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Cytology of a Diploid Bisexual Spider Beetle

XIII.—The Cytology of a Diploid Bisexual Spider Beetle, *Ptinus clavipes* Panzer and its Triploid Gynogenetic form *mobilis* Moore. By Ann R. Sanderson, Department of Natural History, Queen's College, University of St Andrews. Communicated by Professor A. D. Peacock.

(MS. received October 31, 1959. Revised MS. received January 9, 1960.
Read February 1, 1960)

SYNOPSIS

The chromosome constitution of the bisexual beetle *Ptinus clavipes* Panzer (2n = 18) and of its gynogenetic form *P. clavipes f. mobilis* Moore (3n = 27) have been investigated. The triploid *mobilis* lives in close association with the bisexual species and mates freely with the males. Eggs from virgin females never pass beyond the metaphase stage and successful completion of maturation is dependent on the presence of sperm. An endomitotic split is apparent in prophase chromosomes and leads to the formation of twenty-seven pseudobivalents which undergo a pseudomiotic division. Only one maturation division has been observed. Although the fate of the activating sperm has not been resolved it is thought that sperm nuclei give rise to isolated haploid nuclei in some eggs and interfere with normal cleavage in others. Since the triploid form may also mate with males of *Ptinus pusillus* Sturm and *P. fur* L. with reduced numbers of progeny the affinities of the gynogenetic form is discussed. This is the only known case of gynogenesis in Coleoptera.

INTRODUCTION

The spider beetles *Ptinus hirtellus* Sturm and *Ptinus latro* Fab. formerly were considered to be separate species until a special type of parthenogenesis was discovered in *Ptinus latro* and it was suggested that these should be regarded as dimorphic forms of one species (Moore, Woodroffe and Sanderson 1956). This discovery, made at the Pest Infestation Laboratory, Slough, led to reconsideration of the nomenclature, and, as a result of intensive investigation, Moore (1957) found that the correct name for *Ptinus hirtellus* is *Ptinus clavipes* Panzer and that the name *Ptinus latro* Fab. (1775) must now be regarded as a synonym of *Ptinus fur* Linnaeus (1758). Moore (loc. cit.) has given the name of *Ptinus clavipes f. mobilis* to *Ptinus latro*, auct., and in this paper the nomenclature adopted by Moore will be used throughout. For the sake of brevity the two types will be referred to as *clavipes* and *mobilis*. Reasons for considering these as polymorphic forms of a single species have been advanced.
Males and females of *clavipes* occur naturally as dry-litter scavengers in nests of wasps, birds and rats in warehouses. The parthenogenetic form *mobilis* has not been recorded from any entirely natural habitat and is confined to some warehouses where it lives in close association with the bisexual *clavipes*. Outside this country *mobilis* has been reported from Europe and North America.

The beetles are easily reared under normal laboratory conditions but each generation requires the best part of a year to complete its development (Howe 1957, 1958). An account of breeding experiments to investigate the mode of reproduction is given by Woodroffe (1958). To produce fertile eggs the *mobilis* females have to mate with the males of the bisexual *clavipes* or, less successfully, with males of *Ptinus pusillus* Sturm or *P. fur* L. (1758). The sperm only activates the egg (pseudogamy) and reproduction therefore involves a special type of parthenogenesis, *viz.* gynogenesis.

**Material**

The initial stock of *clavipes* and *mobilis* for this investigation was obtained from the Pest Infestation Laboratory, Slough, and has been supplemented from time to time by courtesy of Mr Woodroffe of that department.

**Technique**

Aceto-orcein squash technique has been used throughout on all material, supplemented by Feulgen-stained squashes and sections of material fixed in modified Kahle (Smith 1943). Most stages of spermatogenesis can be found in the full-grown male larva and white pupa; oogonial stages are best obtained from the very young female pupa. Both oocytes and nutritive cells pass through early prophase stages and are to be found in teneral adults. Females lay eggs singly at a rate of about four per day, but as oviposition usually occurs overnight it is difficult to determine the exact age of the eggs. Maturation has been investigated for the most part from aceto-orcein squashes although a small number of eggs have also been sectioned in order to observe the position of the spindle.

For study of maturation the chorion of eggs requires to be freed from all particles of food and this is best done by thorough washing in Ringer's fluid. Somatic counts are readily obtained from follicle and blastoderm cells.
Ptinus clavipes Panzer and Triploid Gynogenetic form mobilis Moore

Photographs were taken with a Leitz camera attachment and 1/12 or 1/7 Fluorite oil immersion objective but are reproduced to the same scale unless otherwise indicated.

**Cytological Observations**

*Ptinus clavipes* Panzer, male. In somatic and spermatogonial cells there are eighteen chromosomes often lying in pairs (Pl. I, fig. 1). In primary spermatocytes the nine bivalents include at least one ring bivalent and an XY parachute in which the X member is distinctly metacentric (Pl. I, figs. 2 and 3). Disjunction occurs at the first meiotic division. Two second spermatocyte telophases with the equational division of the X (left) and the Y (right) are shown in Pl. I, fig. 4. Sperms are typical of ptinids, having the chromatin distributed along a central axis (Pl. I, figs. 5 and 10) as earlier demonstrated by Dlugosz and Harrold (1952) using electron microscopy.

