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A MODIFIED COMSTOCKIELLA CHROMOSOME SYSTEM IN THE OLIVE SCALE INSECT, PARLATORIA OLEAE (COCCOIDEA: DIASPIDIDAE)

By

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With 36 Figures in the Text

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Introduction

A very unusual chromosome system was described in several armored scale and palm scale insects (BROWN 1957 and 1963, BROWN and NUR 1964). BROWN called the system the Comstockiella system after the genus in which it was first discovered. This system is believed to be derived from the lecanoid chromosome system and to have given rise to the diaspidid chromosome system (BROWN 1963).

In the Comstockiella system both males and females have the same number of chromosomes. However, in males one set of chromosomes becomes heterochromatic in early embryogeny and remains so until spermatogenesis. In these aspects the Comstockiella chromosome system is similar to the lecanoid system, but the two systems differ in the course of spermatogenesis. During spermatogenesis in the lecanoid system, the members of the euchromatic set do not pair with their homologues in the heterochromatic set. Both sets divide equationally in the first division, while, in the second, the euchromatic and heterochromatic chromosomes segregate to opposite poles, and only the euchromatic derivatives form functional sperm (HUGHES-SCHRADER 1948). In the Comstockiella system, BROWN described spermatogenesis to consist only of a single division. During very early prophase, all but one of the heterochromatic chromosomes become euchromatic and pair with their euchromatic homologues. In the anaphase that follows, the two homologues segregate to opposite poles and each division product forms a sperm. In the one pair of chromosomes whose heterochromatic member retains its heteropycnosis, the two homologues usually do not pair. The members of this pair, termed the D pair, divide equationally during anaphase, and, as a result of this division, $n+1$ chromosomes begin movement toward each pole. However, the products of the heterochromatic member of the D pair are soon eliminated because of lagging at anaphase, elimination at telophase, or post-telophase. Each spermatid thus contains a normal haploid set of chromosomes.

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All the species studied by BROWN (1963) were reported to have only one D pair per spermatocyte. In some of the species the D pair was reported to be a fixed entity, while, in others, different pairs could assume the D role. The variation in the D pair was always cyst-specific; in all the cells of the same cyst the same pair of homologues acted as the D pair. The variant of the Comstockiella system in which different pairs may play the D role was termed the variable-D Comstockiella system (BROWN 1963).

In the present report a variant of the Comstockiella system will be described in which one, two, or three pairs may assume the D role simultaneously. This variant is also cyst-specific and will be referred to as the *multiple-D variant* of the Comstockiella chromosome system.

Evidence will be presented which suggests that in *Parlatoria oleae*, and probably in other species with the Comstockiella system, the chromosomes undergoing pairing usually do not replicate prior to pairing, while those playing the D role usually do replicate but do not pair.

The significance of the multiple-D variant of the Comstockiella system as an intermediate between the lecanoid and the Comstockiella systems and its bearing on the evolution of the Comstockiella system will also be discussed.

Materials and Methods

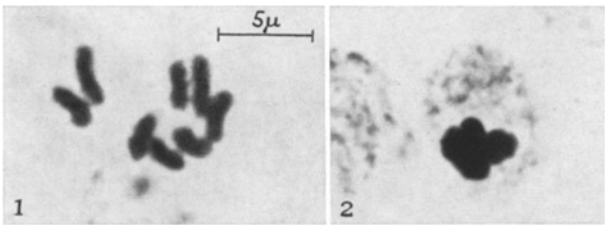
Fixed second instar males of *Parlatoria oleae* (COLVÉE) were made available by Mr. G. W. ROBISON of the Department of Genetics, University of California, Berkeley, to whom the author is very grateful. The males were collected from cultures maintained on potatoes (*Solanum tuberosum*). They were fixed overnight in 1:3 acetic:alcohol and stored in 70% alcohol. The males were stained in bulk in alcoholic HCl-carmine (SNOW 1963) for 48 hours and then washed in 70% alcohol. The testes were then dissected out and squashed in a drop of a mixture of 10 parts of Hoyer's mounting medium (LEE 1937) to one part of alcoholic HCl-carmine. This procedure gave well-stained permanent preparations from which all observations and photographs were made.

Observations

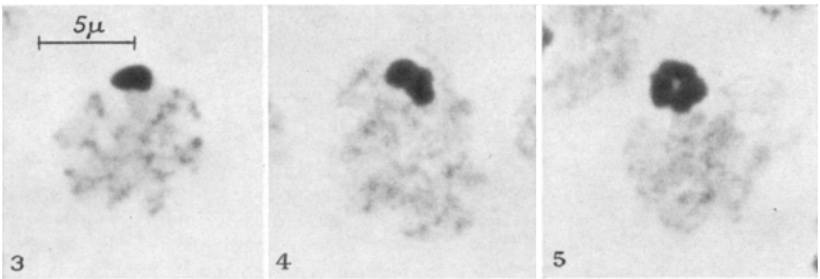
In both males and females of *Parlatoria oleae*, the diploid chromosome number is $2n = 8$ and all the chromosomes are of about equal size (Fig. 1). As in other coccids with the Comstockiella chromosome system, the chromosomes in the females are isopycnotic (euchromatic). In the males, one set of chromosomes appears euchromatic (the "E" set), while the other set appears heteropycnotic and will be referred to as the heterochromatic or "H" set. In the testes of very young second instar males each primary spermatocyte exhibited a chromocenter which was made of four H (heterochromatic) chromosomes (Fig. 2). The primary spermatocytes were organized into cysts, each with 16 primary spermatocytes.

