THE CYTOLOGY OF PARTHENOGENESIS IN TENTHREDINIDAE

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I. INTRODUCTION

Two of the characteristic features of Hymenopterous parthenogenesis are, firstly, that the male arises from an unfertilised egg, and

secondly, that the first spermatocyte division is abortive. These have led to the hypothesis that the male is a haploid organism, but according to modern canons, as will be discussed presently, the cytological evidence for this conclusion is insufficient, particularly in regard to the somatic cells. This paper deals primarily with the problem of male and female chromosome constitution in the *Tenthredinidae*, these insects having been chosen because their cytological phenomena are more amenable to study than are those of more specialised *Hymenoptera*. By the investigation of this more generalised group it was hoped to throw light on certain problematical conditions existing in groups more specialised, and on the evolution of these conditions.

After making a preliminary examination of several saw-fly species it was decided that it would be a better plan to investigate thoroughly one species with a view to establishing a standard for future work. The species chosen, *Pteronidea* (*Nematus*) *ribesii* SCOP. is particularly suitable for this because it reproduces by arrhenotokous parthenogenesis and is easily reared in the laboratory.

II. HISTORICAL REVIEW

1. *Biological*

Since DZIERZON in 1845 first formulated his theory that drones in the honey-bee, *Apis mellifica*, are developed from unfertilised eggs, the problem of parthenogenesis and sex determination in *Hymenoptera* has attracted many workers. The theory "dass die Drohneneier eine Befruchtung nicht bedurfen, die Mittwirkung der Drohnen aber schlechterdings notwendig ist, wenn Arbeitsbienen erzeugt werden sollen" may be looked upon as the starting point of the theory of Parthenogenesis. It met with much adverse criticism and was for long the centre of a long and ardent controversy. The purpose of the following biological review is, however, not to discuss the whole question of parthenogenesis but to focus attention on the conditions of parthenogenesis in the different groups of *Hymenoptera*, particularly in the light of the more recent experimental work.

Apidae. DZIERZON based his theory on the results of biological observation only, but the truth of the theory was revealed later, in 1854,

by VON SIEBOLD, who made an anatomical investigation of the genital organs of the queen bee, and found spermatozoa in the spermatheca. Later, in 1856, he found that sperms were present in the worker eggs from a fertilised queen, and absent in drone eggs. Although these observations were made by the rather crude method of puncturing the eggs at the posterior pole and allowing some of the yolk to escape, VON SIEBOLD states that he saw living sperms in the anterior end of the egg even $22\frac{1}{2}$ hours after oviposition. The accuracy of these observations was questioned by BUTTEL-REEPEN who found the sperm nucleus rounded off in the yolk within 20 minutes from the time of entrance. The laws of VON SIEBOLD and LEUCKART, (1885) were supported by GERSTÄCKER, BESSELS and others, but met with narrow acceptance and were openly opposed by many. The most obstinate opponent of the theory was DICKEL (1898) who maintained that the eggs laid by the queen bee were all fertilised alike and whether the egg should develop into a male or a female depended not on fertilisation but on the secretion of the salivary glands of the worker nurse bees. Three kinds of thoracic glands were known to exist and it was thought that the three secretions of these glands determined the sex — drone, queen or worker! The tedious discussion which followed even after the DZIERZON theory had been confirmed by PETRUNKEWITSCH (1901) has been extensively reviewed by DALLA TORRE (1910) and NACHTSHEIM (1913) and need not be further discussed here. The majority of the objections to the DZIERZON theory were proved practically valueless, but at the same time it should be remembered that some of these objections were well founded on peculiar results obtained by certain hybridisation experiments. These hybridisation experiments afford confirmation of the theory, for, as all males develop from unfertilised eggs, they ought to carry the maternal characters only; as witness BERLEPSCH (1856) EUGSTER (in VON SIEBOLD, 1864), and more recently NEWELL (1914). Yet another series of experiments, however, yielded unexpected results and even DZIERZON himself had occasion to doubt the exactitude of his theory. In 1854, in a cross between an Italian drone and a German queen he obtained a few males carrying the paternal colouration. Analogous results were obtained by LOWE (1867), ARVISET (1878), MATTER (1879) and PEREZ (1876). The last-named, from an Italian queen and a French drone, obtained 300 males, of which 151 were of the mater-

nal type, 83 of the paternal type and 66 showed various gradations between Italian and French varieties. SANSON (1878) criticised the experiment and claimed that the unlooked-for results were due to atavism. GERARD (1878) suggested that the hybrid drone eggs had been laid by the hybrid workers while COOK (1878) suggested that the queen used was probably a hybrid. PEREZ, however, was able to counter all objections. In the opinion of PHILLIPS (1903, p. 288) racial characters are very inconsistent even in pure drones and "any attempt to use them as tests of hybridism is not warranted". More recently CUÉNOT (1909) records similar results.

In gynandromorph bees we have also striking confirmation of the DZIERZON Law. The so-called EUGSTER gynandromorph bees (VON SIEBOLD, 1864) result from a cross between an Italian queen and a German drone. The male parts are of pure Italian type and the female parts possess hybrid character. MEHLING (1915) and BOVERI (1915) still maintain that these verify the original hypothesis of BOVERI (1888) that a gynandromorph results from retarded fertilisation, the sperm nucleus fusing with a blastomere nucleus. These observations, however, do not in any way refute the DZIERZON theory which was built on facts established under normal conditions and with a European race of bees, which is apparently purely arrhenotokous.

Recently, however, convincing evidence is to hand that workers and even queens can develop from unfertilised eggs, i.e., parthenogenesis is of the thelytokous or deuterotokous type. In every case reported these eggs are laid by fertile worker bees, but we have no evidence as yet that unfertilised queens do not also possess the faculty of producing workers. It has long been known that worker bees on occasion can lay viable eggs, but these eggs develop into drones only. HEWITT (1892), working with a race of Punic bees, found that laying workers could give female progeny, and, more recently, similar observations have been made with regard to a South African race of bees, *Apis mellifica unicolor*, var. *intermissa*, by G. W. ONIONS (1912). These results, although attracting but little attention, received a certain amount of adverse criticism by VAN WARMELO, and the work was repeated under expert supervision (R. W. JACK, 1916). The chief facts are as follows: 1. Laying workers of the Cape variety produce workers parthenogenetically; 2. Worker eggs develop into queens as readily as do the fertilised eggs of ordinary queens; 3. The

spermatheca is unusually well developed in the Cape worker though it has never been found to contain sperms; 4. A few small drones are produced at times but these are never reared in special drone cells; 5. The spermatheca is rudimentary in the closely allied Rhodesian bee *Apis mellifica unicolor*, var. *adansoni*; 6. The Cape bee crosses readily with the Rhodesian bee, in which workers have never been known to yield anything but drones.

These observations were evidently well founded, but other instances of the production of females by virgin mothers cited by FABRE (1879—80) and ARMBRUSTER (1916) for *Osmia* and *Halictus* (in WHEELER, 1928) have proved incorrect. FABRE believed that the over-wintering female in *Halictus* produced females only in the spring and that these produced both sexes by parthenogenesis in the autumn. According to ARMBRUSTER there are three generations, the parthenogenetically produced spring and summmer generations and bisexual autumn generation. That these erroneous observations were due to misinterpretation of the facts has been shown by DESCY and by STOCKERT (in WHEELER, 1928) who find that reproduction is purely by arrhenotokous parthenogenesis, the females which emerge in the spring being fecundated females which hibernate in the autumn. Thus, in these species at least there is no infraction of the DZIERZON Law.

Vespidae. In all wasps as far as is known parthenogenesis is of the arrhenotokous type.

Formicidae. The conditions of parthenogenesis in ants are similar to those in *Apis mellifica*, males arising from eggs laid by virgin females, either worker or queen, and females from fertilised eggs. Yet, although sources of evidence are not numerous, there is reason to believe that thelytoky exists in certain species. FOREL (1874), LUBBOCK (1883), WASMANN and VIEHMEYER (in CRAWLEY, 1911) held that worker eggs give males only, but REICHENBACH's experiments with *Lasius niger* carried on for three years (1899—1902) seemed to prove that females also arise parthenogenetically from worker eggs. TANNER (1892, in WHEELER, 1903) reared both males and females from virgin workers of the fungus-raising ant *Sauba*; Miss FIELDE (1905), with a view to disproving the occurrence of thelytoky, reared progeny of virgin females in the species *Campanotus pictus*, *Formica argentata*, *F. pallidafulva fuscata* and *Crematogaster lineolata*, and

found males only. In 1912 CRAWLEY and, later, BUTTEL-REEPEN confirmed REICHENBACH's experiments with *Lasius niger*, and added further evidence from dissections of the fertile workers. As no *receptacula seminales* were found thelytoky may be taken as established in this one species at least.

Ichneumonidae. Parthenogenesis in Ichneumons, Braconids and Chalcids, as a rule, is of the arrhenotokous type but thelytokous and, to some extent, deuterotokous parthenogenesis occur also. The literature dealing with the biology of the group is very scattered but WINKLER (1920) brings together the known facts in some 66 species. Of these 13 species are thelytokous and 2 are deuterotokous. To the former list VANDEL (1931) is able to add another 17 species, and he again draws attention to the fact that nearly related species or even geographical races of the same species behave differently as regards parthenogenesis, e.g., in European *Hemiteles aerator* parthenogenesis is arrhenotokous while the American race *H. tenellus* is thelytokous.

The investigation of the biology of polyembryonic Chalcids has attracted many workers (see LEIBY 1922). Broods of these parasites are very often of both sexes and the explanation offered is that more than one egg is deposited in the host caterpillar, females coming from fertilised eggs and males from unfertilised eggs. The preponderance of one sex is probably due to the abortion of one germ. PATTERSON (1917) suggests that males in mixed broods may arise through mitotic irregularities in the polygerm, as, for example, by abnormal behaviour of the sex chromosomes during cleavage. The risk of contamination in these experiments is very great however, and as far as is definitely proved the DZIERZON Law holds good. Broods derived from eggs laid by fertilised females are composed of either males or females.

Recent researches in the Braconid, *Habrobracon juglandis* and *H. wesmaeli* however appear to constitute an exception to the law of haploidy in males. In a series of experiments in the genetics of these flies A. R. and P. WHITING (1921—8) have demonstrated the occurrence of biparental patroclinous males and uniparental females. Crosses were obtained between normal individuals and others bearing a mutant character in eye colour. In a cross between a normal black-eyed male and a female homozygous for the mutant orange-eyed character, there appeared a large percentage of black-eyed males. It

was at first postulated that in the egg fertilisation had been incomplete and that cleavage of male and female nuclei had taken place without syngamy. Later, however, with the occurrence of further mutations, this theory of mosaic haploid males was abandoned. In 1927 crosses were obtained between females homozygous for both dominant and recessive characters and males carrying the allelomorphs of those factors. The sons were entirely dominant showing that they had derived factors from both parents. The latest theory with regard to the problem is that the biparental males were diploid for all chromosomes save one, the sex chromosome, and thus were males resembling the females in appearance. It is to be noted that these were often abnormal and usually sterile or yielded sterile daughters.

The cytological investigation is not complete but it appears that in spermatogenesis one division is abortive in both normal haploid and in biparental diploid males. Hence in the latter the diploid condition could be expected in the spermatozoa so that haploid eggs fertilised with such spermatozoa would produce triploid daughters, and this might account for their abnormality and sterility (A. R. WHITING, 1928).

P. W. and A. R. WHITING (1927) obtained two cases of "undoubted thelytoky", but, as both females produced were freaks, this evidence cannot be accepted as proof that thelytoky occurs in normal fashion. In fact, the whole series of experiments was carried out with abnormal material. The results are in no way contradictory to the theory of DZIERZON, for under normal conditions all males are derived from unfertilised eggs.

Cynipidae. Of all *Hymenoptera* the *Cynipidae* exhibit the greatest diversity in mode of reproduction. In addition to arrhenotoky and thelytoky there exists heterogony (LEUCKART) — the alternation of two successive generations, one parthenogenetic (agamic) and the other bisexual. Heterogonous reproduction is typical of Rotifers, *Cladocera* and Aphids, but in the gall-flies heterogony in every case known is monocyclic and regular, i.e., in one year there occurs only one sexual generation which alternates regularly with an agamic generation. A very excellent and up-to-date summary of parthenogenesis in this group is given by VANDEL (1931).

Those gall-flies, in which both sexes appear in about equal numbers,

probably propagate by the usual facultative method of most *Hymenoptera* — by arrhenotoky, but it is not yet definitely known whether impregnated females can lay both kinds of eggs or whether unimpregnated females alone can lay unfertilised eggs (PATTERSON, 1928). Really very little is known of the biology of these species beyond the fact that they generally attack and form galls on herbaceous plants. In most primitive species males and females appear in about equal numbers (KIEFFER, in PATTERSON, 1928), but many species are known only from the females, the males being entirely unknown or exceptionally rare, e.g., *Andricus, Ceroptres, Cynips, Diastrophus, Phanacis, Rhodites* (see WINKLER, 1920). Many species, however, at one time listed as purely agamic, have proved to be heterogonous (TASCHENBERG, 1892), and further biological investigation will no doubt reveal many more such species. It is worthy of note that in the well known agamic species *Rhodites rosae* males are generally considered to be of rare occurrence, but, recently, KUZNETZOV-UGAMSKIJ (1928) reports that in Turkestan males appear in as great numbers as females. We have already seen how, of two very closely allied species, one may be arrhenotokous and the other thelytokous but KUZNETZOV-UGAMSKIJ does not give details of his experiments so that we do not know if males are functional and parthenogenesis is arrhenotokous or whether parthenogenesis is purely thelytokous. If it be thelytokous then these males are atavistic and represent a 'throwback' to the more primitive bisexual condition.

Heterogony appears to have come about with the disappearance of the males in one of the two bisexual generations. In one species *Neuroterus-lenticularis-baccarum* the agamic generation emerges in the spring, and in the allied species, *Dryophanta erinacea* the agamic females emerge in the autumn. These agamic females differ from the sexual females not only in mode of reproduction but also in external morphology, the most conspicuous difference being in the ovipositor which in the summer females is quite short, being only about one fifth the length of that of the agamic females (DONCASTER, 1910). These modifications are obviously adaptations to seasonal conditions and no doubt existed in the females of both sexual generations at the more primitive stage when there were two sexual generations each year. This dimorphism was first discovered by WALSH (1864) in *Cynips aciculata-spongifica* and since then many gall-flies at one time

referred to different genera, have proved to be dimorphic individuals of alternating generations. TASCHENBERG in 1892 gave a list of species then known to exhibit heterogony, but this list has been greatly augmented and no doubt will be further extended as investigations in the biology of gall-flies proceed. The species whose life history is most perfectly known is *N. lenticularis* whose summer bisexual generation was originally described as *Spathegaster baccarum*. The late Professor DONCASTER reared about 10,000 of these flies under very thoroughly controlled experimental conditions. From records of sex-ratios and pedigrees he arrived at the following conclusions: 1. Any individual of the agamic spring generation produces either male or female offspring but not both; 2. All the eggs laid by sexual females produce females only in the spring generation; 3. Any sexual female produces almost exclusively ♂-producing or ♀-producing daughters, but not both; 4. Females of the sexual generation do not reproduce parthenogenetically. It will be seen from 3 that the grand-children of any sexual female are of one sex only so that the determination of sex of the summer generation is dependent not on the nature of the eggs laid by the agamic female but on that of those laid by the sexual female. Thus the determination of sex is referred further and further back in the course of evolution of this type of heterogony. In the summer generation the sexes are produced in about equal numbers and DONCASTER was able to prove cytologically that males arise in the summer generation from reduced eggs — arrhenotokous as in all other *Hymenoptera*. But in this case parthenogenesis is obligatory and not facultative. Females of the sexual generation arise from diploid eggs by thelytokous parthenogenesis. In no case did DONCASTER rear a male from any of the over-wintering galls and was led to conclude that all the summer eggs require fertilisation. Recently, however, PATTERSON (1928) reared 10 males and 231 females from galls produced by sexual females of *Neuroterus contortus* and 3 males and 94 females of *N. rileyi* var. *mutatus*, which is only known from the spring agamic generation. No pairings were obtained between these males and females nor, in the case of *N. contortus*, between these males and agamic females. Four of the *N. contortus* females were delayed in emergence (Feb. to April) by being submitted to low temperatures, and were placed with males of the sexual generation, but again no pairings were obtained. Thus PATTERSON concludes that the

agamic females have lost the mating instinct and that the aberrant males have nearly lost the instinct and are sexually functionless. The latter represent a remnant of a more primitive bisexual condition in the generation which has now become agamic. In a later paper PATTERSON (1928) was able to throw still more light on the evolution of parthenogenesis in *Cynipidae* by results obtained from breeding experiments in a wide range of species. In a large number of these heterogonous species the agamic females are of two kinds, ♂-producers and ♀-producers, e.g., *Neuroterus irregularis albipleurae*. In many species, however, the agamic female lays both kinds of eggs, e.g., *Andricus operator sustrior*, while in others there is a varying tendency to lay but one kind of egg. PATTERSON finds a gradual transition from those in which the agamic females reproduce by deuterotoky to those in which parthenogenesis is purely thelytokous or arrhenotokous. Among heterogonous species the function of laying one kind of egg by the agamic female has probably evolved from a condition in which such a female laid both ♂-producing and ♀-producing eggs.

Tenthredinidae. The *Tenthredinidae* are regarded as the most generalised and perhaps the most primitive of *Hymenoptera*. They are in many ways admirably suitable for biological investigation, for the following reasons: 1. The flies lay readily in captivity; 2. They are easily reared; 3. They exhibit within the groups different types of parthenogenesis.

In the majority, parthenogenesis is arrhenotokous as in the *Aculeata*, but in some thirty species parthenogenesis is thelytokous. In a third group certain species exhibit 'gemischte Parthenogenesis' (TASCHENBERG), but it is not known how far these are normal in behaviour. ENSLIN (1918, pp. 29 and 720) gives classified lists of species according to the types of parthenogenesis they display, but these lists will no doubt be increased considerably as further results in breeding experiments are obtained (see WINKLER, 1920; VANDEL, 1931).

Of the species which reproduce by thelytokous parthenogenesis, only in the following have rare males not been produced in the course of breeding experiments: *Amauronematus semilacteus, A. puniceus, Croesus brischkei, Pachynematus obductus, Phyllotoma nemorata, Ph. aceris*. Aberrant males are exceedingly rare in some species as for example in the gooseberry saw-fly *Pristiphora pallipes* which has been reared for a number of years by PEACOCK (unpublished). In

this species thirteen males have appeared in twenty-eight generations. They are usually small and show great unwillingness to mate. In cases where a pairing has been obtained however, insemination has apparently been ineffectual, the offspring differing in no way from those of the virgin females. OSBORNE (1884) however, obtained a pairing which he believed caused an increased viability in the cocoons of fertilised eggs and an increase in the number of males in the second generation, indicating "a necessity for recurrence occasionally to sexual reproduction". A pairing was also obtained by VON SIEBOLD (1884) but the results are unknown. VAN ROSSUM (1904) crossed a *Cimbex lutea* female (arrhenotokous) and a rare male of *Cimbex fagi* but the progeny were all males showing that fertilisation of the egg had not taken place.

These aberrant males, as far as reproduction is concerned, are therefore functionless, and have in most cases lost the mating instinct. Such males may represent a remnant of a more primitive bisexual condition in species which have now become almost exclusively thelytokous.

2. Cases where females have been known to arise parthenogenetically in arrhenotokous species are exceedingly rare. VON SIEBOLD (1871, 1884) reared 1621 males and 13 females from unfertilised eggs of *Pteronidea ribesii*, but he supposed that these were introduced with the plant food. FLETCHER (1880) claims to have reared an aberrant female in a parthenogenetic generation of *Nematus curtispina*, while VAN ROSSUM (in WINKLER, 1920) obtained a female *Thrinax mixta* parthenogenetically, which later laid eggs from which hatched seven larvae. PEACOCK (1925) records the finding of a female in a parthenogenetic brood of *P. ribesii*. The explanation offered is that a haploid male ovum may have become diploid during early segmentation. Gynandromorphs which occasionally appear in parthenogenetic strains (PEACOCK, 1925) are regarded as 'halfway' connecting types, and such somatic abnormalities are explained in the same way, viz., one blastomere in the early cleavage becomes diploid through the daughter chromosome groups being retained in one cell during mitosis. These aberrant females usually appear as solitary individuals and are not due to hybridity as are the Eugster bees, but always occur in parthenogenetic broods.

Mixed Parthenogenesis. The occurrence of deuterotokous partheno-

genesis ('amphoterotoky', TASCHENBERG) has been reported in *Ametastegia equiseti* and *Pseudoclavellaria amerinae*. Both species were reared by VAN ROSSUM but again no pairings were obtained. In the first generation of *P. amerinae* he obtained eight females and three males, but it should be noted that these were not derived from one female, but were the progeny of three different females. WINKLER (1920) failed to appreciate this and therefore the statistics which he submits are misleading. It would seem, however, from VAN ROSSUM's own figures, that one sex, the male, predominates in parthenogenetically produced broods.

Parthenogenesis in the fern saw-fly *Thrinax macula* KLUG., resembles in some measure that existing in the agamic generation of *Cynipidae*. The species, thoroughly investigated by PEACOCK (1928), is for the most part thelytokous (twenty seven out of thirty one observed) but some females have been reared which are predominantly male-producers. It is thought that two kinds of stem-mothers occur, thelytokous (female-producers) and (arrhenotokous male-producers), but occasionally both types of parthenogenesis exist in the same female, so that parthenogenesis in these is really of the deuterotokous type.

Unfortunately the males appeared some days before the females so that matings were not obtained. The males were active however, and an attempt was made to obtain a crossing with *T. mixta* females. Artificial insemination was resorted to but again without having any effect on the progeny, which consisted entirely of *T. mixta* males.

Dissection of some of the *T. macula* males revealed well developed testes. Material was obtained for the investigation of spermatogenesis and a preliminary examination revealed that the maturation differs but slightly from spermatogenesis in other species and was therefore not pursued further, pending completion of work in *P. ribesii*.

It is interesting to note that the two types of parthenogenesis may exist in two closely allied species, e.g. *Thrinax macula* (thelytokous) and *T. mixta* (arrhenotokous), *Allantus pallipes* SPIN. and *A. calceatus* KLG. That thelytokous species have evolved from bisexual species is indicated by the occurrence of aberrant males and by the persistence of the spermatheca in certain thelytokous females, e.g., in *T. macula*, *Allantus pallipes* and *Pristiphora pallipes* LEP.

To summarise: from the foregoing review it is apparent that in *Hymenoptera* parthenogenesis is predominantly arrhenotokous, being typical of *Apidae, Vespidae* and *Formicidae*. In lower groups, *Ichneumonidae* and *Tenthredinidae*, thelytoky is common while the *Cynipidae* are peculiar in that they are heterogonous. In the 'Hymenopteran type' of sex-determination males and females are produced from fecundated females by the non-fertilisation or fertilisation of the egg, but it is not known how the female is able to control the flow of sperm from the spermatheca. VERLAINE (1926, in WHEELER, 1928) maintains that males of social *Aculeata* in general are the offspring of workers. He admits that old bee queens whose spermathecae are exhausted may produce drones, but he is emphatically of the opinion that drones are the offspring of the workers, even in hives where the queen is young and vigorous. WHEELER sees in this hypothesis the elimination of the problem regarding the functioning or non-functioning of the spermathecal pump in adaptation to worker or drone cells. VERLAINE's hypothesis, however, does not cover all the cases for the problem still remains in the non-social *Hymenoptera*. For example, in the arrhenotokous saw-flies, there is no differentiation into castes, and the eggs are deposited in apparently similar nidi. There are three conclusions possible at the moment regarding sex-determination by fertilisation: 1, that it is accidental, 2, that it is under the control of the female, or 3, that it is capable of a mechanical explanation, as in the case of the honey bee.

In thelytokous parthenogenesis sex-determination depends on the chromosome behaviour in the egg nucleus itself. Exceptional cases of thelytoky which we find in *Apidae* (Cape bee) and of mixed parthenogenesis as found in *Thrinax macula*, contribute nothing to the problem of sex-determination which cannot be better investigated in other groups.

2. *Cytological*

Parthenogenesis in *Hymenoptera* having been established by biological experiment as either arrhenotokous or thelytokous, investigations were undertaken to demonstrate the cytological phenomena involved, particularly during gametogenesis. In the majority, parthenogenesis is facultative, and oogenesis follows the normal course,

while spermatogenesis presents unusual phenomena, correlated with the peculiar mode of sex-determination — the so-called "hymenopteran type" (HERTWIG). The literature on the cytology of parthenogenesis in *Hymenoptera* was reviewed by NACHTSHEIM in 1913 and by VANDEL in 1931 but for the purposes of this paper it must be discussed here in some detail. The earliest contributions deal with *Aculeata* but more recently investigations have been confined to the parasitic *Hymenoptera*. In the following review the different groups are discussed separately, special attention being directed to the form and behaviour of the chromosomes. For details regarding chromosome numbers and reduction see Tables III—VII.

Apidae: BLOCHMANN (1889) studied the maturation of eggs of *Apis mellifica* from drone and worker cells and found in both that the nucleus undergoes two maturation divisions. Of the two polar nuclei liberated, the second divides again, so that three groups of chromosomes arise. These break up into granules and lie in a large 'vacuole' of protoplasm. PAULCKE's results (1899) agreed in the main with those of BLOCHMANN, with the exception that he found the two peripheral groups of chromosomes were daughter nuclei of the first polar body. The earliest worker to study maturation and chromosome behaviour with modern technique was PETRUNKEWITSCH (1901 and 1903) and his results may be summarised as follows. In the metaphase plate of the egg he found 16 chromosomes and thus was the first to present a chromosome count in this group. The first polar nucleus is separated by an equatorial division and at the second division the chromosomes of both oocyte and polar nuclei are reduced to eight. In the segmentation nuclei in eggs from both drone and worker cells he again found the number to be 16 and advanced the supposition that in the case of the unfertilised egg the number had been restored by a longitudinal splitting of the chromosomes. His drawings are clear, although presented rather diagrammatically, but, as NACHTSHEIM (1913) has pointed out, his interpretation was erroneous. NACHTSHEIM's view is that the chromosomes of the first plate are already in the form of bivalents, and the apparent reduction to 8 is due merely to a secondary temporary coupling of single chromosomes. PETRUNKEWITSCH (1901), however, on finding that the number of chromosomes increased to 64 in blastoderm cells of both sexes, suggested that the chromosomes were of different values in the drone and worker eggs —

univalent in the ♂ and bivalent in the ♀. From a further investigation (1903) he arrived at the erroneous conclusion that the male germ cells were derived from the "Richtungskopulationkern" which arises from the fusion of the two inner polar nuclei. As the first polar and second oocyte nuclei both undergo reduction, this fusion nucleus contains the diploid number and therefore the ♂ germ cells are diploid. He did not investigate spermatogenesis but he believed that the number was reduced to 8 during maturation of the sperm. With the exception of the work of DONCASTER (1906*a*), who found about 16 chromosomes in the young queen ovarian cells, no further papers on chromosome behaviour in oogenesis in the bee appeared until 1912, when NACHTSHEIM reinvestigated the maturation of the egg. In 1913 he published a more detailed account of the maturation processes in both ♂ and ♀in which he brought forward the hypothesis of the formation of "Sammelchromosomen" to explain anomalous results obtained by other workers. In the polar spindle of the egg he finds 8 dyads (dumb-bells), which, after the second division, separate into single chromosomes — 8 going to each daughter nucleus, as found by PETRUNKEWITSCH. These single chromosomes are considered of double value ('doppelwertig') because prior to segmentation each separates into two components. The unfertilised egg therefore develops with 16 single 'einwertig' chromosomes and the fertilised egg with 32. Thus he assumes that the diploid and haploid numbers are 32 and 16 for the ♀ and ♂ respectively, but as in the oogonia only 16 appear, he is forced to conclude that "coupling" has already occurred. Similar phenomena occur in spermatogenesis, which was first investigated by MEVES in 1904 and later in 1907. The main result of MEVES' investigation was the establishment of the fact that the chromosome number 16, found in the spermatogonia, is not reduced during maturation. A vestigial first maturation division occurs, the primary spermatocyte making an abortive attempt to divide, with the result that there follows the separation of an anucleated cytoplasmic bud, termed a 'polar body'. The second spermatocyte division is equational as regards chromatin but unequal as regards cytoplasm, for there is formed a second 'polar body' which disintegrates also. The chromosomes are in the form of "Doppelkügeln" and exhibit a tendency to unite to form false tetrads. At other times the chromosomes may break up into granules.

DONCASTER (1906) found not 16 single chromosomes in the spermatocytes but 8 double chromosomes, the two members of a pair being alike in size, and separating at the second division. His Figure 1, however, conveys the impression of 16 chromosomes lying near each other, but later (1907*a*) in agreement with MEVES, he found in the metaphase of the second division 16 double (dumb-bell-shaped) chromosomes.

Urged by the necessity of ascertaining the correct number of chromosomes, NACHTSHEIM made a partial reinvestigation of spermatogenesis in order to find out if any kind of 'Sammelchromosomen' were formed in the ♂ germ cells. He found that the 16 of the first spermatocyte are already split and in the form of dyads in preparation for the second spermatocyte division. Thus each spermatid receives 16 single chromosomes (half-dyads). Sometimes the chromosomes have a paired arrangement and thus there appear to be only 8 in each daughter group, as found by DONCASTER, but "the chromosome coupling is in most cases not so close that one cannot prove morphologically the doubleness of the individual chromosomes" (p. 213). NACHTSHEIM has expressed these phenomena in diagrammatical form and has drawn up a scheme to illustrate chromosome behaviour in the bee.

In Text Figure 8 is given an abbreviated scheme of the chromosome cycle, with figures to illustrate NACHTHEIM's conceptions of the morphology and 'value' of the chromosomes. It will be noticed that he uses the term 'chromosome' to designate a single body, irrespective of its value, but if the chromosome is morphologically double he uses the term 'dyad'.

A major criticism of NACHTHEIM's hypothesis of coupling is that supporting cytological evidence is in many places lacking. For instance, at no time does he figure 32 chromosomes in the primitive germ cells of the ♀ to prove that the 16 in the oogonia are actually of double value. Nor can he demonstrate 16 chromosomes in the oocyte of the first order. With regard to the ♂, at no time have 16 single undivided chromosomes been clearly shown in spermatogonia. In MEVES' figures the chromosomes always show a longitudinal split or are composed of two closely applied spheres. The latter are probably the rounded-off daughter halves, but even in the telophase the chromosomes appear as dyads. A further criticism of the hypothesis will be given later.

VANDEL (1931) submits a similar scheme to illustrate the chromosome cycle in the bee, but he omits one very important feature, viz. — coupling in male germ cells.

This phenomenon of coupling has led to erroneous conclusions in many cases. In *Osmia cornuta*, according to ARMBRUSTER (1913), the chromosome number in the spermatocytes is reduced from 16 to 8. This author at first refused to accept NACHTSHEIM's explanation of this apparent reduction and emphatically maintained that at no time did a split occur in the chromosomes, the 16 merely separating into two sets of 8 on the spindle. However, later (1919), because of the rudimentary nature of the first division and his failure to find synaptic phenomena, he was satisfied that reduction does not occur (NACHTSHEIM, 1921).

In *Xylocopa violacea*, investigated by GRANATA (1909), there is no question of the formation of "Sammelchromosomen", the chromosomes being large and hooked. In his first paper on spermatogenesis he illustrates the abortive first and the second divisions, but the question of reduction is left open to doubt, as he shows no figures of second anaphase divisions. In 1913, however, he submits figures to show that the original number 16 found in the first spermatocyte persists in the spermatid, thus proving that reduction does not occur.

Vespidae. Oogenesis and maturation of the egg in this group have not been investigated. Spermatogenesis has been studied in *Vespa germanica* (MEVES, 1904), *V. maculata* (MARK and COPELAND, 1907), and *V. crabro* (MEVES and DUESBERG, 1908), but in no one species is the investigation complete. MARK and COPELAND's figures nowhere demonstrate clearly the chromosome number and merely suffice to show that the first division is abortive and gives rise to a cytoplasmic bud composed chiefly of the "interzonal body". Spermatogenesis of *V. crabro* is very similar to that of the bee, but here again no spermatogonia are figured and the chromosome number is undetermined. The authors, however, believe that the number is higher than 16 and their Figure 9 certainly indicates a number nearer 20, although in the anaphase of the second division only about 10 are present (Fig. 21). As in the bee, the chromosomes first appear as split rods (about 20) which round up into double spheres. Allowing for the fusion of the two components of the dyad, or even for their separation on the spindle of the second division, one cannot easily account for the re-

duced number, unless by postulating that coupling has occurred. The maturation process in wasps differs from that in bees in that only the first division is unequal as regards cytoplasm, the second division resulting in the formation of two equal spermatids.

Formicidae. The cytology of parthenogenesis in this group is comparatively unknown. HENKING (1892), SCHLEIP (1908), and HOGBEN (1920) so far, are the only authors who have investigated oogenesis and maturation. HENKING finds in *Lasius niger* that two polar bodies are already formed when the fertilised egg is laid and that the second maturation division occurs immediately after oviposition. Ten chromosomes occur in the polar spindle and from this he concludes that there will be 10 in the oocyte nucleus also. After fertilisation 15 to 20 chromosomes are present in the segmentation nuclei, but in the unfertilised egg only polar nuclei are capable of division and sooner or later, abnormalities occur, the unfertilised egg as a result, being incapable of development.

SCHLEIP in his investigation of maturation in *Formica sanguinea* examined eggs from the fertilised queen and the unfertilised worker but did not examine the eggs of the normal unfertilised queen. He is unable to determine exactly the number of chromosomes, but about 24 are present (Fig. 2). This number is not reduced, for at later stages, when both second polar and second maturation spindles are formed, he is able to count 40 to 50 chromosomes on the late metaphase plate. In cleavage cells in the unfertilised egg he finds 20—21 and in the blastoderm not more than 24. Thus he finds no doubling of number in the unfertilised egg such as HENKING believed to take place. On the other hand in the unfertilised egg the number of chromosomes is about 48 in the first cleavage nucleus. On analogy with other work he assumes that the 24 chromosomes of the first maturation plate are bivalent, an assumption that is supported by the fact that certain of the chromosomes are ring-like, but he is unable to state at which division the separation of the bivalents occurs. The main result of this investigation is the establishment of the thesis that if reduction occurs it does so in the same way in parthenogenetic as in fertilised eggs. The fairness of this comparison is open to question, however, as he is dealing on the one hand with queen-laid eggs and on the other with worker-laid eggs. HOGBEN (1920), in the course of his investigation of synapsis in *Lasius flava*, found about 24 dot-like chromosomes

in the oogonia and about 11 thread-like chromosomes in the pachytene stage — thereby indicating that reduction would occur.

The investigation of spermatogenesis in ants was first studied by MEVES and DUESBERG but these authors did not make an exact study because of the extreme smallness of the chromosomes. They were, however, able to state that the process of maturation is of the same type as that of the wasp. This result was confirmed by LAMS (1908) in the ant *Campanotus herculeanus*. There is, however, some doubt as to the correct number of chromosomes owing to the phenomenon of coupling. It seems that the chromosomes appear as 16 dots and later, during the abortive first division, fuse to form 8 larger masses. In the resting stage previous to the second division the chromosomes appear as threads about 8 in number which in some cases show a split. Later, the double number of 16 occurs, and these arrange themselves on a spindle side by side. In the metaphase they appear as 8 doubles and in the anaphase as 8 singles. These single chromosomes, according to the figures shown, are half the size of the doubles and therefore appear to have arisen by a separation of the paired elements. If 8 is the haploid number, then the 16 chromosomes represent the precociously split halves of the original chromosomes which will later separate to the poles in the second division. If 16 is the number in the male germ cells, then the 8 doubles represent bivalent chromosomes, and if these separate at the second division, then the division is meiotic, but if the 8 doubles split lengthwise, as LAMS at one point indicates (Fig. 20 & Fig. 26), then the division is equational. Here again the author is more concerned with demonstrating the abortive first division and subsequent cutting off of a cytoplasmic bud. However, on analogy with the bee and in the light of NACHTSHEIM's work, it seems highly probable that the maturation does not involve reduction.

