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A Radiation Analysis of a Comstockiella Chromosome System: Destruction of Heterochromatic Chromosomes during Spermatogenesis in *Parlatoria oleae* (*Coccoidea: Diaspididae*)* ** ***

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Abstract. In the males of the olive scale insect, *Parlatoria oleae* ($2n=8$), the paternal set of chromosomes becomes heterochromatic during late cleavage or early blastula and remains so until spermatogenesis. Immediately before the onset of meiosis in the males one or more heterochromatic chromosomes disappear from each primary spermatocyte. At prophase four euchromatic and from one to three heterochromatic chromosomes are present in each cell. The disappearance of the heterochromatic chromosomes before meiosis could be due either to the dehetero-chromatization of the heterochromatic chromosomes and their subsequent pairing with their euchromatic homologues, or to the destruction of the heterochromatic chromosomes. — The alternative interpretations of spermatogenesis in *P. oleae* were tested by using chromosome aberrations, which had been induced in the hetero-chromatic set by paternal X-irradiation, as genetic markers in breeding tests of about 400 X_1 males. Meiosis was examined in X_1 males which showed conspicuous chromosomal rearrangements in their somatic cells. The absence of either hetero-morphic chromosome pairs or multivalents at spermatogenesis and the failure of the X_1 males to transmit any form of chromosome aberration induced by paternal irradiation is strong evidence that the heterochromatic chromosomes are destroyed in *P. oleae*. — The evolutionary relationships of the chromosome systems in the coccids are considered. Models are outlined for the derivation of a Comstockiella system involving chromosome destruction either from a lecanoid sequence or from a hypothetical Comstockiella sequence involving chromosome pairing. Problems concerning the control of chromosome destruction are discussed.

Introduction

The chromosome systems described in the coccids are among the most unusual and complex known from the animal kingdom. Three different

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*** Dedicated to Dr. Sally Hughes-Schrader on the occasion of her seventy-fifth birthday.

systems, the diaspidid, lecanoid and Comstockiella systems, have been described in the armored and palm scale insects. The characteristic chromosome behavior of each system is shown only by the male; females display the typical aphid-coccid type of inverted meiosis (Hughes-Schrader, 1948).

The diaspidid system is the most common chromosome system in the sexual species of the armored scale insects (Brown, 1965). In this system, the paternal set of chromosomes is lost during cleavage divisions in the males. Spermatogenesis consists of a single mitotic division, and yields two functional sperms (Brown and Bennett, 1957; Bennett and Brown, 1958).

In both the lecanoid and Comstockiella systems, one set of chromosomes becomes heterochromatic during late cleavage or early blastula in the males and remains heterochromatic in many tissues throughout development. The Schraders compared the lecanoid system with true haplodiploidy in which males arise from unfertilized eggs and suggested that the heterochromatic set is paternal in origin and genetically inactive (Schrader and Hughes-Schrader, 1931; Hughes-Schrader, 1948).

In the lecanoid system, first described by Schrader (1921), spermatogenesis consists of two divisions during which chromosome pairing does not occur. The first division is equational for both the euchromatic (E) and heterochromatic (H) sets; in the second division the E and H sets segregate, and only the euchromatic derivatives form functional sperm (Hughes-Schrader, 1948; Brown and Nur, 1964).

In contrast to the lecanoid system, spermatogenesis in the Comstockiella system consists of a single division. The haploid number of H chromosomes present in the primary spermatocytes is reduced to a single H chromosome prior to the onset of meiosis in the typical Comstockiella system. At prophase, $n + 1$ elements, consisting of n E chromosomes and a single H chromosome are present in each cell. All chromosomes divide at anaphase and the derivatives of the H chromosome are eliminated later from the early spermatids, either by lagging at anaphase or by post-telophase ejection.

The Comstockiella system is the most complex of the three chromosome systems studied in the coccids (Brown, 1963; Kitchin, in press). In several species of armored scale insects, both the Comstockiella and lecanoid systems may occur in the same individual. In other armored scale species, variations may exist in the identity of the H chromosome which remains at prophase and in the mode of elimination of the H chromosome. Another variation, the multiple-D variant (Nur, 1965), has been described in the armored scale insect discussed in this paper, *Parlatoria oleae*, which Brown (1967) considered an evolutionary inter-

mediate between the Comstockiella and lecanoid systems. In this variant, four E chromosomes and a variable number of H chromosomes are present in prophase and metaphase cysts. In each variant of the Comstockiella system, the number and identity of the H chromosomes present in each prophase cyst is cyst-specific.

According to Brown's (1957, 1963) interpretation of the Comstockiella system, all but one of the H chromosomes become euchromatic and pair with their homologues in the E set; these bivalents later divide reductionally at anaphase. The remaining H chromosome, referred to as the D^H chromosome, and its euchromatic homologue, the D^E, undergo an additional compensatory replication, and divide equationally at anaphase. The D^H chromosome is eventually eliminated. Thus on Brown's interpretation, males should breed as sub-diploids, and transmit some, but not all, members of their H set to their offspring (Fig. 46, p. 190). Another explanation is that the loss of the H chromosomes prior to meiosis is the result of destruction rather than deheterochromatization of the H chromosomes. The males would then breed as haploids and transmit to their offspring only the members of their E set (Fig. 47, p. 193).

In the present study the alternative interpretations of the Comstockiella system, chromosome pairing vs. chromosome destruction, were tested in *Parlatoria oleae*, the only Comstockiella species available in laboratory stock culture. If the pairing interpretation is correct, then males should transmit markers in their H set to their offspring. Due to the lack of genetic markers in *P. oleae*, it was necessary to induce chromosome aberrations as markers in the H set of the males by X-irradiation, and to follow their transmission and behavior through the immediate and subsequent generations. Since coccid chromosomes are holocentric, and even very small fragments are capable of anaphase movement (Hughes-Schrader and Ris, 1941), this procedure has been used successfully in studies of both the diaspidid (Brown and Bennett, 1957) and lecanoid systems (Brown and Nelson-Rees, 1961; Nelson-Rees, 1962).

The results of these experiments indicate that the heterochromatic set in the Comstockiella system, like that in the lecanoid system, is of paternal origin and is genetically inactive. An exhaustive search for reversion of rearranged chromosomes from the heterochromatic to the euchromatic state during spermatogenesis, or the transmission of rearranged heterochromatic chromosomes to the next generation gave completely negative results. It is concluded that the H set in *Parlatoria oleae* males is eliminated during spermatogenesis by two separate mechanisms: (1) the destruction of some of the H chromosomes in each cell prior to meiosis, and (2) the ejection of the remaing H chromosomes from each cell after telophase.

Materials and Methods

Laboratory Stocks and Culture Methods. The laboratory stock culture of *Parlatoria oleae* (Colvée) was originally obtained from Mr. G. L. Finney, Division of Biological Control, University of California, Berkeley. This stock has been maintained since 1962 on the Irish potato, *Solanum tuberosum*, in a dark, ventilated growth chamber kept at 24 to 26° C and 50 to 75 percent relative humidity. A more detailed description of the life cycle and culture methods of these insects in our laboratory has already been presented (Robison, 1965; Kitchin, 1969).

A small portion of the potato surface was infested with first instar crawlers. The potatoes were then placed in pint-size mason jars for one week to allow the crawlers to become permanently fixed to the surface of the potato. All males were removed from the potatoes 25 to 30 days after infestation, and some were fixed for later use as controls in the studies of spermatogenesis. All potatoes were carefully examined at least twice for contaminating males before they were used in any experiment. The virgin females used in these experiments were 40 to 55 days old.

Paternal Irradiation Experiments. Groups of 15 to 25 adult males were irradiated while in gelatin capsules, and then removed and placed *en masse* on a single potato infested with untreated virgin females. The potatoes were then sealed in mason jars and placed in a dark growth chamber. In order to obtain a high yield of fertilized females several successive matings were made over a period of 7 to 10 days.

Several gravid females were fixed from each potato 4 to 8 days after the last mating, and their X_1 (F_1) embryos examined cytologically. In several experiments oogenesis was examined in adult X_1 females for the presence of chromosome aberrations.

Single-male matings were made by transferring adult X_1 males singly to potatoes infested with virgin females. In many cases, the initiation of the first mating was observed, and the mated female was marked and later fixed and examined separately. All gravid females collected from one potato 5 to 10 days after their mating to an X_1 male were assumed to have been inseminated by the same male, and their embryos, the X_2 generation, were examined.

The X-ray doses used in these experiments were between 1,000 and 12,000 r; these doses were lower than the minimum dose found to induce lethality in the males of the mealybug, *Planococcus citri* (Brown and Nelson-Rees, 1961).

Maternal Irradiation Experiments. Adult virgin females were irradiated and immediately allowed to mate with untreated males. In order to increase the number of successful matings, the same females were allowed to mate again with untreated males at 3, 5 and 7 days after irradiation. Several gravid females were collected from each potato 4 to 8 days after the last mating, and their X_1 embryos examined. In some experiments, adult X_1 females were fixed and examined for chromosome aberrations during oogenesis.

Classification of Aberrations. In both the maternal and paternal treatment series embryos were classified as *normal*, *mosaic* and *rearrangement* embryos after an examination of about five to six cells, although in some cases as few as one or as many as twenty division figures were analyzed. In those embryos classified *mosaic*, the chromosomes of one or more division figures were indistinguishable from the normal complement but one or more other figures showed chromosome aberrations. Mosaic embryos could result either from a spontaneous post-zygotic change, or from an occasional loss of a small X-ray-induced fragment during cell division (Chandra, 1963; Brown, 1960a, b; Nelson-Rees, 1962). Embryos were classed as *rearrangement* embryos only if each cell analyzed showed chromosome rearrangements. Although chromosome rearrangements can occur spontaneously, most of the

X_1 embryos classified as rearrangement embryos presumably were the result of paternal or maternal X-ray treatment.

