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Translocations Between Eu- and Heterochromatic Chromosomes, and Spermatocytes Lacking a Heterochromatic Set in Male Mealy Bugs*

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Abstract. In male mealy bugs the chromosomes of paternal origin become heterochromatic (H) early in embryogeny while those of maternal origin remain euchromatic (E). First instar *Pseudococcus obscurus* Essig males which were irradiated with 3000 r of X-rays carried translocations between E and H chromosomes [T(E;H)'s] in their spermatocytes. In the T(E;H)'s the border between the E and H segments was usually quite sharp, but occasionally short E segments may have become either partially or completely heterochromatic. During the second meiotic division the E and H sets normally segregate to opposite poles. The T(E;H)'s often formed bridges in AII and TII, but in most of the cells they did succeed in reaching one of the poles. The segregation of the T(E;H)'s depended on the relative size of their E and H segments. When the E and H segments were of the same size, the T(E;H)'s segregated more often with the E chromosomes, even though the latter have been observed to be attached to their pole with fewer spindle fibers. Thirty-five of the 173 males analyzed had sectors in their testes which lacked an H set. The number of cysts per sector suggested that each sector was derived from a single irradiated cell. The karyotypes observed in some of the sectors indicated that the lack of an H set was the result of reversal of heterochromatization and not due to the loss of the H set and the endoduplication of the E set. Most of the cells lacking an H set divided normally during the first meiotic division. The second division, however, was abortive and resulted in the production of diploid sperm. Two possibilities for the origin of spermatocytes lacking an H set are considered: (i) that these spermatocytes resulted from X-ray induced reversal of heterochromatization in spermatogonia, and (ii) that these spermatocytes originated from presumptive cyst wall cells whose H set had undergone reversal prior to the irradiation.

Introduction

In male mealy bugs with the lecanoid chromosome system, one haploid set of chromosomes becomes heterochromatic (H) in early embryogeny while the other haploid set remains euchromatic (Schrader, 1923; Hughes-Schrader, 1935); in females both sets remain euchromatic (E). Brown and Nelson-Rees (1961) showed that the H set is of paternal origin and that it possesses little or no genetic activity. The lack of genetic activity by the H set was confirmed by Berlowitz (1965) who observed that the synthesis

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of RNA was apparently restricted to the E set. Further confirmation came from Nur (1967a) who found that in some of the tissues of the male the H set may undergo reversal of heterochromatization, and that on its return to the euchromatic state this set resumes its genetic activity. The analysis of interspecific hybrids indicated that the maintenance of the paternal set in the H state and its reversal are under the control of the E (maternal) set.

In spermatogenesis the E and H sets divide equationally during the first, and segregate from each other during the second meiotic division. Following meiosis two of the meiotic products thus contain only E chromosomes and two contain only H chromosomes. The E derivatives form functional sperm and the H derivatives degenerate. The second meiotic division of male mealy bugs was described first by Schrader (1921) and more fully by Hughes-Schrader (1935). Schrader (1923) considered the possibility that during this division only the H set moved to its pole and that the E set stayed behind. Recently Nelson-Rees (1963) demonstrated that the E set also moved actively to its pole.

In the present study translocations were induced between E and H chromosomes by X-ray treatment of first instar males with 3000 r. In the translocations the E and H segments usually retained their identity. The behavior of the translocations indicated that each of the two sets does move actively to its pole during the second meiotic division and that the forces acting on the E set causing it to move to the pole are apparently stronger than those acting on the H set.

Unexpectedly, some of the spermatogonia and spermatocytes of the irradiated males lacked an H set. These cells, which now contained two E sets, underwent meiosis and spermiogenesis and produced diploid sperm. The possible mechanisms through which the irradiation could have led to the absence of the H set are discussed.

Materials and Methods

The culture of *Pseudococcus obscurus* Essig employed was derived from that used earlier (Nur, 1962). Newly laid eggs were collected daily and placed on sprouting potatoes (*Solanum tuberosum* L.) in one pint wide mouth Mason jars. The eggs were kept at 8° C (to arrest embryonic development) until a sufficient number of eggs had been collected. The jars were transferred to 24° C and hatching occurred about 9—11 days later. The potatoes with the larvae were X-irradiated with a dose of 3,000 r on either the 15th day (first series) or on the 18th day (second series) after the jars were placed at 24° C. At these ages all the larvae were still in the first instar. The target distance was about 80 cm. Other conditions of the irradiation were similar to those used in an earlier study (Nur, 1967a).

For cytological analysis late second instar males were fixed in 1 : 3 acetic ethanol. They were stained with aceto-carmine and the testes were then squashed in Hoyer's mounting medium to which a few drops of ferric acetate in propionic acid were added. The material was examined and photographed with phase contrast optics.

Observations

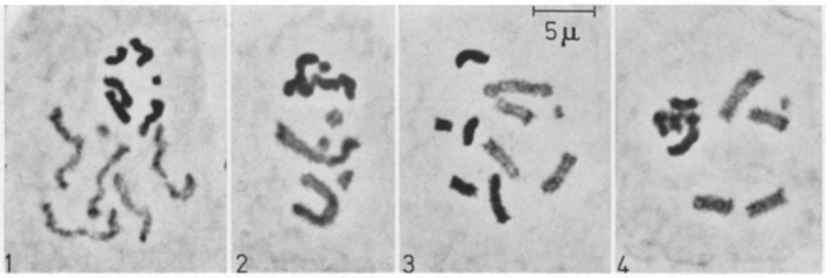
Cysts with Abnormal Karyotypes

The males used in the cytological study came from the first irradiation series. They were irradiated as first instar larvae, 15 days after the eggs had been placed at 24° C. At the time that the males were irradiated, each testis contained about 100 spermatogonia. At the time of meiosis each testis is normally made up of about 150 cysts, each with 16 primary spermatocytes or their division products. Thus, in normal males each of the 100 spermatogonia present in a testis on the 15th day after laying is expected to give rise to one or more whole cysts. In normal males at the end of the second instar some of the cysts are usually undergoing meiosis, some have not yet entered meiosis, and the rest are in various stages of spermiogenesis. Among the 173 irradiated males analyzed, in about 25 males at least one testis was quite small and did not contain any cells in meiosis or later stages. The fate of the small testes is not known, and its is not clear whether the development of these testes stopped completely or only slowed down.