*Ptinus clavipes* Panzer, female. Somatic and oogonial cells have eighteen chromosomes (Pl. I, figs. 6 and 7); the X chromosomes have not been identified. In the bouquet stage of the young oocyte about nine zygotene loops can be counted but during growth stages only the paired heterochromatic regions of the chromosomes are recognizable. The maturation spindle forms in the unaided eggs (Pl. I, fig. 8) and, if oviposition is delayed the division may proceed as far as telophase, even in the absence of sperm (Pl. I, fig. 9). Normally the metaphase plate persists for a short time in the laid egg, but after about 1 hr. nine chromosomes pass into the polar nucleus and quickly consolidate. A second division follows, but the formation of the second polar nucleus has not been observed. Full-length sperms have been found in the yolk of eggs in cleavage (Pl. I, fig. 10) and sometimes as many as nineteen sperms have been observed in one egg, but their subsequent behaviour has not been followed. Cleavage plates in blastoderms have eighteen very long chromosomes.

*Ptinus clavipes t. mobilis* Moore. There are twenty-seven chromosomes in oogonial and cleavage cells and all are metacentric (Pl. II, fig. 11). Thus the all-female form *mobilis* is triploid. Sex chromosomes have not been identified but presumably three X chromosomes are present. In the young pupal ovary groups of cells usually divide synchronously. In one group of eight cells the chromosomes show a complete separation of chromatids at a stage which appears to precede metaphase contraction (Pl. II, fig. 12). Separation of chromatid arms is often observed in isolated follicle cells in both types of females (cf. Pl. I, fig. 6), but in *mobilis* these divided oogonial chromosomes appear to be rather more elongated, and it
may well be that this condition is a critical stage marking the beginning of endomitosis. Later, during the development of the ovarioles, all the central cells in the egg-tube pass through a bouquet stage in which the number of loops appears to be triploid (Pl. II, fig. 13). The next stages are difficult to analyse and there is no criterion by which one can distinguish very early oocytes from follicle and somatic cells. It is unlikely that all the central cells in the ovariole become oocytes and it is the writer’s belief that many of these become nutritive cells near the distal end of the tube, where later there occurs a great mass of cells in which the chromatin forms hundreds of very fine leptotene-like threads. During growth of the oocyte and enlargement of the germinal vesicle the bouquet stage gives place to a more diffuse stage in which heterochromatic nodules appear. These are arranged in pairs and at a later stage are distinctly elongated (Pl. II, fig. 14). Although exact enumeration is not possible it seems that the number of pairs is triploid; certainly the number of paired bodies far exceeds that found in the *clavipes* oocyte. Thus, in order to form the un-reduced number of paired loops, the chromosomes must have undergone an endomitotic split since their last mitotic division. In a later stage corresponding to diplotene no chiasmata are visible however.

In the un-laid egg the maturation spindle forms but does not proceed beyond metaphase (Pl. III, fig. 16) and in laid virgin eggs is blocked indefinitely (Sanderson and Jacobs 1957). The spindle is much wider than in the *clavipes* egg and can be found with the greatest of ease in both squash and sectioned preparations. It is close to and at right angles to the egg margin. The chromosome complement consists of twenty-seven large blocks (Pl. II, fig. 15) which in their orientation and appearance are remarkably like normal bivalents. It would appear that these have been able to form as a result of some previous endomitotic splitting of the chromosomes and that they are in fact pseudobivalents. The maturation plate persists for a variable length of time according to the age and fertility of the egg. During anaphase, which may be found in the egg from 3 to 30 hr. old, the chromosomes, each composed of two chromatids, clump together and cannot be easily counted (Pl. III, fig. 17). In telophase twenty-seven doubles pass to each end of the spindle and the polar nucleus consolidates as in *clavipes* (Pl. III, fig. 18). The inner plate remains more open and the paired chromatids of each chromosome are visible. The formation of a second maturation spindle has not been observed though subsequent to the completion of the above-mentioned division, plates have been seen which questionably may pertain to second metaphase (Pl. III, fig. 19). The amount of evidence available does not permit a decision as to whether the chromatids seen in these plates separate during a second division or during the first remain near the margin of the cleavage chromosomes. nucleus with a very large number of polyplodont nucleus is consis- for in another parthenogene in the weevil *Listrodes cost.* derivatives tend to increase.

The first cleavage spindles oviposition, and after 5 hr. Blastoderm nuclei begin to 24 hr. after oviposition. I elongated (Pl. III, fig. 20).

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It has been shown that it induce oviposition and that of fertile eggs, except un Woodruffe and Sanderson its entirety. Occasionally but it is possible that these making the squash prepara sperms adhering to the shell the flow of sperms fro con-sequent later-depletion of hand, although no sperms overlooked. The growth o in insect eggs at any time and it is not surprising th-
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division or during the first cleavage division. While the chromosomes remain near the margin of the egg they have not the elongated form typical of cleavage chromosomes. Occasionally in developing eggs a marginal nucleus with a very large number of chromosomes has been seen. Such a polyplid nucleus is considered to be a derivative of the polar nucleus, for in another parthenogenetic coleopteran with which the author is familiar, the weevil Listeroderes costirostris Schonh. (Sanderson 1952), polar nuclei derivatives tend to increase their chromosome number.

The first cleavage spindles form deeper in the yolk about $3\frac{1}{2}$ hr. after oviposition, and after 5 hr. as many as thirty nuclei may occur in the yolk. Blastoderm nuclei begin to line up around the periphery of the egg about 24 hr. after oviposition. In these the twenty-seven chromosomes are very elongated (Pl. III, fig. 20).