Because coccid chromosomes are holocentric, chromosome aberrations were usually classifiable only on the basis of their size. The only exceptions were ring chromosomes, which were detected by their shape. Chromosome rearrangements were classed as *long* chromosomes if they resulted in an increase greater than approximately 40% of the length of the normal chromosomes, or as *short* or *fragment-sized* chromosomes if they resulted in a corresponding decrease. Since these rearrangements usually resulted from unequal reciprocal translocations (see below), even the *short* or *fragment-sized* chromosomes were probably most often the result of translocation rather than simple breakage.

Spermatogenic Studies. Spermatogenesis was studied in untreated second instar males collected as controls, and in second instar X_1 males which had been collected 45 to 90 days after either maternal or paternal X-irradiation.

Cytological Techniques. Specimens for cytological study were fixed in Bradley-Carnoy solution (Bradley, 1948) to which one drop of a saturated solution of ferric acetate in propionic acid was added as a mordant. All specimens were transferred to fresh fixative 24 to 48 hours later and stored at 4° C.

Females were squashed and stained in acetocarmine, and photographs, camera lucida drawings and free hand sketches made from temporary preparations. Males were stained in HCl-carmine (Snow, 1963), and then transferred to acetocarmine for one to two minutes. Testes were dissected and squashed in Hoyer's mounting medium (Ward's Natural Science Establishment, Rochester, N. Y.). Camera lucida drawings and photographs of spermatogenesis were made from permanent slides.

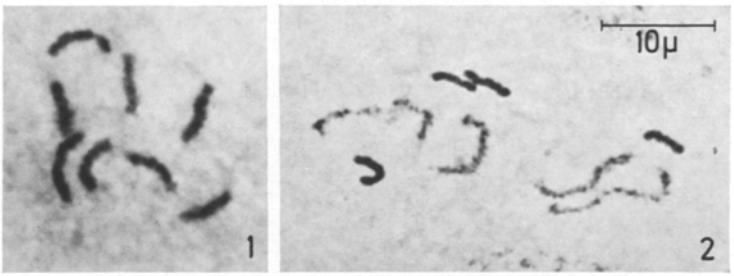
Observations

Cytology of X_1 Generation after Paternal or Maternal Irradiation

In order to test the pairing interpretation of the Comstockiella chromosome system it was first necessary to determine both the parental origin of the H set and the frequency of cytologically detectable chromosome rearrangements induced in the X_1 males after various parental X-ray treatments. The cytology of the X_1 embryos after paternal or maternal irradiation is described below. For clarity, the generations after paternal treatment will subsequently be designated X_1^p and X_2^p and correspondingly, after maternal treatment, X_1^m and X_2^m.

In *Parlatoria oleae* the diploid complement of both sexes consists of eight chromosomes of approximately equal size. A nucleolus organizer is present subterminally in one chromosome pair of the diploid set. As in other coccids characterized by the lecanoid and Comstockiella systems, the chromosomes in *P. oleae* females are isopycnotic (Fig. 1). In males, one set of chromosomes becomes heterochromatic (Fig. 2) during the 32- or 64-cell cleavage stage, and forms a heteropycnotic chromocenter at interphase in the cells of most tissues.

Paternal Irradiation Series. The percent of X_1 embryos with altered chromosomes after paternal or maternal irradiation is presented in Table 1. A relatively low frequency (0 to 0.9%) of the embryos examined

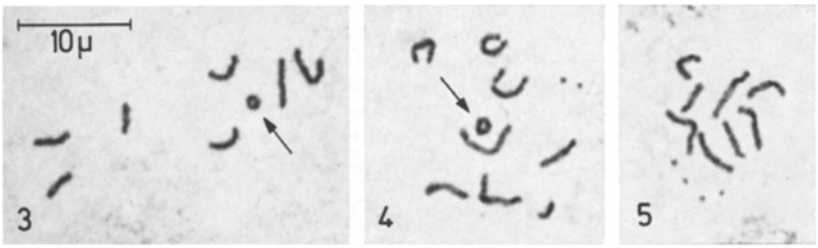


Figs. 1 and 2. Normal chromosome complement in *Parlatoria oleae*. Fig. 1. Prophase division figure from a female embryo with 8 chromosomes. Fig. 2. Prophase division figure from a male embryo with 4 heterochromatic (H) and 4 euchromatic (E) chromosomes

in the control series were rearrangement embryos. More than 95% of all X_1^p embryos examined after 4,000 r paternal irradiation showed abnormal karyotypes, a yield similar to that found in *Planococcus citri* after 8,000 r paternal irradiation by Brown and Nelson-Rees (1961). Although the total percent of rearrangement X_1^p embryos recovered after paternal irradiation usually increased with increasing paternal dose, this increase was not linearly proportional to the dose given. The marked increase in rearrangements from 1,000 to 2,000 r gives a slope of about 2 on log-log plotting; this result suggests that most of the apparent fragmentation may instead be the result of a "two-hit" effect, such as translocation. Although the dosage-response relationship obviously needs more detailed investigation, the two-hit interpretation corresponds with the high frequency of translocations observed at oogenesis in X_1^p females (see below).

Fragment-sized chromosomes were the most frequent chromosome aberration observed in X_1^p embryos. The behavior of radiation-induced fragments in *P. oleae* was similar to that of spontaneous fragments occurring in other armored scale insects (Brown 1960b). In general, they appeared to be quite stable during division. However, fragments were sometimes observed to lag during telophase, and some of these may have been lost. Size appeared to be an important factor affecting the successful movement of a fragment at anaphase. Although small micro-fragments were observed in some division figures in older embryos, they were also found frequently in the cytoplasm of adjacent cells.

Considerable cell to cell variation in the chromosomal complement was found in several X_1^p embryos after 12,000 r paternal X-irradiation. For example, in one embryo seventeen cells could be analyzed; fourteen different cell karyotypes were observed in this embryo, of which three are shown (Figs. 3, 4 and 5). Some embryos were mosaic for relatively



Figs. 3—5. Three prophase division figures from an X_1^p female embryo. Fig. 3. Cell with seven normal chromosomes and a ring chromosome (arrow). Fig. 4. Cell with seven normal chromosomes, one ring chromosome (arrow), one short chromosome and two microfragments. Fig. 5. Cell with eight normal chromosomes and four microfragments

long chromosomes as well as for ring chromosomes and small fragments. Because chromosome bridges and lagging chromosomes were rarely encountered, the instability of long chromosomes may reflect some phenomenon other than the mitotic behavior of the chromosome. For example, if the chromosomes were double at the time of irradiation, as they are in post-anaphase spermatocytes (see below), and rehealing of the broken ends was complete before the first zygotic division occurred, only two basic cell karyotypes should be observed in an X_1^p embryo. The results are too complicated, however, to be explained by two basic types followed by loss of the small chromosomes.

Maternal Irradiation Series. Within each experimental series an increase in the maternal dose usually resulted in an increase in the percent of affected X_1^m embryos (Table 1). At the same dose a considerably higher frequency of affected X_1 embryos was recovered following treatment of the father than after treatment of the mother.

Two of the doses used in these experiments were also used by Brown and Nelson-Rees (1961) in a radiation analysis of a lecanoid chromosome system in *Planococcus citri*. They found that 41.7 and 62.2% of all X_1^m embryos (recalculated from their data) had altered chromosome sets following maternal treatment of 4,000 r and 8,000 r, respectively. In *P. oleae*, 40.6 and 73.4% of all X_1^m embryos had altered chromosome sets after maternal X-ray doses of 4,000 r and 8,000 r, respectively.

The types of abnormalities observed in X_1^m embryos after maternal irradiation consisted largely of fragmentation and the gain or loss of entire chromosomes. The number of chromosomes which were lost appeared to be dependent largely upon the dose administered. After maternal treatment of 10,000 r, embryos could be found from which as many as three chromosomes had been lost. In these individuals, and in several others from which one or more chromosomes had been lost,

Table 1. *Frequency of X_1 embryos with chromosome rearrangements after paternal or maternal X-irradiation*

X-ray treatment	Series	Total embryos examined		% X_1 embryos with chromosome rearrangements				Total % rearrangement embryos
				rearrangement embryos		mosaic embryos		
		♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
Control	A	66	67	0	1.5	3.2	6.0	0.8
Control	B	62	48	0	2.2	0	0	0.9
Control	C	93	79	0	0	5.4	5.1	0
Paternal X-irradiation:								
1,000 r	A	198	191	15.1	26.8	2.0	3.1	20.8
2,000 r	A	158	203	65.3	66.6	2.5	4.9	65.8
2,000 r	B	6	15	66.7	80.0	16.7	0	76.2
3,000 r	A	21	27	76.2	74.1	0	3.7	75.0
4,000 r	B	20	22	95.0	100.0	0	0	97.7
5,000 r	A	29	36	96.6	97.2	2.8	0	97.0
5,000 r	B	40	63	75.0	90.5	0	0	84.4
12,000 r	B	22	20	100.0	100.0	0	0	100.0
Maternal X-irradiation:								
250 r	B	17	16	0	9.3	0	0	5.3
1,000 r	A	14	29	0	33.3	0	0	25.0
2,500 r	A	11	24	40.0	34.8	0	0	35.7
3,000 r	A	18	14	42.8	27.3	0	0	33.3
4,000 r	B	17	20	30.8	47.4	0	0	40.6
6,000 r	B	11	9	33.3	50.0	0	0	41.2
8,000 r	B	6	13	75.0	72.7	0	0	73.4
10,000 r	B	9	17	100.0	78.6	0	0	85.7

large areas of embryonic tissue appeared necrotic. These embryos presumably failed to undergo normal development and died.