Since in male mealy bugs prophase I is the stage most amenable to cytological analysis, the search for translocations between E and H chromosomes was restriced to this stage. In the treated males approximately half of the cells in prophase I appeared to have the normal karyotype. The normal karyotype consists of five E and five H chromosomes, and the five chromosomes of each set are of about equal length. In other cells, chromosomes were present which were either shorter or longer than the normal chromosomes. For convenience, the former will be referred to as broken chromosomes and the latter as translocations.

Cells with abnormal karyotypes included some with broken E chromosomes, some with broken H chromosomes, and some with both types of broken chromosomes (Fig. 1). There were also cells with translocations between E chromosomes (Fig. 2), between H chromosomes, with both types of translocations (Figs. 3 and 4), and with translocations between E and H chromosomes (Figs. 5—14). Those of the last type will be referred to as T(E;H)'s. Of special interest were cells lacking an H set (Figs. 15—21, 27—31 and 44). These will be described later.

The exact number of cells with a given karyotype could not be determined because most of the cells were usually in stages in which cytological analysis was not possible. A minimum estimate, however, is available for cells with the karyotype in Fig. 2, for those with the T(E;H)'s (Table), and for the cells lacking an H set (to be described later). The rather distinct karyotype of Fig. 2, which included three short and two very long E chromosomes, was present in over 190 cells in prophase I and metaphase I. Since each cyst usually contains 16 primary spermato-



Figs. 1—4. Prophase I. Broken euchromatic (E) and heterochromatic (H) chromosomes, and translocations. ×1,600. Fig. 1. One short E and one short H chromosome. Fig. 2. Two E translocations (long E chromosomes) and three short E chromosomes. Figs. 3 and 4. Two cells from the same cyst with E and H translocations (long E and H chromosomes), and broken E and H chromosomes

cytes, these 190 cells must have represented at least 12 cysts. In addition to these 190 cells, this karyotype was present in many cysts in later stages and it was estimated that cysts with this karyotype made up about half of one testis. Because of the distinctness of this karyotype it is possible to conclude that all the cysts carrying it originated from a single irradiated cell. This observation, and similar ones, suggested that the cysts of a given testis originated from relatively few cells and not from all of the approximately 100 spermatogonia present at the time of the irradiation. Thus, many of the irradiated cells apparently died or gave rise to cysts whose cells later died. Cysts with degenerating cells whose nuclei exhibited heteropycnosis could be observed in the testes of many males and were especially common in the small testes mentioned earlier.

The number of cells with the karyotype in Fig. 2, as well as that of cells with several other karyotypes, was not a multiple of 16. When individual cysts remained intact, however, they always had the same karyotype. This observation, and the relatively small number of cells which gave rise to all the cells in the testes, suggested that each of the surviving irradiated cells gave rise to one or more cysts. The fact that cell counts were often not an exact multiple of 16 probably resulted from cell loss during squashing. For this reason, in all subsequent discussion, the number of cells with a given karyotype will be expressed as the minimum number of cysts which could have given rise to the observed number of cells.

In addition to causing cell death, the irradiation also caused the death of a high proportion of the males. The data suggest, however, that the irradiation had little or no effect on the viability of the females. The frequency of males in the strain employed fluctuates somewhat but is

usually near 50 per cent (Bregman, 1968; Nur, unpubl.). In the irradiated cultures the frequency of males was 7.3 per cent; 426 males were collected as second instars and 5444 females were collected as adults a few weeks later. In order to check what appeared to be a greater mortality of males, the irradiation was repeated. This time, however, it was performed on the 18th, rather than the 15th day after the eggs were placed at 24° C. In this second irradiation series 1,191 males and 4,003 females were collected, giving 23.3 per cent males. In the jars which served as control for the second irradiation series there were 1,357 males and 1,646 females, giving 45.1 per cent males. It is clear, therefore, that the irradiation did cause an appreciable amount of male mortality. Female mortality, if present, was apparently very slight. The eggs placed in each jar were not counted, but an attempt was made to place approximately an equal mass of eggs per jar. The number of females per irradiated jar (392 ± 26; $N = 10$) was not significantly lower than that in the control jars (412 ± 46; $N = 4$). On the other hand, the number of males per irradiated jar (119 ± 9) was much lower than that in the control jars (339 ± 44). The much lower frequency of males in the first irradiation series (7.3 per cent), as compared to the second series (23.3 per cent), resulted at least in part from the difference in age at the time of irradiation. The first series was irradiated when the males were at a younger age, and other observations suggested that the sensitivity of males to irradiation decreases with age. The reasons for the greater sensitivity of the males to the irradiation will be considered in the Discussion.

Euchromatic-heterochromatic Translocations

Translocations between E and H chromosomes, T(E'H)'s, were observed in prophase I cells of six males (Table). The origin of such translocations requires at least two breaks, one in an E and one in an H chromosome, and the subsequent joining of one of the E with one of the

