REVERSAL OF HETEROCHROMATIZATION AND THE ACTIVITY OF THE PATERNAL CHROMOSOME SET IN THE MALE MEALY BUG[1]

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IN male mealy bugs with the lecanoid chromosome system, one haploid set of chromosomes becomes heterochromatic early in embryogeny, whereas both haploid sets of female nuclei remain euchromatic. The heterochromatic set was first described by SCHRADER (1921). Later, HUGHES-SCHRADER (1935) showed that this set was not transmitted by the male to its offspring. SCHRADER and HUGHES-SCHRADER (1931) suggested that the heterochromatic set was inactive genetically and HUGHES-SCHRADER (1948) suggested that it was of paternal origin. BROWN and NELSON-REES (1961) analyzed chromosomes for aberrations in embryos which developed after paternal and maternal irradiation and demonstrated that the heterochromatic set was indeed of paternal origin. They also found that, after maternal irradiation of 8000r, very few sons and daughters survived. On the other hand, after paternal irradiation with up to 30,000 rep, all the zygotic daughters died, but all the sons survived. The death of the daughters after either maternal or paternal irradiation must have resulted from the induction of dominant lethals in the irradiated chromosome set. The survival of sons after paternal irradiation with up to 30,000 rep indicated that the induced dominant lethals were not expressed. BROWN and NELSON-REES' interpretation of these results was that the paternal set was genetically inactive when it was in the heterochromatic state.

However, following paternal irradiation with doses above 30,000 rep, the survival of the sons declined sharply, suggesting some residual activity of the heterochromatic (H) set. This residual activity was studied further by NELSON-REES (1962) who showed that the sons surviving high doses of paternal irradiation were sterile. He also showed that at doses above 30,000 rep the irradiated paternal set was highly rearranged and contained fragments and translocations. Loss and nondisjunction of the irradiated chromosomes caused the amount of heterochromatic chromosomal material to vary greatly in young embryos, and only male embryos possessing an approximately normal bulk of the heterochromatic material survived. NELSON-REES's results suggested that the H set performed a function which was essential for male fertility and that the residual activity of the H set expressed itself as a bulk requirement for the H set.

Another observation which suggested that the H set may be genetically active

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came from the study of interspecific hybrids (NUR and CHANDRA 1963). In three interspecific crosses in which male and female embryos were produced, all the offspring died before or soon after hatching. The death of the male embryos was taken as an indication that the paternal set in the male embryos was at least partially active. If the paternal set were completely inactive genetically, the hybrid males would be expected to survive and to resemble the males of the maternal species.

It was previously reported that the cells in several tissues of the male mealy bug did not have an H set (BROWN and NUR 1964). In this paper observations will be presented which indicate that the absence of the H set in these cells resulted from the return of the paternal chromosomes to the euchromatic state. The return of H chromosomes to a euchromatic state was probably first observed in the X chromosome of short-horned grasshoppers. In this group the X chromosome appears positively heteropycnotic, or heterochromatic, in prophase I of spermatogenesis (WHITE 1954); whereas it appears isopycnotic, or euchromatic, in the nuclei of female embryos following fertilization.

In coccids the return of the H set to a euchromatic state was previously referred to as deheterochromatization (CHANDRA 1963b), euchromatization (NUR 1966), and reversal of heterochromatization (BROWN and NUR 1964). In this paper the last term will be shortened and this process will be referred to as *reversal*. It will be shown that most, if not all, the functions previously attributed to the H set are the result of the genetic activity of the paternal set in those tissues in which the H set has undergone reversal.

MATERIALS AND METHODS

The *Planococcus citri* (Risso) (Homoptera: Coccoidea) culture used in this study was derived from that used by BROWN and NELSON-REES (1961) and NELSON-REES (1962). The culture was made available by DR. S. W. BROWN, to whom I am very grateful. The cultures of *Pseudococcus gahani* Green and *Pseudococcus obscurus* Essig were those previously used for interspecific hybridization by NUR and CHANDRA (1963). Material for cytological analysis was fixed in Bradley-Cornoy: 4 chloroform : 3 ethanol : 1 glacial acetic acid. Embryos were strained and squashed in aceto-carmine. First, second, and third instar males were stained in HCl-carmine (SNOW 1963). In the preparation of slides, the testes, the digestive tract, and the Malpighian tubules were dissected out and squashed in Hoyer's mounting medium. In those experiments which involved paternal irradiation, adult males were given a dose of 60,000r with a Picker X-ray machine at 15 ma and 250 kv, with a 0.5 mm aluminum filter and at a target distance of 17.5 cm. The irradiation was performed by MRS. FLORENCE VAN SLYKE of the Department of Radiation Biology. Her cooperation is gratefully acknowledged.

OBSERVATIONS

Reversal of heterochromatization: In male mealy bugs with the lecanoid chromosome system the euchromatic (E) and the heterochromatic (H) sets segregate from each other during meiosis and only the meiotic products with the E set form sperm (HUGHES-SCHRADER 1935). Thus, the chromosome set which is contributed by the sperm is euchromatic. In those eggs which are going to develop into males, the paternal set becomes heterochromatic shortly after the cleavage

