

# HETEROCHROMATIN AND GENETIC ACTIVITY IN MEALY BUGS

## I. COMPENSATION FOR INACTIVE CHROMATIN BY INCREASE IN CELL NUMBER

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IN male coccids displaying the lecanoid system of chromosome behavior one of the haploid sets of chromosomes (the paternal contribution) heterochromatizes early in embryogeny, and in most tissues remains inactive throughout the life history of the organism (reviewed in BROWN and NUR 1964). This heterochromatin is inactive genetically (reviewed in BROWN and NUR 1964) and the inactivity is the result of repression of RNA synthesis (BERLOWITZ 1965).

Female mealy bugs do not exhibit this alteration of chromosome structure. Indeed heterochromatization of the paternal set is the only known chromosomal dimorphism that distinguishes the sexes in these species.

Until late in the second larval instar, male and female mealy bugs are the same size and morphologically indistinguishable; however, cytologically they are readily distinguishable. The males possess physiologically haploid tissues, and yet up to this time apparently keep up metabolically with normal, diploid females. Under these conditions one would expect to find increases either in the amount of genetic material or in the activity of the genes in male mealy bugs to compensate for the repression of a haploid set of chromosomes. Towards the end of second instar, a rather profound sexual dimorphism ensues (NELSON-REES 1960), detected in its earliest manifestation as a color difference in males and females.

In this paper experiments are described in which the amount of DNA/g in males and females was determined biochemically. Further experiments were designed to determine if the observed greater amount of DNA in males can be attributed to an increase in cell number in organs composed of cells displaying a heterochromatic set.

### MATERIALS AND METHODS

Analysis of DNA was performed on the mealy bug *Planococcus* = (*Pseudococcus*) *citri* (Risso). Cells were counted in this species and in the related mealy bug, *Pseudococcus obscurus* Essig; both of these species have a diploid chromosome number of 10. The males possess 5 heterochromatic chromosomes (the paternally derived haploid set) and 5 euchromatic chromosomes in many of their organs. The organs used in this study were the thoracic ganglion (Figure 1A, B), the supraesophageal ganglion (brain) (Figure 1C, D), and the Malpighian tubules of second instar larvae. All the cells in the male ganglia contain heterochromatic chromosomes. In the Malpighian tubules, on the other hand, the cells are polyploid and no heterochromatic chromosomes are present. Embryos selected were in the stage after gastrulation when the germ layers

