CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF
THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD
COLLEGE. E. L. MARK, DIRECTOR.— No. 160.

NATURAL AND ARTIFICIAL PARTHENOGENESIS.¹

ALEXANDER PETRUNKÉVITCH.

WITH the discovery by Oscar Hertwig, in 1875, of the essential nature of fertilization as the union of the nuclei of the two parental germ-cells, the line of research in the branches of biology dealing with reproduction became more clearly defined. While on the one hand this discovery put an end to all guess-work, on the other hand it threw light on the part taken in development and heredity by both egg and sperm. To this was added later the knowledge of the behavior of the centrosomes in fertilization, the whole bringing us nearer to an understanding of the otherwise incomprehensible phylogenetic separation of living beings into two sexes, impelled by the necessity of amphimixis. Again, the theory of the individuality of the chromosomes and the study of the process of reproduction in maturation afforded us an insight, incomplete though it be, into those forms of reproduction which are not accompanied by fertilization. New questions were gradually evolved, but since they had their source in facts recognized by everyone, they were so precise, that when, five years ago, I entered upon the study of parthenogenesis in the honey-bee, I had before me a very definite problem.

All this I purposely mention here. By the time I had published the first part of my research and had gone on in the study of natural and artificial parthenogenesis, there began to appear objections not only to the results of my own work, but even to statements hitherto undisputed, as well as to the most sober interpretations of facts. These objections were raised

¹ Read before Section F—Zoölogy—of the A. A. A. S. at the 54th annual meeting in Philadelphia, 1904.

not only by people unacquainted with the facts won by science and with the methods of scientific research, as bee-keepers for instance, but also by men of very considerable scientific education, especially physiologists. It seems to me therefore seasonable to review briefly these objections, in order to determine what, if anything, has been gained through this controversy, so that we may know how the question of parthenogenesis now stands.

Our knowledge of fertilization and parthenogenesis, acquired in the study of many different animals, may be recapitulated in the following words :

1. Both the egg ready for fertilization and the mature sperm show a reduction in the number of chromosomes of their nuclei to one half of that found in somatic cells.
2. No matter how many spermatozoa succeed in entering the egg, the nucleus of only one of them, under normal conditions, fuses with the egg-nucleus, thus restoring the chromosomes to their original number. All other spermatozoa are absorbed.
3. The centrosome of the egg disappears after the second polar cell is formed, its functions being assumed by the centrosome of the spermatozoön.
4. In most parthenogenetic eggs no reduction of chromosomes takes place — only one polar cell being formed — and the egg-centrosome remains active.

Now, all of the objections alluded to above are directed against one or more of these four propositions. As, however, it is in these propositions that our present understanding of parthenogenesis is epitomized it is of urgent importance to discover how much of truth these objections contain.

First of all I shall consider the objections brought forward by the physiologist Bethe and the zoölogist Bresslau. Both claim to find the essence of fertilization not in the union of the sperm-nucleus with the egg-nucleus, but in the entrance of spermatozoa into the egg. Bethe (:04) says explicitly : "I consider an egg fertilized when a spermatozoön has entered it. What becomes of this spermatozoön later on is another question." He says, further, that not alone the chromosomes of the nucleus enter the egg, but also other substances, of whose fate we know

nothing, and that we keep in sight a part, only, of the whole phenomenon when we speak of fertilization as the union of nuclei. Bresslau (:04) supports nearly the same view, and distinguishes between "Besamung," or the entrance of spermatozoa into the egg, and "Befruchtung" or fertilization in the sense of Hertwig, Weismann, and others, *i. e.*, the union of the nuclei.

In one respect Bethe and Bresslau are right, that is, as to the necessity of distinguishing between these two phenomena; but it remains to decide which of the two phenomena exerts the greater influence upon the development of the egg and may therefore be considered the one essential to fertilization. This question seems to me easy of solution in the light of recent experiments and observations. There can exist no doubt as to the importance of the union between sperm- and egg-nuclei. The normal number of chromosomes is indispensable to normal development. The paternal hereditary characters are transmitted to the descendant by the chromosomes of the sperm-nucleus. Even if the protoplasm of the spermatozoon, in those cases where it succeeds in entering the egg (for it is frequently cut off by the suddenly formed yolk-membrane), should possess the power of transmitting by heredity certain paternal qualities, yet, in the presence of the egg-centrosomes, it loses that power. Nor can there be doubt as to the importance of the part taken by the sperm-centrosome. It gives the stimulus to development and controls the successive divisions. Without it there can be no development; when hindered in the normal performance of its functions, abnormal development results.