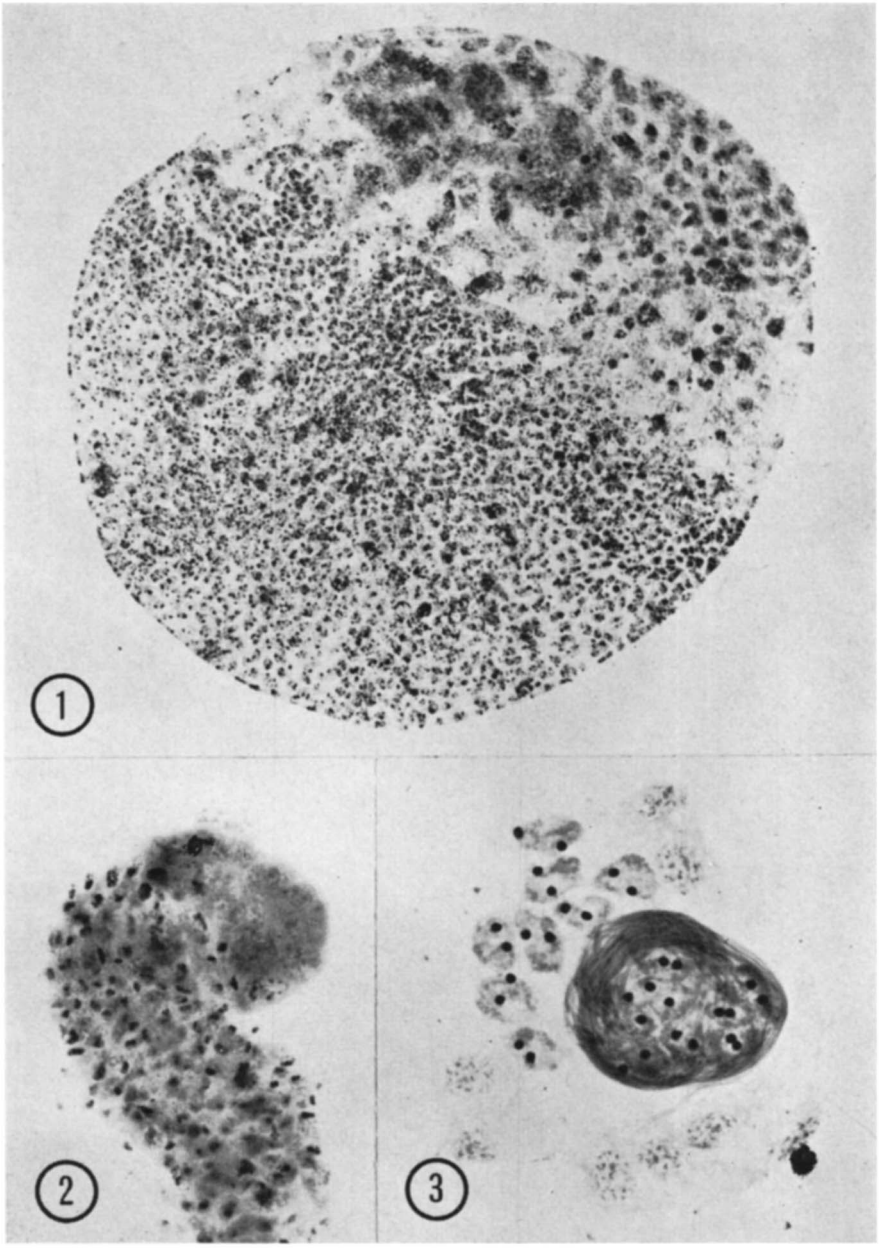
nuclei migrate from the center of the egg to the surface. SCHRADER (1923b) described the migration of the cleavage nuclei to the surface as taking place after the fifth cleavage. In this study the paternal set was first observed to be heterochromatic in embryos with 64 nuclei, i.e., after the sixth cleavage.

In males of the mealy bug *Planococcus citri* cells of the following tissues have been found not to have an H set: The yolk cells in the embryo, the mycetocytes, some of the oenocytes (NUR 1966), some of the cells of the skeletal muscles, some of the cells of the intestinal tract, the cells of the Malpighian tubules, and the cyst wall cells of the testes. In males of other mealy bug species, some of the serosa cells, the testis sheath cells, the cells of the auxiliary glands of the testes and the cells of the hypodermis may also lack an H set.

At present it appears that with the possible exception of the mycetocytes, the lack of an H set in male tissues is the result of the reversal of heterochromatization in the paternal H set. The mycetocytes form the mycetome, house intracellular symbionts, and do not have an H set. Their origin in *Pl. citri* and several other species as worked out by SCHRADER (1923b) is as follows. After fertilization the diploid polar body I fuses with the haploid polar body II to form a triploid polar nucleus. The polar nucleus usually divides two or three times at the surface of the egg prior to the migration of the cleavage nuclei to the surface. The derivatives of the polar nucleus then fuse with each other and also with some of the blastoderm cells, giving rise to the mycetocytes. The fusion of the blastoderm cells with the polar nucleus derivation seems to occur after the heterochromatization of the paternal set in the blastoderm cells. However, it is not clear whether this heterochromatization also occurs in the blastoderm cells which undergo fusion.

When reversal takes place in some of the cells of a given tissue it usually occurs in these cells at about the same time. However, in cells of different tissues the H set may undergo reversal at different times. The first cells in which reversal was observed were the yolk cells and the serosa cells. The yolk cells originate from cleavage nuclei which do not migrate to the surface of the egg (SHINJI 1919; SCHRADER 1923a). In *Pl. citri* the yolk cells of male embryos in early stages of blastoderm formation did have an H set, while those in late stages of blastoderm formation did not. As there was no indication that during this time interval the H set was eliminated, one may conclude that it had undergone reversal.