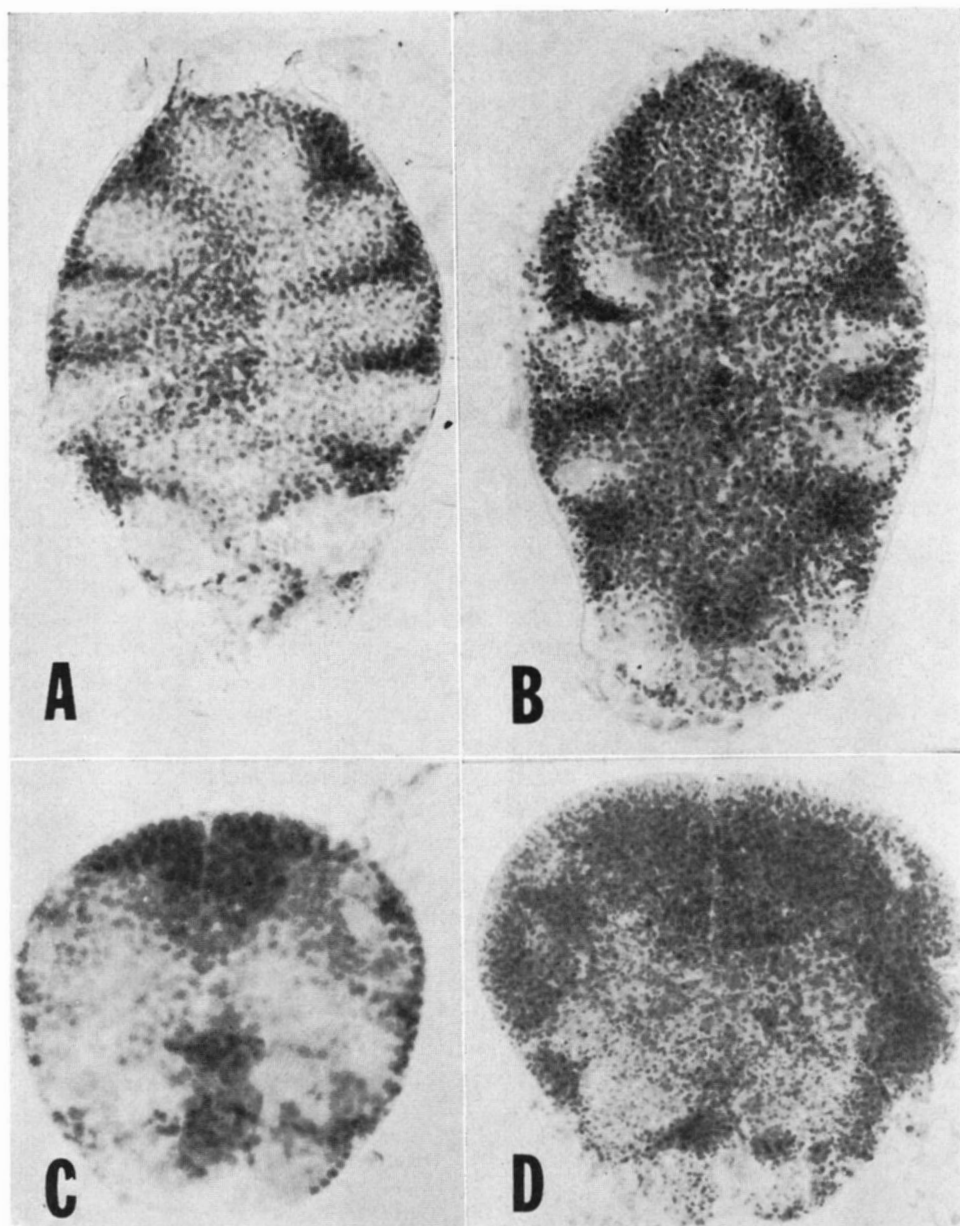


FIGURE 1.—Cell number in second instar mealy bugs; whole mounts of hollow organs. A. and B. thoracic ganglia, female and male; C. and D. brains, female and male; note greater cell number and visible heterochromatin in male as compared to female organs. ( $\times 500$ )

have involuted and are in a straight line within the ovariole (see Figure 89 of SHINJI 1919). Immediately after this stage the long axis of the germ band of the embryo begins to form the sigmoidal convolutions (SCHRADER 1923). Only embryos in the early straight line stage (0.280–0.300 mm) were utilized.

Brains and thoracic ganglia were dissected in aceto-carmin and squashed to a monolayer. Embryonated eggs were usually recovered from egg masses, though some were taken from gravid females. Egg masses were first fixed in chloroform-acetic acid (4:1) and then transferred to aceto-carmin. After penetration of the stain the germ band of the embryo was removed, measured, and squashed. Negatives were made with phase-contrast optics on  $4 \times 5$  plates at a magnification of  $160\times$ . From 2 to 5 frames were required for each organ and 2 frames for each embryo. Negatives were printed to a final magnification of approximately  $300\times$  and mosaic pictures were made from the frames. To avoid error the cells were marked off as they were counted. The Malpighian tubules contain approximately 60 polyploid cells each and could be counted easily when viewed in the phase contrast microscope.

For DNA extractions males and females were selected from synchronous cultures just after color differences were apparent. One hundred mealy bugs were weighed and used for each separate extraction. DNA was extracted from whole, second instar males and females by the method of SCHNEIDER (1957) and DNA determined on the hot TCA extracts by the diphenylamine reaction. All spectrophotometric analyses were performed in microcuvettes of 0.5 cc capacity, to permit the analysis of one-tenth the usual amount of material.

#### RESULTS

Results of the DNA analyses are shown in Table 1. Second instar males had 1.47 times as much DNA per gram as females. This result suggested that males may be compensating for their heterochromatin by producing more copies of their active genetic material.

