless. But with these very facts all the phenomena of sexual reproduction in the Metazoa can be shown to be in harmony.

With the supposed occult influence of the spermatozoon mystery and metaphysics are introduced, where simplicity rules. By rejecting the existence of this power a road leading to error is for ever closed. And by accepting the plain and obvious facts concerning the fourfold gametes of the Metazoa a step is taken along the pathway of truth.

IV. The Manifestation of Sex.

There has been at least one attempt to base the "origin of binary sex" in the former existence of three sorts of gametes, termed by Hartog micro-, meso-, and mega-gametes (M. M. Hartog, Some problems of reproduction, in: Quart. J. microsc. Sc., 1891, p. 72). The identity in number is, however, the sole resemblance between Hartog's theory of the "origin of sex" and the account of the determination of sex, given here. Moreover, Hartog makes the meso-gametes disappear as useless [2]) organisms, and thus he reduces the actual number to two, which is not in accordance with the facts. Nor is any attempt made to show how the differentiation is carried on from generation to generation. It has been indicated here how the male-eggs as a rule only provide for a male embryo and its set of male gametes, the spermatozoa: whereas the female-eggs make provision

1) With very few exceptions, and of these *Ascaris megalocephala* and *Salamandra maculosa* may be named, there is hardly a dioecious animal yet investigated, of which it can with certainty be said, that a second form of sperm is completely absent. In the two apparent ones mentioned the very numerous degenerative spermatogonia and spermatocytes suffice to account for the missing second non-functional sperm.

2) He writes "less useful" (op. cit. p. 72).

not only for a female embryo and a set of female-eggs, but also for a new batch of male-eggs.

In this way the male-eggs, like the Metazoan individuals (WEISMANN), are on their way to a cul-de-sac, out of which part of their progeny, the spermatozoa, can only extricate themselves by union with another egg, male or female in destination. The real responsibility for the continuance of the species thus falls upon the females.

An attempt must be made to indicate where in the life-cycle the differentiation into male- and female-eggs must be conceived as taking place. As unlike gametes, which, however, are incapable of conjugation even with the corresponding unlike gametes of another female individual, their direct forerunners must be differentiated, as in certain Protozoa[1]) in the final, or in one of the last divisions of BOVERI's oogonia, i. e., just prior to the period, where these become oocytes in BOVERI's sense. To put the matter differently, BOVERI's oocytes must be differentiated into the forerunners of male-eggs and female-eggs, or into two sorts of oocytes, respectively destined to give rise to male and female organisms (Fig. A). It may be very

1) The fact is not forgotten, that in the Protozoa the divisions here referred to as preceding conjugation do not yield unlike gametes. To take a well-known instance, that of *Paramoecium* according to MAUPAS' diagram, in *P. caudatum* the micro-nucleus in each conjugating individual divides twice, forming four nuclei. Of these three are abortive, while one divides once more into the stationary and the wandering nucleus. This latter is not, I take it, to be looked upon as representing in any way a division into what might become unlike gametes in the sense of those of the Metazoa; sex not being really represented here. The exchange is one of equivalent gametes, comparable, if at all so, to what happens in the reciprocal hermaphroditism of the snail, or, better, to instances of parthenogenesis. If unlike gametes here arose — gametes of four kinds — their formation would date from the first two divisions of the micronucleus. The so-called "polar bodies" — the three abortive nuclei arising from these two divisions — are, strictly speaking, not comparable to the abortive cells of the like name in Metazoan oogenesis, but are similar to abortive forerunners of gametes, as an example, to abortive spermatocytes. A differentiation of unlike gametes, however, is witnessed in *Vorticella,* according to the researches of MAUPAS. His diagram, showing the formation of the forerunners of the micro-gametes, reveals an additional mitosis in the individual about to give rise to the forerunners of the latter, in fact, this individual divides into two, and each of these undergoes the before-mentioned changes in its micronucleus.

difficult, or even impossible, to demonstrate the truth of this, but the analogy of other cases points to its correctness.

It was the recognition of the probability of the incompleteness of BOVERI's diagram [1]) of oogenesis in this respect, which caused the delay in the publication of the diagram of the life-cycle of the skate. The writer is now convinced, though he cannot at present demonstrate it in the skate, that the actual differentiation of sex, its determination, goes back to the final divisions of the oogonia, in all probability to the very last one itself, before these become oocytes. This must be so, because the germ-cells are unicellular organisms, and because their life-cycles are those of such. For this reason the differentiation of direct forerunners of unlike gametes must happen as it would do in the Protozoa themselves.

As additional evidence in the like direction the well known eggs, of two kinds and sexually differentiated, of *Hydatina senta, Phylloxera,* and *Dinophilus apatris = gyrociliatus* may be cited (Figs. 6 and 7). The small eggs here are destined to produce males, the large ones females. As the size of the egg will naturally be attained during the oogenesis, it would seem to follow, that here the destination of the oogonium must be determined prior to the final phenomena of the reduction and of the ripening, for these latter would not appear to possess any influence on the size of the egg itself. In other words, the size of the egg and its prospective destination, male or female, must be "arranged" prior to the ripening and reduction, i. e., prior to the two final mitoses.

This epoch — the final division of the oogonia — is the latest one, at which the determination of sex can conceivably happen. It is in all probability the usual final point, but in the present state of our knowledge it would be unwise to exclude the possibility of an earlier occurrence in some cases. As instances such hermaphrodite animals as Tunicata and leeches may be cited. From JULIN's researches [2]) on the oogenesis and spermatogenesis of *Styelopsis grossularia* it would seem to follow, that the separation of two categories of germ-cells, the forerunners of female-oocytes and of spermatocytes, must be relegated to an earlier epoch than the final division prior to the

1) As MEVES' researches (to be subsequently referred to) prove, the like is also true of the diagram of spermatogenesis, at any rate in certain cases, such as *Paludina* and *Pygaera*.

2) in: Bull. sc. France, p. 25, 1893.

ripening. The topography of the reproductive organs in the common leech points in a like direction [1]).

Unless the procedure have been modified in some such way as just indicated in order to meet special needs, the final division, prior to ripening, has every appearance of being the turning-point of sex-differentiation. There is, so it would seem, a very close and significant connection between the determination of sex and the phenomena of the reduction. The one follows naturally upon the other. A germ-cell, which has had the stamp of sex placed upon it; that is to say, one whose destination has been decided in a certain direction, must of necessity go through the procedure of formation of gametes — entailing the phenomena of reduction — before it can fulfil its assigned office. Looked at in this light the following words of RÜCKERT acquire a special significance: "It (the reduction of chromosomes) is initiated before the ripening, perhaps already in the oö- and spermatogonia, by the omission of a transverse division of the chromatin-coil, as a result of which each two chromosomes remain linked together" (in: Ergebn. Anat. Entw., V. 3, 1894, p. 582).

The above had been written before MEVES' recent paper [2]) was

1) In connection with this question section VIII of the present writing should, however, be considered. In this portion of the text the writer has acted largely upon the assumption, that everything in the development must be effected by cell-division. Without question the separation of two categories of unlike gametes must originally have been carried out by mitosis. But after such separation there is nothing to prevent the postponement of the revelation to later periods by the acquirement of the faculty of going through further mitoses. As will appear anon, it is concluded, that the reduction is the outward manifestation of the determination of sex. Granted the possibility of the separation along two lines some time prior to the reduction and manifestation of sex, of such a kind, that all the cells produced along either line will have a certain fixed sexual destination, such as would appear to obtain in the leech and *Styelopsis*, the determination of sex would consist of, or be divisible into, two periods, one of separation by cell-division, the other of manifestation by the reduction. My final conclusion, therefore, is, that determination of sex by cell-mitosis is rather a theoretical postulate, than an actual fact, although originally it may have been so effected. Actually, it is brought about without mitosis, but it either succeeds a certain mitosis, or, what is the same thing, a certain number of such.

2) MEVES, F., Ueber den von v. LA VALETTE ST. GEORGE entdeckten Nebenkern (Mitochondrienkörper) der Samenzellen, in: Arch. mikrosk. Anat., V. 56, 1900, p. 553—606, 2 pl., l. c., p. 555.

consulted. Apart from minute details concerning certain aspects of spermatogenesis — which do not concern us here — it contains brief references to the history and morphology of the two sorts of spermatozoa in *Paludina*, and also in one of the Lepidoptera, *Pygaera bucephala*, in which MEVES has likewise found two kinds of spermatozoa to be present. His promised memoir on these forms will be looked forward to with much interest, especially after his statement, that, unlike previous observers, and in particular M. VON BRUNN, he finds a complete parallelism in the development of the two kinds.

According to this observer, the order of the generations of cells, which precede the spermatids, or forerunners of both kinds of spermatozoa, has hitherto been wrongly described. As in other animals, one can divide the development of the spermatozoa in *Paludina* into the three well-known periods. These are, one of increase in number, one of growth, and one of ripening. The first period, in which the spermatogonia or primitive sperm-cells increase by repeated divisions, is common to the ancestral cells of both sorts of spermatozoa in *Paludina*. The development diverges, when these divisions have ceased, on the entry into the period of growth. Here the one set of cells attains a less size than the other. In both sets of cells the usual two divisions take place in the ripening period, and of the spermatocytes, which enter this period, the smaller give rise to the hairlike or ordinary form of spermatozoa, the larger ones to the wormlike (Fig. B).