Brown and Nelson-Rees (1961) also reported the loss of entire chromosomes following maternal irradiation in the mealybug. They observed some bridges during oogenesis and suggested that bridge formation may have resulted in chromosome loss. No bridges were observed in the rather limited number of embryos and oocytes examined in the present experiments. However, simple chromosome fragmentation could also lead to chromosome loss if small fragments fail to pair normally during meiotic prophase and behave irregularly at anaphase I and II.

Cytology of Adult X_1 Females and Their Progeny. In this experiment X_1 males and females were allowed to mate among themselves after either maternal or paternal irradiation. Since both the X_1 males and females could have had chromosome rearrangements, the chromosome

Table 2. *Cytological examination of adult X_1 females after maternal or paternal X-irradiation*

X-ray treatment and series	Number of adult X_1 ♀♀ examined[a]	Number of X_1♀♀ examined with		Number of X_1 ♀♀ with		% rearrangement adult X_1 ♀♀
		normal oocytes	abnormal oocytes	normal embryos only	rearrangement embryos	
Maternal X-irradiation:						
2,500 r A	16	7	0	14	1	6.2
3,000 r A	16	11	0	13	0	0
4,000 r B	17	11	0	15	0	0
10,000 r B	3	1	0	3	0	0
Paternal X-irradiation:						
3,000 r A	13	1	2	2	11	84.6
4,000 r B	29	0	17	4[b]	24	96.6
5,000 r A	20	1	12	0	20	100.0

[a] Some females provided either one or more analyzable oocytes of one of more analyzable embryos, but not both.

[b] Includes three females in which multivalents were observed during meiosis.

aberrations found in their offspring could have been transmitted by either parent. Adult X_1 females were classified as *rearrangement* females only if chromosome rearrangements were detected in an oocyte or in two or more X_2 embryos from a single X_1 female; if chromosome rearrangements were not found, the female was considered *normal*.

Close agreement was found between the percent of rearrangement X_1^p female embryos (Table 1) and the percent of adult rearrangement X_1^p females after paternal irradiation in all of the series examined (Table 2). Although untreated females were not examined as a specific control for the data in Table 2, translocation pairing configurations were not found in the oocytes of over one thousand untreated females during this investigation. On the other hand, since complex translocation pairing configurations, including rings or chains of four, six or eight, often were found in the X_1^p females after paternal irradiation, the frequency of two-hit aberrations must have been quite high even after the lowest X-ray dose used.

Over fifty adult X_1^m females were examined after maternal X-ray doses that had resulted in the production of chromosome rearrangements in 27.3 to 76.8% of all X_1^m female embryos examined (Table 1). Chromosome aberrations were found in the embryos of only one X_1^m female; no abnormal oocytes were detected (Table 2). The failure to recover adult females with chromosome changes only after maternal irradiation may be attributable to the fact that there are four damaged to one normal set

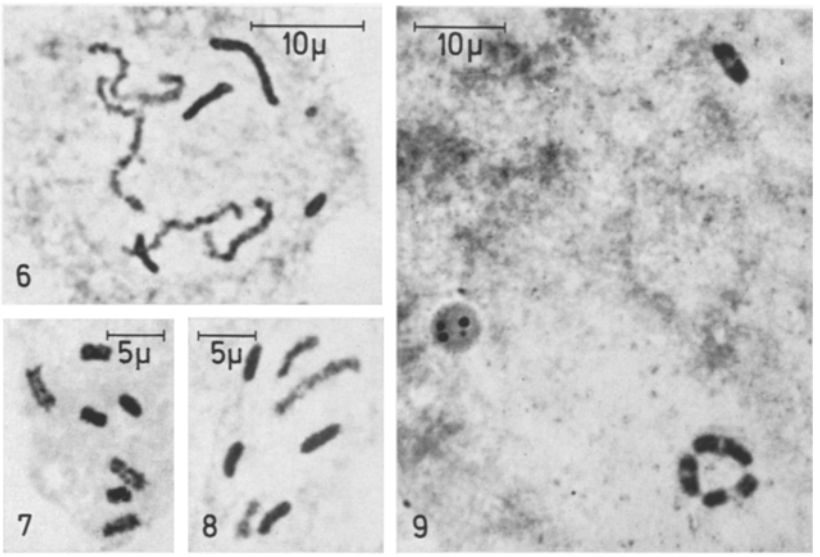
Table 3. *Percentage of male embryos with altered chromosome sets after maternal or paternal X-irradiation*

Treatment and series	Number of mothers examined	Number of male embryos	% Male embryos with aberrations in		
			H set	E set	not distinguishable
Control A	34	66	3.0	0	0
Control B	28	62	0	0	0
Control C	44	93	3.2	1.1	1.1
Maternal X-irradiation:					
4,000 r B	8	13	7.7	23.1	0
8,000 r B	8	6	0	0	50.0
Paternal X-irradiation:					
5,000 r A	25	29	96.6	0	0
5,000 r B	20	40	75.0	0	0
Male embryos from X_1^p adult females:					
3,000 r A	13	10	0	40.0	0
4,000 r B	29	46	0	65.2	13.2
5,000 r A	20	35	0	68.6	8.6

of chromosomes in the pentaploid mycetocytes, whereas the ratio is reversed in females surviving after paternal irradiation. This idea, however, remains to be verified.

Origin of the Heterochromatic Set. In the control series chromosome aberrations were observed in both H and E set in male embryos (Table 3). Chromosome aberrations were induced only in the heterochromatic set of male embryos after paternal irradiation (Fig. 6); after maternal irradiation, the aberrations were present primarily in the euchromatic set (Fig. 7). The only exceptional cases were three X_1^m males in which both sets had been altered after maternal irradiation. In one of the exceptional embryos there were large areas of necrotic tissues, suggesting that some form of physiological or genetic imbalance resulting from the loss of part or all of one or more euchromatic chromosomes had led to further fragmentation affecting both sets of chromosomes.

A cytological study of embryos from adult X_1^p rearrangement females provided an independent verification of the maternal origin of the euchromatic set of chromosomes in the male. Chromosome aberrations were found only in the E set (Fig. 8) of the male offspring of those X_1^p females which had conspicuous chromosome rearrangements in their oocytes (Fig. 9).



Figs. 6—9. Somatic cells and oocytes from X_1 and X_2 individuals after paternal or maternal X-irradiation. Fig. 6. Prophase from an X_1^p male embryo with a normal E set and an altered H set. Fig. 7. Prophase from an X_1^m male embryo with a normal H set and an altered E set. Fig. 8. Prophase division figure of a male embryo with a normal H set and an altered E set. The mother of this embryo had chromosome rearrangements in her oocytes. Fig. 9. Primary oocyte from an X_1^p female showing a ring-of-six and a bivalent at metaphase 1

Cytological Examination of the Offspring of X_1^p Males for Evidence of Transmission of Heterochromatic Chromosome Markers. In order to test whether or not X_1^p males could transmit chromosome markers in their H set to their offspring the following criteria were established: in experiments in which X_2^p embryos are examined, the same chromosome aberrations should be recovered in the X_2^p embryos of two or more females which had been allowed to mate with the same X_1^p male; in experiments in which mature X_2^p females are examined, chromosome aberrations should be detected in the oocytes of two or more X_2^p female offspring of the same X_1^p male.

During this investigation 389 single-male matings were made; X_2^p embryos were recovered from 274 of these crosses. Therefore, the majority of X_1^p males tested were fertile. The failure to recover progeny from about 30% of the single-male matings was apparently due to two major factors: (1) the females used in some single-male matings were not sexually mature, and (2) in other cases, the potatoes became rotten with mold before the X_2^p generation could be collected.

Table 4. *Mating tests of X_1^p males following paternal X-irradiation: summary of a cytological examination of X_2^p embryos for evidence of transmission of heterochromatic chromosome aberrations by X_1^p males*

Treatment and series	Number of adult X_1^p ♂♂ tested	Number of X_2^p embryos examined		% X_2^p embryos with chromosome aberrations				% X_1^p ♂♂ showing transmission of rearranged chromosomes
				rearrangement		mosaic		
		♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
Control[a] D		247	301	0	0	0	0.7	
1,000 r A	57	262	390	0	0.8	0	0.3	0
2,000 r A	148	1,005	1,320	0.5	1.4	0.8	0.3	0.6[b]
4,000 r B	16	63	41	0	0	0	0	0
5,000 r A	53	515	365	0.4	0.3	0.2	0	0

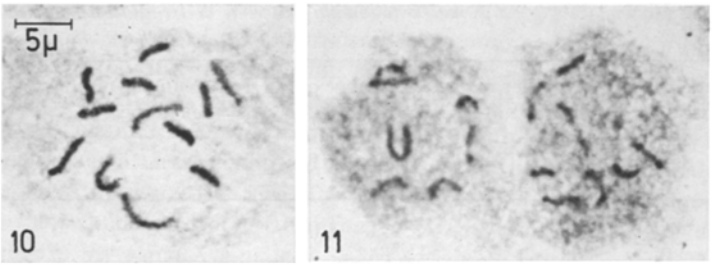
[a] Controls were gravid females collected from mass stock cultures.

[b] Diploid, triploid and hypertriploid X_2^p embryos were recovered from a single X_1^p male tested (see text).

Slides were prepared from both the control and experimental series and coded before examination. Approximately five cells were examined in each embryo, although as few as a single cell, or as many as twenty cells were studied in other embryos. The slides were decoded and the results tabulated only after the cytological examinations were complete. The results are summarized in Table 4.