In some batches of mobilis eggs the writer was puzzled to find the egg nucleus still in the first metaphase even 3 days after laying. The chromosomes were deeply stained and apparently healthy, but the yolk globules were less cohesive and gave the impression of degeneration. It was later found that these infertile eggs were laid by females in cultures where the number of males was limited, or by ageing females in which the supply of sperm was exhausted or in which the sperms has ceased to be functional. Dissection of the spermatheca of old females revealed that although a plentiful supply of sperm was still present, the sperms appeared to be no longer motile. The failure of the infertile eggs to develop thus appears to be due initially to the failure of these old sperms to stimulate the egg to complete its maturation. It is concluded therefore that the completion of maturation of mobilis eggs depends upon the presence of healthy clavipes sperm.

It has been shown that insemination of the mobilis female is essential to induce oviposition and that healthy sperms are necessary for the production of fertile eggs, except under conditions of extreme crowding (Moore, Woodroffe and Sanderson 1956), but the role of the sperm is not known in its entirety. Occasionally full-length sperms have been seen in the yolk, but it is possible that these have got into the yolk during the process of making the squash preparation, for some developing eggs have clusters of sperms adhering to the shell (Pl. III, fig. 21). It seems that during oviposition the flow of sperms from the spermatheca is not fully regulated, with consequent later depletion of the supply. In sectioned eggs, on the other hand, although no sperms have been seen they could easily have been overlooked. The growth of the male pronucleus is difficult to demonstrate in insect eggs at any time—at least the writer finds it so in Hymenoptera—and it is not surprising that the sperm has not been followed in this case.
On two occasions there was observed within the yolk a peculiar body (Pl. III, fig. 22) which bears a striking resemblance to the developing male nucleus in the frog lung-fluke *Haplometra cylindracea* (Zeder). In trematodes generally the sperm is of the “all-head” type, as in the ptinid beetles, and within the egg it swells up to form a spindle-shaped body of considerable size (Sanderson 1959). No confirmation of the origin of the sperm-like body shown in Pl. III, fig. 22 is possible, but there is some evidence that one or more sperms may enter the egg and earlier or later form nuclei within the yolk. In several eggs in stages of normal cleavage there occur isolated cytoplasmic patches containing nine single straight chromosomes (Pl. IV, fig. 23). Sometimes these lie on a spindle as in Pl. IV, fig. 24, where the two smallest chromosomes shown may be the Y chromosome and the small autosome.

The rate of cleavage is variable but is slower in *mobilis* than in *clavipes*. In the most advanced *mobilis* egg about 100 nuclei have been observed 24 hr. after oviposition whereas in the same time about 500 nuclei develop in the *clavipes* egg. In some dividing *mobilis* eggs irregular distribution of the chromosomes on the cleavage spindles occurs (Pl. IV, figs. 25 and 26). At first it was thought that the chromosomes had been displaced in making the aceto-orcein squash preparation, but similar spindles were subsequently found in squashes of fixed material and in sectioned eggs. In some spindles nine lagging chromosomes can be counted (Pl. IV, fig. 26), and here one is tempted to consider them derivatives of a haploid sperm. Eggs with this type of irregular cleavage are unhealthy and the writer learned to detect them by their instant excessive swelling in aceto-orcein. Their degeneration was not due to lack of sperms in every case for in some eggs sperms were visible around the chorion.

Another irregularity which has caused some concern is the occurrence of maturation plates with less than the triploid number of pseudobivalents. Normally the females of both *clavipes* and *mobilis* deposit four eggs overnight. Females which have not mated withhold their eggs and as many as twenty-eight mature, and apparently healthy eggs can be dissected from one *mobilis* female. The majority of these eggs have normal plates (Pl. III, fig. 16) but a few were found in which the maturation plates have either eighteen or nine bivalents only. (Pl. IV, figs. 27 and 28). One telophase spindle found in a laid egg not more than 6 hr. old had eighteen and nine chromosomes in the inner and outer plates respectively. One can only speculate that these unusual plates have arisen through some prematuration elimination during prophase. At no time have *clavipes* females arisen in any of the writer’s cultures of *mobilis* nor in any of the large experimental cultures reared at Slough by Mr Woodroffe. In some overcrowded *mobilis*-ci proportion of eggs from unrim and a very small proportion ready to hatch (Woodroffe 19 has not been examined but it cytological examination at a

The egg of *mobilis* is cons fig. 31a and b). Perhaps this the larger, and the number 0 the same. The increase in b to the relatively larger cells r: The oogonal plates of the tr diploid, as is to be expected, triploid (cf. figs. 7 and 11). be found with great ease on a is exceedingly difficult to loc: The individual bivalents are r and tend to remain well sepa closely together, especially in

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some overcrowded mobilis cultures Mr Woodroffe has observed a small proportion of eggs from unmated females which proceed to development and a very small proportion which reach the stage of fully-formed larvæ ready to hatch (Woodroffe 1958). The chromosome complement of these has not been examined but it is hoped that such eggs may be available for cytological examination at a future date.

Body and Cell Size

The egg of mobilis is considerably larger than that of clavipes (Pl. III, fig. 31a and b). Perhaps this is to be expected since the body of mobilis is the larger, and the number of ovarioles and the rate of egg production is the same. The increase in body size in the triploid form is probably due to the relatively larger cells rather than to increase in the number of cells. The oogonal plates of the triploid are distinctly larger than those of the diploid, as is to be expected, but the chromosomes also are bigger in the triploid (cf. figs. 7 and 11). The maturation plate in mobilis can always be found with great ease on account of its size whereas the plate in clavipes is exceedingly difficult to locate in both squashes and sectioned material. The individual bivalents are much bigger in mobilis (Pl. IV, figs. 29 and 30) and tend to remain well separated whereas in clavipes they tend to stick closely together, especially in full metaphase in the laid egg.

