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MEIOTIC PARTHENOGENESIS AND HETEROCHROMATIZATION IN A SOFT SCALE, PULVINARIA HYDRANGEAE (COCCOIDEA: HOMOPTERA)

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With 16 Figures in the Text

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Cytologically, parthenogenesis can be classified into ameiotic and meiotic (WHITE 1954). In ameiotic parthenogenesis oogenesis consists of but a single mitotic-like division; in the meiotic type two divisions usually occur and different methods are then employed to restore the diploid number of chromosomes.

In coccids the *Lecaniidae* is the only family for which meiotic parthenogenesis has so far been reported (THOMSEN 1927, SUOMALAINEN 1940). The cytology of two species showed that each was composed of two cytological races, a pure parthenogenetic race in which oogenesis consisted of a single division, and a sexual-parthenogenetic race in which oogenesis consisted of two meiotic divisions leading to a haploid egg pronucleus. Sperm when available fertilized the egg to yield both males and females. In the absence of sperm, the second polar body fused with the egg pronucleus thus restoring the diploid chromosome number; only female offspring were produced.

In the family *Lecaniidae*, or soft scales, many species are known to have no or very few males (THOMSEN 1927, THEIM 1933). The chromosome system of this family, and that of several other related families including the mealy bugs, is the lecanoid chromosome system (HUGHES-SCHRADER 1948, BROWN 1959). In this system both males and females have a diploid chromosome number but in the males one set of chromosomes becomes heterochromatic early in embryogeny and remains so throughout life.

In the mealy bugs the heterochromatic set was recently shown to be of paternal origin (BROWN and NELSON-REES 1961). In a species with a lecanoid chromosome system, the presence of males in one generation would thus seem to depend on their presence in the previous generation. In populations of some soft scale species like *Lecanium corni* BOUCHE less than one percent of the individuals are male (THEIM 1933). It is of

interest to know how these males are maintained in such low frequencies without being lost due to chance fluctuations in population size and sex ratio.

In another soft scale, *Pulvinaria mesembryanthemi* VALLOT, males were also quite rare and PESSON (1941) suggested that they were produced parthenogenetically. He noticed that the males, when they did occur, appeared in groups and that each group seemed to be the offspring of a single female. PESSON observed that the mature males ignored the females and that the spermathecae of the females in areas where males were present did not contain sperms. He concluded that both males and females could be produced parthenogenetically; each sex by a different type of female. These observations were not followed by cytological studies, and were unfortunately not discussed in several reviews of parthenogenesis which also covered the coccids.

In the present report a new type of parthenogenesis is described for a species of *Pulvinaria* in which the haploid egg pronucleus first divides and its two products then fuse. Some of the embryos thus produced showed heterochromatization of one chromosome set and were most probably male embryos which were produced parthenogenetically.

Materials and Methods

Pulvinaria hydrangeae STEINWEDEN grows on an ornamental species of *Hydrangea* in California and has one generation a year. The females overwinter on the roots of the plants and the lower parts of the stems. During the last week of May, they move to the upper stems and leaves where they lay eggs. The new generation develops on the leaves but moves to the lower stems and roots just before the leaves are shed in the fall.

For the present study females were collected at the end of May in 1959 and 1960. The were identified by HOWARD L. MCKENZIE, Department of Entomology, University of California, Davis, to whom the author is grateful for his cooperation.

Collections were made in three localities in Berkeley and one in Oakland. In 1959 females were collected in Berkeley from what will be called population A. Since the site was under construction some of the egg-masses were transferred to another *Hydrangea* bush (population A′). In 1960 females were collected from population A′ as well as population B in Berkeley and C in Oakland.

The females were fixed in Bradley-Carnoy, 4 parts chloroform: 3 absolute alcohol: 1 glacial acetic acid, by volume, and stored in the fixative under refrigeration. One day before squashing, a few drops of a saturated solution of ferric acetate in propionic acid was added to the fixative. Staining was done in aceto-carmine.