I have quoted his interesting account at length, because it appears to be clear from it, that the separation into direct forerunners of unlike gametes here takes place at the latest in the final division of the spermatogonia, where these pass into the growing period, and become spermatocytes. The result is not effected by the consideration, that the wormlike spermatozoa are non-functional. There are here two sorts of gametes — as in the female skate — and their direct forerunners would appear, from MEVES' observations, to be differentiated exactly at the point previously suggested for those of the two sorts of eggs [1]). From his brief reference to the two kinds of spermato-

1) It is commonly believed, that the number of divisions of the germ-cells to their "maturations" is always very many. A little consideration will show the absurdity of this assumption. In the development of a female-egg in *R. batis* the primitive germ-cell divides nine times, yielding 512 primary germ-cells. Seven further divisions of 512 cells would result in 65536. (In a child's ovary ALLEN THOM-

zoa of *Pygaera* if (p. 565—566) it may be gathered, that here it is the homologue of the wormlike form of *Paludina*, which is functional.

From MEVES' interesting finds I am inclined to mention one or two other ideas, which have cropped up in the course of the work. His find in *Pygaera*, if I have correctly understood him, seems to support the idea of the functional nature of the wormlike spermatozoon or its equivalent in many or all Crustacea, as well as in some other Arthropods. Even the so well known spermatozoa of *Ascaris* may be of this variety.

Probably the instances of two sorts of unlike gametes in male Metazoa are far more numerous than we have hitherto supposed [1]). Sometimes the one sort may be the functional one in the male, sometimes the other: but nothing goes to demonstrate two kinds of functional gametes in the male.

One may, indeed, safely go further and conclude, that in every spermatogenesis in dioecious forms a second form of spermatozoon — a dimorphism of spermatozoa — will, on investigation, be in evidence; or that some reason for its absence, such as degeneration of "giant-spermatogonia or spermatocytes", will present itself. As a matter of fact, twofold spermatozoa are, even on the results of researches not made in search of such, very common in various divisions of the animal kingdom. They are now known, and not in isolated

SON calculated that there were about 70000 ova.) Four additional mitoses of this number of germ-cells would give 1048576. With only twenty mitoses from the primitive germ-cell we reach this enormous number. Probably in the female skate there are from the period of the primary germ-cells to the ripening of the eggs nothing like ten additional mitoses.

From the above calculations it would follow, that in the human female embryo there must be seventeen divisions from the primitive germ-cell to the oocytes, yielding at the most somewhere about 131000 of the latter.

1) Apparently another case of this kind is to be met with in *Cicada*, as recorded in a memoir by E. V. WILCOX (in: Bull. Mus. comp. Zool., V. 27, No. 1, 1895, p. 7—8). Here giant spermatogonia and spermatozoa are described. From his account it seems to be clear, that these are mainly in a condition of degeneration, and that such an atrophy may happen at any stage in the development from the spermatogonium to the spermatozoon. It would appear doubtful, if any of the larger germ-cells really become perfect spermatozoa, and thus *Cicada* would represent a further step in the "degradation" of the wormlike spermatozoon of *Paludina*.

instances, in Mollusca, Rotifera, Insecta, Amphibia, Aves, and Mammalia, even in the human subject.

Regarding their forms and occurrence K. VON BARDELEBEN [1]) has furnished interesting data for monotremes, marsupials, the hedgehog, and man himself. Considerations of space preclude further citation from his memoir, but it may be noted, that the author fully recognises the dimorphism, that he compares the two forms of mammalian spermatozoa with those of *Paludina*, and that he describes the second form as often rudimentary and always non-functional. More recently a second form of spermatozoon of *Bombinator* has been fully described by IVAR BROMAN [2]).

The subjoined list of instances of dimorphic forms of spermatozoa, already recorded, with the names of the observers, who have noted or described them, may be of interest:

Mollusca: *Paludina vivipara* (Figs. 3 and 4, VON SIEBOLD, 1836, F. LEYDIG, L. AUERBACH, M. VON BRUNN, R. V. ERLANGER, F. MEVES), (Fig. 5, P. KÖHLER, 1892), *Murex brandaris* (L. SCHENK, M. VON BRUNN).

And the following all by M. VON BRUNN: *Ampullaria* (sp.?), *Murex trunculus, Cerithium vulgatum, Nassa mutabilis, Fusus syracusanus, Murex erinaceus, Columbella rustica, Marsenia* (sp.?), *Aporrhais pespelecani, Cassidaria echinophora, Dolium galea, Tritonium cutaceum, Tritonium parthenopeum, Vermetus gigas, Murex trunculus. Cypraea caput-serpentis* (Fig. 5 A), *Pteroceras lambis* (Fig. 5 D), *Strombus lentiginosus* (Fig. 5 E, J. BROCK, 1887), *Typhobias* (J. E. S. MOORE, 1898, in: Quart. J. microsc. Sc., V. 41, p. 192).

Rotifera: *Notommata sieboldi* (F. LEYDIG, 1854).

Arthropoda: *Asellus aquaticus* (W. ZENKER), *Oniscus murarius?* (F. LEYDIG), *Cicada tibicen* (E. V. WILCOX), *Pygaera bucephala* (F. MEVES), *Staphylinus* (NILS HOLMGREN), *Pyrrhocoris* (HENKING, 1891), *Anasa* (PAULMIER, 1899).

Nemertea: *Tetrastemma* (BOLLIES LEE, 1887).

Amphibia: Various species of *Bufo*, *Hyla* (Fig. 2), *Rana esculenta* (VON LA VALETTE ST. GEORGE), *Bombinator igneus* (IVAR BROMAN).

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1) VON BARDELEBEN, K., Dimorphismus der männlichen Geschlechtszellen bei Säugetieren, in: Anat. Anz., V. 17, 1897, p. 564—569, 6 figs.

2) BROMAN, IVAR, Ueber die Histogenese der Riesenspermien bei Bombinator, in: Verh. anat. Ges. 1900, (Pavia), p. 157—164, 19 figs.

Aves: *Larus*, *Tadòrna* (E. Ballowitz, 1890).

Mammalia: The following by K. von Bardeleben: *Ornithorhynchus*, *Echidna*, various Marsupials, and *Erinaceus europaeus*, and, lastly, *Homo sapiens* (Fig. 1, von la Valette St. George, K. von Bardeleben, von Widersperg).

Spermatogonia of large size are described by Montgomery (1900) in *Peripatus*.

In Anat. Hefte 1902, in dealing with the sperms of man and *S. maculosa*, Ivar Broman finds, that is describes, no giant forms, but apparently forerunners of such.

Other instances may occur hidden away in the literature of spermatogenesis. The majority of the above cases of twofold spermatozoa rest upon a very firm basis of evidence, and many of them have even been figured.

Dimorphic forms of eggs have as yet actually been described in only four divisions of the animal kingdom, viz., Rotifera, the aberrant genus *Dinophilus*, Insecta, and Pisces. The instances are: *Hydatina senta* and various other Rotifera, *Dinophilus gyrociliatus* (Fig. 6), *Phylloxera vastatrix* (Fig 7), and *coccinea*[1]), and *Raja batis*. To this tangible evidence in support of their existence may be added that afforded by like-twins for the Mammalia (B. S. Schultze), and the important facts yielded by those instances of the production parthenogenetically of young of both sexes.

With the above testimony, pointing to the existence of two categories of eggs, may be placed that recorded; but, while never yet refuted, universally ignored in the literature of embryology, by von Jhering (in: Biol. Ctrbl., V. 6, p. 532—539, 1886) concerning the invariably like sexes of all the embryos found in one chorion in *Praopus hybridus*, an Edentate.

Among the cases of twofold spermatozoa, already worked out and recorded, there are very few, in which degenerative changes do not

1) The discovery of the facts in *Phylloxera coccinea* (*quercus*) is due to Balbiani in 1873 (vide: Observations sur le Phylloxera du chêne, in: Ann. Sc. nat., V. 19, Art. no. 12, p. 8—9). This is confirmed by Victor Lemoine (in: Biol. Ctrbl., V. 4, 1884—85, p. 554). Both observers state, that the male-eggs are easily recognised by their lesser size and red-brown colouring, while the female ones are of greater dimensions and of a pale brown tint. The whole of the literature relating to *Phylloxera vastatrix* was not at the writer's disposal.

come into play at some period or other of the developmental history. This is quite what might have been expected, for, as already noted, no single case of two sorts of functional spermatozoa is known.

In *Paludina vivipara,* from the statements of M. v. BRUNN and MEVES, the wormlike spermatozoa appear to be as numerous as the ordinary form. Other instances only approach without reaching this full development; indeed, from the literature it would not be difficult to put together a list of cases, showing all sorts of retrogressive stages from those, like *Bombinator* (IVAR BROMAN) with fully formed "giant-spermatozoa" and spermatocytes in degeneration, to others with little or nothing else to represent the second form of spermatozoon except degenerate spermatogonia.

In some cases this faculty has been retained by the male. This retention of a second form of male gamete, interesting and significant though it be to the embryologist, to all appearance has no functional import. It serves, however to indicate, that the conditions were originally alike in the two sexes; and in this way its existence removes difficulties in the way of a true and proper appreciation of the facts in the female. The circumstance, that in some instances, at any rate, the male also possesses the faculty of differentiating two sorts of gametes, throws another light on the production of two kinds of eggs by the female. This loses much of its apparently teleological character.