Preprophase

With the approach of meiosis the number of H chromosomes in the chromocenters of the primary spermatocytes decreased and became variable in the various cells of each cyst. Later, the number of H chromosomes was stabilized within each cyst, usually at one H chromosome per cell (Fig. 3). However, in some of the cysts, all the cells showed two



Figs. 1 and 2. 2500×. Fig. 1. Late prophase with 8 chromosomes from a region of the testis that does not exhibit heterochromatization. Fig. 2. A primary spermatocyte with a chromocenter which resulted from the association of the 4 H chromosomes



Figs. 3—5. 2500×. Primary spermatocytes at preprophase showing one, two, and three heterochromatic chromosomes

or even three H chromosomes (Figs. 4 and 5). This stage is referred to as preprophase.

An analysis of 175 preprophase cysts from 16 males is given in the Table. Most of the cysts could be classified readily into those having one, two, or three H elements, but, in a few, the H elements, were fused into a mass and in these cases the cysts were classified according to the size of the H mass. As can be seen from the Table on the following page, 77.7% of the cysts had one H element, 20.0% had two, and 2.3% had three H elements. Cysts with more than one H element were common in some males but rare or absent in others.

Prophase

During prophase, the primary spermatocytes of most of the cysts contained four euchromatic (E) and one heterochromatic (H) element (Figs. 6 and 7). However, in a few of the cysts, 4E + 2H elements were

Table

The number of cysts with one, two, or three heterochromatic (H) elements at preprophase and with five, six, or seven elements in prophase and metaphase of spermatogenesis

Male	No. of H elements at preprophase			*N*	No. of elements at prophase or metaphase			*N*
	1	2	3		5	6	7	
1	3			3	13*			13
2	1			1	11	3	1	15
3	19	5		24	3*			3
4	14	14	4	32	3	4		7
5	10	4		14	5	1		6
6	5			5	3			3
7	7	2		9	4			4
8	1	1		2	9	1		10
9	4	2		6	5	6		11
10	1	1		2	3			3
11	1			1	11*	2	1	14
12	21			21	17			17
13	9	3		12	8	2		10
14	13	2		15	9	2		11
15	10	1		11	2			2
16	17			17	1			1
17—26					13	4*	1*	18
Sums	136	35	4	175	120	25	3	148
Means	77.7%	20.0%	2.3%		81.1%	16.9%	2.0%	

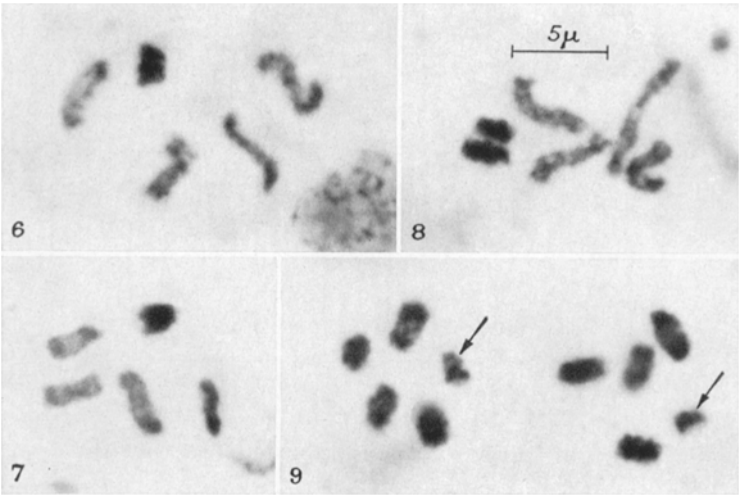
* Including a cyst with a heterochromatic element of only about half the normal size.

present (Fig. 8). In one cyst at late prophase in which all the cells had 5 elements, one of the elements was only about half the size of the other elements (Fig. 9). Since none of the other four elements showed any heteropycnosis, the small chromosome was probably the H element.

The presence at prophase of spermatogenesis of a haploid number of E elements plus a single H element is typical of the Comstockiella chromosome system (BROWN 1957 and 1963). The simplest way to explain the presence of $n+1$ elements at prophase is to assume that $n-1$ elements are bivalents and two are univalents, and this is, indeed, BROWN's interpretation of prophase in the Comstockiella system. The bivalents are believed to be formed by the pairing of three E chromosomes with their H homologues which had changed to a euchromatic state at preprophase. The pair of homologues which does not undergo pairing is referred to as the D (differential) pair, and the E and H members of this pair are referred to as the D^E and D^H elements (BROWN 1963).