Terebrants. Of the chromosome cycle in Terebrants very little is known. The earlier papers deal with the appearance of the so-called germ cell determinant or "oosoma" — a cytoplasmic inclusion found in the ripe ovarian egg and in certain blastoderm cells. The only case where haploidy has been investigated is in *Paracopidosomopsis floridanus* (PATTERSON and PORTER, 1917). Spermatogenesis is of the type found in the wasp. The chromosomes are long and hooked and in the male germ cells and soma are 8 in number. The maturation of the egg is equally satisfactorily worked out, and it is worthy of note

that the figure of the first oocyte nucleus is, so far, the only one in investigated *Hymenoptera* which shows the diploid number of univalent chromosomes in the first metaphase plate. Although no diploid counts are given for oogonia, somatic cells in blastoderm and adult females show consistently 16 chromosomes. Other workers in parasitic *Hymenoptera* are chiefly concerned with the formation of a precocious and interrupted maturation spindle in the ovarian oocyte. The nucleus goes through the usual synaptic phases then forms an asterless spindle in which the chromosomes line up and finally clump. The existence of this spindle does not imply a reductive division in the subsequent formation of the polar bodies, although LEIBY (1922) was able to demonstrate reduction in the liberation of the first polar nucleus in *Copidosoma gelechiae*. It is rarely, however, that the chromosomes can be enumerated, as the maturation divisions generally appear to be atypical and amitotic. The following is a list of parasitic *Hymenoptera* in which abortive spindles have been demonstrated: *Ageniaspis fuscicollis* (MARTIN, 1914), *Copidosoma gelechiae* (HEGNER, 1914; LEIBY, 1922), *Trichogramma evanescens* (GATENBY, 1918), *Orthopelma luteolator* (HOGBEN, 1920), *Apanteles glomeratus* (HEGNER, 1914), *Diastrophus erinaceae* (HEGNER, 1915), *Andricus punctatus* (HEGNER, 1915), *Neuroterus lenticularis*, *Cynips kollari*, and *Rhodites rosae* (HOGBEN, 1920). In all these in which maturation has been followed through to the liberation of polar nuclei, mitoses appear to be atypical. In *Platygaster dryomyiae*, SILVESTRI (1915) does not record finding a condensation spindle. Three polar nuclei are formed in the unfertilised and fertilised eggs of the following: *Litomastix truncatellus* (SILVESTRI, 1906), *Ageniaspis* (SILVESTRI, 1908; MARTIN, 1914), *Copidosoma buyssoni* (SILVESTRI, 1914), *Paracopidosomopsis* (PATTERSON, 1917), *Copidosoma gelechiae* (LEIBY, 1922). In short, the process of egg maturation involves, 1, the formation of an asterless spindle in the late ovarian oocyte; 2, the concentration of the chromosomes; 3, the formation of two atypical maturation spindles by which three polar nuclei are liberated; 4, reduction in chromosome number, at least in arrhenotokous species. What may be the significance of the abortive spindle has not been determined, but in all probability it constitutes the initial stages of diakinesis. The fact that similar processes occur in the *Cynipidae* (DONCASTER, 1911 and HOGBEN, 1920) would indicate that the phenomena are in some way related to the

peculiar biological conditions of parasitism and gall formation.

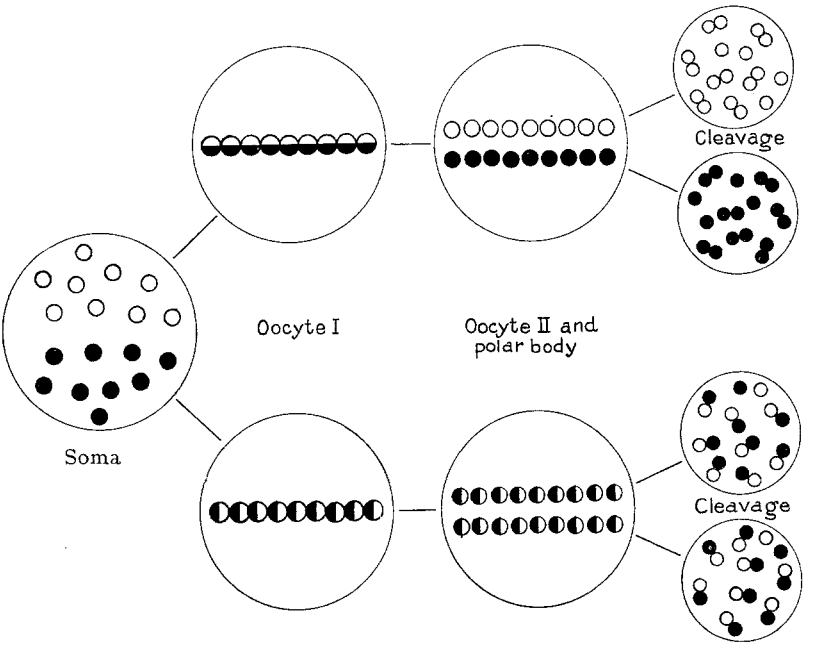
Cynipidae. The cytology of parthenogenesis in *Cynipidae* was first investigated by HENKING (1892) in the thelytokous gall-fly *Rhodites rosae* but with regard to chromosome reduction during maturation he could arrive at no definite conclusion. When the egg is laid the chromosomes in the nucleus appear as clumps, about 9 in number. In the first maturation spindle he figures 9 chromosomes passing to one pole, and in the second spindle, which forms immediately after the first is completed this number still obtains. The nucleus then sinks into the yolk and later segmentation nuclei show that the number of chromosomes has been doubled. This number, 18, is considered by HENKING to represent the normal number, but he is unable to decide whether the 9 found in the first spindle are true bivalents or are merely paired chromosomes which have come together temporarily. He appends a schematised diagram of polar body formation in which he shows how the chromosomes would be shared out according to the nature of the divisions — equational or reductional. If reduction occurs and is followed by a splitting of each chromosome into two in cleavage nuclei, then only 9 chromosomes will be represented in the soma, although 18 (two of each chromosome) are present. If, however true reduction does not occur and the formation of bivalents is temporary only, then, after two equational divisions each of the original 18 chromosomes will be represented in the cleavage nucleus. Thus the dyads found in early cleavage nuclei may have arisen in one of two possible ways, as shown by HENKING. This will be more evident from a study of the diagram appended below — Text Fig. 1.

This difficulty of distinguishing cytologically the two kinds of dyads is still before us, but so far this problem seems to have received but little attention.

The fate of the polar bodies was not followed out. SCHLEIP (1909) in the same species, *Rhodites rosae,* found that reduction does not occur in the egg. There are two equational maturation divisions which result in the formation of four nuclei, each with about 12 chromosomes. The segmenting egg-nucleus retains for a time the normal number of 12, but later in the blastoderm only 6 chromosomes occur, "but whether these 'doppelwertig' chromosomes exist only in the somatic cells, or also in the germ[1]) cells and split into their components later, remains an open question".

[1]) early?

HOGBEN (1920) who studied synapsis in certain *Hymenoptera* in relation to the production of females from virgin eggs, reinvestigated oogenesis and maturation in *Rhodites* and was able to corroborate and add to HENKING's conclusions. In the oogonia 18 chromosomes are present but in the young oocyte and nurse cells parasynapsis occurs whereby the number is reduced to 9. This haploid number of bivalents in the oocyte condenses on an asterless spindle, and later, the chromsomes reappear as 18 filamentous threads (Fig. 32). Thus



TEXT FIGURE 1. Diagram to illustrate two possible ways of dyad formation (after HENKING).

the chromosomes undergo a temporary separation between synapsis and mitosis. The significance of this precocious abortive maturation spindle, which has been found in the ovarian oocyte of several other parasitic *Hymenoptera,* is not understood, but is thought to be the initial stage in the process of reduction. HOGBEN has not investigated the maturation of the egg in *Rhodites,* but of the rival conclusions of HENKING and SCHLEIP he is inclined to accept those of HENKING, on the ground that SCHLEIP's preparations were unsatisfactory. Neither HENKING nor SCHLEIP studied oogonial or somatic mitoses in de-

veloping pupae. HOGBEN examined nerve cells, wing epithelium, and follicle cells, which in *Hymenoptera* are germinal in origin, and found 18 chromosomes, thus showing that the number is in some way doubled subsequent to maturation and early development. HOGBEN's explanation of this "Verdoppelung" of the chromosome number is that: 1, either the polar mitoses are equational and doubling is by disjunction of bivalents; or, 2, the chromosomes which pair in synapsis are equivalent daughter halves of a single chromosome which has divided at some previous time.

Similar phenomena occur in *Cynips kollari, Synergus rheinhardii,* and *Orthopelma luteolator* (HOGBEN, op. cit.). In the oogonia of *Cynips* there are about 20 irregular chromosome masses which, later, in the oocytes preparatory to their differentiation, form 10 pachytene threads. After a short period of separation, these again pair endwise, on a precocious abortive maturation spindle. The somatic number found in pupal epithelium and follicle cells is 20, so that here again the chromosomes are restored to the normal number.

The most satisfactory account of maturation phenomena in *Cynipidae* is that of DONCASTER (1910, 1911, 1916) who investigated chromosome conditions in the cyclic species *Neuroterus lenticularis-baccarum.* In the spring generation there exist two kinds of parthenogenetic females which lay eggs differing in their behaviour as regards maturation (1910). In eggs laid by one class of female no reduction occurs and no polar chromosomes are ever found. The oocyte nucleus, which appears to have 20 chromosomes, reaches the prophase only, then sinks into the yolk and forms the first cleavage spindle parallel to the egg margin. No chromosome counts were obtained from oogonia, but DONCASTER (1910) states that in an egg from an egg-tube of a young pupa 20 chromosomes were seen in the nucleus, and later (1911) that there are "almost certainly more than 10". We do not know whether these females were male-producing or female-producing as DONCASTER was not then aware that these could be classified according to their origin from different types of sexual females. In the egg nucleus of eggs of this type the number appears to be 20, so that it was concluded that no reduction occurs. In eggs laid by the second class of female the nuclear divisions result in the formation of an inner egg-nucleus and three groups of polar chromosomes which degenerate. In the primitive ova about 20 chromosomes are found,

and, although no actual chromosome count was obtained in the oocyte of the first order, the fact that these eggs segment with only the haploid number 10 shows that reduction has occurred. These two types of eggs give rise to the bisexual generation, the first type giving females and the reduced type giving males. The maturation of the summer egg was worked out by DONCASTER (1910) but, on discovering, by breeding experiments, that the sexual females of the summer generation give exclusively either ♂-producing (arinophorous) or ♀-producing (thelyophorous) agamic females he was led to reinvestigate the matter. In the maturation of the summer eggs he finds indications that two distinct types occur, although these differences are not considered sufficiently important to justify their correlation with sex phenomena. In all cases the nucleus approaches the egg margin and there enlarges, while the differences found are as follows. In one type a top-shaped spindle forms and on it the chromatin is arranged at the inner and outer poles. In the second type the nucleus remains spherical and contains a well-marked reticulum, and later the chromosomes appear as double threads which separate into two groups. A second division occurs but the nature of this is not determined. In the first type three distinct groups of polar chromosomes are formed, and in the second type only two groups. These peculiar mitoses which occur in maturation divisions of both sexual and parthenogenetic eggs of *Neuroterus* are compared by DONCASTER to those found by HENKING in *Rhodites*. The final result of division in the parthenogenetic summer egg of the first type is *three* groups of polar chromosomes and an egg nucleus which probably contains 10 chromosomes, as does the pronucleus in the ♂-producing egg of the agamic generation. Syngamy of ♂ and ♀ pronuclei restores the diploid number 20, which appears in segmentation nuclei.

In the germ cells of the male the haploid number persists. DONCASTER figures 10 chromosomes on the spermatogonial spindles and shows that the first spermatocyte division is abortive as in the bee. The second division is equational and gives rise to two equal spermatids each with 10 chromosomes. In second spermatocytes there is present a prominent chromatoid body, which at mitosis passes to one cell only, so that only half of the spermatids receive this body. Certain somatic nuclei in the male also retain the haploid number, a fact which lends additional support to the suggestion that it is the

reduced eggs of the spring generation which give rise to the males.

The spermatogenesis of a closely allied gall-fly *Dryophanta erinacea* has been studied by WIEMAN (1915) whose results differ in many points from those of DONCASTER in *Neuroterus*. He finds no definite interkinetic resting stage between first and second spermatocyte divisions, nor does he describe any small extranuclear body. The chromosome number also is different, the American author finding 12 instead of 10 chromosomes in the ♂ germ cells. A stage which resembles the bouquet stage occurs before the abortive division but no special significance was attached to this. As the number of chromosomes is unreduced during maturation of the sperm he assumes that 12 is the haploid number. But the number in the female appears to be very much less than the expected number 24. He finds in the follicle cells of the female only 13 or 14 chromosomes, which leads him to suggest that the males and females of the bisexual generation develop from eggs whose chromosomes have undergone reduction in maturation. "The slightly larger number of chromosomes found in the female somatic tissues may or may not be of significance, but if sex determination has its basis in the chromosomes, a difference in the method of distribution of the chromosomes in the maturation may explain why some of these eggs develop parthenogenetically into females and others into males". This species seems to be well worth further investigation as the chromosomes are large and are not mere morphologically 'similar dots' like those of *Neuroterus*.

Tenthredinidae. It is to be regretted that the cytological investigation of parthenogenesis in saw-flies has hitherto received so little attention. The group presents convenient material for the study of arrhenotoky and thelytoky, the cytology of which (particularly the latter) is comparatively unknown. The first to make a cytological study of the group was DONCASTER who in 1906 investigated the maturation of unfertilised eggs of *Pteronidea* (*Nematus*) *ribesii* SCOP., *P. melanaspis* HGT., (*N. lacteus*), *Empria abdominalis* F., (*Poecilosoma luteolum*), *Pteronidea pavida* LEP. (*N. pavidus*), *Croesus varus* VILL., and *Hemichroa crocea* GEOFFR. (*H. rufa* PANZ.) [1]).

DONCASTER (1906) found that in the polar mitoses of all species the chromosomes numbered 8, and he was led to conclude that reduction

[1]) Specific names in brackets are those used by DONCASTER.

does not take place, as at no time did he find any evidence of tetrad formation. He was, however, willing to allow the possibility that synapsis had already occurred, in which case the chromosomes appearing on the spindle would be in the reduced form. In 1907, however, work on *P. ribesii* led him to state that two types of maturation occur: 1. In some eggs successive equational divisions give rise to an egg-nucleus and three polar nuclei each with 8 chromosomes; 2. The second type of egg undergoes reduction at the second maturation division, the ripe egg-nucleus containing but 4 chromosomes. DONCASTER believed that these reduced eggs only were capable of fertilisation, this view gaining some measure of support from his findings in spermatogenesis in 1907. In the spermatogonia he found 8 chromosomes but in the spermatocytes these appeared as 4 "gemini" at the onset of the meiotic phase, the resulting spermatids having but 4 chromosomes each. DONCASTER could not demonstrate the actual conjugation of ♂ and ♀ pronuclei but believed that "true fertilisation may take place". Thus fertilised and non-reduced unfertilised eggs develop with the diploid number of 8 chromosomes, this number being found in segmentation and blastoderm nuclei, in spermatogonia and oogonia. In the mitoses of the ovary sheath, however, he found a number greater than 8 and suggested that the germ cells are possibly compound, as asserted by PETRUNKEWITSCH for the bee.

The fate of the reduced unfertilised eggs he was able to follow only as far as the blastoderm. As in the somatic cells at this stage sometimes only 4 chromosomes, but more commonly 8 occur, DONCASTER makes the suggestion that the chromosome number in the reduced egg may be restored by division of the compound chromosomes.

As to the fate of the three polar nuclei he found differences in the eggs of arrhenotokous and thelytokous species. In all cases the outer polar nucleus (daughter half of the first polar nucleus) degenerates. In the three arrhenotokous species *P. ribesii*, *P. lacteus* and *P. pavida* the two inner polar nuclei approach and lose the nuclear membranes, and may give rise to two groups of chromosomes, which may unite into one group, while each chromosome may split into two. Finally the chromosomes scatter and are lost. In thelytokous species, on the contrary, the inner polar nuclei lie further apart, move to the egg margin and disintegrate. In *Empria abdominalis* one group persists for a long time, while in *C. varus* only the inner polar nucleus resolves

into chromosomes, each of which splits into two. DONCASTER did not consider that this contrast in the two kinds of eggs supplied sufficient evidence regarding the causes which determine sex, but he believed "that some indication is offered of the direction in which the solution of the problem is to be sought".

DONCASTER's findings were at variance with those obtained by MEVES (1907) in the bee, and as a result of his subsequent reinvestigations, he published a corrective note (1909), pointing out that in spermatocytes in the saw-fly only one division occurs, the chromosome number being 8, while in the spermatogonia, although not determined exactly, it appeared to be about 16. This result, however, was not in accord with results obtained from oogenesis and maturation, and so he left the matter *sub judice*. The investigation was not resumed and the problem was left unsolved.

PEACOCK (1925 and 1928) investigated spermatogenesis in the arrhenotokous species *Pteronidea melanaspis* (*Nematus lacteus*) whereby he definitely established (1925) that spermatogenesis resembles that of the bee in that no reduction occurs and later (1928) that the primary spermatocyte makes an abortive attempt at division.

2a. SUMMARY OF TABLES III—VII

1. *General.* From the foregoing text and tables it is apparent that most workers in Hymenopterous cytology have investigated chromosome conditions during spermatogenesis or oogenesis only, especially during the period of maturation. The chromosome number has not always been determined exactly, for in most cases the chromosomes are extremely minute spherical bodies which frequently tend to unite in pairs during certain phases. This phenomenon of "coupling" has led many authors to erroneous and contradictory conclusions. For example, ARMBRUSTER in *Osmia cornuta* shows sixteen spherical bodies in the second spermatocyte and only eight in the spermatid (Figs 39, 42) certainly indicating reduction. PETRUNKEWITSCH figures an apparent restoration of the "diploid" number after maturation and reduction in the egg of *Apis mellifica*. He was of the opinion that the sixteen found in the oocyte of the first order represented the diploid number, whereas MEVES (1907) found sixteen in the haploid drone. To explain such anomalous results NACHTSHEIM (1913) advanced the

hypothesis of the formation of "doppelwertig" chromosomes, whereby he seeks to prove that chromosomes unite temporarily in pairs. The phenomenon is not wholly confined to the auxocytes, for NACHTSHEIM finds "Sammelchromosomen" already formed in the oogonia.

Strangely enough, the importance of chromosome enumeration in gonia seems to have been overlooked, spermatogonia and oogonia having been examined in but few species. Spermatogenesis has been well explored, particularly the first abortive division and the fate of the spermatids. In many cases, however, the formation of one or two polar bodies is set forth as evidence of non-reduction without details of chromosome number being given, e.g. *Apis mellifica* and *Vespa maculata* (MARK and COPELAND); *V. crabro* (MEVES and DUESBERG). Similarly, undue importance has been attached to the formation of polar nuclei in the egg as evidence of reduction. Counts in oocytes of the first order are difficult to obtain, the most successful results being derived from studies in the synaptic phases in *Neuroterus* (DONCASTER, 1916), *Cynips*, *Orthopelma* (HOGBEN, 1920), *Copidosoma* (LEIBY, 1922). PATTERSON alone in *Paracopidosomopsis* (1917), has been able to demonstrate the diploid number of bivalent chromosomes in the prophase. Oogonia provide a useful and convenient source for obtaining diploid counts, but as already stated, these have been investigated in but few species. Reduction appears to occur in the first maturation division, although PETRUNKEWITSCH found the second to be the meiotic division.

2. *Maturation and meiosis.* The first maturation division in spermatogenesis is abortive in every species examined, resulting in the extrusion of a small cytoplasmic bud — the so-called polar body. The second division is equational, except in *Osmia* (ARMBRUSTER) and in *Apis* (according to JEGEN, 1920). LAMS leaves the point undecided. In the *Apidae*, *Osmia*, and *Xylocopa*, only one functional sperm is produced from each primary spermatocyte, the second division being again unequal as regards cytoplasm. In the wasps, ants, gallflies, sawflies (*N. melanaspis*) and the chalcid *Paracopidosomopsis* two functional sperms are produced, the second division being equational as regards cytoplasm and chromosomes. JEGEN (1920) attaches importance to the inclusion of a chromatoid body in one of the two spermatids. He considers that the second "polar body" does not disinte-

grate but becomes a functional sperm differing from the larger one in sex-determining property.

In the female, reduction has been definitely proved in the following: *Apis*, *Lasius*, *Neuroterus* (♂-producing agamic female), *Copidosoma* (LEIBY) and *Paracopidosomopsis*. The evidence of SCHLEIP in *Formica* and *Rhodites* is unconvincing, while HOGBEN, although corroborating HENKING's results, is yet unable to state whether chromosome reduction occurs or not. DONCASTER's evidence in *P. ribesii* is inconsistent and incomplete; his work on other species of saw-flies, especially in ♀-producing species, likewise requires revision. DONCASTER built a theory of sex-determination round the behaviour of the polar chromosomes which in many cases fuse and undergo division, but in all cases except in polyembryonic parasitic chalcids, where they form a trophic "paranucleus", the polar chromosomes degenerate. The condition in the ♀-producing parthenogenetic egg of *Neuroterus*, where no polar chromosomes occur, is unparallelled in any other animal, as far as is known.

3. *Somatic.* With regard to chromosome enumeration in somatic cells, very few tissues have been examined expressely for this purpose. Most authors are content with counts obtained from follicle cells, but these are not always reliable. DONCASTER (1910), was the first to investigate mitotic cells in developing pupal tissues. WIEMAN, PATTERSON, and HOGBEN have followed his lead and in every case very clear figures are obtained. Evidence from cleavage and blastoderm nuclei is more abundant, but figures from this source are never very clear, and counts accordingly not very accurate, the chromosomes for the most part being extremely small. Generally, however, diploid and haploid cells can be distinguished, although only an approximation of the numbers is possible.

4. *Evidence of haploidy and diploidy.* Evidence for ♂ haploidy and ♀ diploidy is very restricted. NACHTSHEIM has made a fairly complete study of chromosomal conditions in the honey-bee, but his cytological evidence in regard to ♀ diploidy is weak. It will be observed that the diploid chromosome number in the ♀ is derived solely from cleavage and blastoderm nuclei. In the oogonia, follicle and nurse cells the number appears to be 16, the same as in the ♂ germ cells. The latter numerical anomaly he explains by advancing his theory of coupling. The theory is sound enough and is built on accurate obser-

vation, yet in some ways it does not always fit the facts (see page 402).

DONCASTER presents fairly complete evidence of haploidy in the cyclic *Neuroterus lenticularis*, the haploid number 10 persisting throughout in spermatogonia, sperm, embryo and adult ♂ cells. The progeny of the agamic ♀ is of two kinds as witnessed in cleavage and developing wing cells, the haploid individuals with 10 being male and the diploid individuals being the females of the bisexual generation. In 1910 DONCASTER on finding 20 in ♂ somatic cells suggested that the chromosomes in the germ cells were bivalent, but he failed to appreciate that the anomalous counts in developing nerve and wing cells were due to polyploidy. It is worthy of note that WIEMAN found the same number, 12, in ♂ and ♀ tissues of *Dryophanta*, but in this case both are evidently haploid (?) organisms. His figures and his arguments in favour of haploidy in the bisexual ♀ are convincing enough but on his own showing the evidence on this point is insufficient to lead to any definite conclusion regarding the origin and significance of this condition.

PATTERSON and PORTER present conclusive evidence for haploidy and diploidy in the chalcid *Paracopidosomopsis*, the only lacunae occurring in regard to counts in oogonial and male embryonic cells.

With regard to *Tenthredinidae*, DONCASTER's results were so conflicting that the whole question of haploidy was left *sub judice*.

5. *Chromosome numbers*. It will be noticed in Tables III—VII that the diploid numbers, as established in the thirteen species of bees, wasps, ants and saw flies, are multiples of the basic number eight. In the gall flies the haploid number varies from nine to twelve, being definitely established as ten in *Neuroterus* (DONCASTER), and *Cynips* (HOGBEN).

6. *Polyploidy*. It has been suggested by VANDEL (1931) that males of *Hymenoptera* and Rotifers may have a diploid soma and a haploid germplasm. He bases his argument on the findings of PETRUNKEWITSCH (1901) and TAUSON (in VANDEL, 1931, p. 231). The former found 64 chromosomes in both ♀ and ♂ blastoderm cells in the bee. DONCASTER, MEVES and ARMBRUSTER likewise found multiple numbers in blastoderm and follicle cells. The explanation offered by PETRUNKEWITSCH is that the 16 chromosomes (diploid number), which appear in the segmentation nuclei of the fertilised and unfertilised eggs, are of multiple value — "vierwertig". In blastoderm

nuclei of drones from worker-eggs he found 32 chromosomes and came to the conclusion that these were "doppelwertig". MEVES concludes that "im Lauf der Entwicklung die 16 Chromosomen des Drohneneies in den somatischen Zellen jedes in vier, die 32 chromosomen dagegen, die nach meiner Annahme im befruchteten Ei vorhanden sind, jedes in zwei zerlegt werden". DONCASTER suggested that in all probability the chromosomes in the gonads are compound. NACHTSHEIM applies his theory of coupling and fragmentation by which the chromosomes in the male would be regarded as "vierwertig" as against the "doppelwertig" chromosomes in the female. The fact that NACHTSHEIM never found more than 16 in the male and 32 in the female blastoderm shows that one must be very careful to avoid polyploid cells in submitting evidence of chromosome counts.

III. THE BIOLOGY OF PTERONIDEA RIBESII SCOP.

Pteronidea ribesii SCOP. is an arrhenotokous species of saw-fly in which males and females appear in about equal numbers. The larvae live on the leaves of the currant or gooseberry in which they eat irregular holes. Flies lay readily in captivity whether fertilised or not, and while engaged in egg-laying the female takes up a position on the under side of the leaf, with the head towards the base of the lamina. The eggs are laid in rows along the veins, each egg being clipped and held in position by the sides of the nidus which the fly makes in the vein by means of the saws. The average number of eggs produced is 115 but these rarely, if ever, all develop.

There are as many as four generations per year, the rate of development depending on the season. The period of eclosion extends from 6 to 10 days and the larval period from 13 to 26 days, during which the larvae moult four times. At the last larval ecdysis the mottled larval skin is cast off and the body of the larva assumes a jade-green colour, with the exception of the head and tail regions, which are pale yellow. In the winter generation the larva lies dormant within the cocoon until more favourable conditions obtain in the spring, about the end of April, but in the summer broods, the pupal period, from the last larval ecdysis to the emergence of the adult fly, extends from 15 to 18 days only.

The adult flies if fed regularly on syrup solution live for about 10

days. The male is much smaller than the female during both larval and pupal instars, and it is worthy of note that the male develops relatively more quickly than the female.

Sometimes great difficulty is experienced in obtaining matings, the flies frequently showing no sexual instincts whatever. Males usually mate but once, but one case is on record where four consecutive pairings were obtained with the same male at intervals of two hours and on two separate days, with the interesting result that, of the four females fertilised, three yielded females only. The second female inseminated failed to lay. Unfortunately a record of the mortality ratio was not kept so that the experiments are not critical.

IV. MATERIAL AND METHODS

1. *Material.* The species of saw-fly used in these investigations was *Pteronidea* (*Nematus*) *ribesii* SCOP. The majority of the insects and larvae were bred in the laboratory but many, particularly the larvae of the first spring generation, were collected in the field. Female larvae can be distinguished from male larvae of the same age in healthy mixed broods, by their larger size and somewhat longer larval life.

Preparations have been made in several other species with a view to checking and supplementing results obtained in *P. ribesii*. It was found necessary on occasion, to turn to these, especially when difficult points such as order of seriation in maturation of the spermatocytes had to be decided. Only in one species (*P. ribesii*), however, does the information available permit a complete report.

I have been fortunate in having at my disposal the cytological preparations of the late Professor DONCASTER. Of these a few are on the early gametogenesis in both male and female but the bulk of the collection consists of serial preparations on the maturation of the egg, in all, over 300 slides with about 630 eggs. In addition there are several slides on early cleavage, blastoderm and embryo formation. The necessary acknowledgement of the part these have played is made later.

2. *Technique.*

a. *Fixation.* It was found more satisfactory to dissect out the gonads in RINGER Solution before fixation, as, when the gonads were fixed *in situ*, the solution did not penetrate quickly enough, and

clumping of chromatin resulted. Different fixatives were used but by far the best results were obtained with BOUIN's Picro-formol with Urea, especially in preparations where chromosome counts were desired. Corrosive Acetic Solution is rather harsh and causes undue shrinkage, but it was found necessary to fix much of the material by this fixative for subsequent staining by FEULGEN's 'Nuclealreaktion' method (see below). When gonads were dissected from still colourless pupae in order to obtain maturation stages, the thorax with the wing buds was also preserved in Bouin. Good preparations of adult tissues were difficult to obtain owing to the difficulty of striking the right stage for fixation.

For the examination of blastoderm and embryonic tissues, eggs were fixed at intervals from $1\frac{1}{2}$ to 8 days' incubation, and, as at this stage there are no gross differential characters to distinguish the sexes, control preparations of male tissues only were taken from eggs laid by virgin females.

For the study of the maturation in the egg, virgin and inseminated flies were set up with gooseberry twigs under belljars and the times of commencement of oviposition noted. As the fly lays eggs in sequence, at intervals of about one minute, along the vein, beginning at the distal end, one can obtain a series of eggs at different stages by fixing the whole series on the leaf at one time. At first, eggs were fixed from $1\frac{1}{2}$ to 3 hours after oviposition, but as it was found that the nucleus may complete the first maturation division in the first five minutes, absolutely fresh-laid eggs had also to be fixed. Experience also proved that Bouin is not suitable for eggs, as it is too difficult to wash out, and as a result renders the eggs very brittle.

The best fixative tried, in so far as eggs treated by it are less prone to fragment in sectioning, is PETRUNKEWITSCH's modification of GILSON's Fluid, recommended by DONCASTER (1906). In my opinion this fixative tends to cause abnormal swelling and is detrimental to certain nuclear inclusions (see under oogenesis, p. 389), but, on the other hand, as it seems to have no bad effect on the chromatin (DONCASTER's very excellent material was fixed by this method) and as only in a small percentage of the eggs is the embedding defective, this disadvantage is to be overlooked. Corrosive Acetic is rather harsh and causes shrinkage and even greater fragmentation of the eggs than Bouin.

b. *Embedding.* Gonads as a rule were cleared in xylol and embedded in paraffin wax, M.Pt. 45° or 56° C., but with large objects oil of cedar wood was used. The chief difficulties encountered were those of embedding the eggs, but, as pointed out above, success depends greatly on the nature of the fixation. After dehydration eggs were placed in the oil for over 24 hours, then immersed in xylol for a few minutes to remove the surplus oil and then were embedded in wax (M.Pt. 56° C.) for at least 3 hours. More recent experience shows, however, that far better results are obtained when the eggs are left in the clearing agent for three days at least and that a longer period than three hours in the embedding oven is in no way detrimental.

With regard to the late blastoderm and embryo preparations it was found that quicker and better penetration of wax resulted if the hard chorion was removed. The method of embedding under an electric lamp (McCLUNG, 1929), was tried out on several series of eggs on the leaf and was quite successful. Small objects which cannot be handled with forceps require to be placed in small paper carriers, so that they can be removed to the final embedding chamber.

Sections of all material were made 5 μ in thickness. Regarding the eggs, at first, a great many were sectioned longitudinally, having been freed from the leaf previous to being embedded, but these sections were exceedingly prone to fragment. Transverse sectioning gave better results, but in the end it was found best to embed the eggs on the leaf. Eggs embedded in this way offer a better resistance to the knife-edge and therefore do not break up so readily. Moreover, the little protoplasmic patch which contains the nucleus and polar chromosomes is to be found in the anterior third of the egg, so that, even if the orientation of the egg on the leaf is not known, only the first and last thirds of the sections need be examined.

c. *Staining.* As in most cytological preparations destined to show chromatin behaviour, HEIDENHAIN's Fe. Haematoxylin gave the best results. In my opinion the short method (10 minutes in Fe. Alum, 5%; 20 minutes in Fe. Haematoxylin, 5% solution) is often to be preferred to the long method, on the following grounds: 1. In every case where experiments have been done to compare results by the two methods, preparations stained by the long method are invariably more shrunken in appearance, especially in the case of the cytoplasm, due no doubt to the prolonged action of the mordanting solution;

2. Chromosomes stained by the short method show up an intense black in contrast to the relatively pale cytoplasm; 3. Cytoplasmic inclusions do not stain so darkly by the short method, but one can obtain rapid differentiation between these and the chromatin, without the prolonged destaining necessary in the longer method; 4. In the investigation of synapsis in the early oocyte the chromatin threads show a well pronounced split between the conjugants by the short method, but by the long method these are rendered "fuzzy" and indistinct; 5. During the maturation process in the egg, the yolk takes on an intense black colour if stained for a long period, but, if rapidly stained in a fresh strong solution for 20 minutes, almost all the stain will be given up by the yolk in the differentiating solution before the chromatin loses its stain.

Of course the short method is only to be recommended in cases where chromatin only is the objective, as other cell inclusions do not retain the stain. The Haematoxylin solution must be fresh and strong and care must be taken also to remove all traces of the differentiating Fe.Alum, otherwise the stain will fade. I have some preparations which were made over a year ago and these still retain the original intensity and I do not consider that they are at all likely to fade.

In most cases, when the sections were being dehydrated, a faint counterstain of "Licht Grün" in 90% alcohol was given. Thionin and Gentian Violet were tried when looking for nuclei in the yolk but these stains gave no particular advantage, as they were too easily washed out by the alcohols and were only selective when allowed to act for a few minutes.

MALLORY's Phosphomolybdic-Fuchsin stain was used on sections of the pupal thorax to obtain differentiation of the different tissue cells, but with poor success. This investigation did not warrant the expenditure of the time required to master the technique of so capricious a stain and so the method was abandoned. Other stains which I was led to use while trying to trace the history of the chromatin through the growth phases in the oocyte were AUERBACH's Methyl-Green-Fuchsin and MANN's Methyl-Blue-Eosin. Of these the latter gave good results, but with the former great difficulty was experienced in picking out the basophil structures.

d. *Feulgen's "Nuclealreakion"*. The use of FEULGEN's "Nuclealreaktion" method for the absolute differentiation of the chromatin

has met with marked success. The reaction is based on SCHIFF's test for aldehydes and it provides a precise chemical test for chromatin. When the correct procedure is employed no other structures in the cytoplasm except the chromatin gives the characteristic colour reaction. Of the suitable fixatives recommended by LUDFORD (1928) the only one tried was Sublimate Acetic but, although excellent results have been obtained with material thus fixed, I find that even a brighter reaction is obtained with material fixed in CARNOY's solution. This is probably due to the action of the Chloroform in the fixing fluid which removes certain cytoplasmic inclusions which tend to mask the chromatin. Results obtained after fixation in BOUIN were open to doubt.

The method has been employed to determine the true nature of the chromatoid bodies in male and female germ cells and also to trace the history of the chromatin during the so-called diffuse stage, during the growth phase of the oocyte. The technique involves hydrolysis at 60° C. which causes many of the sections to float off the slides, particularly those of ripe ovaries and eggs.

It has been found in numerous cases during maturation and subsequent stages that the yolk globules take on a pale pinkish tint and consequently the technique was thought to be incorrect. No eggs, however, have yet given the correct(?) reaction and the explanation may be that the yolk at this stage contains a large amount of nucleic acid, which gives the reaction. Possibly this is derived from the large quantity of chromatin passed into the oocyte with the nurse cell nuclei. This must undergo some chemical change after the egg is laid, for the yolk is uncoloured in all eggs within the ovariole. Unfortunately, all series of sections of eggs during maturation have been incomplete, so that as yet no chromosomes have been found by this method, and I am therefore unable to check the accuracy of the technique.

V. CYTOLOGICAL OBSERVATIONS

1. *Spermatogenesis*

(1) External morphology of the testis.

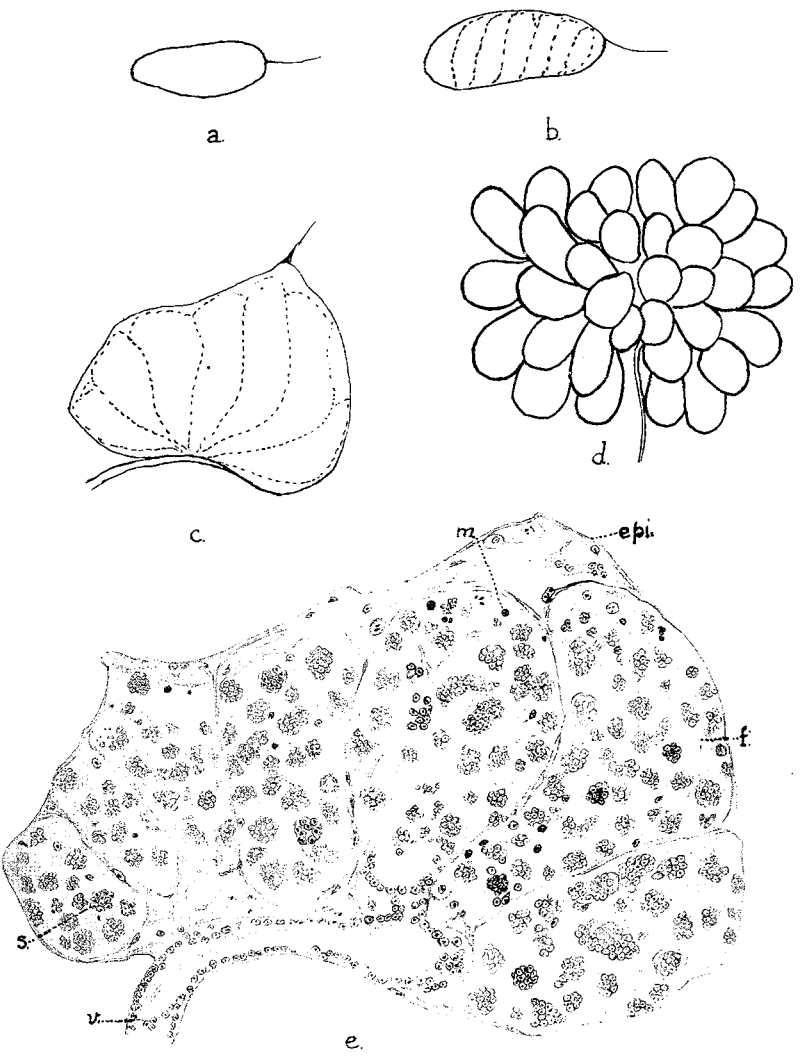
The paired testes are situated on the dorsal side of the 5th abdominal segment, embedded in a sheath of fat. In young larvae they

are difficult to find but in larvae which have reached the third instar they can easily be dissected out with the aid of a binocular microscope. They are white translucent bodies measuring 5 mm. and easily distinguished from the more opaque fat spherules. At this stage they contain primordial germ cells and primary spermatogonia (Text Fig. 2*a*). In older larvae, just before the last ecdysis, the testes are kidney-shaped and measure about 1 mm. in length. The follicles at this stage (Text Fig. 2*c*) are already apparent through the thin epithelial membrane as eight tapering bands running across the testis on each side. This is the condition found in the overwintering larva, and serial sections of these reveal that the follicles now contain numerous rosettes of closely packed spermatogonia. When the larva has attained the pupal form, the follicles at the completion of spermateleosis separate into 16 blunt finger-like projections, remaining joined together at the base by their ducts which merge into a common vas deferens. The two testes meet in the middle line so thata large compact body results measuring 1.25 mm. in diameter (Text Fig. 2*d*). In the early stages, the so-called "Endfaden" form a conspicuous prolongation of the anterior pole, but with subsequent growth and development of the follicles and the bursting of the investing epithelium this structure disappears. It is usually lost in dehydration and clearing processes in the young gonad, but in such serial sections as it does occur it consists of a core of elongated cells with granular nuclei within a clear investing membrane, which is continuous with the epithelium covering of the testis.