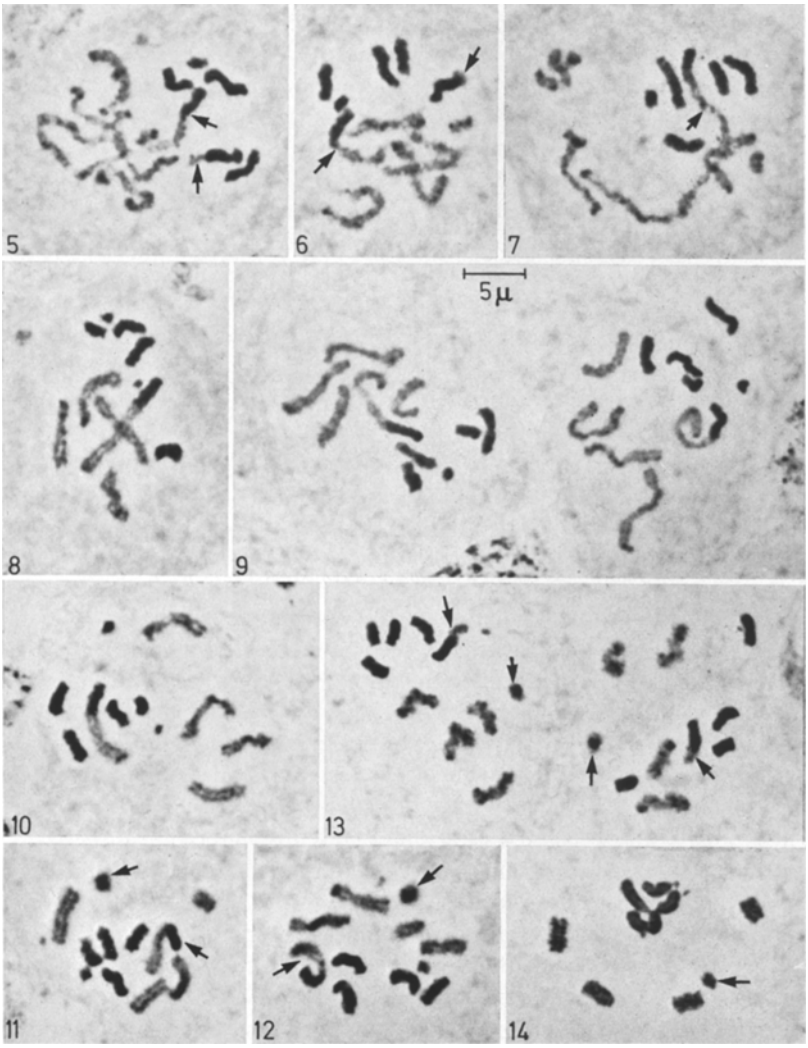
Table. *Data from the six males with translocations between euchromatic and heterochromatic chromosomes [T(E;H)'s]*

Male	Figures	Chromosome elements			Cysts	
		E	H	T(E;H)'s	Prophase	Later stages
1	5—7	4?	4	2	2	
2	8—10, 32—35	4	6	1	8	2
3	11, 12	5	6?	2?	1	2
4	13, 14	4	4	2	1	1
5	36—39	?	5?	1	1	2
6	—	4?	6	1	1	

H segments. The remaining E and H segments could either join also to give rise to a second T(E;H), or should be present in the nucleus as chromosomes which are shorter than the normal chromosomes. Thus, each nucleus with one T(E;H) should contain either a second T(E;H) or at least five E and five H chromosomes in addition to the T(E;H). Fewer E and H chromosomes could be present, however, if some of the chromosomes were lost during the mitoses preceding meioisis.

In each of the six males of the Table, one T(E;H) was readily observed. Careful examination of this material revealed that in Males 1 and 4 these cells apparently contained a second T(E;H) (Figs. 5—7, 13 and 14), and that in Male 3 such a translocation may be present also (Figs. 11 and 12). In Male 5 (Fig. 36) and in Male 6 the exact number of E and H chromosomes could not be determined. In Male 1 a second T(E;H) made up of a long and a short E segment was readily apparent in only one cell (Fig. 5). In a few other cells one end of one of the H chromosomes was more diffuse (Fig. 6) and this end may represent the short E segment of the second T(E;H). In other cells of the same cyst (Fig. 7) the translocated E segment could not be identified readily. It seems reasonable, however, to assume that all the cells did carry the second T(E;H) and that the inability to recognize the short E segment resulted from its precocious condensation. Such precocious condensation, or heterochromatization, of E segments after they are placed adjacent to heterochromatic segments is well known in the polytene chromosomes of *Drosophila* and is believed to be responsible for variegated type position effect exhibited by other cells (Schultz, 1965). Heterochromatization of E segments is also assumed to occur in female mice on the basis of the variegated phenotype produced by certain loci when these are translocated onto the X-chromosome (Russell, 1963).

In Male 4 one short chromosome seemed to be mostly heterochromatic but to carry diffuse material at either one or both ends (Fig. 13). During metaphase I this chromosome was found at the periphery of the metaphase plate together with the E chromosomes rather than at the center, with the H chromosomes (Fig. 14). This arrangement of the E and H chromosomes on the metaphase I plate is typical for males with the lecanoid chromosome system (Hughes-Schrader, 1935). The appearance of the short H chromosome with the diffuse end (or ends) in prophase I and its behavior in metaphase I suggested that it represented a second T(E;H) in which a very short E segment was translocated onto an H segment of about one quarter the size of an H chromosome (Fig. 13). The orientation of the other T(E;H)'s during metaphase I could not be studied because of the lack of material in this stage. In Male 3 the appearance of one of the short chromosomes seemed to be intermediate between that of E and H chromosomes (Figs. 11 and 12); this chromosome may also represent a second T(E;H).



Figs. 5—14. Prophase I and metaphase I. Cells with either one or two translocations between E and H chromosomes [T(E;H)'s]. ×1,600. Figs. 5—7. Male 1. Three cells from the same cyst, with one T(E;H) made up of a large E and a large H segment, and a second T(E;H) whose small E segment is variable in its appearance. Fig. 5. Two T(E;H)'s (arrows). The small E segment of the second T(E;H) (lower arrow) is clearly euchromatic. Fig. 6. The second T(E;H) is not readily identified. Its E segment may be represented by the diffuse end of one of the H chromosomes (upper arrow). Fig. 7. The second T(E;H) cannot be identified. Figs. 8—10. Male 2. Four cells with a T(E;H) in which the E and H segments are about the size of whole chromosomes. Figs. 8 and 10 are of cells from one cyst and Fig. 9 from another cyst of the same testis. In the cells in Fig. 9 the shortest H fragment present in Figs. 8 and 10 is missing. Figs. 11 and 12. Male 3. Two cells from the same cyst in which

The best cytological material was available from Male 2 (Figs. 8—10). In this male only one T(E;H) could be observed even though only four E chromosomes were present. As mentioned earlier, it must be concluded either that in these cells one E chromosome was lost or that one of the H chromosomes actually represented a second T(E;H). The possibility of a loss is strengthened by the observation that a very short H chromosome which was present in seven prophase I cysts from this male (e. g. Figs. 8 and 10) was absent from one cyst (Fig. 9) apparently as a result of a loss. The possibility that one H chromosome was actually the second T(E;H) must also be considered. In Male 2 there were eight cysts in prophase I, and these were unusually favorable for analysis and yet none of the H chromosomes showed any sign of carrying E material. In the long T(E;H) of each of the six males the border between the E and H segments was fairly sharp. By contrast, the appearance of the short E segments in those cells with a second T(E;H) was quite variable. This observation suggested that the E and possibly also the H segments exhibited their characteristic appearance only when they were longer than some minimal size. It is possible, therefore, that a second T(E;H) was also present in the cells of Male 2 but that its E segment could not be observed because it was too short.