DISCUSSION

The observations recorded in this paper have bearings on the following matters relating to parthenogenesis in general: meiosis, activation and the role of the sperm, pseudogamy, polyplody, and the geographic range of polyploids. These matters may now be discussed.

Meiosis

In the bisexual form clavipes there is a well-developed XX/XY sex chromosome mechanism. In the male meiotic cells a distinct XY parachute is formed and disjunction occurs at the first spermatocyte division. In the female, maturation involves two divisions, the first of which is also reductive. In the eggs of the triploid mobilis no reduction in the ordinary sense is attempted, otherwise irregular trivalents should be detectable. Chromosomal doubling occurs before the oocyte enlarges and the triploid number of bivalents (pseudobivalents) form. Each bivalent consists of
four chromatids, but it is not known whether it is the sister or non-sister members which separate at the maturation division. On the maturation spindle congression and disjunction appear to be entirely regular, resulting in two triplod plates in which the chromosomes show a marked dyad structure. No second maturation spindle has been seen and it is probable that these chromatids separate during the first cleavage division.

The evidence relating to the endomitotic division is not complete. Separated chromatids have been seen in the eight cells of an oogonal cyst (Pl. III, fig. 25) but no subsequent abortive mitosis has been found, and no cell count within a rosette has been possible beyond this stage. Endomiosis followed by a pseudoreduction is known in other parthenogenetic animals. In many polyploid earthworms Omodeo (1952) records the occurrence of a “restitutional oogonal division” followed by condensation of the abnormally short and condensed chromosomes within a nuclear membrane. This prernotic restitution which allows of a pseudoreduction occurs not only in anisopolyploid races (3n, 5n, etc.) but in tetraploid and hexaploid races. In the parthenogenetic diploid blattid Pycnoscelis surinamensis Matthey (1945) describes what he terms “endomeiosis” in the young oocyte. During leptotene each chromosome divides by a precocious division of the centromere but the two chromatids remain attached terminally. Homologous “double” chromosomes undergo conjugalion a distance then after pachytene deconjugate. The diploid number of “double” chromosomes line up on the maturation spindle. Two equational divisions follow in which each chromosome again exhibits a precocious division of the centromere and terminal attraction of chromatids. In the thelytokous Australian Vegetable Weevil Listroderes antirrostris Schonh. pseudobivalents similar to those of mobilis occur in the single metaphase of maturation (Sanderson 1953), and here also one is tempted to speculate that there is a connection between centromeric antipatation and parthenogenesis. Early repulsion of chromatids in dividing follicle cells is a common feature in both testes and ovaries of many insects and in follicle cells of both clavipes and mobilis separation of the chromatids is often very striking (Pl. II, fig. 11). The centromeres are not divided, however, whereas in the oogonal cells shown in Pl. II, fig. 12, the separation of chromatids seems to involve their centromere also.

In eggs from virgin mobilis females development is usually blocked at the first metaphase (Sanderson and Jacob 1957). In unactivated eggs the chromosomes retain their stainability and their discrete arrangement on the plate for as long as five days after oviposition. But their bivalent composition is not so clear and they begin to round off into spheres and thereafter degeneration sets in.

Ptinus clavipes Panzer and Trij

Activation and the Role of the S

In order to investigate the mobilis, Woodruffe (1958) sterilized doses of gamma radiation (5000 clavipes and mobilis females. mobilis a little later, and in no. Thus, not only is insemination present of active living sperm. In clavipes the sperm nucleus diploidy presumably in the mobilis the sperm does not of the sperms might be traced in t Jacob and Sirlin (1958) inject grown male clavipes larvae and radiographs of the testes. At unfortunately the experiment with mobilis females with label occurrence of haploid groups of (Pl. IV, figs. 23 and 24) would nuclei within the egg. If oosyngamy with the unreduced unbalance between autosomes is Y-bearing. This may well t degenerating cleavage nuclei (se lag on the spindle. This spin position of the lagging chromosome during the making of the pre spindle may show irregular di and displaced chromosomes at must enter the egg and attach it is unlikely that sperm choral into daughter cells. On the other always occur in a small percent since a breakdown in develop of eggs in both bisexual and Jacob (1957) reported a low pr view is not supported by mor disturbed cultures which show females is about 80 per cent at hatch in clavipes eggs (Woodr
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Activation and the Role of the Sperm in Ptinus clavipes and other Animals.