According to BROWN's interpretation of prophase in the Comstockiella system, three of the four E elements in *P. oleae* are considered to be

bivalents, while one of the E and the single H element are considered to be univalents. The four E elements in prophase of *P. oleae* are of about equal size (Figs. 6 and 7) and the D^E cannot be distinguished from the three E bivalents. In some of the cells, careful focusing will show that all four euchromatic elements are bipartite; therefore, in the three bivalents, the homologues must be paired side by side, and the single D^E must also be divided longitudinally. The E bivalents are formed by the pairing of two homologues, while the E univalent (D^E) must consist



Figs. 6—9. Prophase. 2500×. Figs. 6 and 7. Cells with 1 H and 4 E. Most of the E chromosomes appear to be split longitudinally. Fig. 8. A cell from a cyst with 2 H + 4 E. Fig. 9. Cells from a cyst in which one chromosome (probably the D^H) is smaller than the other chromosomes

of two chromatids rather than chromosomes. Chiasmata are not associated with pairing in *P. oleae* or other species with the Comstockiella system and pairing probably does not involve crossing over.

In some of the cysts, 4E + 2H elements were present (Fig. 8), and these cysts are interpreted as having two D pairs so that two of the E elements are believed to be bivalents while the other two are believed to be D^E chromosomes. Here again the D^E chromosomes appear to be of about the same size as the E bivalents and both the bivalents and the D^E elements are clearly subdivided longitudinally.

Metaphase

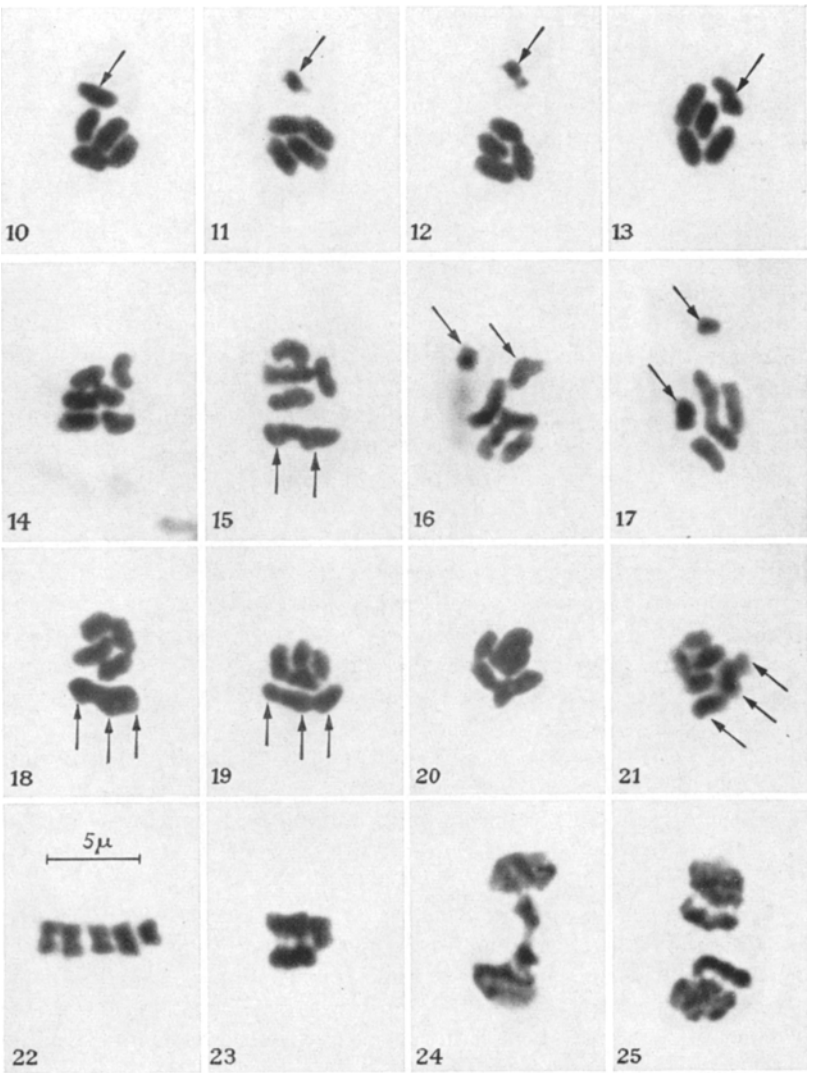
Cyst to cyst variation occurred with regard to the number and size of the chromosomes on the metaphase plate. Most of the cysts had five elements, some had six, and a few has seven elements on the metaphase plate. In the majority of cysts with five elements, all the elements were