(2) Internal morphology of the testis.

The testis in the youngest stage examined may be described as a syncytium in which follicles have been partitioned off by a very thin protoplasmic membrane. At first the primitive spermatogonia have no definite cell walls, consisting, to all appearances, of chromatin only, embedded in a clear fluid-like network of protoplasm. The latter is arranged in very faintly defined chambers, attached to the walls of which are a few larger cells, which will ultimately go to form the wall of the primary cysts. Scattered throughout the testis are to be seen very compact rosettes of small cells, with here and there larger rosettes in which the cells are arranged more loosely.

a. *Spermatogonia.* The youngest cells observed in the testis are



TEXT FIGURE 2.

Stages in the development of the testis in *Pteronidea ribesii*.

a. Testis from larva at third instar: contains primordial germ cells and primary spermatogonia.

b. Testis from larva at beginning of fourth instar; follicles marked off.

c. Testis from overwintering larva.

d. Testes from pupa showing about 32 follicles.

e. Section through testis at stage *c*. 'morula' stage at *m*: *s* cyst of spermatogonia in mitoses; *f* follicle; *epi*. epithelium of testis; *v* vas deferens.

those which compose the "morulae" or primary rosettes. They take the stain heavily but are much too small to allow of accurate description. In the growth phase which follows, the cells begin to swell out and separate, while the chromatin granules are arranged round the periphery of the nucleus, much as in the later growth phases of spermatocytes. In each rosette of this size there appear to be about 16 cells, and doubtless the scattered cells arranged in twos and threes in the primary cyst chambers have been derived from these. In a very few rosettes separation into the components cells does not take place till after the next mitosis, so that 32 cells can be counted in each rosette; but these cases are rare. In the rosette cells the resting nucleolus appears as a solid round body, but at the beginning of growth it is marked by the appearance of a central pale-staining area. When these cells undergo division the chromosomes lie free in the cytoplasm, there being no cell walls. Bands of cytoplasm link up the chromosome groups in each primary cyst (Fig. 5, Pl. 1). Eight chromosomes can be counted in metaphase plates (Figs. 5 and 8), but the precise morphology of the chromosomes is difficult to discern. The sequence is now rather difficult to follow. Figure 5 shows five of a group of eight cells in the growth phase with definite cell walls, but whether these are formed from the fusion of all the cells within the primary cyst, or by division of one only, is not clear. As larger chromosome plates occur than those figured in Figs 3, 5 and 6 it may be that the cells in question do not remain attached, but separate, lose their cell walls and again undergo division. In any case the actual spermatogonial cell which gives rise to the compact cyst of 32 spermatocytes, described later, must be one of at least the fourth generation.

It has been mentioned above that the nucleolus in some of the "morula" cells is dark-staining and spherical, while in others it is vacuolated. In many it appears to be budding but what the significance of this phenomenon may be has not been determined. The explanation that it is due to the rapid separation of chromatin from the plasmosome may not be erroneous, for the presence of several dark-staining bodies outside the nuclear membrane (Figs, 1*c*, 7) would indicate great nucleolar activity. Unfortunately, this stage has not been investigated by FEULGEN's special technique to ascertain the correctness of this suggestion.

b. *Spermatogonial mitoses.* Good mitotic figures of spermatogonia are difficult to find. Fig. 22 shows a cyst of resting gonia in which the

cells are loosely arranged and about to prepare for the final spermatogonial division. Each cell has a prominent dark-staining nucleolus and several scattered chromatin clumps. Preparations stained by FEULGEN's method show that the nucleolus consists of a central non-chromatinic plasmosome surrounded by a karyosome which in section appears as a dark purple ring, from which radiate threads bearing chromatin knots. As the chromatin threads lengthen and increase in thickness the nucleolus decreases in size until finally only the pale-staining plasmosome remains. The chromosomes first appear as irregular swellings on the filaments (Fig. 15, Pl. II) and then gradually assume their characteristic shapes. The plasmosome is now no longer visible and the nuclear membrane appears to persist until the metaphase plate is formed (Fig. 17). The average number of chromosomes is eight (Fig. 17) but plates with nine (Fig. 9) and some with seven only are common (Fig. 11, Pl. I and Fig. 23, Pl. II). These anomalous counts may be due to the splitting and separation of chromosomes in the anaphase or to the fact that one or more of the chromosomes has been lost in the sectioning. In the cytoplasm also there occur one or two spherical bodies of varying size, which may appear to lie on the spindle, according to the plane of the section. These bodies, the so-called chromatoid bodies, are particularly prominent in FLEMMING-Fe. Haematoxylin preparations (Figs. 17 and 18) but they are definitely not chromatinic as proved by their absence from material stained by FEULGEN's technique. In the telophase the chromatin clumps, and in the daughter cells (the primary spermatocytes) the nucleolus again makes its appearance.

c. *The Spermatocytes.* If the spermatocytes undergo a period of rest, the nucleolus remains small and the chromatin network stains but lightly, but when growth begins in the prophase the nucleus increases in size and chromaphility. At this stage the chromatin appears as small irregular blocks near the nuclear membrane, as in the corresponding phase in the spermatogonia. The spermatocytes however are broader than in the gonia, and about this time also they become pear-shaped, the nucleus remaining in the broad end of the cell.

d. *The abortive first maturation division.* The chromatin masses, without undergoing a process of synapsis, are now converted into chromosomes, but it is only occasionally that these can be counted, as in Fig. 24. A true metaphase plate is never formed and the chromosomes come to occupy a position in the centre of the nuclear space.

Only one centrosome is present, situated close to the nuclear membrane which is never dissolved and, consequently, a complete spindle is never formed. In Fig. 28 is shown a cell in which a second centrosome suspends a half-spindle of fibres, but such figures are extremely rare. The chromatin travels to the distal or broad end and forms an irregular clump. The very varied and bizarre shapes assumed by the chromatin at this stage cannot wholly be due to the action of the fixative, for comparable results are obtained in other species and by different fixatives. When viewed from above, the chromatin appears like a polar cap over the spindle area. The most arresting phenomenon of the abortive spindle, however, is the appearance of one or more thin darkly-staining threads which seem to grow out from the main chromatin mass towards the periphery of the nucleus. A small dark granule, whose history will be traced in this paper, lies close to the nuclear membrane and in the cell shown in Fig. 31 a similar but larger body occupies a position in the apex of the cell. This appearance approaches most closely to what is found in the spermatocytes of the Wood Wasp *Sirex cyaneus* (PEACOCK and GRESSON, 1931) but in *P. ribesii* it rarely takes up this position. It may be no more than an unusually large chromatoid body which has been carried into the apex on elongation of the cell. At this stage most workers in *Hymenoptera* record that a small cytoplasmic bud is nipped off at the pole opposite to that near which the chromatin is massed. Despite repeated search I have not been able to satisfy myself that such occurs in sawflies. Appearances such as shown in Fig. 44 would indicate that cytoplasmic buds are freed, but closer examination reveals that these bodies are merely sections through the cytoplasmic bridges by which the spermatocytes are bound together, the "Zellkoppelungen" and "interzonal bodies" of German and American authors respectively. These bodies are particularly well shown in FLEMMING preparations and in certain sections they occur as greyish-brown bodies, generally, although not universally, towards one end of the cell. The elongation of the spermatocytes is not as a rule towards the centre of the cyst but the cells in Fig. 44 appear to have been orientated so that all the "Zellkoppelungen" are proximal. Fig. 32, which is drawn from a testis in which fixation has been almost perfect, shows the more usual arrangement of the cells.

A resting phase is intercalated between the abortive and the second maturation divisions. The chromatin mass opens out into a twisted

tangle of thick threads which gradually thin out and spread throughout the nuclear vesicle (Figs.32,33). The nucleus at this stage, as in resting spermatogonia and spermatocytes, is pale-staining, but it differs from those of the latter in that the chromatin has more the appearance of a spireme. Moreover, the cells may still retain the elongated form assumed during the abortive division. The greatest difficulty is experienced at this stage in placing the different phases in the correct sequence, as all the cells in any one cyst, with rare exceptions, divide synchronously. The cell shown in Fig. 36, taken from DONCASTER's material, is a case in point. It occurs as an exception in a cyst of cells in which the chromatin forms the irregular end cap, but whether it is a laggard, or is in advance of the others, is difficult to say. The chromatin is in the form of a thick twisted thread which bears eight double swellings, some of which are separate and others of which are in process of pairing or dividing. This cell is exceptional also in that no other cell in any other cyst has been found in which the chromosomes are arranged thus. It may be a case of precocious division into the daughter chromosomes so that the cell is a spermatocyte of the second order, or it may represent synapsis in a first spermatocyte which has in some way acquired the diploid number of chromosomes. In my opinion the first explanation offered is the more acceptable.

e. *Second maturation division.* Spermatocyte mitoses are recognisable from spermatogonial divisions by their denser and more closely arranged metaphase plates. In the primary spermatogonia the chromosomes are usually elongated and narrow while in the spermatocytes they are broader (cf. Fig. 23 and Fig. 38). In the spermatocytes in Fig. 34 the cells have again assumed the spherical state and the chromosomes have emerged from the resting state. The chromatoid body, or bodies, is still conspicuous in the cytoplasm and may pass to either of the daughter cells. Fig. 42 shows two spermatids which have each received a small body. That these are not centrosomes is evident from the fact that a definite centrosome is still to be seen in the left member of the pair. The spindles, all of which generally lie in a direction tangential to the cyst wall, are usually very clearly defined in the mitoses, but only rarely are both centrosomes found in one section. The number of chromosomes present is still eight, as counted in both metaphase and anaphase stages (Figs. 39 and 40), proving that reduction has not occurred during spermatogenesis at least. In the telophase the chromatin forms a solid knot, which, after the formation of

a nuclear membrane, immediately opens out and passes into a resting phase prior to the formation of the sperm.

f. *Spermateleosis*. Characteristic cytoplasmic changes in the shape of the spermatids now take place. The cells elongate at right angles to the direction of the last division spindle, i.e., towards the centre of the cyst. Of the behaviour of the nucleus at this stage I am not quite certain, and there may be some question as to correct seriation. The nucleus lies in the broad end of the cell, and in certain cases, the chromatin breaks up into little clumps which lie scattered throughout the vesicle and are connected by very thin filaments. This pale-staining phase (Fig. 47) recalls the resting stages of the spermatogonia and spermatocytes but the spermatid differs from these in that it shows no trace of the nucleolus. As cysts of these spermatids are not very common, this resting phase, if such it be, is probably of short duration.

With further elongation of the cell, the chromatin takes up a position round the inner surface of the nuclear membrane. Sometimes it is in the form of granules (Fig. 52), but in all cases it is most aggregated at the side nearest to the elongating tail (Fig. 51).

In most cells at this stage a dark-staining granule is present, sometimes in the head region near the nucleus, and sometimes in the cytoplasm of the tail. No doubt it is the same chromatoid body whose history can be traced from the spermatogonia through maturation stages. It is probably waste material, as it is apparently sloughed off with the residual protoplasm of the tail (Fig. 51). Fig. 49 shows two such bodies.

The axial filament, which has its origin in the centriole near the posterior pole of the nucleus, is more prominent in material fixed with FLEMMING's than with BOUIN's Solution. At first, while the nucleus is still vesicular, the axial filament is short and does not project beyond the cytosome, but with the progressive drawing out of the whole posterior portion of the spermatid, it elongates with the surrounding cytoplasm, and finally, in part, forms a naked flagellum.

The nucleus meanwhile also elongates, and the separate granules lining the membrane gradually come together and form a continuous layer of chromatin which becomes drawn out into a blunt point at the anterior and posterior ends. Later changes consist in the collapse of the vesicle, with consequent formation of a deeply-staining rod of

chromatin. The cortical layer of cytoplasm moves backward and is cast off with the residual cytoplasm of the tail region.

In DONCASTER's material (FLEMMING Fe. Haematoxylin), I have found spermatids which have in the cytoplasm of the tail region a large greyish spheroidal body, not unlike the "interzonal" body. Special staining methods will have to be employed to decide the true nature of this body, but in all probability it is the chondriosome body or "Nebenkern", previously mistaken for the spindle remnant by the earlier writers (WILSON, page 371).

One stage, which occurs in certain preparations (Fig. 48), I have been unable to place. The nucleus is in the vesicular stage and has attached to it at one side a smaller and more darkly stained body. The apparent lack of cytoplasm in some of the cells may be due to the section passing obliquely through the neck region. The small extranuclear body, by virtue of its position, may be an acroblast, but so far this body has not been observed in longitudinal sections.

g. *The Sperms.* The ripe sperms within the cyst come to lie in conical bundles with their heads all pointing one way, not necessarily towards the sperm duct (Fig. 54*e*). Sperms fixed with Bouin and stained with Fe. Haematoxylin measure 5.3 μ and 29.7 μ in the head and tail respectively. Measurements have not been made of living *ribesii* sperms but those made of *Sirex* sperms reveal that fixation causes excessive shrinkage of the chromatin in the head. The mature testis of the latter species was teased out in RINGER's Fluid and stained lightly with Methyl Green. The sperms remained together in bundles like those of Fig. 54*e* and by rhythmic undulations of the flagella made slow forward progress through the medium. The measurements of head and tail were 11 μ and 26 μ respectively, as against 3.7 μ and 26 μ in the fixed and stained sperms.

1a. *Summary of Observations on Spermatogenesis*

1. The general morphology and development of the testis is described with notes on the ages of larvae in which the different stages are to be found.

2. Spermatogonia are traced from their liberation from the primary rosettes up to the final generation. The chromosomes, in plates of eight, at first lie free in a syncytial cytoplasm but later each chromo-

some plate is bound by a definite cell wall. Certain of these cells go to form follicle and cyst walls while others give rise to compact cysts with about thirty-two spermatocytes.

3. In the resting nucleus, part of the chromatin surrounds a prominent central plasmosome. In the early spermatogonia this nucleolus appears to undergo a process of budding, but the significance of this has not been determined. Prior to the formation of the chromosomes the chromatin again flows out from the nucleolus and appears as blocks on the linin threads throughout the nuclear space.

4. The first maturation division of the spermatocytes is abortive. In the prophase the nucleus increases in size and chromophility, but no synaptic phases occur. The cell becomes pear-shaped, an incomplete half-spindle is formed, but the nuclear membrane does not dissolve. A true metaphase plate is never found; the chromosomes which emerge from the growth phase clump at one side of the nucleus and several dark-staining threads issue from the main chromatin mass. Only one centrosome has been observed at this stage.

5. Between the abortive and second divisions the nucleus undergoes a resting period in which the chromatin is dispersed throughout the nucleus.

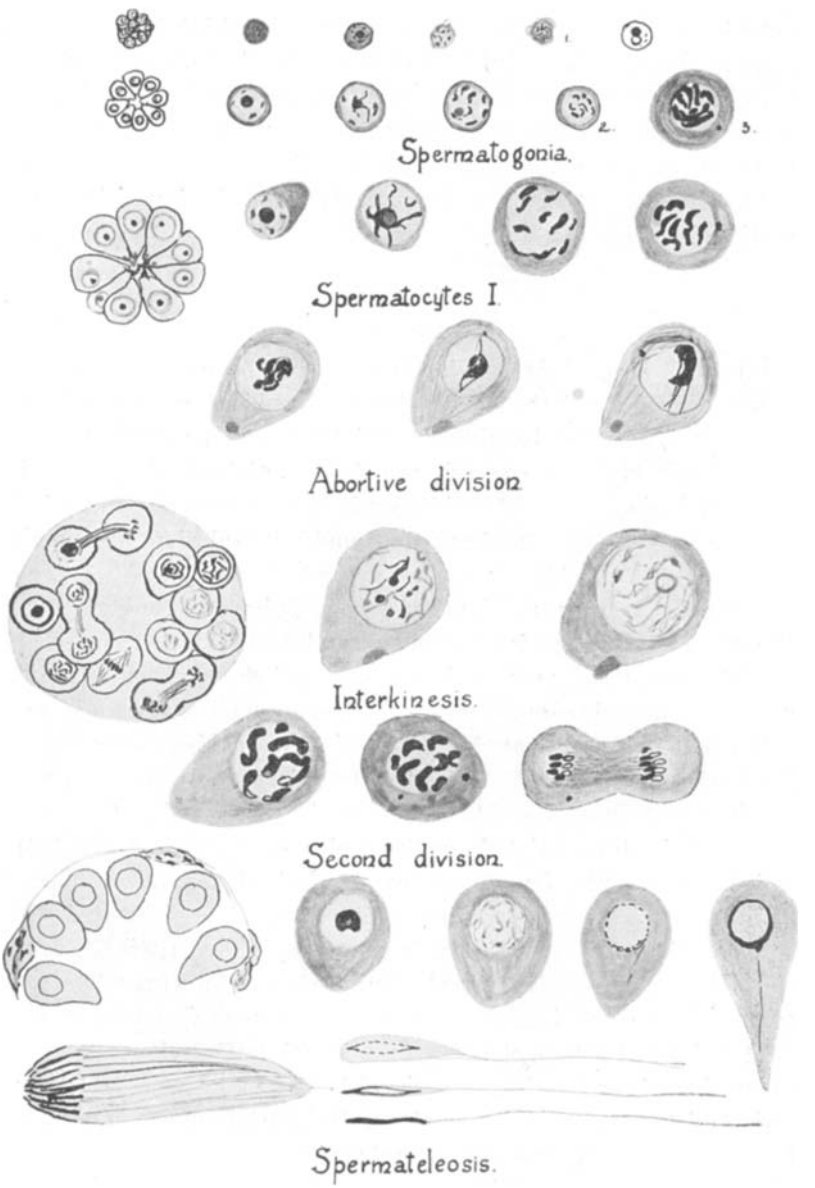
6. The chromosomes in the second spermatocytes are shorter and broader than those in the spermatogonia. The second maturation division is equational both as regards cytoplasm and chromosomes, eight entering each spermatid.

7. Between the second maturation division and spermateleosis an interkinesis is intercalated, in which the pale-staining chromatin is dispersed throughout the vesicle much as in the growth phases. Later it becomes arranged in granules and forms a thin peripheral layer inside the nuclear membrane. The formation of the sperm has been followed only as far as the chromatin is concerned, and no special technique has been employed.

8. In certain BOUIN-Haematoxylin preparations there is present in the developing sperm a peculiar dark-staining body outside the nuclear vesicle. Its origin and fate have not been determined but it is suggested that it may be an acroblast.

9. A peculiar greyish body prominent in the tail region in FLEMMING-Haematoxylin preparations is thought to be a "Nebenkern".

10. One and sometimes two chromatoid bodies are present in the



TEXT FIGURE 3.

Diagrammatic representation of events in spermatogenesis from the primary spermatogonia up to the formation of the ripe sperms. The figures on the left show the arrangement of the cells within the cysts: the numbers 1, 2 and 3 refer to successive generations of spermatogonia.

cytoplasm of the spermatogonia. They can be traced through spermatocytes to the spermatids where they are sloughed off with residual cytoplasm of the tail region. The nature of these bodies has not been determined, but it has been proved by FEULGEN's microchemical test that they are definitely not chromatinic.

11. The sequence of events in spermatogenesis is shown diagrammatically in Text Fig. 3.

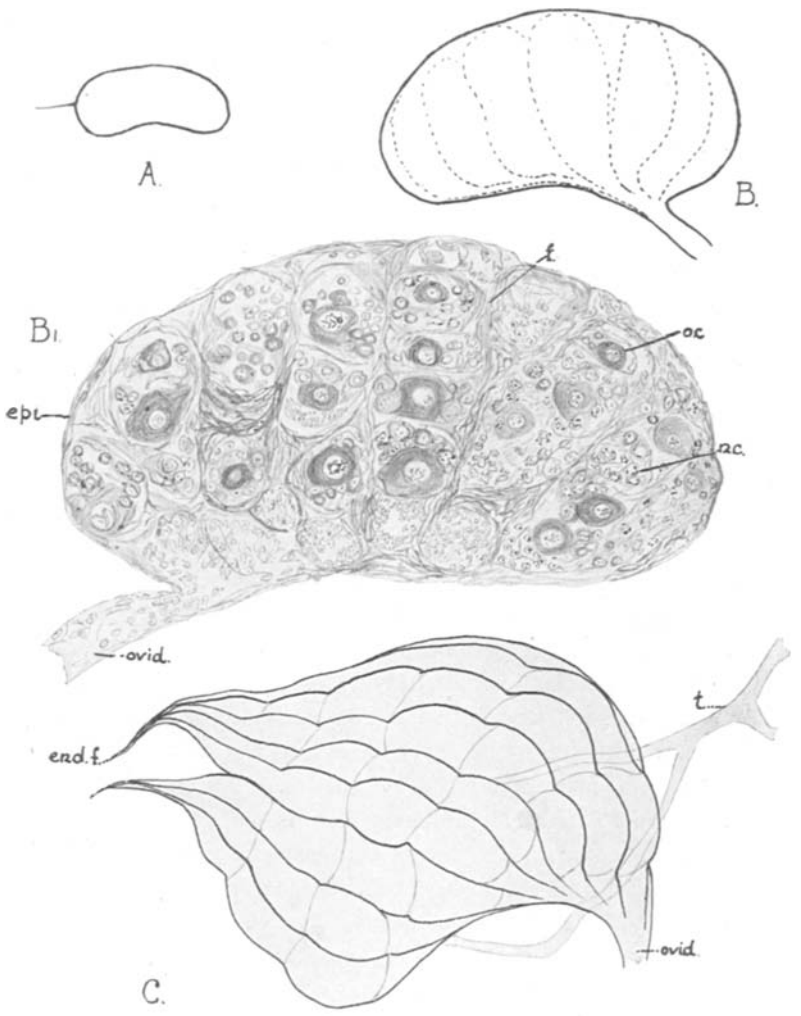
2. *Oogenesis*

(1) External morphology of the ovary.

In the early stages the ovaries closely resemble the testes and only the gross differential size of the larva itself provides a clue to the sex of the individual. The ovary lies in the 5th. abdominal segment, is embedded in a layer of fat and is closely applied ventrally to a patch of oenocytes. A large trachea passes under it and gives off a main branch into either side.

No very early stages with primary oogonia have been obtained, as the ovary at this stage is exceedingly small and difficult to handle if dissected out, and if preserved *in situ* is useless for chromosome enumeration. Rapid oogonial multiplication takes place during the last instar of larval life and at this stage the ovary is kidney-shaped, like the testis in stage *b* (Text Fig. 4*A*). At the last moult, the egg tubes can be seen through the thin covering epithelium, and at this stage they contain differentiated oocytes and nurse cells (Text Fig. 4*B'*). This is the condition found in the overwintering larva. With the onset of metamorphosis, and while the pupa is still unpigmented, the ovary begins to elongate towards the posterior end until the form shown in Text Fig. 4*C* is assumed. There are 16 ovarioles in each ovary, arranged 8 on each side. The nurse chambers are at first very prominent, being larger than the oocytes, but with further elongation and separation of the ovarioles a reversal in these relative sizes occurs, owing to the growth of the oocytes at the expense of the nurse cells (Text Fig. 5, p. 375). Each tube contains about three well-formed eggs with yolk globules, and three smaller eggs nearer the anterior end. The tip of the ovariole, the "Endkammer", consists of a zone of small irregular undifferentiated cells, followed by a patch of epithelial and nurse cells surrounding a young oocyte. Differentiation occurs just

before the last larval ecdysis, and as a rule, no mitoses occur in oogonia later than this (stage B) unless, perhaps, in a few laggards in the



TEXT FIGURE 4.

Stages in the development of the ovary in *Pteronidea ribesii*.

A. Ovary from larva at last instar; contains undifferentiated oogonia.

B. Ovary from larva at last ecdysis; contains ovarioles with differentiated oocytes.

B'. Section through ovary at stage *B* showing ovarioles, oocytes, *oc*, nurse cells, *nc*, and oviduct *ovid.*: *epi* epithelial layer.

C. Ovary from pupa; 16 ovarioles, 8 on each side; *end. f* the 'Endfaden', *t* the trachea and *ovid.* the oviduct.

ovary of summer generation larvae, where rapid elongation takes place *pari passu* with rapid differentiation.

The "Endfaden" are composed of tiers of very much elongated, thin-walled cells which traverse the space between the walls of the peritoneal epithelium. The end threads of each ovary are fused together and serve to convey nourishment to the ovarioles from the pericardial sinus, in which they are inserted (WEYER, 1928, p. 358).

(2) Internal morphology of the ovary.

a. *The Oogonia.* In the earliest stage examined the ovary is composed of large cells, loosely arranged in cysts. The ovariole walls consist of thin epithelium which resembles the follicle walls in the testis. As in all *Hymenoptera* and insects generally, the cells are bound together in rosettes by "Zellkoppelungen" (Fig. 59) which stain very deeply in actively dividing cells. In section, they are greyish oval bodies which lie in the cytoplasm of the inner pointed ends of the cells. In the resting stage they seem to swell out and stain more lightly. Fig. 57 shows a section from the anterior end of the ovary in which cysts are being laid down. Each is composed of eight resting nuclei lying free in the general cytoplasm. The cells round and between the cysts are young cyst-wall cells,while the large resting cell is in the ovary wall. The nuclei of resting and growing oogonia undergo changes very similar to those of the spermatogonia. Figures 55 and 56 show two such stages drawn from cells preparing for the final mitoses. Chromatoid bodies are visible in the growth phase and also during spindle formation (Figs. 56 and 61).

b. *Oogonial mitoses.* Metaphase plates in polar view give very clear chromosome counts as the chromosomes are set transversely on very wide spindles, and the nucleoplasm is pale. The chromosomes are mostly bent rods and on an average number 16. Very often more than 16 bodies appear, but one can never be sure but that some of the chromosomes may have split or that a single rod has not been cut more than once. Moreover, in Haematoxylin preparations, the chromatoid bodies may appear to lie within the spindle area, and only in FEULGEN preparations can one be sure that chromosomes only are visible. Clear figures are rarely obtainable by the latter method, but in certain cases 16 chromosomes can be counted with considerable accuracy (Figs. 67 and 68).

Counts are not easily obtained from profile views of spindles owing to the hooked nature of the chromosomes and the fact that they lie very close together. Further, a peculiar condition is found in many spindles. The chromosomes appear to lie in pairs with the long axes parallel to that of the spindle, but where counts are possible they very often exceed the haploid number. In a preliminary paper on haploidy and diploidy in *P. ribesii* (PEACOCK and SANDERSON, 1930) it was suggested that the double appearance was possibly due to a side-by-side pairing of two chromosomes, but subsequent investigation proves this not to be the case. I have given this matter considerable attention and have come to the conclusion that the phenomenon does not constitute a true "coupling" of chromosomes. It is apparent from polar views of equatorial plates that chromosomes of the same size show a decided tendency to lie near each other, but the members of each pair, presumably synaptic mates, are never very closely approximated. The largest of the chromosomes are distinctly hooked, and they take up a position on the spindle with their apices pointing inwards. Now, the free ends of the large chromosomes will point outwards and when seen in profile give the impression of being closely apposed, while the smaller, more dumb-bell-shaped chromosomes will appear as single dots. On the other hand a single sphere may represent a section through the apex of a hooked chromosome. The full details of subsequent behaviour have not been followed but the apparent paired arrangement persists in the anaphase. The above explanation seems to fit the facts as observed in numerous instances. Figure 69 shows 23 bodies of which 9 are single and 14 (representing 7 hooked chromosomes) lie in pairs.

The spindle area is distinct, the individual fibres being very fine and closely set, so that in transverse section they appear as a continuous line, and give the impression that the nuclear membrane is still undissolved. In the telophase the fibres have little median thickenings which mark the line of the cleavage between the cytoplasm of the daughter cells. The black body seen in Fig. 71 probably arises by the fusion of these. The centrosomes in oogonial spindles are more prominent than in those of the spermatogonia. After the final oogonial division the chromosomes do not appear to form an intense clump of chromatin as in earlier stages, but take up a position near one side of the reconstituted nuclear membrane. A prominent nucle-

olus appears and the chromatin then undergoes synizesis. Events are somewhat masked by this clumping, but in the clearest preparations the chromatin is arranged in radiating loops which bear thickened areas at intervals.The nucleus is large and clear and almost completely fills the cell. All cells undergo synizesis and functional oocytes cannot yet be distinguished from nurse cells until after synapsis. Differentiation takes place about the time of the last larval ecdysis, and is accompanied by increased growth and elongation of the ovarioles. The cells of the oviducts now multiply rapidly in the posterior region of the ovary.

c. *The Oocytes.* (1) *The morphological relations of the oocyte and associated accessory cells.* The young oocyte, at first, differs from the surrounding nurse cells only by a slightly increased amount of cytoplasm (ooplasm). The follicle cells remain small, elongate and partially surround the egg cell with its associated cluster of nurse cells. As the oocyte grows it becomes pushed to one side of the egg tube and soon after is cut off with several nurse cells by a complete layer of follicle cells. Finally, the egg is separated from the nutritive chamber by a second layer of follicle cells and thus the follicle wall round the egg is completed. In the early pupa the rapid elongation is due to the enormous increase in the size of the nurse cells, while the oocyte remains comparatively small, and wedged in between two adjacent nurse chambers. As a rule, seven eggs develop in each ovariole and as these are at different stages of growth, the sequence of events in nuclear behaviour can be followed throughout.

(2) *The history of the chromatin from differentiation to maturation.* (a) *Synapsis.* The synaptic phase is passed through while the ovary is still unelongated as in stage B (Text Fig. 4, p. 369). After a short period in synizesis the nucleus again forms a prominent karyosphere from which radiate the chromatin threads. It seems that the chromatin knot loosens up and the nucleolus, by a process of budding, gives rise to several other nucleoli which take up their position on the chromatin network. Each nucleolus has a dark-staining outer layer with a pale-staining core, and sometimes the nucleolus appears to be budding. These phases are best shown in FLEMMING-Haematoxylin preparations (Fig. 79).

The chromatin threads take origin in the dark-staining part of the nucleus and seem to grow at its expense. The threads are spongy in

texture and bend sharply in zig-zag fashion. At intervals, slight thickenings take the form of paired knots and these, coupled with the open texture of the thread, give the impression of longitudinal duality (Fig. 78). In FEULGEN preparations the nucleus shows very faint diplotene threads round a pale nucleolus (Fig. 83). In certain BOUIN-Haematoxylin preparations, of a later stage, the chromatin forms a continuous post-synaptic spireme of spongy threads, bearing here and there small thickenings, the chromosomes now being in the pachytene stage. No nucleoli were observed in these preparations. In later stages the spireme is cut up into small segments which tend to collect in the centre of the nucleus. The diplotene follows, in which the segments become somewhat thinner and lie in pairs (Fig. 82).

In BOUIN preparations the synaptic mates are not closely apposed and can be seen more distinctly than in FLEMMING preparations, in which the threads swell and often appear to be single. Each thread is irregular in outline and in many cases shows a longitudinal split — the forerunner of the homotypical division. It has not been found possible to make chromosome counts during any of the synaptic phases described above, as the nucleus generally is distributed over about 5 sections.

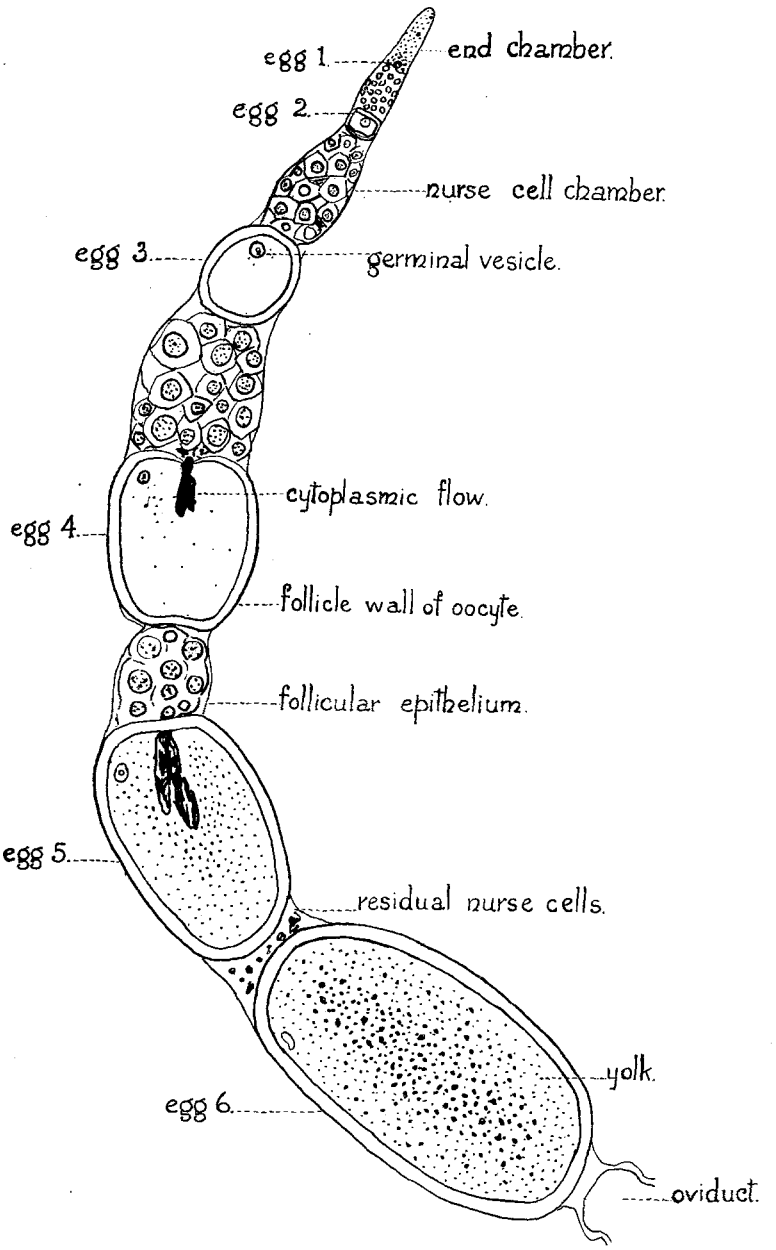
The germinal vesicle is now large and conspicuous, and contrasts sharply with the dark-staining homogeneous ooplasm. The latter partly invests the associated nurse cells and it may be that the egg derives some nutritive material from these. The history of the nurse cells however, will be considered later. A few solitary black corpuscles occur in the ooplasm, each surrounded by a clear halo, and these are probably the products of nucleolar activity (PEACOCK and GRESSON, 1928). Figure 80 shows one of these apparently passing through the nuclear membrane entire, but this appearance may be due to the plane of the section.

(b) *The Growth period.* (Stage C). From this point the chromatin enters the so-called diffuse stage in which, owing to the deconcentration and partial or complete loss of chromophility, it is particularly difficult of demonstration. In order to trace its history a range of fixatives and stains has been employed, and with a certain amount of success.

In Fe. Haematoxylin preparations the most prominent feature of the germinal vesicle is the presence of the nucleolus, which is mark-

TEXT FIGURE 5.

Ovariole from ovary of immature pupa, showing six oocytes, the oldest being at the posterior end and next to the oviduct. The nurse chambers alternate with the oocytes and decrease in size as the nurse cell nuclei pass into the ooplasm in the cytoplasmic flow. The end chamber contains differentiated oocytes but only one of these will mature.


egg 1.
end chamber.
egg 2.
nurse cell chamber.
egg 3.
germinal vesicle.
cytoplasmic flow.
egg 4.
follicle wall of oocyte.
follicular epithelium.
egg 5.
residual nurse cells.
yolk.
egg 6.
oviduct.

edly chromatinic in appearance. With increased growth of the oocyte, the nucleolus enters upon a period of great activity, during which numerous buds are liberated. Material fixed in Corrosive Acetic and stained by MANN's Methyl-Blue-Eosin shows that these nucleolar bodies are of two kinds, basophil and oxyphil. In the growing oocyte the nuclear sap consists of a fine oxyphil meshwork, but in the mature yolk-laden egg, it undergoes a rapid change to basophility. Hitherto it has generally been accepted that during the growth phase, the nucleus enters a "diffuse" stage in which the chromatin elements lose their visible identity, but by means of FEULGEN's microchemical test I have been able to trace the chromatin throughout the growth phase. The reaction was carried out on ovaries of different ages, which had previously been fixed in Carnoy or Corrosive Acetic. Light Green was used as a counterstain and several sections of each series were stained with Fe. Haematoxylin as a control. A positive reaction was obtained in every case. The chromatin takes on an exceedingly pale pinkish tint which is apt to be overlooked.

For convenience, the course of events in the stages of development will be considered according to the age and position of the eggs in the ovariole. A complete egg tube is shown in Text Figure 5 and the eggs are numbered 1—6, the youngest being designated number 1.