At the time of gastrulation, some of the cells are left at the surface of the egg and make up the outer embryonic membrance, the serosa. In *Pl. citri* and several other species the cells of the serosa have a typical H set. In *Pseudococcus obscurus* and a few other species the majority of the cells of the serosa do not have an H set. The serosa cells in *Ps. obscurus* originate from those blastoderm cells which are near the polar bodies and their derivatives. By the use of a supernumerary chromosome, it was possible to show that in this species the derivatives of the polar bodies contributed few if any cells to the serosa. In a cross between a female with one supernumerary and a male without supernumeraries, the triploid polar nucleus always receives one supernumerary from the diploid polar body I. The zygote, on the other hand, may or may not receive a supernumerary, depending



FIGURES 1 to 3.—Tissues lacking a heterochromatic (H) chromosome set in male mealy bugs. FIGURE 1.—A hybrid male embryo from the cross *Ps. obscurus* × *Ps. gahani* in an advanced stage of blastoderm formation. 150 ×. In this embryo, as in male embryos of *Ps. obscurus*, most of the large serosa cells (above and right) lack an H set. In the small blastoderm cells (below and left), the darkly straining bodies represent the H set. FIGURE 2.—Cells lacking an H set at the caudal tip of a *Pl. citri* embryo. 600 ×. The embryo is at a stage in which the appendages are partially formed. The cells at the caudal tip later contribute to the formation of the midgut. FIGURE 3.—A cyst from the testis of a third instar *Pl. citri* male. 600 ×. The cyst is made up of

on whether the supernumerary segregates into the egg pronucleus or into polar body II (NUR 1962b). Examination of embryos from such a cross indicated that, in embryos whose germ band nuclei did not have a supernumerary, the serosa nuclei also did not have a supernumerary. Thus, the serosa cells are almost certainly of zygotic origin. At the time of their reversal, the future serosa cells are situated about midway between the anterior and posterior poles. Later, these cells move together along the surface of the egg toward the anterior pole and form a cap over that pole (Figure 1). In the course of gastrulation, the serosa expands and covers the entire surface of the embryo.

The next tissue to undergo reversal is the midgut rudiment. At the time when the appendages first appear the midgut rudiment consists of a group of cells situated at the caudal tip of the embryo. After the appendages have increased in size, the H set in the cells of the midgut rudiment undergoes reversal (Figure 2). Later. this group of cells participates in the formation of the midgut (SHINJI 1919; SCHRADER 1923a). The H set of the cells which give rise to the Malpighian tubules also undergoes reversal prior to hatching (Figure 8).

At the time of hatching each testis is made up of only a few cells, all of which seem to have an H set. By the time of mid-second instar the testes are organized into cysts and each cyst is surrounded by eight cyst wall cells. The cyst wall cells at this stage and all subsequent stages lack an H set (Figure 3); their H set must have undergone reversal during either first or early second instar.

Some of the cells of the hindgut undergo reversal between hatching and the end of the second instar. At the time of hatching all the cells of the hindgut are small and each have an H set. But by the end of the second instar some of the large cells of the rectum lack an H set.

The exact time of reversal in other tissues is not known. For some of these tissues there is also species to species variation in the types of cells in which reversal may occur. For example, in *Phenacoccus gossypii* reversal occurs in all the testis sheath cells. In *Ps. obscurus* it occurs in only the large testis sheath cells; and in *Pl. citri* it does not occur in any of the testis sheath cells (NUR 1966). In these cells, however, the H set does not replicate while the E set undergoes several cycles of endoreplication. In the majority of the oenocytes of *P. citri* the E and H sets behave similarly, while in a few of the oenocytes the H set does undergo reversal (NUR 1966).

Reversal often seems to occur in diploid cells which later undergo endopolyploidy. However, endopolyploidy apparently does not always follow reversal. For example, the cyst wall cells are relatively small and may be diploid. Reversal usually does not take place in the cells of the hypodermis. However, in *Rhizoecus falcifer* reversal does take place in these cells prior to hatching (NUR 1962a). Yet these cells remain small and are probably diploid.

