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Diploid Arrhenotoky and Automictic Thelytoky in Soft Scale Insects (*Lecaniidae*: *Coccoidea*: *Homoptera*)*

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Abstract. Parthenogenesis is reported in three soft scales with $2n = 16$. In the unfertilized eggs of all three, oogenesis is normal and diploidy is restored by the fusion of the division products of the haploid female pronucleus. In *Lecanium putmani* Phillips 12 of 13 uninseminated females collected in the wild produced only males. The 21 inseminated females produced 15% males. The males were diploid but contained one euchromatic (E) and one heterochromatic (H) chromosome set. Most of the eggs produced by the inseminated females contained sperm but a few did not. It was concluded, therefore, that females develop from fertilized eggs and males from unfertilized eggs and that the species was diploid arrhenotokous. In *L. cerasifex* Fitch only 18 of 56 females collected in the wild had been inseminated. The frequency of males among their embryos was 22%. The males were again diploid with one E and one H set of chromosomes. Among the 38 uninseminated females, 27 produced only males, and 10 produced only females. All the female producers contained needle-like bacterial symbionts. Most of the male producers, and most of the inseminated females contained no symbionts; the rest contained rod-like symbionts. It was concluded, therefore, that the females of *L. cerasifex* studied belonged to two races, a diploid arrhenotokous race and an obligate automictic thelytokous race. *Eucalymantus tessellatus* (Signoret) is obligate automictic thelytokous. All the females examined were uninseminated and produced only females.

Introduction

Phillips (1965b) has reported unusual types of parthenogenesis in two soft scales. In *Lecanium putmani* uninseminated females were reported to produce only haploid males and parthenogenesis was thus haploid arrhenotokous. In *L. cerasifex* uninseminated females were reported to produce both males and females, and parthenogenesis was thus deuterotokous. Phillips assumed that in *L. cerasifex* the males were functional and concluded that the species was facultative deuterotokous. Phillips' report was of interest because haploid arrhenotoky was unknown in the family *Lecaniidae* and in related coccid families (Hughes-

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Schrader, 1948; Nur, 1971). Deuterotoky has been previously reported only from one other coccid, the soft scale *Pulvinaria hydrangeae*, but in that species the males were not functional (Nur, 1963), and deuterotoky was thus obligate rather than facultative.

A preliminary examination of material provided by Phillips, confirmed Phillips' report of arrhenotoky and facultative deuterotoky (Nur, 1971), but established that in both species the males were diploid. The examination also established that in parthenogenetically produced males and females diploidy is restored by the fusion of the two haploid division products of the females pronucleus.

The present report presents the details of the cytological analysis of *L. putmani* and *L. cerasifex*, as well as that of an obligate thelytokous soft scale, *Eucalymantus tessellatus*, in which diploidy is restored in the same way as in the two *Lecanium* species. This more detailed study revealed that *L. cerasifex* is apparently not deuterotokous. Instead, it is made up of two races: a diploid arrhenotokous race, and an obligate thelytokous race. The separation of the species into two races is based in part on the finding that the uninseminated females of *L. cerasifex* are either male producing or female producing, and that the two types differ in the bacteria-like symbionts which they may harbor.

Materials and Methods

Collection data for each species will be presented in Observations. All individuals were fixed in 4 parts chloroform, 3 parts 100% ethyl alcohol, and 1 part glacial acetic acid, for one day. The material was then transferred to 70% alcohol and kept under refrigeration until used. Staining was carried out in alcoholic-HCl-carmine (Snow, 1963), and the material was squashed in aceto-carmine. In order to determine whether the females were inseminated, the reproductive system was dissected out, and the oviducts and spermatheca were examined for the presence of sperms and sperm bundles. In the three species examined, as well as in other species of this family, the spermatheca is spherical and is attached to the common oviduct at the point where the two oviducts join to form the common oviduct. Part of the way through the study of *Lecanium putmani* and *L. cerasifex*, it was discovered that some of the females contained bacteria-like symbionts. From that time on the reproductive system of each female and some of the tissues attached to it were mounted and squashed in Hoyer's mounting medium, and the examination was then carried out using both regular and phase contrast optics. The slides so produced were then kept as a permanent record.

As in some other soft scales, each mature female deposits her embryos in a cavity beneath her body. The sex ratio of the offspring was determined by staining these embryos with aceto-propiono-carmine. Only embryos in which gastrulation had begun, or older embryos, were analyzed. Embryos were classed as males when they exhibited darkly staining heterochromatic chromosomes in most of their cells. Females lack such chromosomes altogether and their interphase nuclei contain only lightly staining chromatin distributed fairly uniformly. Most of the *L. putmani* females had only a few associated embryos. Only those females with larger numbers of embryos were chosen for analysis and all the embryos of each

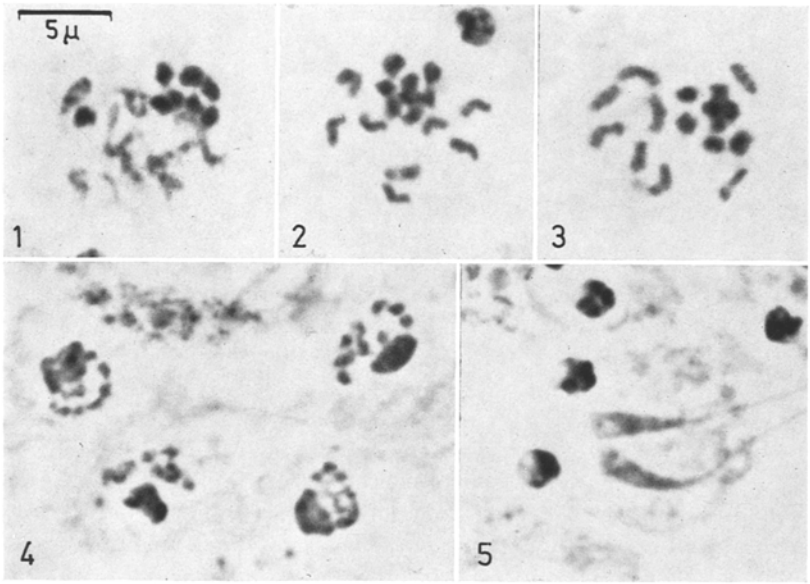
of these females were analyzed. In general, *L. cerasifex* females had more associated embryos than those of *L. putmani*. In *L. cerasifex* some of the females with smaller numbers of embryos were also studied, and when many embryos were present only about 100 were analyzed.