Egg 1. In the youngest egg the chromatin appears to be still in the diplonema, the stage found in the resting winter larva. Nuclear changes take place very rapidly in the oocytes in the "Endkammer" of the ovariole and at a later stage than those which go to form the first laid eggs. This is not surprising when one considers that the ovary of the wintering larva has to provide at least seven eggs for each of the sixteen ovarioles. Certain of the eggs at this stage give the impression that the chromatin is again forming a clump apart from the large nucleolus, although it is still attached to the latter by a few threads (Fig. 77). Unfortunately I have not been able to identify this stage in FEULGEN preparations, the stages observed being in the diplotene phase such as is shown in Figure 83.

From this point, the chromatin cannot be identified in preparations stained with Haematoxylin owing to the large amount of nucleolar material in the germinal vesicle. The varied and problematical results obtained by different methods will be discussed later (p. 392) and only such evidence as has been obtained by the use of FEULGEN's technique will be given here.

Egg 2. The egg is now almost completely surrounded by follicle cells. The nucleus, which is large and central, is filled with a homogeneous fluid which stains lightly with the green counterstain employed. In the centre of the nucleus a very pale-staining pinkish mass marks the position of the chromatin. The nucleolus is exceedingly thin-walled at this stage and is rarely seen (Fig. 86).

Egg 3. In eggs of this age the follicle cells completely separate the oocyte from the nutritive chamber. The germinal vesicle lies towards the anterior side of the egg, and contains a prominent thin-walled nucleolus which lies in association with the bean-shaped chromatin mass (Fig. 87).

Egg 4. The nucleus is now in the anterior end of the egg, and towards one side. The chromatin appears as a kidney-shaped body and in several oocytes is distinctly separate from the nucleolus (Fig. 91). The latter appears to have reached its greatest size and is beginning to show signs of collapse. Very often faint vesicular bodies can be seen inside the nucleolus and occasionally several similar spheres can be seen in the nucleoplasm, outside the nucleolus. The fluid-like substance in the nucleolus appears to be of the same consistency as that of the nucleus.

Egg 5. In the egg at this stage albuminous and fatty yolk spheres now make their appearance in the ooplasm and at the same time nurse cell nuclei begin to flow into the oocyte. These nuclei are loaded with granules of chromatin which stain dark purple, contrasting deeply with the paler cytoplasm. The albuminous globules stain light green and contain highly refractive granules, while the fatty globules are homogeneous. The germinal vesicle is no longer spherical but is elongated in the direction of the long axis of the egg, and is pressed flat against the follicle cells. The chromatin appears as a trilobed body (Fig. 92), but there is no appreciable increase in mass. Sometimes a clear central area can be made out, which gives the impression of budding in the chromatin body. The nucleolus is bounded by a thick homogeneous fluid-like substance which stains darkly in Light Green.

Egg 6. The last and most advanced egg in the ovariole is much longer and narrower than those preceding, and the germinal vesicle lies about one third along from the anterior pole, having been pushed backwards by the narrowing of the egg. It shows signs of collapse in

the side next to the follicle wall. The nucleolus is much shrunken, and the chromatin still persists as a kidney-shaped body of the same size as that found in the younger oocytes (Fig. 94).

Later stages than this do not usually occur in mature pupae but in ovaries dissected from adult flies which have not been allowed to lay, later stages may occur. These, however, will be dealt with in the next section on maturation of the egg.

3. *Maturation of the Egg*

The investigation of egg maturation has necessitated the examination of a considerable amount of material. DONCASTER, who investigated the matter first, was concerned chiefly with the fate of the polar bodies and consequently in only a very few of his 600 egg maturation preparations are found stages previous to spindle formation. My own material was therefore prepared with a view to obtaining the early prophase and the first metaphase plate, and to demonstrating the entrance of the sperm. To this end over one hundred eggs were sectioned but with only a limited amount of success.

The nucleus lies in the anterior third of the egg on the dorsal side, i.e., on the side away from the leaf. The nuclear membrane is dissolved on oviposition and the nucleoplasm escapes and forms a pale-staining radiating patch close under the yolk-free layer of protoplasm which underlies the chorion. In the earliest stage found the chromatin forms a compact mass (Fig. 95) but in a later stage the chromatin is irregularly arranged in little granules on a very much twisted thread. In Figure 100 long thread-like chromosomes with thickened ends make their appearance. The nucleus is in two sections but the number of threads can be estimated and is somewhere about 16. They lie perpendicular to the wall and in the direction of the spindle fibres which appear to be continuous with the thin thread-like ends. This is the only case in which this phase has been found, but the parallel arrangement of the chromosomes would suggest that they are preparing for diakinesis.

The first spindle is formed within five minutes after oviposition and the metaphase is passed through very rapidly. A fortunate circumstance has enabled me to demonstrate this phase. Eggs were dissected out from the abdomen of a virgin adult fly which for some reason or other, after several days, had failed to lay. These eggs were

found to be undergoing maturation and division, and some had even developed as far as the early blastoderm. An equatorial plate with about 16 separate elements was found in several eggs (Fig. 102) and their nuclei were, in general, peculiar in that there was present on either side of the chromosomes an enveloping layer of dark-staining material. These eggs were fixed with Bouin, and one explanation may be that the dark mass is of mitochondrial material which has not been destroyed by the fixative. Its constant appearance, however, also leads one to suggest that it may play some part in the dissolution of the yolk spheres round the nucleus. These invariably become smaller in the region of the polar nuclei and around the egg nucleus as it sinks further into the yolk. In a later phase found the chromosomes appear to be lying in pairs. The section is incomplete, but from 5 to 8 pairs of chromosomes are present. Only one good anaphase has been found (Fig. 96). Eight chromosomes are travelling to either end along very thick and dark-staining spindle fibres. There are no centrosomes. The chromosomes can still be counted in the telophase, polar view, and each plate is composed of eight double spheres, closely applied to each other. The dyad character of these is due to the splitting of the single chromosomes in preparation for the second division. Sometimes the nuclei undergo a period of rest in which the chromosomes appear as short segments with thickened ends. Curiously enough this interkinesis is regularly found in eggs laid by virgin females and only rarely in those laid by inseminated flies. Another peculiarity in eggs laid by virgin flies is that the chorion very often shows a deep indentation exactly opposite the dividing nucleus (Fig. 101). The significance of this has not been determined, but it may be due to some attraction which the nucleus exerts if no sperm affects an entrance into the egg. Another possible explanation is that it may mark the position of the micropyle, which is therefore rendered conspicuous only in the unfertilised egg. DONCASTER stated that the sperm enters at the anterior end, but he was unable to demonstrate either the sperm within the egg or the micropyle.

The second division spindles are also formed perpendicular to the wall but they are much narrower than the first division spindles. Both are frequently obtained in one traverse section and between them there lies a small reticulated body which represents the remains of the spindle fibres of the first division. In the eggs laid by one particu-

lar virgin female a solid dark-staining body remains on the equator of the spindle (Fig. 104). It probably originates by the fusion of all the median thickened areas on the fibres, but whether this represents elimination chromatin such as appears as an equatorial accumulation during maturation in certain *Lepidoptera*, cannot be determined without employing more specific staining methods.

In the telophase of the first division the chromatids show different degrees of separation in preparation for the next division, which is completed about half an hour after oviposition. In many cases the two divisions occur simultaneously but sometimes the polar nucleus and sometimes the oocyte nucleus divides first (Fig. 97). The eight dyads arrange themselves on the spindle with their long axes at right angles to the fibres. When the maturation is completed the four daughter nuclei take up various positions relative to each other. Each nucleus forms a nuclear membrane and enters a resting stage. The four vesicles may swell out enormously until they meet, as shown in Fig. 99. Occasionally only the inner nucleus of the outer division spindle and the outer nucleus of the inner spindle come in contact and fuse. In other eggs the ♀ pronucleus remains separated from the polar nuclei by the spindle fibres, while the three polar vesicles fuse. The outermost of these is very often pressed sightly against the chorion and in many cases bears a slight concave curvature on the outer surface. In these nuclei the chromatin is scattered throughout the vesicle as large lumps on a thin pale-staining thread — exactly as in a resting somatic nucleus, but with this difference that in the polar nucleus no nucleolus is present. This stage is reached about three quarters of a hour after oviposition and the polar vesicles may persist for another hour or so.

The protoplasmic patch elongates *pari passu* with the progress of the divisions, and as the ♀ pronucleus sinks into the yolk, the globules in its path are pushed aside or break up into smaller globules. This feature of the developing egg is of considerable value in locating the egg nucleus once it has migrated from the polar area. Once one is familiar with the pale-staining protoplasmic radiation round polar and cleavage nuclei, it is a matter of no difficulty to find them with the low powers of the microscope. In fact, I found it advantageous to make the preliminary examination of all sections with the low powers as the protoplasmic areas are more readily found than when the high powers are used.

4. *Fertilisation*

The maturation processes are similar in eggs laid by virgin and by inseminated flies. The latter lay two kinds of eggs — fertilised and unfertilised, but so far the sperm has not been found within the egg nor has syngamy been observed. DONCASTER figured what he considered to be the approach of ♂ and ♀ pronuclei (1907*b*, Fig. 1) but in my opinion DONCASTER was in error. I have examined the preparation from which he made his drawings and I consider that the small body (the ♂ pronucleus) lies too near the edge to be preparing for syngamy, on the following grounds. In all the eggs examined from fertilised females, there seems to be a rapid insinking of the ♀ pronucleus to the centre of the egg. In several eggs I have found two similar nuclei in the centre of the egg in the resting condition. These are either the ♀ and ♂ pronuclei, or the daughter nuclei resulting from the first cleavage. Whichever is the correct reading, it is plain that if fertilisation occurs it must take place deep in the yolk, for it is unlikely that the zygote nucleus would sink into the yolk to undergo segmentation, and no other nuclei have been found which might be derivatives of an earlier division in the region figured by DONCASTER.

Another point worthy of note is that in certain eggs from fertilised females, there occurs a pale, almost yolk-free path in the cytoplasm, which takes its origin in the anterior pole of the egg in a pale protoplasmic patch. DONCASTER considered this to be the point at which the sperm entered. The path has been followed in several cases and it seems to run from the anterior pole along the "ventral" surface of the egg till opposite the point of attachment, where it bends rapidly in towards the centre of the egg and towards the ♀ pronucleus. No nucleus which might represent the ♂ pronucleus, however, has been detected in this path. Similar paths have been observed in eggs whose chorion has been damaged, the yolk globules appearing to run together, leaving a clear path in the ooplasm. The "sperm track" therefore, may be no more than an artifact, but its regular occurrence in eggs from fertilised females and its complete absence in almost all virgin eggs seem to indicate that it is in some way connected with the entrance of the sperm.

Only in two cases have I found what may be ♂ pronuclei. In one of these eggs, probably fertilised, and fixed less than two hours after

oviposition (DONCASTER's material), the polar chromosomes are completing a mitosis and the ♀ pronucleus is deep in the yolk. In the next section but one, and nearer the ventral surface of the egg there is a small body which resembles the head of a sperm (Fig. 107*a*). One of the pointed ends has a small dark-stained cap. It is surrounded by a membrane and in the patch of protoplasm in which it lies there is a small granule surrounded by a clear halo. The other nucleus (the ♀ pronucleus) is also accompanied by a similar centrosome-like body. The relative positions of these nuclei are shown in Fig. 107*c* and it will be noted that although the male pronucleus has not yet become vesicular, it has still a considerable distance to travel.

In another egg from a fertilised female two vesicular nuclei lie deep in the yolk. Each is surrounded by a radiating patch of protoplasm and outside the nuclear membrane in each there is a prominent spherical body which stains somewhat differently from the globules of yolk (Fig. 106*a*, *b*). The polar nuclei in this egg are close to the wall and one is undergoing division.

There is no differentiating feature by which the ♂ pronucleus may be distinguished from the ♀ pronucleus, but on the other hand there is no evidence but that both are cleavage nuclei. If the large bodies outside the membrane are centrosomes such as occur in segmentation nuclei, then the nuclei in question are possibly daughter nuclei of the first cleavage nucleus.

There is great need for further investigation of the problems of the entrance of the sperm and the location of the micropyle; of the history of the ♂ pronucleus and of syngamy. Biological experiments prove that fertilisation occurs in those eggs which give rise to females, but the process is difficult of cytological demonstration.

4*a*. *Summary of Oogenesis, Maturation and Fertilisation*

1. The ovary is composed of sixteen ovarioles in each of which seven eggs are matured.

2. Oogonial divisions occur in larvae up to the time of the last instar. The cells are arranged in cysts and are bound together by prominent "Zellkoppelungen". There are sixteen chromosomes in each oogonium and homologous members exhibit a tendency to lie near each other. A former suggestion that the chromosomes are arranged on the spindle as eight pairs is withdrawn.

3. One or two chromatoid bodies are present in oogonia but their ultimate fate has not been traced.

4. At the last mitosis the spindle fibres have very prominent median thickenings and these fuse to form a cell plate, which persists for a time as a dark-staining body.

5. In the telophase of the last mitosis the chromatin does not clump but passes immediately to one side of the reconstituted nuclear membrane, where it becomes arranged in radiating loops around a nucleolus. This synizesis occurs in all ovarian cells. No leptotene stage has been found.

6. About the time of the last ecdysis the oocyte is distinguished from nurse and follicle cells by increase in the amount of cytoplasm and growth of the germinal vesicle. The chromatin emerges from synizesis as double threads which bear thickened knots at intervals. Several nucleoli appear during the pachytene stage but these disappear during later diplotene stages. In the latter, the chromosomes appear as short segments but the two members of each pair usually lie well apart.

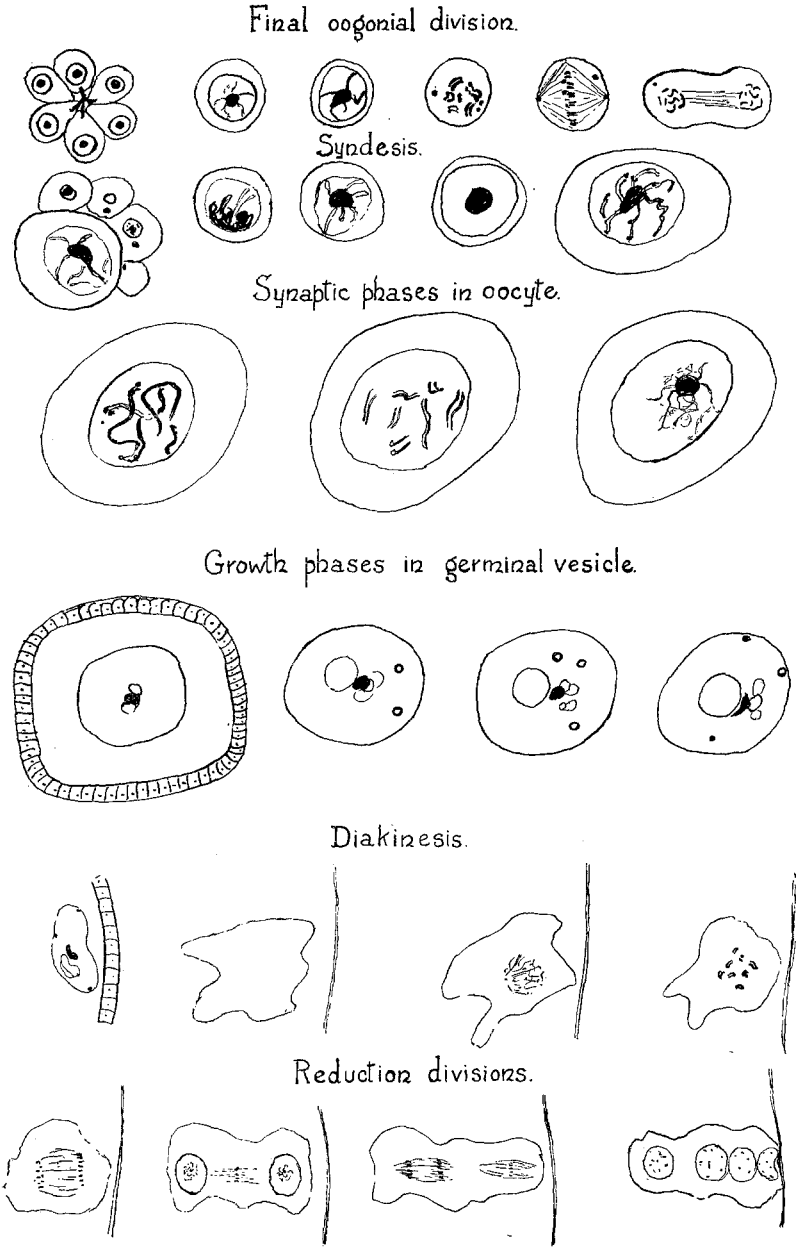
7. Several chromatin segments show a longitudinal split, which is more apparent in BOUIN- than in FLEMMING-fixed material.

8. No exact enumeration has been possible owing to the large size of the germinal vesicle and its distribution through several sections, but it is estimated that about eight pairs of chromosomes are present.

9. During the growth phase the chromatin of the nucleus enters upon a resting phase during which it is only identifiable by FEULGEN's "Nuclealreaktion" method. According to the latter technique the chromatin persists as a compact mass, which is usually found in association with a large thin-walled plasmosome.

10. The nucleolus during the growth phase of the oocyte enters upon a period of great activity. Basophil and oxyphil components can be distinguished by MANN's Methyl-Blue-Eosin, and numerous oxyphil buds are liberated into the germinal vesicle. These appear to pass into the ooplasm and play some part in the formation of yolk.

11. The investigation of the maturation of the egg reveals that polar spindles are formed as in other insects. The chromatin clump in the oocyte nucleus gives rise to small irregular threads which later form about eight bivalent chromosomes. A barrel-shaped spindle is formed during the first five minutes after the egg is laid. Eight chro-



TEXT FIGURE 6.

Diagrammatic representation of events in oogenesis from the final oogonial division to the reduction division in the ripe egg and the formation of the polar nuclei. The chromatin in the germinal vesicle during the growth phase is represented by the solid black mass.

mosomes travel to each end of the spindle and subsequently enter the second division metaphase.

12. The second division is equational; the first polar nucleus also divides and thus four groups of chromosomes arise. These may acquire a nuclear membrane and become vesicular. The three outer polar nuclei may lie free in the yolk or they may fuse: ultimately they disappear.

13. The maturation divisions are completed about $1\frac{1}{2}$ hours after oviposition. In both fertilised and unfertilised eggs the pronucleus sinks into the yolk.

14. The entrance of the sperm has not been observed, but pale paths in the yolk of eggs from inseminated females indicate that fertilisation does occur, although syngamy has not been observed.

15. A peculiar indentation in the egg membrane opposite the polar nuclei was observed in eggs from virgin females, and the suggestion is advanced that this may mark the position of the micropyle.

16. Maturation divisions may occur in unlaid eggs within the abdomen of the female if she fails to lay in the first three or four days after emergence.

5. *Chromosome Numbers in Somatic Cells*

Reference to Section II, 2 (pp. 334—348) and Tables III—VII will abundantly prove that evidence of haploidy and diploidy in *Hymenoptera* has generally been drawn from cleavage and blastoderm cells. In saw-flies, however, these cells are so small that enumeration of the chromosomes can only be approximate.

a. *Cleavage and blastoderm nuclei.* The first segmentation division appears to take place deep in the yolk about the centre of the egg, for although the first spindle has not been observed the daughter nuclei derived from it have been found. These undergo a resting phase in which the chromatin forms a solid knot. The nuclear membrane is indistinct, and near the edge of the protoplasmic area there are one or two granules each in a clear space, which are probably centrosomes. Later, cleavage nuclei within the yolk show many peculiar cytological phenomena. The most striking features of the spindles are the enormously large centrosomes, the large residual equatorial mass and apparent irregularities in chromosome distribution on the spindle

fibres. A few of these appearances are shown in Fig. 108. The daughter nuclei become vesicular in the telophase and the centrosome divides into two in preparation for the next cleavage division. Spindles showing distinct chromosomes are rarely found until several mitoses have occurred. In cleavage cells in virgin eggs about 10 hours old the number of chromosomes is clearly 8 (Fig. 110) but, in eggs from inseminated flies, only one cleavage cell, so far, has been found which shows clearly the diploid number (Fig. 109). In eggs about 15 hours old nuclei begin to accumulate on the side of the egg away from the leaf i.e. on the ventral wall of the developing blastoderm. Blastoderm and early embryo nuclei are extremely small, and even in the best preparations fixed with Bouin the chromosomes are difficult to count. There is no appreciable difference in the size of the cells, nor in the length and breadth of the spindles in the different embryos but the chromosomes appear to be more closely crowded on the spindle in some embryos than in others. This gross differential feature is most obvious in polar views; more accurate counts are generally obtained from profile views of spindles (Figs. 112 & 120). Certain preparations of embryos show consistently about 8 and others about 16 chromosomes. The best preparations were made from eggs of $2\frac{1}{2}$—4 days incubation and in eggs of this age it is better to remove the chorion previous to embedding, to facilitate penetration of the wax. The serosa at this age is composed of very large cells which contain two nucleoli, in the resting phase.

b. *Adult tissues.* The only really satisfactory counts of somatic chromosomes are obtained from developing adult tissues in the unpigmented pupa. The chromosomes at this stage are comparatively large and are more amenable to study than at any other stage of development. The best preparations were made from pupae in which the wings were well formed and extruded externally. There is no criterion, however, by which one can ascertain the best age at which to fix the tissues, as only one pupa in several of the same age may yield mitotic figures. It seems that all mitoses take place synchronously and that a period of activity is followed by a period in which the cells are at rest. This assumption is based on the observation that in every one of a series of sections of a female pupa numerous mitotic phases occurred while in another series apparently of the same age, only a few were found. I have not been so fortunate in obtaining clear mito-

tic cells in the male tissues, although repeated attempts were made. Fortunately, however, observations in these can be supplemented from tissues of other arrhenotokous species, viz., *Allantus cinctus*, *A. calceatus*, *Thrinax mixta* — species in which the haploid number is also 8, as shown in spermatogonia and spermatocytes (see Table I, p. 406).

It is well known that polyploid cells are of common occurrence in certain somatic tissues, especially in insects (WILSON, 1928, p. 871). Reduplication in chromosome number, however, is invariably accompanied by increased cell size, so that only in the smallest cells does the chromosome number remain normal. The largest mitotic spindles occur in the wall of the tracheal tubes and in dividing fat and oenocyte cells. Hypodermal and certain nerve cells are intermediate in size, while the connective tissue, muscle and loose blood cells remain normal.

It is practically impossible to count the enormous number of chromosomes in the largest of these cells, because the chromosomes lie at different depths and the spindle may be distributed over as many as five sections. By very careful scrutiny and estimation of the numbers in anaphase plates, however, I have arrived at the following conclusions, with regard to female tissues:

1. The chromosome number 16 remains normal in all connective tissue and blood cells; 2. Hypodermal cells are tetraploid and have about 32 chromosomes; 3. The largest cells, fat and oenocyte cells, appear to have about 120 chromosomes and are therefore 16-ploid.

I have not obtained such clear evidence in male tissues but from such as I have it is certain that the normal number 8 persists in the connective tissue cells, while the hypodermal cells are diploid (16). The fat cells have, without doubt, a greater number, but at present I am unable to give even a rough estimate of these.

It appears from the above that the haploid-diploid ratio is maintained in male and female adult tissues. Polyploidy is probably due to the great demand for rapid increase in number and size of cells, for the degree of multiplication in number of chromosomes seems to bear a relation to the rate of increase demanded by the rapidly growing tissues. The greatest demand is apparently on the tracheal and fat cells; the connective tissue is of secondary importance, therefore divisions are more leisurely and take place after the main tissues are

laid down. The loose cells found between elongating muscle fibres and those embedded in patches of fat cells, are probably wandering blood cells: these are always normal (Figs. 115 & 117).

c. *The Chromosomes.* The chromosomes in normal somatic plates can be identified in some cases with those of the oogonia. Figure 115 is drawn from connective tissue at the base of the wing and the chromosomes homologise with those of Figure 64 in size and relative positions. This comparison cannot be made so readily in cells from male tissues because in these, strangely enough, the chromosomes seldom lie in one plane.

In the polyploid cells the chromosomes, by reason of their increased number, appear to be smaller, but in reality they are about the same size as those in smaller cells. In the largest cells the chromosomes, as they enter the telophase in the final division, appear to fragment. In this way the chromatin forms a solid granular mass, which is afterwards distributed in several clumps throughout the cell. In the fat cells the chromatin is distributed evenly throughout the characteristic branching nuclei. If only one cell goes to form a fat corpuscle — and appearances certainly indicate this — then only a polyploid cell could provide a sufficiently large amount of chromatin. These granules are particularly well shown in fat cells surrounding gonads which have been submitted to FEULGEN's nuclear reaction technique. It is worthy of note that fat cells seem to be more common in females than in males, presumably because a larger amount of fat is necessary in the female to nourish the large number of ripening eggs, and to provide the energy necessary in egg-laying.

6. *Nucleolar Phenomena during Growth in the Oocyte*

The occurrence of a definite chromatin mass in the oocyte nuclei led me to investigate the staining reactions of the nucleolus and nucleolar emissions. With the stains usually employed, one is unable to identify the chromatin among the numerous substances within the nucleus. In material stained with MANN's Methyl-Blue Eosin certain bodies are shown to be oxyphil (red) and others basophil (blue), but subsequent staining of the material with Fe. Haematoxylin reveals that oxyphil and basophil bodies frequently stain similarly, and, further, that many more bodies are present than appear by the double

stain. Even if a slight difference in density can be detected it is not always possible to distinguish the basophil from the oxyphil bodies, as sometimes the former stain the more darkly and sometimes *vice versa*.

Different fixatives were employed, viz., Bouin, PETRUNKEWITSCH's modification of Gilson, Carnoy and Corrosive Acetic. The last-named has to be used for material which is to be treated by MANN's Methyl-Blue-Eosin, but its reaction is somewhat harsh and causes a slight shrinkage. Gilson-Petrunkewitsch is an excellent fixative for ovaries and yolky eggs but it has the disadvantage that it is detrimental to certain nuclear inclusions. Material thus fixed has an abnormally swollen appearance, which is particularly well shown in the follicle cells round the oocytes (Fig. 130). In BOUIN-Haematoxylin preparations there is present in the germinal vesicle a peculiarly large, thinwalled nucleolus, but this is never found in material fixed by Gilson-Petrunkewitsch. Probably the nucleolus wall is so far distended that it bursts and its contents become lost in the surrounding nucleoplasm. This body is also absent in material fixed by strong Flemming (DONCASTER's material). Bouin gives perfect fixation of all nuclear inclusions, but material thus fixed has been stained in Haematoxylin only and used as a control to other methods. In the following description much of the evidence has been derived from material stained first in MANN's Methyl-Blue-Eosin and subsequently destained and treated with Haematoxylin. Drawings to illustrate the size, position and colour of the nuclear inclusions were made as carefully as possible before the sections were treated with the Fe. Haematoxylin. In this way it was hoped to identify in the Haematoxylin preparations those bodies which showed differential staining by FEULGEN's and MANN's methods.

In the following description Eggs 1—6 correspond in size and position to those of the same designation in the section on growth of the oocyte (p. 375, Text Fig. 5).

In very young oocytes treated with Methyl-Blue-Eosin the nucleolus is strongly basophil. No other structures are apparent by this method but in Haematoxylin preparations the nucleus contains, in addition, a faintly staining chromatin network, which, a little later, appears to collect round the nucleolus. This phase unfortunately was not identified in preparations made by FEULGEN's technique so that the true nature of the nucleolus cannot be determined.

Egg 2. In eggs at this stage the basophil nucleolus is no longer present and the nucleoplasm is composed of a faintly staining oxyphil meshwork containing here and there somewhat denser areas. Figure 84*a* and *b* shows such an egg of this size stained first by MANN's method and then by Fe. Haematoxylin. On the evidence of the Haematoxylin-stained material one would have no hesitation in saying that the black mass (the denser areas in Figure 84*a*) represents the chromatin, which is about to form a clump. If this is correct then the chromatin at this stage is acidophil. No other structures are visible.

Egg 2a. In slightly older eggs there is present a more compact but still indefinite oxyphil body with a bluish tinted mass closely applied to it. This blue colouration of basophil bodies, it should be noted, is never very distinct. In a later stage, Egg 3, this amphophil body is more prominent and appears to consist of three of four hollow oxyphil spheres attached to an irregularly lobed basophil body. The oxyphil bodies appear to be liberated, for several of these occur free in the nucleoplasm. In Haematoxylin, these bodies stain with different degrees of intensity, some appearing to be solid throughout while others are composed of a central pale-staining core surrounded by a layer of darker-staining material. As for the amphophil body, the oxyphil component may stain more darkly with Haematoxylin than the basophil component (Fig. 88) or *vice versa* (Fig. 89). These results, moreover, must be accepted with reserve, as oxyphil bodies apparently vesicular in Methyl-Blue-Eosin may appear as solid bodies when subsequently treated with Fe. Haematoxylin (Fig. 88*a* and *b*). Certain preparations of eggs of this age reveal a large, very thin-walled nucleolus. It gives no reaction with MANN's double stain but appears as a very thin transparent sac. It is prominent in material fixed with Bouin but, as mentioned above, is absent in PETRUNKEWITSCH preparations. The hydrolysis necessary in carrying out Feulgen's nuclear reaction does not appear to have any detrimental effect, for this body is present in all preparations and is rendered conspicuous by the Light Green counterstain. It persists throughout the growth phrases, and in order to avoid ambiguity in the following description, it will be designated the "plasmosome", although at the moment its origin, its ultimate fate and the part which it plays in the metabolism of the oocyte are unknown.

Nucleolar emissions become more numerous within the germinal

vesicle and some appear to enter the ooplasm. In one particular oocyte stained by MANN's method two large clear globular bodies were seen close to the follicle wall. When stained in Haematoxylin they appear to be giving off buds. Regarding their origin, although it cannot be stated wth certainty that they are derived from the nucleolar emissions, yet work on nucleolar phenomena (see GRESSON, 1929*a* and HARVEY, 1929) renders it highly probable that they are products of nucleolar activity which have been extruded into the ooplasm.

Egg 4. The large plasmosome has now reached its greatest size (Fig. 93), and the wall in Haematoxylin preparations appears in certain aspects to be composed of a dark-staining homogeneous substance. With the two stains employed the fluid which fills the large central vesicle is of the same consistency as the nucleoplasm. The pale-staining mass in Haematoxylin appears to be enlarging at the expense of the associated basophil(?) component of the amphophil body which is vacuolated. Several buds are being liberated into the ooplasm but the nature of these has not been determined with certainty. From results obtained by AUERBACH's method these appear to be oxyphil, for in material stained thus these bodies assume a bright red colouration. They are vesicular and of different sizes within the germinal vesicle but those in the ooplasm are all very small (Fig. 90).

Egg 5. Nucleolar buds are now much more numerous in the germinal vesicle and somewhat smaller in diameter, being derived from the larger bodies by a process of budding or possibly fragmentation. Those in the centre of the nucleus are much paler than those near the nuclear membrane and some of the latter appear to be passing through the nuclear membrane entire. This appearance has been recorded by several workers (GRESSON (1929) for *Allantus*; GARDINER (1927) for *Limulus*) and it has also been noted by the writer in the early differentiated oocytes. Although the actual manner in which they pass through the membrane must still be left undecided, the fact that they do enter the ooplasm is beyond question. Their escape from the germinal vesicle synchronizes with the first appearance of yolk spheres (oxyphil, according to MANN's Methyl Blue-Eosin) from which they are almost indistinguishable, but whether the buds are directly converted into yolk or undergo some structural change has not been determined. In certain preparations of oocytes, fixed in Corrosive Acetic and stained in Haematoxylin, a layer of dark-staining

fluid-like material is found along the wall of the germinal vesicle on the side towards the yolk (Fig. 132). This fluid-like material may have been derived from the substance of the nucleolar buds as they pass through the membrane in fluid form; but on the other hand there is undoubted evidence of shrinkage due to the Corrosive Acetic and therefore this accumulation of material may be merely an artifact.

The amphophil body is reduced in size and seems to be less active than in younger stages, if the number of free buds in the nucleus is any criterion. The "plasmosome" is still visible in certain oocytes as a thin-walled sac. However, in other oocytes, (BOUIN-Haematoxylin and Corrosive Acetic-Haematoxylin preparations) it is rendered conspicuous by the presence of a homogeneous fluid-like substance which forms a dark-staining layer round the periphery (Figs. 131 and 140). Within plasmosomes of this type there appear numerous small black rods or granules, not unlike the chromatin rods of the early chromosomes, embedded in a darkish fluid. It is worthy of note that in all nuclei showing this condition the amphophil body is lacking. Unfortunately tnis material was not previously treated with Methyl-Blue-Eosin so that the relation of this fluid to the plasmosome cannot be determined. The absence of the amphophil body, however, would indicate that the dark-staining fluid mass has been derived from that body.

Egg 6. In the ripe egg within the ovariole the nucleoplasm is reticulated and undergoes a change from oxyphility to basophility. The plasmosome is faint and in most cases has collapsed. Only a few small corpuscles remain within the germinal vesicle and those in the ooplasm round the nuclear membrane are likewise less numerous. A much lobulated dark-staining body, presumably the remains of the amphophil body, is found in several eggs, but in others it appears to be wanting. Complete series of sections of the yolky eggs are difficult to obtain, however, and the absence of this mass may be due to its having been lost in the preparation of the sections.

7. *The Identification of the Chromatin in the Germinal Vesicle*

In a previous section (p. 376) it has been shown by means of FEULGEN's technique that the chromatin persists as a definite body during the growth phase of the oocyte. It appears as a homogeneous kidney-

shaped mass lying in association with a large "plasmosome" and in later phases it may become vesicular and lobulated. This chromatin body is easily overlooked but once one is familiar with its position, it can be detected, even under low powers of the microscope. I am convinced that the reaction is correct for the following reasons: 1. Chromatin in neighbouring nurse, follicle and oviduct wall cells appears in the correct shade of purple described by FEULGEN (1924) and LUDFORD (1928); 2. No other structures within the cells, or within the ooplasm, give the reaction; 3. The pinkish mass is prominent in material fixed by the different fluids Corrosive Acetic and Carnoy, the latter fixative having been found to give successful results in oogonial material.

The question arises as to which of the substances in evidence by other staining-methods employed corresponds to this chromatin mass. The chromatin clump in the very early oocyte (Fig. 84) was found by double-staining methods to be acidophil. In my opinion the chromatin in later stages is basophil. This assumption is based on the fact that in Methyl-Blue-Eosin preparations the bluish-tinted body corresponds closely, both in position and density of colouring, to the pinkish body found in FEULGEN preparations. If this assumption is correct then the chromatin is located in the amphophil body, but it has not been possible to identify the basophil component of this with certainty. It has, however, been shown that nucleolar extrusions liberated from the amphophil body are oxyphilic, and therefore, as the extrusions do not give a reaction with FEULGEN's technique, the chromatin is probably located in the small residual mass found in the older oocytes (Fig. 93).

With regard to the peculiar dark-staining matter found round the "plasmosome" in egg 5 (Fig. 131), I have suggested that the basophil component may have become aggregated round the large nucleolus. If this hypothesis is correct, and if the residual mass from which this layer is derived represents the chromatin, then FEULGEN preparations will show the chromatin as a pinkish layer round the "plasmosome". Such a condition has not been found, unless the pink streak shown in Figure 92 can be accepted as evidence of such. This enveloping layer will necessarily be extremely thin and easily overlooked unless the chromatin increases greatly in amount. The other alternative, that the thick layer of fluid round the "plasmosome" is

non-chromatinic, gains some support from the fact that even in the FEULGEN preparation in question, the "plasmosome" wall appears to be composed of a thick layer of non-chromatinic material.

It is difficult to see what purpose would be served by the dispersal of the chromatin over the plasmosome wall, unless the process involves a recombination of elements which have previously been separated at the beginning of growth in the oocyte. WILSON (1928, p. 354), dealing with the transformation of the chromosomes and parallel changes in the nucleoli, suggests that the process "involves a splitting off of the nucleic acid component of the basichromatin and its storage wholly or in part in the nucleoli".

The above hypothesis was put forward somewhat tentatively by Wilson but it seems to gain some support from facts revealed by FEULGEN's microchemical test for chromatin. From the evidence to hand, it appears that the chromatin, or at least the thymus-nucleic acid component, (which according to FEULGEN is the substance which gives the reaction) is associated with a larger body — the amphophil nucleolus. If only the nucleic acid component is stored in the nucleolus, whence, one may ask, does the chromatin derive the other material necessary for the assumption of the "basophilic" character? Can it be that this material is found in the large "plasmosome"? The chromatin body certainly appears to be associated with the "plasmosome", especially during the early stages, but it has not yet been found to be intimately connected with it.

8. *The Nurse Cells*

The rôle of the nurse cells, oocytes and follicle cells has been investigated in four different species of saw-flies by PEACOCK and GRESSON (1928), particular attention being directed to the origin of certain inclusions in the ooplasm and cytoplasm of the nurse cells. One conclusion arrived at, viz., that chromatin is extruded from the nurse cell nuclei and plays a part in the nutrition of the egg, proved to be erroneous, however, when the phenomena were investigated by FEULGEN's technique (GRESSON, 1930).

I have investigated the chromatin behaviour in the nurse cells of *P. ribesii* and find conditions similar to those existing in the four species of saw-flies examined by GRESSON. It has been shown above (p. 372)

that oocytes are differentiated from nurse cells shortly after the final oogonial division, all the cells at first being potential oocytes, and, as such, undergoing synizesis. From this point onwards, however, the nuclei of oocytes and nurse cells behave differently. In the youngest nurse cells the chromatin forms an irregular spireme round one or more prominent nucleoli. In the cytoplasm of nurse cells of this age, especially in those in the anterior end of the elongating ovariole, there are large masses of dark-staining material. The origin of these is not known, but as similar bodies were observed emigrating from the nucleus into the cytoplasm in the young oocyte (p. 373) it is probable that these likewise are nucleolar extrusions. At a later stage the chromatin threads become looser in texture, stain red in MANN's Methyl-Blue-Eosin, and are therefore oxyphil, while the single prominent nucleolus which persists is basophil.

