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# Nuclear DNA Content of the Emu

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*Abstract.* The nuclear DNA content of the emu relative to that of the chicken has been measured by microdensitometry of Feulgen-stained erythrocytes and found to be $1.236 \pm 0.028$ which is within the range of values previously found for birds. The statistical method for obtaining this value and its standard error is given in full.

## Introduction

Because avian red blood cells are nucleated, relative DNA values can be obtained by microdensitometry of fixed, Feulgen-stained erythrocytes (Atkin *et al.*, 1965). Nuclear DNA values for emu, *Dromiceius novae-hollandiae* (Lath.) and canary, *Serinus canaria* (L.) relative to chicken, *Gallus domesticus* (L.) are reported here.

A variety of methods for calculating the DNA value and its standard error has been reported so the statistical method is set out here in full.

## Materials and Methods

### *a) Preparation and Measurements of Cells*

A white Australorp cockerel was used as the control in both measurements. The canary and emu were both of unknown sex, the emu only two days old.

Blood was drawn into a heparinised syringe from wing veins of chicken and canary and from a leg vein of the emu chick. Blood samples from the two birds to be compared were collected within a few minutes of each other and thereafter were treated equally at every stage.

A few drops of blood were suspended in a large volume of hypotonic (0.075 M) KCl at 37° for 7 minutes and then centrifuged at 500 r.p.m. The cells were fixed in 3 parts methanol and 1 part glacial acetic acid for 3 minutes. A single drop of the control (chicken) cells and the cells to be measured (emu or canary) were placed at opposite ends of a slide (less than 1 mm thick) and air-dried. A plasticine strip was placed across the middle of the slide to prevent migration of cells from one drop into the other. Two such slides were prepared; control (X) and test (Y) cells were placed at different ends on each slide and the pair of slides was treated in this order, $\begin{matrix} X & Y \\ Y & X \end{matrix}$, throughout the process to minimise systematic position errors.

Immediately they were dry, the slides were hydrolysed in 1N HCl at 60° for 5 minutes, dipped into cold water and then stained by the Feulgen process (Darlington and LaCour, 1969, p. 165). After dehydration they were mounted in a neutral medium and stored in the dark to prevent fading of the stain.

DNA values were measured on a Barr and Stroud Integrating Microdensitometer GN2 at a wavelength of 5650 Å and only large, well-spread erythrocytes were measured. A measurement was taken as the mean of three sequential readings; previous studies with the instrument have shown this source of variance to be insignificant. Twenty five cells of each species on each of two slides were read in the canary measurement and twenty cells of each species on each of two slides in the emu measurement.

*b) Calculation of the Ratio and Its Standard Error*

After the DNA contents of the two species have been shown to be different by analysis of variance of the microdensitometer readings (Table 1), the ratio of the two values and its standard error can be estimated.

If the DNA content of erythrocytes is being measured, for instance in birds or reptiles, then there will be no difficulty in reading the required number of cells under each coverslip so that a simple balanced analysis can be performed. However, if mitotic cells are being measured such as plant root-tip cells or mammalian lymphocytes, then it may often be necessary to measure different numbers of cells under each coverslip. For this reason the general analysis is explained.

The ratio of the DNA content of species X to species Y and its variance are estimated as shown below. The $x$ values are measurements of species X and the $y$ values of species Y. The method is general for the case where there are $l$ slides and the number of cells under one coverslip is not necessarily the same as the number measured under any of the other $2l-1$ coverslips. The expressions simplify if the experiment is balanced.

| Slide 1 ..... | | Slide $i$ ..... | | Slide $l$..... | |
|---|---|---|---|---|---|
| $x_{1,1}$ | $y_{1,1}$ | $x_{1,i}$ | $y_{1,i}$ | $x_{1,l}$ | $y_{1,l}$ |
| . | . | . | . | . | . |
| . | . | . | . | . | $y_{n_l,l}$ |
| . | . | . | . | . | |
| . | . | $x_{m_i,i}$ | . | . | |
| . | . | | . | . | |
| . | . | | . | $x_{m_l,l}$ | |
| $x_{m_1,1}$ | . | | . | | |
| | . | | $y_{n_i,i}$ | | |
| | . | | | | |
| | $y_{n_1,1}$ | | | | |
| No. cells | $m_1$ | $n_1$ | $m_i$ | $n_i$ | $m_l$ | $n_l$ |
| Mean | $\bar{x}_1$ | $\bar{y}_1$ | $\bar{x}_i$ | $\bar{y}_i$ | $\bar{x}_l$ | $\bar{y}_l$ |
| s.d. | $s_{x_1}$ | $s_{y_1}$ | $s_{xi}$ | $s_{y_i}$ | $s_{xl}$ | $s_{yl}$ |

