Heterochromatization and euchromatization of whole genomes in scale insects (Coccoidea: Homoptera)

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Summary

In several families of scale insects (coccids), the sex of an embryo is determined by the number of genetically active genomes present (one=males, two=females). In mealybugs (Pseudococcidae), both males and females develop from fertilized eggs but, in the embryos that develop into males, the set of chromosomes (genome) of paternal origin (PG) becomes heterochromatic (H) and genetically inactive and is not transmitted to the offspring. The mechanism that reduces the number of active genomes in male embryos may vary between families and even between congeneric species. Thus, in male embryos of most armored scale species (Diaspididae), the PG is eliminated, while in a few species it becomes H. In two genera of soft scales (Coccidae), males develop from unfertilized eggs when one of two identical genomes of maternal origin becomes H. In most male tissues, one genome remains H. However, in several tissues that become polyplid by endoreduplication, the PG becomes E and genetically active. The tissues in which the PG becomes E often vary between species and the analysis of hybrid males demonstrated that whether the PG becomes H or remains E is determined by the genome of maternal origin. The euchromatization of the PG in the haploid sector of mosaic male embryos and the presence of spermatocytes with two E genomes (instead of one E and one H), following the irradiation of young mealybug males, strongly suggest that the maintenance of the H state requires the presence of a genetically active genome.

Key words: heterochromatization, euchromatization, genome, scale insects, coccids.

Introduction

In several families of scale insects (coccids), there are no sex chromosomes and both males and females develop from fertilized eggs. However, in the eggs destined to develop into males, one genome either becomes heterochromatic (H) and genetically inactive, or is eliminated (Brown and Chandra, 1977; Nur, 1980). The differential behaviour of the two genomes has been attributed to 'prior conditioning' (Hughes-Schrader, 1948) or genome imprinting (Chandra and Brown, 1975). This report will review the available information about the types of genome that may become H, some of the factors that might be involved in genome imprinting, and how the H state might be maintained.

The paternal origin of the H genome in mealybugs

The first indication that mealybugs (Pseudococcidae) have an unorthodox chromosome system was the report by Schrader (1921) that in the males of the mealybug Nipaecoccus nipae five of the chromosomes were euchromatic (E) and five were H, but in the females all ten chromosomes were E. A major step in our understanding of the chromosome system of mealybugs was the discovery by Hughes-Schrader (1935) that the males transmit only the E set of chromosomes (genome). Thus, she concluded that males begin to develop with two E genomes, but that the genome of paternal origin (PG) becomes H during embryogenesis. Earlier, Schrader and Hughes-Schrader (1931) proposed that the H genome is of paternal origin and genetically inactive, and that this inactivation is part of the sex determination mechanism of this group.

The paternal origin of the H set in mealybugs was established by Brown and Nelson-Rees (1961) by taking advantage of the fact that coccid chromosomes are holocentric and become attached to the mitotic spindle along their entire length; thus chromosome fragments are rarely lost (Hughes-Schrader and Ris, 1941). Brown and Nelson-Rees (1961) subjected males of the mealybug Planococcus citri to a high dose of irradiation, crossed them to control females and observed that following a high dose of irradiation to the male parent the chromosome fragments present in the sons were H, while following irradiation to the female parent the fragments were E. The observation that following paternal irradiation, all the fragments whose state of condensation could be determined became H, was quite unexpected and demonstrated that the imprinting
process that tags the PG for heterochromatization is not confined to one or a few control centers per chromosome.

The genetic inactivity of the PG in males

Brown and Nelson-Rees (1961) also used radiation to study the genetic activity of the PG. They observed that, even after males received a dose of 150 Gy of gamma irradiation, almost all their sons survived (although their fertility was reduced), but almost all their daughters died. Thus, the damage induced in the PG by the irradiation was expressed as dominant lethality in the daughters (where the PG is E) but not in the sons (where the PG is H). The genetic inactivity of the PG in those cells in which it is in the H state was confirmed later by the use of genetic markers (Brown and Wiegmann, 1969; Nur, unpublished observations). Moreover, the reduction in the fertility of the sons after 150 Gy of paternal irradiation and the reduction in the viability at higher doses of paternal irradiation were later shown to be the result of the euchromatization and genetic reactivation of the PG in certain male tissues (Nur, 1967).