We now turn our attention to the phenomenon of the entrance of spermatozoa into the egg. Does it make any difference in the development of an egg whether one or several spermatozoa enter it? No, for the nucleus of only one spermatozoon is destined to unite with that of the egg. In those cases where polyspermy exists normally, all spermatozoa with the exception of one are gradually absorbed by the egg-protoplasm, and the resulting development is the same whether the spermatozoa are many or few; but when, as sometimes happens, they are not absorbed by the egg-protoplasm, their centrosomes form independent centers for cell-division, thus interfering with the nor-

mal development of the egg, and sometimes even proving fatal. Abnormal development also follows where the egg-nucleus unites with more than one sperm-nucleus.

Thus the entrance of a spermatozoön into an egg cannot be regarded as in itself the efficient stimulus to normal development. Moreover, it is possible by etherizing an egg, to prevent the union of the sperm-nucleus with the egg-nucleus. By this means one half of the egg, that containing the egg-nucleus, develops parthenogenetically, while the other, containing the sperm-nucleus, develops merogenetically. Knowing all this, one must realize that the simple fact that a spermatozoön has entered an egg is relatively unimportant, though it is of course a necessary step in fertilization, rendering possible the union of the two pronuclei.

I consider it therefore perfectly correct to hold to the old definition of fertilization and to call an egg fertilized only when the union of the nuclei is accomplished. This definition does not take into account the behavior of the centrosomes, and rightly, since these constitute only the stimulus and the controlling apparatus of division and are not as important as the chromosomes, *i. e.*, whether the division is accomplished through the influence of the egg-centrosome, or that of the sperm-centrosome, does not change the outcome of the union of the pronuclei.

Applying the same standard to a definition of parthenogenesis, we should expect to say that the latter represents development without fertilization; yet we shall see that such a definition is too broad and that in order to avoid misunderstandings it is necessary to restrict it in certain points.

Development without fertilization takes place normally in many animals of different groups. Leaving out of consideration reproduction by the division of the whole animal, we find these three kinds of propagation: parthenogenesis, pædogenesis, and budding. Under budding we understand development through successive, regular mitotic cell-divisions, proceeding from one or several cells. Parthenogenesis is always development of an unfertilized egg and is introduced by a maturation process, while the nature of pædogenesis is not yet known. The facts that pædogenesis occurs in only a few animals and

that these animals are very rare, make its study exceedingly difficult. We know no more now than was contained in the work of Metschnikoff on *Cecidomyia*, the account of which appeared in 1865, when the maturation divisions of the egg were still unknown. Should further research show that *pædogenesis* is also introduced by a process of maturation in the egg, it must then be regarded as a case of larval parthenogenesis. If, on the contrary, the maturation process is wanting in *pædogenetic* development, it would be more reasonable to place *pædogenesis* in the same group with budding. In any event it is a problem by itself.

The process of maturation in the egg is without doubt equally indispensable for parthenogenetic development and for that resulting from fertilization; yet there is a great difference between the two, the importance of which was at once recognized by Weismann, its discoverer, many years ago. All eggs requiring for their development fertilization, undergo a double maturation division. This results in the reduction in the number of chromosomes by one half. On the other hand, in the majority of parthenogenetic eggs—the single exception being that of certain insects—there takes place but one maturation division, and in consequence, there is no reduction in the number of chromosomes. In this way the opportunities for variation are much restricted. The exception to this rule is found in those insects in which only one sex develops parthenogenetically, and in the male individuals in those cases where both sexes develop parthenogenetically. In such cases two maturation divisions invariably take place with a corresponding reduction in the number of chromosomes. The insects which on account of the possession of this peculiarity have been much studied lately, are the ants and the bees. In these Hymenoptera it is only the males that develop parthenogenetically. I have every reason to re-affirm this, notwithstanding the recent objection raised against it.