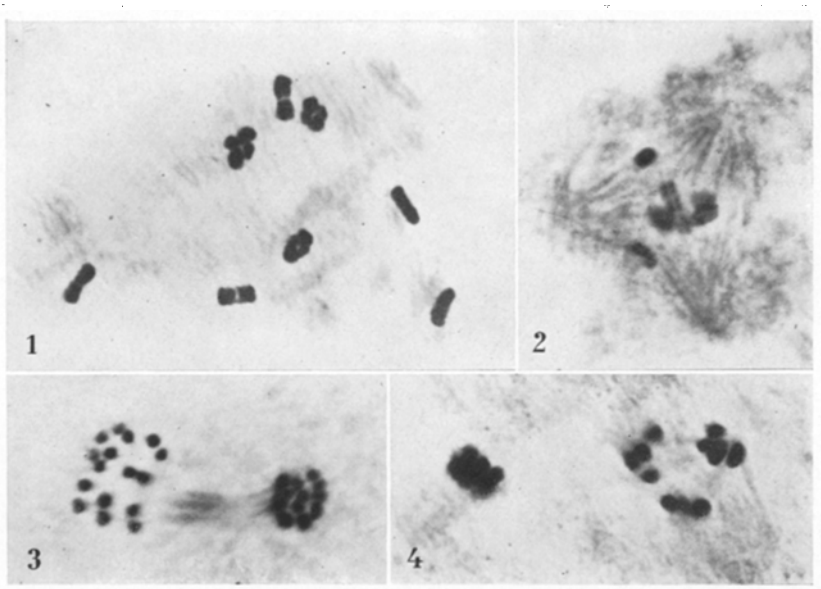
Observations

Oogenesis

Oogenesis has previously been described in three species of soft scales: *Lecanium hesperidum* BURM. (THOMSEN 1927), *Lecanium hemisphaericum* TARG. (THOMSEN 1927, SUOMALAINEN 1940) and *Sphaero-*

lecanium prunastri FONSC. (TREMBLAY 1961). The course of oogenesis in the sexual-parthenogenetic race of each of the first two species was very similar to that of the third species, which was sexual, and the differences which existed were mostly post-meiotic.

Oogenesis in *P. hydrangeae* differed from these other species mainly in the presence of a tripolar spindle at prometaphase I and the mode of division of polar body I.



Figs. 1—4. Oogenesis. 1500 ×. Fig. 1. Diakinesis with 8 bivalents. Fig. 2. Prometaphase with a tripolar spindle. Not all the bivalents visible. Fig. 3. Telophase I; polar body I (P. B. I) to the right and the secondary oocyte to the left. Fig. 4. Metaphase II — Anaphase II. P. B. I dividing into 3 products to the right (not all the chromosomes seen); the secondary oocyte has not yet divided

Diakinesis was the first analyzable stage of meiosis; 8 bivalents were present, some with interstitial and some with terminal chiasmata (Fig. 1). When the spindle fibers first became recognizable at prometaphase I, the spindle was tripolar (Fig. 2). All three poles of the spindle were clearly visible in eight out of the ten cells at this stage and in this species the tripolar spindle is undoubtedly a typical, if not constant feature of prometaphase. At the onset of full metaphase, two of the poles fused, and the bivalents moved regularly to the remaining poles during anaphase I. At telophase I, while the two secondary oocytes were still connected by spindle fibers, one group of chromosomes was more compact than the other and since it was also nearer to the surface of the egg it was identified as polar body I (Fig. 3).

The spindle of the first division was perpendicular to the surface of the egg and after telophase I the outer product (polar body I) was extruded from the egg with some cytoplasm. During the second division polar body I divided first, usually into three (Fig. 4), but sometimes into two

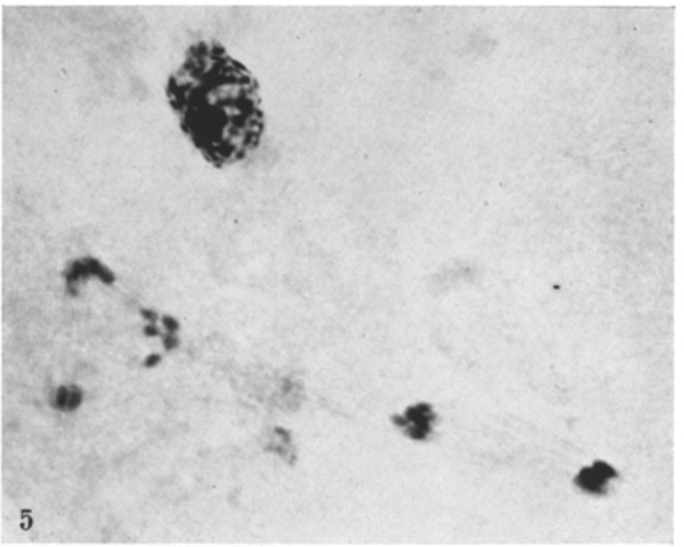


Fig. 5. Telophase II. P. B. I (3 products) to the left, P. B. II and the egg pronucleus (E.P.N.) to the right. A nucleus of a large somatic cell of the type which is later associated with degenerating embryos, above. 1500 ×