A relatively low frequency of mosaic embryos was found in both the experimental and control series. Rearrangement embryos were found only in the experimental series. A total of 29 rearrangement X_2^p embryos was found in the progeny of 274 single X_1^p male matings examined; six showed one or more small fragments, while another had both an extra-long and an extra-short chromosome. Because the other 22 rearrangement embryos were either polyploid or aneuploid, these embryos were obviously not directly due to rearrangements, and were included in this class only as a matter of convenience.

In one single-male mating, X_2^p embryos were examined from eight gravid females. All of the X_2^p embryos studied from three of these females were normal diploids, while all of the X_2^p offspring of two other females were either 3n or 3n + 2. The X_2^p embryos obtained from three other females consisted of a mixture of diploid, triploid and hypertriploid embryos. Since there were 8H and 4E chromosomes in the cells of the triploid male embryos (Figs. 10 and 11), the extra set of chromosomes found in the triploid and hypertriploid embryos of this X_1^p male must have been paternal in origin. Because *P. oleae* females usually mate only once in their lifetime, it seems most likely that in this cross the X_1^p male



Figs. 10 and 11. Somatic cells of an X_2^p male embryo. Prophase division figure with 12 chromosomes (8H and 4E). Fig. 11. Two interphase nuclei with 8H chromosomes. Some of the chromosomes are lighter in the middle or slightly stretched

produced a mixture of functional sperm carrying n, 2n, 2n + 1 or 2n + 2 chromosomes. Although the mode of origin of the diploid and hyperdiploid sperm in this X_1^p male is still unknown, his triploid and hypertriploid offspring do not constitute conclusive evidence that chromosome pairing occurs during spermatogenesis in *P. oleae*.

Gravid X_2^p females were collected from the offspring of 118 single-male matings and their embryos and oocytes were examined for chromosome aberrations. These observations are summarized in Table 5. Chromosome rearrangements were detected in the oocytes and embryos of only one of 904 X_2^p females examined. The oocytes and embryos of the four other X_2^p females recovered in the progeny of the same X_1^p male were normal.

A cytological examination of the X_2^p embryos (see Table 4) and adult X_2^p female offspring (see Table 5) of 392 single-male matings failed to provide any conclusive cytological evidence that male *P. oleae* can transmit members of their H set to their offspring. Several factors were considered which could have affected the outcome of these experiments, including lethality or sterility of the X_1^p males, lethality of duplication and deficiency bearing gametes, or some unusual meiotic event.

Brown and Nelson-Rees (1961), in their radiation analysis of a lecanoid system, did not observe lethality in X_1^p males of *Planococcus citri* at doses below 30,000 rep paternal cobalt irradiation, a dose much higher than any used in the present study. A cytological examination of the mitotic division figures and primary spermatocytes of second instar *P. oleae* males revealed the presence of chromosome aberrations in 80 to 94% of the X_1^p males after 5,000 r paternal irradiation (see Table 6). Since there was no evidence of appreciable lethality in the X_1^p males between second instar and eclosion of the adult males, it is reasonable to assume that most of the adult X_1^p males tested had chromosome rearrangements. Furthermore, most of the X_1^p males tested were fertile.

Table 5. *Mating tests of X_1^p males following paternal X-irradiation: summary of heterochromatic chromosome*

Treatment and series	Number of adult X_1^p ♂♂ tested	Number of adult X_2^p ♀♀ examined	Number of oocytes examined in X_1^p ♀♀	
			normal	abnormal
3,000 r A	62	271	197	0
4,000 r B	23	65	84	2
5,000 r A	33	125	114	0

Table 6. *Analysis of the mitotic division figures of second instar X_1 males after maternal or paternal X-irradiation*

Treatment and series	Total number of X_1 ♂♂ examined	Somatic cell karyotypes present				% analyzable second instar X_1 ♂♂ with chromosome rearrangements
		Number of normal X_1 ♂♂	Number of rearrangement X_1 ♂♂	Number of mosaic X_1 ♂♂	Number of X_1 ♂♂ not analyzable	
Control A	187	64	1	0	122	1.5
Maternal X-irradiation:						
2,000 r A	130	52	0	1	77	0
2,500 r A	20	5	0	0	15	0
3,000 r A	164	58	1	0	105	1.7
4,000 r B	80	44	0	0	36	0
6,000 r B	69	33	0	0	36	0
Paternal X-irradiation:						
5,000 r A	548	28	265	4	251	94.3
5,000 r B	207	18	82	2	105	80.4

Because some evidence had already been obtained that unbalanced gametes are functional in both sexes in *P. oleae* (see above), a detailed study of the behavior of radiation-induced chromosome rearrangements during spermatogenesis in the X_1^p males was carried out.

Spermatogenesis

Spermatogenesis in Control Males. In *Parlatoria oleae*, spermatogenesis consists of a modified Comstockiella system that is believed to be an intermediate between the Comstockiella and lecanoid systems (Nur, 1965; Brown, 1967). For purposes of orientation, spermatogenesis is described below. More details are available from Nur (1965) and Kitchin (1969).

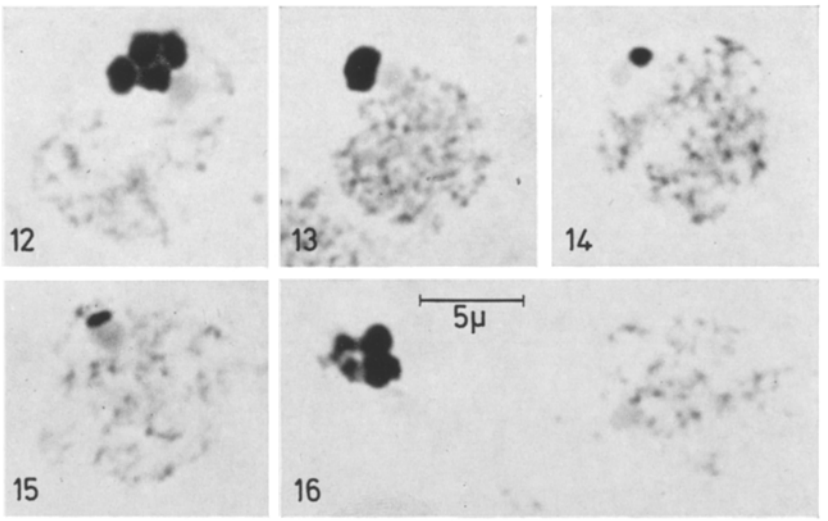
a cytological examination of adult $X_2{}^p$ females for evidence of transmission of aberrations by $X_1{}^p$ males

Number of $X_3{}^p$ embryos examined		% $X_3{}^p$ embryos with chromosome aberrations				% $X_1{}^p$ ♂♂ transmitting H chromosomes
		rearrangement		mosaic		
♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
288	342	0	0.6	0.3	0.3	0
230	179	0.4	1.6	0	0.6	0
163	216	0	0	0	0.6	0

In young second instar males the cysts of the testes were composed of 16 primary spermatocytes. In each primary spermatocyte a chromocenter was present which was made up of four heterochromatic (H) chromosomes of about equal size (Fig. 12). Prior to the onset of prophase the number of H chromosomes in the primary spermatocytes decreased until it was stabilized within each cyst, most often at one H chromosome per cell (Fig. 13). However, some cysts were found in which all cells showed two, three or four H chromosomes. This stage was named *preprophase* by Brown (1963).

In most of the preprophase cysts studied the H chromosomes were of about equal size. However, small fragment-sized H chromosomes of two distinct sizes were found in some preprophase cysts. In a few males, several cysts were found in which a single H chromosome, about half the size of a normal H chromosome, was present in all primary spermatocytes (Fig. 14). Even smaller H chromosomes, about one quarter the size of a normal H chromosome, were found in a few preprophase cysts in two control males (Fig. 15). Nur (1965) also observed small H elements in a few cysts in *P. oleae*, but only during later stages in spermatogenesis. He attributed the small size of the H chromosome to its failure to replicate. If an H chromosome occasionally fails to replicate, then some cells should be found with three normal-sized and one fragment-sized H chromosomes prior to reduction. Such cells, however, have not been observed.

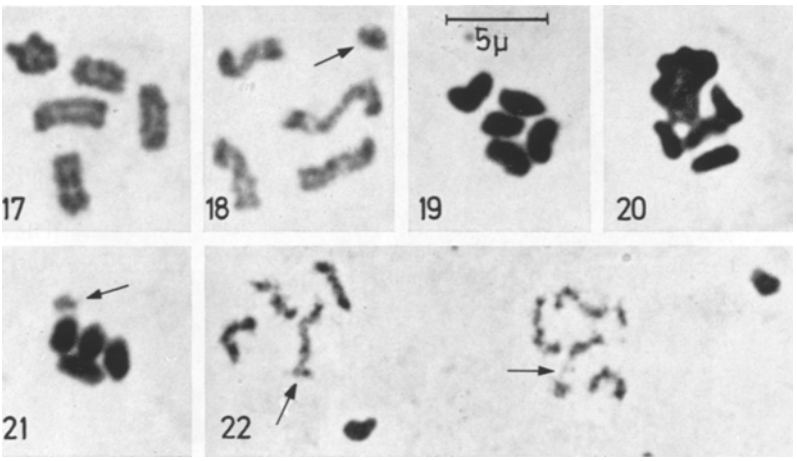
During the transitional stage, when the H chromosomes decrease in number, one or more H chromosomes in some primary spermatocytes appeared to disintegrate into several lightly stained fragments or strands which often lay adjacent to one or more intact H chromosomes (Fig. 16). Because these cells were generally found in testes in which most of the cysts were composed of primary spermatocytes with four H chromosomes at preprophase, they may represent an intermediate stage in the destruction or deheterochromatization of the H chromosomes.