In order to investigate the role of the sperm in Ptinus clavipes f. mobilis, Woodroffe (1958) sterilized clavipes males by exposure to various doses of gamma radiation (5000–15,000 röntgen) and mated them to both clavipes and mobilis females. Eggs of clavipes collapsed very early, mobilis a little later, and in neither case were viable embryos produced. Thus, not only is insemination necessary to induce oviposition, but the presence of active living sperm is essential for activation of the eggs. In clavipes the sperm nucleus fuses with the egg nucleus and restores diploidy presumably in the usual way and determines the sex, but in mobilis the sperm does not effect normal syngamy. It was hoped that the sperms might be traced in the eggs if they were labelled radioactively. Jacob and Sirlin (1958) injected a solution of adenine -8-14C into half-grown male clavipes larvae and about a hundred days later prepared autoradiographs of the testes. All sperm masses were heavily labelled but unfortunately the experiment was not completed and, so far, insemination of mobilis females with labelled sperm has not been attempted. The occurrence of haploid groups of chromosomes within the yolk of some eggs (Pl. IV, figs. 23 and 24) would indicate that the sperms sometimes do form nuclei within the egg. If occasionally a male nucleus should effect syngamy with the unreduced triploid egg then one would expect some unbalance between autosomes and sex chromosomes if the operative sperm is Y-bearing. This may well be the explanation of irregular and probably degenerating cleavage nuclei (see Pl. IV, fig. 26) in which nine chromosomes lag on the spindle. This spindle is in a sectioned egg and therefore the position of the lagging chromosomes cannot be attributed to displacement during the making of the preparation. Within a developing egg several spindles may show irregular distribution of chromosomes. If the lagging and displaced chromosomes are derived from sperms then many sperms must enter the egg and attach themselves to different female nuclei since it is unlikely that sperm chromosomes would divide and be carried over into daughter cells. On the other hand these lagging chromosomes which always occur in a small percentage of eggs may be of no special significance since a breakdown in development occurs regularly in a small percentage of eggs in both bisexual and parthenogenetic forms. Sanderson and Jacob (1957) reported a low percentage of viable eggs in mobilis, but this view is not supported by more recent results from specially reared undisturbed cultures which show that the hatch of mobilis eggs from mated females is about 80 per cent and compares favourably with the percentage hatch in clavipes eggs (Woodroffe 1958).
Thus in the egg stage of *mobilis* mortality may be due to one or other of these causes: total failure of development consequent upon absence of healthy activating sperms, interference by sperms, or simple cessation of development such as normally occurs in a percentage of *clavipes* eggs.

Further evidence that the role of the sperm is solely activatory and that *clavipes* sperms are much more effective as activators of *mobilis* eggs than sperms of other species is furnished by results of breeding experiments using males of two other nearly-related species of ptinids (Woodroffe 1958). Males of *P. pusillus* Sturm had a mating response as vigorous as males of *P. clavipes*, but the culture produced only about 100 *mobilis* larvae as opposed to 2000 in an average *clavipes-mobilis* culture. Males of *P. fur* L. gave a weak mating response with production of only two *mobilis* larvae.

An experiment to activate the mobilis egg artificially was carried out by Sanderson and Jacob (1957). It was found that traumatization by needle puncture caused the maturation division to proceed as far as telophase in over 50 per cent of eggs from virgin females.

Thus, from the evidence to date, it is concluded that Ptinus *clavipes* form *mobilis* is a triploid in which chromosome regulation is assured by premeiotic endomitosis and a pseudomeiotic maturation. Under natural conditions the sperm of the male *P. clavipes* activates the egg to complete its maturation, but the sperm plays no part in syngamy. In the majority of the eggs development proceeds as in other polyploid parthenogenetic forms, the somatic triploid number of chromosomes persisting throughout. At no time has a tetraploid blastoderm been observed, nor any reversion to the diploid *clavipes* condition in any cultures of the beetles.

**Pseudogamy**

Pseudogamy occurs regularly in two other groups of animals, viz. nematodes and planarians. In several species of *Rhabditis* the activating sperms are derived from males of the same species (Belar 1923 and Nigon 1949). *Heterodera trifolii* is normally thelytokous, the male being unknown, but Mulvey (1958) has observed that the female may be impregnated by a male of *H. schachtii* without any change in the sex of the offspring. What role the sperm plays, if any, is still not known. *Heterodera trifolii* has twenty-seven chromosomes and would appear to be a triploid since the allied species *H. schachtii* has eighteen chromosomes. Mulvey's further investigation of this problem is awaited with interest.

In planarians several cases of gynogenseis have been investigated by Italian workers and inheritants (Benazzi-Lentati 1952). In a (1950) found a premeiotic doul and emission of two polar bo *benazzi*, he observed the disint plasmonic bud. A comparable *mobilis* would be unlikely to be with the polar nuclei in an ins.

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In experimental gynogene if only partial, can depend on pronuclei and this is probably. Exceptionally, in *mobilis*, con to the nucleination of the *clav* may interfere with cleavage observed (see p. 338). The d the usual metaphase observ likewise may have been facili

Studies in experimental between heterogenous eggs a distantly related the "parer "Hertwig effect" is discuss therefore enquire whether thi
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may be due to one or other consequent upon absence of sperms, or simple cessation in a percentage of clavipes

is solely activatory and that stimulants of mobilis eggs than slits of breeding experiments of ptinids (Woodroffe 1958). ponse as vigorous as males of about 100 mobilis larvae as is culture. Males of P. fur L. on of only two mobilis larvae. g artificially was carried out bound that traumatization by vision to proceed as far as origin females.

included that Ptinus clavipes some regulation is assured by maturation. Under natural activates the egg to complete in syngamy. In the majority of polyplloid parthenogenetic somes persisting throughout. 1 observed, nor any reversion of the beetles.

ther groups of animals, viz. es of Rhabditis the activating pecies (Bell 1923 and Nigon lytokous, the male being un- it the female may be impreg- ange in the sex of the offspring. t known. Heterodera trifolii spear to be a triploid since the omosomes. Mulvey's further interest.

cis have been investigated by

Ptnus clavipes Panzer and Triploid Gynogenetic form mobilis Moore 343

Italian workers and inheritance has been proved to be purely maternal (Benazzi-Lentati 1952). In a tetraploid race of Polycelis nigra Benazzi (1950) found a premeiotic doubling of chromosomes followed by reduction and emission of two polar bodies. In another tetraploid race, Dugesia benazzi, he observed the disintegration of the sperm in an extruded cytoplasmic bud. A comparable elimination of the pseudogamous sperm in mobilis would be unlikely to be so apparent since no cytoplasm is extruded with the polar nuclei in an insect egg.