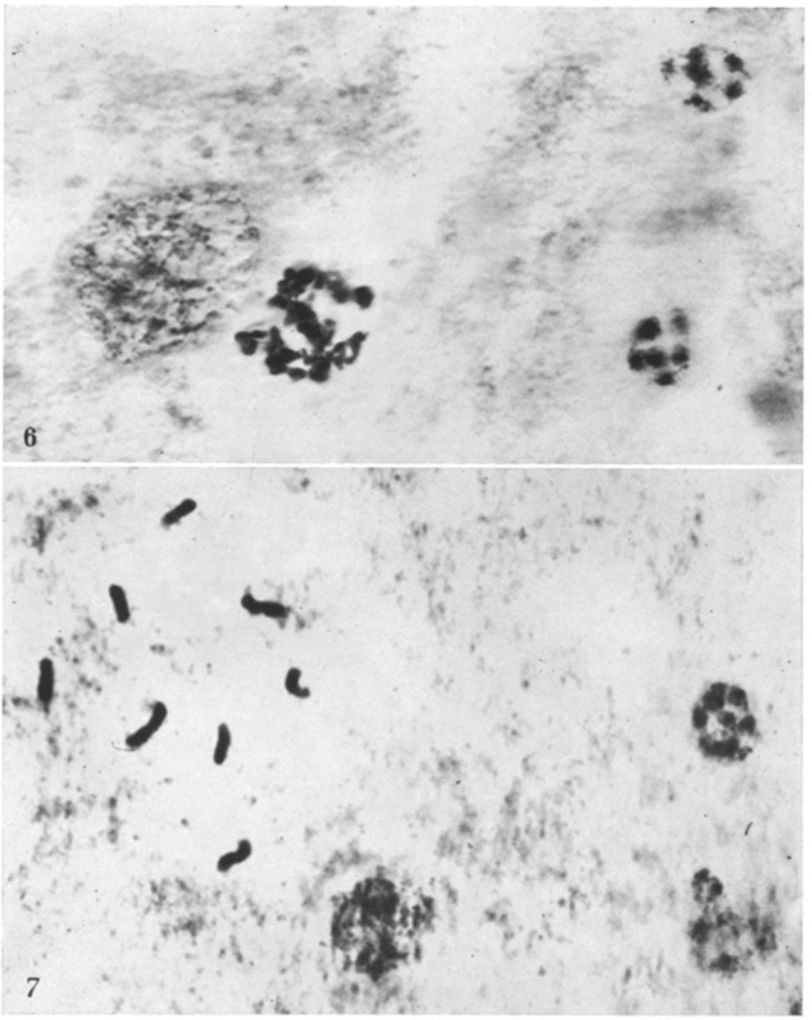
(Fig. 6), four or five products. The number of chromosomes in the products of polar body I varied from one case to another, for example: 6–5–5, 7–5–4, etc. The secondary oocyte then divided into the egg pronucleus and polar body II (Fig. 5), each with 8 chromosomes. After the second division, polar body II was also extruded with some cytoplasm and its cytoplasm then united with that of polar body I.

Reconstitution of diploidy

After telophase II the egg pronucleus enlarged (Fig. 6) and then divided (Fig. 7) to give two haploid nuclei which passed through a resting stage and into prophase. At about this juncture, polar body II also divided, usually into two but sometimes into three products. But in contrast to the anaphase figures of the egg pronucleus, those of polar body II were usually highly irregular and sticky (Fig. 8). After the division of polar body II, the two products of the egg pronucleus arrived at metaphase and the two metaphase complements came together (Fig. 8), finally resulting in a single plate and thus restoring the diploid

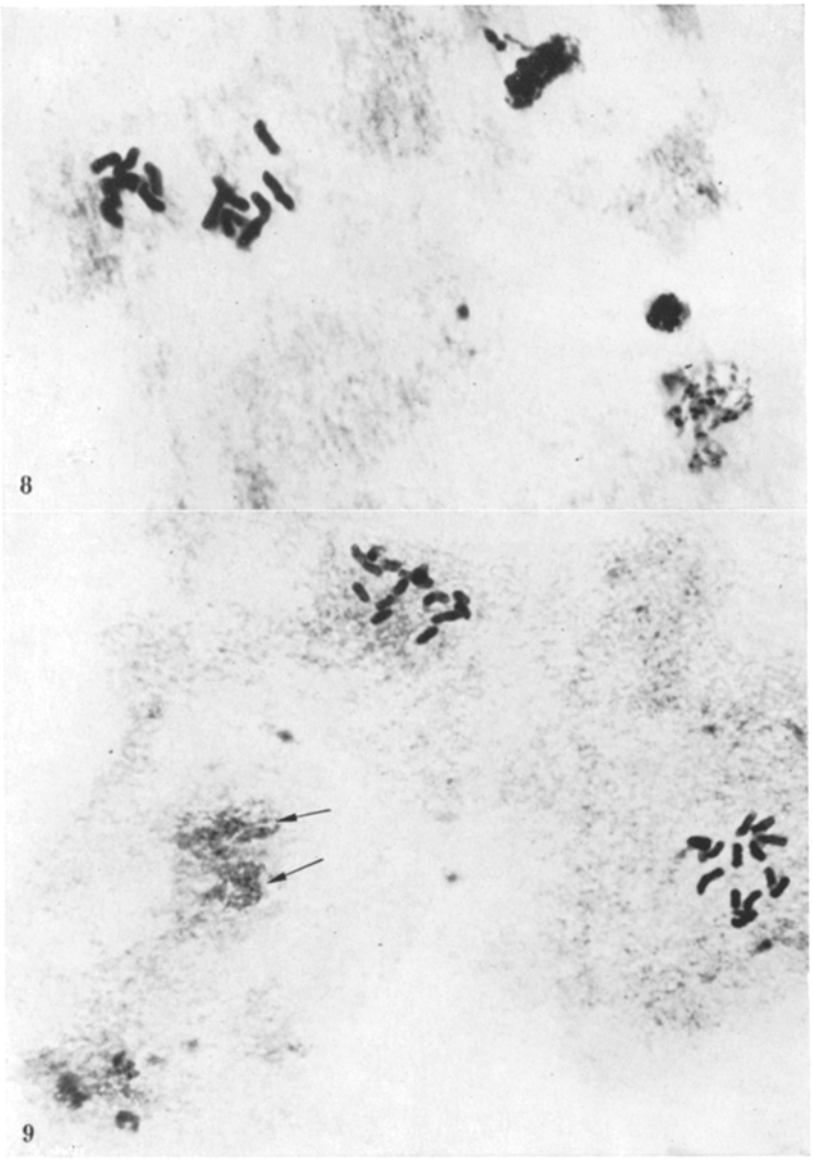
chromosome number. The first division following this union can thus be considered the first cleavage division.