Heterochromatization of a genome of maternal origin

The first evidence that a genome of maternal origin can become H, was the presence of a few embryos with eight E and eight H chromosomes among those produced by uninseminated females of the soft scale insect *P. hydrangeae* (Nur, 1963). All the embryos were produced parthenogenetically from unfertilized eggs. Oogenesis was fairly normal and resulted in the production of a haploid female pronucleus. The pronucleus then divided once and the products fused and formed a diploid zygote substitute. In most of the embryos, both genomes remained E, and these embryos developed into females. In about 5% of the eggs, however, half of the chromosomes became H (Fig. 2), and these embryos either failed to mature or developed into adult males that were nonfunctional.

A more complex situation was described by Phillips (1965) in two other soft scale insects, *Lecanium putumani* and *L. cerasifex*, and examined cytologically by Nur (1972a). Several populations of both species consisted of inseminated females (that produced about 80% females), uninseminated females that produced only males, and uninseminated females that produced either only females or mostly females. In *L. cerasifex*, all the uninseminated females that produced only females carried needle-like bacterial symbionts that were transmitted through the egg; the other two types of female lacked such symbionts. The absence of inseminated females with the needle-like symbionts indicated that the females carrying the bacteria did not mate, and must represent a separate race or form that reproduced only by parthenogenesis. All these observations could
be explained by assuming that the populations of both species consisted of a sexual form, in which females developed from fertilized eggs and males from unfertilized eggs, and a purely parthenogenetic form, such as the one studied in *P. hydrangeae*, in which females developed from unfertilized eggs (Nur, 1972a).

The observation that in *P. hydrangeae* one of two identical genomes of maternal origin becomes H led to the conclusion that in this species the genomes are imprinted according to their position in the egg and not according to their parental origin (Nur, 1963). These, and later observations (Nur, 1972a) also led Chandra and Brown (1975) to postulate that it is unlikely that the mechanisms of imprinting in soft scale insects and in the species in which males develop from fertilized eggs are fundamentally different. Thus, Chandra and Brown proposed the following. (1) In coccids in which males develop from fertilized eggs, the imprinting occurs in the egg after fertilization and before the fusion of the male and female pronuclei. (2) Whether an egg will develop into a male or a female depends on the presence, size and position of the imprinting region. (3) In all the eggs, once a genome is imprinted it will either become H or, in the armoured scale insects, will be eliminated.

The model of Chandra and Brown is consistent with what is known about coccids and helps focus attention on the need to distinguish between imprinting and its consequences. Moreover, it is clearly superior to the model of Sager and Kitchin (1975) which is based on the now invalid assumption that heterochromatization is the result of the action of a restriction endonuclease and that in the males the DNA is not methylated. However, at present there is neither a theoretical nor experimental basis for dismissing the possibility that differences between the sperm and the egg may play a role in imprinting and heterochromatization (as Chandra and Brown did in their model).

**Heterochromatization of genomes derived from the polar bodies**

In most species of mealybugs and armoured scale insects, the polar bodies reenter the egg and contribute to, or give rise to, large polyploid cells (the mycetocytes) that house intracellular bacterial symbionts. The origin of the mycetocytes was first described by Schrader (1923) in several species of mealybug, including *Pl. citri*. Schrader reported that after reentering the egg, polar body I (which is diploid) and polar body II (which is haploid) fused and formed a triploid polar nucleus. The polar nucleus divided several times and then the resulting nuclei fused, either in pairs or with a zygotic nucleus and produced nuclei that varied in the level of polyploidy. In the armoured scale insects, the sequence is fairly similar (Brown and Bennett, 1957; Brown, 1965) except that the polar nucleus fuses only with one nucleus of zygotic origin, thus producing mycetocytes that are pentaploid.

