

Base Ratios in DNA in Male and Female *Pseudococcus citri*

THE mealy bug, *P. citri*, affords a unique opportunity for analysis of biochemical changes underlying chromosome behaviour. In the male embryos, the entire paternal set, that is, one half the total number of chromosomes, remains condensed in the non-dividing nuclei; this facultative behaviour is similar to that of typical heterochromatic elements such as sex chromosomes^{1,2}. Experiments with tritiated thymidine show that, like heterochromatic elements in other organisms^{3,4}, the paternal set in the males reproduces asynchronously from the maternal, or euchromatic, set⁵. As measured by dominant lethality² and mutant genes⁶, the paternal set is genetically inert in the males, but some 'residual' influences can be demonstrated^{7,8}.

Except for a few tissues, the paternal set maintains its heterochromaticity throughout the life of the male; at spermatogenesis it is segregated from the maternal, or euchromatic, set, and is not included in the sperm. In certain related forms, however, the heterochromatic state is reversed during spermatogenesis and the paternal chromosomes are segregated, apparently at random, to the sperm, to be transmitted to offspring of both sexes⁹. It seems likely, therefore, that the chromosomal changes responsible for facultative heterochromatization are ones which block rather than alter or destroy the genetic information. As a beginning we have isolated DNA from male and female insects and determined the base ratios from melting temperature and buoyant density measurements.

Mealy bugs were grown on potatoes, *Solanum tuberosum* L.; the large, gravid females containing both male and female embryos provided abundant material for establishment of experimental procedures; the second instar, prior to the extreme sexual dimorphism of later states, was used for the comparisons of males and females. No attempt was made to separate a yeast-like symbiont, present to about the same extent in males and females of the second instar; the DNA isolated includes symbiont nucleic acid also. DNA was isolated from 1 g of mealy bugs by the method of Marmur¹⁰. Phosphate was determined by the method of Fiske and SubbaRow¹¹, sealed down for the analysis of 10 µg of DNA. The best preparation of DNA had an $E(P)$ of 7,240. Chromatographic analysis was done on 1 mg of DNA from gravid females, hydrolysed 2 h in 70 per cent formic acid at 175° C. No methylcytosine was detected by Wyatt's procedure¹². The average results from six analyses for base content are given in Table 1.

Table 1. BASE CONTENT OF DNA FROM GRAVID, FEMALE MEALY BUGS

Base	Mole (%)
Adenine	34.2 ± 0.7
Thymine	31.9 ± 1.2
Guanine	16.2 ± 0.7
Cytosine	17.8 ± 1.3
Mole (%) guanine-cytosine	34.0 ± 1.1

Table 2. MELTING TEMPERATURES OF DNA FROM MEALY BUGS

Source	T _m	Mole (%) guanine-cytosine
Gravid females	84.4 ± 0.95	37.5 ± 1.0
Second instar males	84.9 ± 0.20	38.7 ± 0.6
Second instar females	84.9 ± 0.28	38.7 ± 0.8

Melting temperature profiles were determined on a DU Beckman spectrophotometer with a heated cell compartment. The solvent used was 0.15 M sodium chloride-0.015 M sodium citrate. The melting temperatures of DNA from gravid females and male and female second instar mealy bugs are given in Table 2. The mole per cent guanine-cytosine is calculated from published results¹³.

One melting profile for DNA of second instar males is given in Fig. 1. The profile of DNA from females is superimposable, but with a hyperchromicity of 1.39. No heterogeneity due to symbiont or heterochromatin appears in these curves.

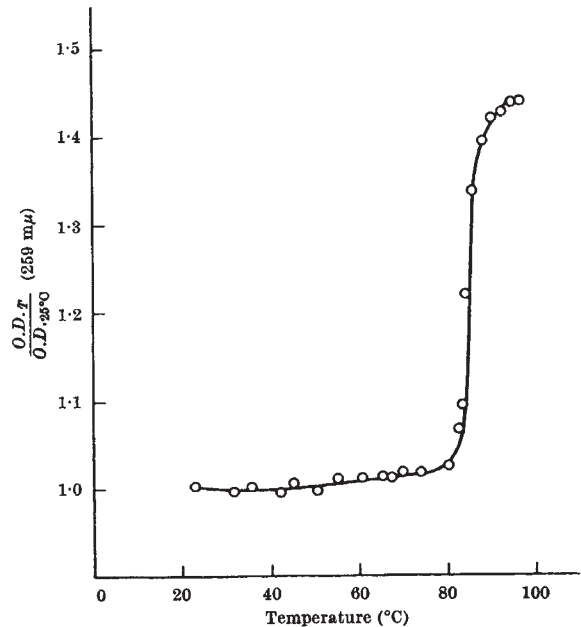


Fig. 1. Melting temperature profile of DNA from male mealy bug. Solvent used was 0.15 M sodium chloride-0.015 M sodium citrate. (Optical densities uncorrected for volume expansion)

The buoyant densities of DNA from both male and female second instar insects was 1.695 ± 0.001 , corresponding to 35.8 ± 1.0 mole (per cent) guanine-cytosine¹⁴.

The difference in base ratios by these three methods is not outside the variation reported in the literature¹⁴. It may be concluded that there is no evidence of a difference in base ratios greater than 1 per cent between DNA from male and female mealy bugs. Although genetic information no doubt could be destroyed without producing grossly detectable changes in base ratios, the fact that such cannot be demonstrated here is certainly in conformity with, rather than opposed to, the independent conclusion, from reversals of facultative heterochromatization, that the genetic information is masked rather than disrupted. Work is in progress to determine what differences may be found in other chromosomal components.

Buoyant densities in Cs¹³⁷Cl were determined by Dr. John Hearst, of the Department of Chemistry, University of California, at Berkeley. The λ phage DNA used as a density standard was a gift of Dr. Monica Reilly, of the Department of Microbiology, University of California, at Davis.

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