It was previously suggested that in tissues undergoing endopolyploidization

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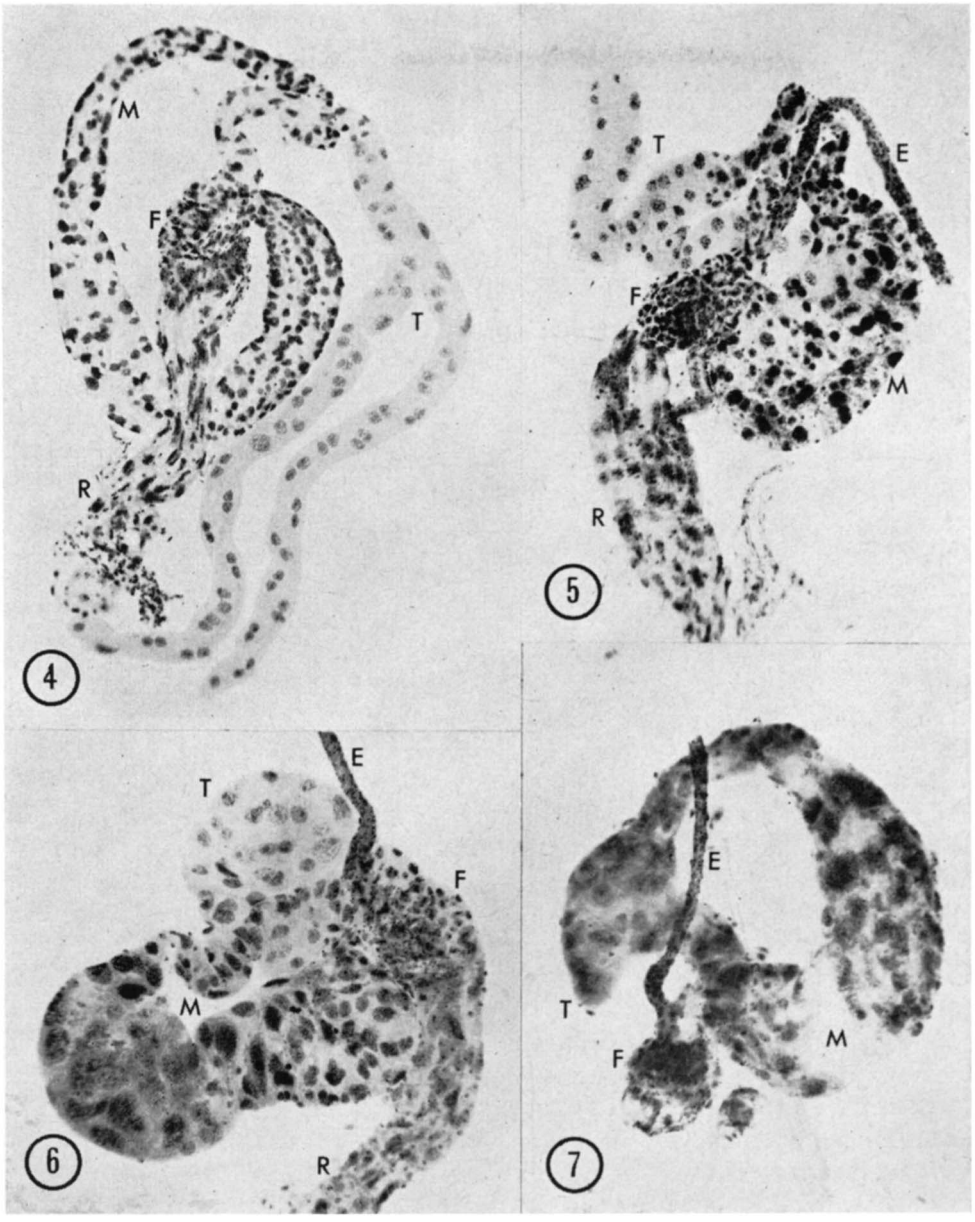
32 long thin sperms and 32 heterochromatic bodies. The latter are embedded in the remains of the cytoplasm of 16 primary spermatocytes. The eight cyst wall cells later play a role in the transformation of the 32 sperms into two long sperm bundles.

the return of the inactive H set to the euchromatic state may be a way to increase the number of active sets in these cells and thus to conserve resources for the organism (NUR 1966). The presence of either a single E set or one E and one H set may be necessary for determining the course of development of these cells, but not for the functioning of these cells.

The effects of paternal irradiation: The observation that the H set in nuclei of several tissues undergoes reversal at various times during development was described in the preceding section. Following 60,000r of paternal irradiation the process of reversal is apparently not affected, and nuclei without an H set appear in all the tissues in which they normally do. If the paternal set were to return to normal activity as a result of reversal, its activity after high doses of paternal irradiation would be expected to greatly upset the development and the function of those tissues in which reversal occurred. Analysis of some of these tissues was undertaken in order to see whether they would exhibit abnormal development. The midgut and the Malpighian tubules were selected for this analysis because of their relatively large size and their distinctive shapes. The digestive tract of mealy bugs (Figure 4, 8) includes the esophagus, the filter chamber, the midgut to which the Malpighian tubules are attached, and the hindgut (Figure 5.3.E in Fox and Fox 1964). The cells of the esophagus and the filter chamber, the epithelial cells of the midgut, and some of the smaller cells of the hindgut all have an H set. On the other hand, as a result of reversal, the large cells of the midgut and hindgut and all the cells of the Malpighian tubules lack the H set (Figure 4, 8, 12).

Following 60,000r of paternal irradiation, the esophagus and the hindgut of third instar males seemed little affected. The midgut, while it still retained its loop shape, was much shorter and parts of it were much wider. The cells of the midgut were fewer in number, and most of them were larger than the cells of the midgut of normal males (Figures 5–7). The most severely affected structures were the Malpighian tubules. The Malpighian tubules of mealy bugs are Y-shaped. The two tubules which form the arms of the Y lie in the abdomen on both sides of the filter chamber and hindgut and along the testes. Each tubule is made up of a relatively small number of large cells which are usually binucleated (Figure 4). Following 60,000r of paternal irradiation, the shape of the Malpighian tubules of third instar males was greatly modified (Figures 5–7). The cells of the Malpighian tubules were abnormally large and few in number, and the shape of the tubules varied greatly in different males. In some of the males the tubules were represented only by a few large cells which were attached to the midgut (Figures 6, 7). In some others they were represented by a single short tubule. In about half of the males the tubules were Y-shaped but much shorter and thicker than they normally are (Figure 5) and were curled up instead of running the length of the hindgut and the testes.

Interspecific hybridization: Reversal of heterochromatization of the paternal chromosome set in some of the male tissues may also explain the death of young hybrid males. It was decided, therefore, to repeat one of the crosses of NUR and CHANDRA (1963) and to study the development of the hybrids in more detail. In a cross of *Pseudococcus obscurus* females by *Ps. gahani* males, some of the



FIGURES 4 to 7.—The intestinal tract and the Malpighian tubules of third instar *Pl. citri* males. 100 ×. FIGURE 4.—Control. The esophagus was lost in the dissection. The midgut (M) forms a loop which begins and ends in the filter chamber (F). The Y-shaped Malpighian tubules (T) are attached to the midgut just before the latter returns to the filter chamber. The hindgut continues from the filter chamber as a wide tube, the rectum (R), and a narrow tube, which then join together. Notice that in this figure as well as in FIGURES 5 to 11, all the cells of the Malpighian tubules, most of the cells of the midgut, and some of the cells of the hindgut lack the small darkly stained body which represents the H set.