Recently an unusual type of pseudogamy has been reported in the enchytraeid worm Lumbricillus lineatus Müll, by Christensen and O'Connor (1958). Two sub-species live in "obligatory coexistence", one a diploid functional hermaphrodite (2n = 26) and the other a triploid male-sterile hermaphrodite (3n = 39). The latter copulated only with the diploid form and "pseudofertilization" of the egg is essential for the successful production of viable embryos. The role of the sperm has yet to be determined, but it plays a somewhat lesser role than in the ptinid where its presence is essential for the completion of maturation and the initiation of cleavage.

Other than P. clavipes f. mobilis only one other case of gynogenesis has been investigated in insects and it parallels that of the ptinid beetle very closely. Narbel-Hoffstetter (1955), for two ecological races of the psychid moth Luffia lapidella Goeze, finds that one race consists of pseudogamic thelytokous females which mate with the males of the neighbouring bisexual race. Regulation is by regression and fusion of first telophase chromosomes; the sperm nucleus fuses with the inner diploid nucleus of the second division, but the embryo develops from the outer diploid nucleus alone and thus maintains diploidy.

In experimental gynogenesis successful activation to development, even if only partial, can depend on the relative rates of development of the two pronuclei and this is probably influenced by a cytoplasmic factor in the egg. Exceptionally, in mobilis, conditions in the ageing egg may be so favourable to the nucleination of the clavipes sperm that the chromosomes of the latter may interfere with cleavage and lead to such irregularities as have been observed (see p. 338). The development of the maturation spindle beyond the usual metaphase observed in the over-ripe un laid egg of clavipes likewise may have been facilitated by cytoplasmic changes.

Studies in experimental gynogenesis have shown that interaction between heterogenous eggs and sperms is the more successful the more distantly related the "parent" species. This paradoxical situation or "Hertwig effect" is discussed by Beatty (1957) at some length. We may therefore enquire whether this has any bearing on the case of mobilis and
clavipes whose relationship is not yet fully resolved. In experimentally induced parthenogenesis in Amphibia the parthenogene (Peacock 1939) (parthenogenone—Beatty) does not survive beyond the early embryonic stage, whereas in the triploid ptinid beetle gynogenesis has been successfully established as the only method of reproduction, the chromatin of the activating sperm being rendered inert under normal conditions. As the mortality rate in mobilis is not above average it might be argued that the potent activatory effect of clavipes sperm on mobilis eggs denotes that the two forms are only distantly related. Continuing this line of argument mobilis would be more closely related to P. pusillus than to clavipes since insemination by the male of the former yields only 5 per cent offspring of a mobilis-clavipes mating. The male of P. fur (L. 1758) yields even poorer results and on analogy would be more closely related to mobilis than pusillus or clavipes. But in insects one cannot exclude such factors as environmental preferences and mating reactions, factors which do not obtain in the artificially induced relationship of the egg and sperm in the amphibian experiments. Woodroffe's observations show that the mating reaction is equally vigorous in both clavipes-mobilis and pusillus-mobilis matings, but produces a reduced number of progeny (5 per cent) in the latter. Assuming that an equivalent amount of sperm is injected in each case then the lessened fertility in the pusillus mating may be due to one or other of the following factors: 1. The failure of the sperms to maintain their mobility in the "foreign" spermatheca; 2. a glandular reaction of the vagina on the introduction of "foreign" sperm. In some species-crosses of Drosophila secretions of the vagina form a reaction mass which obstructs the passage of the eggs (Dobzhansky 1931). If either of these factors is responsible for the reduced fertility then it would appear that there is a closer similarity in the spermathecal fluids of mobilis to the seminal fluids of clavipes than to those of pusillus and fur in that order. All biological evidence to date indicates that mobilis is particularly related to clavipes, pusillus and fur, especially clavipes. Cytological evidence indicates that mobilis is a triploid derivative of a species which has a diploid number 18. Preliminary examination of the chromosome constitution of P. pusillus by the author and of P. fur by Dr D. W. Hall of the Pest Infestation Laboratory, Slough,* indicates that the chromosome number is the same as in P. clavipes, viz. 2n = 18 (Pl. IV, fig. 32). But until more biological and physiological evidence is available and the chromosome constitution of the other two relevant species has been further investigated, the exact status and taxonomic position of mobilis must remain in some doubt.