In subsequent growth phases the nucleolus in the nurse cell nuclei give rise to several clumps by irregular divisions or by a process of budding. These masses, which stain darkly in Haematoxylin, are distinctly basophil (MANN's method), but are non-chromatinic as shown by FEULGEN's technique. By the latter method the spireme threads appear to be set free as very fine granules in the nucleoplasm. These granules sometimes appear to be arranged on a basic meshwork, but close inspection reveals that this erroneous impression is given by the aggregation of the particles of chromatin round the pale-staining nucleolar masses. These particles in Haematoxylin preparations correspond to the somewhat flocculent plasma-like substance in which the nucleolar bodies are embedded. In preparations made by FEULGEN's technique the nuclei of these bodies in the nutritive chamber in the distal end of the ovariole appear as pale-staining vacuoles among the purple chromatin granules, which are now much finer and give a brighter chromatin reaction. The "unstained spaces in the nuclei" found by GRESSON (1930) in *Allantus* appear to be due to the presence of these masses.

As the nurse cell nuclei increase in growth their cell boundaries disappear and finally they come to lie within a homogeneous basophil matrix (Fig. 125). The cytoplasm of the nuclear chamber flows into the egg adjacent on the distal side, and sometimes this flow can be observed in eggs about a third from the anterior end of the ovariole. No nuclei, however, pass into the oocyte until yolk globules appear in

the ooplasm. At least this seems to be the ultimate fate of the nurse cell nuclei, although they have never been observed in the ooplasm of this species, *P. ribesii*. PEACOCK and GRESSON (1928) figure an oocyte of *Pristiphora padi* containing prominent nurse cell nuclei near the posterior pole of the oocyte, but in *P. ribesii* the nuclei appear to undergo some chemical change before passing into the ooplasm, for they only appear in the cytoplasmic bridge between the nurse chamber and the oocyte. Nurse cell nuclei in the yolk would be conspicuous in FEULGEN's preparations, but so far those have not been observed.

When the nutritive chamber contents have passed over into the now almost mature egg, the two last consecutive oocytes come to lie very near each other, separated only by a few basophil masses. The oocytes, however, are still bound together by the epithelial covering of the ovariole. In the most mature oocyte the last of the cytoplasmic flow may persist for a time in the yolk. This is very prominent in MANN preparations.

In the last oocyte the follicle wall shows a slight inward curvature at the posterior pole while the follicle cells are two-deep and appear to be continuous with the epithelium cells of the egg duct (Fig. 129).

SUMMARY OF SECTIONS 5 TO 8

5. *Chromosome Numbers in Somatic Cells*

1. The first cleavage nucleus of the egg has not been found. Subsequent divisions exhibit peculiar cytological features in irregular chromatin distribution on the spindles, enormous centrosomes and the formation of large middle cell plates.

2. Nuclei begin to accumulate on the ventral wall of the egg about 15 hours after oviposition, but the nuclei are extremely small.

3. Blastoderms are of two kinds. In those from virgin females the nuclei have eight chromosomes only, while those from fertilised females have either sixteen or eight, presumably due to whether the egg has been fertilised or not.

4. The chromosomes in developing pupal tissues are large and distinct. Normal cells are found in connective tissue and blood cells, but other cells such as those of hypodermis and tracheae as well as oenocytes and fat cells are usually polyploid. The chromosomes in

normal male and female cells number eight and sixteen respectively, and this haploid-diploid relationship is maintained even in polyploid cells.

6. *Nucleolar Phenomena during Growth of the Oocyte*

1. Material stained first in MANN's Methyl-Blue-Eosin, destained and subsequently stained in Fe.-Haematoxylin reveals that oxyphil and basophil bodies present in the nucleus, stain alike in Haematoxylin.

2. Different fixatives were used. Of these Corrosive Acetic is too harsh and causes shrinkage, while PETRUNKEWITSCH's modification of GILSON, brings about abnormal swelling and dissolution of the plasmosome. Carnoy causes a certain amount of shrinkage but very clear results are obtained in material which is subsequently treated by FEULGEN's "Nuclealreaktion" method. This is due to the removal of certain cytoplasmic inclusions which tend to mask the chromatin.

3. The chromatin in the young oocyte is oxyphil but it later becomes basophil. The nucleolus is composed of two parts — oxyphil and basophil components. By a process of budding oxyphil buds are liberated into the nucleoplasm. They are at first pale-staining in Haematoxylin but when they ultimately pass into the ooplasm they are darker-staining. The basophil component of the nucleus also undergoes a process of budding but the fate of these bodies is not known.

4. A large thin-walled nucleolus, here called the "plasmosome", is found in all stages of growth of the oocyte, and in the later phases a dark-staining fluid appears round the periphery. The fluid contents of the plasmosome are of the same constituency as the surrounding nucleoplasm.

7. *The Identification of the Chromatin in the Germinal Vesicle*

1. The chromatin which appears by FEULGEN's method is thought to be located in the darker-staining component of the amphophil nucleolus seen in material stained by Haematoxylin.

2. It is suggested that the peculiar dark-staining fluid which is found round the periphery of the "plasmosome" in later stages is derived from the basophil component of the amphonucleolus. If the

chromatin is located in this basophil body then its dispersal over the "plasmosome" may constitute a recombination of elements which have previously been separated. FEULGEN's chemical test, however, is only specific for the nucleic acid component of chromatin, so that at present no definite conclusion regarding the relation of the chromatin to the nucleolus can be arrived at.

8. *The Nurse Cells*

1. The chromatin in the nurse cell nuclei prior to differentiation undergoes a process of synizesis. Subsequently it is dispersed throughout the nucleus and appears to increase in amount. In FEULGEN material it appears as very fine granules which stain bright purple.

2. During the growth of the nucleus the nucleolus is very active. It is at first a single body but as development proceeds it undergoes a process of budding. These nucleolar buds are basophil but non-chromatinic.

3. As the nuclei increase in size the cell boundaries gradually disappear and finally the nuclei lie free in the cytoplasm. They are ultimately passed into the egg, but they appear to undergo some chemical change as no entire nurse cell nuclei have been found in the ooplasm.

VI. DISCUSSION

1. *Cyto'ogy*

a. *The chromosomes.* The saw-flies are a particularly suitable group for the type of investigation undertaken because the chromosomes are comparatively large and exhibit recognisable differences of size and shape. They are most clearly seen in metaphase plates, and, although the chromosomes cannot always be identified individually, it is certain that the diploid group contains two chromosomes of each recognisable type and the haploid group but one. The homologous chromosomes in diploid groups exhibit a tendency to lie in pairs, but one can never be sure of their identity as several chromosome pairs are remarkably alike. This is particularly noticeable in male haploid chromosome sets. For example, in most spermatogonial and sperma-

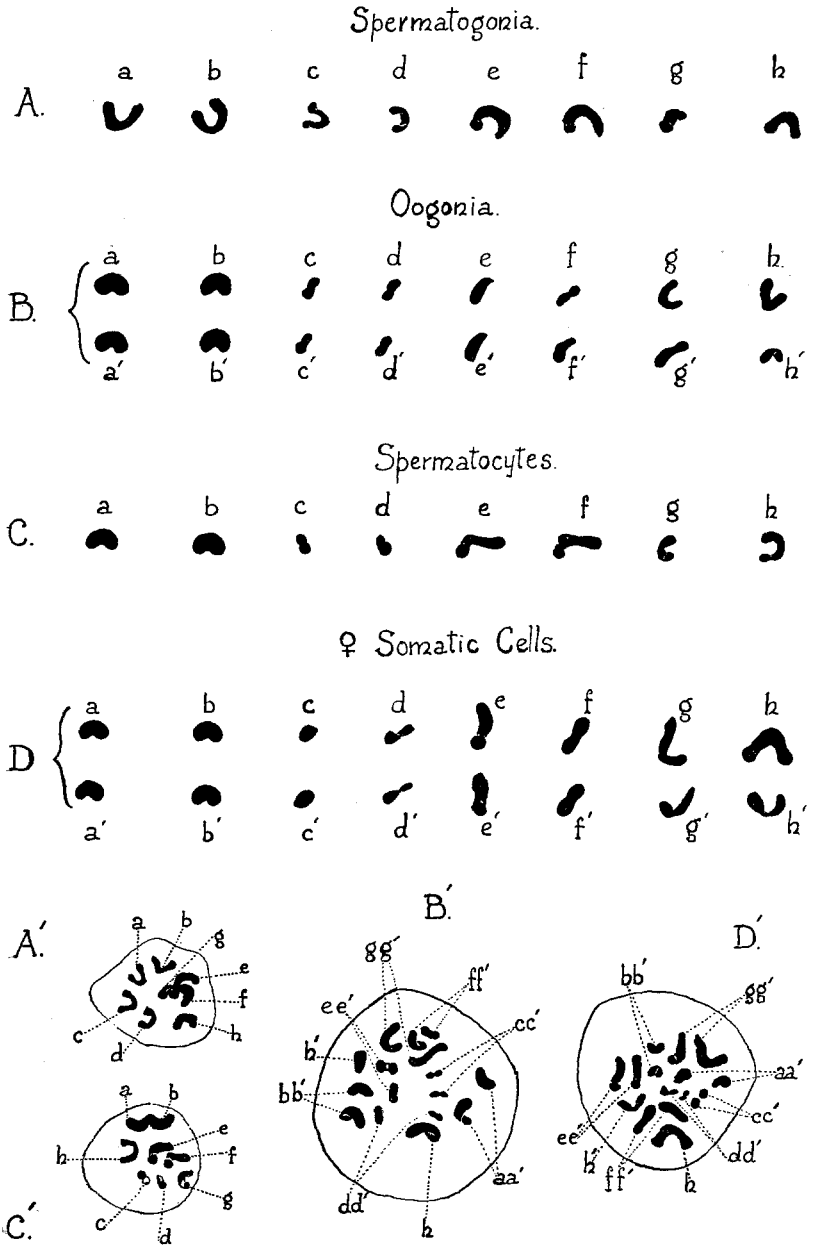
tocyte metaphase plates one pair of similar longitudinally apposed L-shaped chromosomes is conspicuous, and usually two pairs of hooked chromosomes can also be found. If these chromosomes in the male are actually homologous, then the male is really a diploid individual and the female a tetraploid. Only certain of the chromosomes can be paired, however, as will be seen from Text Fig. 7 in which the chromosomes from male and female tissues are shown somewhat diagrammatically. These chromosome sets are typical of different tissues although they are drawn from individual cells. In several cases there are in the male three pairs of chromosomes, *a* and *b*, *c* and *d*, *e* and *f*, and two odd members *g* and *h*. In the female, chromosomes homologous with these can likewise be paired, e.g., in Fig. B., chromosomes *a* and *a'* can be paired with *b* and b', *c* and *c'* with *d* and *d'*, and in Fig. D, *a* and *a'* can be paired with *b* and *b'* and *e* and *e'* with *f* and *f'*. We should expect that the odd members *g*, *g'*, *h* and *h'* in the female could be arranged in two pairs and could be homologised with *g* and *h* of male tissues, but this is rarely possible. Chromosome *h* occurs in both male and female cell but it appears to be present in only one genom in female cells. The explanation may be that the odd chromosomes are concerned with the determination of sex, in which case the female will have four chromosomes bearing sex chromatin and the male but two. ROBERTSON (1930) is able to distinguish homologous chromosomes in cells of both biparental and parthenogenetically produced *Tettigidae*, and he finds that in the biparental individuals homologous chromosomes lie at different sides of the metaphase plate, whereas in parthenogenetically produced individuals homologous chromosomes are closely approximated. On analogy with ROBERTSON's findings we should expect that in the biparental females of *P. ribesii b* or *b'* will be the homologues of *a* and not *a'*. The data to hand, however, is at present too scanty to allow of any definite conclusion regarding the relations and derivation of like chromosomes.

Such phenomena, however, naturally suggest that the chromosome constitution could be explained on a diploid-tetraploid hypothesis, but the presence of these odd members renders this untenable. In a recent paper on hybrid vigour as a factor in the cause of parthenogenesis, ROBERTSON (1931) makes a similar suggestion with regard to the chromosome constitution of the bee, on the assumption that "The

Chromosomes from male and female tissues in *Pteronidea ribesii* arranged to show the marked paired arrangement and similarity.

A. Chromosomes from spermatogonium shown diagrammatically. These can be arranged as three pairs and two odd members.
B. Chromosomes from oogonial metaphase showing two distinct sets of four, two pairs and four odd members.
C. Chromosomes from spermatocytes; three pairs and two odd members.
D. Chromosomes from female connective tissue cell; two sets of four chromosomes, *a*, *a'*, *b*, *b'*, and *e*, *e'*, *f*, *f'*. Chromosome *h* is larger than the others and is usually unpaired: *g* and *g'* could be paired in this cell but usually these two members have very different forms.

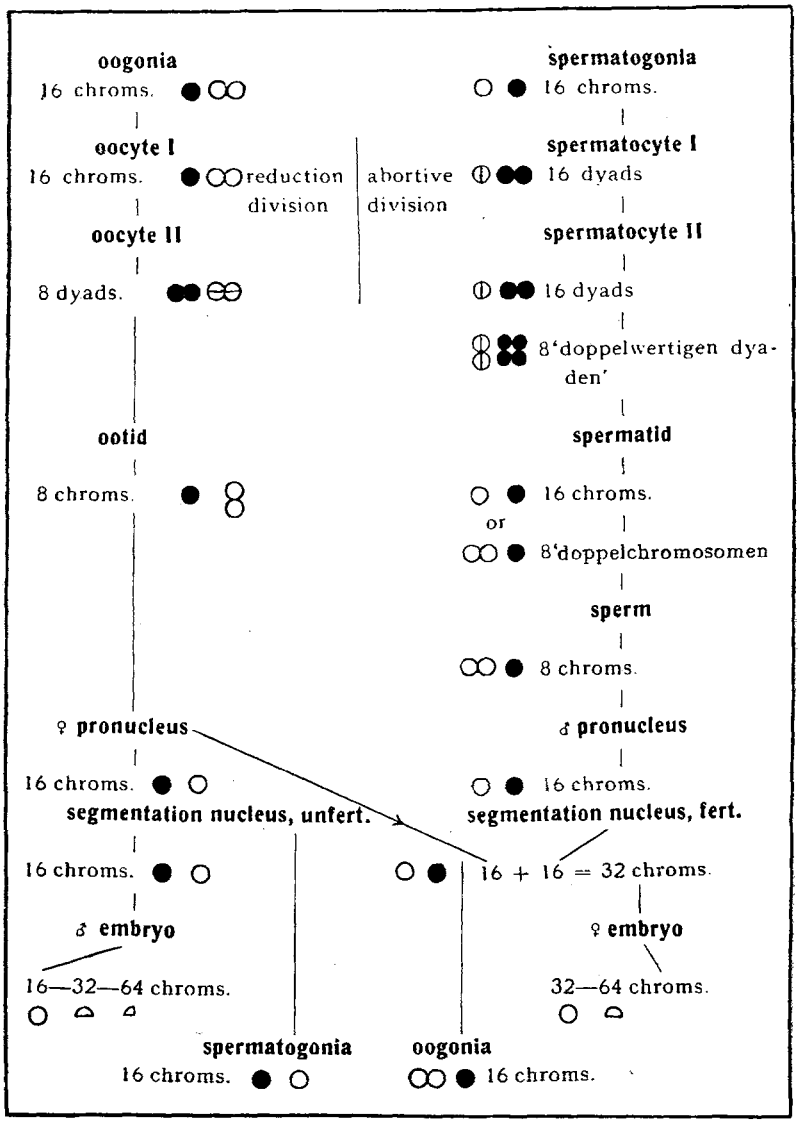
A'B'C'D' show the arrangement of the chromosomes in above cells, and it will be noted that paired chromosomes generally lie together, but it is not known whether these are identical or not in the case of the female cells.



TEXT FIGURE 7.

'Coupling' (NACHTSHEIM, 1913) of like chromosomes in the second spermatocytes and somatic cells of the male and in the oogonial and somatic cells of the female may in some way be a case of the association of homologues similar to that which occurs in the parthenoproduced *Tettigidae* (ROBERTSON, 1930)". In my opinion the evidence is not sufficiently complete for the acceptance of this hypothesis, and, in fact, ROBERTSON's statement in regard to "Coupling" in the somatic cells of the male is incorrect, for NACHTSHEIM in his investigation of male somatic cleavage and blastoderm cells found the haploid number of chromosomes 16 monads and not 8 double chromosomes; further, a similar condition was found in the spermatogonial cells, for he distinctly states (p. 229) that in these no "Sammelchromosomen" are formed. Certainly in the oogonia the chromosomes are apparently present as "doubles", but as previously pointed out, the evidence in regard to chromosome behaviour in the female tissues of the bee is weak. Again, on what grounds is it assumed that the chromosomes which pair are "like"? The investigation of chromosome coupling in *Hymenoptera* is yet far from complete. It is an established fact that in certain *Diptera* the synaptic mates in diploid nuclei may be so closely associated as to simulate the two halves of the longitudinally split chromosome (WILSON, p. 837), but the adherence one to another of chromosomes belonging to different pairs has been observed in a number of cases (see SCHRADER, 1928). In *Hymenoptera* the investigation is rendered particularly difficult owing to the fact that the chromosomes during the stages examined are in the form of very small spheres and at no time exhibit morphological individuality. As they are at one time associated very intimately in pairs and at other times lie apart as separate elements, the question arises as to which is the primary formation of the chromosome, the fused or the separate condition?

NACHTSHEIM (1913) in an excellent diagram has illustrated his conception of the chromosome cycle in the bee (see Text Fig. 8). It is noteworthy, however, that the types upon which the NACHTSHEIM hypothesis of "coupling" is based are bees and wasps, which are restricted in number and specialised in their cytological behaviour. Therefore the application of this hypothesis to other types may likewise be a restricted one. The conception is moreover in some parts purely hypothetical as there is no evidence to prove the doubleness of



TEXT FIGURE 8. The chromosome cycle in the honey-bee.

In the above scheme the solid black spheres represent the morphological, and the open spheres, half- and quarter-spheres the constitutional character of the chromosomes. The terms are those used by NACHTSHEIM.

the chromosomes in oogonia or during maturation of the egg. The cytological evidence he produces shows 16 single chromosomes in the oogonia, 8 dyads in the anaphase of the first maturation spindle and 8 monads in the anaphase of the second spindle — evidence which certainly does not support his view that the diploid number is 32. The hypothesis of coupling gains greatest support from conditions in haploid nuclei in males, but in most species investigated the findings are unchecked against female tissues. The phenomenon of coupling occurs with any regularity only in bees and wasps, for example in *Vespa* (MARK and COPELAND, 1907; and MEVES and DUESBERG, 1908) and in *Osmia* (ARMBRUSTER, 1913), but here again investigation is rendered difficult because the chromosomes are small and morphologically alike. It is to be noted that no "Sammelchromosomen" however, were found in *Xylocopa* (GRANATA, 1909), in which the chromosomes are large and hooked.

SCHLEIP in *Formica sanguinea* found in one case only 13 or 14 chromosomes during the maturation of the egg, and as the diploid number is 48 and the haploid 24, he suggests that the chromosomes "perhaps not all, can fuse side by side". LAMS's figures in *Campanotus* indicate that coupling occurs before the second division in spermatogenesis, but the chromosomes are so small and crowded that the evidence is not convincing. In his earliest papers on the maturation of the egg of *P. ribesii* DONCASTER figured plates with 4 chromosomes which he believed represented the haploid number. As 16 is the diploid number these plates might represent fused pairs of the haploid set of chromosomes, but DONCASTER himself later (1909) showed that these appearances were due to imperfect fixation. I have examined much of his material, both of male and female tissues, and I find that accurate enumeration is possible only in rare instances, as the fixative used (strong Flemming) causes excessive swelling and consequent clumping of the chromosomes. SCHLEIP, likewise, in the investigation of *Rhodites rosae* was led to erroneous conclusions through faulty technique. He found a side-by-side pairing of the chromosomes in the blastoderm, the number thereby being apparently reduced from 12 to 6, but this evidence has been shown by HOGBEN (1920) to be incorrect. The great propensity for union and for breaking up which the chromosomes of *Rhodites* possess has been discussed in a previous section (p. 342). Of the two interpretations

offered by HOGBEN of his own and HENKING's results, the first, that the doubling in the pronucleus is a disjunction of bivalents, fits into NACHTSHEIM's scheme; but the alternative view, that the chromosomes which pair in synapsis are equivalent daughter halves of a single chromosome which divided previous to segmentation is not to be neglected.

The whole question is, "Which is the chromosome, the monad or the dyad?". In most organisms diploid chromosomes are simple monads (WALTON, 1924) which by synapsis and a longitudinal split before or after pairing, give tetrads. Where the dyad is retained as the simple form two semi-independent tetrads or di-tetrads are formed, consisting of eight units, each of which is fundamentally independent. If the plane of division is along the plane of synapsis then the division is reductional, if along the line of the longitudinal split in the original tetrad, then it is equational.

WALTON, for Ascarids, considers the monad form found in the somatic cells to be the primitive condition, so that the chromosome constitution is that of the total number of such monads. In the course of phylogeny a decrease rather than an increase in the numbers of chromosomes has occurred. Thus the chromosomes of the cleavage cells may be elements of less value than those of the germ cells and, while apparently univalent, may be capable of dividing into even smaller elements. NACHTSHEIM, who also believes that the higher chromosome number is phylogenetically the older, considers 32 as the normal diploid number in the honey-bee. He holds that each of the 32 and 16 chromosomes of the female and the male blastoderm cells is a single element, but he is willing to allow (1913, p. 219) that a later increase in number is possible by transverse fragmentation into elements of less value, 'halbwertig' chromosomes. The existence of such would be adverse to NACHTSHEIM's concept of phylogeny which surely implies that if a higher number than 32 exists, then the 32 are compound elements. On the evidence of the Tenthredinid chromosome complement it is more probable that the lesser number is phylogenetically the older, for these reasons: — 1. The chromosomes in the haploid nuclei of the saw-fly can be paired, suggesting that they have arisen from a more primitive condition in which the haploid number was four; 2. A common basic number, 8, (Table I, p. 406) is found in the saw-flies, which are the most generalised of all the *Hyme-*

TABLE I. CHROMOSOME NUMBERS FOUND IN DIFFERENT SPECIES OF SAW-FLIES

Species	Sex	Count	Tissue	Remarks
Pteronidea leucotrocha HTG.	♀	16	oogonia	chromosomes very distinct
	♂	8	sp'gonia	
P. melanaspis HTG.	♂	8	sp'cytes II	
Allantus calceatus KL.	♀	16	oogonia	
	♂	8?	sp'gonia	
A. cinctus L.	♂	8	connective tissue	hypodermis 16 trachea 32 fat ± 60
Thrinax mixta KLG.	♂	8	sp'cytes and blastoderm	
Cladius pectinicornis GEOFFR.	♂	6	sp'gonia	
		6	sp'cytes	
		6—8	connective tissue	
Cladius sp. (on birch)	♂	6?	sp'gonia	
Cladius sp. (on rowan)	♂	6?	sp'gonia	
		8	sp'cytes	
Cimbex femorata?	♂	8	blastoderm	
Sirex cyaneus FABR.	♂	8	sp'gonia	large cells in cysts with 16
Thrinax macula KL.	♂	8	sp'gonia sp'cytes	
	♀	16	oogonia oocyte I	16 in early cleavage cells in yolk

All the above species are arrhenotokously parthenogenetic except *T. macula*, which is thelytokously parthenogenetic though at times producing males. The latter species forms the subject of a later publication.

noptera, and this number will therefore probably be the more primitive for *Hymenoptera*; 3. From the evidence as set down in Table III, it seems that, as 16 and 8 are the commonest numbers found in the female and male honey-bee during maturation of the germ cells,

the 32 and 16 found earlier, and also in the later, developmental stages, give evidence of the evolution of polyploidy, as ROBERTSON has suggested (1930). The true chromosome complement in the female will therefore be 16 and in the male 8 chromosomes. NACHTSHEIM shows two polar plates composed each of 8 dyads, but, as the chromosomes after the second maturation division appear as single elements, it seems quite likely that these dyads are the rounded off halves of the chromosomes which have already divided longitudinally in preparation for the second (equational) division. Further, if the chromosomes are dyad in form, then di-tetrads ought to appear at maturation but, so far, the metaphase of the first maturation spindle has not been figured or observed. In the same way, the 8 dyads found by DONCASTER in the first spermatocytes of the bee may represent an early formation of the two equal halves which will separate on the spindle of the second division.

On the other hand, as the direction of the splitting is not known, the presence of dyad chromosomes in the maturation divisions of the female may represent the true synaptic formation. The second reduction to 8 in this case will find analogy in the 'double reduction' which occurs in the spermatogenesis of birds (GUYER, 1909) and lice (DONCASTER and CANNON, 1920). In the male bee 16 dyads have been found in the spermatocytes of the first order and this certainly points to the number in the primary germ cells being 16 and not 8. The fact that the two parts of the dyad fuse to from a single element may be merely a 'marking time' during the failure of the first division, or it may represent an attempt at synapsis, but against this we have the same phenomena of coupling and splitting *after* reduction has occurred in the female, i.e., if we consider 32 the primary number in the female.

The cytological evidence on chromosome number and morphology, as far as it goes, shows that in the male the haploid number is 16 monad "einwertigen" chromosomes and in the female the diploid number is 16 monad "doppelwertigen" chromosomes. These are elements of different value but as to which is the primary it is difficult to decide. It seems therefore that the true number of chromosomes is to be looked for in the somatic cells where the chromosomes appear in their normal condition. Until this is done the monad or dyad morphology of the chromosomes must remain undecided.

It is noteworthy that chromosomes in the bee have been figured as

small bent rods by PETRUNKEWITSCH (1901) in the metaphase of the egg nucleus (Fig. 18), and by JEGEN (1920) in the anaphase of the second spermatocyte (Fig. 8). From a preliminary examination of one of DONCASTER's preparations of a young queen bee ovary (fixed in Flemming), and of my own preparations of a worker pupa (fixed in Bouin) I am of the opinion that the chromosomes in these cells (oogonia and hypodermis) are not mere dots as figured by NACHTSHEIM but are well-formed and distinct, as in the saw-fly somatic cells. One must, however, be able to recognise and to avoid polyploid cells in submitting chromosome counts of somatic cells. WIEMAN (1915) maintains that "somatic mitoses should not be used as a safe and reliable method of determining the diploid number", but I cannot agree with this author, as in my experience, it is always possible to find a few cells showing the normal haploid or diploid number. DONCASTER (1911) in *Neuroterus* also found "undoubted haploid figures .... and in no male examined are there certainly no such mitoses".

It would be interesting to ascertain how, when and where these polyploid cells arise. FROLOWA (1929) believes that they arise by fusion of two resting cells, or by non-division of the cytoplasm following nuclear division. The chromosomes may be reduplicated in the early differentiation of the tissues in the embryo or during metamorphosis. NACHTSHEIM never found more than 32 chromosomes in the female and 16 in the male bee, while other authors report finding as many as 64 even in the male. This increase in number in *Apis* has been attributed to fragmentation but where the chromosomes are so small this must be difficult of demonstration. In the polyploid cells of saw-flies the chromosomes do not appear to fragment but arise by longitudinal equational splitting. If the phenomenon is correlated with the rapid development and physiological functioning of the cells, then the measure of reduplication may vary with the rate of development of the tissues according to the season and the generation.

b. *The chromatin during the resting stages.* In resting nuclei of oogonia, spermatogonia and spermatocytes the chromatin is scattered in very small masses throughout the vesicle and is also aggregated round a prominent nucleolus. That the latter is an amphinucleolus is shown by its character when the nucleus enters the prophase. In certain Haematoxylin preparations the nucleolus shows a pale-staining core, a plasmosome, with a darker-staining covering layer,

but the latter, as revealed by FEULGEN's technique, is chromatinic, as are also the knots on the linin framework. The chromatin or at least the nucleic acid component, is stored in the nucleolus until required. The plasmosome disappears when the chromosomes are formed and it is probable that it provides the material necessary to the chromosomes for the assumption of their final form. At the close of a mitosis, and during the growth phase of the cells, the nucleolus reappears. The same phenomena were observed in the male germ cells of *Notonecta* by BROWNE (1913) who suggests that the chromatin which flows back into the karyosphere is the idiochromatin which will later form the chromosomes, while the remainder, the trophochromatin, which forms a flaky reticulum in the nucleolus, has a metabolic function during the growth of the spermatocyte. This dualistic conception of chromatin substance is akin to conclusions arrived at by other workers in regard to the "eliminated chromatin" in *Ascaris*, *Dytiscus*, *Lepidoptera* etc. (for general review and references see WILSON, 1928, pp. 323—328). Before a final interpretation of these elimination phenomena can be given, they will have to be reinvestigated by some such technique as FEULGEN has elaborated. At present the most that can be said in regard to the karyosphere is that the distinction between the two kinds of chromatin believed to exist has not yet been demonstrated.

In resting somatic and follicle cells frequently more than one nucleolus is present, probably in accordance with the large size of the cells. It is noteworthy that no nucleolus is present in the spermatid and again one can only advance the supposition that it is intimately bound up with the chromatin of the developing sperm head.

The relations between the chromatin and nucleolus during the growth phase of the egg have already been discussed and reasons given for supposing that the chromatin is associated with the nucleolus. The finding by FEULGEN's technique of a definite chromatin mass in the nucleus of the oocyte throughout the whole growth phase appears to be a novel observation, most workers having obtained a faint colouration only. KOCH (1925) failed to obtain any reaction in the egg of certain chilopods and came to the conclusion that after the last oogonial division the chromatin substance undergoes a profound chemical change. GRESSON (1930) arrives at the same conclusion regarding the chromatin in the older oocytes of the saw-flies *Thrinax*

mixta and *Allantus pallipes*. LUDFORD (1928) in *Limnaea stagnalis* obtained a faint purple colouration from which he concludes that the chromatin is finely dispersed throughout the germinal vesicle. HARVEY (1929) utilised the method in the investigation of oogenesis in *Carcinus moenas* but found that "up to and including the bouquet stage the chromatin was stained red, but that on becoming diffuse again it no longer stained by the method, a fact in keeping with the general inertia of the chromatin of the germinal vesicle". MUKERJI (1930), in an investigation of the chromatin content of the secondary nuclei and germ cell determinant by FEULGEN's technique, found granules of chromatin present in the young oocyte nucleus and absent from the older eggs. Recently GRESSON (1931) in a study of yolk formation in *Periplaneta orientalis* finds confirmation of his former conclusion that the chromatin of the growing oocyte undergoes a chemical change. In a recent paper HILTON (1931) finds that the chromatin during the early growth phase of the oocyte of *Calanus finmarchicus* forms a small pinkish body deeply embedded in a large clear plasmosome. This is interpreted as a stage in the process of fusion of the plasmosome and the karyosome. Indications of the same process of fusion are also obtained by the CHAMPY-KULL method. This result is in accordance with my conclusion that the chromatin in *P. ribesii* persists as a compact mass and is associated with the nucleolus during the growth phases of the oocyte.

The chromatin in the nuclei of growing nurse cells is rendered very conspicuous by FEULGEN's technique, and all writers are agreed that in these the chromatin appears as a mass of very fine granules. GRESSON (1930) believes they form a network but I am of the opinion that this appearance is due to the presence of nucleolar buds, which appear as unstained areas in the nucleoplasm. As the nuclei of nurse cells and oocytes have originally the same amount of chromatin the question arises, "By what process has the chromatin in the nurse cells increased in amount?" The increase is probably brought about through the activity of the nucleolar buds in the nurse cell nuclei for there seems no other way to account for it. As the nucleoli of nurse cells and oocytes are identical up to a certain time it is therefore justifiable to argue that the nucleolus in the oocyte must likewise be capable of increasing the amount of chromatin. But the chromatin does not increase in amount in the germinal vesicle nor is any extruded into the ooplasm.

The nurse cells are in reality aborted oocytes which take no direct part in reproduction, but are highly specialised for the elaboration of food materials for the developing egg. It has been shown by GRESSON (1929*b*) that albuminous yolk in the saw-fly arises by the extrusion of oocyte nucleolar buds into the ooplasm, while the fatty yolk arises through the activity of the Golgi bodies. Hence we arrive at the conclusion that nucleolar activity in oocytes is directed to the production of nutritive material, while the chromatin in the germinal vesicle remains constant in amount. Thus the genetic continuity of the chromosomes is upheld. The nurse cell nuclei do not appear to be passed directly into the oocyte in *P. ribesii*, as in the case of *Pristiphora padi* (PEACOCK and GRESSON, 1928), but appear to undergo some chemical change. Probably the chromatin is dispersed so finely throughout the yolk that at first it fails to give the colour reaction by FEULGEN's method. In older oocytes it appears to increase in amount, for, although not present as visible granules, it nevertheless gives a reaction similar to the chromatin in the germinal vesicle. The actual part which this chromatin plays in the nutrition of the egg is not known.

c. *The abortive spermatocyte division.* In all *Hymenoptera* the first spermatocyte division is abortive but minor differences of detail exist between the different groups and even within closely allied species. In the bees the spindle consists of two complete half-spindles; in the wasps and gall-flies only the distal half-spindle is formed and in the saw-flies spindle fibres are generally poorly defined. Correlated with the extent of spindle formation is the behaviour of the chromosomes. In the higher *Hymenoptera* the chromosomes line up on an equatorial plate and subsequently pass to one of the poles as separate elements. In the saw-flies, however, the chromatin forms an irregular clump which behaves in various ways in different species. In *P. ribesii* the chromatin collects at one side of the nuclear membrane and may send out dark-staining threads of chromatin (Figs. 25—30). The chromatin mass in *Allantus calceatus* breaks up into about three lumps, each of which is shown by FEULGEN's technique to be composed of an outer chromatin layer surrounding a plasmosome. In *Thrinax macula* the chromatin mass is somewhat vacuolated and small granules occur in the nucleoplasm and also in the cytoplasm. The origin of these, however, has not been determined. PEACOCK and GRESSON (1931) note the following about *Sirex cyaneus*. The

chromatin forms a loose skein of threads in which are situated a few dark-staining granules. In many spermatocytes a thick thread of chromatin extends from the main chromatin mass to the nuclear membrane. A small knob of chromatin is sometimes found at the end of this thread inside the nuclear membrane, and sometimes a small granule is present outside the membrane, suggesting that chromatin may be extruded into the cytoplasm. The granules, however, were not observed in material treated by FEULGEN's method. Two centrosomes occur and appearances suggest that a bud containing granules is constricted off the cell process at the more pointed pole of the cell, but "it is very difficult to determine with certainty whether the buds become absolutely free; indeed it is possible that they are not freed".

No buds appear to be liberated in *P. ribesii* nor in any other saw-fly which I have examined, but in the species *Allantus calceatus*, small vesicles similar to the "Bläschen" found in the bee and wasp by MEVES, appear on the cell wall. These, however, are present in the early spermatocyte and cannot therefore correspond to the "polar bodies". The size of the cytoplasmic bud seems to bear some relation to the degree of development of the spindle, for the largest buds appear to be separated off in the higher *Hymenoptera*. In *Neuroterus* (DONCASTER), *Dryophanta* (WIEMAN) and *Paracopidosomopsis* (PATTERSON and PORTER), the cell is drawn out into a fine point and a very small portion of the cytoplasm is nipped off. In these flies the chromatin enters a resting phase, subsequent to the second spermatocyte division. In *Neuroterus* the chromatin is dispersed freely throughout the nucleus in much the same way as in *P. ribesii*. In *Dryophanta* the chromatin recedes to the broad end of the cell and appears to be arranged in loops, as if in a "bouquet stage". WIEMAN believed that this phase occurred before the bud was liberated, but his figures bear a remarkable resemblance to those of PATTERSON and PORTER on *Paracopidosomopsis* in which a definite resting stage has been established.

d. *The second spermatocyte division.* This is normal in saw-flies; a complete spindle is formed and the chromosomes divide equationally and pass to the poles. In the bees *Apis*, *Xylocopa* and *Osmia* the division is again unequal as regards cytoplasm so that a second polocyte is formed, while in all other species two functional sperms are

found. The polar bodies finally disintegrate, although JEGEN (1920) finds that the second polar body in *Apis* undergoes intensive growt and chromatin changes characteristic of the normal developing sperm.

In certain species the second maturation division is peculiar in that lagging chromosomes appear, e.g., *Paracopidosomopsis*. In the bee, according to JEGEN, an unpaired body passes into the smaller spermatid. Thus the sperm, which he believes can develop from the latter, contains 8 autochromosomes and 1 heterochromosome. His theory of sex-determination, based on this observation, will be discussed in a later section.

From the foregoing it is apparent that in *Hymenoptera* we can trace a gradation in the process of abortion of the first spermatocyte division. In the most generalised group, the saw-flies, the abortive division shows no clear metaphase plate, or 'polar body', there is a poorly defined half-spindle and a resting stage is intercalated between the first and second divisions. In the gall-flies, chalcids, and ants an imperfect half-spindle but no clear metaphase plate is found. In the wasps a complete half-spindle is formed, a clear metaphase plate appears and a relatively large cytoplasmic but is cut off. In the bee two half-spindles give rise to an almost complete spindle and a clear metaphase plate is found. In the latter the process approaches most closely to a normal division in the first spermatocyte but apparently the cell is unable to divide normally at the second division for the latter is likewise peculiar in that only one spermatid is formed.

In this aberrant type of spermatogenesis we see an attempt to undergo a maturation division and still retain the haploid number of chromosomes. The abortive division is characteristic of *Hymenoptera* but it bears some resemblance to other aberrant types found in lower groups. A theory of the origin and evolution of these types has been propounded by SCHRADER (1929) and is fully discussed by VANDEL (1931). The following is a brief summary of SCHRADER's observations on certain *Homoptera*.