Observations

Lecanium putmani Phillips

Collection. All the material of this species, as well as that of *L. cerasifex*, was provided by Dr. J. H. H. Phillips, Canada, Department of Agriculture, Vineland Station, Ontario, to whom the author is very grateful. Late second instar and adult males were collected from Japanese plum in Grimsby, Ontario, on April 24, 1965. As that time twigs with young females were brought into the lab and the males from these twigs were removed to insure that the feamles remained uninseminated. Some of these isolated females were fixed on June 4, 1965, after they had begun to lay eggs. Laying females were collected from Japanese plum in the same area on June 10, 1965. According to Dr. Phillips the Japanese plum was infested with three *Lecanium* species. *L. coryli* is readily distinguishable from the other two. However, *L. putnami* and *L. cerasifex* cannot be readily separated at the adult stage. To check whether all the females collected belonged to *L. putmani* Dr. Phillips mounted some of the collected females, and allowed the eggs of some of the others to hatch. The analysis of the mounted females and the first instar larvae (which can be easily identified) indicated that either all or almost all of the laying females belonged to *L. putmani*. Among the young uninseminated females, however, as many as 10% may have belonged to *L. cerasifex*.

Cytology of the Males. Most of the nuclei of the second instar and adult males exhibited a darkly staining body made up of heterochromatic (H) chromosomes. The presence of H chromosomes is characteristic of males of *Lecanium hesperidum* and *L. hemisphaericum* (Thomsen, 1927), as well as of male coccids with the lecanoid and Comstockiella chromosome systems (Hughes-Schrader, 1948; Brown, 1963). In *L. putmani* the behavior of the chromosomes during spermatogenesis (Figs. 1—5) was similar to that of *L. hemisphaericum* (Thomsen, 1927) which was assumed to be of the lecanoid type (Hughes-Schrader, 1948). In the lecanoid type the chromosomes do not pair in prophase I, they divide equationally in anaphase I, and the euchromatic (E) and the H set segregate from each other during anaphase II. Only telophase I is followed by cytokinesis. Following telophase II the pairs of cells produced by the first cytokinesis fuse to form quadrinucleate spermatids. During spermiogenesis only the nuclei derived from the E set form sperm. In *L. putmani* most of the cells in prophase I exhibited the haploid



Figs. 1—5. Spermatogenesis in *Lecanium putmani*. ×2400. Fig. 1. Early prophase I with 8 heterochromatic (H) chromosomes. Figs. 2 and 3. Prophase I with 8 euchromatic (E) and 8 H chromosomes. Fig. 4. Four secondary spermatocytes in interkinesis with E and H chromosomes (phase optics). Fig. 5. The two elongated nuclei, each with an E set of chromosomes, are being transformed into sperm; the four round nuclei, each with an H set, remain inactive (phase optics)

number of eight E and eight H chromosomes (Figs. 1—3). In the cells of some of the cysts, however, only seven H chromosomes could be counted. This observation suggested that while in most of the cysts spermatogenesis was of the lecanoid type, in some it may have been of the Comstockiella type. The Comstockiella type differs from the lecanoid type in that the number of the H chromosomes present during prophase I is less than the haploid number (Brown, 1963), apparently as a result of the destricution of some of the H chromosomes just prior to prophase I (Kitchin, 1970).

Spermatogenesis in *L. putmani* resembled more closely that of the *Eriococcidae* and especially that of *Gossyparia spuria* (Schrader, 1929; Nur, 1967b) than that of mealybugs (Hughes-Schrader, 1948) in at least two aspects. First, during metaphase I the two chromosome sets showed the same degree of condensation. During metaphase I in mealybugs the E chromosomes are somewhat less condensed than those of the H set and form an incomplete circle around the H set. Second, following telophase I the chromosomes of the E set became less condensed (Fig. 4).

and then they condensed again prior to anaphase II. In mealybugs telophase I apparently proceeds directly into prophase II and the E chromosomes do not become diffuse prior to prophase II. It is of interest to note that in the *Eriococcidae* spermatogenesis is of the Comstockiella type (Nur, 1967b) while in mealybugs it is of the lecanoid type (Hughes-Schrader, 1948).