Ptinus clavipes Panzer and Ti

Polyplody and Parthenogenesis

Ptinus

The theory that parthenogenesis evidence from many sources, (Seiler and Schaeffer 1941) and many European majority of parthenogenetic w and some of these have both Ptinus clavipes-mobilis compl bisexual and parthenogenetic. In the ability of an unaid eg its reduction division there parthenogenesis. The manne speculation. Perhaps it arose sperm or by dispermic fertilize the evidence from biological a mobilis is a triploid derivative parthenogenesis preceded pol

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Polyploidy and Parthenogenesis as seen in certain other insects and in
Ptnus clavipes f. mobilis

The theory that parthenogenesis precedes polyploidy is supported by
vidence from many sources, e.g. the psychid moths Solenobia triquetrella
(Seiler and Schaeffer 1941) and Luffia ferchaultella (Narbel-Hoffstetter
1954) and many European weevils (Suomalainen 1949). Although the
majority of parthenogenetic weevils are polyploid a few species are diploid
and some of these have both parthenogenetic and bisexual races. In the
Ptnus clavipes-mobilis complex also there may possibly exist both diploid
sexual and parthenogenetic races, but of this we have no evidence.
In the ability of an unaided egg from a diploid virgin clavipes to complete
its reduction division there may be at best an indication of incipient
parthenogenesis. The manner of origin of the triploid form is a matter of
peculation. Perhaps it arose by fertilization of a diploid egg by a haploid
sperm or by dispermic fertilization of a haploid egg. At the time of writing
the evidence from biological and cytological sources indicate that the form
mobilis is a triploid derivative of Ptnus clavipes, but the question whether
parthenogenesis preceded polyploidy must remain open.

Geographical Range of the Polyploid f. mobilis

The triploid mobilis seems to be successful only in a limited environ-
ment. Its geographical and ecological ranges are limited by its depend-
ence on the males of the bisexual form. As Woodroffe points out (1958)
clavipes and mobilis are sufficiently different taxonomically to warrant
placing them in different species, but the absence of any geographical or
ecological separation precludes this. Woodroffe suggests that mobilis
has arisen accidentally from the bisexual clavipes and has been able
to survive only in a limited man-made environment, viz. certain
warehouses. Other naturally-occurring parthenogenetic polyploids such as
some Solenobia moths, the woodlouse Trichoniscus coelebs (Vandel 1928,
1940) and the weevils of the Otiorychnidæ (Suomalainen 1949 and 1950)
have a wider geographic range than the diploid forms and would appear
to be hardier (see White 1954). But, as Woodroffe points out, in the cases
of P. clavipes and f. mobilis the triploid mobilis does not occur in natural
habitats in locations that are acceptable to clavipes, and it may therefore have
been able to survive only by virtue of a peculiarly favourable environment.

But whatever its origin or potentialities for becoming ecologically more
widely spread through becoming obligatorily parthenogenetic, the form
mobilis is still tied down by its pseudogamous habit to the bridge connecting
amphimixis and parthenogenesis.
SUMMARY

1. The cytology of the bisexual spider beetle *Ptilus clavipes* and of its
gynogenetic female form *P. clavipes f. mobilis* have been investigated.

2. The bisexual form is diploid, $2n = 18$ and the gynogenetic form is
triploid, $3n = 27$.

3. The complement in the male includes an unequal X/Y pair; disjunction
occurs at the first maturation division. As in other ptinids the
chromosomes are distributed along the whole axis of the sperm.

4. In the female *clavipes* maturation involves two divisions the first of
which is reductional. Normally the first maturation division is not com-
pleted in the unfertilized or un laid egg, but it may proceed as far as telo-
phase in the over-ripe un laid egg.

5. Supernumerary sperms may enter the egg, but these do not interfere
with normal development.

6. *P. clavipes f. mobilis* exists only as triploid females.

7. During prophase in the oocyte of *mobilis* an endomitotic doubling of
the chromosomes occurs. It is thought that this takes place after the
last oogonal division by separation of the chromatids following precocious
centromeric division. The bouquet stage has twenty-seven "synaptic"
loops and the growth phase also has the triploid number of paired chromo-
somes. Pseudobivalents, twenty-seven in number, are formed.

8. The maturation plate forms in the un laid egg; disjunction in the
twenty-seven pseudobivalents follows in the laid egg only if it has been
activated by a sperm. Only one maturation division has been observed
and twenty-seven dyads occur in the egg nucleus and the polar nucleus.

9. Although the presence of healthy living sperm is essential for success-
ful completion of maturation it does not affect syngamy and
development is gynogenetic.

10. Haploid groups of chromosomes found in developing eggs probably
arise from sperm nuclei.

11. Cleavage nuclei have twenty-seven chromosomes. Abnormal
cleavage spindles found in unhealthy eggs may be the result of interference
by sperm nuclei.

12. The larger size of the *mobilis* female is believed to be due to increase
in the size of the cells.

13. The origin, relationships and range of *mobilis* are discussed.
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ACKNOWLEDGMENTS

I wish to acknowledge indebtedness to the following: Mr G. V. B. Herford, Director of the Pest Infestation Laboratory, Slough, for laboratory facilities at the outset of the investigation, and to Mr G. E. Woodroffe of the same department who has kindly sent me supplies of beetles as required. I would also express my great indebtedness to Professor A. D. Peacock for his helpful criticism and his continued interest in this work.

REFERENCES TO LITERATURE


Ann R. Sanderson, Cytology of a Diploid Bisexual Spider Beetle,


Pinitus clavipes Panzer and T

Pinitus clavipes.

All photomicrographs are to sa

Fig. 1. Spermatogonial me

Fig. 2. Late diplotene in X/Y parachute.

Fig. 3. Polar view of meta

Fig. 4. Telophase in sister somes in left and right cells resp

Fig. 5. Developing sperms

Fig. 6. Follicle cell from y chromatids; eighteen chromos

Fig. 7. Oogonium showing

Fig. 8. Metaphase plate in

Fig. 9. Telophase of matur
delayed ovivposition.

