Heterochromatic Chromosomes in the Coccids

The process of heterochromatization and the function of heterochromatin in coccid insects are reviewed.

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Cytologically, heterochromatization appears as a change in chromosome behavior: chromosomes, instead of extending themselves and losing their intense stainability at the end of a division, tend to maintain a condensed state in the nondividing nucleus. It is hoped that a consideration of heterochromatin in several coccid insects will add to our understanding of the process of heterochromatization and the function of heterochromatin.

Heterochromatization is apparently one of the ways in which part of a chromosome, a whole chromosome, or an entire set of chromosomes may become genetically inactive during the course of development. Examples of the inception of heterochromatization during embryogeny are less well known than instances in which chromatin regularly appears heterochromatic throughout various developmental stages. The inception of the process no doubt requires a control factor in addition to whatever is necessary to maintain heterochromaticity. The female house mouse, as well as other female mammals, demonstrates the heterochromatization of one of its two X chromosomes during development. Such a female becomes a fairly fine-grained mixture or mosaic of two types of tissue, that in which the heterochromatized X is of paternal origin and that in which it is of maternal origin. The heterochromatized X is recognized as the "Barr body" in nondividing nuclei (*1*), and experimental evidence associates the heterochromatization with the loss of genetic activity of at least some of the loci on the X chromosome (*2*).

The coccids are a relatively small, highly specialized family (or superfamily) of homopteran insects closely related to the more familiar aphids. Within this restricted group the diversity of chromosome systems is rather remarkable: nine different sexual systems are now known (*3, 4*) as well as several types of parthenogenesis. In several of the chromosome systems one or more entire sets of chromosomes become heterochromatized during early embryogeny. The large and constant proportion of chromosome material thus involved in heterochromatization simplifies interpretation of results from both the "evolutionary experiments" discovered in nature and the laboratory experiments set up with suitable subjects.

In this article the information so far gleaned from the coccids is used in an attempt to answer, in whole or in part, three questions: What are the origin and fate of the chromosomes which become heterochromatic? What is the effect of heterochromatization on genetic function? What is responsible for the induction, maintenance, and regulation of the heterochromatic state?

The Mealy Bug System

Coccid cytology began in 1921 with Schrader's (*5*) first study of mealy bug chromosomes. Since then, further work by both Schrader (*6*) and Hughes-Schrader (*3, 7*) has revealed the complete sequence in the mealy bug, or more properly, the lecanoid system of chromosome behavior. In this system one property is of particular significance: in some embryos an entire haploid set of chromosomes becomes heterochromatized and remains heterochromatic during subsequent development.

In the cleavage divisions immediately following fertilization, the chromosomes of all embryos appear to be euchromatic. Shortly thereafter, at blastula, one half of the chromosomes become heterochromatized in those embryos destined to become male. At interphase, the heterochromatic chromosomes clump together to form a conspicuous chromocenter. It is thus possible to tell at a glance the sex of an embryo, or even of a small patch of tissue, long before the gonads show their distinctive aspects. In the female embryo none of the chromosomes are heterochromatized, all of them thus remaining in the euchromatic state, and the further development of the female, including oogenesis, is also rather orthodox.

During the subsequent development of the males the heterochromatic set divides synchronously with the euchromatic set. In the species shown diagrammatically in Fig. 1 the haploid number is five. In one type of division figure, that of the female, all ten chromosomes of the diploid complement are similar. In the figures from the male the five chromosomes of the heterochromatic set are shorter and more densely stained than the five of the euchromatic set. By metaphase, the hetero- and euchromatic sets are no longer distinguishable and metaphase plates look the same in males and females.

The quite unusual sequence of spermatogenesis in the male mealy bug is shown diagrammatically in Fig. 2. The first division is equational, as though this were simply another mitosis for both the euchromatic and the heterochromatic sets. During the second division the two types of chromosomes segregate to opposite sides of the cell. The result of the two divisions is thus the expected four nuclei, but no pairing and no genetic recombination have occurred. Two of the four nuclei are strictly euchromatic derivatives; the other two are strictly heterochromatic. Only the euchromatic derivatives proceed to form sperm. The heterochromatic derivatives gradually disintegrate while the euchromatic are undergoing spermiogenesis. The Schraders (*3, 6, 7*) were thus able to show, from their cytological studies, the fate of the heter-

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ochromatic set. Because of its failure to form sperm, it is excluded from the genetic continuum.

The Schraders also offered two hypotheses in regard to the origin and function of the heterochromatic set, and both have been recently confirmed with the common mealy bug, *Planococcus citri*, as the test subject.

The first hypothesis (*3*) states that the heterochromatic set in the males is of paternal origin. This was confirmed quite simply (*8*). After x-ray treatment of fathers, chromosome aberrations appeared in the heterochromatic set of male embryos; after x-ray treatment of mothers, the aberrations were found in the euchromatic set. Therefore, the euchromatic set of the father becomes the heterochromatic set of the son, since the heterochromatic set degenerates after spermiogenesis.