The sequence of events which led to the production of the diploid zygote is rather complex but it could be followed without too much



Figs. 6 and 7 × 1500. Fig. 6. P. B. I (two products) to the right, P. B. II (in interphase) to the left and the egg pronucleus (E.P.N.) which is preparing for division. Fig. 7. The E.P.N. in metaphase to the left, P.B. II below it and the P. B. I products to the right

difficulty because at the various stages the different nuclei in the egg usually had their own characteristic appearance. After meiosis the products of polar body I could be recognized by their number (usually



Figs. 8 and 9. Fig. 8. The products of the egg pronucleus just before fusion (left); P. B. II has elongated and is beginning to divide (above); P. B. I has a small, darkly stained, product and a large and a small lightly stained product (the latter not clearly seen). 1500 ×. Fig. 9. An embryo with two nuclei each with 16 chromosomes, 2 P. B. II products (arrows) and 3 P. B. I products (lower left). 1000 ×

three) and also by their appearance. Until approximately the time of the first cleavage division they remained in a prophase-like condition

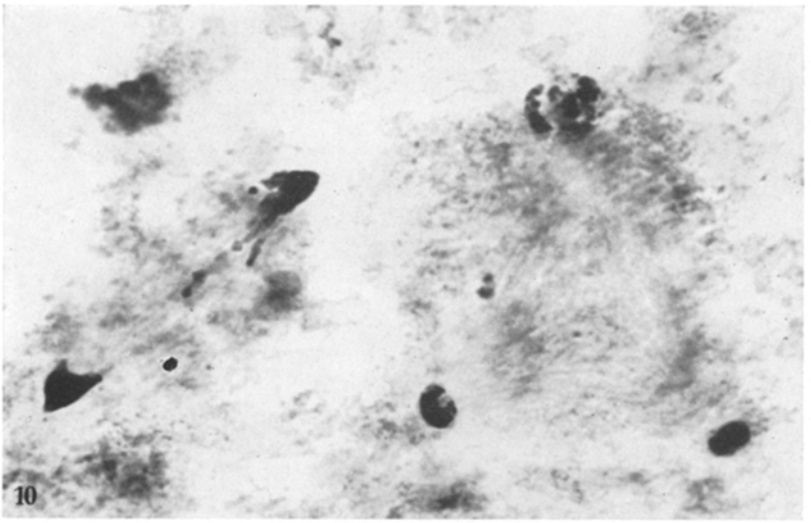


Fig. 10. The products of P. B. I (right) and P. B. II from an embryo at the 16-nuclei stage. The spindle fibers between the derivatives of the polar bodies have reappeared. 1500 ×

in which the individual chromosomes could be seen as spherical and rather fuzzy elements (Fig. 7). Polar body II resembled the egg pronucleus, rather than the products of polar body I, but was usually smaller than the egg pronucleus, lagged behind in its preparations for division, and, when it did divide, produced a sticky anaphase figure.

The most critical stage for the demonstration of the parthenogenetic development of the egg, and that which distinguished it from other types of parthenogenesis, was the division of the egg pronucleus. At this stage a metaphase plate with the haploid number of eight chromosomes (Fig. 7) clearly indicated that the egg pronucleus was not going to fuse with a sperm, nor with polar body II or its products. This citical stage was seen in more than 50 eggs from all the four populations studied and the squashing technique used insured that the entire egg and its content were under observation, and that only one dividing nucleus was present in the egg.

Further cleavage and the fate of the polar bodies

From the end of oogenesis until the first cleavage division the products of both polar bodies were outside the egg. Following the first cleavage, they re-entered the egg where they could be followed until about the 64-nuclei stage. At about the time of the first cleavage division all the products of the polar bodies were in interphase but later at the 8- and 16-nuclei stages, spindle fibers reappeared between the division products (Fig. 10). Also at about this time, one of the two

products of polar body II sometimes reached metaphase, revealing eight condensed chromosomes, but apparently these metaphases were not followed by division, since haploid nuclei were never seen in more advanced embryos. At a later stage the spindle fibers again disappeared and the products of the polar bodies eventually degenerated.