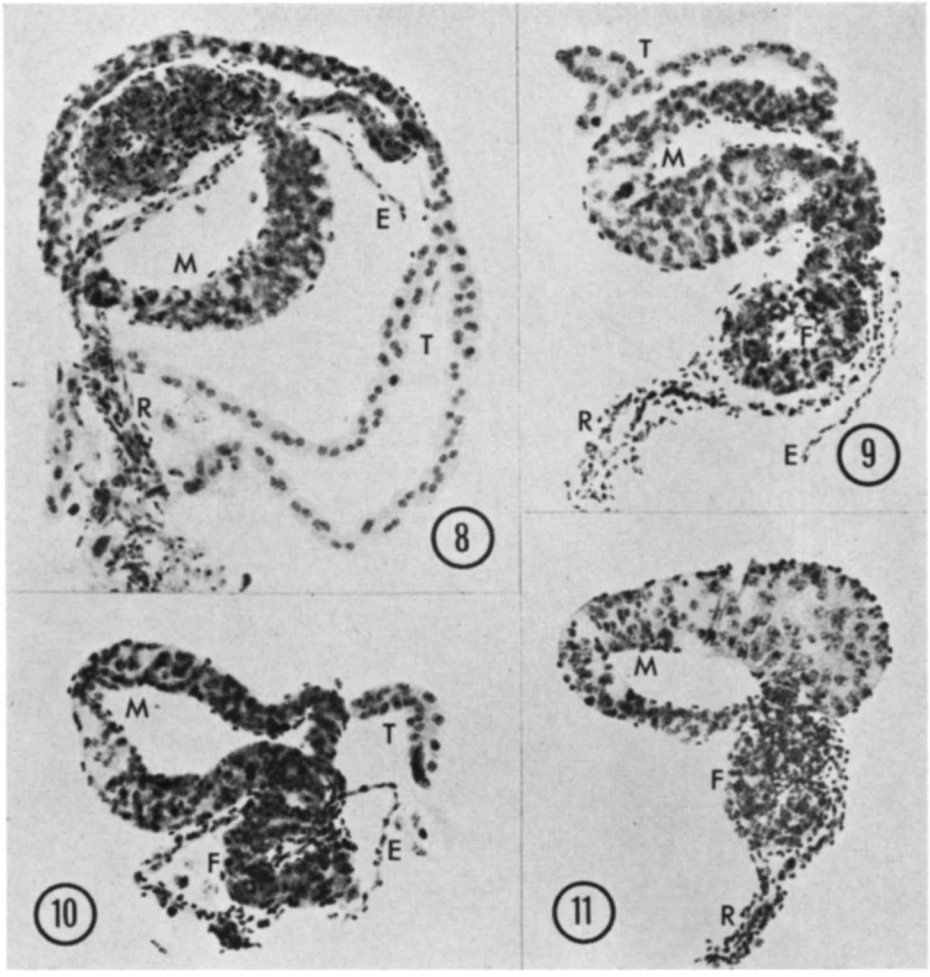
FIGURES 5 to 7.—60,000r paternal irradiation. The Malpighian tubules (T) are poorly developed. The loop of the midgut (M) is shorter and wider and contains fewer, larger cells. The esophagus (E) is present in the three figures. The hindgut was lost in Figure 7.

embryos hatched, but none survived past the first instar. Cytological analysis of the hybrid embryos indicated that, while almost all the hybrid male embryos hatched, none of the hybrid female embryos developed beyond the blastoderm stage. In the female embryos some of the nuclei showed chromosome fragmentation and stickiness. Of special interest was the observation that in some of the nuclei of female embryos the prophase chromosomes could be recognized as two different sets on the basis of their degree of condensation. Thus, it appeared that in the female hybrids, one of the two chromosome sets was affected by a process which resembled heterochromatization. Unlike male embryos, however, in females there were no visible differences between the two sets during interphase.

Hybrid male embryos seemed to develop normally, although more slowly, until hatching. In these hybrid males reversal took place in those tissues in which it normally occurred. In some of the cells, however, the process of reversal seemed to be incomplete. That is, in some of the nuclei parts of the chromatin appeared more coarse and more darkly stained. In a few nuclei of the Malpighian tubules and the midgut the more darkly stained masses of chromatin could be resolved into five chromosomes (Figure 13). These chromosomes were not as condensed as the H chromosomes from tissues in which there is no reversal, and they did not exhibit the latter's tendency to fuse into a single mass (Figure 12). They were, therefore, considered to have resulted from a partial reversion. The presence of a haploid number of more darkly stained chromosomes may serve as one line of evidence that the absence of an H set in a nucleus is the result of reversal and not loss.

The species, *Ps. obscurus* and *Ps. gahani*, differ with respect to reversal of the H set in serosa cells. In *Ps. obscurus* most of the serosa cells do undergo reversal, while in *Ps. gahani* they do not. In the male hybrids, the H set of most of the serosa cells underwent reversal (Figure 1) even though the H set was contributed by a species whose H set does not normally undergo reversal in serosa cells. Thus, the reversal is not due to an independent behavior of the H set, but instead it is under the control of either the chromosomes or the cytoplasm of the maternal species. On the basis of the behavior of the paternal H set in haploid-diploid mosaic embryos, it was concluded earlier (BROWN and NUR 1964) that the maintenance of the H state of the paternal chromosomes is under the control of the maternal chromosomes. The behavior of the H set in the serosa cells of the hybrid males may be similarly explained.