But it is the egg, which invariably develops, and not the spermatozoon. In all animal development an egg is essential, though on occasion a spermatozoon may be dispensed with. The function of the latter is thus reduced to the bringing about of the effects due to amphimixis, and for this reason the development of a second kind of spermatozoon is unnecessary.

Since the above was written the two following papers have appeared: NILS HOLMGREN, Ueber den Bau der Hoden und die Spermatogenese von Staphylinus (a Coleopterous insect, allied to the dung-beetles), in: Anat. Anz., V. 19, No. 18, 27th June, 1901, p. 449—471, 5 figs. In this paper the author records for this insect two sorts of spermatogonia and two categories of spermatozoa. — F. MEVES, Ueber die sog. wurmförmigen Samenfäden von Paludina und über ihre Entwicklung (vorgetragen im physiologischen Verein in Kiel am 20. Mai 1901), Separat-Abdruck aus "Mittheil. f. d. Verein Schlesw.-Holst.

Aerzte", Jahrg. 10, No. 1, 1901, p. 1—11, also under the same title and with 8 figures in Verh. anat. Ges., 1901, Bonn, p. 23—36).

Holmgren's record of two kinds of spermatozoa in one of the Coleoptera suggests the inquiry whether the second form of sperm may not in fact sometimes or often be functional as well as the usual sperm. The order of Coleopterous insects is very large, embracing more than 70 000 species. The Insecta form an immense group, not fewer than 250 000 species being known. Probably in a thousand years the spermatogenesis of not a tithe of either the Insecta, or of its order of Coleoptera, will have been worked out! This reflection bears significantly upon the question of the relativity of human knowledge. Naturally, we cannot wait another thousand years before drawing our conclusions: we can only say, that in our experience a second form is never functional, and that, though it be very often differentiated, there is no great likelihood of its ever being found to be of functional value alongside the usual form of sperm, and in addition to this.

The exceedingly interesting and important recent work of Meves, referred to above, affords additional evidence of the probable truth of this. Meves has studied the spermatogenesis of *Paludina* in much greater detail than any preceding observer. He records, that in the spermatogenesis of *Paludina* there is no reduction of chromosomes in the spermatogonia of the worm-like sperm. A most curious irregular reduction takes place in the first of the two ensuing divisions, for details of which the reader may be referred to the original, and the final result is, that each spermatid, resulting from the second mitosis, contains but one chromosome. The normal number of chromosomes in *Paludina* is, according to Meves, 14, the reduced number 7. Here, therefore, instead of 7 chromosomes each spermatozoon contains but the equivalent of one. In *Pygaera*, according to the same observer, in the formation of the non-functional form of spermatozoon chromatic material, in other words chromosomes, find no place, "in *Pygaera* the second form of spermatozoon is completely destitute of nuclear portion, that is, it is headless" (Meves). Notwithstanding Meves' cautiously expressed opinions to the contrary, there would appear to be no escape from the conclusion, that, even the complete differentiation of the second form of sperm in *Paludina* and *Pygaera* being accompanied by phenomena, only diagnostic as degenerative, it can be of no functional import whatsoever.

When, therefore, I state, that evidences of degeneration are always to be found in the development of a second form of sperm, and that these and other evidences, i. e., actual experience, always and invariably point to its non-functional nature at the present time, this conclusion is in accordance with the strictest canons of scientific investigation. The opposite view would be not only contrary to all experience, but, apparently, incapable of proof. "But", it may be asked, "may not some one or other of the 250,000 species of insects possess two forms of functional spermatozoa?" This is exceedingly unlikely, and it would not fit in with the homogeneity of the reproductive processes of the Metazoa as they now exist. One might as well hope to meet with cases in which the polar bodies of oögenesis were normal functional gametes or eggs. Neither contingency is, of course, impossible, only highly improbable, for what has been in the past may, so far as our very limited knowledge and intelligence extend, happen again in the future. Neither occurrence would merge into the miraculous, as is the case with the supposed conversion of males into hermaphrodites. We may neither limit Nature's powers, nor seek to make her perform miracles.

V. The Basis of Sex.

Sex in its origin in the Metazoa may quite possibly have been bound up with the existence and constant differentiation of four sorts of functional unlike gametes. The evidences of the present existence of such in certain cases seem to point in some such direction as this. An origin of sex based on these facts would grant to the male the property of producing and differentiating two categories of functional gametes. This, however, would not be hermaphroditism. The second kind would not be of the nature and character of what we term an egg. These gametes may have been, and probably were, originally differentiated for union with a certain sort of egg; thus, if they were S and s, the former or B-sperm may have been destined for E, the latter or A-sperm for e, where E and e were the two sorts of gametes of the female.

Previous inquiries into the nature and determination of sex have of necessity proved abortive from failure to grasp the essence of the problem. Many of them have simply regarded it as one of the nature of maleness and femininity, or as one of the origin in past time of two kinds of individuals bearing these attributes. Others again have concerned themselves with the causes, which during development may be supposed to influence the destiny as to sex of the offspring, the embryo.

The idea, that in some mysterious way or other the sex of each individual embryo may be determined in a particular direction during the development, is doubtless responsible for failure to appreciate the actual facts.

The main source of error has, of course, lain in the futile search for a determination of sex during and in the course of the development of some particular individual, long after it had really been decided. As we have seen, sex is not determined during this, but it is already predestined for the next generation long before this actually begins to arise.

Were there some hidden process at work during the development — daemon in embryone — two sorts of gametes, spermatozoa and ova, might suffice. Their office would merely be that of giving origin to an embryo of no predestined sex, or, as some embryologists still believe, one whose nature was in the meantime hermaphrodite. A theory of sex of this kind would seem to postulate some hidden genius in the development. Moreover, it would not furnish any explanation of the facts.

If, on the other hand, sex be really predestined, the question raises itself "how does this come to pass, in what way is the sex of an embryo, ultimately destined to develop, decided before even the egg, set apart to furnish it, has yet reached maturity?"

Where the individuals are all alike, as in the majority of the Protozoa, either like gametes or two sorts of unlike ones are sufficient. If the gametes become unlike, this may ultimately lead to the production of unlike individuals. The case of *Vorticella* with two sorts of unlike gametes but with like individuals shows, that this does not of necessity follow from the former facts. If the individuals become unlike, i. e., of two kinds, this will entail other conditions upon the gametes, from whose union these arise. The conjugation of two sorts of gametes is not calculated to bring about the production of two kinds of individuals[1]). At least three categories of gametes are

1) Ordinary hermaphroditism illustrates the truth of this, for here with two sorts of gametes, (female-)eggs and spermatozoa, but one sort of individual, the hermaphrodite, arises. The commonly accepted view of the primary nature of hermaphroditism — what Fritz Müller ironically described as "die Ursprünglichkeit der Zwitterbildung" — is a complete travesty of the facts; so much so, that one may repeat Fritz Müller's

needed for this result. The simplest arrangement is, however, that under which each individual "produces" two kinds of gametes, and under which these taken together make up four distinct categories. As already pointed out, if these be of such a nature, that their pairings be restricted, discriminated, and selected, the bringing-forth of two kinds of individuals will be secured.

In these lines the individuals have been vaguely spoken of without attaching to them any particular attributes except unlikeness. The conditions are not altered, when they are described as Metazoan ones. The multicellular sterilised individuals, termed Metazoa, are simply dimorphic forms (or including hermaphrodites polymorphic ones), bearing germ-cells, that is unicellular organisms, of a certain category. The problem of sex, therefore, is not one referring directly to the former, for these naturally bear a certain impress, male, or female (or hermaphrodite), from the nature of the germ-cells harboured by them. So that the individuals referred to above may be taken to be the unicellular ones, the germ-cells.

The cycle of life of these is really a double one, for it includes four sorts of gametes, of which two are formed by one sort of germ-cell and two by the other. The determination of sex for the following generation must lie along the one line or the other; there would be no order, were this not fixed and constant. It has come to be along what we term the female line, for it is the egg, which develops, not the spermatozoon. For this reason, although but three sorts of gametes be needful for the continuation of two categories of individuals, two of these must be differentiated along the female line. The third kind remains to be formed along the other line, that of the male. As we have seen, in some instances there are two sorts of gametes also differentiated here, but of these only one is of functional value.

It is owing to this circumstance, that the female has retained the property of forming two sorts of functional gametes, and that

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still unanswered question, "wie konnte eine auf so schwachen Füssen stehende Lehre unter den Zoologen bis heute sich unerschüttert erhalten, ja fast als selbstverständlich hingenommen werden?" (in: Kosmos, V. 2, 1885, p. 333).

It may also be pointed out, that in hermaphroditism, where there obtains a third sort of gamete, the male egg, there arises from the fertilisation of this a second kind of individual, the complemental or dwarf male, e. g., *Sacculina*, *Myzostoma* (some species), certain Cirripedia and Nematoda, etc.

these are the ones, which really develop (for in some instances the one, or the other, or both, may do this without fertilisation), that the determination of sex for the next generation rests with the female Metazoan individual, or, rather, with the germ-cells, of which it is the bearer.

Summing up, the problems of sex thus become those relating to unicellular organisms, of which the individuals are of two kinds. For the continuance of these more than two categories of gametes are needful. Prior to conjugation each kind of individual originally "produced", that is to say, broke up into the forerunners of two categories of gametes, and these together made up four distinct kinds, so discriminated and selected, that their pairings gave rise to but two sorts of individuals (zygotes). Conceivably, the formation of the forerunners may originally have been a mere undoing of the previous union: now, whatever it may have been in its origin, it is something more than this (for a simple separation of the parts originally bound together would but result in two sorts of gametes), and in fact it is such, that four kinds arise instead of but two.