Embryos with heterochromatization

After more than 20 females with several hundred embryos had been studied from population A, and when it seemed that the type of parthenogenesis found in *P. hydrangeae* was clearly understood, an embryo was observed which exhibited a heterochromatic set of chromosomes. In the lecanoid chromosome system, both males and females are diploid, but one of the two haploid sets in the male is heterochromatic. This characteristic picture of males and females had already been observed in several sexual species of this family (THOMSEN 1927, NUR unpubl.). For this reason, embryos showing heterochromatization of one set of chromosomes (Figs. 15 and 16) are believed to be male embryos and will be considered as such throughout the rest of this paper.

Since in the related family, *Pseudococcidae*, the heterochromatic set of the males has been shown to be of paternal origin (BROWN and NELSON-REES 1961), the presence of what appeared to be a male embryo suggested that *P. hydrangeae* might be at least partially sexual. To test this possibility a new collection was made in 1960 from the original population (now population A′, see Materials and Methods) as well as populations B and C. The females from each population were all fixed together, with their ovisacs. At the time of fixation the females in populations A′ and C were beginning to lay eggs while those of B were at the end of egg laying. The latter females were shrunken and each already had a long ovisac.

In coccids, part of the embryonic development is completed before the eggs are laid, and part afterwards; the embryos themselves may thus be referred to as the "laid" and the "unlaid". Three thousand laid and unlaid embryos were examined from population A′. This total must have represented the combined production of at least 8 females (the ovisacs were not fixed individually), and all the embryos were females. In population C over 1000 embryos of five females were also all females. In population B, on the other hand, about two percent of the laid embryos showed heterochromatization (Table). These few male embryos could have been the offspring of one or two fertilized females among the 19 fixed females; therefore, each female was dissected individually and the unlaid embryos inside her analyzed. Four of the females had neither

sexable nor degenerating embryos; the results from the other females are given in the table. Ten of the females had male embryos; the other five had so few embryos that the possibility could not be excluded that they also had produced male embryos. In addition to male and female embryos there was also a large number of abnormal or degenerating embryos, to be described below.

Sperms and sperm bundles of coccids can easily be recognized in fertilized females although sperm bundles have been sometimes mistaken for the sperms themselves (NUR 1962). Several authors have described sperms and sperm bundles in the spermatheca and oviducts of soft scale females (THOMSEN 1927, TREMBLAY 1961, see also NUR 1962). The writer has also observed them in two sexual species of soft scales. In *P. hydrangeae*, the reproductive system, especially the spermatheca, of all the females of populations A′, B and C were studied carefully but sperms were never observed. Nor were sperms ever seen inside the egg even though they can clearly be recognized as such (see Fig. 41 in THOMSEN 1927 and NUR 1962). Since the sequence of events leading to the formation of the diploid zygote in population B was identical to that of the three other populations, the male embryos were apparently produced parthenogenetically and in the same way as the female embryos.

Table. *The types of laid and unlaid embryos from females of population B*

Female	No. of embryos	Type of embryos (%)		
		Males	Females	Degenerating
1	76	1.3	77.6	21.1
2	26	0.0	7.7	92.3[1]
3	11	0.0	100.0	0.0
4	1	0.0	100.0	0.0
5	9	11.1	33.3	55.6
6	3	0.0	33.3	66.7
7	22	40.9	36.4	22.7
8	11	18.2	81.8	0.0
9	99	60.6	0.0	39.4[2]
10	3	0.0	66.7	33.3
11	258	2.3	26.8	70.9
12	3	33.3	66.7	0.0
13	51	5.9	84.3	9.8
14	15	6.7	60.0	33.3
15	8	12.5	87.5	0.0
Means of unlaid embs.		12.8	57.5	29.7
Laid embs.	1141	2.0	97.1	0.9

[1] Mostly type 1 (see text).
[2] Mostly type 3 (see text).

Just before gastrulation, the male embryos werecomposed of small cells showing heterochromatization and large cells, near the yeast-like symbionts, which did not show heterochromatization. These large cells must have originated from the zygote-substitute and were probably polyploid. Similarly, female embryos also had small and large cells; both types again derived from the zygote-substitute.

The fate of the male embryos is not known. Males and females are morphologically indistinguishable until the third instar and no cytological

examination was undertaken to see whether males were present among the first and second instar nymphs. There were no males among the adults, but the males could easily have escaped notice since the species overwinters and the animals reach maturity near or below the ground. But even if some of the males did survive to maturity and fertilized a few of the females, the production of males in the next generation apparently did not depend on these males.