The second hypothesis of the Schraders (*9*) is concerned with function. According to this hypothesis the heterochromatic set in the males is genetically inert; the male mealy bug would therefore be a virtual, or physiological, haploid even though he has a diploid number of chromosomes. This hypothesis was verified by Brown and Nelson-Rees (*8*), who used x-rays and also irradiation from a radiocobalt source, and found a differential survival of male and female progeny following irradiation of the fathers at various dosages. With increasing dosage, the number of surviving daughters declined, as would be expected if the treatment were inducing dominant lethal mutations. At a dose of 16,000 roentgens only 3 percent of the daughters survived. The number of sons did not decline, and there were approximately as many sons after irradiation of the fathers at 30,000 rep (roentgen equivalent, physical) as there were for unirradiated controls. Irradiation above 30,000 rep usually made the fragmented chromosomes sticky, so that at these doses lethality probably resulted from mechanical disruption of very early embryogenesis. But a few sons still survived after the fathers had been irradiated at 60,000 and 90,000 rep. This survival study indicated that dominant lethal genes are not expressed when they are present in the heterochromatic set, thus demonstrating the inertness of the heterochromatic set and confirming the Schraders' second hypothesis.

The foregoing conclusions may be summarized as follows: the male mealy bug receives a set of chromosomes from his mother which remains euchromatic and genetically active, and is transmitted in his sperm; the set which he receives from his father becomes heterochromatic and genetically inert, and is discarded rather than transmitted.

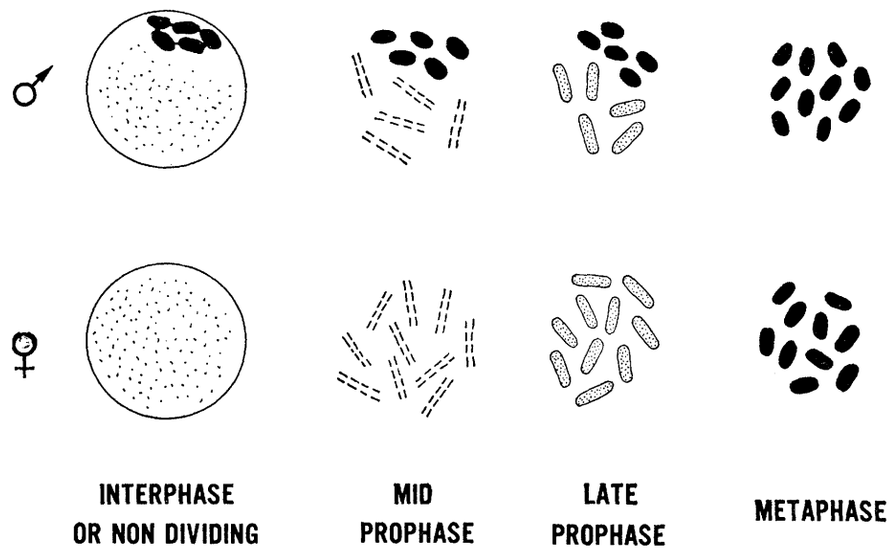

Fig. 1. Mitosis in female and male mealy bugs. The female and the male mealy bug have the same number of chromosomes (ten is the commonest number). The chromosomes of the female (bottom row) remain unchanged during development. Half the chromosomes of the male (top row) become heterochromatized at an early embryonic stage and remain so throughout development. The heterochromatic chromosomes clump together to form a chromocenter at interphase and appear more condensed than the others during prophase. At metaphase all chromosomes of both sexes are equally condensed.

The expression and transmission of all the genetic mutations found so far in the mealy bug conform to this pattern. The males express and transmit only the genes they receive from their mothers. Several of the mutations discovered affect wing morphology and cannot be studied in the female because all female coccids are wingless. Our best examples, therefore, are the several mutations to a lighter eye color, salmon eye, which have occurred independently at several different loci. The mutant genes behave as typical recessives in the female. The male will express the recessive only if he gets it from his mother, and irrespective of whether he receives the dominant or the recessive gene from his father.

So far the mealy bug system seems quite straightforward. The heterochromatic set is the paternal set. It is genetically inert and is eventually discarded. Thus, our questions regarding origin, function, and fate appear to be answered. However, this is by no means the end of the story, since the heterochromatic set does show certain types of activities.