Owing to the constant differentiation either of two forms of gametes, or of the forerunners of such, by every Metazoan individual, and because two categories of gametes arise in every hermaphrodite individual, one may define a Metazoan as *an animal form, in which either two kinds of gametes, or the forerunners of such (males), are constantly differentiated.*

Nature would appear to work her reproductive mill in the Metazoa by means of three sorts of functional gametes: spermatozoa, male-eggs, and female-eggs. With one of these she can dispense in hermaphroditism: with two on occasion, and, perhaps, sometimes entirely, in parthenogenesis. A fourth sort, giving two kinds of functional elements in the male, only appears to obtain as an abortive, futile, or useless sport. Indeed, to all appearance it would be a needless luxury, leading to difficulty, and even to disaster; and the mill can go on, merrily grinding away [1]), without it.

VI. Strasburger's Researches on Dioecious Plants.

With the close of this portion of the present manuscript it has been possible to study Strasburger's recent record of detailed and

1) "The world rolls round for ever like a mill;
It grinds out death and life and good and ill."
James Thomson (B. V.), "The City of Dreadful Night."

prolonged researches, "Versuche mit diöcischen Pflanzen in Rücksicht auf Geschlechtsvertheilung". The part of his memoir concerning us here is that contained in: Biol. Ctrbl., V. 20, 15. Dec. 1900. It is a matter of impossibility to give in a short space anything like an adequate abstract of this elaborate memoir. It may be noted, that in some points the author has reached conclusions similar to some of mine. Thus (p. 766), it is recognised for plants, that during the development nothing can influence the sex, and that this must be already predetermined in the germ. On the other hand, STRASBURGER (p. 771) accepts DZIERZON's theory of the determination of sex in the bee by the omission or the occurrence of fertilisation. Indeed, he goes further, and ascribes to all the eggs of the bee a strong male tendency, which is supposed to be counteracted on fertilisation by a still stronger female tendency on the part of the spermatozoon. On p. 770, however, we read "when I relegate the separation of the characters to within the pollen- and embryo-sac-mother-cells, it is quite clear to me, that this conclusion means as much for every prothallus of the Pteridophyta as the bringing-forth (Erzeugung) of sexual products of the like sexual tendency" [1]). The characters here mentioned are, however, not male and female ones. What they are is plain enough from the context, and to this the reader may be referred. The argument is based upon an analogy; but, on p. 774 the author writes: "the circumstance, that dioecious plants reproduce from the embryonic substance of their growing-points [Vegetationspunkte] quite generally only the one sex, is decisive for the view, that sex is already determined in the embryonic substance." This idea, consequently followed out, might possibly have led the learned author to conclusions for plants, similar to those here adopted for animals. There may well be differences in detail.

Just as there has long been an indication of the zoological way in the two sorts of eggs — of different sizes and destinies — of *Hydatina senta*, *Phylloxera*, and *Dinophilus apatris* (= *gyrociliatus*), so unto the botanists there may have been given a sign in the microspores and macrospores of Marsilia, Salvinia, and the majority of the Lycopodinae.

1) In the original written "Tendenz", not "Bestimmung". From his explanation of the supposed course of events in the bee it is clear, that STRASBURGER believes in the possibility of the alteration of the "tendency" and the final determination of sex — its „Bestimmung" — at fertilisation.

Were there not in plants — as in animals — dioecious, as well as hermaphrodite, species, the differentiation of two sorts of gametes might suffice. The equivalents of male- and female-eggs[1]) would, however, appear to be here also necessary. If my reading of the signs be not erroneous, they go to show the differentiation and determination of sex in plants as happeniug at the formation of spore-mother-cells. As elsewhere shown, the primary germ-cells of animals represent the spore-mother-cells of the Metaphyta. Possibly the determination of sex originally took place at the first division of the former, that is to say, before the Metazoan sexual generation attained any high degree of organisation. Nay, possibly there may still be lowly animals, in which the division of the primary germ-cells into secondary ones at the same time furnishes the determination of sex.

Should it turn out to be the case, that the spore-mother-cells were concerned in the determination of sex, this latter would be in connection with the reduction of chromosomes in both animals and plants.

VII. Significance of the Reduction of Chromosomes.

Finally, the question as to what relation may subsist between the reduction of chromosomes and the determination of sex suggests itself for consideration. Like Strasburger (l. c. p. 769), but independently of him and from different facts and considerations, I arrive at the conclusion of a close connection between the two.

1) From the exceedingly interesting experiments of Gregor Mendel on hybridism between green and yellow peas, cited by Strasburger on p. 767, the existence of four sorts of gametes appears to be here indicated. Indeed, Mendel drew this conclusion, for in his opinion his results pointed to the occurrence of two sorts of male and of female "Befruchtungszellen". In this inter-crossing the yellow is dominant or pre-potent. This manifests itself after the first generation. If such hybrids be fertilised with their own pollen, in the seed-pods there are on the average 3 yellow peas for each one of a green colour. How this serves to indicate the existence of four kinds of gametes may be gathered from Strasburger's memoir. The case, however, is one of hybridism with self-fertilising hermaphroditism, and the four sorts of gametes, if present, would not be sexually differentiated like those of animals. Vide: Gregor Mendel, Versuche über Pflanzenhybriden, in: Ostwald's Klassiker d. exacten Wissenschaften, No. 121. See also Bateson's translation in: J. Roy. hortic. Soc., V. 26, 1901.

With the exception of their products no other cells in the Metazoan developmental cycle have received so much attention as the oöcytes and spermatocytes. From the published researches on oogenesis and spermatogenesis it must be apparent, that there exists a much closer association between the determination of sex and the reduction of chromosomes than has been suggested in the preceding pages. In these, following RÜCKERT, HÄCKER, WEISMANN, VOM RATH, and others, the latter phenomenon has been assumed to happen in the two final divisions of the oocytes and spermatocytes. It now remains to be seen what light the standpoint of BOVERI, O. HERTWIG, GUIGNARD, STRASBURGER, BRAUER, MOORE, MEVES, FARMER and others throws upon the question.

As unlimited space is not at the writer's disposition, one of these observers may be allowed to speak for the rest. The one chosen may be BOVERI, his words certain passages from the article "Befruchtung" (in: Ergebn. Anat. Entw., V. 1, 1891, p. 453—467):

"In oogenesis and spermatogenesis in some cell-generation or other there takes place a reduction of the number of chromosomes to the half."

"A priori there are very different modes conceivable, in which the reduction in number of the chromosomes may be effected, and it appears to be open to question, whether it may be carried out by an act of division."

The author goes on to show, that in general the numerical reduction in chromosomes comes about before the formation of the polar bodies, and at the latest in the oocytes of the first generation. In *Ascaris* it does not happen earlier than this period. "The reduction in the number of chromosomes takes place [erfolgt] in the oo- and spermatocytes of the first generation."

How in these the reduced number is brought to pass neither BOVERI nor any succeeding worker has as yet been able to make out. He remarks, that the critical point, at which observation is brought to a standstill, is the nucleus of the oocyte or spermatocyte of the first generation. According to him, it is not the reduced number in the actual egg, or polar bodies, or spermatozoa, which requires explanation, but this diminished number in the oocytes and spermatocytes of the first generation.

At this juncture the writer would mention, that in the same year (1891) in his already cited memoir (p. 54—58) HARTOG urged several objections to the necessary correctness and validity of current views of the reduction. And in a valuable memoir [1]) J. E. S. MOORE has arrived at BOVERI's conclusion from researches on spermatogenesis. Other zoological researches, such as those of BRAUER and MEVES, and the whole of botanical opinion are in accord with this attitude. For the particular epoch in question MOORE has proposed the convenient term "synapsis".

On the last page of his memoir MOORE writes: "Whatever the synapsis may eventually turn out to be, it is evidently a cellular metamorphosis of a profoundly fundamental character, which would appear to have been acquired before the animal and vegetable ancestry went apart, and to have existed ever since."

We have seen that, according to the testimony of BOVERI, BRAUER, MOORE, GUIGNARD, STRASBURGER, FARMER, and others not named, in the period of the history of the oocytes of the first generation, termed by MOORE the "synapsis", in some at present unknown way the reduction of chromosomes is brought to pass. This, though hardly contradicted by RÜCKERT, HAECKER, WEISMANN and others, it is attempted to explain away. To these authors the process here described is not a real, is not *the* real reduction, and this they relegate to one or other of the later divisions of the oo- or spermatogenesis. The weight of actual fact, both zoological and botanical, seems to be against the correctness of their views. There appears to be no doubt, that in many of the best investigated cases, both in plants and animals, the actual reduction is effected prior to the two divisions forming gametes or spores.

I have deliberately refrained from altering the earlier pages of the present writing, in order to permit the reader to see how the conclusion is finally arrived at, that the oocytes of the first generation are concerned in the determination of sex. The last step may now be taken.

In the oocytes of the first generation, and (probably) in plants in the spore-mother-cells, two important phenomena come to pass. These are the numerical reduction of chromosomes and the determination of sex. Not merely an association, but a close and intimate connection must subsist between the two [2]).

1) MOORE, J. E. S., On the structural changes in the reproductive cells during the spermatogenesis of Elasmobranchs, in: Quart. J. microsc. Sc., V. 38, p. 275—313, 4 pl.