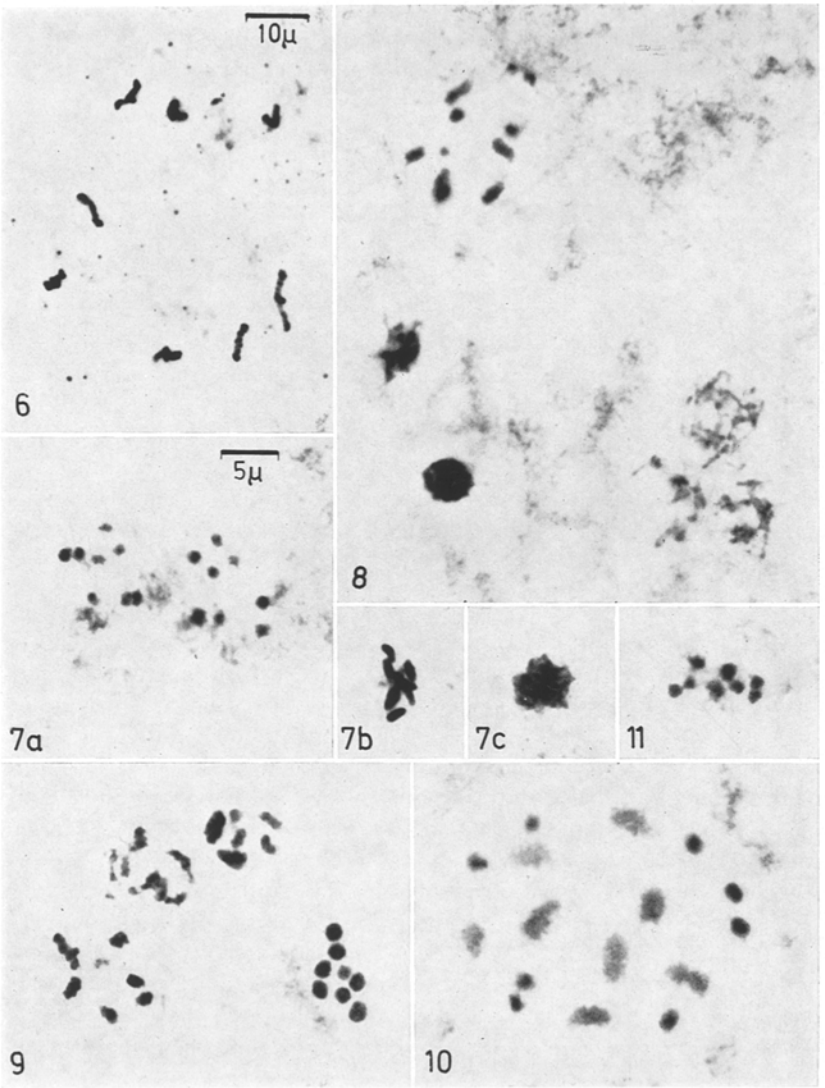
Type of Parthenogenesis. Cytological examination of the embryos laid by 21 of the females isolated by Phillips before they became adults (Table 1, Females 36–56) showed that all the embryos contained nuclei with H chromosomes. Since second instar and adult males contained H chromosomes, while adult females did not, it was concluded that the embryos with H chromosomes were male embryos. The reproductive system of 18 of the isolated females was examined, and none contained sperm or sperm bundles (Table 1). Among the 13 females which were collected in the wild after they had begun to lay eggs (Table 1, Females 23–35), 12 females produced only male embryos and all these females were also found to be uninseminated. These results, as well as the observation that the inseminated females produced mostly females (Table 1, Females 1–22), tended to confirm Phillips' (1965b) conclusion that the species is arrhenotokous. The exception to this conclusion is female 23 (Table 1) which was uninseminated but among whose embryos there were seven females. If these female embryos do not represent a contamination, they may indicate that females of this species may sometimes reproduce deuterotokously.

Cytology of the Uninseminated Females. The cytology of the uninseminated females and their embryos was very similar to that of *Pulvinaria hydrangeae* (Nur, 1963), and to that of the uninseminated females of *L. cerasifex* and *Eucalymantus tessellatus*, to be reported later. The main difference between the four species was the sex of the embryos produced parthenogenetically. In *L. putmani* almost all were males; in *P. hydrangeae* most lacked an H set and were thus considered to be females; in *L. cerasifex* they were either males or females; and in *E. tessellatus* they were all females. Oogenesis and early embryogenesis in the uninseminated *L. putmani* females will now be described briefly. The first stage in which chromosomes can be seen is diakinesis. During this stage eight bivalents may be seen, which often exhibit chiasmata (Fig. 6). The first meiotic division is similar to that observed in other coccids. During the second division the secondary oocyte divides regularly but polar body I (PBI) sometimes divides into three or more products. Following the second division and until early cleavage the chromosomes of the products of PBI remain condensed and appear as prochromosomes (Fig. 7a). As a result the products of PBI may be readily recognized. The haploid female pronucleus divides into two haploid products, and

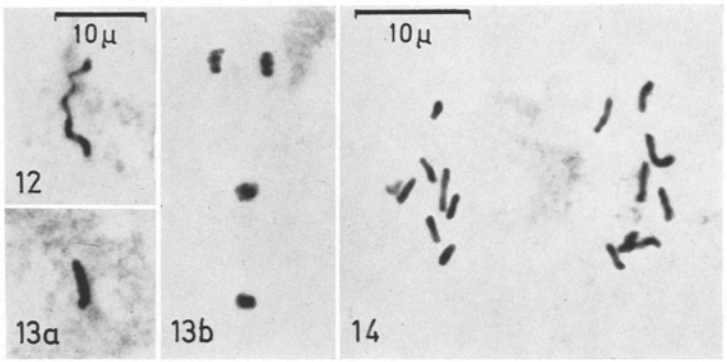
Table 1. *Lecanium putmani*. The frequency of male embryos (sex ratio), insemination, and the presence of bacteria-like symbionts. Blank spaces indicate lack of data. Females 1–35 were collected in the wild, together with their laid embryos. Females 36–56 were collected when young and then kept without males. Females with similar characteristics were grouped together

Female No.	Male Embryos	Female Embryos	Sex Ratio	Insem.	Bact. in Female
1	4	24	0.143	+	+
2	22	100	0.180	+	+
3	7	27	0.206	+	+
4	14	16	0.467	+	+
5	1	15	0.063	+	+
6	11	185	0.056	+	+
7	14	87	0.139	+	+
8	2	6	0.250	+	+
9	7	74	0.086	+	+
10	5	79	0.060	+	+
11	1	90	0.011	+	+
12	13	206	0.059	+	+
13	8	218	0.035	+	+
14	30	142	0.174	+	+
15	0	41	0	+	+
16	2	26	0.071	+	
17	4	15	0.211	+	
18	10	9	0.526	+	
19	3	4	0.429	+	
20	0	28	0	+	
21	5	51	0.089	+	−
22	66	133	0.332		+
Subtotals	229	1576	0.16 ± 0.03	21/21	16/17
23	30	7	0.811	−	+
24–32	580	0	1.0	−	+
33–35	56	0	1.0	−	
Subtotals	666	7	0.985	0/13	10/10
Totals (1–35)	895	1583	0.47 ± 0.07	21/34	26/27
36–49	161	0	1.0	−	+
50	16	0	1.0	−	−
51	13	0	1.0		−
52–54	150	0	1.0	−	
55, 56	57	0	1.0		+
Totals (36–56)	397	0	1.0	0/18	16/18

during the division of these products the two haploid chromosome groups come together and fuse. Thus the first two cleavage divisions produce two diploid nuclei, which later give rise to the entire embryo. The