Figs. 12—16. Spermatogenesis in control males. Preprophase. Figs. 12 and 13. Primary spermatocytes showing four and one H chromosomes, respectively. Fig. 14. Cell from a cyst with a half-sized H chromosome. Fig. 15. Cell from a cyst with a quarter-sized H chromosome. Fig. 16. A cell with two normal H chromosomes and pieces of one or more H chromosomes which presumably are being destroyed

By the end of preprophase the number of H elements within each cyst had stabilized, and the euchromatic (E) elements began to condense. During prophase, four E and one H elements were found in each primary spermatocyte in most cysts (Fig. 17). However, cysts were also observed in which each spermatocyte contained either $4E+2H$, or $4E+3H$ elements. Several prophase cysts were found in which one H element was about half the normal size (Fig. 18). The small H element was often isopycnotic or negatively heteropycnotic when compared to the normal H element.

The E elements continued to condense during prophase and at metaphase appeared isopycnotic with the H elements. The size and number of elements on the metaphase plate varied from cyst to cyst. The spermatocytes of most cysts contained five elements (Fig. 19), although in some cysts all of the spermatocytes had six, seven or eight elements at metaphase (Fig. 20). In most cysts all elements were of about equal size. However, a few cysts were observed in which one element was half the size of the other elements, which were presumably euchromatic. The small element was usually on the periphery of the metaphase plate, and often negatively heteropycnotic and irregular in shape (Fig. 21).

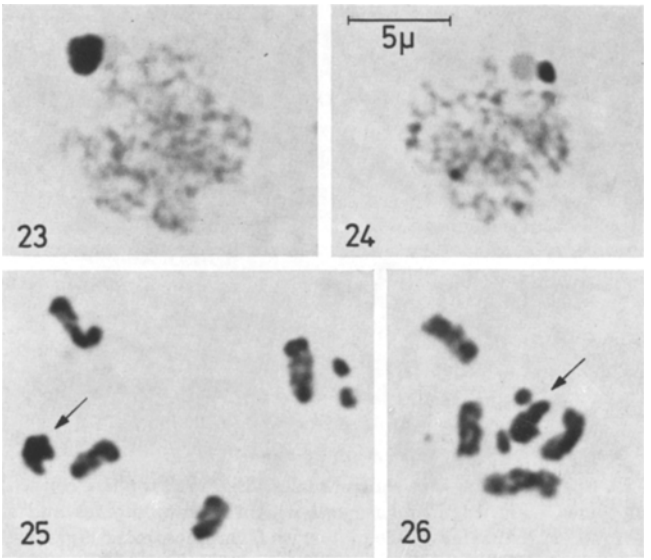


Figs. 17—22. Spermatogenesis in control males. Fig. 17. Prophase cell with 4E and 1H chromosomes. Fig. 18. Prophase cell with 4E chromosomes and a small H element (arrow). Fig. 19. Metaphase plate with 5 chromosomes. Fig. 20. Metaphase plate with 4 densely clumped H chromosomes and 4E chromosomes. Fig. 21. Metaphase plate from a cyst in which the H element (arrow) was small and negatively heteropycnotic. Fig. 22. Pseudoprophase. Each early spermatid contains four bipartite E chromosomes (see at arrows). The H derivatives have been eliminated from the future sperms

At metaphase the H element appeared more condensed than the E elements in side view, and was the last to divide at anaphase. Each telophase nucleus contained four E elements; the H derivatives generally lay between the E derivatives.

After telophase, the more condensed H derivatives appeared to lie inside the nucleus at the edge of the less condensed E set. The H derivatives then moved away from the E set, and were eliminated from the future sperms. Later, four bipartite E chromosomes could be clearly seen in each early spermatid nucleus; the positively heteropycnotic H derivatives lay outside the nuclei (Fig. 22). The size of the H derivative reflects the size (Brown, 1963) and number (Nur, 1965) of H chromosomes eliminated from each cell. Brown called this stage *pseudoprophase*.

Spermatogenesis in X_1^p *Males.* Spermatogenesis was examined in a total of 758 X_1^p males after 5,000 r paternal irradiation. At this dose chromosome aberrations were induced in 75.0 to 96.6% of all X_1^p male embryos (see Table 1). The karyotype analysis of the second instar X_1^p males was based on a study of mitotic division figures found in an area of the testis lacking heterochromatin. Because chromosome rearrangements were induced in the H set in the X_1^p male embryos after paternal

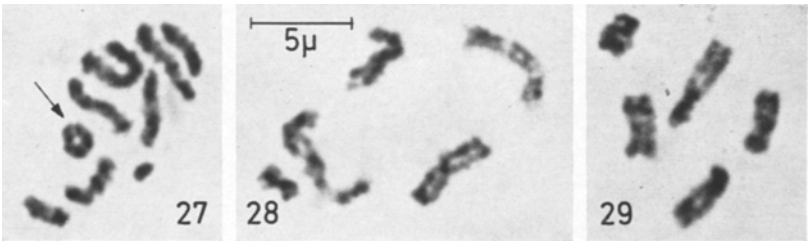


Figs. 23—26. Spermatogenesis in X_1^p males. Figs. 23 and 24. Cells from two different preprophase cysts of an X_1^p male. Each cell in one cyst had a normal-sized H chromosome, while the cells of another cyst had a small H chromosome. Figs. 25 and 26. Prophase. Two small fragments are associated with a normal H chromosome (arrow) in some cells in a cyst; in other cells of this cyst the small fragments were displaced from the H chromosome

irradiation (see Table 3), and were found only in the H set in the testes during spermatogenesis in second instar X_1^p males, it was assumed that the chromosome aberrations found in those mitotic division figures of the X_1^p males in which the H and E set could not be differentiated were also part of the paternal set. Chromosome rearrangements were detected in the mitotic division figures and primary spermatocytes of 86.7% of all second instar X_1^p males studied (Table 6).

Small-sized chromosomes were the most frequently observed chromosome aberration found in the primary spermatocytes of the X_1^p males of *P. oleae* after paternal X-irradiation. Although small chromosomes have been observed in the prophase and metaphase cysts of many X_1^p males, there was no evidence similar to that found by Brown (1963) in *Nicholiella bumeliae* for chromosome pairing during spermatogenesis in *P. oleae*.

In *P. oleae*, the size of the H element frequently varied from cyst to cyst in those X_1^p males which had small fragment chromosomes in their somatic cells. In some preprophase cysts, the H element was apparently of normal size, while in others it was extremely small (Figs. 23 and 24). Variation from cyst to cyst in the size of the H element at preprophase



Figs. 27—29. Spermatogenesis in an X_1^p male with a ring chromosome. Fig. 27. Mitotic division figure with a ring chromosome (arrow). Fig. 28. Cell from a cyst at early prophase with 4 longitudinally split E chromosomes and a small H chromosome. Fig. 29. Mid-prophase cell with 4 E chromosomes and a normal H chromosome

and prophase was observed far more frequently in the X_1^p males than in the controls, demonstrating that not only the *number* of H chromosomes, but also *which* H chromosomes remain at prophase may vary from cyst to cyst in the *multiple-D variant* of the Comstockiella system.

No evidence was found of pairing between the one or more short chromosomes present in a given cyst and their unaltered homologue(s) in the E set at prophase. When a fragment-sized chromosome was found in a cyst, it was either the only H element present, or it was closely associated with other H elements in at least some cells of the cyst (Figs. 25 and 26). In the most favorable prophase cells examined, each euchromatic chromosome appeared to be split along its entire length; there was no evidence of pairing between heteromorphic homologues.

Ring chromosomes were found in the mitotic division figures of two X_1^p males. In one of these males, several cells had seven normal chromosomes, one ring chromosome (arrow), and one or two small fragments (Fig. 27). Several different classes of prophase cysts were found in this male. A small H element, which was obviously bipartite, was found in one cyst at early prophase (Fig. 28), while in another cyst at midprophase the H element in each cell was of normal size (Fig. 29). In each of the prophase cells studied in this male, the E elements were of normal size and clearly bipartite; the ring chromosome was not observed in either the E or H set. One metaphase cyst was found in which six isopycnotic chromosomes, including both a normal-sized and a ring H chromosome, were present in most of the cells analyzed. Four normal E elements were observed in each telophase cell examined in the only telophase cyst found.

During this study, prophase cysts were examined in about 50 X_1^p males which showed extra-long chromosomes in their mitotic division figures. In most of these males at least two easily detectable chromosome aberrations were found in each of the mitotic division figures analyzed (Table 7). The extra-long chromosomes were at least 50% longer than the averaged lengths of the four unaltered members of the E set.

Table 7. *The number of long (L) and short (S) chromosomes present in the mitotic division figures of X_1^p males of P. oleae analyzed at meiosis*

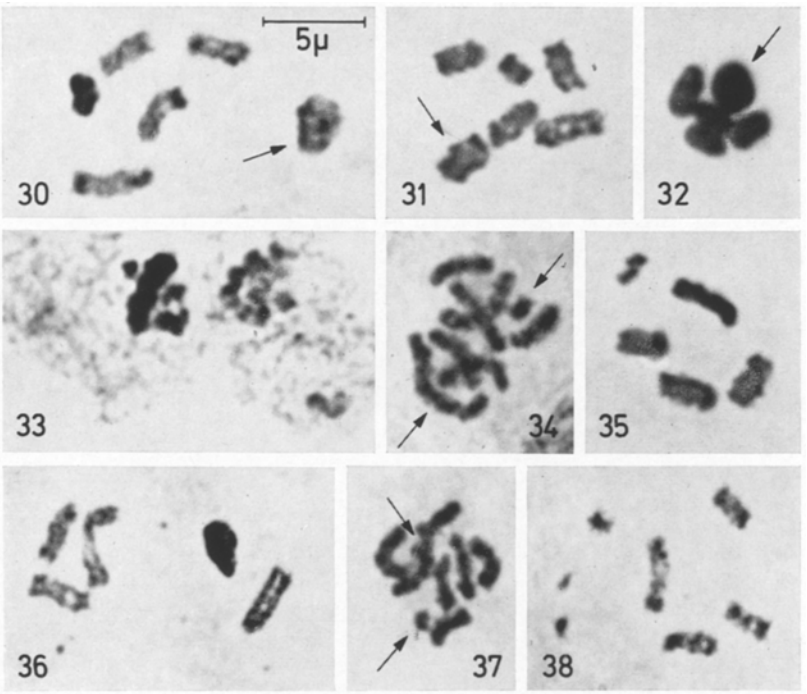
	L	L+S	L+2S	2L	2L+1S	2L+2S	2L+3S
Number of males	1	17	21	0	3	4	3

Extra-long chromosomes were found in only a few of the prophase and metaphase cysts studied (Figs. 30, 31 and 32). Presumably, in most cysts the long H chromosome had either already become euchromatic and paired with its homologues in the E set or it had disintegrated. An apparent example of disintegration is shown in Fig. 33.