After what I have said about fertilization it is clear that microscopic study, and that only, is able to show without error whether an egg is fertilized or not. The development of an aster around the centrosome of the spermatozoön after the lat-

ter has entered the egg, facilitates the finding of the sperm-nucleus, which keeps its place close behind the migrating centrosome. Were it not for the objections which have been raised by men like Pflüger and Bethe, I should scarcely need to mention that this sperm-aster develops in the same way throughout the entire animal kingdom, that in polyspermic bee-eggs similar asters appear in numbers equalling those of the spermatozoa which enter the egg, and that also in the so-called drone-egg an aster would surely develop, should a spermatozöon enter it. The experiments made upon the eggs of bees, consisting in transporting them from one kind of comb-cell into another, in an attempt to change their sex, have proved to be failures. Should similar experiments, however, some day meet with success, it would only show that it may be possible artificially to change sex.¹ The objection that in drone-eggs the aster might not develop because of the influence of the saliva of the workers which take charge of the eggs as soon as they have been laid, and that in consequence a spermatozöon, though present, might easily be overlooked, does not bear critical investigation. Experiments and microscopic preparations alike showed that the saliva of the workers exerts no such influence. For when fertilized eggs are treated with the same saliva, the aster none the less develops. Or would the opponents of parthenogenesis — who believe that a queen can by no reflex be impelled to fertilize or to inhibit the fertilization of her eggs as she lays them in worker-cells or in drone-cells — be bold enough to affirm that the workers treat the eggs with two different kinds of saliva, corresponding to the comb-cells in which these eggs are laid? Yet this is what the opponents of parthenogenesis claim. How

¹ Experiments in attempting to change sex artificially have been made by vom Rath, by v. Buttel-Reepen, by myself, and by others with negative results. Experiments with apparently positive results have been made and often described by Dickel, a German bee-keeper. I desire here earnestly to warn against the attempt to use in any way whatsoever the experiments of Dickel without first verifying them, as he has on several occasions in his scientific work practiced deliberate fraud in an effort to support his theory, for which delinquency he has frequently been called to account by v. Buttel-Reepen and myself. The history of the falsely labelled bottles containing bee-eggs, together with my exposure, of this deliberate trick, made clear the absence of spermatozoa in drone-eggs and thus demonstrated the efficiency of microscopic study.

wonderfully complicated, that reflex would have to be which could stimulate to action at a given time only one of three pairs of glands which possess a single duct common to them all!

There are still other difficulties in the way of these objections. The union of the nuclei, not the presence of an aster, is the essential of fertilization. Now, this union of the nuclei may readily be observed, whether the aster lies in the same microscopic section with the nucleus or not, and even when it does not appear at all. Since there is no union of nuclei in drone-eggs, they are not fertilized. As this is the only reasonable conclusion, it sounds strange to hear Pflüger (:03) say that to claim parthenogenesis for the bee, is to adopt a false and insufficiently supported hypothesis, and that the queen-bee is probably a hermaphrodite in which the male sexual glands have not yet been discovered. Still stranger is the contention of Bachmetjew (:03), who says that the right wing of the drone and the left wing of the worker develop parthenogenetically, while the left wing of the drone and the right wing of the worker are the result of fertilization, on the ground that the number of hooks on their wings is subject to variation, displaying two different maxima of frequency. If the theory of partial parthenogenesis can find any support in facts, it is more likely to be applicable to those cases of bee-monsters which exhibit a marvelous mosaic of male and female characters.

If, however, more than one form of parthenogenetic development can with certainty be recognized, should not microscopical research be able to throw light upon the origin of these forms? The answer to this question may be looked for in the behavior of centrosome and chromosomes. There can be no doubt that in parthenogenetic eggs the egg-centrosome remains active, and in *Artemia salina*, at least, we can easily see that it moves alone toward the center of the egg to await there the nucleus, which after the single maturation division, is destined to become the first cleavage nucleus. Thus does the egg-centrosome in parthenogenetic eggs maintain its individuality through all cell generations. In the fertilized egg of bees the centrosome of the spermatozoön becomes the active centrosome and as such passes in turn into the spermatozoön of the next generation. We may