One way in which males might accumulate more genes per organ than females is by accumulating a greater number of cells per organ. Comparisons of whole organ squashes of second instar male and female thoracic ganglia are shown in Table 2. Male thoracic ganglia possessed about 1.47 times the number of cells as female thoracic ganglia. The close correspondence of this figure and the one derived from DNA analysis of whole organs is probably coincidental. If the male organs were compensating fully for heterochromatization one would expect 2 times more cells in males than in females.

The cell numbers within another organ were compared to determine if similar results would be obtained. Brains were chosen because of their small size and because in males they were comprised of diploid cells showing heterochromatization. Preliminary results indicated a bimodal distribution of cell numbers in both

TABLE 1

*Comparison of the amount of DNA extracted from whole male and female mealy bugs (Planococcus citri) at second instar\**

Extraction	Amount of DNA (mg/g tissue)	Average (mg/g)
1. females	$1.87 \pm 0.08\dagger$	1.86
2. females	$1.85 \pm 0.04$	
3. males	$2.66 \pm 0.03$	2.74
4. males	$2.82 \pm 0.03$	

Ratio of average amount of DNA male/female: 1.47.

\* 100 organisms per extraction.

† average error.

TABLE 2

*Comparison of the number of cells in male and female mealy bugs (Planococcus citri)*

Sex	Organ	Stage	Number	Average number of cells and S.E.	Ratio males/females
M	Thoracic ganglion	Second instar	10	6,480 ± 87	1.47
F	Thoracic ganglion	Second instar	10	4,395 ± 98	
M	Brain	Early instar	5	5,924 ± 440	3.95
F		Second instar			
M	Brain	Early instar	2	1,498 ± 126	
F		Second instar			
M	Brain	Late instar	5	10,005 ± 605	4.46
F		Second instar			
M*	.....	290 μ Embryo	3	3,290 ± 93	
F*		290 μ Embryo			
M*	Malpighian tubule	Second instar	10	60.0 ± 0.8	0.99
F*	Malpighian tubule	Second instar	10	61.0 ± 0.5	

\* Data from *Pseudococcus obscurus*.

† Actual sizes of embryos: Female: 280 μ, 290 μ, 290 μ; Male: 280 μ, 290 μ, 300 μ. The average number of cells in three 400 μ females was 2600, fewer than in the smallest male embryo.

males and females. It was hypothesized that this distribution stemmed from a large increase in brain size during second instar. For this reason just post-molt second instars and very late second instars were selected for separate studies. Table 2 shows that the number of cells in both male and female brains increased considerably over the second instar period, but males maintained about 4 times as many cells as females. No similar change was observed in thoracic ganglia.

If all of the cells in whole organisms were counted, the ratio of male cells to female cells may be expected to resemble the difference in DNA per gram in whole organisms. When 290 μ embryos were counted, male embryos had 1.45 times more cells than female embryos (Table 2).

The results of counting cells from squashes of Malpighian tubules are also

shown in Table 2. For this polyploid, non-heterochromatic material the number of cells in males and females was essentially identical.

#### DISCUSSION

The results of the experiments described in this paper suggest that male mealy bugs can compensate for having a genetically inactive haploid set of chromosomes by having more cells than females of approximately the same larval stage and size.

NUR (1967) has accumulated evidence that in many organ systems of *P. obscurus* the problem of compensation is avoided. He has determined that cells destined to become, for example, oenocytes or Malpighian tubule cells revert to euchromaticity and often become polyploid. In other cells which remain heterochromatized the euchromatin endoreduplicates to produce a differential polyploidy (NUR 1966). Up to 48 copies of the euchromatic set but at most 2 copies of the heterochromatic set are ever found in these cells. The present results suggest that in organs which neither deheterochromatize nor polyploidize males increase the amount of genetically active material by increasing their cell numbers.

These results can be compared to the findings of FANKHAUSER (1952) and GRAHAM (1966) in amphibians. Working with salamanders, FANKHAUSER showed that for organs of equal size a decrease in ploidy from  $4n$  to  $n$  resulted in a compensatory increase in cell number. GRAHAM compared artificially induced haploid embryos of *Xenopus laevis* to normal diploids and found a cell ratio of 2.1:1, haploid: diploid at 24 hours post-fertilization. Organs of male mealy bugs containing a heterochromatic set of chromosomes apparently behave similarly to the haploid organs of amphibians with respect to dosage compensation.

