# DNA content of haploid and diploid nuclei in a male XO type bug

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*Summary.* The DNA content of sperm, spermatide, and brain cell nuclei was determined by Feulgen microspectrophotometry in *Dysdercus intermedius.* Both in haploid and diploid cells 2 classes of DNA content were found. The differences are due to a large X chromosome in this male XO type bug. The absolute amounts of DNA represented by the X chromosome and the complete genome were calculated.

Sex determination of the XO type was discovered in a bug in 1890 by Henking[2] and later described in several insects[3]. In Lepidoptera the female is the ZO type; in Orthoptera, Hemiptera and Heteroptera the male is the XO typed heterogametic sex[4]. It is assumed that such karyotypes evolved from XY ancestors by translocation or Robertsonian fusion of the Y chromosome[5]. In *Drosophila* the small heterochromatic Y chromosome carries only a few genes concerned with male fertility[6]. The transcriptional activity is restricted to spermatogenesis[7,8]. On the other hand, many genes are located in the large X chromosome including loci for sex determination and oogenesis[9]. The X chromosome contributes 18% of the haploid genome DNA in *Drosophila*[9,10] and 17% in *Gryllus*[11]. As no data from Heteroptera are available, we measured the DNA content of haploid and diploid male and female nuclei in the cotton bug, *Dysdercus intermedius,* and calculated the X chromosome value in this male XO type insect.

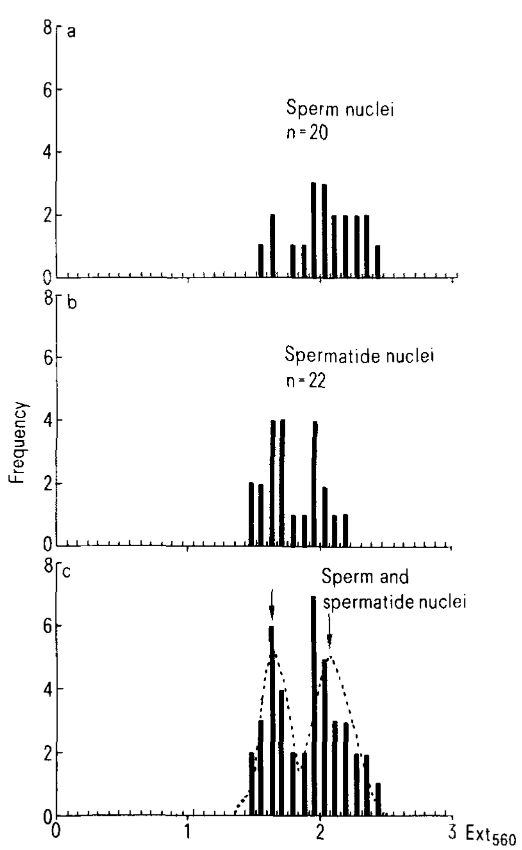


Figure 1. Histograms of DNA measurements of male germ cells in the cotton bug, *Dysdercus intermedius.* Ordinate: absolute frequency of the determinations; abscissa: extinction equivalents in arbitrary units, Zeiss UMSP measurements of Feulgen stained nuclei at 560 nm wavelength, average of 3 measurements/nucleus. Arrows indicate mean values. The existence of 2 classes of DNA content can be seen especially from the combined data plot in c. The values are of normal distribution in both classes.

*Material and methods.* Sperm, spermatides, and brain cells were isolated from adult bugs of the laboratory strain. The cells were lysed in 0.5% Triton X 100 solution to which 200 mg/l spermidine phosphate (Sigma, München) had been added to stabilize the nuclear membranes. The nuclear preparations were air-dried on chromalum-coated slides and postfixed in ethanol/acetic acid 3:1 for 30 min. Hydrolysis was performed in 5 N HCl for 40 min at 21 ± 1 °C. The Feulgen reaction and microspectrophotometric DNA determination at 560 nm wavelength using a Zeiss UMSP II were done as routine techniques[12]. For each type of nucleus two or more bugs were used; 2 slides were made from each bug. Each nucleus was measured 3–4 times and the average extinction was calculated. The data were processed on a Zeiss Kontron IBAS I system.

*Results.* The extinction equivalents in arbitrary units vary from 1.56 to 2.44 in sperm nuclei (fig. 1a) and from 1.46 to 2.19 in spermatide nuclei (fig. 1b). The densely packed DNA in the sperm head nuclei apparently resulted in a somewhat higher extinction[10,12]. The combined data clearly show 2 clusters of values, both of normal distribution, with peaks at 1.63 and 2.08 (fig. 1c) representing 2 groups of male germ cells with different DNA content on the haploid genome level.

In the diploid brain nuclei measured as an example of non-polyploid somatic cells[13], extinction equivalents vary from 3.02 to 4.84 in males (fig. 2a) and 3.18 to 5.24 in females (fig. 2b). Both data groups are of normal distribution. The difference of the means, 3.87 ± 0.40 and 4.22 ± 0.63, is significant according to correlation analysis, U-test and T-test on a 0.90 level of confidence. So the diploid somatic cells also represent 2 classes of DNA content with more DNA in the female nuclei. Only little data could be obtained from the clearly diploid, male germ cells which were first order spermatocytes. The mean of 6 measurements, 3.96 ± 0.34, is pretty close to the corresponding male diploid brain nucleus value.