of about equal size (Figs. 10 and 13). However, in two cysts, one element was only about half the size of the other elements (Figs. 11 and 12). The small element usually lay at the edge of the metaphase plate and this behavior suggested that it was the H element. As was explained earlier, the five elements are interpreted to be three bivalents and two univalents. The fact that the bivalents and the univalents are of the same size indicates that the univalents must have undergone an "extra" replication. This "extra" replication, was termed by BROWN (1964) compensation, and defined as "the extra duplication required to give one or more chromosomes the same status as the remaining members of the complement, thereby preventing aneuploidy". BROWN suggested that in the Comstockiella system compensation may occur either after meiosis, as in *Comstockiella sabalis*, or prior to prophase, as in those cases in which the D^E can not be readily identified. The compensation in *P. oleae* is clearly of the second type and must be taking place either prior to or during preprophase.

The presence of cysts with a small D^H element could not have been the result of a simple fragmentation of the D^H because the chromosomes of *P. oleae*, like those of other coccids, are holokinetic and in these chromosomes fragments are usually capable of orientation and movement on the spindle during cell division (HUGHES-SCHRADER and RIS 1941). Thus following fragmentation we would expect to find two or more fragments rather than one small chromosome. The simplest way to account for the presence of cysts with 5 elements in which the D^H is only about half the size of the other elements is to assume that in these cysts the D^H element failed to undergo the "extra" compensatory replication. This explanation accounts for the fact that in these cysts the D^H is only about half the size of the other elements and also for the fact that the small D^H is cyst-specific.

Among the cysts with six elements on the metaphase plate (Figs. 14 and 15), two of the elements are D^H univalents and they can usually be identified by their irregular shape and their position at the edge of the metaphase plate (Fig. 15). In one cyst with six elements, one of the D^H elements was only about half the size of the other elements (Figs. 16 and 17). Among the cysts with seven elements, three of the elements are D^H elements and they are usually found together at the edge of the metaphase plate (Fig. 18). In one cyst with seven elements, one D^H was of about half the size of the other elements (Figs. 19—21).

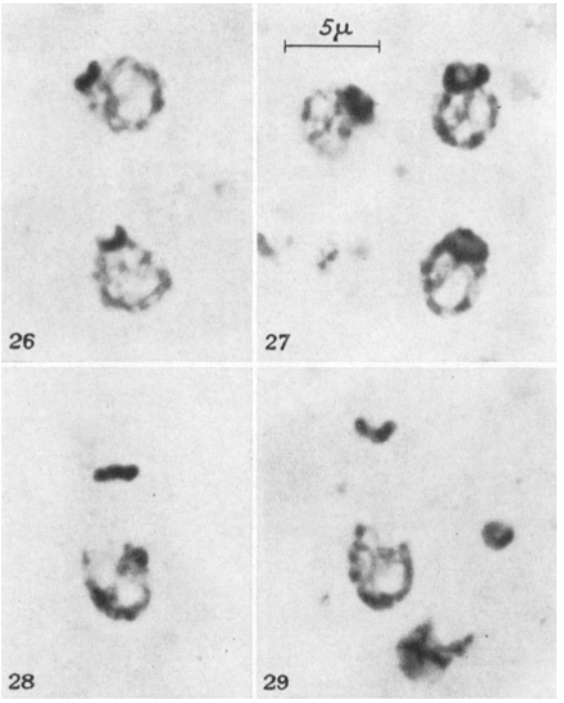
The frequency of cysts with five, six and seven elements at prophase and metaphase is given in the Table. The frequency of the three classes agrees well with the frequency of cysts with one, two and three heterochromatic elements at preprophase. In addition, males which had cysts with two or three H chromosomes at preprophase usually also had cysts with six or seven elements at prophase or metaphase.



Figs. 10—25. Metaphase to telophase. 2500×. Figs. 10—13. Metaphase plates with 5 chromosomes. Fig. 10. The D^H (arrow) is about the same size as the other chromosomes. Figs. 11 and 12. Metaphase plates from 2 cysts in which the D^H (arrow) is only about half the size of the other chromosomes. Fig. 13. The chromatids of the D^H can be resolved. Figs. 14—17. Metaphase plates with 6 chromosomes. Fig. 14. The $2D^H$ chromosomes cannot be identified. Fig. 15. The D^H chromosomes (arrows) are less regular in shape than the other chromosomes. Figs. 16 and 17. Metaphase plates from a cyst with one normal and one half-size D^H chromosomes (arrows). Figs. 18—21. Metaphase plates with 7 chromosomes. Fig. 18. 3 full size D^H chromosomes (arrows). Figs. 19—21. Metaphase plates from a cyst with two normal and one half-size D^H chromosomes (arrows). Fig. 22. Metaphase, side view. The D^H appears more condensed. Fig. 23. Anaphase. Fig. 24. Telophase. The D^H lags in its division. Fig. 25. Telophase from a cyst which probably had $2D^H$ chromosomes