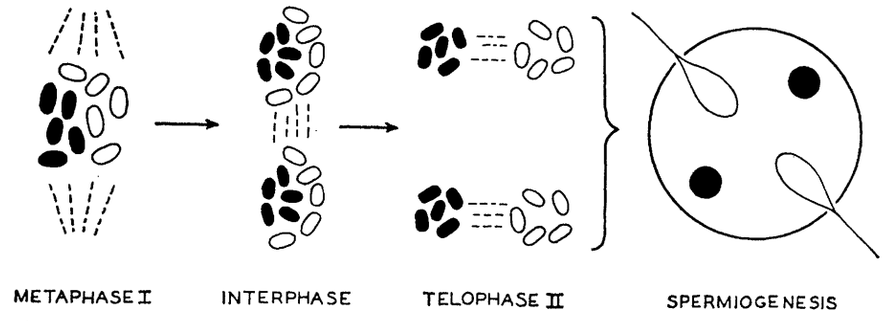

Fig. 2. Spermatogenesis in the mealy bug. The euchromatic and heterochromatic sets both divide equationally during the first spermatogenic division and segregate during the second. Only the euchromatic products form sperm; the heterochromatic products appear as deep staining residues, which slowly degenerate.

Residual Activity of the Heterochromatic Set

About five years ago, during a discussion of coccid chromosome systems at a national meeting, M. J. D. White stated that he felt certain the heterochromatic set was doing something or it would not be there. It has since been learned that White was correct; there are at least three effects attributable to what we may call the residual activity of the heterochromatic set.

Two of these have been recently discovered by Nelson-Rees (*10*). The first concerns what has been referred to as the bulk requirement. After very severe irradiation of fathers (60,000 to 90,000 rep) a few sons are still able to survive to adulthood. No matter how badly damaged or rearranged the heterochromatic set is in these survivors, the bulk of the heterochromatic material is just about the same as it is in the untreated controls. However, if the chromosomes are studied earlier in the embryos, shortly after fertilization, gross disturbances may be observed to occur in the heterochromatic set as the result of the high irradiation. Therefore, only those embryos with an approximately normal amount of heterochromatin survive, regardless of how this set may otherwise have been modified.

The second effect is on fertility. After irradiation of the fathers at 30,000 rep, 100 percent of the sons survive. However, a large proportion of these sons are sterile. The proportion of sterile sons increases with dose, becoming 100 percent at dosages between 60,000 rep and the top limit, 90,000 rep. According to further observations by Nelson-Rees (*10*), the morphological changes associated with sterility occur mainly during sperm formation.

The third effect involves foreign heterochromatin. A heterochromatic set from one species cannot be substituted for that of another. If the heterochromatic set of a species were completely inert, it should be possible to replace it with a heterochromatic set of comparable bulk from another species. In this case, after at least some interspecific crosses, the male offspring would be expected to survive, although the female offspring would not. However, Nur and Chandra (*11*) found that no offspring of either sex survived beyond the first instar stage after interspecific hybridization of several species of mealy bugs, some quite closely related.

In conclusion, therefore, we can say that the heterochromatic set is inert genetically except for certain residual functions. These residual functions manifest themselves as an apparent bulk requirement, an effect on fertility, and a failure of interspecific substitution. None of these are really understood yet, and so their relationship with typical gene action remains quite unknown.

Induction and Control

The last question to be considered concerns the induction and maintenance of the heterochromatic state in the coccids. The chromosomal chemistry involved in such processes is not known, but cytological and autoradiographic studies of heterochromatin have revealed some interesting properties.

Studies of irradiated coccid chromosomes were carried out in an attempt to localize the site within a chromosome which controls the induction of heterochromatization of that chromosome. Coccid chromosomes are holokinetic. Therefore, every chromosome fragment, no matter how small, is capable of some movement on the mitotic spindle. Very small fragments tend to be lost fairly readily, but this can be attributed to their slowness in getting to the poles; they in no way appear to be acentric. When the mealy bug chromosomes are irradiated, all of the fragments behave like whole chromosomes, not only with respect to movement, as would be expected, but also with respect to heterochromatization. That is, after irradiation of the fathers, all of the fragments in the male embryos are heterochromatic; after irradiation of the mothers, all are euchromatic. Therefore, there must not be a single locus or restricted region on each chromosome which controls or regulates the induction of its heterochromatization.

As shown by Nelson-Rees (*10*), there is also no one region within a heterochromatic chromosome that controls its behavior in spermatogenesis. He studied the spermatogenetic behavior of the many small chromosomal fragments resulting from irradiation of the father at a very high dose. These fragments invariably behaved like typical members of the heterochromatic set, always segregating along with the rest of the heterochromatin in the second division.