In the course of a cytological survey of the armoured scale insects, Brown (1965) observed that, in *Phenacaspis pinifolia*, three of the five genomes present in the mycetocytes of both males and females were H. Because at least one of the three genomes had to be of polar body origin, and the polar bodies contributed three genomes, Brown proposed that all three originated from the polar bodies. This case is intriguing because (i) the three genomes became H only after the mycetocytes stopped dividing mitotically (as they do in most other species) and began to increase in size by endoreduplication; (ii) the three genomes became H in eggs in which the PG failed to do so.

**Control of the euchromatization of the PG during development**

In males of mealybugs and of other families in which one genome becomes H, this genome later becomes E (and genetically active) in several tissues or organs. These include the midgut, the Malpighian tubules, the salivary glands, the oenocytes and the serosa (Nur, 1967, 1972b). One characteristic of most of these tissues is that their nuclei later become polyploid as a result of endoreduplication or endomitosis. Euchromatization, however, is apparently not an essential step in the development of these tissues, because the types of tissue involved may vary between congeneric species (Nur, 1967). Moreover, the frequency of cells in which euchromatization occurred sometimes varies between individuals (Nur, 1972b). However, in those nuclei or cells in which the PG remained H, it usually either did not replicate, or replicated once, while the E chromosomes replicated several times (Nur, 1966 and 1972b; Lorick, 1970).

Because it is unlikely that an inactive genome will control its own behaviour, it may be assumed that whether or not in a particular cell the PG becomes E is controlled by the E set present in the cell. That this is indeed the case was demonstrated by the behaviour of the PG in the serosa (outer embryonic membrane) of hybrid males. Thus, when males of the mealybug *Pseudococcus calcicola* (in which in the serosa of male embryos the PG remained H) were crossed to females of *Ps. affinis* (in which the PG became E), the PG in the serosa of the hybrid males became E.

There is now ample evidence that the extent to which the DNA is methylated is different in active and inactive X chromosomes of the mammalian female (reviewed in Riggs, 1990). Thus the level of m5C may play an important role in the establishment and maintenance of the H state. It is of interest to note that in the mealybug *Ps. calcicola* the amount of m5C present in the DNA of males is significantly higher than that present in the DNA of females (0.68±0.02 percent versus 0.44±0.04 percent) (Scarbrough et al. 1984). The level of m5C is also higher in the DNA of males of *Ps. affinis* (1.34±0.13 versus 1.26±0.06 percent), but the difference is not statistically significant. However, these data do not provide information about whether the higher level of m5C observed in the DNA from males is
Heterochromatization and its reversal in mosaic embryos

In his study of the effect of aneuploidy on sex determination in the mealybug *P. citri*, Chandra (1963) analyzed 272 embryos produced by triploid females (with 3n = 15). The number of chromosomes present in these embryos ranged from 6 to 19, and 12 also contained a haploid sector (with five chromosomes). All the embryos were in the blastoderm stage and thus developed past the stage at which heterochromatization usually takes place (the fifth or sixth mitotic division). Thus, all the embryos could be sexed by the presence or absence of an H set of chromosomes.

Of the 12 mosaic embryos, the PG was H in the zygotic sector of seven, and these will be referred to as the mosaic male embryos. However, in five of the seven, the chromatin in the interphase nuclei of the haploid sector appeared diffuse (E). In the remaining two embryos, which were somewhat younger, the appearance of the chromatin in the interphase nuclei of the haploid sector ranged from that typical of euchromatin to that of typical heterochromatin. On the basis of these observations, Chandra (1963) concluded that the genome present in the haploid sector was of paternal origin, and that in both the haploid and the zygotic sectors of the male embryos the PG became H. However, because of the absence of a genetically active genome in the haploid sector, the H set either became E, or was in the process of becoming E.