The T(E;H)'s were observed in six of the 173 males examined. The relative frequency of males with such translocations was actually higher because the cytology of only about 70 of the males was favorable enough to allow the detection of such translocations. As was mentioned earlier, in about half of the prophase I cysts the cells appeared to have the normal karyotype. In the majority of the remaining cells the karyotype was made up of broken and translocated H chromosomes, and sometimes also broken and translocated E chromosomes. Cells with aberrations only in the E chromosomes were quite rare and in general it appeared that the aberrations were more common in the H than in the E set. The finding of many more cells with translocations in both the H and the E set than cells with T(E;H)'s indicated that the joining of broken ends was much

one T(E;H) is clearly identified (lower arrows). Each cell has one very short E and one short H fragment. The third shortest chromosome (upper arrows) is intermediate in its appearance between the E and H chromosomes and may represent a second T(E;H). Figs. 13 and 14. Male 4. Fig. 13. Two cells from the same cyst each with a T(E;H) which is made up of a short E and a long H segment (Upper left and lower right arrows). Each cell also has a short chromosome (arrows in the middle) which is mostly H but shows some E material at one or both ends. This chromosome probably represents a second T(E;H). Figs. 14. Metaphase I cell from a cyst adjacent to that of Fig. 13 and with the same karyotype. The long T(E;H) behaved like an H chromosome and moved to the center of the metaphase plate. The presumed second T(E;H) is at the periphery with the E chromosomes (arrow), indicating that it carries some E material

more likely to occur between chromosomes from the same set than between those from the two different sets. The simplest explanation for this preference is the spacial separation of the two sets in the nucleus. During interphase the chromosomes of the H set are almost always clumped together near the nuclear membrance, while those of the E set occupy the rest of the nucleus. Another possible explanation for this preferential joining is that broken ends join more readily with other ends with a similar degree of condensation.

The division of the T(E;H)'s and of the cells carrying them during the first meiotic division was apparently quite normal. This division is equational and the formation of anaphase I bridges by the T(E;H)'s was neither expected nor observed. During anaphase II and telophase II, the E and H sets normally segregate from each other. During these stages the T(E;H)'s often formed a bridge between the two sets (Figs. 22 to 26). The bridge sometimes persisted into later stages (Figs. 37—39), but more often the T(E;H) eventually joined either the E or the H chromosomes. The movement of the T(E;H)'s was influenced by the relative sizes of the E and H segments, but the E segments were apparently more effective in causing the T(E;H)'s to move toward their pole than H segments of comparable size. For example, in the T(E;H) of Male 2 in which the E and H segments were each of about the size of a normal chromosome (Figs. 8—10), the T(E;H) segregated more often with the E than with the H set. In a group of cells in post-telophase II the segregation of the T(E;H) was as follows: The T(E;H) moved with the E chromosomes in five cells (e. g., Fig. 32); with the H chromosomes in two cells (E. g., Fig. 35); with all the E and one H chromosome in two cells (Figs. 33 and 34); and with all the E and two H chromosomes in two cells. In one cell the T(E;H) moved together with five of the H chromosomes but one H chromosome moved with the E chromosomes. In another cell three E and four H chromosomes moved to one pole and one E chromosome, two H chromosomes, and the T(E;H) moved to the other pole. Excluding the last cell, the T(E;H) moved together with the E chromosomes in 9 out of 12 cells. In 13 other cells from the same group the E and H chromosomes were very near each other, probably as a result of the bridge fromed by the T(E;H); these cells could not be classified. The T(E;H) of Male 2 was apparently the only translocation which tended to upset the segregation of E and H chromosomes. In cells with the other T(E;H)'s individual E or H chromosomes were never observed to segregate with the opposite group.

In the T(E;H) of Male 5 (Fig. 36), in which about half of an H chromosome was translocated to what appeared to be a segment of at least the size of a whole E chromosome, the T(E;H) almost always segregated with the E chromosomes. For example, of the thirteen pairs of post-

telophase II nuclei illustrated in Fig. 39, the T(E;H) segregated with the E chromosomes in ten pairs, with the H chromosomes in two pairs, and kept the two nuclei near each other in one pair.

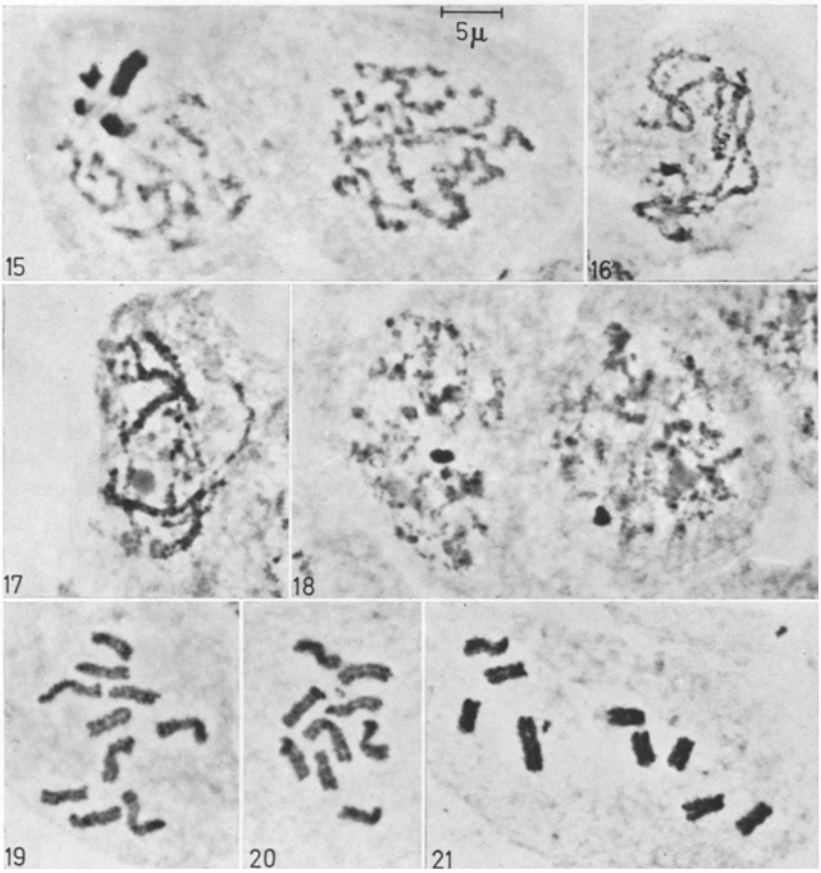
In coccids, telophase II is not followed by cytokinesis. Instead, the two products of a given primary spermatocyte normally fuse to form a quadrinucleated cell with two E and two H nuclei (Hughes-Schrader, 1935). In some of the irradiated males the fused post-telophase II cells contained either only two or only three nuclei, and in these cells one or two of the nuclei contained both the E and the H set. Most of the E + H nuclei were apparently restitution nuclei which resulted from persisting bridges formed by the T(E;H)'s. The involvement of the T(E;H)'s in the formation of E + H spermatids may be seen from Fig. 40, in which next to an E + H nucleus (upper right) there is one H nucleus and an elongating E nucleus containing in H segment (Arrow). The latter is almost certainly part of a T(E;H) which apparently succeeded in moving toward one of the poles in one of the secondary spermatocytes but not in the other.