Figs. 6—11. *L. putmani*. Fig. 6. Eight bivalents in early diakinesis. Some show a chiasma. ×800. Figs. 7—11. ×1500. Fig. 7a—c. Haploid female pronucleus (b), polar body I (PBI) with 16 chromosomes (a), and PBII with condensed chromatin (c) from an egg of an uninseminated female. Fig. 8. Six derivatives of the polar bodies from an embryo with 32 cleavage nuclei from an inseminated female. The derivative at the upper left is apparently haploid, but with broken chromosomes. Fig. 9. Four haploid groups of chromosomes which were probably derived from PBII. The embryo was from an inseminated female and had about 200 other nuclei. Fig. 10. A nucleus in prophase with 8 E and 8 H chromosomes from an embryo in the blastula stage. From an uninseminated female. Fig. 11. A nucleus with 8 H chromosomes. The diffuse E chromosomes cannot be seen but were visible in the original preparation. From an embryo of an uninseminated female

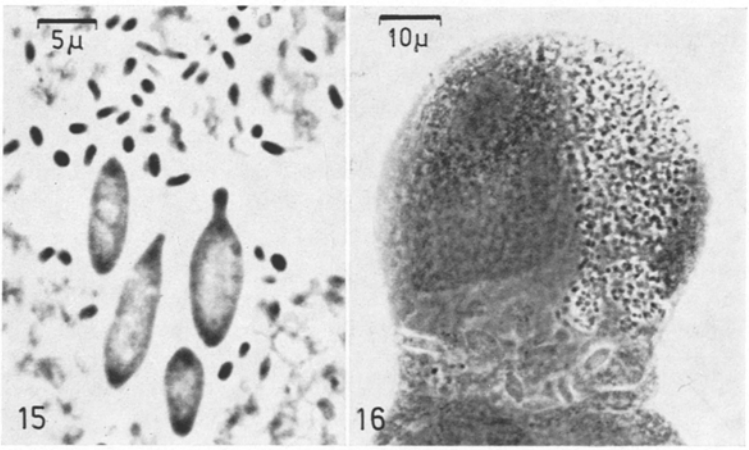


Figs. 12—14. *L. putmani*. Fig. 12. Sperm from an egg in telophase I. ×1200. Fig. 13a. Sperm. Fig. 13b. Four groups of chromosomes in telophase II from the same egg. ×1200. Fig. 14. Two haploid groups of chromosomes from an embryo of an inseminated female. The groups probably represent the sperm and the haploid female pronucleus. The polar bodies could not be seen. ×1500

stage represented in Fig. 7 is crucial for identifying the method of restoring diploidy. In this stage only one nucleus can be seen to divide. From the examination of many embryos at this stage it is clear that the first haploid nucleus to divide is the female pronucleus and not PBII. This conclusion is based on the observation that the dividing group of chromosomes is usually imbedded in an islet of darkly staining cytoplasm which is situated at some distance from the egg surface. In the same eggs PBI and PBII are situated near the surface, and are not imbedded in darkly staining cytoplasm. PBI apparently never divides again. PBII divides once after the second cleavage division, and may divide once again later. Figs. 8 and 9 represent derivatives of the polar bodies in older embryos of inseminated females. Similar nuclei were also observed in embryos of uninseminated females. It is believed that the derivatives of the polar bodies eventually degenerate and do not contribute to the embryo.

In embryos with over 100 nuclei one chromosome set begins to appear more condensed and darkly staining. The exact time of this process of heterochromatization was not established, but in embryos with a few hundred nuclei the H chromosomes appear very distinct (Fig. 10). In lightly stained nuclei in interphase only the H chromosomes are readily seen (Fig. 11), and the presence of such groups of 8 chromosomes may have led Phillips (1965b) to conclude that some of the embryos were haploid.

The Inseminated Females and Their Embryos. Oogenesis in the inseminated females was the same as that in the uninseminated ones



Figs. 15 and 16. *L. putmani*. Phase optics. Fig. 15. Yeast-like and bacteria-like symbionts from an adult female. ×1500. Fig. 16. Ovariole with part of the oocyte (below) the nurse cells (above) and the "neck" connecting them. Bacteria-like symbionts are imbedded in the nurse cell on right. Yeast-like symbionts are imbedded in the neck ×80

until telophase I, at which time sperm could be recognized in most of the eggs (Fig. 12). The sperm remained visible inside the eggs until shortly after telophase II (Fig. 13). Most of the eggs had one sperm but some had two, and some clearly had none. Embryos with a single haploid group of dividing chromosomes were quite rare but a few were observed. On the other hand, embryos with two haploid groups of dividing chromosomes (which were very rare in the uninseminated females) were quite common (Fig. 14).

Among the 21 inseminated females with embryos old enough to be sexed, 19 produced both male and female embryos and two produced only female embryos (Table 1). The mean frequency of males produced by the 21 inseminated and by one female whose frequency of males suggested that she was also inseminated was 0.16 ± 0.03. This low frequency of males and the observation that some of the eggs in telophase I–telophase II did not contain sperm suggested that the male embryos produced by inseminated females developed from unfertilized eggs, and supported the conclusion reached earlier that the species is arrhenotokous.