Let

$$k_x = \frac{1}{l}\sum_{i=1}^{l}\frac{s_{xi}}{\bar{x}_i}, \quad k_y = \frac{1}{l}\sum_{i=1}^{l}\frac{s_{yi}}{\bar{y}_i} \quad \text{and} \quad c_i = 1 + \frac{k_y^2}{n_i}$$

Then an estimate of the ratio, $R = \frac{\text{Mean species X DNA content}}{\text{Mean species Y DNA content}}$ for the $i^{th}$ slide, $\hat{R}_i$, and its variance, $V(\hat{R}_i)$, are given by

$$\hat{R}_i \cong \frac{\bar{x}_i}{\bar{y}_i} \text{ (where } E(\hat{R}_i) = Rc_i) \quad \text{and} \quad V(\hat{R}_i) \cong R^2 \left[\frac{k_x^2}{m_i} + \frac{k_y^2}{n_i}\right]$$

Combining the data from all slides,

$$\hat{R} = \sum_{i=1}^{l} w_i \hat{R}_i \quad \text{and} \quad V(\hat{R}) \cong \sum_{i=1}^{l} w_i^2 V(\hat{R}_i)$$

where $w_i$ are weights chosen such that $\hat{R}$ is the best linear unbiased estimate of R. These are given by

$$w_i = \frac{c_i}{V(\hat{R}_i) \sum_{i=1}^{l} \frac{c_i^2}{V(\hat{R}_i)}} \cong \frac{c_i}{\left[\frac{k_x^2}{m_i} + \frac{k_y^2}{n_i}\right] \sum_{i=1}^{l} \frac{c_i^2}{\frac{k_x^2}{m_i} + \frac{k_y^2}{n_i}}}$$

Having obtained $w_i$, $\hat{R}$ can be obtained and used to calculate $V(\hat{R}_i)$ and hence $V(\hat{R})$. If $m_i = n_i$ for all $i$ and each $m_i$ is sufficiently large, then $c_i \cong 1$ and $w_i = \frac{m_i}{\sum_{i=1}^{l} m_i}$.

In addition, if $n_i = n_{i+1}$ for all $i$, then $w_i = \frac{1}{l}$. A Fortran computer program has been written which performs the analysis of variance and obtains the above estimates.

## Results and Discussion

Analyses of variance for the two comparisons are shown in Table 1. In each case the "between species" MS is highly significant. The "between slides" MS's are also significant, indicating real variations in the staining procedure, but since the "Interaction" MS is negligible for each comparison this is not important.

The DNA value of the canary relative to the chicken is $1.260 \pm 0.012$. This is close to the value of 1.294 reported by Atkin *et al.* (1965) who used a similar technique but larger than the value of 1.184 found by Bachmann *et al.* (1972) who used liver cells and a much longer hydrolysis time. The canary had one of the largest DNA contents of the 23 bird species examined by Bachmann. Our estimate for emu relative to chicken is $1.236 \pm 0.028$, about the same value as the canary. Using Bachmann's calibration this is equivalent to about 4.27 picograms.

We killed the emu chick and examined mitosis in colchicinised bone marrow cells. The karyotype reported by Takagi *et al.* (1972) who found $2n = 80$, was confirmed. It appears that the emu has both a typical avian karyotype and nuclear DNA content.

Table 1. Analyses of variance for nuclear DNA content comparisons

| Source | df | SS | MS | F | *P* |
|---|---|---|---|---|---|
| | (a) Canary versus chicken | | | | |
| Between slides | 1 | 155.7504 | 155.7504 | 172.60 | $< 0.001$ |
| Between species | 1 | 486.6436 | 486.6436 | 539.30 | $< 0.001$ |
| Interaction (slides × species) | 1 | 1.4400 | 1.4400 | 1.60 | |
| Within coverslips | 96 | 86.6264 | 0.9024 | | |
| Total | 99 | 730.4604 | | | |
| | (b) Emu versus chicken | | | | |
| Between slides | 1 | 207.0461 | 207.0461 | 78.57 | $< 0.001$ |
| Between species | 1 | 212.2261 | 212.2261 | 80.53 | $< 0.001$ |
| Interaction (slides × species) | 1 | 0.0011 | 0.0011 | 0.00 | |
| Within coverslips | 76 | 200.2815 | 2.6353 | | |
| Total | 79 | 619.5549 | | | |

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