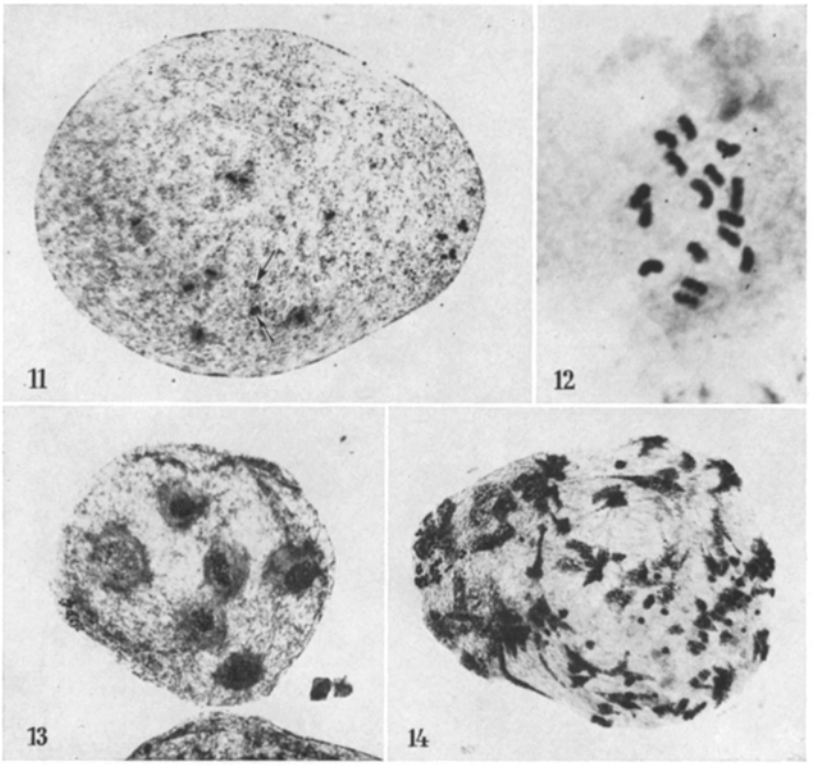
The degenerating embryos

The abnormal embryos which were present among the laid and unlaid embryos could be classified into at least three types: 1. young embryos with a few very large polyploid nuclei, 2. old embryos with many polyploid nuclei, and 3. embryos with small heteropycnotic nuclei (apparently diploid) in the process of degeneration. Type 1 (Fig. 13) apparently developed directly into type 2 (Fig. 14). The fate of type 3 embryos was not clear but some of these embryos had polyploid cells similar to those of types 1 and 2 so that it is possible that like type 1, they also developed into type 2 embryos. Most of the degenerating embryos were of type 2 except for those of female no. 2, which were mostly type 1, and of female no. 9, which were mostly type 3. The type 3 embryos in female no. 9 seemed to be mostly male embryos in different stages of degeneration.

The polyploid nuclei in the degenerating embryos of types 1 and 2 seemed to originate from a certain type of female somatic cells. These cells were large and their nuclei darkly stained and heavily granulated (Fig. 5). One, two, and sometimes more of these nuclei could be seen in eggs at different stages of meiosis and in young embryos, but not all the eggs and young embryos had them. They were usually in interphase but in a few type 1 embryos they were dividing, revealing different levels of polyploidy. In some of these dividing cells the chromosomes were also fragmented.

In the mealy bug *Planococcus citri*, similar cells were described by NELSON-REES (1961) in degenerating eggs. In adult females of this species, the mature ovarioles with the eggs inside them degenerated if the females were aged without being mated. Polyploid cells were found in the degenerating eggs, and NELSON-REES suggested that they may have orginated from the follicular cells which surround the egg. In *P. hydrangeae*, however, the polyploid cells did not resemble the follicular cells; they might represent a special type of cell which is responsible for the destruction of degenerating tissues and which took over development in those eggs and embryos whose development stopped.

In population B, males and degenerating embryos appeared in a higher frequency among the unlaid than among the laid embryos (Table). This difference could have been due to an increase in the production of these classes toward the end of oviposition or to the

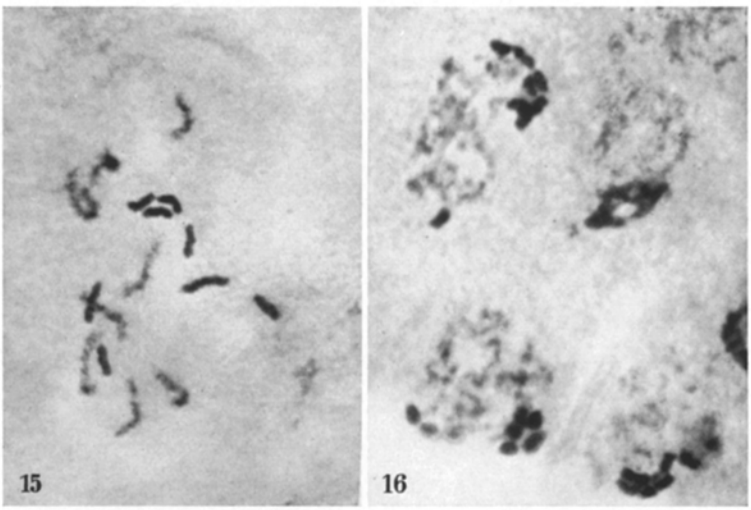


Figs. 11—14. Fig. 11. A normal embryo with 8 nuclei at metaphase, with the products of P. B. I and P. B. II (arrows) and with a few darkly stained symbionts (right). 90 ×. Fig. 12. A metaphase plate from a young embryo, 16 chromosomes. 1500 ×. Fig. 13. A degenerating embryo (type 1) with 6 large, polyploid, nuclei. 90 ×. Fig. 14. A degenerating embryo (type 3) with many polyploid nuclei. 90 ×