In normal, quadrinucleated, post-telophase II cells only the E nuclei elongate and later transform into sperm. In a few cysts, however, some of the H nuclei were also seen to elongate (Figs. 42 and 43, arrows). The reason for this abnormal behavior is not clear. It is tempting to speculate that it was the result of the presence of the E segment of a T(E;H) which segregated together with the H chromosomes.

Nuclei Lacking an H Set

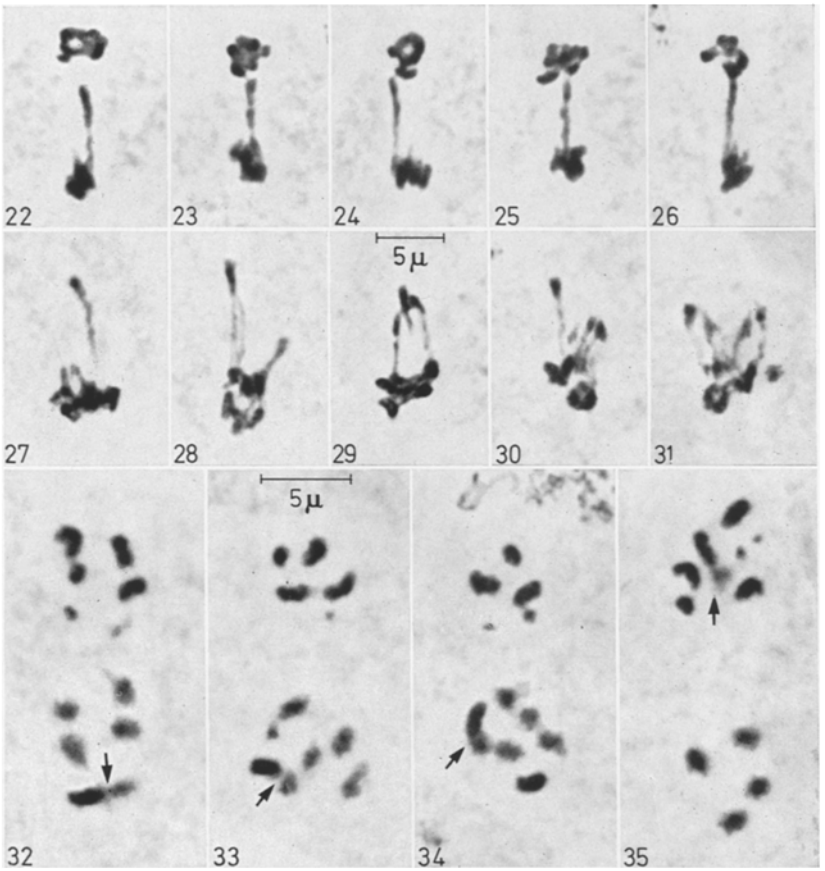
As was mentioned earlier some of the nuclei in premeiotic interphase as well as some of those undergoing meiosis and spermiogenesis lacked an H set (Figs. 15—21, 27—31, and 44). In prophase I and metaphase I these nuclei had either the normal diploid karyotype of 10 E chromosomes or a karyotype which could be derived from it by one or more breaks. In many of these cells the presence of two E sets must have been the result of the return of the H set to the E state and not due to the loss of the H set and the endoduplication of the remaining E set. Endoduplication could be ruled out for those prophase I cells whose karyotype included chromosomes which were either shorter or longer than the normal chromosomes. Such cells had either one long and one short chromosome (Figs. 20 and 21) or only one short chromosome; two of each kind would be expected if the E set had duplicated by endomitosis. The karyotype of Figs. 20 and 21 was observed in one cyst, and another cyst had a similar karyotype. In addition, in several other cysts only a single short chromosome was present.

In a few cysts which were either in interphase or in very early prophase the chromosomes exhibited chromomeres and each chromosome was clearly made up of two subunits. Two of these cysts apparently contained