Spermatogenesis was quite uniform in those X_1^p males showing an exceptionally clear extra-long chromosome in their mitotic division figures. In these males, although the size of the H element often varied from cyst to cyst in the testis, the E set always consisted of four bipartite elements of about equal length. For example, in one X_1^p male, six chromosomes of about normal length, one long chromosome and a small fragment were found in each of the mitotic division figures studied (Fig. 34). In one prophase cyst in this male, the H element present in each cell was very small (Fig. 35), while in another cyst it was of about normal size (Fig. 36). The mitotic division figures of another male showed five chromosomes of about normal length, one very long chromosome and three small fragments (Fig. 37). In one prophase cyst in this male, three heterochromatic fragments were present in most of the cells examined (Fig. 38). No multivalents or associations between heteromorphic homologues were found in any of the prophase cells examined in these X_1^p males, even though these pairing configurations should have been quite striking; the E set always consisted of four chromosomes of about normal length.

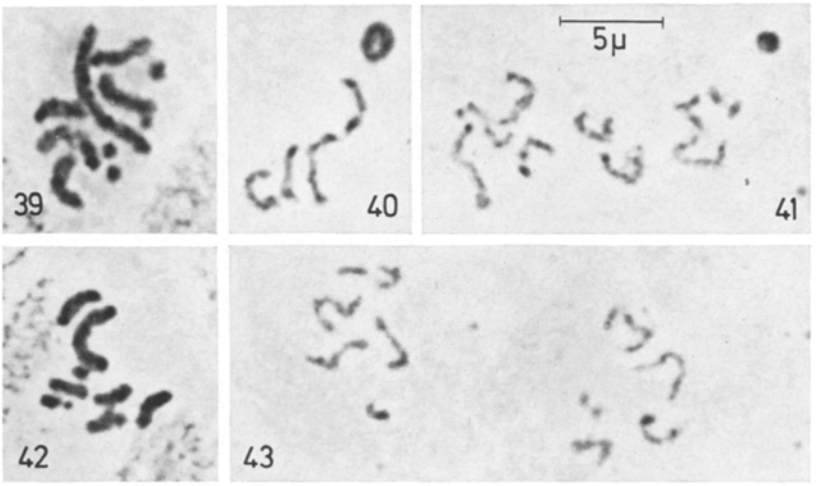
The experiments designed to test if males can transmit some of the chromosomes in their H set to their offspring gave negative results. Two explanations are possible: either (1) X_1^p males do form unbalanced gametes which are non-functional, or (2) X_1^p males do not form unbalanced gametes. Fortunately, there are several meiotic stages in which all four chromosomes in each early spermatid are visible; these stages are telophase and pseudoprophase.

During pseudoprophase, four E chromosomes can be identified in each early spermatid; the derivatives of the H chromosomes have been eliminated from the nuclei. Several exceptionally clear pseudoprophase cells could be studied in a number of X_1^p males which showed obvious chromosomal rearrangements in their mitotic division figures (Figs. 39 and 42). As a rule, although the size of the eliminated H derivatives



Figs. 30—38. Spermatogenesis in X_1^p males. Figs. 30—32. Prophase and metaphase cells with a large H chromosome (arrows). Fig. 33. The chromocenter of one preprophase cell shows one very long H chromosome and three short H chromosomes. The H chromosomes appear to be broken into many small fragments in an adjacent cell in the same cyst. Figs. 34—36. Mitotic division figure and primary spermatocytes at prophase from an X_1^p male. Fig. 34. Mitotic division figure with six normal chromosomes, one long chromosome (arrow), and one small fragment (arrow). Fig. 35. Cell from a cyst with a small H element in each cell. Fig. 36. Cell from a cyst with a normal H element in each cell. Figs. 37 and 38. Mitotic division figure and a primary spermatocyte at prophase from an X_1^p male. Fig. 37. Mitotic division figure with five normal chromosomes, one long chromosome (arrow), and three small fragments (arrow). Two of the fragments are partly obscured by the long chromosome. Fig. 38. Prophase cell with three small H chromosomes

varied considerably from cyst to cyst, each of the early spermatids contained only four euchromatic chromosomes of about normal length (Figs. 40, 41 and 43). Fragments could not be reliably identified in the E set at pseudoprophase, because at this stage the nucleolar satellite frequently resembles a small fragment [see Nur's (1965) Fig. 32]. Because there was no evidence of mosaicism in the mitotic division figures analyzed in the testis of the X_1^p males, these observations seem to be

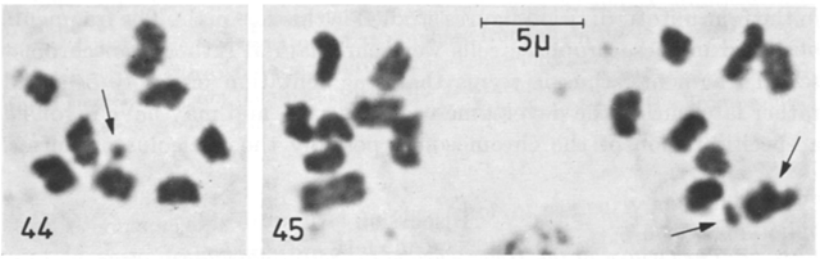


Figs. 39—43. Spermatogenesis in X_1^p males. Figs. 39—41. Mitotic division figure and early spermatids at pseudoprophase. Fig. 39. Mitotic division figure with five normal chromosomes, one very long chromosome and four small fragments. Fig. 40. Early spermatid with four normal E chromosomes; the derivative of the H chromosomes of each cell in this cyst was very large, and probably corresponds to the elimination of either the long chromosome or a normal chromosome and the short fragments from each cell in the cyst. Fig. 41. Each early spermatid in this cyst had four normal E chromosomes; the size of the elimination body probably reflects the loss of several of the short H chromosomes. One of the H derivatives was probably displaced during squashing. Figs. 42 and 43. Mitotic division figure and early spermatids from an X_1^p male. Fig. 42. Mitotic division figure with five normal chromosomes, one long chromosome, and four short chromosomes. Fig. 43. Pseudoprophase. Two early spermatids; each shows four normal E chromosomes; the H residues are small and lightly stained

irreconcilable with the pairing interpretation of spermatogenesis in *Parlatoria oleae.*

Spermatogenesis in X_1^m Males. Meiosis was studied in approximately 400 X_1^m males after maternal X-ray doses ranging from 2,000 to 8,000 r. Although mitotic division figures suitable for karyotype analysis were found in about 200 X_1^m males, only a single X_1^m male showed chromosome aberrations in these cells (Table 6). This male, however, had no meiotic stages available for study.

In comparison to those results obtained after paternal irradiation, maternal irradiation appeared to result in almost complete lethality in the affected X_1^m males. Since in lecanoid males only a single set of chromosomes, the euchromatic set, is genetically active in the cells of most tissues (Brown and Nelson-Rees, 1961; Berlowitz, 1965; Brown, 1969), radiation-induced deletions in the euchromatic set after maternal



Figs. 44 and 45. Spermatogenesis in X_1^m males. Fig. 44. Primary spermatocytes at prophase with 8 chromosomes and a small fragment (arrow). Fig. 45. Two cells from the same prophase cyst. One cell shows 4 E and 4 H chromosomes. A small euchromatic fragment (arrow) was removed from the end of one of the E chromosomes (arrow) in another cell in this cyst

treatment may account for the lethality encountered in the X_1^m males of *P. oleae*.

Spermatogenesis was perfectly normal in the majority of X_1^m males studied. One observation which is worth mentioning, however, was the discovery of four X_1^m males in which the testes consisted of a mixture of both lecanoid and Comstockiella cysts. In three of these males, the few preprophase, prophase or metaphase lecanoid cysts present were distributed apparently at random throughout a testis which consisted primarily of Comstockiella cysts. Because lecanoid cysts were not found in the later stages of spermatogenesis, it was not possible to determine whether *P. oleae* males had retained the potentiality for the second segregational division found in true lecanoid males, or if chromosome elimination, which occurs in most Comstockiella males, has been permanently substituted for it. However, a second segregational division, similar to that found in the lecanoid system, has been observed in several members of the *Eriococcidae*, *Scutare lanuginosa* (Brown, 1967) and *Gossyparia spuria* (Nur, 1967a), which, like *P. oleae*, are characterized by the multiple-D variant of the Comstockiella system.