The Symbionts. Females of all the *Lecanium* species previously examined, as well as those of other soft scales, are known to contain yeast-like intracellular symbionts (Buchner, 1965). Such symbionts were also observed in every female of *L. putmani*, *L. cerasifex*, and *E. tessel-*

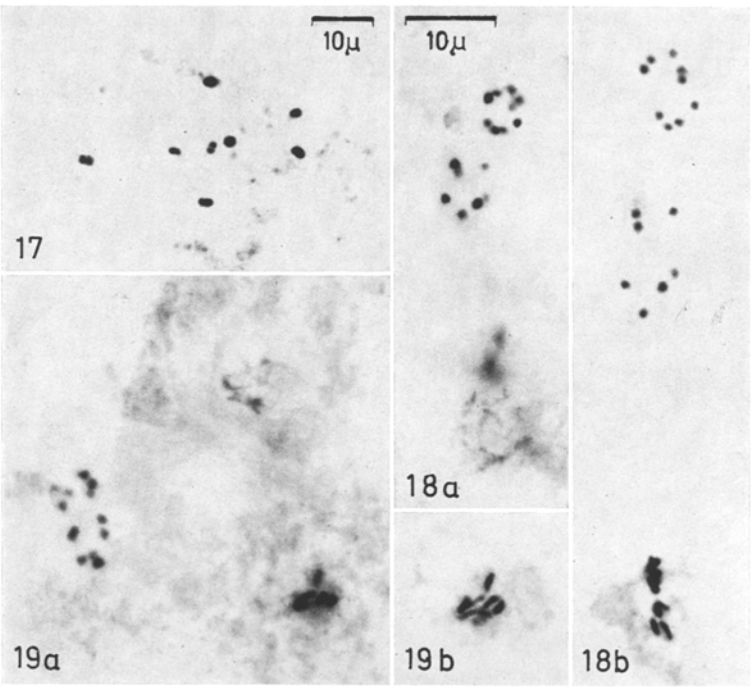
latus (Figs. 15 and 16). The yeast-like symbionts (to be referred to as yeasts) are transmitted regularly to all the offspring. The yeasts accumulate around the "neck" of each ovariole (Fig. 16) and then sink into the egg prior to metaphase I of meiosis. This method of transmission is discussed by Buchner (1965, p. 234) and illustrated for *Lecanium corni*.

Symbionts of a second type were detected only after the reproductive tract of some of the females was examined with phase contrast optics to facilitate the detection of the sperm. The symbionts of the second type are much smaller than the yeasts, do not exhibiting budding, and appear bacteria-like (Fig. 15). These symbionts, which will be referred to as bacteria, were present in various tissues, but were especially common in the fat cells. The bacteria were sometimes found inside one or more of the nurse cells of the ovarioles but appeared not to be present at the "neck". Most of the females carried the bacteria, but one inseminated and two uninseminated females apparently lacked them (Table 1). The transmission of the bacteria to the embryos varied in different females from about 20% to over 90%. There was also an indication that the proportion of embryos with bacteria may increase from early cleavage to late gastrula. When present, the bacteria were found to be distributed throughout the embryo and, unlike the yeasts, were not restricted to the anterior pole. These observations suggested that the bacteria did not enter the eggs through the "neck" and the nutritive cord together with the yeast. The maintenance of the bacteria will be considered in the Discussion.

Lecanium cerasifex Fitch

Collection. Laying adult females were collected by Dr. J. H. H. Phillips June 7, 1965, on maple, *Acer sp.*, in Vineland Station, Ontario, Canada. According to Dr. Phillips all the females almost certainly belonged to that species.

Types of Females. Oogenesis and early embryogeny were very similar to those observed in *L. putmani* (Figs. 17—19). There were two major differences, however. First, some of the uninseminated females produced only female embryos (Table 2); and second, when bacteria-like symbionts were present, they were of two types (Figs. 20 and 21). Phillips (1965b) reported that groups of uninseminated females produced both males and females, and assumed that both types may be produced by the same female. It was surprising, therefore, to find that with the exception of one female, all the uninseminated females produced either only male or only female embryos (Table 2). In the inseminated females most of the eggs in telophase I to telophase II contained sperm but a few did not. Moreover, in a few of the eggs a single haploid nucleus was seen to divide, indicating that these eggs developed without fer-



Figs. 17—19. *L. cerasifex*. Fig. 17. Eight bivalents in late diakinesis. ×800. Figs. 18 and 19. First cleavage of the haploid female pronucleus. PBI is characterized by its condensed chromosomes, and PBII by the diffuse chromatin. ×1200. Fig. 18a. PBII below. The female pronucleus is not in focus. From an embryo of an uninseminated female which produced only males. Fig. 18b. The same embryo after further squashing. PBII cannot be seen. Fig. 19a. PBII above. Fig. 19b. The haploid female pronucleus following further squashing. From an embryo of an inseminated female

tilization. Since the frequency of male among the embryos produced by the inseminated females was about 20% it is reasonable to conclude that in the inseminated females most of the eggs were fertilized, and that these eggs developed into females. About 20% of the eggs remained unfertilized and these eggs developed into males.

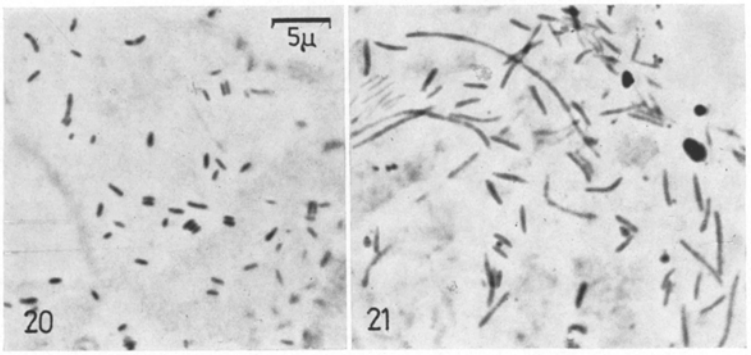
The production of only females by some of the uninseminated females was at first puzzling. The discovery of the presence of bacteria of two types, however, and of a correlation between the type of bacteria present in an uninseminated female and the production of either males or females (Table 2) cleared the puzzle by suggesting that the *L. cerasifex* females belonged to at least two races: a diploid arrhenotokous race

Table 2. *Lecanium cerasifex*. All the females were collected in the wild together with their laid embryos. The presentation of data is similar to that in Table 1. R: Rod-like bacteria. N: Needle-like bacteria. In the last column N and — indicate the presence or absence of bacteria in most of the embryos