An interaction between the hetero- and euchromatic sets has been demonstrated by recent observations of Nur

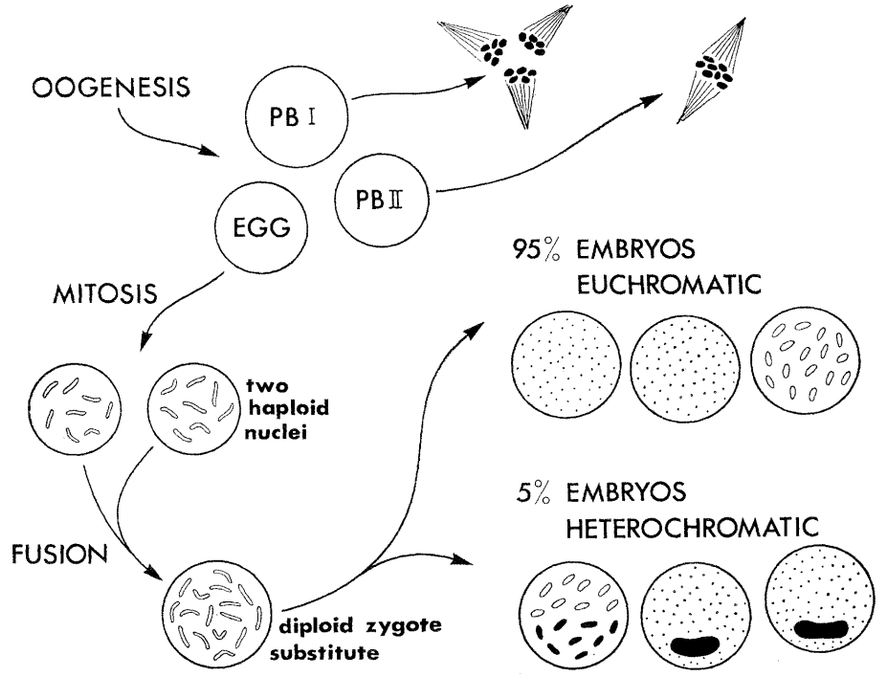

Fig. 3. Origin of embryos in a parthenogenetic soft scale. A typical meiosis yields the usual products. The two polar bodies divide on aberrant spindles and do not participate in development thereafter. The egg nucleus divides once to yield two haploid nuclei, which soon unite to produce a diploid zygote substitute. In about 5 percent of the embryos so produced, one haploid set is heterochromatized and shows the typical chromocenters and division figures.

(*12*) and Chandra (*13*). This interaction is responsible for the maintenance, but not the induction, of the heterochromatic state. The single paternal set of chromosomes present in experimentally produced haploid embryos and haploid embryo sectors was observed first to undergo typical heterochromatization, and subsequently to lose its heterochromaticity. It appears that the heterochromatic set in normal embryos is being suppressed (maintained in the heterochromatic state) by the euchromatic set. However, such suppression is not an interaction between genetic homologues such that chromosome 1 in the euchromatic set is responsible for the suppression of chromosome 1 in the heterochromatic set. Nor is the converse true. When a chromosome is lacking from either set in an embryo, its homologue in the other set nonetheless shows the typical expression of the set to which it belongs (*14*).

Autoradiographic studies by Baer (*15*) on the mealy bug, *Pseudococcus obscurus*, have shown that the heterochromatic set synthesizes DNA asynchronously with respect to the euchromatic set. Lima-de-Faria (*16*), Taylor (*17*), and others had previously found such asynchronous DNA replication in various organisms. In the known examples, the heterochromatic chromosomes (or regions) usually synthesize DNA after the euchromatic ones do. In *P. obscurus*, also, the heterochromatized chromosomes synthesize DNA following the completion of synthesis by the euchromatic set. Thus the same chromosomes which were replicated first in the fathers are replicated second in the sons. The shift in timing may reflect profound changes in chromosome organization concomitant with heterochromatization but no such inference may yet be drawn.