Fig. 10. Sperm in ooplasm

P. clavipes form 1

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Fig. 11. Oogonium shown

Fig. 12. Group of oogonial chromatin, probably endomi

Fig. 13. Young oocyte w “synaptic” loops.

Fig. 14. Germinal vesicle i loosely.

Fig. 15. Side view of met bivalent condition clearly illustr

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Fig. 16. Metaphase plate shown; cf. size with that of cla

Fig. 17. Anaphase of ma chromosomes rather clumped to

Fig. 18. Telophase of matur in each plate; p.n. polar nucleus

Fig. 19. Metaphase II (?) ;

Fig. 20. Cleavage plate fro

Fig. 21. Mass of clavipes mobilis egg.

Fig. 22. Peculiar sperm-lik
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DESCRIPTION OF PLATES

Plate I

Ptilinus clavipes. Spermatogenesis and oogenesis.

All photomicrographs are to same scale, and all from aceto-orcein preparations.

Fig. 1. Spermatogonial metaphase.
Fig. 2. Late diplotene in spermatocytes; eight autosome bivalents and an X/Y parachute.
Fig. 3. Polar view of metaphase plate; X chromosome metacentric.
Fig. 4. Telophase in sister spermatocytes II with lagging X and Y chromosomes in left and right cells respectively.
Fig. 5. Developing sperms showing elongated heads.
Fig. 6. Follicle cell from young ovariode showing premetaphase separation of chromatids; eighteen chromosomes.
Fig. 7. Oogonium showing eighteen chromosomes.
Fig. 8. Metaphase plate in unladen egg; nine bivalents.
Fig. 9. Telophase of maturation spindle in unladen egg from virgin female with delayed oviposition.
Fig. 10. Sperm in ooplasm of laid egg; no clearly demarcated tail region.

Plate II

P. clavipes form mobilis. Oogenesis and maturation.

All figures from aceto-orcein preparations; figure 12 to smaller scale.

Fig. 11. Oogonium showing twenty-seven chromosomes.
Fig. 12. Group of oogonial cells forming a rosette; premetaphase separation of chromatids, probably endomitotic phase.
Fig. 13. Young oocyte with approximately triploid number of double "synaptic" loops.
Fig. 14. Germinal vesicle in oocyte, chromosomes elongated but still paired loosely.
Fig. 15. Side view of metaphase plate in egg 42-44 hr. after oviposition; bivalent condition clearly illustrated.

Plate III

P. clavipes form mobilis. Maturation and cleavage.

All figures from aceto-orcein preparations; figure 21 to smaller scale.

Fig. 16. Metaphase plate in unladen egg; twenty-seven pseudobivalents shown; cf. size with that of clavipes shown in Pl. I, fig. 8.
Fig. 17. Anaphase of maturation spindle, 30-80 hr. after oviposition; chromosomes rather clumped together.
Fig. 18. Telophase of maturation, 1â2 hr. after oviposition; triploid number in each plate; p.n. polar nucleus.
Fig. 19. Metaphase II (?) and polar nucleus; chromatids clearly visible.
Fig. 20. Cleavage plate from blastoderm; twenty-seven chromosomes.
Fig. 21. Mass of clavipes sperm adhering to chorion (ch.) of developing mobilis egg.
Fig. 22. Peculiar sperm-like body staining like chromatin in aceto-orcein.
Plate IV.

Figures 23-31. Haploid and irregular nuclei in mobilis eggs; size comparison in claviges and mobilis.

Figure 32. Chromosomes of Pinus fur. L.

All figures except fig. 26 from aceto-orcein preparations.

Fig. 23. Isolated group of nine chromosomes in ooplasm.

Fig. 24. Haploid group of nine straight chromosomes in isolated spindle-like area in developing egg with at least one normal nucleus with twenty-seven chromosomes. The two smallest chromosomes may be the smallest autosome and the Y chromosome. Egg not more than 24 hr. old.

Fig. 25. Cleavage spindle in telophase with laterally-attached group of five chromosomes.

Fig. 26. Irregular chromosome division in cleavage spindle; nine chromosomes lagging on spindle. From a Kahle-Feulgen squash preparation.

Fig. 27. Maturation plate in unlaid egg from virgin female; about eighteen bivalents observed.

Fig. 28. Maturation plate from same virgin female with nine bivalents.

Cf. fig. 16 from same female.

Fig. 29. Photomicrograph and outline drawing of maturation plate (side view) in egg of mobilis—to same scale as fig. 30. Not all twenty-seven pseudobivalents shown.

Fig. 30. Photomicrograph and outline drawing of maturation plate (side view) in egg of claviges.

Fig. 31. (a) Egg of claviges;

(b) Egg of mobilis.

Fig. 32. Oogonial plate of Pinus fur; eighteen chromosomes. (By courtesy of Dr D. W. Hall.)

(Issued separately September 16, 1960)
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drawing of maturation plate (side view)

Not all twenty-seven pseudobivalents
drawing of maturation plate (side view)

eighteen chromosomes. (By courtesy

(ember 16, 1960)
In recent years research has shown that the Department of Agriculture was making a faunistic survey of the Clyde sea area. The results of this survey have been published in the Department's publications dealing with coastal and offshore water life. The results of this survey have been published in the Department's publications dealing with coastal and offshore water life.

From a faunistic survey in the north-west coast and in the deep sea, new species of polychaetes were discovered. Seven of these species are new recordings for the area.

* This paper was assisted in Universities of Scotland.