2) Since the discovery of the reduction, there has been an ever greater tendency — at any rate among zoologists — to connect it

In plants, so far as is known, the reduction of chromosomes precedes the origin of the sexual generation; in animals it succeeds the coming-into-being of the latter. In the one case the reduction happens prior to the formation of spores, in the other of gametes. On the view (apparently held by STRASBURGER) of the occurrence of sex-determination at the formation of spore-mother-cells the immediate sequel is the production of a sexual organism, the gametophyte, with its germ-cell or cells. In animals the next consequence of the differentiation of sex in the oocytes and spermatocytes is the formation of gametes. Moreover, it is only after the union of such gametes, after the development of an asexual generation, or phorozoon, or larva, and after the formation of a new set of germ-cells, that from one of these latter a sexual organism arises.

Not only, therefore, is the period of the reduction of chromosomes associated with that of the determination of sex, but the said reduction is immediately in both animals and plants followed by direct manifestations of sex, in the one instance the gametes, in the other the gametophyte. The two are thus linked together. Where the one is found at a certain point in the life-cycle, there also is the other to be encountered. This point is apparently not the same in animals and in plants [1]).

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directly with the formation of gametes; nay, even to regard the bringing into existence of these as the real reason of the occurrence of a reduction. Nor was it at all obvious, that it might be associated with anything else. HARTOG has urged, that the numerical reduction of chromosomes is certainly not universal in the formation of gametes (op. cit. p. 54). If the explanation of the meaning of the reduction current among zoologists be the correct and only one, why should an apparent diminution in the number so long precede the actual accomplishment of the process? Why should two nuclear divisions be necessary to effect it?

Since the reduction precedes the two final mitoses, resulting in the production of gametes, the appearance of the latter is the consequence, not the cause, of the reduction.

1) The difference, however, is more apparent than real. In both cases the beginning is made between the asexual generation, the sporophyte and phorozoon respectively, and what follows this. To put the matter in another way, the commencement immediately succeeds the asexual generation. In plants the reduction and determination are at once brought to a finish, prior to any new appearance: in animals the production of a gametozoon intervenes between the commencement of the determination and reduction, and the finish.

The only logical conclusion would appear to be, that the reduction is the outward manifestation of the determination of sex. Whether the latter be brought to pass by the reduction of chromosomes, or, as is quite possible, the reduction in the number be a consequence of the determination of sex; which of these be the cause and which the effect is a question for research. The determination of sex is certainly the more important item, and possibly for this reason the reduction[1]) may be looked upon as following naturally in its train.

But, perhaps, the two are inseparable and indivisible; and, therefore, it may be futile to inquire into the exact nature of their mutual relationships.

VIII. The Determination of Sex.

The tracing-backwards of the sex in the embryo to earlier and earlier periods has long occupied the attention of the writer. Two years ago or thereabouts it had been followed (for the embryo — not for the next generation) with greater or less certainty to the primitive germ-cell, from which the primary germ-cells take their origin. But the fact, that in the development of *Raja batis* there must be two sorts of eggs, producing 512 and 256 primary germ-cells respectively, was the starting point for the present inquiry. The like fact weighed heavily in the scale in leading to the conclusion, that the oogonia, or their last division, or the oocytes, were concerned in the determination of sex for the following generation.

The obvious interpretation of the two numbers was, that the smaller related to eggs, which at some earlier period had undergone an additional mitosis. For the lack of one division in the early development, where the products in the two cases were of the like sizes, naturally meant, that it must have been carried out at some previous epoch. It was irrational to suppose, that the missing division had taken place among the early cleavage ones prior to the formation of the primitive germ-cell. Therefore, it must have happened in the oogonia. This is as much as saying, that before the final division, giving rise to the female oocytes, there was an additional one in the forerunners of the male oocytes, as diagrammatically depicted in

1) To prevent misconception it may be noted, that, notwithstanding anything to the contrary in the earlier pages of this writing, the author does not for a moment maintain the view, that the reduction of chromosomes is ever effected by cell-division.

Fig. A. As we have seen, theoretically the determination of sex must in all probability originally be referred to the division of the primary germ-cells into secondary ones. In previous pages this was regarded as the original point. This idea may be reverted to, for the facts

Fig. A.

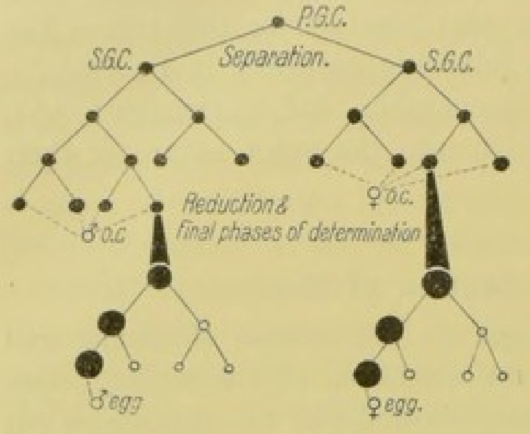

Fig. B.

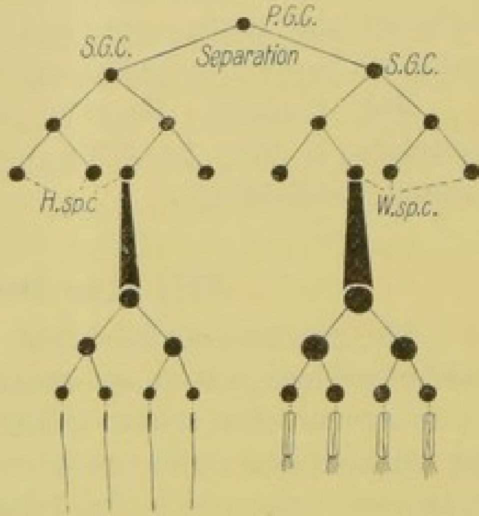

Fig. C.

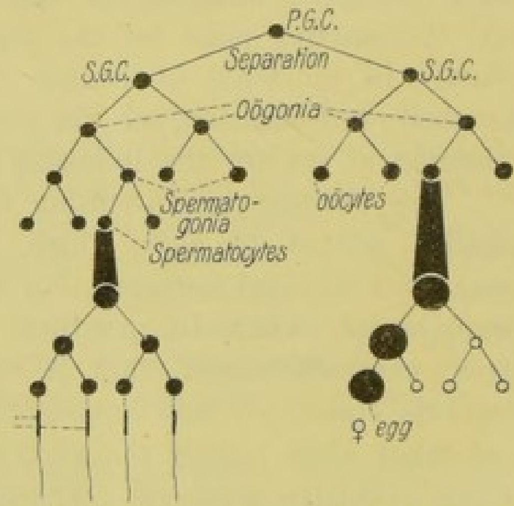

Fig. A. Oogenesis and determination of sex in *Raja batis*, the smooth skate. At *P. G. C* is shown a primary germ-cell, which by division gives rise to two secondary germ-cells, *S. G. C.* By the word "*Separation*" it is indicated, that at this point is the parting of the ways, which will culminate along the one line in female oocytes, along the other in male ones. The oocytes are labelled *O. C.* From the separation to the final phases of the determination and reduction there are shown n mitoses along the female line, n+1 along the male line. The remainder of the figure depicts the "ripenings" of a male- and of a female-egg.

Fig. B. For comparison with the preceding figure there is here shown the probable course of spermatogenesis and determination in *Paludina*, *Pygaera* etc. The lettering as before, except *H. Sp. C* and *W. Sp. C*, which denote the oöcytes destined to form hairlike and wormlike spermatozoa respectively. From the line of the reduction to the formation of the two kinds of spermatozoa after F. Meves.

Fig. C. Course of oogenesis and spermatogenesis in pure hermaphroditism. For comparison with Figs. A and B.

seen in some hermaphrodite animals, such as the leech and Ascidian, require elucidation.

It has been pointed out, that the final division of the oogonia into oocytes is the latest period, at which the determination of sex can happen. I now fall back upon the division of the primary germ-cells into secondary ones as the point, at which the first step in the determination is taken. The initiation of the process here would account for the inability of the latter to undergo independent development. Moreover, it would explain how in the leech in certain gonads only (female-)eggs were produced, in others the equivalents of male-eggs, spermatocytes etc.[1]). Many other developmental facts are cleared up by this reference, such as long successions of eggs of a certain category in some instances of parthenogenesis.

In the diagrammatic Fig. A an attempt has been made to depict what must be the actual course of events in the oogenesis of *Raja batis*.

To Fig. A another of the spermatogenesis of cases with two forms of spermatozoa has been added in Fig. B. This will complete the comparison, but a lengthy description of the figure will hardly be called for. As oogenesis and spermatogenesis must and do follow along parallel lines, the details of Fig. B closely resemble those of the preceding figure of oogenesis.

Lastly, the course of events in hemaphroditism is depicted in Fig. C.

It will, I trust, be clear to the reader how in oogenesis, as depicted in Fig. A, along with the degeneration and suppression of germ-cells anywhere along either of the lines leading to male and female oöcytes, Nature is able to regulate the sexes in any way she finds fit. The two means adapted to carry out this end are variation in the number "2^n" and degeneration of germ-cells. In the skate the number "2^n" is apparently greater by a unit along the line of the male oöcytes than along that of the female ones. In cases of parthenogenesis with complete suppression of the males "2^n" need be only 1 along the male line, and even this may as a rule be suppressed.