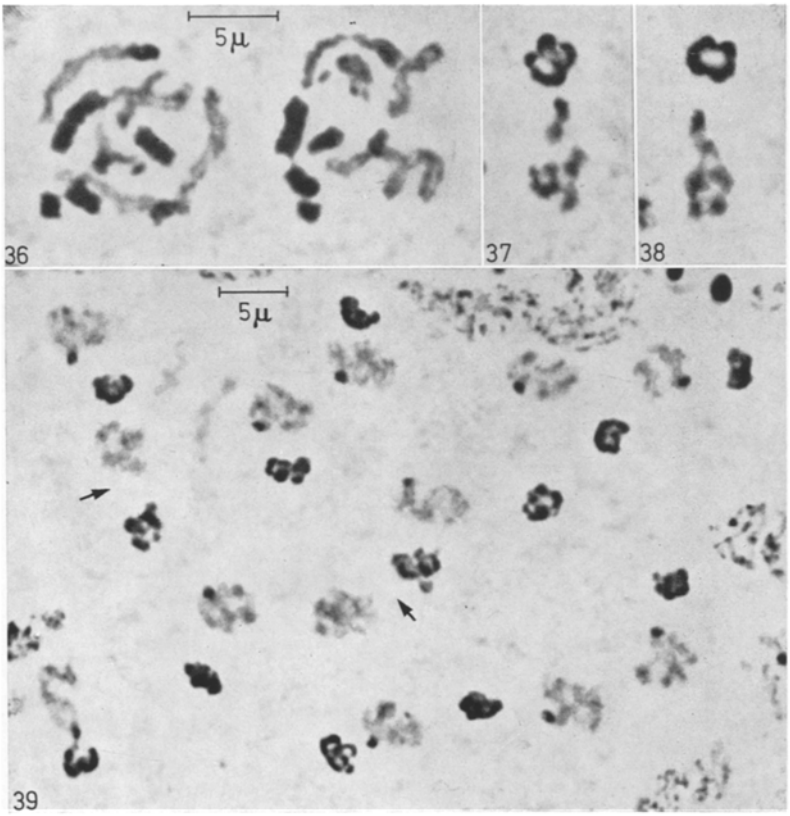
Figs. 15—21. Cells lacking an H set. ×1,600. Fig. 15. A cell with an H set and a cell lacking it. The very long H chromosome in the cell at left resulted from at least two translocations between H chromosomes. Fig. 16. A cell which appears to have only 5 E chromosome elements; one 8-shaped and one V-shaped above, one below, and two stretched between those above and below. Notice the chromomeres, and the clear subdivision of each chromosome element into two subunits. Fig. 17. A cell with paired and unpaired E segments. Fig. 18. Cells with an H chromosome segment. Fig. 19. Ten E chromosomes. Figs. 20 and 21. Two cells from the same cyst with nine E chromosomes and one E fragment. The long E chromosome represents a translocation between E chromosomes. The fragment in the upper right corner in Fig. 21 is from another cell

only five E elements (Fig. 16). In these cysts the H chromosomes were either lost, or had undergone reversal of heterochromatization which was followed by pairing between the homologues. The possibility that pairing may have been involved was supported by the observation that in several cysts the chromosomes exhibited chromomeres but were not paired, and



Figs. 22—35. Anaphase II and Post-telophase. Figs. 22—31. Anaphase II ×1,800. Figs. 22—26. Cells from the same cyst, each with a bridge formed by a T(E;H). The H set above and the E set below. Figs. 27—31. Cells lacking an H set and exhibiting bridge-like projections. All from the same cyst, and from the same testis as Fig. 2. Figs. 32—35. Male 2. Post-telophase II. Segregation of theT(E;H) (arrows) of Figs. 8—10. Most of the H chromosomes above and the E below. ×2,400. Fig. 32. The T(E;H) segregated with the E chromosomes. Fig. 33. The T(E;H) and one short H chromosome segregated with the E chromosomes. Fig. 34. The T(E;H) and one H chromosome segregated with the E chromosomes. Fig. 35. The T(E;H) segregated with the H chromosomes

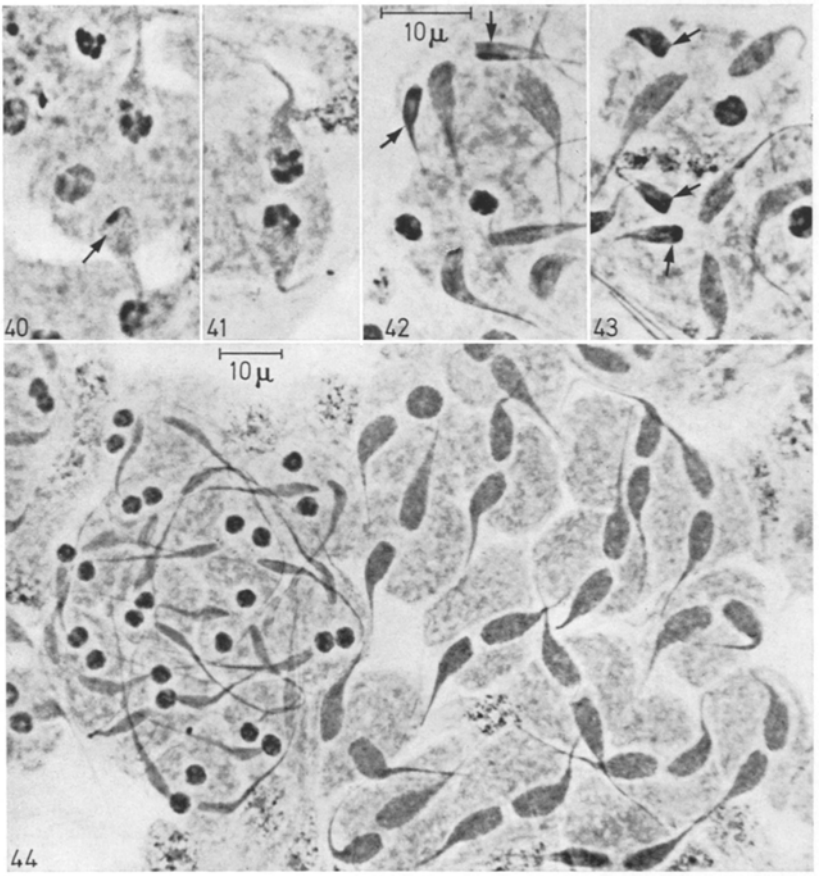
that in others, both paired and unpaired chromosome segments were present (Fig. 17). As will be discussed later, the size and the distribution of sectors lacking an H set suggested that most of these sectors were derived from a single irradiated cell. In several males which possessed cells lacking an H set, cells with chromosomes exhibiting distinct chromomeres were present in the same area of the testis with cells lacking an H set which



Figs. 36—39. Male 5. A T(E;H) and its behavior. Fig. 36. The T(E;H) (above) is made up of approximately one whole E chromosome and about one-half of an H chromosome. ×2,400. Figs. 37 and 38. Post-telophase II. ×2,400. The T(E;H) is situated between the H set (above) and the E set (below). Fig. 39. Post-telophase II. Preferential segregation of the T(E;H) into the E products. ×1,800. The T(E;H) formed a bridge between the E and H products in one cell (lower left); segregated with the E set in ten cells, and segregated with the H set in two cells (arrows). In those cells in which the T(E;H) segregated with the E set, the H segment can be recognized as a dark dot

were undergoing meiosis and spermiogenesis. This observation suggested that the two types of cells may have been derived from the same irradiated cell.