1. In one class, exemplified in *Pseudococcus*, there exist two kinds of chromatin in the spermatocyte; the first division is equational but at the second division, which is reductional, the two kinds of chromatin are separated. Two kinds of sperms are formed and these determine the sex of the zygote.
2. Another type of spermatogenesis which forms a link between

haploid and diploid types is furnished by *Gossyparia spuria* in which there are also two kinds of chromatin. There are two maturation divisions and in the first an abortive unipolar spindle is formed in which two kinds of chromatin are separated. One class of sperm (that with the heteropycnotic chromatin) degenerates. Only fertilised eggs develop, but the male is thought to be physiologically although not morphologically haploid through the degeneration of the heteropycnotic chromatin during development.

3. In the coccid *Icerya purchasi* there is no abortive division, the single maturation division being equational. Males are haploid and arise by arrhenotokous parthenogenesis.

The *Hymenoptera* therefore represent a condition intermediate between *Gossyparia* and *Icerya*. In the latter the reductional division is completely aborted. In *Hymenoptera* it is represented by the formation of an anucleated 'polar body'. VANDEL (1931) attributes the abortive division not to the possession of the haploid set but to the absence of the other haploid set of chromosomes — the chromosomes determining the male sex. Extending this hypothesis he deduces that, if diploid males exist, then spermatogenesis in these will be of the same type as in the haploid males. He finds support to his argument in the observation of A. R. WHITING (1928) that in the diploid male of *Habrobracon* spermatogenesis is apparently of the usual hymenopteran type.

e. *The question of cytoplasmic 'polar body' formation.* The above hypothesis does not account for the origin of the second abortive division in the bee. We do not know how a large quantity of cytoplasm can be extruded from the first spermatocytes without upsetting the karyoplasmic ratio. The cells are already haploid and do not undergo a further reduction in chromosome number and therefore should not be expected to show a cytoplasmic reduction. If the cytoplasm were to be halved then a growth phase would require to be intercalated before the next division. The cytoplasmic phenomena relating to the abortive division in *Hymenoptera* are summarised in Table VIII, and it will be seen that the species which undergo a resting phase do not in every case extrude a 'polar body' so that the main purpose of the resting phase cannot be to make good any cytoplasmic loss by a period of growth. Yet the second division also is abortive in the bees

and in these it will be noted that the second spindle is formed along the axis of the first. It may be that the second division follows rapidly, with consequent failure of the cells to make good the loss of cytoplasm incurred by the formation of the first 'polar body' and so a second 'polar body' is formed with very little cytoplasm.

f. *Synapsis and reduction.* The occurrence of synapsis in the oocytes and its entire absence in the spermatocytes furnishes another proof of the haploid constitution of the hymenopteran male. This fertile source of cytological evidence in *Hymenoptera* has hitherto received but little attention, the phenomenon having been investigated in a few species only. What little is known of reduction has been derived chiefly from the study of polar and cleavage mitoses. PAULCKE (1900), MARSHALL (1907), NACHTSHEIM (1913), HEGNER (1915), BUCHNER (1918) and more recently WEYER (1928) and PEACOCK and GRESSON (1928) have studied the process of differentiation of the oocytes, but the only clear piece of work on the behaviour of the chromosomes at this period is that of HOGBEN (1920). He investigated synaptic phases in the ant *Lasius flava* and in certain agamic gall-flies, viz., *Cynips* and *Rhodites*, and arrhenotokous parasitic Ichneumons, viz., *Synergus* and *Orthopelma*. The main conclusions we may deduce from this investigation are: 1. The process of synapsis is similar in thelytokous and arrhenotokous species; 2. The process does not differ from that of forms with normal bisexual reproduction; 3. There is formed in the parasitic species a post-synaptic abortive maturation spindle in which the chromosomes undergo a second syndesis and pair telosynaptically.

Synaptic phases similar to those described by HOGBEN have been demonstrated in *P. ribesii* (Figs. 78—84). No leptotene stage was found, the chromosomes apparently passing directly from the oogonial telophase to the 'bouquet' stage in syndesis. The chromosomes go through the usual pachytene and diplotene phases but at no time were chromosome counts obtainable, for, as already stated (p. 373) the nucleus is distributed over several sections. In the diplonema the synaptic mates are distinctly separated and in some a secondary split was observed. Later the chromatin undergoes a second clumping, and comes together to form a compact mass in the centre of the nucleus. During the growth phase the diminution in staining capacity was observed by HOGBEN to coincide with the appearance of granules

on the membrane. HOGBEN believed that these were chromatinic, but that this view is erroneous has been shown by GRESSON (1930) and this paper supports the latter writer. The chromosomes after oviposition do not emerge as bivalents but appear as scattered granules which finally give rise to chromosomes. Observation so far shows that the chromosomes are arranged in pairs, the two partners in each pair lying longitudinally apposed, but this stage has not been found with sufficient frequency to allow of a precise statement as to whether the individual chromosomes are longitudinally split. In a later stage DONCASTER found a comparable nucleus ("abnormal") in which the chromatin was arranged like "iron filings". From the fact that in these the polar patch is small, I am led to suppose that these were similar stages to those which I have taken to represent the early formation of the metaphase plate (cf. Fig. 100 of this paper and DONCASTER's Fig. 23, 1907).

PATTERSON figures the formation of tetrads in *Paracopidosomopsis* and is the only worker who has up to now clearly demonstrated this stage.

In *P. ribesii* the chromosomes are extremely small and at no time show the shape characteristic of the auxocytes and somatic cells. This is a feature commonly found in other organisms but the cause of this diminution in size is not understood. Spindle formation follows the usual plan; centrosomes are absent and the spindle fibres consequently do not converge towards the poles. The chromosomes on the second polar spindle are in the form of dyads, the line of cleavage between the two single elements marking the line of separation in the subsequent division.

The saw-flies differ from other *Hymenoptera* investigated in the formation of large polar vesicles. These are not always found, however, and it is probable that they are in some way dependent on the behaviour of the female pronucleus. It has been noted that in virgin eggs the two daughter nuclei of the first spindle may undergo a period of rest (p. 379) and it is here suggested that this may be consequent upon the non-entrance of a sperm. In the same way the polar nuclei of the unfertilised egg may pass through a "waiting period" in interkinesis. A similar observation, that the fertilisation of the egg, or the presence of spermatozoa in the egg, influences the behaviour of the polar nuclei, was made by DONCASTER, and this suggestion is fur-

ther supported by DONCASTER's findings in the thelytokous species *Croesus varus*, *Empria abdominalis* and *Hemichroa crocea*, in which polar vesicles are regularly formed.

With regard to the question of reduction, certain of DONCASTER's findings have been shown to be erroneous. His misinterpretation of chromosome behaviour, was due to faulty technique, as he himself stated in 1909. His conclusions were: 1. The chromosome number in the virgin egg is 8; 2. Reduction to 4 occurs in fertilised eggs; 3. Unfertilised reduced eggs can develop as far as the blastoderm. It has been shown by the writer that the number in the unreduced oocyte is 16, and that the number is reduced to 8 in all eggs from virgin and inseminated flies alike. DONCASTER's observations on thelytokous species have not been checked, but in view of the above-mentioned erroneous observations in *ribesii* his findings must be accepted with some reservation.

It has been found that eggs of agamic and thelytokous females may retain the diploid number and give females, as in *Neuroterus* (DONCASTER), or they may undergo reduction and develop with the haploid number and give males (*Neuroterus*, DONCASTER), or they may develop by diploid parthenogenesis as in *Rhodites* (HENKING; HOGBEN). Synapsis was not investigated by DONCASTER in *Neuroterus* but, later, HOGBEN (1920), quoting unpublished work of his own, states that synapsis follows the same plan as that of other gall-flies investigated. Thus in all probability the number is temporarily reduced and is restored in the same way as in *Rhodites*. DONCASTER's interpretation of maturation spindles in thelytokous species of sawflies is that 8 is the diploid number, but this view is not substantiated by any knowledge of the counts in oogonia, segmentation nuclei or somatic cells. From HOGBEN's observations an alternative interpretation would be that the diploid number in these thelytokous sawflies is 16. There is here an interesting problem, the solution of which lies in the investigation of oogonial and synaptic phases.

g. *Fertilisation and development.* Of all cytological investigations undertaken here, the demonstration of the fertilisation of the egg is the least satisfactory. No sperms have been found within the egg nor has the position of the micropyle been demonstrated. In the bee the latter is situated at the anterior pole and its position is rendered conspicuous by a patch of yolk-free protoplasm. This protoplasmic patch

is present in the saw-fly egg but it does not seem to differ in any way from that found at the posterior pole. On the evidence of biological experiments, however, it may be confidently accepted that the sperm enters certain eggs and effects fertilisation. Syngamy of male and female pronuclei has not been observed with certainty, owing to the difficulty of distinguishing between pronuclei and cleavage nuclei in the yolk.

In my examination of DONCASTER's material I sometimes formed the opinion that the particulars regarding the age, noted on the slides, were not always correct. In several instances in eggs supposedly about $1\frac{3}{4}$ hours old I found what I considered to be blastoderm nuclei, for the occurrence of such nuclei in eggs of this age is certainly exceptional although it has been shown (p. 379) that eggs are capable of development within the abdomen of the female if oviposition is delayed. On the other hand the possibility is admitted that these resting nuclei are male pronuclei consequent upon polyspermy. Each is surrounded by a small protoplasmic radiation, but the latter is never of such large proportions as those figured by NACHTSHEIM in the sperm nucleus of the bee. DONCASTER (1906) found "indications that the spermatozoa develop into nuclei which disintegrate without ever conjugating with the egg nucleus" but he submits no cytological evidence in support of this statement. NACHTSHEIM finds from 3—7 sperms in the worker egg, and this is explainable by our understanding of the "sperm-pump" of the spermatheca. NACHTSHEIM finds that the complete sperm enters the yolk, but this cannot be the case in the saw-fly, otherwise the sperms could scarcely have evaded detection thus far.

The peculiar types of spindles found during cleavage in the egg are not unknown in other insects. The large body which is found midway between two daughter cells is evidently the "Zellplatte" which has arisen by the fusion of the thickened areas of the spindle fibres. Its fate has not been followed but it is probable that it may acquire a membrane and persist for some time. Unusual nuclei have been found which may be derived from bodies of this type and which may give rise to the "Dotterzellen" found by numerous authors.

These thickened fibres are prominent in final oogonial divisions and give rise to a similar large body which remains for a time in the connecting protoplasmic bridge. It probably passes into one of the two

potential oocytes formed and may persist for a time in the cytoplasm. It cannot, however, be identified among the numerous extranuclear bodies in differentiated oocytes and nurse cells.

h. *The chromatoid body.* It is far from certain whether all bodies which go under this name are of the same nature. The chromatoid body is never very large in the male germ cells, appearing in the spermatocytes as a single granule. Occasionally more than one body appears, as in *Sirex* (PEACOCK and GRESSON, 1931). It may take up a position near the nuclear membrane or may lie close to the edge of the cell. As it usually lies opposite the broadest part of the spindle, it may approximate so closely to the chromosomes in metaphase plates that it becomes indistinguishable from them. In the second spermatocyte division it passes into one or other of the two spermatids, but if it is present in duplicate then one granule may enter each. This inconstancy of number goes to prove that it takes no part in the differentiation of two kinds of sperms as has been attributed to it previously (DONCASTER, 1910; and JEGEN, 1920). The chromatoid body is sloughed off with the residual protoplasm of the tail, so that, if it has any physiological function, such must be performed not later than the spermatocyte divisions. As WILSON has suggested, it is in all probability a by-product.

A similar body, or bodies, occurs in the oogonia but here again the origin and function is unknown. To the best of my knowledge this is the first time that such a body has been reported in the female reproductive tissues. As to the nature of the chromatoid body, the most that can be said of it is that it is definitely not chromatinic, since it does not appear in material treated by FEULGEN's "Nuclealreaktion" method. With regard to the chromatoid body in *Sirex* spermatocytes, a similar observation was made by PEACOCK and GRESSON (1931).

2. *Male Haploidy and Female Diploidy in Saw-flies*

In the foregoing record of observations on the chromosome cycle in *P. ribesii*, it has been shown that the chromosome number is 8 in male tissues and 16 in female tissues. At certain stages in the life cycle, e.g., in blastoderm and embryonic cells, absolutely exact counts are not always possible, because of the very small size of the chromosomes. This circumstance, however, does not invalidate the main con-

clusion, viz., that the male is a haploid and the female a diploid organism. The evidence on this head is conveniently summarised in the following table.

TABLE II. CHROMOSOME NUMBERS IN P. RIBESII

	Tissue	Male	Female
Germinal	Gonia	8 (Fig. 17)	16 (Fig. 62)
	Gametocyte I	No count possible	16 (circa) in synapsis metaphase (Fig. 82).
	Gametocyte II	8 (Fig. 39)	8+8 (Fig. 102) in metaphase of 1st. division spindle; 8 (Fig. 103) and 8 (Fig. 96) anaphase.
Somatic	Cleavage	8 (Fig. 111)	16 (Fig. 109)
	Embryonic	8 (Fig. 112)	16 (Fig. 119)
	Pupal		
	Conn. Tissue	8 (Fig. 114)	16 Fig. (115)
	Hypodermis	16	32
	Fat cells, oenocytes etc.	32—64	64—120 (Figs. 121, 123)

a. *The chromosome number*. The most convincing evidence of male haploidy is obtained from a comparison of chromosome counts in male and female gonial cells, for in these the number remains normal always. In the somatic cells there is a tendency towards polyploidy,

but it has already been pointed out that even in these abnormal cells the haploid-diploid ratio apparently is maintained.

b. *Synapsis.* Perhaps the most striking evidence of haploidy lies in the absence of any formation of synaptic threads in the prophase of the spermatocytes. The absence of this phase is due to the fact that only one complete set of chromosomes is present. The attempt which the cells make to divide is rendered all the more remarkable because the initial phase of maturation, synapsis, is absent. The peculiar behaviour of the haploid cell during maturation is as yet unexplained. The formation of a "polar body" is generally believed to be "a vestigial process which represents the remnant of a formerly complete reduction division" (WILSON, p. 75).

c. *Body size.* Correlated with the haploid chromosome constitution of the male is a marked reduction in gross morphology. This difference in size is noticeable in the larva, but is more pronounced in the adult. Measurements of *ribesii* larvae are not to hand, but those of the fern species *Thrinax mixta* will serve to make this clear. Larvae were removed from their burrows during the winter and as at this season they are dormant, fairly exact measurements were possible. Differences in size exist between members of the same sex, but as a rule, these are not sufficiently pronounced to render the sexes indistinguishable. The males measure on an average 6 mm. and the females 8 mm., the proportion of male to female length being 3 : 4, and this is in accordance with observations made in the adult insects.

d. *Cell size.* According to BOVERI's law of karyoplasmic ratio, the size of the cell, or, more correctly, the size of the nucleus, is proportional to the number of chromosomes. Thus we should expect to find marked differences in the cell measurements in corresponding male and female tissues. In order to obtain a fair comparison, it is essential that the tissues examined should be subjected to the same treatment. Unfortunately, the only material which has been thus treated is germinal. Gonial cells from male and female gonads were fixed in the same fluid, dehydrated, embedded and sectioned together, and finally mounted and stained on the same slide. The difference in cell diameter is not marked, the average ratio of female to male cells being 123 : 100. If we consider the volume of these cells, assuming that they are spherical, then the ratio is as the cube of the diameter, viz., 186 : 100. The expected volumetric ratio is 200 : 100 and the

diametric ratio would be 126 : 100. It will be seen from these figures that the actual measurements are not far from the expected, and, when we consider the expected diametric ratio, it is not surprising that the cells do not appear to differ very much when sectioned.

The above observations were made on cells in the resting condition in order to obtain a fair comparison. It must be added, however, that even with the precautions taken, the results can only be approximate, as even gonial cells differ markedly in size in different individuals of the same sex.

To summarise, it is abundantly proved that the male is a haploid organism, which differs from the female morphologically, cytologically and genetically.

3. *Parthenogenesis in Hymenoptera*

Haploidy. Since DZIERZON first formulated his theory of sex-determination on the honey-bee, the subject of parthenogenesis has attracted many workers. From the results of biological, cytological and experimental research our knowledge of the phenomenon is fairly extensive although far from complete.

It is now commonly accepted that sex in arrhenotokous species depends on fertilisation, the female developing from a fertilised egg with the diploid number of chromosomes and the male from an unfertilised egg with the haploid number. But the question raised by HERTWIG (1920) as to whether the haploid number is retained in the soma is still regarded by many as unsettled. As NACHTSHEIM pointed out (1921) HERTWIG put too much value on the evidence of MEVES and PETRUNKEWITSCH with regard to the chromosome number in the soma. At that time, however, the question was pertinent, as the whole problem was befogged by the lengthy controversy on the chromosome constitution of the bee, which is in many ways an "exceptional creature" (WHEELER, 1928, p. 162). Today, with such evidence as we have from species more amenable to cytological examination, such as saw-flies, coccids and rotifers, the question may be regarded as settled. Nevertheless, certain authors, notably VANDEL and TAUSON suggest the tenability of the hypothesis that although the germ-plasm may be haploid, the soma may become diploid by "des régulations secondaires". VANDEL likewise bases his arguments on the chromosome

complement in the bee, but in my opinion these chromosome counts of MEVES and PETRUNKEWITSCH are not decisive because they are obviously not normal, my saw-fly observations leading me to believe that they are derived from cells which are either polyploid or have acquired this increase in number by chromosome fragmentation. VANDEL further supports his argument with evidence from cell-size measurements, but here again the evidence is unreliable. OEHNINGER (1913) and NACHTSHEIM (1913) made measurements in drone and worker tissues but found no differences in cell size. But there again the fairness of the comparison may be questioned for: 1. Neither author seemed to take into consideration the volume of the cell, so that slight differences in diameter may have been neglected; 2. NACHTSHEIM himself never found more than the usual 32 chromosomes in the worker and 16 in the drone, which shows that the cases of polyploidy cited must be irregular in occurrence and hence, unless one is very familiar with the behaviour of the cells within the different tissues, random measurements may give an erroneous result; 3. It may be asked, "Is the drone bee normal as regards size?". His relatively large body is probably developed as a result of a more generous supply of food than that given to the worker. If the increase is brought about by an increase in the number of cell divisions then the relative size of the cells will remain normal, but if the increase is brought about by increased growth of the cells, then the comparison of male and female tissues is unjust. In all probability the increase in body size is brought about by the increased cell size for it has been shown above that chromosomes are capable of growth (c.f. blastoderm and connective tissues cells). VANDEL attributes the similarity in cell size in the sexes to a doubling in the chromosome number in the male at some time previous during segmentation. He finds greater support for this hypothesis (p. 229) in the evidence of TAUSON (1924) and SCHRADER (1927). The former records a doubling before the first segmentation division in the male egg of a certain rotifer and a precocious reduction in the primary spermatogonia. A similar precocious reduction was found by SCHRADER in the male germ gland of a hermaphrodite (modified female) coccid.

Although it would seem that these males possess a diploid soma and a haploid germ-plasm, yet in the bee cases of diploidy in the soma are not sufficiently well-established to be critical, and, as

already pointed out, the chromosomes behave so irregularly that evidence from the honey-bee must be regarded with caution. In species where the chromosomes retain their morphological individuality there is no question of a regulative doubling in number. In SCHRADER's work on parthenogenesis in the coccid *Icerya* and the mite *Tetranychus*, we have indubitable evidence of male haploidy. In fact, the former species, in which the normal number of chromosomes is four and the haploid two, exhibits one of the clearest examples of arrhenotokous parthenogenesis which has yet been demonstrated.

Exceptional cases. Exceptional cases of female production by virgin females such as are known in certain races of bees, do not constitute a refutation of the DZIERZON theory. In all probability these are aberrant cases, but where the females habitually produce females by parthenogenesis then is the species not truly arrhenotokous but amphoterotokous. I would recall only ONIONS' work on the S. African race of the honey-bee, in which workers are capable of producing parthenogenetically other workers or even queens. A cytological investigation has not yet been undertaken but in all probability it will be found that the females so produced are normal diploid individuals. It is worthy of note that PETRUNKEWITSCH (1901) found a difference between the maturation of queen- and worker-laid eggs. In his Figure 18 he shows an equatorial plate of the first maturation spindle of a worker egg where there are 32 chromosomes. His explanation is that the chromosomes are of different value, viz., bivalent instead of quadrivalent. NACHTSHEIM's explanation is that the suppression of chromosome coupling is due to the rudimentary state of the ovary of the worker mother.

Another difference which PETRUNKEWITSCH finds lies in the relative rates of maturation processes. In queen eggs maturation is over in 30—40 minutes but in worker eggs the process takes up to two hours. Not much weight can be placed on the latter observation, however, as the material which PETRUNKEWITSCH used was sent to him by DICKEL, whose observations, according to NACHTSHEIM, are unreliable (NACHTSHEIM, 1913, p. 182). In his first observation PETRUNKEWITSCH does not state whether the worker eggs in the three cases cited were from the same female or not. This circumstance does not detract from the suggestion that eggs of this type give rise to exceptional females such as occur in the Cape bee. These aberrant females are

developed thelytokously. There is as yet however no very clear evidence as to how the diploid chromosome number is maintained in Hymenopteran eggs in the thelytokous species, but it has the possible explanations: 1. The maturation divisions are equational; 2. Reduction occurs and is followed by a doubling of the number of chromosomes by some means; *a.* either by the fusion of a polar body with the egg nucleus as in Echinoderms, or, *b*, by a regenerative doubling of number in segmentation nuclei, as in *Rhodites*.

It is difficult to see how an egg from a virginally-produced mother can undergo true synapsis, as the nucleus contains only maternal chromosomes, but HOGBEN has shown that the eggs of thelytokous species, e.g., *Rhodites*, do undergo synapsis. That the chromosomes are present in duplicate is suggested by the fact that rare males occur in *Rhodites* (KUZNETZOV-UGAMSKIJ, 1928) but the chromosome constitution of the latter is not known. I have had opportunity to make a preliminary examination of the spermatogenesis of rare males in the saw-fly *Thrinax macula* and I find that the maturation phenomena do not differ very much from those of *P. ribesii*. The chromosome number is eight in the male and current investigations show sixteen to be the chromosome number in the female (p. 406). It is hoped that such an investigation of oogenesis will throw some light on the mode of sex-determination in thelytokous species of *Hymenoptera*.

Recently ROBERTSON (1930) has shown that in parthenogenetically produced *Tettigidae* diploidy is restored by an equational splitting of the chromosomes. The two members derived from each single chromosome in the haploid set, lie near together, but in bi-parental individuals homologous members lie at different sides of the metaphase plate. On analogy with ROBERTSON's findings it is justifiable to suggest that if reduction occurs in thelytokous species of saw-flies, and is followed by a regenerative doubling, similar chromosomes will lie together on the plate, while if no reduction occurs, in all probability the chromosomes will differ morphologically from each other, i.e., only one complete set of chromosomes will be present. On the other hand two genoms may be present in thelytokous species, but although synapsis occurs we have no proof that it is followed by reduction in number of chromosomes. Investigations, similar to those of ROBERTSON, on the arrangement of the chromosomes on the metaphase plate may yield a clue to the solution of the problem of the

chromosome constitution of thelytokous species of *Hymenoptera*.

Sex-determination. Sex is determined in arrhenotokous *Hymenoptera* by the fertilisation or non-fertilisation of the egg. In most bisexual non-parthenogenetic species sex is determined by the nature of the spermatozoon, but in *Hymenoptera* all sperms have the same sex-determining qualities (NACHTSHEIM, 1913). This is proved by the fact that all fertilised eggs give females (PEACOCK, 1925*a*). Several authors have advocated the existence of two kinds of sperms in *Hymenoptera*, notably DONCASTER (1910) and JEGEN (1920). The latter based his argument on cytological evidence that at the second division one spermatid received an unpaired body. The smaller of the two spermatids (the second "polar body") he believed to be capable of development. He believed that all progeny of normal queens develop from fertilised eggs, the sex being determined by the kind of sperm; that the ♂-determining sperms are active only under certain conditions which exist at the time of laying of the drone eggs; that drones from these fertilised eggs differ from drones produced by virgin queens in that the former are smaller. NACHTSHEIM in a criticism of this work (1921) pointed out that this difference between drones does not exist, and further, the second "polar body" does not develop into a sperm.

Evolution of Parthenogenesis. The manner in which the types of parthenogenesis vary in different groups of animals has been very fully discussed by VANDEL (1931). Of these, the most specialised is the haploid arrhenotokous type exhibited by the *Hymenoptera*, *Hemiptera* and *Rotifera*. Diploid parthenogenesis (thelytokous) is characteristic of certain Rotifers, Crustaceans and Aphids. The many hypotheses which have been advanced to explain the evolution of these different types will not be entered into here further than to mention briefly the most outstanding of these (VANDEL, 1931).

1. The absence of fertilisation due to the disappearance of males may have induced parthenogenesis, but of this there is no proof.

2. Parthenogenesis may have brought about the disappearance of the males; in which case parthenogenesis must have arisen spontaneously.

3. Apogamy and fertilisation may be in some way connected with hybridisation, as was first suggested by ERNST (1918) as a result of his studies in the stone-worts, and was later developed by a number of others (see ROBERTSON, 1931, p. 166). Cases of the occurrence of par-

thenogenesis in hybrid animals are not very common, being confined to the geometrid moths (HARRISON and PEACOCK, 1925) and the grouse locust (*Tettigidae*) (NABOURS and his school), groups which are normally bisexual.

Most polyploid species arise by hybridisation and they are generally sterile. But cases where such hybrids have been capable of reproducing parthenogenetically are known. Parthenogenesis in these has been attributed to hybrid vigour but the exact cause of the latter is not known. It manifests itself in such ways as rapidity of growth, increased ultimate size, and fecundity, with consequent increase in cell size and rate of cell division (ROBERTSON, 1931, p. 166). The cause of increased fecundity is likewise as yet a matter for speculation only, but ROBERTSON's suggestion on this head is worthy of attention. The hybrid vigour is expressed in the oocyte of the hybrid in such a a way as to advance the growth and consequent splitting processes in the chromatin threads during synapsis and succeeding stages. This impulse is sufficient to bring about the maturation divisions of the egg and thus its development parthenogenetically.

VII. GENERAL SUMMARY

1. The literature dealing with biological experimentation in *Hymenoptera* is reviewed, particular attention being directed to the most recent experiments.

2. The literature relating to chromosome behaviour in all species of *Hymenoptera* hitherto investigated has been reviewed and the results are summarised in tabular form. Cytological evidence of male haploidy and female diploidy has been shown to be incomplete in most species of *Hymenoptera* examined.

3. The problem of the formation of "Sammelchromosomen" and the apparent occurrence of reduction in the male germ cells of other *Hymenoptera* is discussed in some detail. The NACHTSHEIM hypothesis of "coupling" applied to the chromosomes of the bee is shown to be insecurely based. The nature of the chromosomes in the tissues of the bee requires to be reinvestigated from somatic cells. There are indications that the chromosomes in the bee are not mere dots as figured by most workers but have recognisable shapes, particularly in oogonia and pupal cells.

4. The species used in this investigation is the currant saw-fly *Pteronidea* (*Nematus*) *ribesii* SCOP. The cytological material was obtained from larvae reared in the laboratory or collected in the field. Eggs from virgin females yield males only; inseminated females lay both fertilised and unfertilised eggs.

5. The chromosome cycle is investigated in the somatic and generative tissues of both sexes during all stages of the life-history. Gametogenesis is followed from gonial stages in the larvae up to the formation of the ripe gametes and it is shown that, compared with the female, the male is a haploid organism.

6. Plates of eight chromosomes lie free in a syncytial cytoplasm in the immature testis; rapid mitoses give rise to compact rosettes of cells which are bound together by "Zellkoppelungen", which may appear in section as free buds in the cytoplasm.

7. Spermatogenesis follows the usual Hymenopteran plan in so far as the first maturation division is abortive and no chromosome reduction occurs. No cytoplasmic bud appears to be liberated during the first maturation division, and in this feature *P. ribesii* differs from the majority of *Hymenoptera* investigated. The nuclear membrane persists and an attempt is made to form a half spindle of achromatic fibres. An imperfect metaphase plate is formed, and later, the chromatin clump sends out thin threads towards the nuclear membrane.

8. The second maturation division is equational both as regards chromatin and cytoplasm, eight chromosomes entering each spermatid.

9. One and sometimes two chromatoid bodies, present in the spermatogonia, can be traced to the spermatids from which they are sloughed off with the residual cytoplasm of the tail. Similar bodies appear in oogonia but their fate has not been followed. It has been proved by FEULGEN's "Nuclealreaktion" method that these bodies are definitely not chromatinic.

10. In each ovariole seven eggs are developed. The oogonia are arranged in cysts like the spermatogonia and are also bound together by cytoplasmic bridges. The cells are large and the chromosomes, sixteen in number, are very distinct.

11. At the last instar all ovarian cells undergo synizesis, and oocytes cannot be distinguished from nurse cells and follicle cells until after synapsis.

12. The chromatin in the germinal vesicle passes through all synaptic stages with the possible exception of the leptotene, which stage has not been found.

13. It has been shown by FEULGEN's technique that the chromatin during the growth phase of the egg is not diffused throughout the germinal vesicle but persists as a compact mass.

14. Nucleolar phenomena during the growth of the oocyte have been investigated by special staining methods in order to identify the chromatin among the numerous bodies which stain similarly with the majority of stains usually employed. During the growth phase the nucleolus consists of an oxyphil and a basophil component. Oxyphil buds are liberated into the nucleoplasm and finally appear in the ooplasm, where they are utilised in the production of albuminous yolk. From the results of staining methods it is deduced that the chromatin is located in the basophil component of the amphophil body. In FEULGEN preparations it usually lies in association with a very large thin-walled plasmosome. The origin and nature of this peculiar nucleolus has not been determined.

15. The chromatin in the nurse cell nucleus increases in amount through the activity of the basophil nucleolus. The contents of the nurse chamber pass into the oocyte but the nuclei disintegrate before passing into the ooplasm.

16. It is suggested that the pale pink colouration obtained in the cytoplasm of ripe oocytes treated by FEULGEN's "Nucleal-reaktion" method is due to the presence of nucleic acid which has entered the egg from the nurse cell nuclei.

17. The study of egg maturation has been greatly assisted by the examination of material prepared by the late Professor DONCASTER.

18. Different fixatives were used but the one which gave the best results in so far as sectioning of the eggs was concerned was GILSON's modification of PETRUNKEWITSCH's fluid. This fixative causes abnormal swelling but it does not appear to have any detrimental effect on the chromatin.

19. The first maturation division of the egg is reductional, the second equational. The first polar nucleus divides synchronously with the second division of the egg nucleus and thus four nuclei are formed, the egg pronucleus and three polar nuclei. They become vesicular and the three outer nuclei may fuse, but ultimately they

disintegrate. The egg nucleus sinks into the yolk before undergoing cleavage.

20. Fertilisation appears to take place in certain eggs from inseminated females, but the entrance of the sperm and syngamy of male and female pronuclei have not been observed.

21. Embryos which develop from unfertilised eggs have only eight chromosomes and are male, while those from presumably fertilised eggs have sixteen chromosomes and are female.

22. The male remains haploid throughout the entire life period. In somatic mitoses in the developing pupal tissues the chromosomes are large and well formed. Polyploid cells occur in tracheal, hypodermal, oenocyte and fat cells, but connective tissue and blood cells generally remain normal as regards chromosome number. The haploid-diploid relationship is maintained in corresponding cells in both male and female tissues, whether the cells be normal or polyploid.

23. The chromosomes in somatic cells can be homologised with some degree of certainty with those in germ cells, and in cells of female tissues two complete sets of chromosomes can be distinguished. The two members of each pair appear to lie near each other but one cannot be sure of their identity owing to the fact that three sets of four similar chromosomes are present. The remaining four chromosomes cannot as yet be paired. The occurrence of similar pairs of chromosomes is even more striking in male haploid cells than in female diploid cells. The chromosomes can be arranged in three pairs and two odd members, but it is only rarely that these odd members can be homologised with the odd members in the female cells. The same phenomena have been found in cells of other species of sawflies. These raise important issues, viz., 1. That the odd members in male and female cells are probably sex-chromosomes; 2. That in chromosome constitution the male is really a diploid organism and the female a tetraploid. Further work is obviously necessary.

VIII. CONCLUSIONS

1. In *Pteronidea ribesii*, a saw-fly which is arrhenotokously parthenogenetic, the male arises from an unfertilised egg.

2. In the male the number of chromosomes is eight and in the female the number is sixteen. These numbers are found throughout all stages of the life history, viz., at segmentation, in the blastoderm and in the adult somatic and germ cells. Following the usual hypothesis for *Hymenoptera* this may be interpreted as showing a haploid-diploid relationship of male and female.

3. Polyploid cells in the male and female adult tissues still retain the haploid-diploid ratio.

4*a*. Homologous chromosomes can be distinguished in male and female cells and their number and arrangement indicate the possibility that the male is really a diploid organism and the female a tetraploid.

b. The shape and arrangement of other chromosomes (two in the female) indicate the possibility that sex chromosomes occur.

These possibilities are likely to be of great importance in interpreting parthenogenesis not only in *Tenthredinidae* but in *Hymenoptera*.

5. All eggs undergo synapsis and reduction during maturation. Maturation divisions may occur in the unlaid egg.

6. Spermatogenesis is of the usual Hymenopteran type, no synapsis occurring during the maturation of the germ cells and the first division being abortive.

6. During the growth phase of the egg, the chromatin is not diffused throughout the germinal vesicle but persists as a definite compact mass, associated closely with the nucleolus.

7. The so-called chromatoid bodies, which occur in both male and female germ cells, are not chromatinic.

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XI

TABLES III—VIII

TABLE III. CHROMOSOME NUMBERS IN APIS MELLIFICA

Author	Sex	Germinal		
		Gonia	1st. Spermatocyte and Oocyte	2nd. Spermatocyte and Oocyte
PETRUNKEWITSCH 1901	♂	—	—	—
	♀	—	**16.** Fig. 3	**16.** Fig. 13
MEVES 1907	♂	**16.** mostly dumb-bells. Fig. 11	**16** (8 pairs). Fig. 30 **6** tetrads and **4** doubles. Fig. 29	**16** monads. Figs. 36 and 37 **16** dyads. Fig. 75
DONCASTER 1906	♂	—	**8** pairs. Fig. 1	**8** dyads. Fig. 2
	♀	about **16,** in the young queen ovarian cells	—	—
1907	♂	—	—	**16** dumb-bells: division by a transverse split
MARK & COPELAND 1906	♂	—	**16** dumb-bells. Fig. 5	**16-32-16.** Figs. poor
NACHTSHEIM 1913	♂	—	**16** dyads. Fig. 56	**16** dyads. Fig. 58
1912	♀	—	—	**8** dyads. Fig. 2
1913	♀	**16** monads. Fig. 63	—	**8** dyads. Figs. 1, 2, 3
JEGEN 1920	♂	**16,** in abnormal drone from worker egg. Figures?	**32** single rods in normal drone. Fig. 3	**16** doubles. Fig. 5 **16** + **1.** Fig. 6

TABLE III

	Somatic		
Spermatid and ripe Oocyte	Cleavage	Blastoderm	Adult
8. Fig. 9	**16.** Fig., 16 c	**64.** Fig. 17 **64.** No figures	— —
16 monads Fig. 46	—	more-than **60.** No figures	more than **60,** in follicle cells. Figs. 5, 6
8 monads. Fig. 4, anaphase **16,** anaphase.	— — —	— — —	— — —
16 monads; no figures	—	—	—
8 dyads. Fig. 59, anaphase **8** monads. Fig. 60 **8** doubles. Fig. 3 —	**16** monads. Fig. 29 **32,** mostly in pairs. Fig. 36, *a, b*	**16** monads. Figs. 51, 52 **32** monads. Fig. 55	— **16** in follicle and nurse cells. No figures
a **8.** Fig. 8 b **8** + **1**	—	—	—

TABLE IV. CHROMOSOME NUMBERS IN APIDAE, VESPIDAE, FORMICIDAE

Species	Author	Sex	Germinal		
			Gonia	1st. sp'cyte and oocyte	2nd sp'cyte and oocyte
Xylocopa violacea	GRANATA 1909	♂	**16** hooked. Fig. 9	**16** can be paired. Fig. 25. 1st. division abortive	Division equational; no countable figures
	1913	♂	**16** doubles. Fig. 1	**16.** Fig. 6	**16.** Fig.?
Osmia cornuta	ARMBRUSTER 1913	♂	**16** 13, Fig. 5 17, metaphase, Fig. 6 14 dumb-bells, Fig. 8	**16** some arranged in pairs. Figs. 25 & 34; no division	**16** spherical; paired; division is reductional
Vespa maculata	MARK & COPELAND 1907	♂	—	not less than **16.** 1st. division abortive	Figures very poor. 2 equal spermatids
Vespa crabro	MEVES & DUESBERG 1908	♂	—	more than **16.** 1st. division abortive	No polar views of equatorial plates
Lasius niger	HENKING 1892	♀	—	—	**10.** Fig. 283
Campanotus herculeanus	LAMS 1908	♂	—	**16** spheres of which 6 might be paired. Fig. 5 **7** large. Figs. 8 and 10	**6** doubles. Fig. 12 **8** singles. Fig. 13
Formica sanguinea	SCHLEIP 1908	♀♂	—	**24.** Fig. 2	**24.** Fig. 3, early anaphase **40-50,** in metaphase. Figs. 6*a*, *b*
		♀	—	**24.** Fig. 17*a*, *b*, 1st. polar body	
Lasius flava	HOGBEN 1920	♀	**24** spheres in primitive germ cells	**11** in pachytene. Fig. 55	

TABLE IV

	Somatic		
Sp'tid and Ootid	Cleavage	Blastoderm	Adult
No figures countable; only one sperm	—	—	—
	—	—	—
8 monads. Figs. 39, 42; one sperm only	—	—	Number in follicle cells more than in germ cells More than **20** in telophase. Fig. 47
2 equal sperms	—	—	—
—	—	—	—
8. Fig. 303, pro-n. **10** in polar spindle	**19** or **20** in fert. egg. Fig. 323	**26** dots in Fig. 310	—
8 or **16**? non-reduction not certain	—	—	—
24. Fig. 7*b*	♂ **20-21** unfert. Fig. 10	chromosomes sickle-shaped. Fig. 12: no doubling of number	remains haploid up to laying down of germ cells. No evidence
24. Fig. 16	♀ more than **30,** fert. Fig. 23	—	—
—	—	—	

TABLE V. CHROMOSOME NUMBERS IN CYNIPIDAE

Species	Author	Sex	Germinal		
			Gonia	1st. Sp'cyte and Oocyte	2nd. Sp'cyte and Oocyte
Neuroterus lenticularis	DONCASTER 1910	♂	**10.** Fig. 4; probably bivalent	**20** irregular masses in early 'cytes **10** bands. Fig. 6	**10.** Figs. 10, 11, 12 and 14
		♀P	**20** in primitive egg-tube. Fig. ?	(*a*) — (*b*) —	— —
		♀S	**20** in primitive ova. Fig. 19	—	**10** approx. after 1st. maturation division. Fig. 25*a*, *b*
	1911	♀P	—	(*a*) No count Fig. 12 (*b*) —	no count. Fig. 13 —
	1916	♀P ♀S	— —	**20.** Fig. 21 No definite first plate	— **10.** Fig. 11
Rhodites rosae	HENKING 1892	♀	—	**9** doubles. Fig. 248	**9** singles; anaphase of 1st. Div. Fig. 250
	SCHLEIP 1909	♀	—	**10-12.** Fig. 4	**10-12.** No clear figure
	HOGBEN 1920	♀	**18** Fig. 19	**9** bivalents. Figs. 26—29 **18** diffuse. Fig. 32 **9** dumb-bells. Fig.34	—
Dryophanta erinacea	WIEMAN 1915	♂	—	**12.** Fig. 8 (shows about 8)	**12** hooked. Fig. 9; polar view of metaphase
		♀S	—	—	—

P = parthenogenetic spring generation.
S = sexual summer generation.