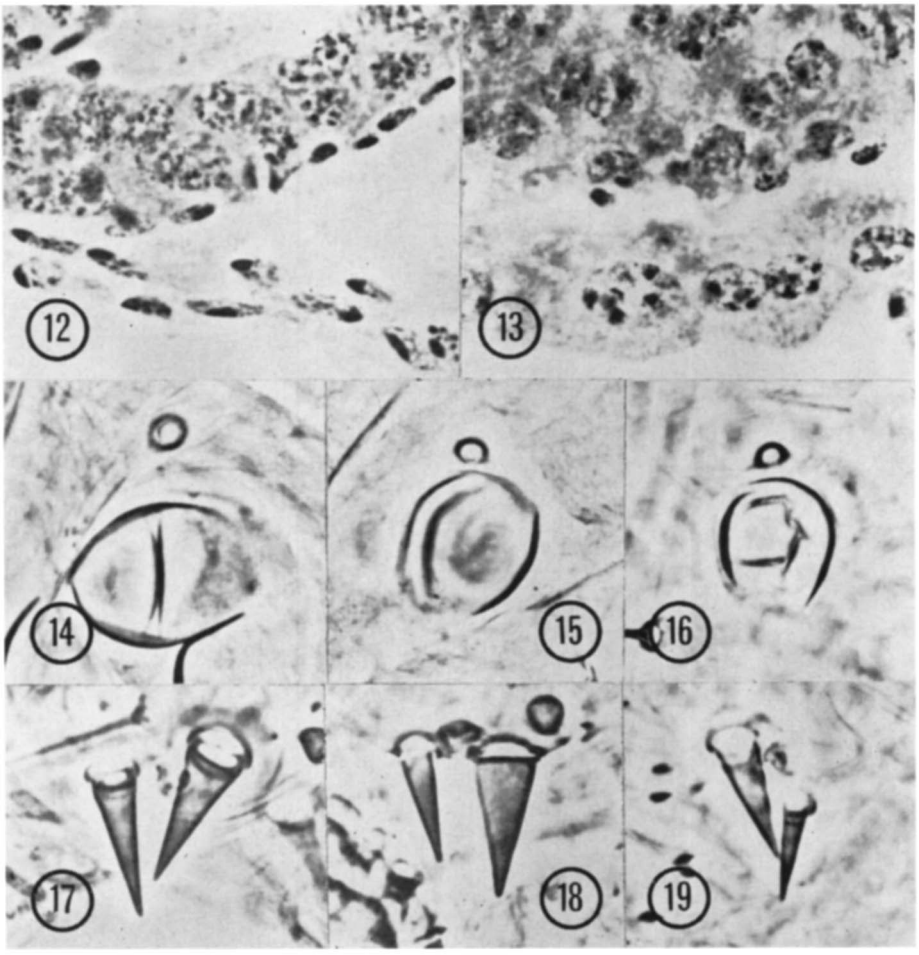
The digestive tract and the Malpighian tubules of newly hatched *Ps. obscurus* and *Ps. gahani* males are quite similar and except for size, they also resemble closely those of third instar *Pl. citri* males (Figures 4, 8). A comparison between the midgut and the Malpighian tubules of males of the parental species and those of newly hatched hybrid males revealed changes in the midgut and Malpighian tubules which resembled those found after 60,000r of paternal irradiation. The loop of the midgut of the male hybrids was shorter and seemed to contain fewer cells (Figures 9–11). As in males developing after 60,000r of paternal irradiation, the Malpighian tubules seemed more strongly affected than the midgut. Out of 13 newly hatched hybrid males analyzed, eight lacked any structure which might



FIGURES 8 to 11.—The intestinal tract and the Malpighian tubules of newly hatched males. Labeling of parts as in Figures 4 to 7. 240 ×. FIGURE 8.—*Ps. obscurus*. FIGURES 9 to 11.—Male hybrids of the cross *Ps. obscurus* × *Ps. gahani*. The Malphighian tubules (T) are poorly developed in Figure 9, are only loosely attached in Figure 10, and are absent in Figure 11. The midgut loop (M) is shorter and probably contains fewer cells than in Figure 8.

be considered to represent the Malpighian tubules (Figure 11). It is possible that in these males cells representing the Malpighian tubules were present but failed to become attached to the midgut. In one male a single short tubule was loosely attached to the midgut (Figure 10), and in four other males the Malpighian tubules did appear Y-shaped but were very poorly developed (Figure 9).

The external skeleton of insects is made up of cuticle and is secreted by the hypodermis (Fox and Fox 1964). Examination of the hypodermis of the hybrid males showed that it was made up of small cells whose nuclei had an H set. It was of interest, therefore, to compare the external morphology of the hybrid males



FIGURES 12 and 13.—The esophagus, midgut, and Malpighian tubules of newly hatched males. Phase contrast, 1200 ×. FIGURE 12.—*Pseudococcus obscurus*. A section of the midgut (above) and of the esophagus (below). The large nuclei in the midgut lack an H set. The esophagus is represented by two rows of small nuclei each with an H set. FIGURE 13.—*Ps. obscurus* × *gahani*. An enlarged section of Figure 9 with the midgut (above) and a Malpighian tubule (below). The nuclei of the Malpighian tubule and some of those of the midgut contain a darkly stained nucleolus, and in addition, darkly stained masses of chromatin. These dark masses represent partially reverted H chromosomes. Five of these masses and a nucleolus can be seen in the large Malpighian tubule nucleus to the right of the number.