therefore say that in all cases in which either both sexes, or even one sex, invariably develop from fertilized eggs, every centrosome (even that of the egg, which is destined to disappear) is a descendant of a sperm-centrosome—a *male* centrosome, if I may so call it. There is apparently a difference between this and pure parthenogenesis, where every centrosome is a descendant of the egg-centrosome and could be called a *female* centrosome. Does such a difference in reality exist? If we believe with Wedekind (:02) that parthenogenesis is an original, primary mode of development, we must admit that it does. But this would mean that animals which are closely related to each other differ in their phylogenetic origin. Is it not simpler to admit that even in pure parthenogenesis the ostensibly female centrosomes are descendants of a remote ancestral male centrosome? It seems, indeed, as though in animals where fertilization occurs only as a rare exception the males had gradually disappeared yielding to hermaphroditism and parthenogenesis, as Maupas (:00) has so beautifully shown for certain nematodes.

Still more important biological problems are connected with the behavior of the *chromosomes* in parthenogenesis. In pure parthenogenesis only one maturation division takes place and the number of chromosomes remains normal; the individuality of the chromosomes is preserved and thus the questions of variation and hereditary transmission are greatly simplified. Not so in the case of that form of parthenogenesis in which the number of chromosomes is reduced. I have elsewhere (Petrunkewitsch, :02) pointed out that the great difficulties here encountered by the theory of Weismann—which maintains not an essential, but a *qualitative* difference only between the chromosomes—grow out of the cessation of hereditary transmissible variability. There seem to be fewer difficulties in the way of the theory of Sutton and Boveri,—who affirm that the chromosomes are *essentially* different,—since I have shown—and still maintain after a careful re-examination of my sections, in opposition to the objections of Doncaster (:04)—that the number of chromosomes in the first cleavage nucleus of the drone-egg again becomes normal, probably through longitudinal splitting without a corresponding division of the cytoplasm. I thought to find a support for

Weismann's theory in my observation that in parthenogenetic eggs the polar cells, after a copulation similar to that of the pronuclei, give origin through successive divisions to many cells which may be easily traced as far as the gastrulation stage of the embryo. My attempt to trace them still farther and to show that the male sexual glands in bees develop from these cells, has met with much criticism and requires confirmation. Doncaster, however, was able to prove in *Nematus* the accuracy of my observations as far as the beginning of the blastoderm stage. Although I am still, and until convinced of error shall remain, an adherent of Weismann's theory, I admit that the Sutton-Boveri theory would be strengthened, if it could be shown with certainty that I was at fault in my observations, and that the sexual glands of the drones develop not from polar cells but from the pronucleus. Future research will reveal the truth, but one thing is already certain: in all parthenogenetic eggs hitherto studied, with possibly the one exception of *Nematus* (according to Doncaster), the number of chromosomes in the first cleavage nucleus becomes in some way equal to that in the somatic cells. This is not limited to animals, but applies equally well to plants¹ and is consequently of fundamental importance. Yet it is not impossible that in *Nematus* there is no reduction in spermatogenesis, which would lead to the same thing.

It is clear that to reach a solution in the problems connected with the two theories of which I speak, we ought not to limit our research to observation of normal parthenogenesis alone, but rather to supplement such observation by the use of experiment. Here two methods are possible, of which one is cross-breeding and inbreeding, the other, artificial parthenogenesis and merogeny. Much light could be gained by experiments in the former upon animals like the bees, since such experiments would have many advantages over similar ones made on animals developing by fertilization only. Although this method promises much, it has never yet, I regret to say, met with scientific application.

By fertilizing enucleated eggs we obtain development by mer-

¹ Compare the observations of J. B. Overton (:04) on *Thalictrum purpurascens*.