TABLE V

Somatic			
Sp'tid and ripe Ootid	Cleavage	Blastoderm	Adult
10. Fig. 15, telophase	**10,** probably bivalent. Figs. 47, 48	—	**10** in nerve cells **20** in follicle cells; no figures **20** in wing cells of old larva. Fig. 1
	(*a*) **10** (above)	—	**20** in egg follicle cell. Fig. 3
	(*b*) **20.** No polar chromosomes Figs. 44*a*, 44*b*, 46	—	
20 Fig. 26. Figs. 36-38	—	—	**20** dots in developing wings. Figs. 1, 2
20. Fig. 18, polar chroms.	(*a*) **10.** Fig. 15	—	(*a*) **10** pairs. Figs. 26-28
—	(*b*) **20** (about). Figs. 8, 9	—	**20.** Fig. 29 (*b*) diploid: no figures
—	—	—	—
Fig. 251, **9** and **9** in polar nucleus	Fig. 252, **9-18-20** in segmentation nucleus	—	—
10-12. Fig. ?	no more than **12**	**6** 'doppelwertig'. Fig. 10	—
—	—	—	**18** in follicle cells; minute, Fig. 18
—	—	—	**12** hooked, in wing. Fig. 19 **12** in follicle cells. Figs. 20, 21, 22

TABLE VI. CHROMOSOME NUMBERS IN CYNIPIDAE (contd.) AND ICHNEUMONIDAE ETC.

Species	Author	Sex	Gonia	1st. Sp'cyte and Oocyte	2nd. Sp'cyte and Oocyte
Andricus punctatus	HEGNER 1915	♀	—	**6** doubles. Fig. 84; abortive spindle	—
Diastrophus nebulosus	HEGNER 1915	♀	—	abortive spindle in early oocyte	—
Cynips kollari	HOGBEN 1920	♀	**20** irregular masses in rosette. Fig. 4	**10** pachytene. **20.** Fig. 11 **10** in abortive spindle. Fig. 13	—
Ageniaspis fuscicollis	MARTIN 1914	♀	—	**8-12.** Figs. 5, 6,7	**4.** Fig. 11
Copidosoma buyssoni	SILVESTRI 1914	♀	—	**12** dots. Fig. 11, abortive spindle	**12.** Fig. 21; telophase 2nd. division
Copidosoma gelechiae	HEGNER 1914, 1915	♀	—	**12** paired. Fig. 58 **6** doubles. Fig. 59	—
	LEIBY 1922	♀	—	**12-16.** Fig. 23, ovarian oocyte: **8** doubles. Fig. 27, in maturation spindle	**8.** Fig. 32
Paracopidosomopsis floridanus	PATTERSON & PORTER 1917	♂	**8** long, hooked. Fig. 1	**8.** Figs. 6, 7, 8	**8.** Figs. 10, 11
	PATTERSON 1917	♀	—	**8** bivalents in prophase. Fig. 7	**8.** Figs. 9, 10
	1921	♀	—	**16.** Fig. 26	**8.** Fig. 27
Apanteles glomeratus	HEGNER 1915	♀	—	**12.** Fig. 76 **6** doubles. Fig. 78, abortive spindle	—
Orthopelma luteolator	HOGBEN 1920	♀	**22.** Fig. 42	**11** pairs. Fig. 48, abortive spindle	—

TABLE VI

Sp'tid and ripe Ootid	Cleavage	Blastoderm	Adult
—	—	—	—
—	—	—	**12**? follicle cells
—	—	—	**20.** Epithelium and nerve cells, Fig. 1; follicle cells, Fig. 2; chroms. filamentous
5. Fig. 12. Two polar bodies; syngamy, Fig. 18	—	**9.** Fig. 24, fertilised?	—
—	—	—	—
—	—	—	—
8. Fig. 34; three polar nuclei	—	**16.** Fig. 60, fertilised	—
8. Fig. 15 anaphase; two spermatids	—	—	**8.** Figs. 5, 6, central nervous system
8. Fig. 11 **8.** Fig. 29	**16.** Fig. 17, metaphase, fertilised —	**16.** Fig. 18 —	**16.** Figs. 3, 4, central nervous system —
—	—	—	—
—	—	—	—

TABLE VII. CHROMOSOME NUMBERS IN TENTHREDINIDAE

Species	Author	Sex	Gonia	1st. Sp'cyte and Oocyte	2nd. Sp'cyte and Oocyte
Pteronidea ribesii	DONCASTER 1906	♀	—	**8.** No figures	**8.** Fig. 24
	1907	♀	**8** masses. Figs. 12, 15	—	(*a*) **8.** Fig. 18 (*b*) **4.** Figs. 20-21
		♂	about **8** masses. Figs. 6*a*, 6*b*	**4** double or quadruple masses. Figs. 7*a*, 7*b*.	**4.** Fig. 8*a*
		♂	**16.** No figures	**8** no figures	
Pteronidea melanaspis	PEACOCK 1925	♂	**8.** Fig. 1	**8.** Fig. 3.	**8.** Fig. 5
Nematus lacteus	DONCASTER 1906	♀	—	—	**8.** No figures 3 polar nuclei
Nematus pavidus	DONCASTER 1906	♀	—	—	**8.** Fig. 14. 3 polar nuclei
Poecilosoma luteolum		♀	—	—	**8.** Fig. 29, in second mitosis
Hemichroa rufa		♀	—	—	**8.** Fig. 21, inner polar spindle
Croesus varus		♀	—	—	**8.** Fig. 23, inner polar nucleus
Sirex cyaneus	PEACOCK & GRESSON 1931	♂	—	—	**8.** Figs. 14-16
		♀	**16.** Fig. 19	—	—

TABLE VII

Sp'tid and ripe Oocyte	Cleavage	Blastoderm	Adult
8. Fig. 25, anaphase	—	—	—
(*a*) **8.** (*b*) **4.** No figures	—	**8** in fertilised egg. No figures	**16** in ovary sheath. Fig. 16 f **8** in follicle cells. No figures
—	—	—	—
—	—	—	—
—	—	—	—
—	—	—	—
—	—	—	—
—	—	—	—
Three polar nuclei are formed in both ♂-producing and ♀-producing species			
—	—	—	—
8. Fig. 17, anaphase	—	—	—

TABLE VIII. CYTOPLASMIC PHENOMENA DURING SPERMATOCYTE

Species	Nuclear membrane in Sp'cyte I	Metaphase Plate in Sp'cyte I	Spindle in Sp'cyte I
Apis mellifica	persists	clear	two half-spindles
Osmia cornuta	persists	well-formed chroms. distinct	two half-spindles
Xylocopa violacea	persists	chroms. distinct	full spindle
Vespa crabro	persists through two divisions.	chroms. countable but plate not so clear as in bee	one half-spindle
Vespa maculata	indistinct in I, dissolved in II	indistinct	one half-spindle; intranuclear fibres
Campanotus herculeanus	persists; wavy outline	chroms. in irregular clump	spindle not clear
Paracopidosomopsis	persists	chroms. closely massed	imperfect
Dryophanta erinacea	irregularbut entire	scattered	'reticular' indication of spindle
Neuroterus lenticularis	persists	not clear; in broad end of cell	fine fibres connect with bud
P. ribesii	persists	no plate; chroms. clump	one half-spindle; not clear
Sirex cyaneus	persists	no plate	two half-spindles

DIVISIONS IN HYMENOPTERA. TABLE VIII

Abortive division in Sp'cyte I	Interkinesis	Spindle in Sp'cyte II	Second Sp'cyte division
large bud with centrosome	—	along axis of first: free in cytoplasm	unequal in cytoplasm: only one sperm
no bud found	—	along axis of first	unequal, one sperm only
large bud contains chromatoid body	—	along axis of first	unequal, one sperm only
bud larger than in bee	—	at right angles to first	equational; two sperms
bud chiefly of interzonal body	—	?	equational; two sperms
?	figures indicate a resting stage	?	equational; two sperms
filar process: centrosome cut off in bud	chroms. clump in broad end of cell	?	equational
filar process: bud distinct	resting bouquet stage	along axis of first	equational
very small bud with centrosome	comparable to growth phase of sp'cyte I	?	equational
no bud	comparable to growth phase	at right angles	equational
cell elongated; bud formed	—	?	equational

XII

PLATES I—XIX

DESCRIPTION OF PLATES

Drawings were made with a Reichert camera lucida apparatus and a Spenser microscope, using a $^1/_{12}$th Reichert Oil Immersion Objective and a x 10 Ocular. With the exception of the drawings of growth phases in the oocyte, the majority, made by the above method, have been magnified $2^1/_2$ times and are therefore to the same scale. Scale measurements are given on each plate.

ac	acroblast	*n* or *n'*	nucleolar buds
amph	amphophil body	*nc*	nurse cells
b or *b'*	basophil bodies	*nc. gr*	granules from nurse cells
c	chromatoid body	*nm*	nuclear membrane
cb	body in cytoplasm	*o* or *o'*	oxyphil bodies
ch	chromatin mass	*oc*	oocyte
chor	chorion	*ovid*	oviduct
ch. gr	chromatin granules	*p*	plasmosome
chr	chromatin threads	*pn*	polar nucleus
cs	centrosome	*r*	residual body
cw	cyst wall cell	*ros*	rosette of cells
dc	degenerating cell	*s*	solitary cell
epi	epithelial cells	*sp. b*	body formed from spindle fibres
f	follicle epithelium cells		
fb	foreign body	*uc*	undifferentiated cells
gv	germinal vesicle	*ya*	albuminous yolk
m	mitochondrial body	*yf*	fatty yolk
N	nucleolus	*z*	'Zellkoppelung'

PLATE I

All figures are from BOUIN-Haematoxylin preparations and are to same scale.

Fig. 1 a. Primary spermatogonium showing budding of nucleolus.
b. Group of spermatogonia showing budding of nucleolus.
c. Group of larger cells, one with extranuclear body.

Fig. 2 Spermatogonial 'morula' stage beginning to break up.

Fig. 3 Early spermatogonium metaphase plate: chromosomes about 7 in number lying free in general cytoplasm.

Fig. 4 Later spermatogonial plate showing 8 chromosomes.

Fig. 5 Part of epithelial wall of testis showing outline of developing cyst with five chromosome plates linked up by bands of cytoplasm: *ros* rosette of larger cells in growth phase: *s* a solitary cell probably a developing cyst or follicle wall cell.

Fig. 6 Cyst of spermatogonial plates.

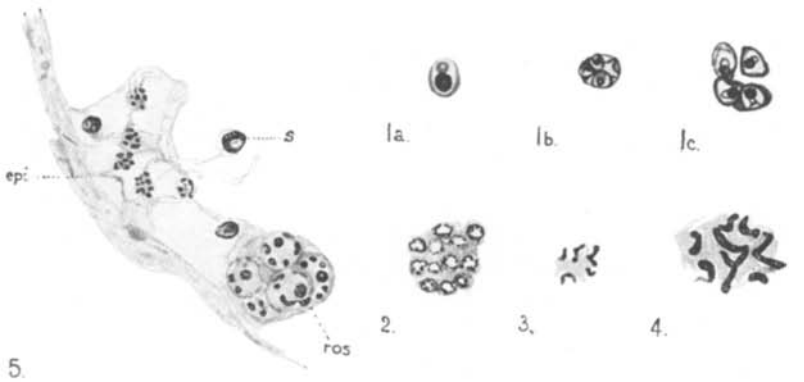
Fig. 7 Developing cyst wit groups of primary spermatogonia: extranuclear body in cell on right.

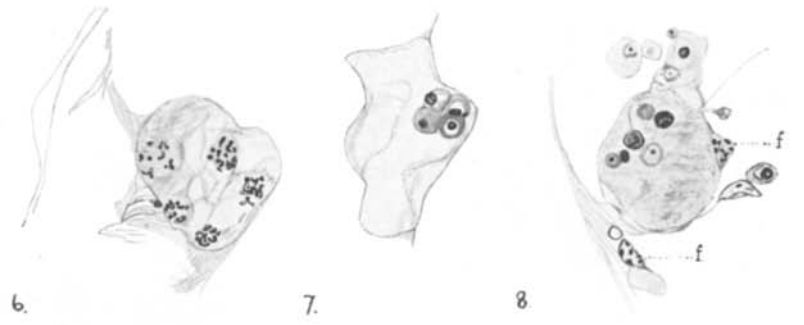
Fig. 8 Cyst with small cells which have just been liberated from 'morula'; two follicle cells shown at *f*.

Fig. 9 Spermatogonial metaphase showing 9 chromosomes, and two chromatoid bodies: *fb* peculiar body possibly foreign.

Fig. 10 Spermatogonium in early anaphase showing 17 chromosomes of which three pairs are still incompletely separated.

Fig. 11 Spermatogonial metaphase plate showing 8 chromosomes.





10 U.

PLATE II

Spermatogonia. Figures 12–21 are drawn from DONCASTER'S FLEMMING Haematoxylin preparations. Figures 22 and 23 are from BOUIN-Haematoxylin material.

Figs. 12—15 Growth phases of spermatogonia.

Fig. 16 Spermatogonium showing about 9 chromosomes.

Fig. 17 Spermatogonium showing 8 chromosomes: two small and one large chromatoid bodies in cytoplasm.

Figs. 18 & 19 Spermatogonial plates with two chromatoid bodies.

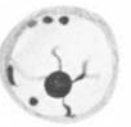
Fig. 20 Stage in the breaking up of the chromatin clump.

Fig. 21 Nucleolus sending out chromatin threads prior to formation of chromosomes.

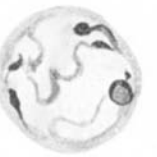
Fig. 22 BOUIN-Haematoxylin preparation: cyst of spermatogonia from testis of winter larva, showing "Zellkoppelungen".

Fig. 23 Spermatogonial plate showing 7 chromosomes.

12.



13.

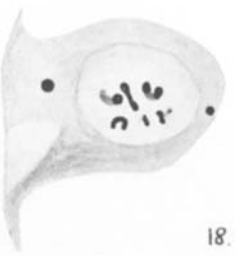


14.

15.

16.

17.



18.

19.

20.

21.

22.

23.

FIG. 22 100

FIGS. 12-21-23 100

PLATE III

The abortive first maturation division: FLEMMING-Haematoxylin preparations from DONCASTER's material. Figures 26—32 are drawn to smaller scale than Figures 24, 25 and 33; the latter have been enlarged $2^1/_2$ times the former.

Fig. 24 Spermatocyte I showing formation of about 8 chromosomes.

Fig. 25 Stage previous to that of Fig. 24.

Fig. 26 Chromosomes on abortive spindle, a stage rarely found.

Fig. 27 Abortive spindle: chromatin sending out irregular threads to nuclear membrane: *z* "Zellkoppelung".

Fig. 28 Complete half-spindle of fibres: "Zellkoppelung" on side of cell: two centrosomes *cs* shown.

Figs. 29 & 30 Chromatin approaching distal or broad end of cell.

Fig. 31 Elongated cell showing a large chromatoid body towards one end.

Fig. 32 Group of spermatocytes II in resting phase showing "Zellkoppelungen".

Fig. 33 Spermatocyte II at beginning of resting phase after abortive division.

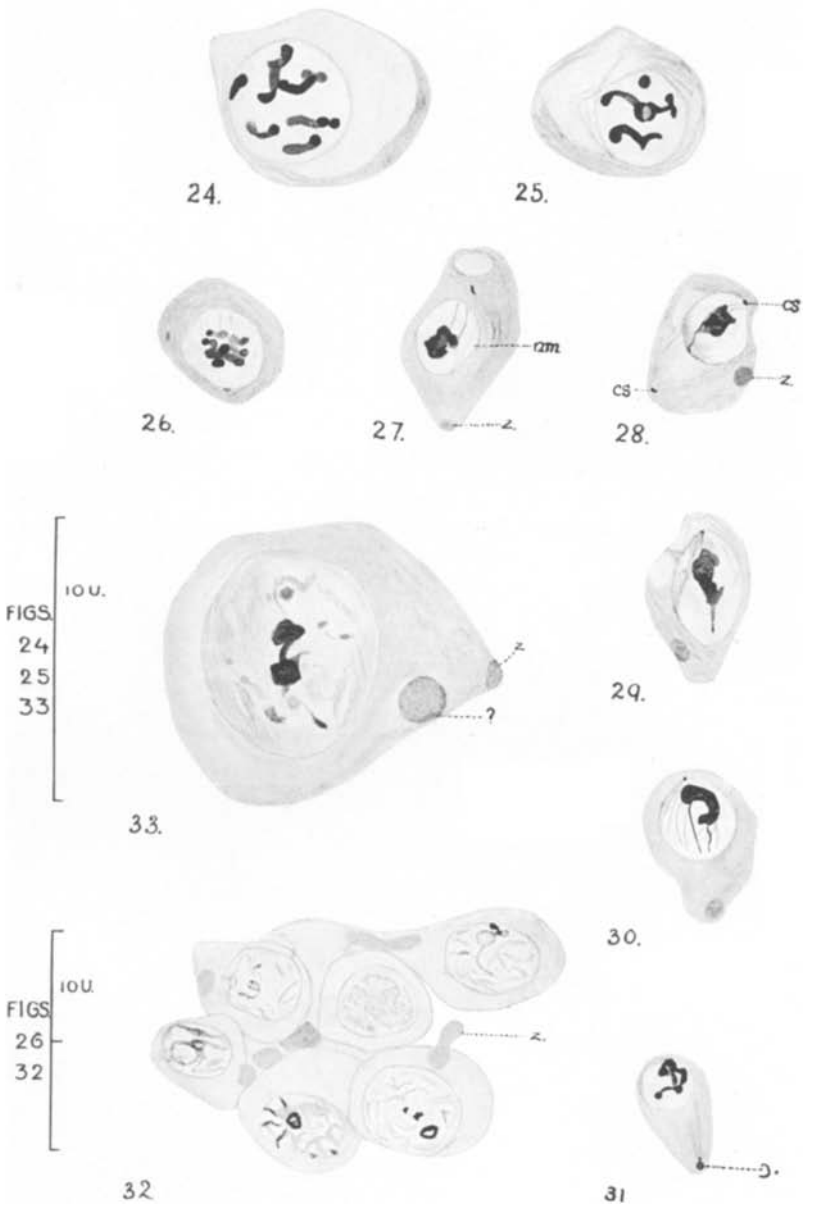

24.
25.
26.
27.
am.
z.
28.
cs
cs
z.
FIGS.
24
25
33
100.
z
?
33.
29.
30.
FIGS.
26
32
100.
z.
32
31
c.

PLATE IV

Second spermatocyte division: All figures with the exception of Figures 36, 42 and 44 are BOUIN-Haematoxylin preparations. Figure 44 is to a smaller scale.

Figs. 34 & 35 Spermatocyte II: emergence of chromosomes prior to formation of second division metaphase plate.

Fig. 36 FLEMMING-Haematoxylin preparation: peculiar cell considered to be spermatocyte of second order: chromatin in twisted thread with about 8 paired swellings.

Fig. 37 Second spermatocyte early metaphase plate showing 8 chromosomes: chromatoid body in cytoplasm.

Fig. 38 Metaphase plate with 7 chromosomes, 6 of which can be paired.

Fig. 39 Metaphase plate with 8 chromosomes of which 6 can be paired.

Fig. 40 Anaphase of second division showing I large and 7 small chromosomes at lower pole of spindle and 7 at upper pole.

Fig. 41 Polar view of anaphase showing 8 chromosomes and one large body *c*, probably a chromatoid body.

Fig. 42 FLEMMING-Haematoxylin preparation showing telophase of second division: a chromatoid body in each spermatid.

Fig. 43 Spermatid showing chromatin in a clump.

Fig. 44 Cyst of second spermatocytes showing in the centre free "buds" which are sections of the cytoplasmic bridges: one of these has a dark-staining granule.

34.

35.

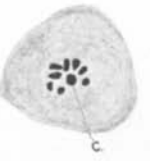
36.

37.

38.

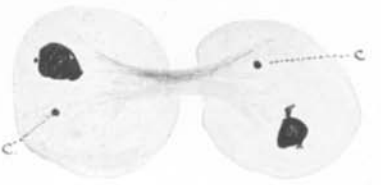
39.

40.



41.

43.



42

44.

10 U.

FIGS. 34-43.

10 U.

FIG. 44

PLATE V

Spermateleosis: Figures 45—47 are FLEMMING-Haematoxylin, figures 48—52 BOUIN-Haematoxylin preparations.

Fig. 45 — Spermatid just after second spermatocyte division.

Figs 46 & 47 — Chromatin of spermatid entering resting phase.

Fig. 48 — Stage in formation of sperm: chromatin in granules around nuclear membrane: *ac* peculiar body outside membrane, probably an acroblast.

Figs 49—51 — Stages in the development of flagellum; in Fig. 49 two chromatoid bodies and one near posterior end in Fig. 51.

Fig. 52 — Group of developing sperms: nucleus still spherical and chromatin in granules.

45.

48.

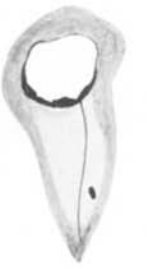
46.

52.

47.

49.

50.



51.

PLATE VI

Fig. 53 FLEMMING-Haematoxylin preparation showing cyst of spermatids close to testis wall: "Zellkoppelung" still attached to cell: *f* enlarged follicle cell.

Fig. 54 a—d, stages in the development of sperm; BOUIN-Haematoxylin. e, a bundle of sperms.

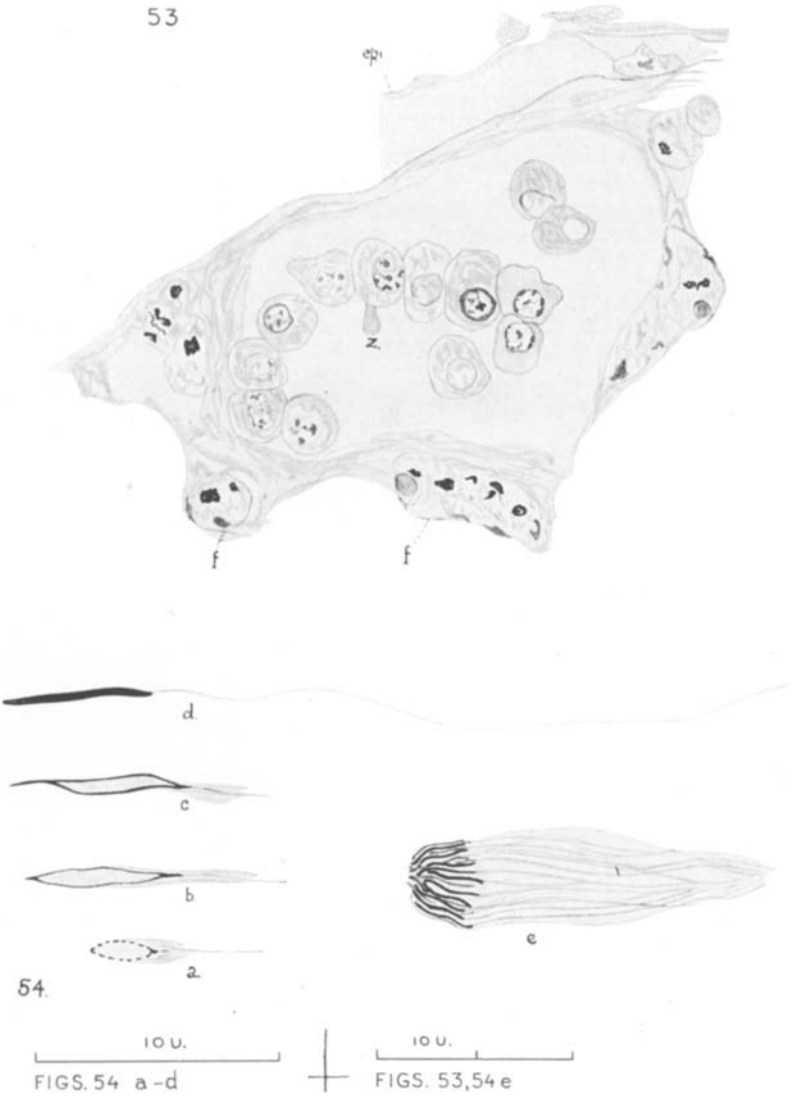

53
ep
N
f
f
d
c
b
a
54
e
100.
FIGS. 54 a-d
100.
FIGS. 53,54 e

PLATE VII

Oogonia: BOUIN-Haematoxylin preparations. Figures 57—60 are drawn to smaller scale.

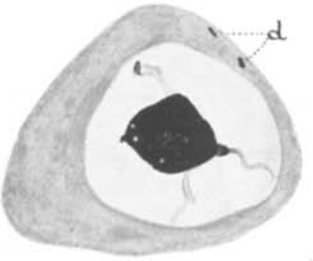
Figs 55 & 56 Oogonia showing growth phases: Fig. 56 with two chromatoid bodies in cytoplasm; nature of bodies at *d* doubtful.

Fig. 57 Cysts of oogonia in growth phases: *cw* small cyst wall cells; *f* follicle cell.

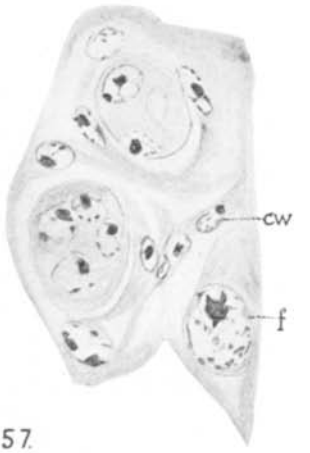
Fig. 58 Oogonial spindles; clumped chromatin due to fixation. Numerous granules in cytoplasm probably of same nature as those in Fig. 56.

Fig. 59 Four oogonia from a rosette showing "Zellkoppelungen".

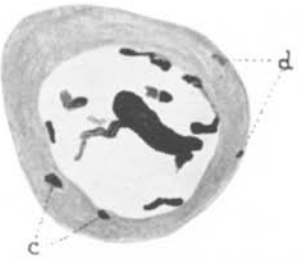
Fig. 60 A cyst of resting oogonia showing arrangement of cells and prominent "Zellkoppelungen" in section.



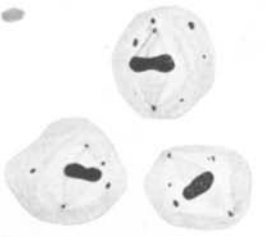
55.



57.

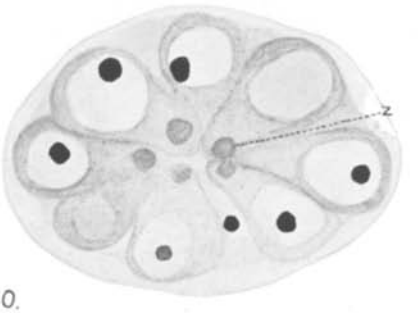


56.



58.

59.



60.

100.

FIGS. 55, 56

100.

FIGS. 57-60

PLATE VIII

Oogonial mitoses: Figures 61—65, 69 and 70 are from BOUIN-Haematoxylin preparations. Figures 66—68 are reproduced from drawings made in correct colourings of material stained by FEULGEN'S "Nuclealreaktion" method and counter-stained with Light Green.

Fig. 61 Stage previous to spindle formation: chromosomes forming *cb* bodies in cytoplasm.

Fig. 62 Metaphase plate showing 16 chromosomes and one chromatoid (?) body *c*.

Fig. 63 Metaphase plate showing 21 chromosomes. Those on the left do not appear to belong to this cell.

Fig. 64 Cell with 18 chromosomes.

Fig. 65 Oogonium showing 15 or 16 chromosomes several of which are arranged in pairs: a chromatoid body in the cytoplasm.

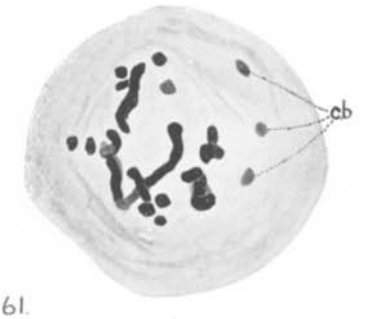
Fig. 66 Profile view of spindle showing 14 chromosomes.

Fig. 67 Metaphase plate with 16 chromosomes.

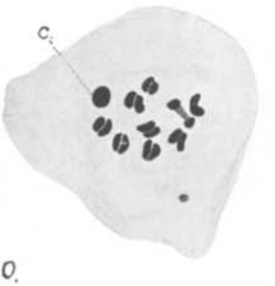
Fig. 68 Metaphase plate with 15 chromosomes.

Fig. 69 Early anaphase viewed obliquely.

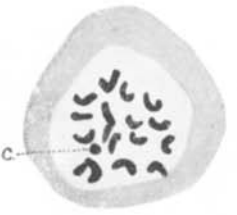
Fig. 70 Metaphase plate showing 16 chromosomes 14 of which are paired. One large spherical chromatoid body.



61.

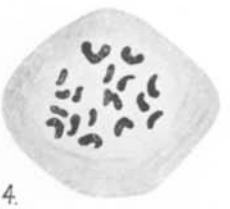


70.



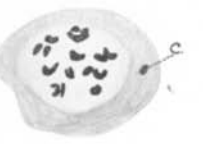
62.

63.



64.

66.



65.

67.

69.

68.

10 U.

PLATE IX

Syndesis: Figures 71—76 are from BOUIN-Haematoxylin preparations. Figure 77 is from CARNOY-Haematoxylin material. Figures 73, 76 and 77 are to smaller scale.

Fig. 71 Telophase of last oogonial division; chromosomes becoming thread-like prior to syndesis.

Fig. 72 Oogonium in prophase; chromosomes forming at expense of nucleolus.

Fig. 73 Three potential oocytes; chromatin in syndesis: *sp b* body formed by fusion of spindle fibres.

Fig. 74 Early stage in syndesis; chromatin threads clumping.

Fig. 75 a. Cell in syndesis.
b. Polar view of similar cell.
c. Cell with two loops of chromatin extending from the main mass to the nuclear membrane. Bodies in cytoplasm may be residual cell plate.

Fig. 76 Small scale drawing of oocyte and four nurse cells; nucleolus large and prominent; linin threads pale; body *n* in cytoplasm probably derived from nucleolus, or may be remains of cell-plate; cf. *sp b* Fig. 73.

Fig. 77 To same scale as cells in Fig. 76. Oocyte from end chamber of ovariole; chromatin appears to be clumping near large nucleolus; nature of dark-staining body in cytoplasm not determined.

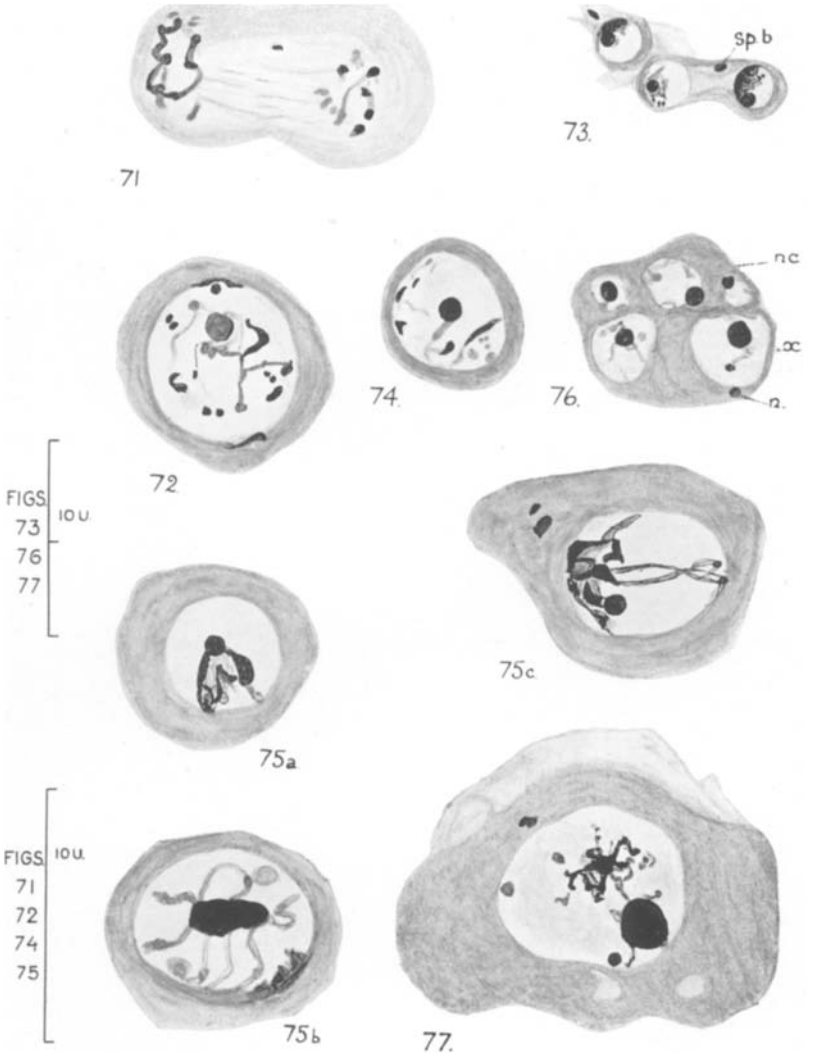

71
73.
sp. b
72
74.
76.
n.c
.oc
n.
FIGS
73
76
77
10 u
75c
75a
FIGS
71
72
74
75
10 u
75b
77.

PLATE X

Synaptic phases in germinal vesicle. Figures 78—79 are drawn from DONCASTER'S material. All figures to same scale. Figures 83 and 84 are reproduced from coloured drawings.

Fig. 78 Young oocyte with nurse cell: nucleolus *N* in germinal vesicle sending out chromatin in paired threads: *n* is probably a nucleolar extrusion.

Fig. 79 Post-syndesis phase: three nucleoli present and one of them is budding: chromatin in pale-staining threads.

Fig. 80 Stage not often found; chromatin thread is single and very thin; nucleolar body is budding and appears to be passing entire through the nuclear membrane.

Fig. 81 Post-synaptic spireme; paired chromatin threads closely approximated; two bodies in the ooplasm.

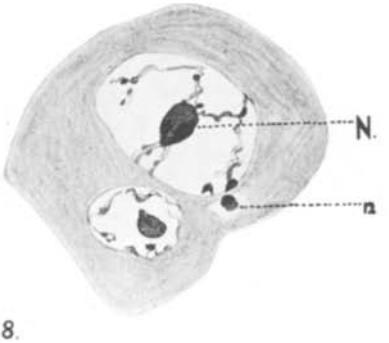
Fig. 82 Diplonema; chromatin thread in paired segments which are thin and bear paired knots.

Fig. 83 FEULGEN preparation; chromatin (purple) in early diplotene; nucleolus stained green.

Fig. 84 *Egg I* from anterior chamber of ovariole.

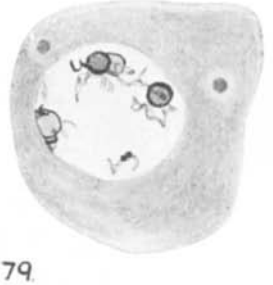
a. MANN'S Methyl-Blue-Eosin preparation; chromatin as a pinkish body.

b. Same section treated with Haematoxylin: chromatin is apparently oxyphil.



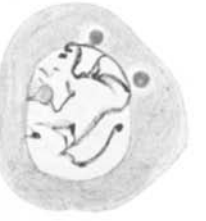
78.