A haploid/diploid mosaic male embryo was also observed in the mealybug *Pseudeococcus affinis* (Fig. 3). As in the mosaic male embryos studied by Chandra (1963), the haploid sector was also of paternal origin (as indicated by the presence of a B chromosome, which the father carried and the mother did not) but the PG was E. An additional feature was the presence in the haploid sector of larger, diploid nuclei with ten E chromosomes and two B chromosomes (Fig. 3F,G). Thus, these nuclei apparently resulted from the fusion of two haploid nuclei.

In his 1963 paper, Chandra also reported that among the 272 embryos analyzed, eight received from the egg fewer than five chromosomes (the haploid number). On the basis of whether the PG was E or H, three were females and five were males. Among the five males, one had only one E chromosome and two had two E chromosomes. Because most of the embryos were males, there was no reason to assume that one or more of the female embryos is a male embryo in which the H set had become E. Thus, Chandra (1963) concluded that the presence of a single E chromosome was sufficient to maintain the PG in the H state. This conclusion, however, now seems unlikely, because it implies that the E chromosome is genetically active and carries the appropriate locus or loci necessary for maintaining the PG in the H state. However, the genetic activity of only about 1/5 of the genome would be expected to block the development of such an embryo soon after it was initiated. Thus, it is much more likely that in the male embryos with one to four E chromosomes the PG remained H because the absence of a complete set of E chromosomes prevented these embryos from proceeding in their development to the stage at which in the haploid sector the H set became E. Moreover, the assumption that the mosaic embryos proceeded in their development further than the male embryos with one to four E chromosomes is consistent with Chandra’s statements that the development of the latter was arrested fairly soon after the formation of the blastoderm, while that of the aneuploid mosaic male embryos was arrested at a late blastoderm stage.
The ability of the PG to become H in the haploid sector of at least two of the mosaic male embryos indicates that the genome of maternal origin does not play a role in the process of heterochromatization. Thus, this process must involve an interaction between the PG and something in the egg cytoplasm.

Did irradiation cause the euchromatization of the PG in primordial spermatogonia?

A study that resulted in the production of translocations between E and H chromosomes also led to the suggestion that the maintenance of the H state may be disrupted by the inactivation of only one or a few genes (Nur, 1970). In that study, first instar males of the mealybug *P. affinis* were treated with 3000 rep of gamma irradiation, and spermatogenesis was examined at the late second instar stage. Unexpectedly, however, the testes of 35 of the 173 males examined contained, in addition to spermatocytes with one E set of chromosomes and one H set, also whole cysts, or larger regions of the testes, in which all the spermatocytes contained two sets of E chromosomes. Moreover, the development of these spermatocytes was fairly normal, except that it resulted in the production of diploid spermatids.

In several of the cysts or regions with two sets of E chromosomes, the karyotype was asymmetrical, e.g. it included only one large and/or one small chromosome. As was pointed out earlier, cisocid chromosomes are holocentric, and fragments are rarely lost (Hughes-Schrader and Ris, 1941). Thus, one can rule out the possibility that in the spermatocytes with two E sets, the entire H set was lost and that, in some way, the number of chromosomes was doubled. This apparently leaves only two alternative explanations for the presence of spermatocytes with two E sets: (i) as a result of the irradiation, the H set became E in some of the primordial spermatogonia and these gave rise to the cysts or regions with only E chromosomes, or (ii) other cells in the embryo, in which the H set had already become E prior to the irradiation, dedifferentiated, and then redifferentiated as primordial spermatogonia. Because the second explanation requires the occurrence of two unlikely events (dedifferentiation and redifferentiation of many cells), I believe that the most likely explanation for absence of the H set is that the radiation caused the inactivation of a gene(s) on the E set which is essential for the maintenance of chromosomes in the H state. This explanation in combination with Chandra's (1963) conclusion that in the haploid sectors of the mosaic males the PG became E, strongly suggests that as in the case of the facultative heterochromatization of the X chromosome in the mammalian female, the default state is activity (Riggs, 1990). It may be noted, however, that the evidence that suggests that euchromatization can occur by default, tells us very little about whether the euchromatization of the PG in certain male tissues also occurs by the inactivation of a locus (or loci) involved in the maintenance of the H state, or whether in these tissues the MG plays an active role.

References


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