In general, in any given cyst the whole H set was either present or absent. In one cyst, however, only one short H chromosome or chromosome segment was present (Fig. 18). The same testis also contained several cysts lacking the H set entirely. The possible origin of the short H chromosome will be considered in the Discussion.



Figs. 40—44. Elongating spermatids. Figs. 40 and 41. Five spermatids (two above and one below in Fig. 40 and two in Fig. 41) with both E and H chromosomes. ×1,200. These spermatids apparently resulted when a T(E;H) formed a bridge in AII. Same male as the AII bridges of Figs. 22—26. The T(E;H) managed to segregate into the lower spermatid in Fig. 40 (arrow) and may be recognized by the dark dot representing the H segment. Figs. 42 and 43. Quadrinucleated cells in which some of the H products started to elongate (arrows); presumably because of the presence of the E segment of a T(E;H). ×1,200. Fig. 44. Part of a normal cyst (left), and of two cysts lacking an H set (right). ×800. Notice that in the two on the right the spermatids are larger (diploid) and that the round H products are absent

Most of the cysts lacking an H set proceeded through meiosis and completed spermiogenesis regularly but apparently more slowly than the normal cysts. All the cells lacking an H set which entered meiosis had two E sets. They divided normally during the first meiotic division but

their second meiotic division was usually abortive. During anaphase II and telophase II the chromosomes were usually clumped together closer to one side of the cell but formed bridge-like projections toward the other side of the cell (Figs. 27—31). During sperm elongation each cell contained only two nuclei (Fig. 44), instead of the expected four, indicating that the second meiotic division was abortive and that the bridge-like projections did not lead to the segregation of any of the chromosomes away from the rest. The elongating spermatids of cysts lacking an H set were about twice as large as normal, haploid, spermatids. This greater size was clearly the result of the failure of the second meiotic division and the inclusion in each spermatid of two sets of chromsoomes. The fate of these diploid spermatids is not known.

Of the 173 males examined cytologically, 35 contained one or more cysts with cells lacking an H set. In almost all these males these cysts were present in only one of the two testes. Four males had one such cyst; six males had two cysts; three males had 3—5 cysts; eight males had 6—10 cysts; and 13 males had more than ten of these cysts. The frequencies of the cysts resembled those of the cysts with the various T(E;H)'s (Table). This similarity suggested that all the cells lacking an H set in a given testis usually originated from a single irradiated cell. The mechanism by which the irradiation may have led to the presence of cells lacking an H set will be considered in a later section.

Discussion

Differential Sensitivity to X-rays

Earlier in this report it was shown that the males were much more sensitive to the X-irradiation than the females. The simplest explanation for the greater sensitivity of the males to irradiation is that the X-rays produced many recessive lethal mutations. The males, with only one active (E) set in many of their cells, are expected to be sensitive to these mutations while the females with two active sets are not. The assumption that the X-rays produced many recessive lethals, however, seems to be at variance with the finding of Brown and Nelson-Rees (1961). They irradiated adult *Planococcus citri* females with doses ranging from 1,000 to 8,000r and observed that while the survival of both the sons and the daughters decreased with dose, there was very little difference in the survival of the two sexes. This lack of difference may be interpreted to mean that recessive lethals did not make a large contribution to the mortality of the sons. The differences between the two results may be explained, at least in part, by the differences in the types of cells, which were irradiated, i. e., oogonia and oocytes in adult female versus somatic and germ cells in first instar larvae.

The Nature of the Lecanoid Second Division

As was mentioned in the Introduction, Schrader (1923) considered the possibility that during anaphase II (AII) the E set was passive and that the H set moved away from it. Recently, however, Nelson-Rees (1963) showed that in living cells the E set did move actively to its pole, but that in fixed and stained material the E set was attached to its pole with fewer spindle fibers. He also showed that following irradiation, bridges were present in both AI and AII, and that some of the spermatids contained both E and H chromosomes. Nelson-Rees interpreted the AII bridges to be translocations between E and H chromosomes and concluded that the formation of these bridges indicated an active movement by both the E and the H chromosomes.

The present study demonstrated more clearly the production of such translocations [the T(E;H)'s]. The observation that the T(E;H) of Male 2, which was made up of an E and an H segment of about equal length, segregated in most cases with the E set was quite unexpected. It indicated that the forces bringing about the movement of the E chromosomes are apparently stronger than those acting on the H chromosomes, even though the E chromosomes are apparently attached to their pole with fewer spindle fibers.

The position of the T(E;H)'s during AII and TII seemed to depend on the sum of the forces operating on each T(E;H). When the forces operating on both the E and the H segment were sufficiently strong to form a bridge the final segregation apparently depended also on the relationship between the T(E;H) and the other chromosomes. At the end of TII, when the forces stretching the bridge disappeared, the stretched T(E;H) apparently contracted and then often withdrew into either the E or the H nucleus, depending on how strongly it was "stuck" to the E and the H chromosomes.

Cells with an H Segment

Reversal usually affected all the H chromosomes of a given cell. In one cyst, however, all the cells had only one H element, which was shorter than other H chromosomes. This H element will be referred to as the H segment. The exact origin of the cyst carrying the H segment is not known, but since it was found adjacent ot several cysts whose cells lacked the H set completely, it probably originated from the same cell as the adjacent cysts. The presence of the H segment can be explained in at least four different ways. 1) The H chromosomes of this cyst were lost and only part of one H chromosome remained. 2) The H set was either lost or had undergone reversal; a short H segment (telomere ?), however, replicated several times to give rise to the observed H segment. 3) In the cell giving rise to this cyst the H set had undergone reversal, except for

one H segment which failed to do so. 4) The cells with the H segment originated from a cyst wall cell which already lacked an H set. In this cell one chromosome segment became heterochromatic, apparently as a result of the irradiation.

In considering the merits of the first two explanations, it may be pointed out that in the present study there was no evidence that the irradiation caused the loss of either whole, or broken H chromosomes except for a few very short H chromosomes (fragments). The loss of such fragments however, could not have been detected unless the loss occurred in only some of the cells. Indeed, such a partial loss was observed in Male 2.