Anaphase-Telophase

During metaphase the H element (or elements) usually lay at the edge of the metaphase plate. In side view, it appeared more condensed than the other elements and its division into two subunits was less pronounced (Fig. 22). During anaphase the D^H (or D^H's) was the last



Figs. 26—29. Post telophase stages. 2500×. Fig. 26. The E chromosomes appear as a diffused mass; the D^H lies at the edge of the E mass. Fig. 27. Same as Fig. 26, but from a cyst which probably had $2D^H$ chromosomes. Figs. 28 and 29. The elimination of the D^H. Notice the basket-shape appearance of E mass

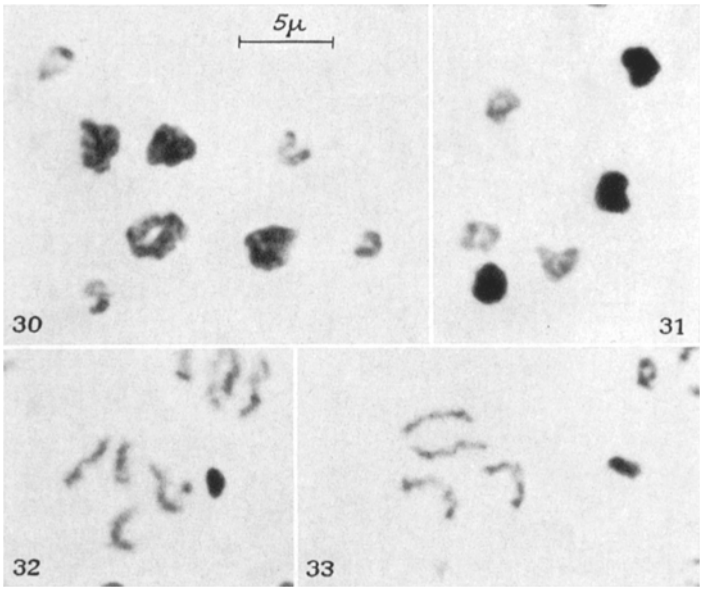
element to divide (Fig. 23). The lagging of the D^H element at anaphase moved it from the edge of the metaphase plate toward the center (Fig. 24), so that at telophase the divided D^H products now faced each other (Fig. 25).

Post-telophase stages

After telophase, the E elements became more diffused while the D^H derivattives remained more condensed, appeared somewhat curved, and usually lay at the edge of the E masses (Fig. 26). In some of the cysts, the D^H derivatives were considerably larger than those of the majority of the cysts (Fig. 27). The larger D^H derivatives probably consisted of

the division products of the D^H elements from cells with two or three D pairs.

At a later stage, the D^H derivatives were eliminated from the future sperms when they segregated from the diffused E masses which now assumed the shape of an open basket (Figs. 28 and 29). With the methods of staining and squashing employed, it was not possible to determine

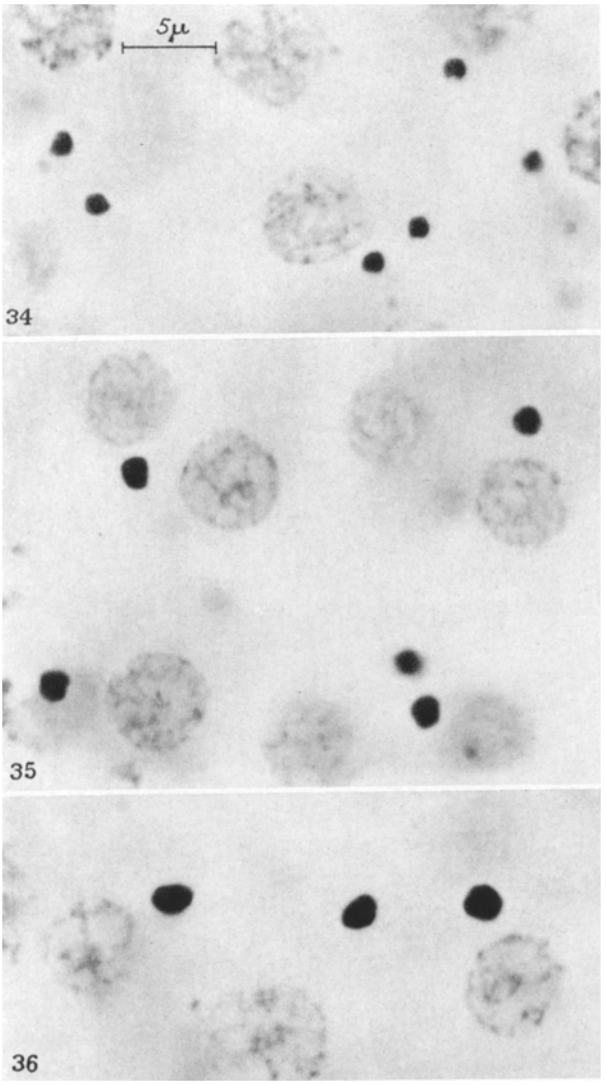


Figs. 30—33. Post-elimination stages. 2500×. Fig. 30. The large E masses start condensing; the eliminated D^H chromosomes are now negatively heteropycnotic. Fig. 31. The E masses fully condensed; the eliminated bodies are negatively heteropycnotic and each is probably made of the division products of $2D^H$ chromosomes. Figs. 32 and 33. Pseudoprophase. The E mass can now be resolved into 4 chromosomes; the eliminated D^H product is positively heteropycnotic. In comparison with the E chromosomes of prophase (Figs. 6—8) the E chromosomes now appear as single, unsplit chromatids