82.



79.

80.



81.

83.

84.

10 U.

PLATE XI

Nuclear changes during the growth phase of the oocyte.

These figures have been reproduced from drawings coloured according to the stains employed. All are drawn to same scale.

Fig. 85 *Egg 2* Germinal vesicle stained first with MANN's Methyl-Blue-Eosin (a), destained and subsequently treated with Fe. Haematoxylin (b).

a. Nucleoplasm oxyphil with darker-stained area *ch* in centre.

b. Dark-staining lobulated mass in centre *ch* is probably chromatinic.

Fig. 86 *Egg 2* Stained by FEULGEN'S method; chromatin as a compact mass in the centre of the germinal vesicle.

Fig. 87 *Egg 3* FEULGEN preparation; chromatin mass *ch* closely applied to the large plasmosome *p*.

Figs. 88 & 89 *Egg 3* Germinal vesicles of two different eggs stained first by MANN'S double stain (a) and then by Haematoxylin (b).

Fig. 88 a. Large plasmosome *p*, oxyphil body vesicular at *o*, basophil component of the nucleolus at *b*.

b. Plasmosome more distinct than in Methyl-Blue-Eosin. Oxyphil body *o'* stained intensely black; basophil paler, *b'*; numerous black bodies *n'* liberated from nucleolus, free in nucleoplasm.

Fig. 89 a. Large plasmosome not found; basophil body *b* stained dark in (b); oxyphil vesicles free in nucleoplasm appear solid in b at *n'*.

b. Small granules approaching nuclear membrane not shown in (a).

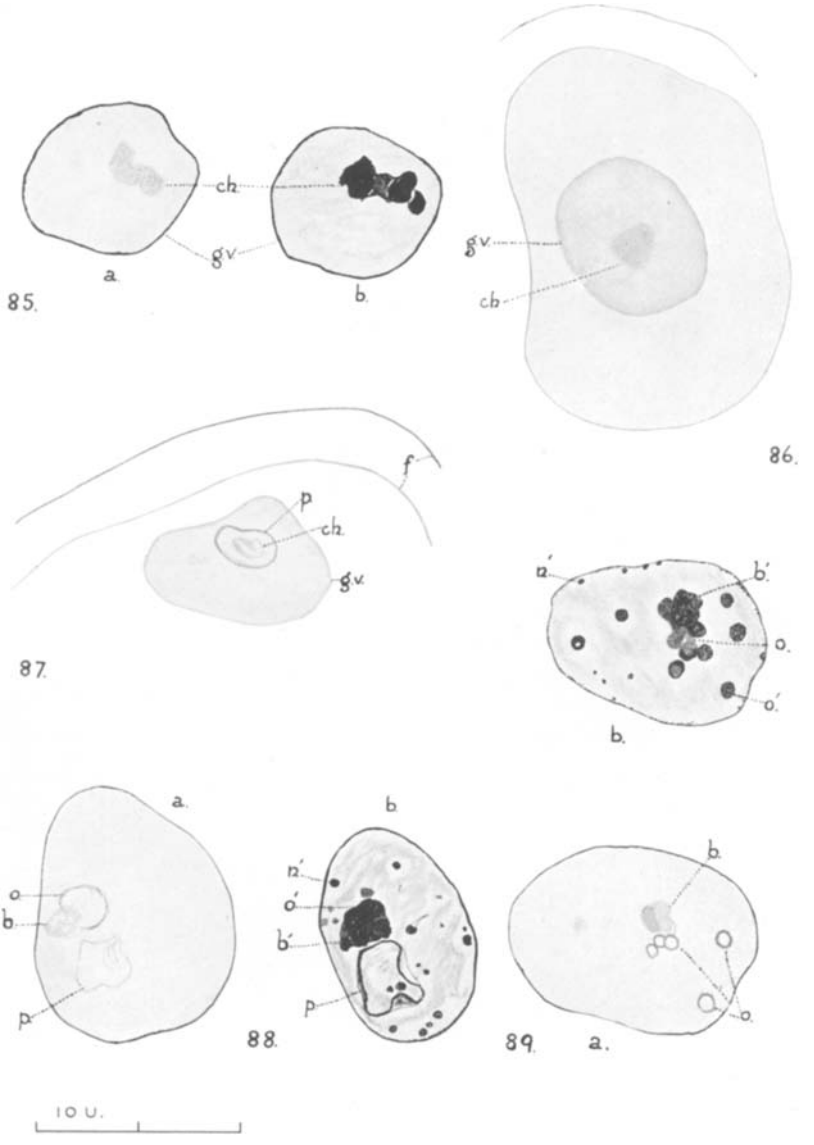

ch.
g.v.
a.
b.
85.
g.v.
ch.
86.
f.
p.
ch.
g.v.
87.
n'.
b'.
o.
o'.
b.
a.
o.
b'.
p.
b.
n'.
o'.
b'.
p.
88.
b.
o.
89.
a.
10 U.

PLATE XII

Nuclear changes in Eggs 4, 5 and 6; Figures 91, 92 and 94 are reproduced from coloured drawings.

Fig. 90 *Egg 4* from BOUIN-Haematoxylin preparation: the plasmosome *p* is large and appears to be budding; oxyphil and basophil bodies not distinct; nucleolar buds *n* in ooplasm.

Fig. 91 *Egg 4* FEULGEN preparation; chromatin mass *ch* appears to be vacuolated; plasmosome vesicular.

Fig. 92 *Egg 5* FEULGEN preparation; chromatin mass is budding and appears to be extending over the wall of the plasmosome which is composed of a homogeneous fluid-like substance.

Fig. 93 *Egg 4* BOUIN-Haematoxylin preparation; plasmosome very large; basophil body reduced in size; oxyphil body enlarged; body *n* in nucleoplasm derived from nucleolus, probably oxyphil.

Fig. 94 FEULGEN preparation of *Egg 5;* germinal vesicle now elongated and parallel to follicle wall *f*; plasmosome appears to be collapsing; chromatin as a kidney-shaped mass not now associated with the plasmosome.

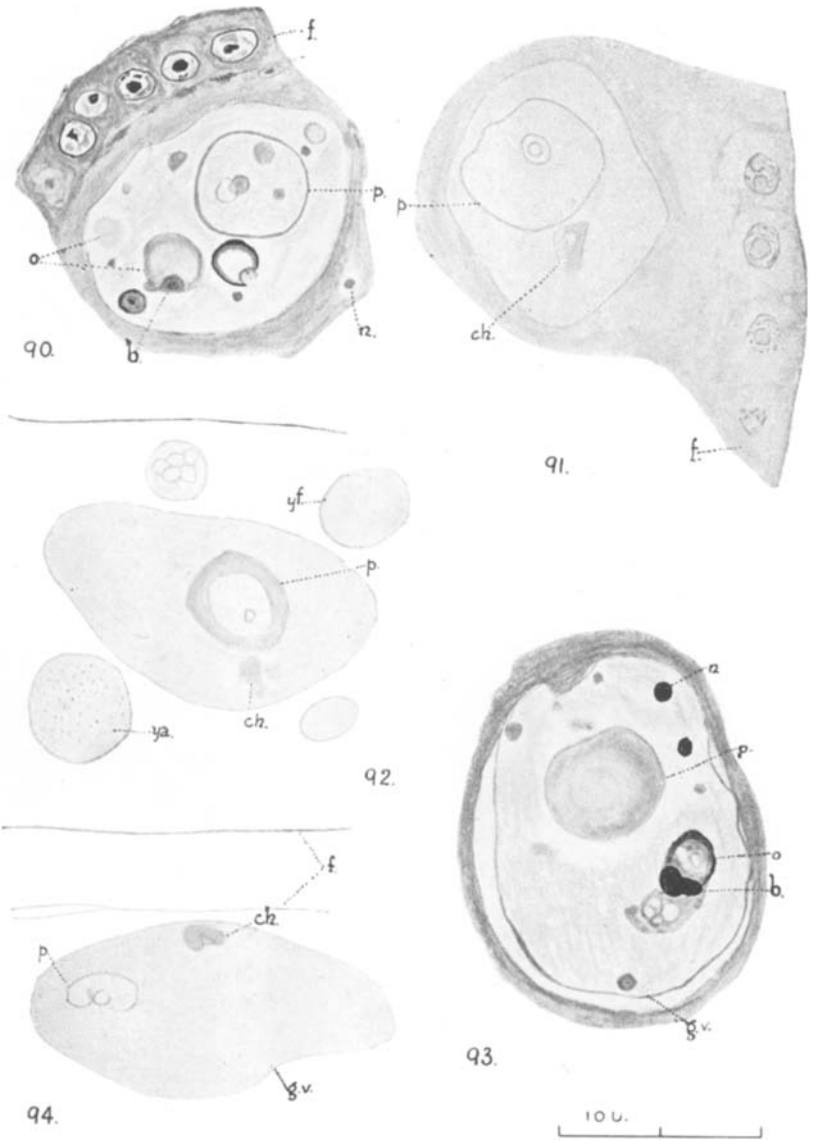

f.
P.
o
b.
n.
90.
p
ch.
f.
91.
yf.
p.
ch.
ya.
92.
n
p.
o
b.
g.v.
93.
f.
ch.
p.
g.v.
94.
100.

PLATE XIII

Maturation and reduction phases in the egg. Figures 95 and 96 are drawn from BOUIN-Haematoxylin preparations, figures 97—99 from DONCASTER'S PETRUNKEWITSCH-Haematoxylin material. All figures are to same scale.

Fig. 95 Nucleus in freshly laid egg; nucleoplasm in radiating patch; chromatin as a compact clump; small vesicle in protoplasmic patch.

Fig. 96 Anaphase of first maturation division of unfertilised egg from egg-bound female, showing 8 chromosomes (four in high and four in deep focus) at the upper pole, and 7 chromosomes at the lower pole (four in high and three in deep focus); the missing chromosome evidently belongs to the deep focus judging by the course of the spindle fibres; the latter are very prominent between the daughter chromosomes; the appearance *m* is probably mitochondrial.

Fig. 97 Oocyte from virgin female, not more than $^3/_4$ hour old, showing first polar nucleus with 8 large chromosomes arranged mostly in pairs, one of which *a* is possibly dividing, and the egg nucleus undergoing second maturation division, 6 chromosomes having divided and 2 appearing undivided. The large size of the chromosomes of the first polar nucleus becomes lost when the chromosomes divide to form two daughter polar nuclei.

Fig. 98 Oocyte from virgin female: not more than $^3/_4$ hour old: early first polar nucleus showing 16 bodies, 7 pairs and 2 singles prior to formation of two daughter polar nuclei each with 8 chromosomes. The second oocyte nucleus is missing but the area *a* marks its position.

Fig. 99 Oocyte from virgin female about one hour old, showing four nuclei in the vesicular stage; the inner is the reduced female pronucleus beginning to sink into the yolk; the others are polar and the two outer appear to be about to fuse.

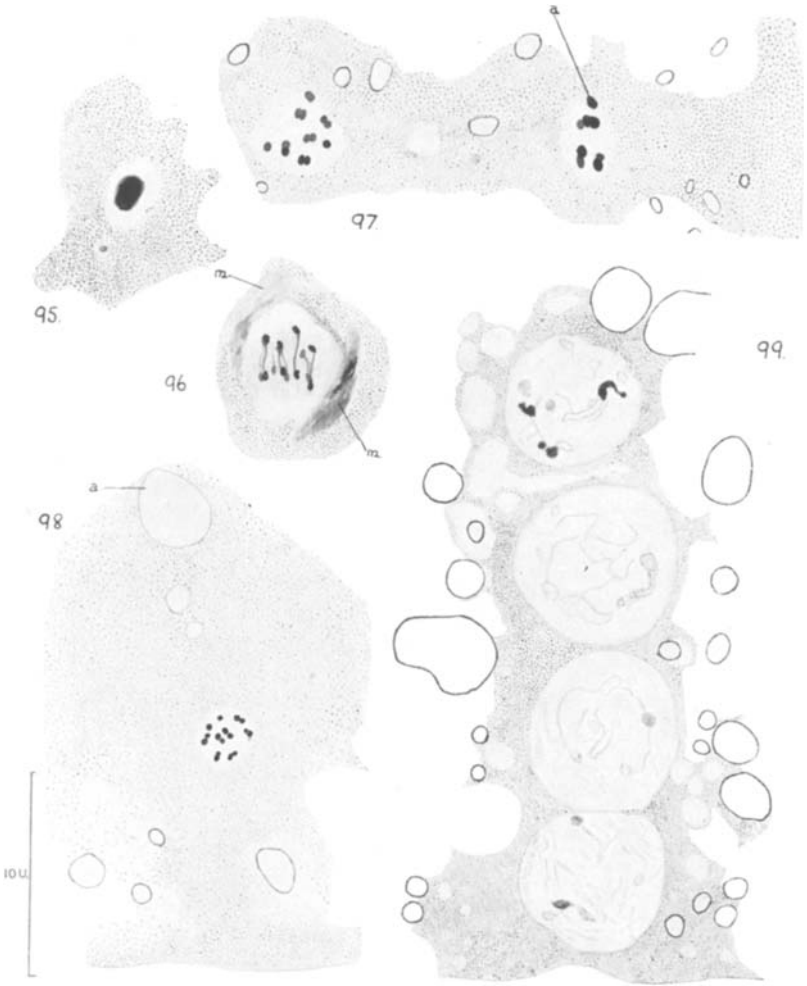

a
97
95
m
96
m
99
a
98
10 u

PLATE XIV

The maturation divisions in the oocyte. Figs. 100—101 and 104—5 are from PETRUNKEWITSCH-Haematoxylin material; Figs. 102 and 103 from BOUIN-Haematoxylin. All drawings to same scale, with the exception of 101 a.

Fig. 100 Egg from virgin female, fresh. Stage prior to the formation of the chromosomes on the first metaphase plate. The chorion *chor.* has become separated.

Fig. 101 Egg from virgin female, fresh. First maturation spindle in telophase: shows indentation in egg margin characteristic of several unfertilised eggs.
a. Small scale drawing of same egg to show position of nucleus relative to the point of attachment of the egg in the leaf.

Figs. 102—3 From unlaid eggs of fly which was not allowed to lay.

Fig. 102 First metaphase plate; about 15 chromatin masses present: *m* is thought to be mitochondrial.

Fig. 103 First anaphase plate showing 8 chromosomes in high focus.

Fig. 104 First polar spindle: all eggs from this female have such a peculiar residual mass on the spindle.

Fig. 105 Egg from virgin female less than 2 hours old; first division completed, nuclei in resting stage: *pn* first polar nucleus.

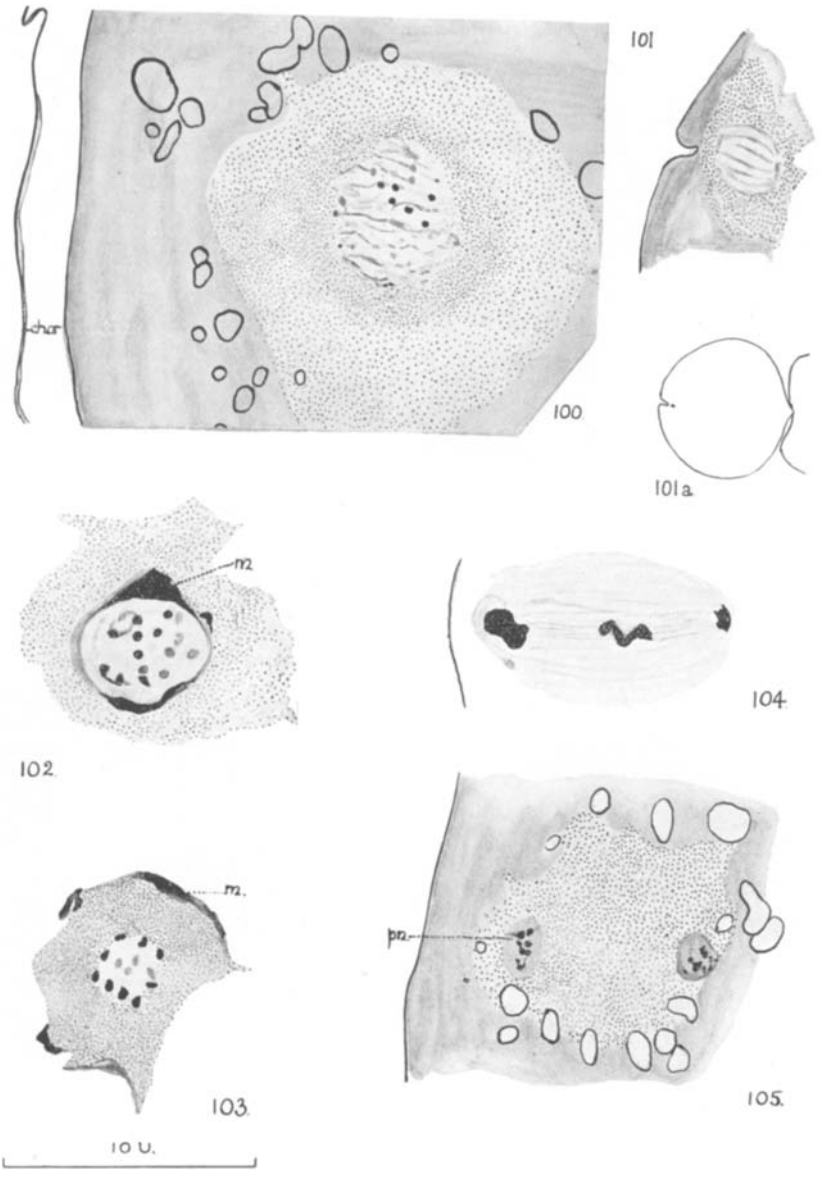

chr
100
101
101a
m2
102
104
m2
103
pn
105
10 U.

PLATE XV

Fertilisation and cleavage.
All figures are drawn from DONCASTER'S material.

Fig. 106 a and b are two resting nuclei in the yolk, which were found in two consecutive sections. Peculiar dark bodies in cytoplasmic patch may be centrosomes, and nuclei may be the result of division of the egg pronucleus: from the position of these nuclei one is thought to be the ♂ pronucleus and the other the ♀.

Fig. 107 a, b and c. From a fertilised egg $1^1/_2$—2 hours after oviposition.

a. Peculiar sperm-like body in a protoplasmic radiation deep in yolk.

b. Resting nucleus found in yolk separated from (a) by five sections. Polar chromosomes are present near the egg margin and it is thought that the nuclei in the yolk represent the ♂ and ♀ pronuclei.

c. Shows the relative positions of these nuclei and it will be seen that the ♂ nucleus has yet some distance to travel to the ♀ pronucleus.

Fig. 108 Peculiar cleavage nuclei found in the egg 6 hours old; *r* residual middle body.

Fig. 109 Diploid cleavage nucleus in yolk of early blastoderm from an egg 6 hours old laid by a fertilised female; about sixteen chromosomes are shown though the hooked shape of some renders precise counting difficult.

Fig. 110 Haploid cleavage nucleus (in yolk) of early blastoderm from a virgin egg 6—$10^1/_2$ hours old; metaphase, polar view, showing eight chromosomes and one centrosome.

Fig. 111 Haploid cleavage nucleus in early blastoderm from a virgin egg showing eight chromosomes and a very large centrosome.

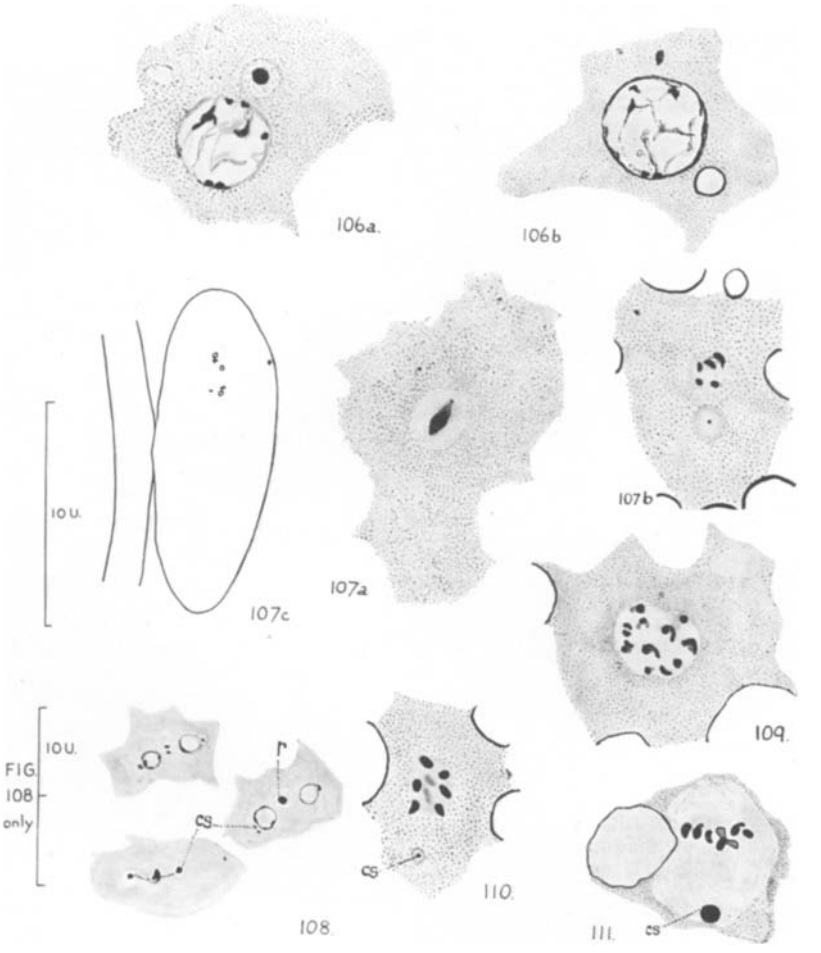

106a.
106b
10 u.
107c
107a
107b
109.
FIG. 108 only
10 u.
cs
108.
cs
110.
111.
cs

PLATE XVI

Somatic nuclei: blastoderm and developing adult tissues. All figures are from BOUIN-Haematoxylin material. Figs 112—4, 120, from male tissues. Figs 115—119 from female tissues.

Fig. 112 Cell from embryo, about $2^1/_2$ days old, developed from egg of virgin female; metaphase in oblique profile; about 8 chromosomes.

Fig. 113 Connective tissue cell from the same male pupa as Fig. 114; early anaphase showing different stages of splitting of the individual chromosomes of the original 8; the hooked character of certain of these renders counting difficult.

Fig. 114 Connective tissue cell from same male pupa as Fig. 113; late anaphase with spindle viewed obliquely; the upper pole shows 4 distinct chromosomes on the right and a close cluster of 4 on the left; the body marked *a* is distinctly outside the spindle and is not considered to be a chromosome; the lower pole shows 8 chromosomes closely grouped, the V on the right representing 2 chromosomes, and the lowermost mass also 2 chromosomes lying in contiguity.

Fig. 115 Connective tissue cell from female pupa; metaphase plate showing about 16 or 17 chromosomes most of which are arranged in pairs; the exact number in all probability is 16 as the two bodies at *a* (joined by a thin line) are most likely to be a single large chromosome with the centre region poorly shown.

Fig. 116 Giant cell from female hypodermis, to same scale as Fig. 115; Telophase showing over 50 single elements; the cell is therefore octoploid.

Fig. 117 Loose cell between muscle fibres in female pupa; 16 chromosomes present of which 10 can be paired.

Fig. 118 Connective tissue cell from same female as Figs 116, 117; early anaphase showing some of the original 16 chromosomes divided into daughter chromosomes; 27—29 daughter and original chromosomes are figured.

Fig. 119 Cell from embryo, about $2^1/_2$ days old, developed from egg of fertilised female; early anaphase; the hooked structure *a* is considered to be 2 chromosomes; the nature of the 2 bodies at *b* has not been determined but they do not appear to be chromosomes; the number of chromosomes shown is about 22.

Fig. 120 Cell from embryo, about $2^1/_2$ days old, developed from the egg of a virgin female; early anaphase showing 16 chromosomes derived from 8 which have split; the bodies at *a* and *b*, being outside the spindle are not chromosomes.

Fig. 121 Tracheal cell from female pupa; about 52 chromosomes at the poles. Small scale drawing.

Fig. 122 Polyploid follicle cell from male testis showing 15 chromosomes.

Fig. 123 Large loose cell, probably oenocyte, from female pupa, showing about 50 chromosomes. Small scale drawing.

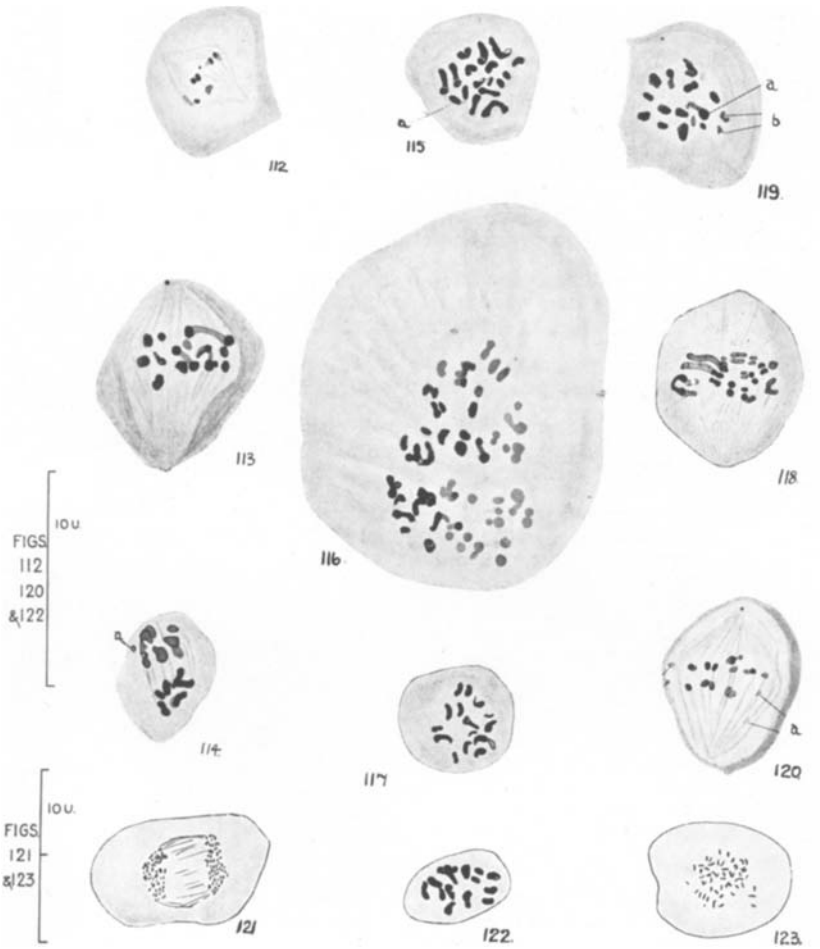

112.
115
p
a.
b
119.
113
116.
118
10 u.
FIGS.
112
120
&122
p
114
117
a.
120
10 u.
FIGS.
121
&123
121
122.
123.

PLATE XVII

Nurse cells and differentiation of oocytes.

Fig. 124 Anterior end of young ovariole: FLEMMING-Haematoxylin preparation showing differentiation of oocytes, their separation with a cluster of oocytes and the growth of the follicular epithelium.
a Peculiar nurse cell (or oocyte) which seems to be disintegrating: *e* epithelium layer round ovariole.
dc Degenerating cells in which chromatin or nucleolar material is broken up into granules.
oc young oocyte: *d* oocyte with associated nurse cells *n* and follicle cells *f* beginning to surround lower wall of oocyte: *g* group of nurse cells entirely separated off with oocyte; numerous dark-staining bodies free in cytoplasm are probably nucleolar extrusions.

Fig. 125 Anterior end of *Egg 5* showing cytoplasmic flow from nurse cell chamber into ooplasm; *nc. gr* granules free in cytoplasm resulting from breaking up of nurse cell nucleus.

Fig. 126 FEULGEN preparation showing first differentiated nurse chamber with nurse cell *nc* separated from undifferentiated cells *oc* by follicle cells *f*.

Fig. 127 Nurse cell from 5th nurse cell chamber, a BOUIN-Haematoxylin preparation; *ch. gr* granules of chromatin surrounding nucleoli in the nurse cell nucleus.

Fig. 128 Nurse cell from a similar chamber to that of Fig. 127. FEULGEN preparation showing the granules of chromatin surrounding the non-chromatinic nucleoli.

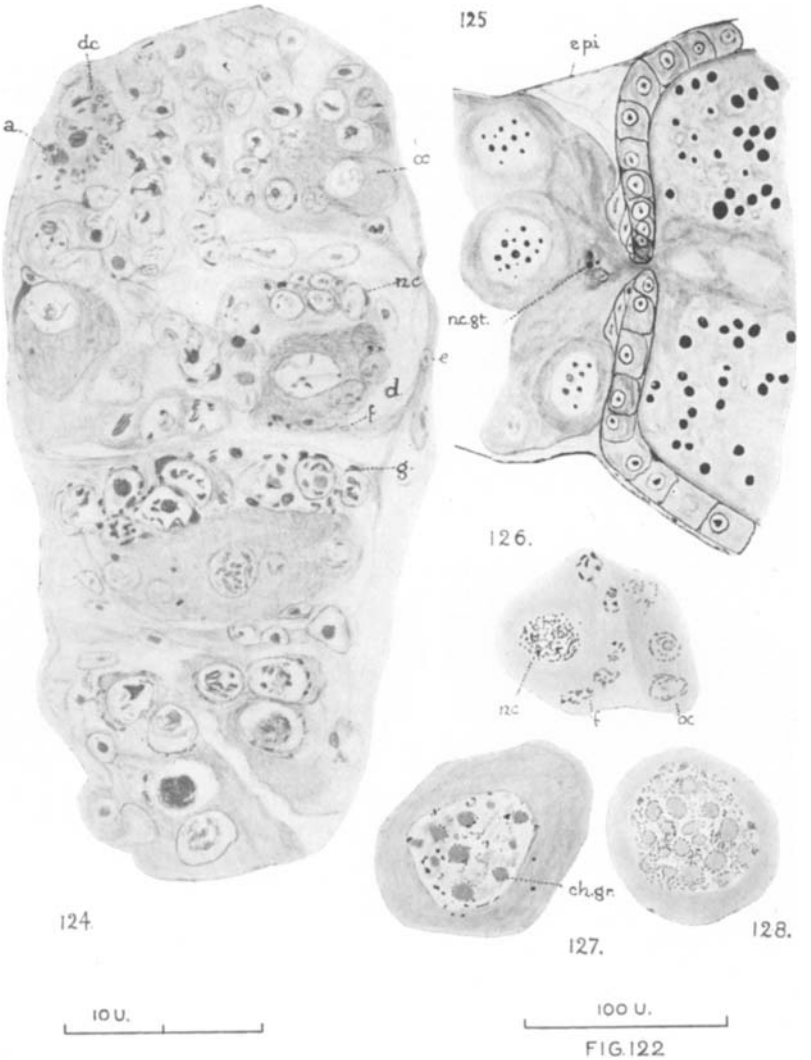

dc
a
cc
nc
e
d
f
g
124
125
epi
nc.gt.
126.
nc
f
bc
ch.gr.
127.
128.
100 U.
100 U.

FIG. 122

PLATE XVIII

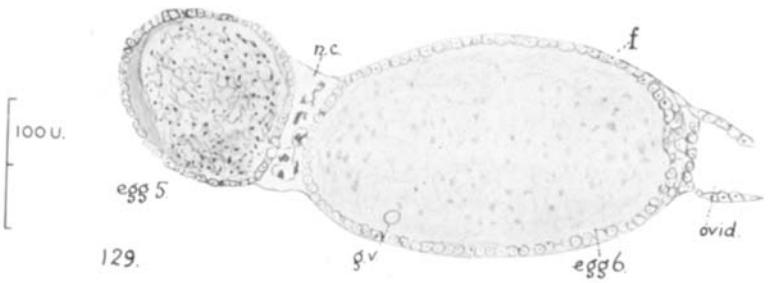
Oogonia in late growth phase. Figures 129 and 130 reproduced from coloured drawings.

Fig. 129 PETRUNKEWITSCH-MANN preparation showing last egg *(Egg 6)* in duct: *nc* remains of nurse cell nuclei from nurse chamber: *Egg 5* shows more oxyphil nature of yolk—oblique section.

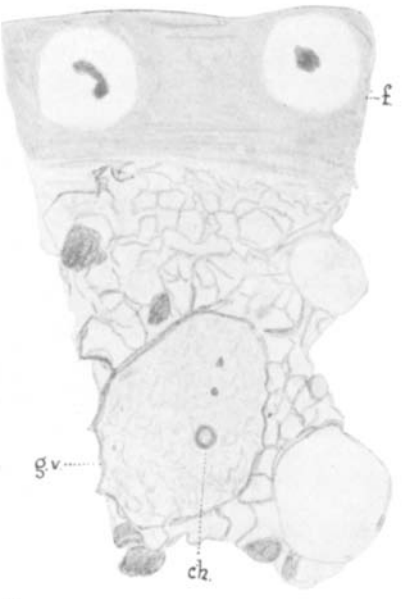
Fig. 130 Part of wall of ripe egg in ovariole to show excessive swelling caused by PETRUNKEWITSCH fixation; germinal vesicle *gv* with chromatin mass (?) *ch.*

Fig. 131 *Egg 5* Germinal vesicle showing large plasmosome with thick layer of fluid substance round periphery. Small nucleolar buds in nucleoplasm; *chr* chromatin-like threads in plasmosome.

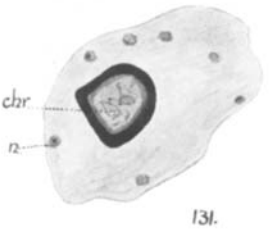
Fig. 132 *Egg 5* Germinal vesicle with layer of fluid substance on side next yolk; plasmosome collapsed; several small nucleolar extrusions near periphery of nucleus.



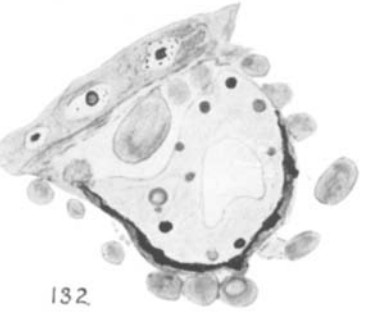
129.



130.



131.



132.

10 D.

FIGS. 127-129

PLATE XIX

Untouched photographs from Fe. Haematoxylin preparations of *P. ribesii* and *Cladius pectinicornis* taken with a Spenser microscope and Reichert Oil Immersion Objectives. Figures 133—134 taken with a No. 3 Merker Objective and a x10 Ocular.

Fig. 133 Longitudinal section of ovary from overwintering larva, showing ovarioles with oocytes: the oviduct is on the left.

Fig. 134 Longitudinal section of testis from overwintering larva, to same scale as Fig. 133; the dark-stained nuclei are spermatogonia undergoing division.

Fig. 135 Polyploid tracheal cell.

Fig. 136 First maturation division in oocyte showing 8 chromosomes, the reduced number.

Fig. 137 Oogonial metaphase or early anaphase plate showing more than the normal number of chromosomes, probably owing to several having split. To same scale as Fig. 136.

Fig. 138 Two spindles from female pupal somatic tissues. The large spindle 1 with chromosomes in late anaphase is a polyploid fat cell; the small spindle 2 on the left is probably a connective tissue or blood cell with the normal number of chromosomes.

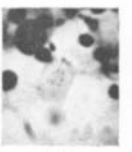
Fig. 139 Fat cell from female showing lagging chromosomes.

Fig. 140 Nucleus of oocyte 5 showing dark fluid material round plasmosome; cf. Fig. 131. The body in the pale nucleoplasm is foreign. To same scale as Fig. 136.

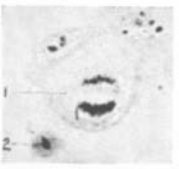
Fig. 141 Nucleus of Egg 4 showing pale plasmosome on left and amphophil body on right. Follicle cells on left margin. To same scale as Fig. 36.

Fig. 142 Polyploid tracheal cells.

Fig. 143 Second spermatocyte metaphase plates of *Cladius pectinicornis*. This genus differs from the others examined in that only six chromosomes are present. Magnification the same as Fig. 137.


136


137



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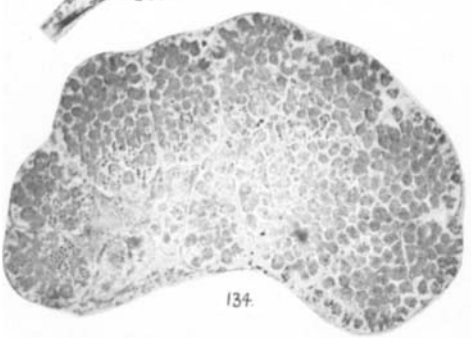
139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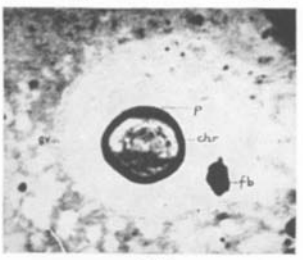
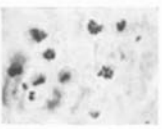
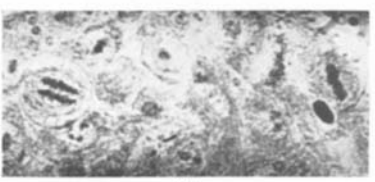


140


135.


143.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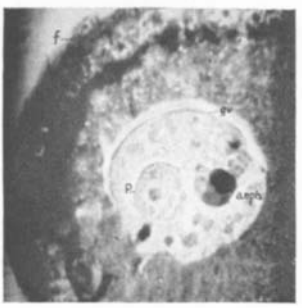

141.