ogeny, which is characterized by a reduced number of chromosomes, and by the presence of the active sperm-centrosome. In stimulating unfertilized eggs by chemical or physical means to develop, we get what is called artificial parthenogenesis. As I have elsewhere (Petrunkewitsch, :04) tried to show, this development is due not to a new formation of centrosomes,¹ but to the stimulation to new life of the egg-centrosome, which is otherwise destined to disappear. According as we apply the stimulus before or after the second maturation division, we obtain different results; if before, we get development with the normal number of chromosomes, if after, then with the reduced number. Now, it is interesting that both parthenogenetic development with the reduced number of chromosomes and merogenic development show abnormalities when compared with the development of fertilized eggs. These abnormalities increase in inverse proportion to the number of chromosomes left in the egg, an indication of which is given in the experiments of Stevens, which consisted in a dissection of the fertilized egg in the amphiaser stage into two parts containing unequal numbers of chromosomes. All this goes to show that neither merogeny nor artificial parthenogenesis with a reduced number of chromosomes can be regarded as equivalent to natural parthenogenesis. I call, therefore, these two forms, *artificial, pathological, uni-parental development*. On the other hand, artificial parthenogenesis with the normal number of chromosomes leads to apparently normal development. I therefore call it *artificial, true parthenogenesis*.

If we could cause an egg to develop by applying a stimulus before the first maturation division, the gap between parthenogenesis and budding would be filled. Again, if we could bring about artificial parthenogenetic development through several successive generations by stimulating the eggs at the three different moments of their maturation, we might obtain valuable

¹If there are such *de novo* formations of centrosomes, as seems to be again emphasized by the research of Yatsu (:04), it is nevertheless probable that in experiments with artificial parthenogenesis, the divisions are due to the egg-centrosome. This being present in every egg, is stimulated to new life, and counteracts possible teratological action on the part of the artificial centrosomes.

results connected with the questions of individuality of chromosomes, determination of sex and variation. These are the problems to which my own study of parthenogenesis has naturally led me and to which I am now seeking solutions in experiments. In this way parthenogenesis, an important problem in itself, becomes at the same time a method for the study of other and greater problems.

BIBLIOGRAPHY.

BACHMETJEW, P.

- :03. Ein Versuch, die Frage über die Parthenogenese der Drohnen mittels der analytisch-statistischen Methode zu lösen. *Allg. Zeit. f. Entomol.*, Bd. 8, no. 2-3, pp. 37-44.

BETHE, A.

- :04. Entgegnung auf den Aufsatz von v. Buttel-Reepen in Nr. 4 dieser Zeitschrift. *Bienenwirtschaftliches Centralblatt*, 1904, no. 11, 2 pp.

BRESSLAU, E.

- :04. See 'Diskussion.' *Verhandl. Deutsch. Zool. Gesellsch.*, 14. Jahresvers. zu Tübingen. Leipzig, 1904, pp. 66-72, 73-77 *passim*.

DONCASTER, L.

- :04. On the Early Development of the Unfertilized Egg in the Sawfly, *Nematus ribesii*. *Proceed. Cambridge Philos. Soc.*, vol. 12, part 6, pp. 474-476.

MAUPAS, E.

- :00. Mode et formes de reproduction des nématodes. *Arch. zool. exp. gén.*, sér. 13, tom. 8, no. 3, pp. 463-624, pl. 16-26.

OVERTON, J. B.

- :04. Ueber Parthenogenesis bei *Thalictrum purpurascens*. *Berichte Deutsch. Bot. Gesellsch.*, Bd. 22, Heft 5, pp. 274-283, Taf. 15.

PETRUNKEWITSCH, A.

- :02. Das Schicksal der Richtungskörper im Drohnen. Ein Beitrag zur Kenntniss der natürlichen Parthenogenese. *Zool. Jahrb., Abth. f. Anat. u. Ontog.*, Bd. 17, Heft 3, pp. 481-516, Taf. 11-13.

PETRUNKEWITSCH, A.

- :04. Künstliche Parthenogenese. *Zool. Jahrb., Suppl. vii, Festschrift Weismann*, pp. 77-138, Taf. 8-10. *Also separate*, 62 pp. 3 Taf., 1904.

PFLÜGER, E. F. W.

- :03. Ueber die jungfräuliche Zeugung der Bienen. Arch. f. d. ges. Physiol., Bd. 99, Heft 3-4, pp. 243-244.
Also in Münchener Bienenzeitung, no. 20, 1903.

WEDEKIND, W.

- :02. Die Parthenogenese und das Sexualgesetz. Verhandl. 5. Internat. Zool. Congr. zu Berlin, pp. 403-409.

YATSU, N.

- :04. Aster Formation in Enucleated Egg-fragments of Cerebratulus. Science, vol. 20, no. 521, pp. 889-890.