whether the segregation of the D^H derivatives from the E masses was due to the movement of the E masses, the D^H derivatives, or both, and whether spindle fibers were associated with the process. The process of segregation of the E masses and the D^H derivatives was similar to that observed by BROWN (1963) in *Nicholiella bumeliae* and termed by him "post-telophase ejection". At the time of elimination, the D^H derivatives often appeared V-shaped and bipartite and the two subunits are believed to be chromatids.

After the elimination of the D^H derivative or derivatives, the E mass gradually became more condensed and the eliminated D^H derivatives became more lightly stained, or negatively heteropycnotic. (Figs 30 and 31). This stage is somewhat similar to the second telophase of the

lecanoid chromosome system. The condensed E mass then became less condensed again and at this stage four E chromosomes and an eliminated D^H derivative could be clearly recognized (Figs. 32 and 33). This stage of the early spermatid nucleus in *P. oleae* is similar to that described



Figs. 34—36. Spermatids and heterochromatic residues (elimination bodies). 2500×. Fig. 34. Small H residues. Fig. 35. Medium-size H residues. Fig. 36. Large H residues. The small medium, and large H residues are believed to be the result of the elimination of the division products of one, two and three D^H chromosomes, respectively

by BROWN (1963) in *Aonidia lauri* and termed by him "pseudoprophase". The four E elements at this stage are of about the same length as the chromosomes at prophase except that at pseudoprophase the chromosomes do not seem to be split longitudinally. The appearance of the E chromosomes as unsplit at pseudoprophase tends to support the idea that at prophase the chromosomes in the bivalents are paired longitudinally. Continued extension of the chromosomes then transformed the pseudoprophase into the typical interphase condition (Fig. 34); the heteropycnotic elimination bodies, after the transient decondensation shown in Figs. 30 and 31, again became condensed. The sequence of stages from the elimination of the D^H derivatives through the condensation of the E mass to pseudoprophase and then to the interphase condition of the E mass was established on the basis of a close seriation of intermediate stages.

During the stage at which the E mass showed the typical interphase condition, at least four sizes could be recognized among the elimination bodies (Figs. 34—36): 1) a small residue of about half the size of a metaphase chromosome, 2) a medium size residue of about the size of a metaphase chromosome, 3) a large residue of about one and a half times the size of a metaphase chromosome, and 4) a very small H residue. The first three types were probably the result of the elimination of one, two and three D^H derivatives. The frequency of cysts with these types of residues agrees well with the frequency of cysts with one, two and three H elements at metaphase. The fourth type was only about half the size of the small type and was very rare. It probably represented the division and elimination products of the half size D^H chromosomes.

Discussion

The relation between pairing and replication

The most common type of metaphase plate in *P. oleae* had five elements of about equal size and on the basis of BROWN's (1963) interpretation of the Comstockiella system they were considered to be three bivalents and two univalents. The fact that all the elements were usually of the same size suggested that the univalents underwent an "extra" compensatory replication. Since the homologues which formed the bivalents separated at anaphase and were included in the sperm without ever undergoing division, the simplest interpretation for the similarity in size between the univalents and the bivalents is to assume that the chromosomes which underwent pairing did not replicate prior to pairing and that those chromosomes which did replicate, did not pair. Thus pairing in *P. oleae*, and probably also in other species with the Comstockiella chromosome system, apparently takes place between unreplicated chromosomes. This type of pairing between unreplicated

chromosomes apparently does not involve chiasma formation, and seems to be related to the secondary pairing of coccids rather than to pairing as it usually takes place in the early stages of the first meiotic division.

Coccids have been shown to have an inverted type of meiotic sequence (HUGHES-SCHRADER 1948, CHANDRA 1962) and during the course of meiosis, chromosomes usually pair in both the first and the second prophase stages of meiosis. During a typical coccid meiosis, chromosomes pair and form chiasmata at prophase I and auto-orient at metaphase I. Anaphase I separation is "equational" in a cytological sense (HUGHES-SCHRADER 1955), and the segregation is chromatid from chromatid rather that chromosome from chromosome. The two chromatids forming each dyad first move to one of the poles and then fall apart during interkinesis, only to reassociate at prophase II in what HUGHES-SCHRADER (1948) termed secondary pairing. Secondary pairing or *prophase II pairing* is thus a non-chiasmatic type of pairing which involves unreplicated chromosomes.