tendency of these embryos to be retained inside the females. In female no. 9 many of the degenerating embryos appeared to have been male embryos, so that at least part of the increase in degenerating embryos toward the end of oviposition could be due to the increase in the frequency of males at this time. An increase in the production of males toward the end of oviposition would not be unexpected. NELSON-REES (1960) has reported a case of partial sexual dichronism in another species with a lecanoid system, the mealy bug *P. citri*. Young and aged mothers produced mostly sons during the first few days of ovipositing but

mothers aged only a short time produced mostly daughters at first. In addition, aged mothers also produced a preponderance of male offspring as had previously been reported by JAMES (1938).

Male embryos were present in most of the females of population B, in none of population C and in one female of population A in 1959 but in none in 1960 (A'). This difference cannot be attributed entirely to differences in the stages in oviposition during which the females were



Figs. 15 and 16. Fig. 15. A prophase nucleus from a young male embryo with 8 heterochromatic and 8 euchromatic chromosomes. 1500 ×. Fig. 16. A few cells from a male embryo showing nuclei with heterochromatic chromosomes and diffuse euchromatin. 1500 ×

fixed. In population B, males in advanced stages of development were present among the laid embryos, indicating that male production had already started early in oviposition. The differences in the frequency of males in the three populations might indicate genetic differentiation among the populations or differences in environmental conditions.

Discussion

Comparison with other types of parthenogenesis

The type of parthenogenesis found in *P. hydrangeae* has not been previously described in other animals (SUOMALAINEN 1950, WHITE 1954), although it was suggested for the parthenogenetic race of the white fly *Trialeurodes vaporarium* (THOMSEN 1927). A quite similar type, however, is that found in the moth *Solenobia triquetrella*. In this species the chromosome number of the eggs of the tetraploid parthenogenetic

race is restored after the egg pronucleus divides several times and some of the products then fuse in pairs (SEILER and GESSNER 1950). In the tetraploid parthenogenetic race of *S. trequetrella* and in *P. hydrangeae* all the offspring are expected to be fully homozygous for all loci, irrespective, of course, of the duplicate condition of the tetraploid.

The behaviour of the polar bodies

Oogenesis in *P. hydrangeae* has already been compared with that of other soft scale species. The various species differ greatly in the behaviour of polar body II and in the method of restoring the diploid chromosome number. Polar body II was seen to divide only in *P. hydrangeae*; in the sexual species *Sphaerolecanium prunastri* it remained inactive outside the egg (TREMBLAY 1961); in the sexual-parthenogenetic races of *Lecanium hesperidum* and *L. hemisphaericum* (THOMSEN 1927) it reinvaded the egg in the absence of sperm and fused with the egg pronucleus. The fate of polar body II in the fertilized eggs of these species is not known. In *L. hesperidum* the two products of polar body I apparently fused and then divided and THOMSEN (1927) believed that these cells later became the mycetocytes on acquisition of the yeast-like symbionts. In *S. prunastri* (TREMBLAY 1961) and in *P. hydrangeae* the mycetocytes were the derivatives of the zygote or the zygote-substitute and not the polar bodies.

The origin of the male embryos

Heterochromatization in the male embryos began usually after the fifth or sixth cleavage division and was not fully expressed for a few more divisions. The products of the polar bodies could be clearly observed only during early cleavage and were not seen beyond the 64-nuclei stage. Thus it would have been very difficult to observe the polar bodies in an embryo showing heterochromatization, and due to the scarcity of male embryos in the appropriate stage, a male embryo with all the polar bodies derivatives was not observed. However, there is good evidence that the male embryos were formed in the same way as the female embryos, that is, by the fusion of the products of the egg pronucleus. The sequence of events leading to the formation of the zygote in the females of population B, containing male embryos, was identical with that in the females of the other two populations. Moreover, female no. 9 had inside her only male and degenerating embryos and since the proportion of male embryos increased in the course of oviposition, the eggs and young embryos in female no. 9 were most probably going to develop into male embryos. Yet these eggs and young embryos exhibited the same sequence of events found in females from the populations in which males were not observed.

Sex determination in the lecanoid system

In the lecanoid system, all the chromosomes of the female are euchromatic, while in the male one haploid set is heterochromatic and the other euchromatic. SCHRADER and HUGHES-SCHRADER (1931) suggested that the heterochromatic set is genetically inert so that the male is a physiological haploid. Recently, BROWN and NELSON-REES (1961) demonstrated that the heterochromatic set is virtually inactive genetically since dominant lethals could not be produced in males after paternal irradiation of up to the relatively high dose of 30,000 r. However, NELSON-REES (1962) showed that the heterochromatic set is necessary for survival and plays a role in male fertility because sons surviving after high dosage paternal treatment are all sterile. In spermatogenesis there is no pairing and crossing-over and the sperm is produced only from the euchromatic set; all sperms from one male are thus genetically identical, barring mutations. The sex of the embryo must therefore be determined in some way by the female and is revealed early in development by heterochromatization of the paternal chromosome set in some embryos but not in others. Sex ratio in species with a lecanoid system is known to fluctuate greatly and to depend to a large extent on environmental factors (JAMES 1938, NELSON-REES 1960). However, the hypothesis that the female is heterozygous for some sex determining genetic factors and that the environment affects the segregation of these factors at oogenesis has not been disproved. The production of embryos with and without heterochromatization in *P. hydrangeae* by females which were completely homozygous for all loci proved conclusively that heterochromatization and apparently also sex determination do not depend on the segregation of genetic factors.