Extra replication of only part of an H chromosome is not known from mealy bugs but was reported from tobacco where it gave rise to long H segments (Gerstel and Burns, 1967). The third explanation, if true, would imply that as a result of the irradiation a segment of an H chromosome somehow lost the ability to undergo reversal. The possibility raised in the fourth explanation that spermatocytes may originate from cyst wall cells, will be discussed later. So far, H segments have never been observed following the irradiation of females, or of those male tissues whose cells lack an H set. The absence of such H segments tends to favor the first three explanations.

Cells Lacking an H Set

As was mentioned earlier,in some of the nuclei which lacked an H set the chromosomes exhibited chromomeres and a clear division into two chromatids. Such nuclei were never observed in mealy bug males with the lecanoid chromosome system. Cysts with such nuclei, however, were observed in the eriococcid, *Gossyparia spuria*, whose males also possess an H set but whose spermatogenesis is of the Comstockiella type (Nur, 1967b). In that species, as well as in the present study, these cysts were observed in males of various stages, including those in which all the other cysts had already completed meiosis. It may be concluded, therefore, that at least some of these cysts always remained in the same stage and did not undergo meiosis.

All the cells lacking an H set which underwent meiosis had two E sets. In those cells with chromosome aberrations each particular aberration was represented only once, ruling out the possibility that in these cells the H set was lost and that the diploid condition was restored by the endoduplication of the remaining E set. It was concluded, therefore, that the lack of an H set resulted from the reversal of heterochromatization. Since such sectors were never observed among more than a thousand non-irradiated males of this species, it may be safely concluded that the presence of spermatocytes lacking an H set was caused by the irradiation. Two ex-

planations for the lack of the H set will be considered: (i) that the spermatocytes lacking an H set originated from other cells whose H set had undergone reversal prior to the irradiation, and (ii) that the irradiation caused the reversal of the H set in some of the early spermatogonia.

Origin of the H-lacking Spermatocytes from Presumptive Cyst Wall Cells

At the beginning of meiosis the cells of the testes of male mealy bugs are organized into cysts. In *P. obscurus* each cyst contains sixteen primary spermatocytes and is surrounded by eight cyst wall cells which lack an H set. The presence of eight cyst wall cells per cyst and the absence of the H set were first reported for the mealy bug *Planococcus citri* (Nur, 1967a). In that report it was stated that at the time of hatching the testes seemed to contain only cells with an H set. Recently, however, Mr. Chung-chia Huang (personal communication) observed that in newly hatched *P. obscurus* males each testis contained several cells lacking an H set in addition to at least 11 cells with an H set. The exact number of the former could not be established but there were fewer cells lacking an H set than those having it. Since the cyst wall cells also lack an H set, it seems reasonable to assume that the cells lacking an H set present in the testes of newly hatched males were presumptive cyst wall cells. The observation that at the time of irradiation cells lacking an H set were present in the testes raises the possibility that the irradiation may have caused some of these cells to give rise to the spermatocytes lacking an H set.

A different origin for the cyst wall cells was suggested by Węglarska (1966 and 1968), on the basis of her studies of another coccid, the armored scale insect *Quadraspidiotus ostreaeformis*. She reported that in the males of this species there were sixteen primary spermatocytes and sixteen cyst wall cells, and that these two cell types originate from sixteen spermatogonia, as a result of the last mitosis preceding meiosis. Such an origin, however, cannot apply to *P. obscurus* and *Planococcus citri* since in both species there are sixteen primary spermatocytes, but only eight cyst wall cells. Węglarska's suggestion about the origin of the cyst wall cells also does not apply to all armored scale insects, since cell counts in the testes of the olive scale, *Parlatoria oleae* (Colvée), indicated that in this species there are sixteen primary spermatocytes per cyst but only four cyst wall cells.

X-ray Induced Reversal of Heterochromatization

According to the second explanation the irradiation caused the H set of some of the spermatogonia to revert to the E state. The direct effect of the irradiation, however, may have been on the E set, and the rever-

sal of heterochromatization by the paternal set may have been only an indirect result of the irradiation. This possibility follows from the observations, to be discussed next, that the maintenance of the paternal set in the H state is under the control of the E set.

In haplo-diploid male mosaics whose haploid sector is of paternal origin, the chromosome set of the haploid nuclei first becomes heterochromatic and then undergoes reversal (Chandra, 1963; Nur, 1962). The initial heterochromatization of the paternal set may be attributed to a "heterochromatizing substance" which was produced prior to fertilization by those eggs which later develop into males. The subsequent reversal of the paternal set in the haploid sector has been attributed to the lack of an E set, and it was concluded that the maintenance of the paternal set in the H state is under the control of the E set (Brown and Nur, 1964). The control of the H set may also be attributed to a "heterochromatizing substance", which may or may not be the same as that produced by the egg.

Similar conclusions about the role of the E set may be reached from the study of interspecific hybrids. In the mealy bug *P. obscurus* the H set undergoes reversal in a group of embryonic cells at about the time that these cells differentiate into the serosa (Nur, 1967a; Bregman, 1968); in *P. gahani* the cells forming the serosa do not undergo such a reversal. In the hybrid formed by the mating of *P. gahani* males with *P. obscurus* females, the paternal set in the serosa does undergo reversal. Since in the paternal species (*P. gahani*) the H set does not undergo reversal in these cells, the paternal set could not have supplied the information for its own reversal and this information must have been provided by the E set. These results also indicate that the maintenance of the H state is under the control of the E set.

The observations which have just been reviewed indicate that the H set is under the control of the E set, and it seems reasonable to assume that this control is mediated by a heterochromatizing substance (or substances). One possible explanation for the X-ray induced reversal would thus be that the irradiation damaged either the locus specifying the structure of this substance or a locus controlling its production. Other possible explanations may include an indirect effect of the irradiation on the level of this substance, the induction of a substance causing reversal, as well as others not directly involved with such substances. Since there is no evidence indicating that the irradiation did cause the reversal of the H set, the various explanations of how such a reversal could have come about will not be discussed.

At present both explanations for the origin of the spermatocytes lacking an H set, e. g. origin from presumptive cyst wall cells, and X-ray induced reversal, seem to be equally successful in explaining the

observed results. Until additional information on these cells is available, however, it is not possible to favor one over the other.

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