Female No.	Male Embryos	Female Embryos	Sex Ratio	Insem.	Bact. in Female	Bact. in Embryos
1	26	98	0.210	+	R	—
2	187	172	0.521	+	R	—
3	18	110	0.141	+	R	—
4	9	61	0.129	+	—	—
5	59	145	0.289	+	—	—
6	28	147	0.160	+	—	—
7	23	156	0.128	+	—	—
8	28	77	0.267	+	—	—
9	8	117	0.064	+	—	—
10	9	130	0.065	+	—	—
11	79	261	0.232	+	—	—
12	218	100	0.686	+	—	—
13	3	37	0.075	+	—	—
14	4	8	0.333	+	—	—
15	0	140	0	+	—	—
16	29	78	0.271	+		—
17	11	51	0.177	+		—
18	14	73	0.161	+		—
19	1	113	0.009			—
Subtotals	754	2074	0.21 ± 0.04	18/18	3/15	0/19
20–22	129	0	1.0	—	R	—
23–42	2636	0	1.0	—	—	—
43–46	633	0	1.0	—		—
47	149	0	1.0		—	—
48, 49	62	0	1.0			—
Subtotals	3609	0	1.0	0/27	3/24	0/30
Totals	4363	2074	0.69 ± 0.06	18/45	6/39	0/49
50	0	99	0	—		
51	1	56	0.018	—	N	N
52–58	0	563	0	—	N	N
59, 60	0	190	0	—		N
Totals	1	908	0.002	0/11	8/8	10/10

and an obligate thelytokous race. The two races will be discussed further in later sections.

The Symbionts. All the females contained yeasts but only a few contained bacteria. In the inseminated females with bacteria the bacteria

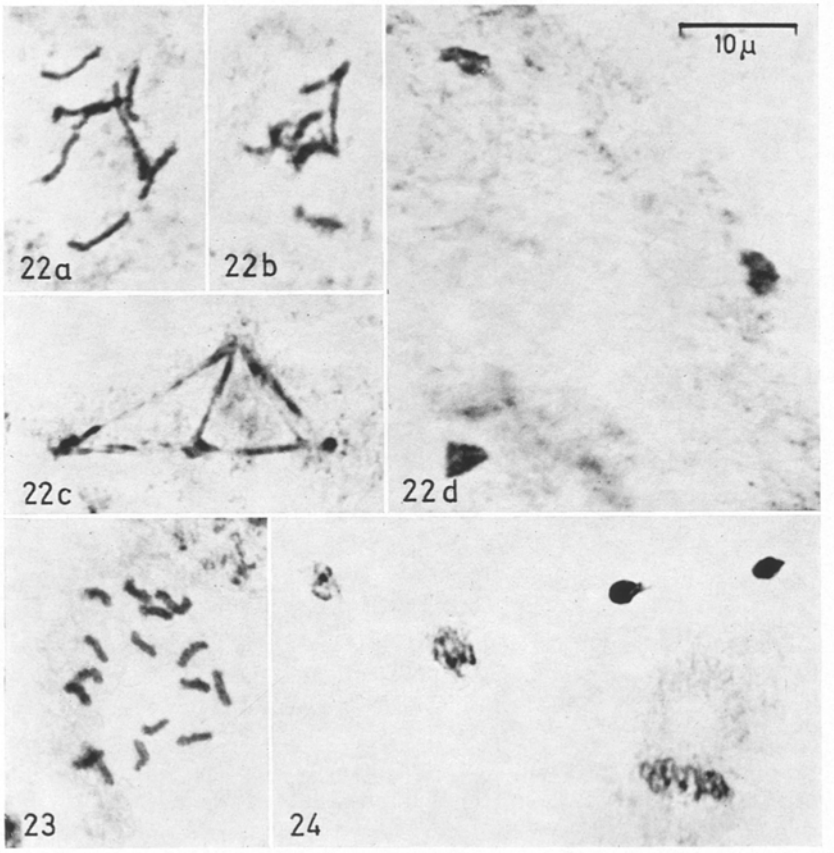


Figs. 20 and 21. *L. cerasifex.* Bacteria-like symbionts. Phase optics. ×1500. Fig. 20. Rod-like symbionts from an uninseminated female which produced only males. Fig. 21. Needle-like symbionts from an uninseminated female wich produced only females

were short and appeared rod-shaped (Fig. 20). They were apparently not transmitted to any of the embryos. Because of the small size of these bacteria, however, the possibility that a few bacteria were transmitted to a small proportion of the embryos could not be ruled out. The male producers among the uninseminated females resembled the inseminated females (Table 2) in the frequency of females with bacteria, in the type of bacteria present, and in the lack of transmission of the bacteria. On the other hand, those female producers among the uninseminated females, which were examined after the bacteria were discovered, all carried bacteria which were much longer and somewhat thicker than the rod-like bacteria present in the other females (Fig. 21). Moreover, these needle-like bacteria were present near the "neck" of the ovarioles in diakinesis, and could be observed inside all the eggs at metaphase I and later stages, as well as in all the embryos. In young embryos these bacteria were always together with the yeast near the anterior pole. It appears, therefore, that the needle-like bacteria entered the egg together with the yeast near the nutrient cord of the ovariole. Neither the needle-like nor the rod-like bacteria were present inside the nurse cells of the ovarioles, and in this respect as well as in their shape and their rate of transmission they differed from the bacteria present in *L. putmani.*

Euclaymantus tessellatus **(Signoret)**

Laying females were collected April 24, 1960, on ivy, *Hedera helix*, in Berkeley, California. The 16 females examined were uninseminated and produced only female embryos. Oogenesis and early embryogeny