HUGHES-SCHRADER (1948) suggested that the euchromatic set on its passage through the male is conditioned in such a way that it becomes heterochromatic during early development in those embryos destined to become males. Passage through the male, however, is not the only way in which a chromosome set can be conditioned toward heterochromatization as can be clearly seen in *P. hydrangeae*. The heterochromatization of one of two identical sets of chromosomes with a similar developmental history is at first surprising. However if one considers the fact that polar body I and the secondary oocyte are genetically identical and yet they behave differently and that similarly the egg pronucleus and polar body II are also genetically identical, it becomes clear that the behaviour of the nuclei does not depend on their genetic composition. What determines the behaviour and fate of the different products of meiosis at least in part is their position in the egg cytoplasm, and the two products of the egg pronucleus must obviously differ, if only slightly, in their position.

In sexual species with the lecanoid system the females apparently produce two types of eggs in only one of which the paternal chromosome set becomes heterochromatic. In *P. hydrangeae* the females still produce eggs in which heterochromatization can take place, but whether all the eggs of this type show heterochromatization we do not know. In those eggs in which heterochromatization does take place the relative position of the two egg pronucleus derivatives probably determines which one will become heterochromatic.

The role of the males

In the lecanoid chromosome system parthenogenetic production of males has so far been reported only in the genus *Pulvinaria*. In *P. mesembryanthemi* (PESSON 1941) the males reached adulthood but were apparently non-functional. In *P. hydrangeae* their fate beyond the stage of advanced embryos is not known but since none of the females studied were fertilized, they probably either died before becoming adults or were non-functional. Thus these parthenogenetic males might be only an insignificant by-product of parthenogenesis in this group. On the other hand, even if only a few of the parthenogenetic males were functional they might be of great importance to the species in enabling it one day to return to sexuality.

Three types of parthenogenesis have so far been described in soft scales: 1. ameiotic (THOMSEN 1927), 2. that in which the egg pronucleus fuses with polar body II (THOMSEN 1927), and 3. that involving fusion of the products of the egg pronucleus, described in this report. The first type leads towards an increase in heterozygosity (WHITE 1954), the second to an increase in homozygosity, and the last type to complete homozygosis. Thus the three types differ in the advantages and disadvantages that they might confer on a species. In several soft scale species meiotic and ameiotic parthenogenetic races are found in the same locality (THOMSEN 1927, 1929), and presumably compete with each other. The advantage of the ameiotic type of parthenogenesis might be in its ability to maintain heterozygosity. That of the meiotic type might be in its facultative nature and only meiotic types are expected to be able to produce males parthenogenetically. If parthenogenetic males would prove to be at least partially functional, species with these types of parthenogenesis might be able to capitalize on the increased reproductive potential of parthenogenesis without paying for it by the complete loss of sexuality and genetic recombination. The maintenance of a small percentage of males could satisfy this condition; whether or not parthenogenetically produced males, as described in this report, are ever functional remains, however, to be demonstrated.

Summary

Meiotic parthenogenesis of a type not previously described was found in *Pulvinaria hydrangeae* STEINWEDEN. During diakinesis 8 bivalents were formed. At prometaphase the spindle was tripolar but anaphase I was bipolar and normal. After completion of division of the primary oocyte, the following sequence occurred: 1. polar body I divided, usually into 3 products; 2. the secondary oocyte divided to yield the egg pronucleus and polar body II; 3. the egg pronucleus divided into its two haploid products; and 4. the second polar body divided. The products of the egg pronucleus fused while dividing to restore the diploid chromosome number; this division may be equated to the first cleavage division. The products of the polar bodies did not take part in the formation of the embryo proper or the mycetocytes.

Among the embryos produced by females of two out of the three populations studied some of the embryos showed a heterochromatic chromosome set, characteristic of males in this and related families. The reproductive system of the females as well as the eggs did not contain any sperm; thus the male embryos were apparently produced parthenogenetically.

The euchromatic and heterochromatic chromosome sets were genetically identical, since they both originated from the egg pronucleus by mitosis. The heterochromatization of one set but not the other might be due in part to a previous difference in their position in the cytoplasm.

The females were completely homozygous yet they produced male and female embryos. Thus it appears that sex determination in the group does not depend on the segregation of genetic factors in either males or females.

In addition to male and female embryos, three types of degenerating embryos were observed. It is believed that these embryos were formed by polyploid somatic cells which invaded abnormal eggs and embryos and took over development.

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