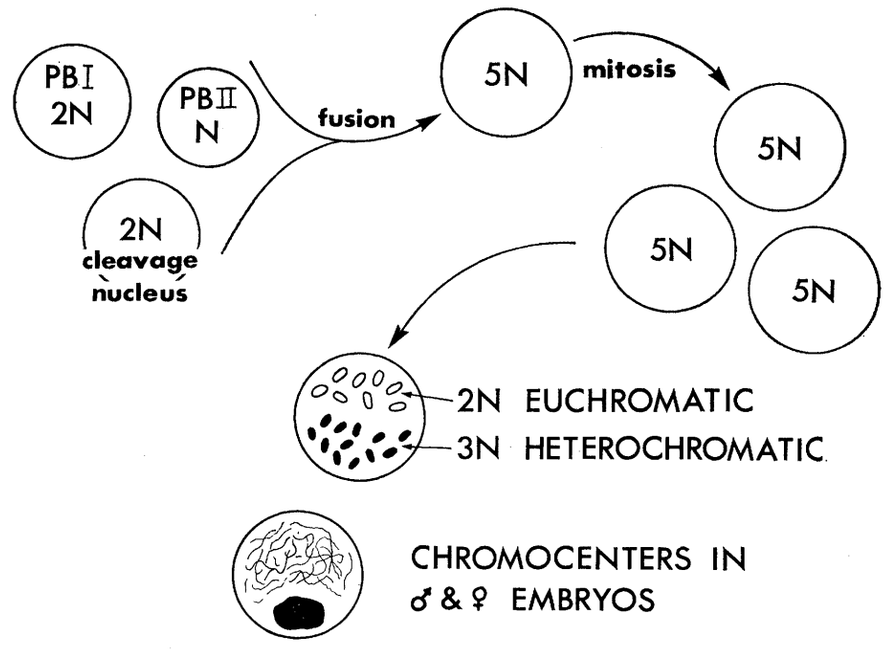

Fig. 4. Chromocenter formation in an adjunct tissue. A typical pentaploid (*5N*) tissue is formed. Later, when each of these pentaploid nuclei undergoes an abortive endomitosis, three of the five chromosome sets become heterochromatic and two remain euchromatic. The heterochromatic chromosomes clump together to form a chromocenter. The process occurs in both male and female embryos.

Lack of Genetic Determination of Sex

Observations on parthenogenetic and sexual mealy bugs indicate that sex is not determined genetically. Since heterochromatization occurs in one sex and not in the other, we might at first assume that sex is determined genetically, and that heterochromatization is simply the product of male development. There is no evidence, however, that female embryos differ genetically from male embryos in the sexual species, and in the parthenogenetic species there is proof that they do not.

Even though there is no question that the heterochromatic set is of paternal origin in the male of the sexual species, are these male embryos genetically different from the female embryos? Considerable circumstantial evidence suggests that the mother determines sex by producing two types of eggs, different in substance but genetically equivalent. For example, the sex ratio fluctuates widely from female to female and is subject to environmental influence (*18*). Sex ratio is markedly altered in favor of males by aging the mother prior to mating. There is partial sexual dichronism—that is, the male and female embryos tend to be deposited at different times during oviposition—and the sexual dichronism can also be altered in pattern by maternal aging. However, these results may still be explained in terms of directed segregation, during oogenesis, of chromosomes bearing the sex factors.

Recent studies rule out this latter explanation for the parthenogenetic soft scale insect, *Pulvinaria hydrangeae* (*19*), in which embryos of both types, with and without heterochromatization, can be produced parthenogenetically by completely homozygous females. This chromosome system is shown diagrammatically in Fig. 3. Oogenesis is meiotic. The two polar bodies are eliminated by division on abnormal spindles. The egg nucleus divides once, and its products unite to form a diploid zygote substitute. The large majority of the embryos are female, with both sets of chromosomes euchromatic, whereas the remainder of the embryos have the heterochromatization characteristic of males. Except in the case of mutation, the males as well as the females must be strictly homozygous. Adult males have never been reported, and the development of the male embryos has not been followed beyond the late cleavage stages. However, the total absence of sperm from spermathecae and ovarian ducts of adult females indicates that if males do develop further, they are not functional. In a related European species, *P. mesembryanthemi*, which has not yet been studied cytologically, males which are apparently produced parthenogenetically survive to adulthood but never mate (*20*).

In this soft-scale insect there are hence no genetic differences between the class of embryos in which heterochromatization occurs and the class in which all the chromosomes remain euchromatic. And in the male embryo there can be no genetic differences between the set which becomes heterochromatic

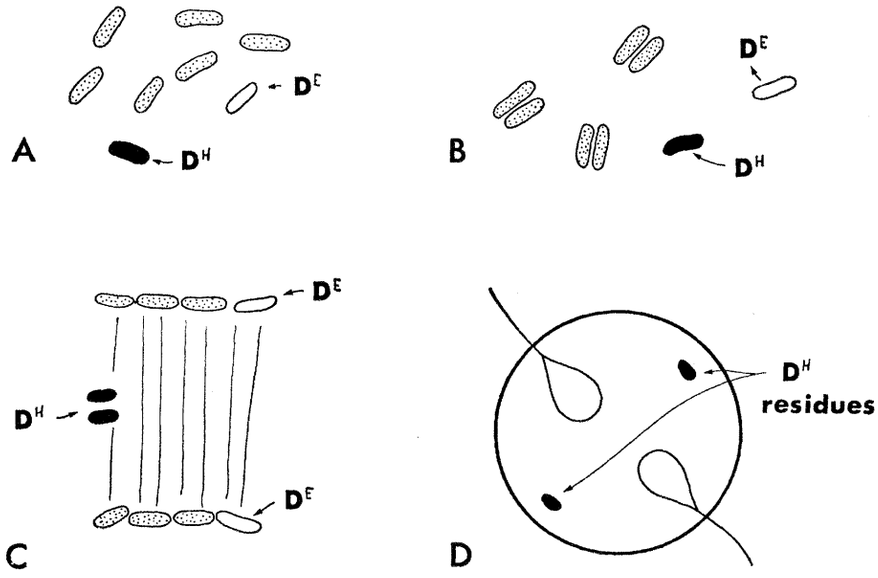

Fig. 5. Spermatogenesis in the Comstockiella system. The euchromatic and heterochromatic sets lose their difference, except for the D pair of homologues; pairing begins between an early, or preprophase, stage and mid-prophase (*A*) and is complete at late prophase (*B*). At anaphase the members of the pairs simply separate to opposite poles. One pair of chromosomes, the D pair, behaves differently. Usually the two members of this pair do not associate closely. The member derived from the heterochromatic set, D^H, is eliminated at anaphase or telophase, and its division products form two residues which slowly disintegrate. The D^E chromosome, from the euchromatic set, divides equationally; thus, each sperm is genetically complete.