Figs. 22—24. *Eucalymantus tessellatus*. ×1500. Fig. 22a—d. Two haploid female pronucleus derivatives (a and b), PBII which is dividing irregularly (c), and three division products of PBI (d), all from the same embryo. Fig. 23. Dividing nucleus with 16 chromosomes from an embryo in the blastula stage. Fig. 24. Pairs of PBI (left) and PBII (right) derivatives from an embryo with 16 pairs of cleavage nuclei in telophase. One such nucleus is shown at lower right

were very similar to those in *P. hydrangeae* and the two *Lecanium* species just discussed. The diploid chromosome number is $2n = 16$ (Fig. 23) and diploidy is again restored by the fusion of the two haploid products of the female pronucleus (Fig. 22). PBI divided about as often into two as into three groups of chromosomes (Figs. 22 and 24). PBII usually divided into two groups but in Fig. 22 it may have been dividing into three or more groups. Many embryos were observed with a divided PBI, an undivided PBII and a dividing haploid pronucleus, but it was

not possible to obtain a good photograph of this stage. The products of the polar bodies persisted into early cleavage but apparently did not contribute to the embryo. There is no information about the presence of bacteria. At the time that this species was studied the author was unaware of the possibility that bacteria might be present.

Discussion

Biological Races in Soft Scales

On the basis of insemination, sex of offspring produced, presence of bacteria, and type of bacteria, the females of *L. cerasifex* may be classified as belonging to seven different types. As was pointed out earlier, however, the simplest interpretation of the situation is to assume that all belonged to just two biological races: a diploid arrhenotokous race and an obligate automictic thelytokous race. In the diploid arrhenotokous race the females may carry rod-like bacteria which are apparently not transmitted to the offspring. Uninseminated females produce only diploid males and inseminated females usually produce both males and females. The males develop from unfertilized eggs and the females from fertilized eggs. Biologically, this race closely resembles *L. putmani*. It differs from it only in the lower frequency of females with bacteria, the type of bacteria present, and the lack of transmission of the bacteria. The two species are also very similar morphologically, and were separated only recently by Phillips (1965a), who has shown that they will not hybridize (1965b). The second race of *L. cerasifex*, may be classified as obligate automictic thelytokous (Nur, 1971). The presence of one male embryo among the embryos of one female of this race may be due to contamination. The possibility cannot be ruled out, however, that a few of the females of this race are deuterotokous.

The presence of two biological races which are morphologically indistinguishable, but differ in their method of reproduction has also been reported from other coccids. Several mealybugs and armored scale insects contain both a purely bisexual race and an obligate thelytokous race (Nur, 1971). *L. cerasifex*, however, resembles more closely *L. hesperidum* and *L. hemisphaericum* because, according to Thomsen (1927, 1929), both species are made up of two different parthenogenetic races: an obligately parthenogenetic race, and a facultatively parthenogenetic race. According to Thomsen, oogenesis of the obligate parthenogenetic races of both *L. hesperidum* and *L. hemisphaericum* consists of but a single division and all the offspring are females. In the facultatively parthenogenetic races all the females raised by Thomsen were uninseminated and produced only females. Oogenesis was normal and diploidy was restored by the fusion of the haploid female pronucleus

with the haploid PBII. Populations of the facultative parthenogenetic race of both species sometimes contained a few males. Thomsen assumed that the males were functional and developed from fertilized eggs, and referred to this race as the bisexual parthenogenetic race; however, he did not perform the necessary experiments to support these assumptions.

The Maintenance of the Bacteria

Most coccids possess symbionts of one type but a few possess two distinct types (Walczuch, 1932; Buchner, 1965). In the family *Lecaniidae*, two types were previously reported only in *Lecanium corni* (Benedek and Specht, 1933). The symbionts of this species resembled those of *L. putmani* and *L. cerasifex* in two ways. First, one type was yeast-like and the other bacteria-like. Second, while the yeast were present in all the *L. corni* individuals examined, the bacteria were present only in some individuals (about 50%). The presence in a given locality of individuals with and without bacteria raises the question of whether the individuals examined do not belong to two different species. In *L. putmani* only three of the 45 females examined lacked the bacteria. Since, according to Phillips, as many as 10% of the females collected as *L. putmani* may have been *L. cerasifex*, and most of the females of the latter species lacked bacteria, the possibility cannot be ruled out that the three females lacking bacteria belonged to *L. cerasifex*.

In *L. putmani* the bacteria did not appear to be transmitted to all the embryos. Since either most or all the laying females contained bacteria it is necessary to conclude either that the bacteria may be acquired after hatching, or that females lacking them do not survive to adulthood.

In the diploid arrhenotokous race of *L. cerasifex* the females with the rod-like bacteria apparently did not transmit them to any of their offspring. Thus, it is necessary to assume that some of the females acquire bacteria after hatching. In this species, however, it is clear that the presence of the bacteria is not essential for survival.

Frequency of Males

The inseminated females of *L. putmani* produced about 16% males and those of the arrhenotokous race of *L. cerasifex* about 21% males. The two species also differed in the frequency of laying females which were uninseminated. In *L. putmani* the frequency of uninseminated females was about 38%, while in *L. cerasifex* it was about 60%. The remarkably high frequency of uninseminated females observed in *L. cerasifex* is apparently due to the lack of adult males. According to Phillips (1965a): "When second instar larvae wintering on maple were

examined numerous males were found but these failed to develop beyond the second instar." It is of interest to note that an arrhenotokous species cannot maintain a fixed frequency of inseminated females (different from 1.0) generation after generation. Instead, this frequency is expected to oscillate between 1.0 and that frequency which may be inseminated by the males produced in those generations in which all the females are inseminated (Hamilton, 1967). The frequency of males is expected to oscillate in a similar manner. According to Hamilton: "If each haploid male can mate with k females and if mated females produce sex ratio e, some females fail to mate if $k<(1-e)/e$. Such cases finally have a regular alternation of sex ratios between e and $1-ke$." In the future, it would be of great interest to establish whether in the two species the frequencies of males and of the inseminated females do oscillate in this manner.