FIGURES 14 to 19.—Discoidal pores and cerarian spines in newly hatched males. Phase contrast, 1500 ×. FIGURE 14.—*Ps. gahani*. The discoidal pore (small circle) is at some distance from the eye (large circle). FIGURE 15.—*Ps. obscurus*. The discoidal pore is near the eye. FIGURE 16.—*Ps. obscurus* × *gahani* hybrid. The discoidal pore is near the eye as in the maternal species (*Ps. obscurus*). FIGURE 17.—*Ps. gahani*. The two cerarian spines of the anal lobe are of about equal size. FIGURE 18.—*Ps. obscurus*. A large and a small cerarian spine. FIGURE 19.—*Ps. obscurus* × *gahani* hybrid. A large and a small cerarian spine as in the maternal species (*Ps. obscurus*).

to those of the parental species. Examination of first instar males of the two parental species revealed only two clear differences between them. In *Ps. obscurus* a discoidal pore is present near the eye (Figure 15), while in *Ps. gahani* this pore is usually found at some distance from the eye (Figure 14). The second difference involves the cerarian spines of the anal lobes. The first instar larvae of both species possess two pairs of these spines. In *Ps. obscurus* one spine of each pair is large, and the other is small (Figure 18). In *Ps. gahani* the two spines of each pair are of about equal size (Figure 17). In the first instar hybrid males these two characters differed from both parents but resembled much more closely those of *Ps. obscurus*, the maternal species (Figures 16, 19; Table 1). Although the ratio between the diameters of the spines was quite similar to that of the maternal species, the actual diameter of the spines was considerably smaller than that of either of the parental species (Table 1). The combined length of the tibia and the tarsus of the hind legs was also smaller than that of the parental species.

In the parental species each anal lobe usually contained two cerarian spines. In the hybrid males 11.5% of the lobes had three, instead of two, spines. In some of the hybrid males the additional spine was large while in others it was small. In 12 out of 61 hybrid males, three spines were present in one lobe and two in the other. In one male both lobes had three spines. The cerarian spines are enlarged setae, or bristles, and like them they are produced by two cells, a seta-forming cell and a socket-forming cell (Fox and Fox 1964). These cells are usually much larger than other neighboring hypodermis cells. In an examination of squashed preparations of newly hatched males it was not possible to identify the seta- and socket-forming cells on the basis of greater size. It was also not possible to find any nuclei in the hypodermis in the area adjacent to the spines which lacked an H set. It is believed, therefore, that the H set of the seta and socket cells forming the spines did not undergo reversal.

DISCUSSION

The first evidence for the genetic inactivity of the H set came from the inability to induce lethality in males through paternal irradiation with doses up to 30,000 rep (BROWN and NELSON-REES 1961). Additional evidence came from the be-

TABLE 1

Data on several characters of newly hatched larvae of Pseudococcus gahani, Ps. obscurus, *and their male hybrids*

	Distance, pore to eye	Diameter of large spine (L)	Diameter of small spine (S)	Ratio S/L	Hind legs: tibia + tarsus	Anal lobes with 3 spines
Pseudococcus gahani	7.9 ± .3	4.2 ± .1	3.2 ± .1	0.76 ± .02	127.8 ± .5	0.7% (150)*
Ps. obscurus	1.0 ± .1	4.7 ± .1	2.8 ± .1	0.59 ± .01	138.1 ± .9	1.8% (110)
Ps. obscurus × *gahani*	1.4 ± .1	3.7 ± .1	2.0 ± .1	0.54 ± .01	119.0 ± .6	11.5% (122)

* Number of lobes examined.
Linear dimensions are given in microns and are based on 50 measurements. Spines from anal lobes with three spines were not measured.

havior of a recessive mutant for eye color and from the study of RNA metabolism. Females heterozygous for the mutant *sal* (salmon eye color) exhibit the wild-type phenotype. Heterozygous males show the wild-type phenotype if the wild-type allele is in the euchromatic set but show the mutant phenotype if the wild-type allele is in the heterochromatic set (BROWN and NUR 1964). Thus, the wild-type allele seems to be inactive when it is in the H set. A study of RNA metabolism in spermatogonial cells indicated that the incorporation of H^3-uridine into RNA was restricted almost entirely to the euchromatic set (BERLOWITZ 1965). In the light of the present knowledge about gene function, the lack of RNA synthesis by the H set can be considered as a further indication of the inactivity of the H set.

The sterility or lethality of males following high doses of paternal irradiation and the lethality of hybrid males were previously interpreted as indication that the H set was at least partially active genetically. The observation reported in this paper, however, indicated that the tissues most severely affected were those lacking an H set. The midgut and the Malpighian tubules were chosen for the study of the effects of high doses of paternal irradiation and of interspecific hybridization because the nuclei of these organs lacked an H set. The abnormal development of these two organs following paternal irradiation and hybridization clearly indicated that the paternal set was still present in the cells of these organs and was genetically active. On the other hand, the external morphology of the males seemed not to be too adversely affected by high doses of paternal irradiation (NELSON-REES 1961) and by hybridization. As was pointed out earlier, the external skeleton of insects is secreted by the hypodermis. In the males of the species used in this study, as well as in the hybrid males, the nuclei of the hypodermis always had an H set. Thus, there seemed to be good agreement between the presence of a paternal H set in a given tissue or organ and the resistance of that tissue or organ to the harmful effects of paternal irradiation and hybridization.