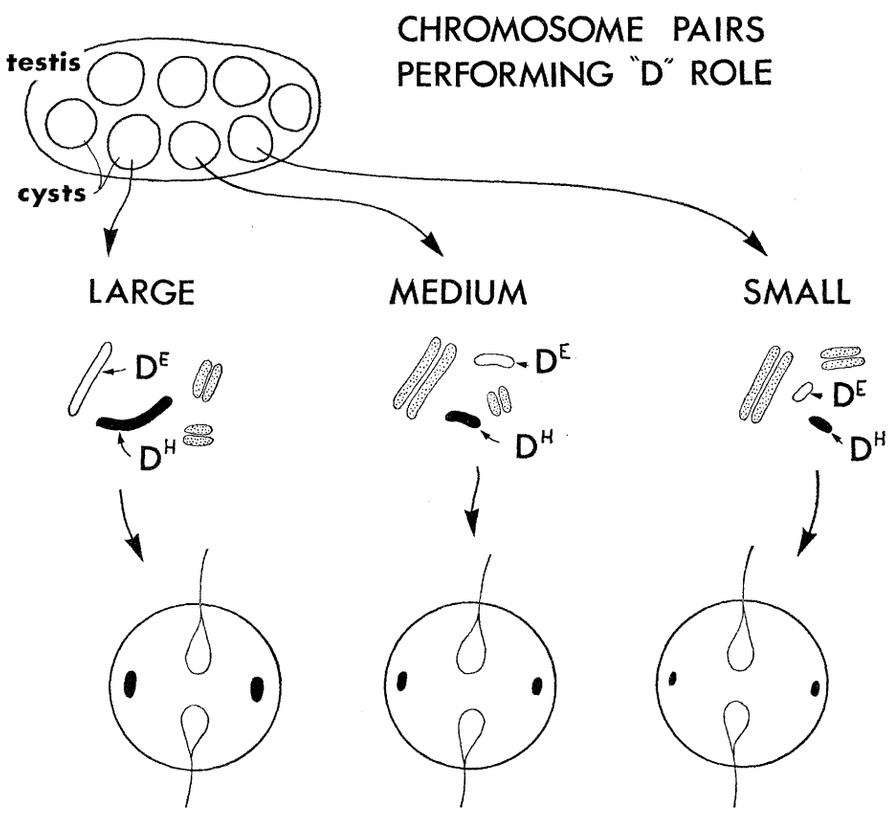

Fig. 6. Variation in the D chromosomes. In *Ancepaspis tridentata* there are only three pairs of chromosomes, and these "take turns," from cyst to cyst, in appearing as the D chromosomes. Because of the several different types of modification going on simultaneously, the difference between the medium-sized and the small chromosomes is frequently obscured at prophase of spermatogenesis. However, the three size classes of the D^H residues are clearly apparent in the early stages of spermiogenesis.

and the set which remains euchromatic. Male embryos tend to be produced near the end of oviposition, so it may be assumed that the female produces two types of eggs, a more numerous type in which no heterochromatization occurs and a less numerous type in which heterochromatization does occur. Prior passage of a chromosome through a male is not, in this case, a requisite for its heterochromatization. Given an almost identical origin of the two haploid sets, how can heterochromatization be induced in only one set? For the present, only speculations can be offered. The process of induction of heterochromatization may greatly amplify very minute differences in the chromosome which, in turn, stem from very minute differences in past developmental history. One of the two haploid nuclei may pick up some substance localized in the egg cytoplasm, with the consequence that its chromosomes become markedly altered.

It seems likely that similar events may go on in the sexual mealy bug, and that there are no genetic factors determining sex. But more data are needed before we can rule out the slight possibility that the parthenogenetic species has evolved an entirely different mechanism to produce males, and useless ones at that.

Heterochromatization and Sex

In the examples given above, heterochromatization has been associated with the male sex, but this relationship is not a strict one either. In some other mealy bug species we find the typical heterochromatization restricted to the germ line and just part of the soma of the male. The remainder of the tissues are similar to those of the female; both sets are euchromatic, and there are no chromocenters. There is thus considerable variation in heterochromatization in the males. In some distant relatives of the mealy bug, the armored scale insects, most of the species demonstrate a genetic system in which the paternal set is completely eliminated from those embryos destined to become male. The father's chromosomes are discarded at late cleavage, and the male continues development thereafter as a conventional haploid.