Heterochromatin and Sex Determination

The results of this study suggest that in *L. putmani* and in the arrhenotokous race of *L. cerasifex* fertilized eggs develop into females and unfertilized eggs into males. In the fertilized eggs the two chromosome sets remain euchromatic, and the embryos develop into females. In the unfertilized eggs one of the sets becomes heterochromatic (H) and, apparently as a result, the embryos develop into males.

The presence of an H set in males but not in females is typical of several families of coccids with the lecanoid (Hughes-Schrader, 1948) and the Comstockiella chromosome system (Brown, 1963). The H set was first observed by Schrader (1921), who believed that it played a role in sex determination similar to that of a Y chromosome. Later, Schrader (1929) discovered that the H set does not form functional sperm and suggested that it is genetically inactive. Schrader and Hughes-Schrader (1931) then suggested that in species in which the males possess an H set sex determination is of the haplo-diploid type; the male is cytologically diploid but genetically and physiological haploid. The genetic inactivity of the H set of mealybugs (*Pseudococcidae*) was demonstrated by Brown and Nelson-Rees (1961), by Berlowitz (1965), by Nur (1967a) and by Brown (1969). Brown and Nelson-Rees also demonstrated that in mealybugs the H set is of paternal origin, and Kitchin (1970) demonstrated that it has a similar origin in armored scale insects (*Diaspididae*) with the Comstockiella chromosome system. In both families the male transmits only euchromatic chromosomes which then become heterochromatic in the embryos developing into males but not in those developing into females.

The first evidence that in males of the family *Lecaniidae* the H set may be of maternal origin was the observation (Nur, 1963) that some

of the embryos produced by uninseminated females of the soft scale, *Pulvinaria hydrangeae*, contained an H set. The present observations on *L. putmani* and *L. cerasifex* extend the evidence about the maternal origin of the H set to two more species of this family and demonstrate that the H set is not transmitted by the male even when it is of maternal origin.

In *P. hydrangeae*, *L. putmani* and *L. cerasifex* the set which becomes heterochromatic is one of two sets which must be genetically identical and also appear to have very similar developmental history. The mechanism responsible for the heterochromatization of only one set and the reason why the haploid maternal set does not become heterochromatic in the presence of the sperm, are at present unknown.

The Maintenance of Males in Facultative Parthenogenesis

In many soft scales males are rare. As mentioned earlier, Thomsen (1927, 1929) suggested that these males developed only from fertilized eggs and that in populations in which males were present parthenogenesis was facultative thelytokons. He explained the lack of males in some of the populatious by pointing out that once the males were lost they could not reappear. Thomsen's postulated origin of the rare males seemed to receive support from the results of breeding experiments in *Eulecanium* (=*Lecanium*) *corni*, since according to Habib (1956) in this species males were produced only by inseminated females. Habib reported that unmated females of *E. corni* produced only females and mated females produced both males and females, but that in the latter "the percentage of males was always very low". He also reported that each male died after mating with only one female, and that in the populations of this species on *Cotoneaster microphyla* the frequency of males was about 4%. It is clear, however, that under the conditions described by Habib the males could not have been maintained, and should have disappeared within very few generations.

For species in which uninseminated females produce only females, the percentage of males can be maintained only when the product of the number of females inseminated by each male and the fraction of offspring of these females which develops into males equals or exceeds unity. For example, when only 20% of the females are inseminated and each produces 20% males, the frequency of males in the population will be 4%. In order to maintain this percentage each male must inseminate an average of about five females, or else the number of males will decline from generation to generation. However, even in species in which the product just discussed is unity, the percentage of males in the population is not expected to remain stable, but rather to increase until all the females are inseminated, or to decrease to zero. Any per-

turbation which results in either fewer or more males in a given generation is expected to change the frequency of males in the next generation in the same direction. For example, when for some reason the frequency of males decreases in a given generation, fewer females will be inseminated, and thus there will also be fewer males in the next generation. Following a series of perturbation, the males will either be lost, or the frequency of males will increase until there are enough males to inseminate all the females and the populations will no longer reproduce parthenogenetically.

The fact that in many soft scales males are maintained at a low frequency suggests that other explanations are needed for the origin and the maintenance of these males. One such an explanation assumes that at least some of the males are produced parthenogenetically. This explanation has the advantage that the males will be maintained even when they are of low viability or when they can inseminate only one or a few females. The absence of males from some of the populations, will then be interpreted as due to the loss of females which can reproduce deuterotokously.

The explanation just discussed can also account for the presence of males in species like *Pulvinaria hydrangeae* and *P. mesembryanthemi* in which the males are either inviable or non-functional, even though it does not explain why such males continue to appear. In *P. hydrangeae* (Nur, 1963) males were not observed and all the females analyzed were uninseminated. Some of the females, however, produced a few male embryos. In *P. mesembryanthemi* adult males were present but according to Pesson (1941) they were non-functional, and thus, had to be produced parthenogenetically.

The Types of Facultative Parthenogenesis

It may be of interest to consider the types of parthenogenesis which may be expected in those species in which the males are at least partially functional. In such species it is expected that at least some of the fertilized eggs will develop into females. The advantage of sexual reproduction is that it permits recombination. In males with an H set, however, there is neither chromosome segregation nor crossing over. Thus, it is unlikely that any form of sexual reproduction will be maintained unless at least some of the fertilized eggs developed into females. It may be concluded, therefore, that in species with facultative parthenogenesis the fertilized eggs must develop either into females or into both males and females. In the preceding section it was concluded that in order to maintain the males, at least some of the unfertilized eggs had to develop into males, and thus that species with rare males are probably not facultative thelytokous. These conclusions suggest that

there can be only three basic types of facultative parthenogenesis: diploid arrhenotokoy, and two types of deuterotoky. In one type of deuterotoky all the fertilized eggs develop into females, and in the other they develop into both males and females. The last type is of special interest because of the possibility that in the males developing from fertilized egg the H set may be of paternal origin, and thus that the H set may have different origin in different males. The first of the three types was described in this report. Whether any of the soft scales with rare males belong to the other two types is not known. At present, it is also not known whether some soft scale are purely sexual. It appears, therefore, that additional studies on this group should prove rewarding.

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