Pairing during prophase I is not a prerequisite for secondary, or prophase II, pairing. In the males of the monophlebine coccid, *Aspidoproctus maximus*, the autosomes often fail to pair in prophase I, yet they pair regularly during prophase II (HUGHES-SCHRADER 1955). Similarly, the supernumerary chromosomes of the mealy bug, *Pseudococcus obscurus*, do not pair during prophase I of oogenesis but still pair regularly in prophase II (NUR 1962). The behavior of the supernumeraries of the coelostomidine coccid, *Nautococcus schraderae*, may suggest that prophase II pairing is not a necessary result of the presence of two homologous unreplicated chromosomes at prophase II. In the males of *Nautococcus schraderae*, the supernumeraries do not pair in either the first or the second meiotic prophases although the autosomes do pair during prophase of the first meiotic division and undergo secondary pairing in prophase II (HUGHES-SCHRADER 1942). Because the supernumeraries of the latter species do not pair and are present in at least two sizes, one does not know to what extent all the supernumeraries of this species are homologous. However, supernumeraries are quite rare in coccids, so that in a species with supernumeraries, at least some of them are expected to be homologous. Thus, if the presence of two homologous unreplicated chromosomes during prophase II always leads to pairing, the supernumeraries of *N. schraderae* should have exhibited pairing in at least some of the males with more than one supernumerary.

In *Parlatoria oleae*, several cysts were observed in which the D^H element, or one of the D^H elements, was only about half the size of the other elements. The small size of the D^H in these cysts was attributed to the failure of the D^H to replicate. The small D^H element is not expected to pair with its D^E homologue because the latter is twice as large and must have replicated previously.

The similarity between pairing in spermatogenesis of the Comstockiella system and prophase II pairing in meiosis of other coccids suggests that in the establishment of pairing in spermatogenesis of the Comstockiella system the mechanism of prophase II pairing was utilized rather than that of prophase I.

Another kind of pairing, which involves the pairing of heterochromatic chromosomes during spermatogenesis, was reported by SCHRADER (1929) in *Gossyparia spuria*. *G. spuria* was shown to have a diploid chromosome number of 28, and in the somatic cells of the males 14 euchromatic and 14 heterochromatic chromosomes were present. SCHRADER reported that during spermatogenesis only 21 chromosomes, 14 euchromatic and 7 heterochromatic, were present. He interpreted spermatogenesis to be of the lecanoid type and suggested that the presence of only 7 heterochromatic chromosomes was the result of pairing among the H chromosomes. However, recent studies showed that members of the family *Eriococcidae*, to which *Gossyparia* belongs, are characterized by some form of the Comstockiella system (BROWN, unpublished). This observation was also extended to *Gossyparia spuria* (NUR, unpublished), so that the reduction in the number of chromosomes in spermatogenesis in *G. spuria* is probably due to pairing between euchromatic chromosomes and their heterochromatic homologues which had previously undergone deheterochromatization, and not to pairing among the heterochromatic chromosomes.

The origin of the multiple-D variant

Two variants of the Comstockiella chromosome system have already been previously described by BROWN (1963): 1) the simple Comstockiella system, and 2) the variable-D variant. In the first, the D pair is a fixed entity, while, in the second, any one of the pairs may play the D role, but there is only one D pair per cell at any given time.

In the variant of the Comstockiella system which has just been described in *Parlatoria oleae*, more than one pair may play the D role. For this variant of the Comstockiella system, the name multiple-D, is suggested. In *P. oleae* the four pairs of chromosomes are of about the same size so that we cannot determine whether all the chromosomes are capable of playing the D role. However, the presence of cysts with three D pairs clearly indicates that the D role can be played by at least three of the four chromosome pairs.

There is good evidence from both morphology and cytology on which to conclude that the Comstockiella system was derived from the lecanoid system (BROWN 1963). In spermatogenesis of the lecanoid system, the entire E and H sets divide equationally in the first division and then the two sets segregate from each other in the second division. In the

Comstockiella system, only the D pair (or pairs) divides equationally in the first division. The D^E and D^H derivatives then segregate from each other by the elimination of the D^H. The behavior of the D^E and D^H chromosomes in the Comstockiella system closely resembles the behavior of the E and H chromosomes in the lecanoid system and for this reason the D pair may be considered to be a remnant from the lecanoid system (BROWN 1963).