In *P. oleae*, a case of fragmentation was encountered in a single X_1^m male which was similar to that described by Brown (1963) in *Nicholiella bumeliae* and cited as evidence for chromosome pairing. In one prophase cyst, a small euchromatic fragment was present in four of the fourteen cells examined (Figs. 44 and 45). The euchromatic fragment in each cell was about the size of the nucleolar satellite of this species. Although a gap was found in a bipartite E element in one cell with a euchromatic fragment, it could not be due to the failure of the fragment to pair with its homologue, because four normal-sized H chromosomes were present in the chromocenter of each cell in the cyst. No fragments were present

in the four mitotic division figures studied in this X_1^m male. The fragments observed in these prophase cells were chromatidal rather than chromosomal fragments, thus it seems that fragmentation probably occurred rather late during the development of this cyst, and may have involved a specific region of the chromosome, possibly the nucleolus organizer.

Discussion

The Origin of the Heterochromatic Set in Parlatoria oleae

The Schraders compared the lecanoid system with true haplodiploidy and suggested that the heterochromatic set of chromosomes in the male is paternal in origin and genetically inactive (Schrader and Hughes-Schrader, 1931; Hughes-Schrader, 1948). The Schraders' hypotheses have since been confirmed in the mealybug, *Planococcus citri*, by several investigators (Brown and Nelson-Rees, 1961; Brown and Nur, 1964; Berlowitz, 1965; Nur, 1967b; Brown, 1969).

Our understanding of the Comstockiella system was based mainly through analogy with the better understood lecanoid system. The present study is the first experimental analysis of the Comstockiella chromosome system.

In *Parlatoria oleae*, paternal irradiation induced chromosome aberrations only in the heterochromatic set of the X_1 male embryos; after irradiation of the mothers, induced aberrations were usually confined to the euchromatic set. In addition, the male embryos from X_1 females which showed chromosome aberrations in their oocytes had chromosome rearrangements only in their euchromatic set. Lethality was very low in both the X_1 males and females after moderate (1,000 r to 5,000 r) X-irradiation of their fathers. However, similar doses delivered to their mothers almost always resulted in lethality in the X_1 embryos of both sexes. These results indicate that the heterochromatic set is also paternal in origin and genetically inactive in the Comstockiella chromosome system.

Male coccids apparently may compensate for having a genetically inactive set of chromosomes. For example, although a lecanoid chromosome system is present in the male of the mealybug, *Planococcus citri*, cells in the Malpighian tubules, hindgut, midgut, skeletal muscles, some of the oenocytes and the cyst wall cells of the testis lack heterochromatin (Nur, 1966, 1967b). Nur (1967b) has shown that this is due to the reversal of the H set to the euchromatic state and its return to genetic activity, and that the time of reversal may vary from tissue to tissue.

In the males of *Parlatoria oleae*, which show a Comstockiella system, the testis sheath cells, accessory glands of the testis and the cyst wall cells also lack heterochromatin. The presence of chromosome aberrations

in cells which lacked heterochromatin in males after paternal irradiation is the best evidence yet available that the lack of an H set in some male tissues is due to reversal and not the loss of the H set.

Spermatogenesis in Parlatoria oleae

One or more heterochromatic chromosomes disappear from most primary spermatocytes just before meiosis begins in the males of *Parlatoria oleae*. The sudden loss of the H chromosomes could not be due to simple chromosome ejection prior to prophase, since the H chromosomes, or their residues, are not found in the cytoplasm at any stage of meiosis. The disappearance of the heterochromatic chromosomes before meiosis, however, could be due either to the deheterochromatization of the heterochromatic chromosomes and their subsequent pairing with their euchromatic homologues (Brown, 1963), or to the destruction of the heterochromatic chromosomes.

The alternative interpretations of the preprophase events in *P. oleae*, chromosome pairing vs. chromosome destruction, were tested by using chromosome aberrations, which had been induced in the heterochromatic set by paternal irradiation, as genetic markers in breeding tests of approximately 400 X_1 males. A careful cytological examination of the X_2 progeny revealed no evidence of transmission of heterochromatic chromosomes from the X_1 males to their offspring.

Spermatogenesis was carefully studied in about 50 X_1^p males which had unusually long chromosomes in their somatic cells. Although the size of the H elements varied from cyst to cyst, the euchromatic set always consisted of four longitudinally split chromosomes of about equal size. Translocation pairing configurations or unequal bivalents were never found at prophase, even though these chromosome associations should have been quite striking. During pseudoprophase in these males the size of the eliminated derivatives of the H chromosome(s) varied from cyst to cyst, but each early spermatid contained only four normal sized chromosomes.

The absence of either heteromorphic chromosome pairs or multivalents at spermatogenesis and the failure of the X_1^p males to transmit any form of chromosome aberration induced by paternal irradiation indicates that the H set must be eliminated in *P. oleae* by two separate mechanisms: (1) the destruction of some of the H chromosomes in each cell during preprophase, and (2) the ejection of the remaining H chromosomes from the cells after telophase.

The Evolution of the Comstockiella System

On the basis of the best taxonomic and cytological evidence available, Brown (1963) suggested that the Comstockiella system arose from

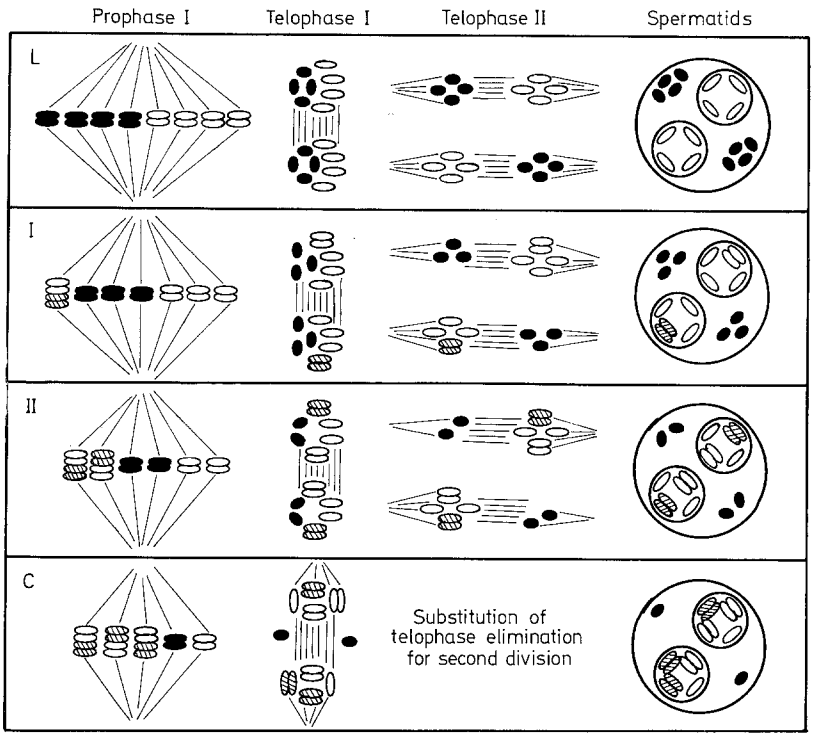


Fig. 46. Diagram of Brown's (1963) model for the derivation of a Comstockiella (C) sequence involving chromosome pairing from a lecanoid (L) sequence. Both the euchromatic (hollow) and heterochromatic (solid) chromosomes divide equationally during the first division of the lecanoid sequence. The E and H sets segregate from each other during the second division, and only the euchromatic derivatives are incorporated into the sperm. Brown proposed that during the first step (I) in the derivation of the Comstockiella system one H chromosome becomes euchromatic (cross-hatched) and pairs with its euchromatic homologue. This pair separates during the first division and both division products move with the E set during the second division. Since this bivalent divides reductionally while each univalent divides equationally, the univalents must undergo an additional replication to make the division products of the bivalent and univalents equivalent. Brown called this process "compensation". From this diagram it is apparent that if compensation does not occur prior to meiosis aneuploidy will result. The number of bivalents gradually increases (II) until all but one of the H chromosomes are involved in pairing. A true Comstockiella sequence (C) results when the second division of the lecanoid sequence is replaced by some form of telophase elimination. According to this interpretation, Comstockiella males should breed as sub-diploids and transmit some members of their paternally derived H set to their offspring

the lecanoid system, and eventually led to the diaspidid system. Brown constructed a model outlining the gradual evolution of a Comstockiella sequence from the lecanoid system. His scheme is shown in Fig. 46.

Brown suggested that at first one heterochromatic chromosome became euchromatic and paired with its euchromatic homologue. This bivalent divided during the first division and both division products moved with the euchromatic set during the second division in the lecanoid sequence. At this stage some form of suppression, which Brown called *compensation*, must occur to make the undivided chromosomes of the bivalent equivalent to the univalents which divide equationally at anaphase I. If compensation does not occur, an aneuploid gamete would be produced and the evolutionary sequence abruptly ended. A gradual increase in the number of bivalents was assumed to occur until all but one of the H chromosomes were involved in pairing. At this time some lagging or ejection mechanism was substituted for the second divison.

Brown (1963, 1964) showed that a mutation "responsible for a certain change in the life cycle of an organism will itself be automatically increased in frequency, with selection and random fluctuations remaining inconsequential". He called this phenomenon *automatic frequency response* (AFR), and suggested that AFR could account for the evolutionary sequence of the chromosome systems in the coccids. Brown assumed that a mutation could appear in the lecanoid system which, when present in the heterochromatic state, causes one or more members of the H set to become euchromatic and pair with their homologues in the E set. This mutant gene will be transmitted to the next generation not only when it is present in the E set, but it will also be transmitted in some of the sperm whenever it is present in the H set. Such a mutation would switch chromosome behavior from lecanoid to Comstockiella, and would increase in frequency until it replaces its normal allele in spite of considerable selection against it (Brown, 1964).