An example of heterochromatin in female coccids is provided by studies of polyploid tissues. In most of the armored scales, as in many other coc-

cids, the polar bodies combine with cleavage nuclei to form an adjunct tissue of large polyploid cells. Like plant endosperm, this adjunct tissue is genetically a dead end. Its function, as Schrader (*21*) demonstrated, is to house intracellular symbionts. The course of events in certain species of the armored scale insect is illustrated in Fig. 4. The two polar bodies combine with one cleavage nucleus to give a pentaploid nucleus, which then undergoes mitosis to produce a pentaploid sector in the embryo. During the course of division, three of the five sets of chromosomes become heterochromatic, and these eventually clump together to form a persistent chromocenter. This process occurs in both male and female embryos, constituting a clear-cut example of heterochromatization not associated with sex.

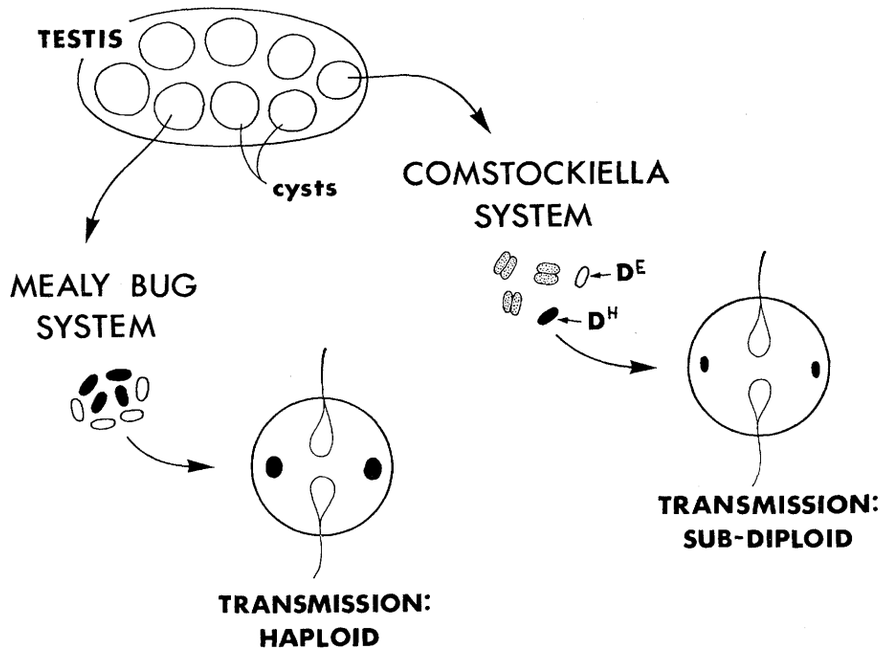

Fig. 7. Combination of mealy bug and Comstockiella systems. Both systems occur in several species of armored scale insects, frequently in the same testis, but never in the same cyst. The mealy bug cysts transmit only the euchromatic chromosomes, and the transmission from these is thus haploid. The Comstockiella cysts demonstrate a subdiploid transmission in that they transmit all the chromosomes except the D^H. An individual male can thus breed as a haploid and subdiploid.

Reversals of the Heterochromatic State

In the cases mentioned so far, the heterochromatic chromosomes have been discarded, either during spermiogenesis or in the expiration of a dead-end tissue. The next example, from the mealy bug *Pseudococcus obscurus* (*22*), shows how supernumerary chromosomes can add another dimension to the mealy bug system. The normal chromosomes vary little in size, and the supernumerary is only slightly smaller than the smallest of them. The number of supernumeraries ranges from zero to as high as six in some natural populations. They are heterochromatic in some tissues of the female, and in the male they behave like typical members of the heterochromatic set until spermatogenesis. At this stage they suddenly "change sides" and become even less condensed than the euchromatic chromosomes. They divide in the expected equational fashion during the first spermatogenetic division and segregate with the euchromatic set during the second. Their inclusion in the sperm is not always effected, however, since about 10 percent lag between the euchromatic and the heterochromatic derivatives or segregate with the heterochromatic set. The supernumerary chromosomes thus show that the heterochromatic state does not necessarily lead to a dead end in the mealy bug system.