In a specialized, differentiated cell only a portion of the genome is active. For example, a ganglion cell has active specialized genes for neural function, and also, in common with other cells, has active genes for maintenance functions. Presumably many genes, particularly those involved with the specialized products of other differentiated cell types, are inactive in ganglion cells. Thus, despite the fact that specialized cells require amplification of only a portion of their DNA in any given cell, male mealy bug cells uneconomically amplify their entire DNA complement. This seems to hold true also for the haploid organisms described above. Evidence suggesting that mealy bugs do not amplify their genome through differential polynemy is presented in a subsequent paper (BERLOWITZ and PALLOTTA (in preparation)).

The male thoracic ganglion has 1.47 times more cells than the female, while the male brain has about 4 times more cells than the female. The differences in ratio between male and female ganglia and male and female brains may reflect a difference in the activity of these organs during development. As in other insects, the brain in second instar mealy bug males probably plays a hormonal role in preparing for male metamorphosis in later instars. The female continues to molt in later stages but does not undergo the striking metamorphosis of the male (NELSON-REES 1960). She matures as a sub-imago and remains sedentary.

The mature male, on the other hand, is a highly mobile organism with developed sensory organs for flight and for locating females. These functions must also require an increased brain output when compared to females. Therefore, at least some of the increased cell number in male brains is probably attributable to preparation for sexual dimorphism rather than to heterochromatization. O'BRIEN (1956) has shown a similar phenomenon in the haploid-diploid, sexually dimorphic coccid, *Steatococcus tuberculatus* Morrison. In this species there is a four-fold increase in DNA in the wax glands of males as the result of endoreduplication of the haploid complement to tetraploidy. This is correlated with the onset of secretion of the cocoon in the early third instar. In the female which does not form a cocoon, there occurs no change in the nuclear content of DNA. The increased cell number in 290  $\mu$  whole embryos, on the other hand, most likely results entirely from compensation for heterochromatization. Metabolic levels from this stage through the active crawler period are probably very similar for males and females.

That the Malpighian tubules, a non-heterochromatic, polyploid organ, does not show a greater cell number in the male is supporting evidence for the contention that the observed increase in other organs is a form of compensation for heterochromatization.

It is possible to estimate the percentage of heterochromatic material in males from the amount of DNA per gram in males and females. If all cells in males were heterochromatic and diploid, and males, therefore, had to compensate for a genome half turned-off when compared to the female genome, the expected ratio of DNA males: females would be 2:1 rather than 1.47:1. This expectation is based on the following assumptions: (1) that the metabolism of the euchromatic male set is equal to one-half the metabolism of the diploid female set and (2) that the mealy bug normally uses all copies of its available active genes, that is, a hidden genetic reserve (extra homologous, polynemic, or serial copies of the same allele) is unavailable. Evidence for the validity of these assumptions is presented in a subsequent paper (BERLOWITZ and PALLOTTA, in preparation). If the assumptions are correct, then the deviation from the expected 2:1 ratio should be a good indication of the amount of cells with heterochromatic chromosomes in the male. The ratio 1.47:1 would correspond to an estimate of 47% of the cells in the male containing heterochromatic chromosomes.

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#### SUMMARY

It has been determined biochemically that male mealy bugs (Coccoidea: Homoptera) have 1.47 times more DNA than female mealy bugs. From these data it is estimated that 47% of the cells in males have heterochromatic chromosomes. Male mealy bugs have many organs containing cells in which one set of chromosomes is heterochromatic and genetically inactive (NUR 1966). When

all cells were counted in the germ band of male and female (290  $\mu$ ) embryos it was determined that males had 1.45 times as many cells as females, suggesting that the greater amount of DNA in males is the result of increased cell number. It was hypothesized that such an increase in cell number is a way in which male mealy bugs compensate for having organs containing cells with only a single complement of active euchromatic chromosomes. This hypothesis is supported by evidence that male brains and thoracic ganglia had from 1.47 to 4 times more cells than their female counterparts, while Malpighian tubules (containing, in both sexes, cells with polyploid, euchromatic chromosomes) showed no differences in cell numbers.

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