The application of AFR to the lecano-diaspidid evolutionary sequence is limited in at least one important respect: AFR will not occur in a haploid-to-haploid change (Brown, 1963, 1964). According to Brown (1964), the lecano-diaspidid evolutionary sequence (XX—XO, lecanoid, Comstockiella, diaspidid) alternates diploid, haploid, diploid, haploid. However, in the only experimentally analyzed Comstockiella species, *Parlatoria oleae*, the males breed as haploids and not, as Brown (1964) and Nur (1965) suggested, as sub-diploids. There is no conclusive evidence of bivalent formation in any of the other Comstockiella species studied.

If chromosome destruction occurs during spermatogenesis in all species with a Comstockiella sequence, then the lecano-diaspidid evolutionary sequence essentially would consist of a series of modifications of a basic genetic system involving male haploidy. AFR could have acted during the derivation of the lecanoid system from a diploid genetic system similar to those which occur in the genus *Puto* (Hughes-Schrader, 1948; Brown and Cleveland, 1968), which shows the least specialized

chromosome system among the coccids studied, since the change would have been from diploid to haploid. However, after the appearance of the lecanoid system AFR could not play a role in the later evolution of the Comstockiella and diaspidid systems, since all subsequent changes would involve haploid-to-haploid changes. Although some positive selective pressures must have been operative during the transition from the lecanoid to the Comstockiella chromosome system, the reason for this change is still unknown.

A Comstockiella system involving chromosome destruction can easily be derived from the lecanoid system (Fig. 47). Let us assume that a mutation occurred in the euchromatic set which caused the destruction of one or more heterochromatic chromosomes at preprophase. The remaining H chromosomes then divided equationally during the first division and were segregated from the E set in the second division as in the lecanoid system. As the number of H chromosomes remaining at prophase was reduced, a mechanism involving chromosome ejection or lagging was substituted for the second division of the lecanoid sequence. AFR is not involved in this change, since the mutant gene will be transmitted to the next generation only when it is present in the euchromatic set.

In several respects, this model is much simpler than that suggested by Brown (1963). First, a single genetic change would immediately alter a chromosome from lecanoid to Comstockiella behavior. It would not be necessary to assume the development of a complex compensation mechanism, because only univalents divide at anaphase. The replication patterns at spermatogenesis in the Comstockiella system could be identical to those in the lecanoid system. Second, the change from lecanoid to Comstockiella behavior could utilize DNase and various proteases, enzymes which are present in most organisms, to effect chromosome destruction. The problem then is essentially one of packaging and releasing these enzymes in the primary spermatocytes during preprophase, and limiting their action to particular chromosomes.

It should, however, be noted that a Comstockiella system involving chromosome destruction can also be derived from a hypothetical Comstockiella sequence in which chromosome pairing occurs (Kitchin, 1969; Beardsley and Brown, *in preparation*), if, in fact, such species do exist. For example, let us assume that a mutation occurs in a Comstockiella sequence in which chromosome pairing takes place that when present in the E set causes the destruction at preprophase of all heterochromatic chromosomes except the D^H. This mutation would show exactly the same AFR relationship as outlined for a Comstockiella to lecanoid change, and would switch a Comstockiella sequence in which chromosome pairing occurs to one involving chromosome destruction. This gene

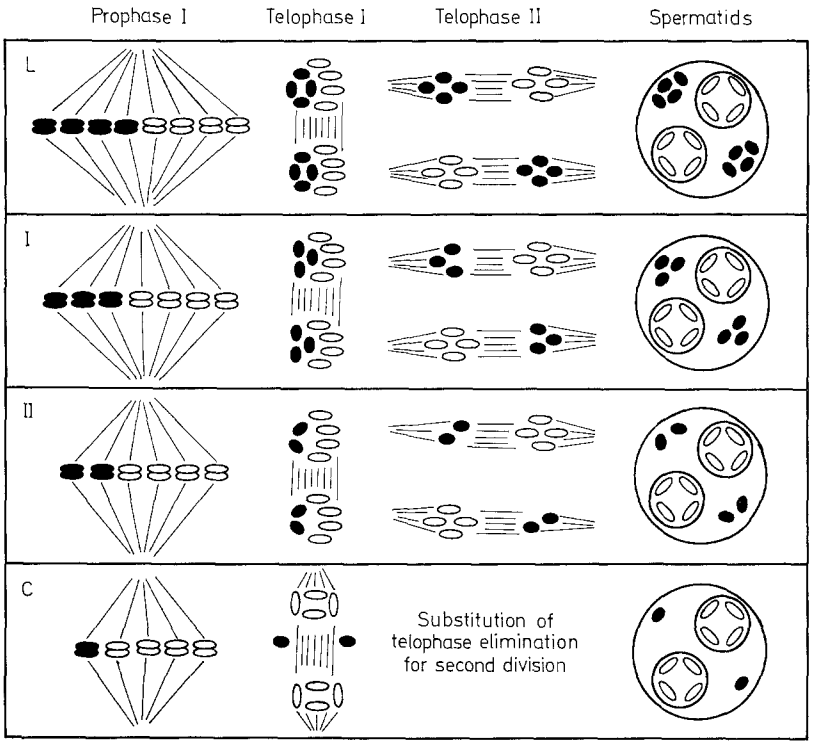


Fig. 47. Diagram of a model for the derivation of a Comstockiella (C) sequence involving chromosome destruction from a lecanoid (L) sequence. During the first step (I), one of the H chromosomes is destroyed at preprophase. The remaining H chromosomes divide equationally during the first division and move away from the E set during the second division of the lecanoid sequence. Since all chromosomes divide equationally in this scheme, compensation is not required. The number of H chromosomes which are destroyed at preprophase gradually increases (II) until only a single H chromosome remains. A true Comstockiella sequence (C) results when the second division of the lecanoid sequence is replaced by some form of telophase elimination. According to this interpretation of spermatogenesis, Comstockiella males breed as haploids and transmit only the members of their maternally derived E set to their offspring

would automatically increase in frequency since it would be present in about half the sperm whenever it is present in the H set, and would be transmitted in all of the sperm whenever it is present in the E set.

The Control of Chromosome Destruction

In *Parlatoria oleae*, both the number of H chromosomes and the particular H chromosomes which are destroyed during spermatogenesis are cyst-specific characteristics; the remaining H chromosomes persist

through spermatogenesis until they are eliminated by post-telophase ejection. At no time were complete Comstockiella cysts with no H chromosomes found, and complete lecanoid cysts with four H chromosomes were rarely observed. Although chromosome aberrations of any size, including even very small fragments, seemed to be capable of remaining heterochromatic through spermatogenesis in the X_1 males, there was no correlation between the size of a rearranged chromosome and the frequency with which it remained heterochromatic during the later meiotic stages. These observations raise several intriguing questions concerning the control of chromosome destruction. First, how are those chromosomes chosen which will be destroyed during spermatogenesis, and when does this selection take place? This process shall be referred to as *determination*. Second, how are the chromosomes differentiated so that only certain H chromosomes are destroyed? The answers to these questions must still be considered highly speculative.

Since each cell in a cyst always showed the same number of H chromosomes at prophase and metaphase, determination probably occurs at least four mitotic divisions before the onset of spermatogenesis, when each cyst is represented by a single gonial cell. Determination may occur relatively early in some species. For example, both lecanoid and Comstockiella systems occur in *Nicholiella bumeliae*, often within the same male (Brown, 1963). Since one testis often consists almost entirely of lecanoid cysts, while the other testis is mostly Comstockiella, determination in *N. bumeliae* may occur very early during the development of the testes, perhaps as early as the two or four cell stage. In *Ancepaspis tridentata* (Brown, 1963) and *Parlatoria oleae*, different classes of cysts could be distinguished by the size of the H residues after telophase elimination. Because the mixture of the different classes of cysts within the testes was completely at random in *A. tridentata* and *P. oleae*, determination in these species probably occurs relatively late during development.

The mechanism of determination is probably quite complex in species like *P. oleae* in which both the *number* and *identity* of the H chromosomes which are destroyed at spermatogenesis may vary from cyst to cyst. Brown (1963) has suggested that controlling elements capable of moving from chromosome to chromosome, similar to those described by McClintock (1956) in maize, may also occur in some Comstockiella species. If the incorporation of a controlling factor at a specific attachment site on a chromosome allows that chromosome to remain heterochromatic through meiosis in *P. oleae*, then there must be at least one, and only rarely as many as four controlling elements in each cell when determination occurs. Furthermore, since even very small fragments could remain heterochromatic, the attachment sites for these controlling

elements must be spread along the length of each chromosome. However, long H chromosomes were rarely present in the X_1 males by prophase of spermatogenesis, in spite of the presumably greater number of attachment sites on these chromosomes, thus controlling factors of this type may not occur in *P. oleae*.

Some structural or chemical differences between the heterochromatic chromosomes may exist which predispose some chromosomes to enzymatic destruction while adjacent ones remain unaffected. On the other hand, the association of a heterochromatic chromosome with the nucleolus or the nuclear membrane may play some role in determination or in the differentiation of the heterochromatic chromosomes.

These observations indicate that a complex mechanism exists for the control of chromosome destruction during meiosis in the males of *P. oleae*. The development of a mechanism for the destruction of chromosomes during spermatogenesis must have been advantageous when some heterochromatic chromosomes are destroyed, but not when all of the heterochromatic chromosomes are destroyed. What advantage chromosome destruction may be to species with a Comstockiella chromosome system is still unknown. Although chromosome pairing does not occur during spermatogenesis in *P. oleae*, pairing may occur in other species with a Comstockiella system. A careful examination of the replication pattern of chromosomes prior to spermatogenesis in *P. oleae* and other species with a Comstockiella chromosome system may resolve the present doubt concerning the pairing interpretation of the Comstockiella system.

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