It must be pointed out, that the like is true of spermatogenesis. Although in some or many instances, as apparently would be the

1) The instance of the leech or earthworm would seem to suggest the probable production of only one sort of egg in any particular gonad of a segmented animal, such as a dioecious worm. In all probability the existing arrangement of the gonads of the common leech, for example, has been inherited from some dioecious ancestor, in which the representatives of the present ovaries gave rise to female-eggs, the equivalents of the existing testes being the seat of "origin" of male-eggs. It may be concluded, that in any segmental arrangement of ovaries in dioecious Metazoa certain of these give rise to male-eggs, others to female eggs only.

case in the skate, two sorts of spermatozoa may not be fully differentiated, the procedure of forming these must of necessity invariably be initiated. But, as in some instances of oogenesis, such as in parthenogenesis with complete suppression of the males, it may be arrested at any point after the separation [1]). This will simply mean, that the cell or cells, set apart to form a second kind of male gamete or spermatozoon, will undergo degeneration at some point or other prior to the differentiation of gametes. This may happen somewhat after the manner already described for *Cicada* (WILCOX), or the degeneration may take place at still earlier periods.

It is concluded, that the beginning of the determination of sex takes place in the division of the primary germ-cells into secondary ones by a separation along two lines, as shown in the diagrammatic figure (Fig. A), and that the final step in the procedure, the actual determination of sex, is taken by the reduction of the chromosomes in the oocytes and spermatocytes.

The peculiar phenomena, associated with the reduction, with the first appearance of the diminished number of chromosomes, become intelligible from the above. Although, according to the testimony of BOVERI and others, the reduction be not brought to pass by a mitotic division, it happens in the closing phases of one such, where the oogonia, for example, become oocytes [2]). In its origin the determination of sex must have been brought about in the course of some cell-division; but now, owing to the intercalation of new mitoses, it follows a certain one, and in such a way as to appear to be a part of it [3]).

1) This explains how the occasional neglect to suppress one or more of the forerunners of male-eggs in certain cases of parthenogenesis with the usual absence of male individuals but with their rare and sporadic appearance, as described by WEISMANN and others, may lead to the production of a male-egg, capable of development.

2) From this it would seem to follow, that the four spermatozoa are alike in all their fundamental characters (in their hereditary features), and the same must be true of the actual egg and its three polar bodies. That is to say, the four gametes, derived from any particular oöcyte or spermatocyte, must be identical.

3) WOLTERECK has already interpreted the synapsis as a suppressed mitosis. He writes: "Gleichwohl scheint auch die Synapsis des Cyprisovars mit einer Theilung in Verbindung zu stehen, welche jedoch nicht mehr, wie dies HÄCKER für *Canthocamptus* annimmt, zur Durchführung kommt, sondern vielleicht eine u n t e r d r ü c k t e m i t o t i s c h e T h e i l u n g d a r s t e l l t." (Zur Bildung und Entwicklung des Ostracodeneies, in: Z. wiss. Zool., V. 64, p. 596—623, 2 plates, l. c. p. 615.)

Though the determination of sex be not effected directly by cell-division, there must be in every developmental history some point at which the separation of the forerunners of unlike gametes is carried out by mitosis. The phenomena of the reduction only complete the process initiated at this.

In the formation or differentiation of two sorts of gametes from the starting point of a primary germ-cell, it may be insisted, it is probably mainly a matter of the number of mitoses from the start to the finish. To put the point differently, apart from its different destination, but probably in some way correlated with this, in the divisions leading to the formation of a male-egg (Fig. A), as already pointed out, there is obviously one more mitotic division than along the line resulting in a female-egg. This in the skate is evidenced by the lesser number of primary germ-cells, formed by a male-egg, than by a female one. But, as the primary germ-cells are of the like sizes in the two cases, as obviously from this fact and from the number 512 of primary germ-cells formed by the female-egg the missing division has been made good in the cleavage of the latter, and the like starting point has been reached in both cases in the primary germ-cells.

In addition, were an oocyte, destined to give rise to a male-egg, to be turned aside from the final phenomena of the ripening, and were it instead thereof to undergo some additional mitoses, perhaps only two, the result would be the production of spermatozoa, instead of a male-egg.

Similarly, it is probable, that in spermatogenesis with two categories of spermatozoa, as in *Paludina*, the number of mitoses in some way causally related to the kind of sperm resulting, is a different one along the line of the hairlike sperm (probably a greater one), than along that of the wormlike one. And these two numbers again may or may not be identical with those in the parts of the cycle leading to the differentiation of two sorts of eggs.

Summing up, the differentiation of a particular form of gamete is, apart from other possible factors, probably a function of the number of mitotic divisions from the starting point of the primary germ-cells to the ending at the oocytes and spermatocytes respectively.

IX. Concerning the Origin of Sex in the Metazoa.

The sexual reproduction of the Metazoa is doubtless a modification of an original isogamy. It would appear to be derivable from such through a former heterogamy of twofold gametes, micro- and macro-gametes. Perhaps it would be futile to speculate whether the actual heterogamy of fourfold gametes arose prior to the formation of sterilised Metazoan persons. On this question the writer expresses no opinion. One obvious result of the production of such an individual, as an incident of the developmental cycle, has been to reduce, or to keep short, the period of gametic union.

As already elsewhere recorded, in the skate the union of the nuclei of the two gametes is what may be termed a loose one; for, exactly as already described by RÜCKERT and HÄCKER in other and similar cases, paternal and maternal parts of the nuclei remain distinct. This is so for that portion of the cycle of the germ-cells, which culminates in the formation of what have been termed the primary germ-cells. That is to say, the duplication and loose union are retained, until the germ-cells of like generation and equivalence with that which formed the embryo, after the long resting phase — during which the embryo is in course of evolution — divide and form secondary germ-cells; or, in other words, until the initiation of the ensuing sex-determination.

We may, perhaps, look upon the reduction and sex-determination as originally merely an undoing of the previous union, such that, if the gametes were E and e, and S and s [1]), where the two unions resulted in ES and es as the zygotes, then these latter at the ensuing reduction may have separated into E and S, e and s, but while E and s retained their nature as female-egg and hair-like spermatozoon respectively, S became the male-egg and e the second form of spermatozoon. Putting the matter in tabular form, and assuming the continuity of the conjugating nuclei from generation to generation, we obtain the following result:

	Female-egg		Male-egg	First sperm		Second sperm
First generation	E		e	S		s
Zygotes		ES			es	
Second generation	E		S	e		s
Zygotes		Ee			Ss	
Third generation	E		e	S		s

1) E and e being the two forms of eggs, S and s the spermatozoa, respectively arranged and destined to unite with them.

That is to say, in the "grand-child-generation" we revert to the original condition. Whatever basis of truth there be in this conclusion, it is none the less of interest, because from other considerations there are reasons for attaching more importance to the grandparental conditions than to the parental ones.

For the present the above may be regarded as a "Selbstgespräch", and it is merely intended to show how out of simple beginnings present conditions could conceivably have been derived. But, in fact, matters are complicated by two things, 1) the non-functional nature of the second form of spermatozoon, in most cases at any rate, and 2) the fact, that reduction is now usually not merely an undoing of the previous union, for female traits may be handed over to the male-egg etc.

The mingling of characters, due to the union of egg and sperm, termed by Weismann "amphimixis", is to all appearance only complete for the embryo, which thereafter arises, and, strictly speaking, not for the primary germ-cells of the latter. As already recorded, these retain their nuclear duplication, as long as they are primary germ-cells: they could make the mingling more complete, were they to develop like the embryo. But with their division into secondary germ-cells they initiate the reduction. Were this a halving in Galton's sense, then all the consequences drawn by him might follow. But it is doubtful, whether the matter be carried out with such mathematical precision. In any case, after the reduction is complete, we are not dealing with half cells, for in the forerunners of both male and female gametes there are usually two further divisions to form the latter. As these two mitoses cannot possibly be qualitative — in the absence of a "reducing division" — the egg and its three polar bodies must be identical gametes in all essential characters (except size), and the like must be true of the four spermatozoa formed from one spermatocyte. The amount of variation, of variety, assumed by Weismann to obtain in the gametes, and to be induced by a reducing division, is far greater than is required by the facts. Indeed, it may be doubted, whether under it „offspring" could bear any great resemblance to their immediate "ancestors" [1]).

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1) Owing to the facts, concerning the course of the development from one generation to the next, and to the mode of formation or evolution of the embryo from one primary germ-cell, as revealed by the writer's researches, the words "offspring" and "ancestor" possess no

Since the foregoing was written, Häcker[1]) has published further studies upon the persistence (the autonomy, as he now would term it) of the paternal and maternal nuclear elements. Without citing his paper at length it may be noted, that he speaks of a possible competition between the two halves and its importance for the question of the nature of the prepotency of one parent. In embryological lectures I have also pointed this out, and in conversation with other zoologists. It had not been my intention to enter further into the question at this juncture. What was written in the earlier part of this section appeared to suffice for the present, and, perhaps, until more light had been found. But science moves apace, and Häcker's paper dispels further reserve.

The conceptions of the writer concerning this matter are briefly as follows.

1) The union of the nuclei of the two gametes in fertilisation is the joining of two individualities. In Häcker's terminology their autonomy is retained along the germinal track and in the primary germ-cells.