The evolution of the Comstockiella system from the lecanoid was considered by BROWN (1963) who pictured the change as a gradual process. First, in one pair of chromosomes, the heterochromatic member became euchromatic and paired with its euchromatic member to form a bivalent. This bivalent divided in the first meiotic division and its two products segregated with the other euchromatic chromosomes in the second division. Later, two or more pairs behaved in the same way and finally all but one pair were involved in pairing. At this stage the elimination of the single H chromosome was shifted to telophase or post-telophase and involved the ejection of the H chromosome rather than its segregation on a spindle. The steps just described would derive the simple Comstockiella system from the lecanoid system. BROWN (1963 and 1964) suggested that this change may have taken place not because it was beneficial to the species undergoing the change, but rather because it may have tended to increase the frequency of those genes which were responsible for the change. Such an evolutionary force that comes into play "when genes responsible for altering the life cycle, including meiosis, are thereby increased or decreased in frequency" BROWN (1963 and 1964) called automatic frequency response (AFR). Automatic frequency response can explain some of the changes from one chromosome system to another as, for example, from diploidy to male haploidy, and from diploidy to the lecanoid system; changes which are otherwise difficult to explain. Applying the idea of AFR to the lecanoid and Comstockiella systems, BROWN (1963 and 1964) showed that AFR can explain a change from the lacanoid to the Comstockiella system, but also a return from the Comstockiella system back to the lecanoid. BROWN's explanation of the role played by AFR in the evolution of the Comstockiella system from the lecanoid system will now be summarized. In the lecanoid system, only the chromosomes of the euchromatic set are transmitted to the next generation. Let us consider now what would happen when a mutant gene appears on one of the chromosomes which, when this chromosome is in the heterochromatic state, would cause both homologues to pair and segregate in the first division, so that both would be transmitted to the next generation. Such a mutant gene would be transmitted in the normal way when it happens to be in the euchromatic state, but, in addition, it will also be transmitted when it is in the

heterochromatic state by becoming euchromatic and pairing with its homologue. Such a gene would tend to increase in frequency until it replaces its allele completely. If genes of this type appeared and affected all but one of the chromosome pairs, the results would be to produce the simple Comstockiella chromosome system. The retention of one pair with a lecanoid behavior (the D pair) was attributed to its indispensability. BROWN suggested that the D^H functions in the heterochromatic residue and that this function is essential for male fertility. A change from the Comstockiella back to the lecanoid system can also be explained by AFR. In a species with the Comstockiella system, genes may appear which would change chromosome behavior back to lecanoid when the genes are on the E set. Such genes would then be transmitted exclusively whenever they are on the E set, at the expense of their alleles on the H set, and this would again tend to increase their frequency. As there is no *a priori* reason to assume that the genes pushing toward a lecanoid behavior are either stronger or weaker than those pushing toward a Comstockiella behavior, one would also expect to find intermediate stages between the two systems. A mixture, but not an intermediate stage was described by BROWN (1963) in *Nicholiella bumeliae*. In this species, both the lecanoid and the Comstockiella systems appear in the cells of different cysts of the same male.

In a species with the multiple-D variant of the Comstockiella system, the number of H chromosomes which are not transmitted to the next generation varies from cyst to cyst, according to the number of D^H chromosomes. This number may range from the whole H set to only a single H chromosome. The former case is typical of the lecanoid system while the latter is characteristic of the simple Comstockiella system. The multiple-D variant may be considered, therefore, to be an intermediate between the lecanoid and the simple Comstockiella systems and its presence in *Parlatoria oleae* would thus tend to support BROWN's suggestion that automatic frequency response played a role in the evolution of coccid chromosome systems, in general, and in the evolution of the Comstockiella chromosome system, in particular.

Summary

A modified Comstockiella chromosome system is described in the olive scale insect, *Parlatoria oleae* (COLVÉE), in which one, two, or three pairs of chromosomes may play the D role during spermatogenesis.

In the male, one set of chromosomes became heteropycnotic in the embryo and remained so until spermatogenesis. Prior to the meiotic prophase, some of the heteropycnotic (H) chromosomes became euchromatic and the number of these chromosomes was cyst-specific. Those

chromosomes of the H set which remained heteropycnotic are referred to as D^H elements and these chromosomes together with their euchromatic (E) homologues are called the D pairs.

During prophase and metaphase, 4 E and from one to three H elements were present. The number of H elements was considered to be an indication of the number of pairs which did not undergo pairing (D pairs). Thus in cysts with 1 H and 4 E elements, 3 of the E elements were considered to be bivalents while 1 E and 1 H elements were considered to be univalents.

All the chromosomes at metaphase were of about the same size. This was taken to mean that the univalents replicated prior to meiosis while the bivalents did not.

During anaphase, all the elements divided, with 4 E and from one to three D^H derivatives going to each pole. In the stages which followed telophase, the D^H derivatives segregated from the 4 E chromosomes. During spermiogenesis, only the nuclei with the E chromosomes formed functional sperm.

On the basis of the analysis of pairing in the Comstockiella system and in other coccids, it is suggested that in the Comstockiella system, and probably also in other coccids, when pairing is not associated with chiasma formation, the homologues entering pairing are unreplicated chromosomes.

The modified Comstockiella system found in *P. oleae* is called the multiple-D variant and is considered to be an intermediate between the lecanoid and the simple Comstockiella systems. The presence of such intermediates was predicted by BROWN (1963 and 1964) who considered the evolution of the Comstockiella system to be the product of automatic frequency response.

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