NELSON-REES (1961), who studied the effects of high doses of paternal irradiation on *Pl. citri* males, observed that in these males spermatogenesis, spermiogenesis and sperm bundle formation were highly abnormal and that after doses of 60,000 rep or higher the surviving males were completely sterile. He attributed the sterility to disturbances in the function of the H set and suggested that the H set performed a function which was necessary for male fertility. Insemination in coccids involves the transfer of sperm bundles rather than individual sperms. The cyst wall cells are apparenty responsible for the organization of the sperms of each cyst into two sperm bundles, and for the formation of a sheath around each bundle (NUR 1962a). The observation that the cyst wall cells lacked an H set opened the possibility that the abnormalities were due to the activity of the paternal set in the cyst wall cells and not to the activity of the H set in the germ cells.

If one accepts the interpretation that the reversal of heterochromatization leads to the return of the paternal set to normal genetic activity, then the survival of some males following high doses of paternal irradiation becomes quite remark-

able. The survival is due at least in part to the holokinetic nature of coccid chromosomes. Following fragmentation the fragments are still capable of movement on the mitotic spindle (HUGHES-SCHRADER and RIS 1941), so that chromosome breakage does not lead automatically to the loss of genetic material. BROWN and NELSON-REES (1961) reported that the survival of males following high doses of paternal irradiation started to decline only after 30,000 rep. Above this dose the irradiated chromosomes started to exhibit bridge formation and nondisjunction (CHANDRA 1963a), and as a result of bridging and nondisjunction the developing embryos varied greatly in the amount of paternal chromosomal material which they possessed. NELSON-REES (1962) showed, however, that only those with an approximately normal amount of paternal chromosomal material survived. NELSON-REES concluded that a bulk requirement existed for the heterochromatic material. However, the bulk requirement is probably nothing more than a requirement for euploidy of the paternal set in cells in which it undergoes reversal. After doses below 30,000 rep, bridge formation and nondisjunction were rare, so that following reversal each cell still contained two full sets of chromosomes. At doses above 30,000 rep, bridge formation and nondisjunction led to aneuploidy for large blocks of genes, and, as a result, lethality was greatly increased.

In males, the presence of a chromosome set from another species of the same genus seemed to be more harmful than paternal irradiation of 60,000r. In the former case all the sons died shortly after hatching, while in the latter case at least some survived to adulthood. The hybrid males also showed greater abnormalities in their Malpighian tubules than males developing after paternal irradiation. It seems, therefore, that the death of the hybrid males could have been the result of the absence or malfunction of the Malpighian tubules, as well as, the malfunction of the abnormal midgut and all the other tissues in which reversal had taken place.

In the hybrid males, the ratio between the diameter of the spines, and the distance between the eye and the discoidal pore were quite similar to those of the maternal species (Table 1). It was previously pointed out that the features of the external morphology are determined by the cells of the hypodermis. In the nuclei of these cells the paternal set is in the heterochromatic state. Thus, the similarity between the hybrid males and those of the maternal species also tends to support the idea of the genetic inactivity of the H set. It should be pointed out that in these two characters, as well as in all the others studied, the hybrids were not identical with the maternal species. In the hybrid males, the smaller diameter of both spines, the shorter hind legs, the high frequency of anal lobes with three spines, and the slower rate of development can be considered as indications that the development of these males was in some way upset. This upset could be explained by a low level of genetic activity of the H set. Other explanations, however, also seem just as plausible. At least part of this upset could have been the result of the abnormal development and function of those tissues in which the H set had undergone reversal. Another cause for this upset could have been an incompatibility between the paternal and maternal chromosome sets, and

between the paternal set and the maternal cytoplasm. Such an incompatibility, which may result in many chromosome changes when the chromosomes replicate in a foreign cytoplasm, is known to occur following nuclear transplantation in frogs (HENNEN 1963).

In conclusion it may be stated that in order to explain the results of paternal irradiation and hybridization it is not necessary to assume that the H set is genetically active in any way other than in its own replication. Nevertheless, the evidence for the complete inactivity is not conclusive, and future work may show that a low level of genetic activity does exist.

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SUMMARY

In mealy bugs (Coccoidea: Homoptera) with the lecanoid chromosome system, the haploid set of chromosomes which is contributed by the father becomes heterochromatic in male embryos. It was previously observed that cells in some of the male tissues lacked the heterochromatic (H) set. It is shown that this lack of an H set is the result of the reversal of heterochromatization, and the return of the H set to a euchromatic state. The types of cells in which this reversal takes place may vary from species to species, and the time of reversal of the H set may vary from tissue to tissue. Most of the evidence from previous work suggested that, when the paternal set becomes heterochromatic, it is no longer genetically active. However, sterility and lethality of males after high doses of paternal irradiation and lethality of hybrid males produced by interspecific hybridization were previously interpreted as indications that the H set was at least partially active. In the present study, it is shown that the activities previously attributed to the H set can be explained by the activity of the paternal set following its return to a euchromatic state in some of the male tissues. Examination of several tissues of third instar males developing after 60,000r paternal irradiation and hybrid males from the cross *Pseudococcus obscurus* × *Ps. gahani* indicated that developmental abnormalities occurred mostly in those tissues in which the H set had previously undergone reversal. The external morphology of males is determined by cells in which the paternal set is heterochromatic. The hybrid males resembled males of the maternal species in their external morphology, supplying additional evidence that the H set is genetically inactive.

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