2) In the primary germ-cell, which unfolds itself as an embryo, this autonomy is shattered, with the resulting conquest of the stronger, i. e. more potent portion. As each nucleus is made up of a series of characters or qualities, this conquest may be on the part of all those of one nuclear half, or of the greater or less number than half of those of either portion. That is to say, the offspring will reflect the sum-total of the half of all the characters of the two lines, paternal and maternal, represented each by a unit in each of the gametes. For clearness, if all the paternal characters contained in the sperm, and, therefore, in the spermatic half of each nucleus along the germinal track, be represented by a red pack of cards, and if all the maternal characters of the egg be symbolised by a blue pack of cards, the offspring will be made up of characters, which taken together make up a complete pack of cards, (not two such), red or blue in any proportion. The duplication in the nuclear elements is equivalent to a doubling of all the characters handed down along the two lines of the egg and sperm. The "embryo" can only contain half of these characters.

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morphological meaning, or that commonly attached to them in daily life becomes nonsensical, when applied to the facts of development.

1) Häcker, V., Ueber die Autonomie der väterlichen und mütterlichen Kernsubstanz vom Ei bis zu den Fortpflanzungszellen, in: Anat. Anz., V. 20, 1902, p. 440—452, 11 figs.

3) As already indicated, the reduction of chromosomes must originally have been merely an undoing of the previous conjugation, such that each separated half became a gamete, or gave rise to such. This separation originally took place at the division of the primary germ-cells into secondary ones. By the intercalation of new mitoses it has been delayed until the end of this intercalated series, until the division of the oogonia into oocytes, for example. The result of this has been, that the reduction is no longer effected by cell-division, but it appears in the closing phases of such a division.

4) The one half of each nucleus, which originally like the rest itself gave rise to gametes, owing to the separation (Figs. A and B), due to the intercalation of new mitoses, is thus lost in and during the reduction. The separation along two lines to form gametes having taken place once at an earlier point, it cannot be repeated.

In considering the reduction and allied questions, such as those of heredity, two factors must not be overlooked. These are, that, on the one hand, the line of "ancestry" ends in the "embryo", which forms a termination of two series of "ancestors", paternal and maternal, and that, on the other, it is continued onwards for the germ-cells *without passing at all through the embryo*. This has hitherto, owing to the nature of prevailing conceptions of the relationships of embryo and germ-cells, been ignored.

Moreover, a reduction in the number of chromosomes is the ultimate consequence of the previous conjugation of two cells. This might seem to be too obvious for mention. But, if it be put in another way, the need of insisting upon it may be clear. As reduction of chromosomes now precedes conjugation of gametes, it has been assumed, that this was always so, even at the start of the process. Strasburger rightly pointed out some years ago, that the reduction might be regarded as a reversion to what obtained before conjugation was initiated, and, perhaps, this expresses what has been urged above.

The result of this is, that in conjugation we can never have a union of half entities or individualities, for the process must originally have started with whole ones, and with such it must be continued.

This conclusion throws some new light upon Galton's law of ancestral inheritance.

X. The Regulation of Sex in Nature.

Properly speaking, the problem of the mode or modes, by which Nature may be supposed to regulate the proportions of the wo sexets is beyond the scope of the present enquiry. With the facts recorded here it will in future be impossible to regard the regulation of sex as, strictly considered, an embryological problem. But it may be permissible to draw attention to one or two of its aspects, and to state some conclusions concerning these.

From the preceding pages it would, of course, follow, that the males of a species have as little influence on the regulation of sex for the following generation as upon its determination. In other words, the regulation of sex, like its determination, must lie with the females. The proportion of the two sexes, produced by any given female, and in most instances the order of development of male and female embryos do not belong to the question, for these two factors may be determined by the "ancestry" of the particular individual, and Nature, as TENNYSON wrote, is "careless of the single life". The individuals of a species stand in a relation to her, similar to that occupied by the common soldiers of a great army to the Field Marshal Commanding, the difference being, that in Nature's vastly greater army of the race the individual is far more insignificant than in the human machine.

What Nature concerns herself with is the regulation of the proportion of the sexes for the race as a whole: any and, indeed, every individual case may be an exception. Eliminating the males, and disregarding the cases of individual females, from known facts and factors and from the recognition, that sex and what appertains to it are decided very early in the life of one generation for the next following one, certain conclusions may be reached.

It must first of all be noted, that the normal proportion of the sexes varies enormously in different species. Thus, in some *Daphnidae*, according to the researches of WEISMANN, already cited, male individuals either never occur at all, or only at long intervals. This is an extreme case, but it will serve to demonstrate, that the proportion of the sexes is largely due to hereditary influences. This becomes certain from WEISMANN's discovery, that in some instances they appear only in fixed predetermined generations.

Apart from such exceptional examples it would appear to be a general rule in very many portions of the animal kingdom — and among plants — that the first offspring should be either predominantly male, or even entirely so. Additional evidence in the like direction is furnished by hermaphroditism. Zoologists have long recognised in very many cases of this, that the first "sexual cells" to ripen are the spermatozoa. Such forms are spoken of as "protandrous hermaphrodites". As we have seen in earlier pages, the spermatozoa of hermaphrodites are products of transformed male-eggs. So that here again we are confronted with an earlier ripening of male-eggs or their products, and this is the same thing as the production of males before the ripening of female-eggs.

The ripening of more male- than female-eggs in the first instance is of very great importance, for owing to the rôle played by the female in sexual reproduction it converts the regulation of the sexes into a self-adjusting one[1]). This was also Hensen's and Düsing's[2]) conclusion, though the latter failed to recognise the very simple way, in which it could be, and probably is, brought to pass. He believed, that, for example, where the males predominate, there is a greater probability of an early fertilisation of the eggs, and that, therefore, there would result a much greater number of female young. The consequence would, however, probably be the opposite, a still greater preponderance of the males.

In dealing with this problem attention may not be fixed upon but one generation. With such any self-regulating arrangement has not time to operate. Many generations may be required, before an equilibrium can be established. A preponderance of males is only important for the generation, in which it happens, for the males have no influence on the sexes of succeeding generations. What is the deciding factor would appear to be a decrease of either the males or the females of a race, such that the total number of individuals was seriously affected. A decrease in the number or proportion of males is hardly likely to cause a like diminution in the number of young born: if it do so, it will probably bring the self-regulating mechanism into action by the decrease in the number of females in the way to be presently indicated. A diminution in the number of females will in all probability bring about an increase in the number of young produced by each and all the females. As a result, owing to the presence of more female-eggs than male ones in those later ripened, there will, in the first instance, be something of an increase in the number of female young. As the first progeny of these latter will once again be predominantly male, in the third generation there will

1) Probably in plants also.

2) Düsing, C., Die Regulirung des Geschlechtsverhältnisses bei der Vermehrung der Menschen, Thiere und Pflanzen, in: Jena. Z. Naturw., V. 17, 1883.

be more males than in the preceding generation. The oscillations will go on, until equilibrium be reached. The decisive factors in the whole question are 1) the fact, that with the female and not the male the determination and regulation of sex for the succeeding generation lie, and 2) the circumstance, that the proportion of females apparently increases with the number of offspring[1]).

A sudden preponderance in the number of the males is not of gravity, for this can easily be put right in the next generation. A decrease in the males will at first lead to an increase in the females, and, finally, from these to an increment in the males. A sudden augmentation in the females will be followed by the production of more males, and thus indirectly it may lead to a decrease in the females. A diminution in the number of females must be followed by the bringing forth of more young by the remaining ones, and from this an increment in the females will ensue.

There is much statistical evidence, going to show, that at first the offspring in man embrace more males than females. Tables contradictory to this result also exist, and for fuller information the reader may refer to other works treating of the problems of sex, such as HENSEN's[2]) "Zeugung". As we have now disposed of any supposed influence of the male, these tables must be differently interpreted than hitherto. Thus, where it is said, that the father was older than the mother, it must be assumed, that the mother was young, and so on[3]).

Whether the aforesaid self-regulating arrangement[4]) be or be not

1) Large families will, therefore, increase the population in succeeding generations, because of the greater number of females thereby arising: small families tend to produce the opposite result, because of their unfavourable effect on the number of females.

2) HENSEN, V., Die Physiologie der Zeugung, Leipzig 1881, p. 203—207.

3) These tables prove nothing, as HENSEN has already insisted. The introduction of the father's age as a factor, and the vague statements, as to the actual and relative ages of the chief agents, the mothers, completely vitiate them.

4) Objections will doubtless be raised as to the validity of the conclusion. Of such two very obvious ones may be noted: the queen-bee sometimes in later life only lays male-eggs, i. e., when her store of sperms is used up, and among the Chinese, where female infanticide is practised, the race shows no signs of decrease. Regarding the first it is in reality a diseased condition, and it leads to disastrous results, the death of the hive. Under normal conditions the queen-bee produces

that employed by Nature in both animals and plants it must be obvious, that one simpler and better calculated to suit the end is hardly conceivable.

Summary of Chief Conclusions.