A second example, from another chromosome system in the armored scale insects, indicates that the regular members of a newly heterochromatized chromosome set can undergo a similar reversal. This system, which occurs in a minority of armored scale insects, is called the Comstockiella system, after the genus in which it was first discovered (*4*, *23*). It is quite like the mealy bug system until spermatogenesis. Spermatogenesis consists of but a single division (Fig. 5). Immediately prior to the prophase of this division there is a reversal of heterochromatization such that the distinction between the euchromatic and the heterochromatic sets breaks down. Homologous chromosomes pair, and then simply separate from each other at anaphase, apparently at random with respect to parental origin. Two nuclei result, and both form sperm. One pair of chromosomes, however, always behaves differently from the remainder; it has been called the D pair (*4, 23*). The D pair usually retains some chromatic difference, so that the euchromatic D homologue and the heterochromatic D homologue can often be recognized at late prophase. Both of the D homologues divide equationally while the paired chromosomes are separating from each other reductionally. Immediately thereafter the heterochromatic D chromosomes are eliminated, sometimes by lagging at anaphase, but more often by ejection during or after telophase. We might conclude that the D chromosome is a genetically fixed entity, in this way analogous to a sex chromosome which may often show differential behavior during meiosis. This may be true in certain species, but in others it is definitely not true. The situation in *Ancepaspis tridentata*, an armored scale from Mexico, is shown in Fig. 6. The haploid number is three, and each chromosome can be recognized by its length. In some cysts the long chromosome pair is elected to perform the D role; in others, the middle-sized pair, or the small pair. In any one cyst there is uniformity, but variation in the testis is completely random. On checking the sizes of the residues during early spermiogenesis, when each cyst forms a discrete entity, it is possible to observe a completely haphazard arrangement.

The fate of the heterochromatic set in the Comstockiella system is the opposite of that in the mealy bug system; instead of being discarded it remains in the genetic continuum, except, of course, for the one D member. When the D member varies from cyst to cyst, that chromosome which is eliminated in one cyst will be transmitted by the adjoining cyst, which in turn eliminates a different one.

Variable Transmission

The election of different pairs of homologues to the D role is one of the two cases known in the coccids in which the hereditary transmission varies from cyst to cyst of the same animal. The D chromosomes exhibit an essentially mealy bug type of behavior, with retention of only the euchromatic member, and Brown (*4*) has suggested that the D type of behavior has thus persevered during the evolution of the Comstockiella from the mealy bug system. The rotation of the D role among the various homologues adds a further complexity. We have no information as to how the D pair is chosen; since both the D^{H} and the D^{E} chromosomes must simultaneously alter their behavior from that of their respective fellows, it seems reasonable to presume that the mechanism depends in part on genetic homology. The synchronization of the D^{E} with the D^{H} chromosomes, or vice versa, cannot be simply a consequence of pairing failure, as the D homologues will pair in some instances without modification of subsequent behavior.

Several species of armored scale insects have both the mealy bug chromosome system and the Comstockiella system frequently occurring together in the same testis, but again cyst-specific (*4, 24*). As previously mentioned, these two systems appear to be the same until spermatogenesis. Either one system or the other occurs in each cyst (Fig. 7), again at random with respect to position within the testis. From those cysts in which the mealy bug system occurs, only the maternal chromosomes are transmitted; in the others, those with a Comstockiella system, all but the heterochromatic D chromosome get into the sperm. Thus, a single animal breeds as both a haploid and a subdiploid. Developmental differences, however slight, among the individual cysts must be determining this marked difference in chromosome behavior and hereditary transmission.

Conclusions

How can we use this various and diverse information in an attempt to resolve our questions regarding the origin, function, and fate of heterochromatin in coccids?

With respect to origin, all products of gametogenesis undergo heterochromatization in one system or another. In the sexual system of the mealy bug, it is the sperm. In the parthenogenetic system of the soft scale, it is an immediate product of the egg nucleus. In the adjunct tissue of certain armored scales, the three sets becoming heterochromatic may be from the two polar bodies, but at least one polar body must make a contribution, since a cleavage nucleus can contribute only two sets.

With respect to fate, the heterochromatic chromosomes can either be excluded from the genetic continuum or, as in the Comstockiella system, lose their heterochromaticity and take their place in the continuum.

With respect to genetic function, the only information available comes from the sexual system of the mealy bug. However, it seems reasonable to assume that heterochromatization elsewhere also represents a change to a generalized inertness.

In the life cycle, heterochromatization seems to be geared to gross developmental differences and gross differences in hereditary transmission. The developmental differences, to summarize, are male versus female, germ line versus soma, and adjunct tissue versus the regular embryo. The gross differences in hereditary transmission are, in the primitive mealy bugs, haploid transmission by the diploid male versus diploid transmission by the likewise diploid female. In some armored scales the heterochromatic chromosome which is not to be transmitted (the D^{H}) varies from cyst to cyst, and in other armored scales, which have both the Comstockiella system and the mealy bug system, haploid and subdiploid transmission may both be found in the same testis.

There are only a few hints as to the induction, regulation, and maintenance of the heterochromatic state. But it is obvious, from the parthenogenetic soft scale and the cyst-to-cyst variation in the armored scales, that the developmental differences associated with the inception of heterochromatization are at present as obscure as the later differences, associated with the heterochromatic state, are gross and obvious.

The only general conclusion that can be safely stated at present is that the potentiality for heterochromatization has provided a means whereby further differential chromosome behavior can be elicited, and that this potentiality has been exploited during the course of evolution in the establishment of a contrasting assortment of chromosome systems.

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