Reviewing briefly the ground covered in preceding pages, it is seen, that sex in its origin from a primeval isogamy was bound up with the constant differentiation of fourfold gametes. These were such, that two of them, the female- and the male-eggs, were ultimately formed within a sterilised Metazoan person, the female, while the development of the remaining two, the two kinds of spermatozoa, was alloted to a similar but not identical person, the male. The gametes of the female, that is, the two forms of eggs, possessed functions different from those of the two kinds of spermatozoa of the male, and for this reason alone a sexual difference between the male and the female, a dimorphism, was bound to follow. Of the twofold gametes of the male it is to all appearance rare at the present time to find the full and complete differentiation of both in any given case, but this is known to happen in *Paludina vivipara*, *Pygaera bucephala*, and a few other instances. In others one form of male gamete undergones more or less complete suppression in the course of the spermatogenesis, thus in *Cicada*, *Bufo calamita*, *Hyla*, *Rana esculenta* etc., the degree of degeneration varying in different cases. Though never of functional value — unless it take the place of the ordinary form of sperm — the second kind of spermatozoon is probably always represented by something in every Metazoan spermatogenesis, its development must at least be initiated, but it may be arrested anywhere in the history of the spermatogonia, or in the spermatocytes. It is the task of the functional spermatozoon to bring about the effects due to amphimixis.

Since it is the egg, which develops, and not the sperm, the burden of providing for the continuance of the race falls upon the female Metazoon, or rather upon the germ-cells, of which it is the host. To carry out this duty the differentiation of twofold gametes, the male- and the fëmale-eggs, is needful. The germ-cells of the female thus make

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in later life a greater proportion of female-eggs than of male ones. If female infanticide were very general in China, the race would infallibly die out, for no females would be left; those murdered are such as, owing to the prevailing social conditions, would produce no offspring. Obviously, this example proves the truth of the thesis up to the hilt.

provision not only for a new batch of female-eggs, but also for one of male-eggs. The determination of sex for the next generation thus lies with the germ-cells of the female Metazoan organism.

In all dioecious Metazoa three kinds of functional gametes are constantly needed and differentiated, of these two arise in the female, one in the male.

The actual determination of sex is initiated at the division of the primary germ-cells into secondary ones: it is completed at the formation of the oocytes and spermatocytes, and its manifestation is accomplished by the numerical reduction of the chromosomes in these. The determination does not come about in the primary germ-cells, for if one of these undergo independent development alongside the embryo, the result is the bringing forth of identical twins. All known cases of like-twins are of the same sex, and from this it follows with absolute certainty, that the primary germ-cells are alike in sexual potentialities as in other respects. From a host of evidence it is certain, that the determination of sex does not take place later than the formation of the oocytes and spermatocytes. The history of the two sorts of eggs of *Raja batis, Hydatina senta*, *Phylloxera*, und *Dinophilus gyrociliatus*, and that of the twofold spermatozoa of *Paludina vivipara* and *Pygaera bucephala*, suffice to demonstrate the truth of this.

Hermaphroditism is associated with the partial or complete suppression of one form of gamete, the male-egg: parthenogenesis, on the other hand, entails the occasional, or the cyclical, arrestment of one or other of the two gametes of the female. If it ever become acyclical with the consequent disappearance of the males, with these there vanish the male-eggs, which produce them, and the spermatozoa. That is, in such instances the only functional form of gamete left is the female-egg, with which there remains no other form of gamete to unite. (Actually, there is union with a polar body [Boveri] — a form of isogamy.)

Of very great importance for many questions is the recognition, that any particular form of gamete may undergo suppression at any period of the life-history; thus, in some instances of the rare production of male persons their occasional appearance is undoubtedly due to the omission to suppress one or more of the forerunners of male-eggs. Similarly, the rarity or the apparent absence of a second form of spermatozoon is readily explicable.

Since there is no qualitative mitosis subsequently to the formation of oocytes and spermatocytes, all four products of any one of these latter, that is to say, the four spermatozoa, or the egg and its three polar bodies, must be identical gametes. In spite of the non-functional nature of the second kind of spermatozoon, a greater percentage of abortive gametes may still obtain in oögenesis than in spermatogenesis.

The total of the females of the race occupy a relation towards the regulation of sex in nature, similar to that filled with regard to its determination by the individual. In other words, the regulation of sex appertains to the total of the females, and the males possess no influence whatever upon it.

The regulation of sex would appear to be effected by a self-adjusting arrangement, as HENSEN and DÜSING insisted without identifying its character. This method of self-regulation is explained more fully in the text: it would appear to be based in the predominance of female over male offspring, in both animals and plants, in the later born young.

Any interference with, or alteration of, the determination of sex is absolutely beyond human power. To hope ever to influence or modify its manifestations would be not less futile and vain, than to imagine it possible for Man to breathe the breath of Life into inanimate matter.

For the workings of Nature in sex merge in her revelations of Life itself.

Description of Plate.

Plate 45.

Fig. 1. The two forms of spermatozoa, the hairlike and the giant-sperm, of man (*Homo sapiens*). After von WIDERSPERG, from WALDEYER, 'Die Geschlechtszellen', in: HERTWIG's Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbelthiere. 1000 : 1.

Fig. 2. The two forms of spermatozoa of *Hyla arborea*. After VON LA VALETTE ST. GEORGE. From the same source as Fig. 4.

Fig. 3. Three stages in the spermatogenesis of the wormlike sperm of *Paludina vivipara*. After F. MEVES. From KORSCHELT & HEIDER, Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere, Allgemeiner Theil, 1902.

Fig. 4. A the wormlike, and B the hairlike spermatozoa of *Paludina vivipara*, and C the two ends of the former under high magnification. After F. MEVES. From KORSCHELT & HEIDER as before.

Fig. 5. Wormlike spermatozoa, A of *Cypraea caput serpentis*, B and C of *Murex brandaris* (B unripe, und C completely formed), D of *Pteroceras lambis*, E of *Strombus lentiginosus*, and F hairlike or ordinary sperm of the latter species. B and C after R. KOEHLER, the rest after BROCK. From KORSCHELT & HEIDER as before.

Fig. 6. Cocoon of *Dinophilus apatris* = *gyrociliatus*, with large female- and small male-eggs. After KORSCHELT. From KORSCHELT & HEIDER as before.

Fig. 7. The life-cycle of *Phylloxera vastatrix* after LEUCKART & NITSCHE and after RITTER & RÜBSAMEN, from WEISMANN, Descendenz-theorie, 1902. The figure is reproduced to illustrate the female-, E^1, and the male-, E^2, eggs. F^1 and F^2 are respectively the female and male forms arising from these.

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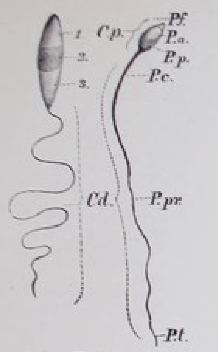
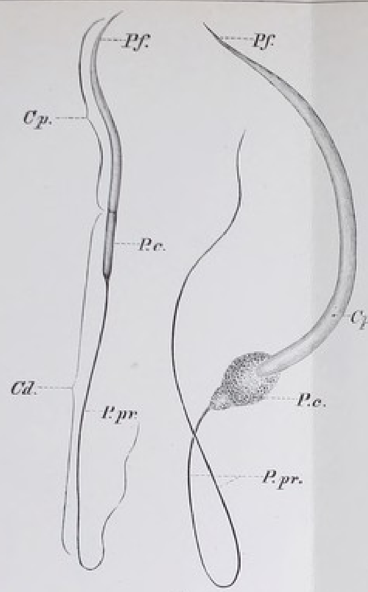


Fig. 1.



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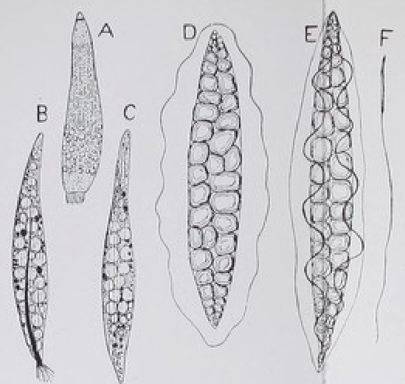

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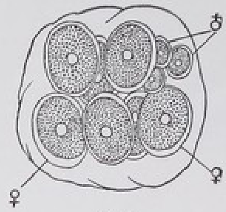

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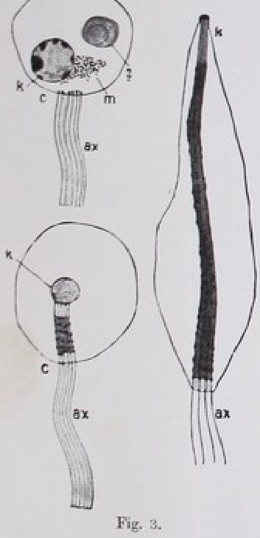

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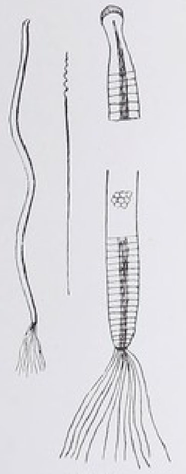

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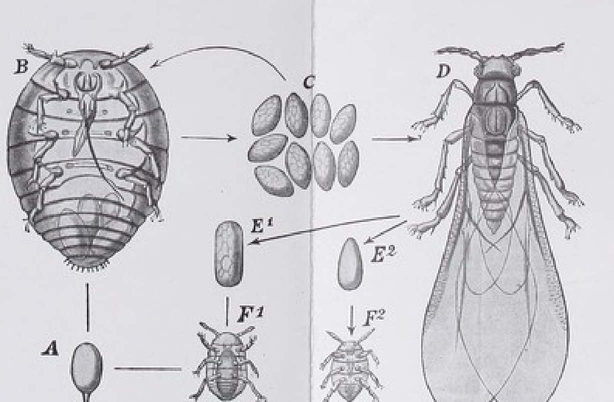

Fig. 7.

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