The amount of DNA corresponding to our arbitrary $E_{560}$ equivalents was determined using red blood cells of the domestic fowl, *Gallus domesticus,* as a reference of $2.30 \times 10^{-12}$ g DNA/nucleus[14]. The nuclei measured (fig. 3) averaged 5.83 ± 0.39 extinction equivalents. One arbitrary extinction unit in our measurements therefore corresponds to 0.39 pg of DNA.

Table 1. DNA contents in haploid and diploid tissues in *Dysdercus intermedius*

| Type of nucleus | DNA haploid (pg) male-producing | DNA haploid (pg) female-producing | DNA diploid (pg) |
|---|---|---|---|
| Sperm | 0.62 | 0.82 | |
| Spermatide | 0.64 | 0.78 | |
| Combined data | 0.64 | 0.81 | |
| Male brain | | | 1.51 |
| Female brain | | | 1.65 |

The calculated DNA contents of the haploid and diploid *Dysdercus* cells measured are given in table 1. The assumption that in male germ cells a low DNA content is normal for male-producing sperms without an X chromosome, and a high DNA value for female-producing sperms with an X chromosome, is in good accordance with the data obtained from male and female diploid brain cells. Assuming that egg nuclei, which could not be measured for technical reasons, would have the same DNA content as female-producing sperms, the DNA value of a male zygote is 1.45 pg and of a female one 1.62 pg. Both calculations are confirmed by the male and female brain cell data (table 1). The sex specific difference in the DNA content is 0.17 pg in the germ cell and 0.14 pg in the brain cells. Within the given variation of the measurements the two values can be regarded as the same. This difference in the DNA content of haploid as well as diploid cells must be contributed by the X chromosome. So in diploid XX female cells about 17% and in diploid XO male cells about 9% of the nuclear DNA content is contributed by the large X chromosome in *Dysdercus intermedius*. In haploid cells with an X chromosome this contributes 21% to the genome (table 2).

*Discussion.* Among insects, those with a panoistic follicle type have high species-specific DNA values, and those with a polytrophic-meroistic type lower ones[11,15]. The few data available from insects with telotrophic-meroistic ovarioles are intermediate[11]. Our *Dysdercus intermedius* determinations fit very well into this general picture (table 2).

It can be concluded that despite the absolute size of the genome the relative amount of insect X chromosomes tends to be about 20% of the haploid chromosome set including an X chromosome. This is true both for XY (i.e. *Drosophila*) and XO types *(Gryllus, Dysdercus)* of sex determination, regardless of the size of the X chromosome. There seems to be no evolutionary interference between the genes located on insect X chromosomes, the existence of Y chromosome, and the follicle type. On the other hand, there are several correlations between the species-specific DNA content, the type of oogenesis, and the time required for embryonic development in insects[11,16].

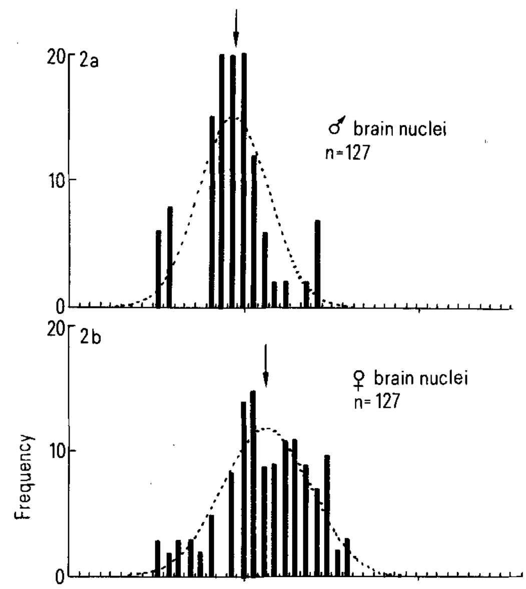


Figure 2. Histograms of DNA measurements of male and female diploid brain nuclei in *Dysdercus intermedius*. Both data groups are of normal distribution. The mean values differ, indicating a higher DNA content in the female cells.

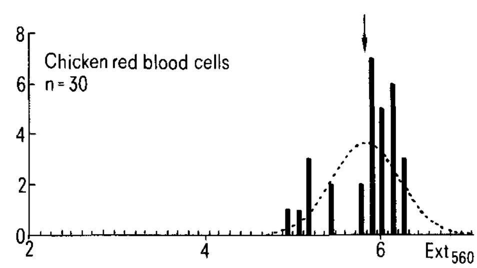


Figure 3. Histogram of DNA measurements of red blood cell nuclei in the domestic fowl, *Gallus domesticus*, used for the calibration of the arbitrary units. Ordinate, abscissa, and procedure as in figure 1.

Table 2. Comparison between the haploid DNA content and the size of the X chromosome in insects with different types of ovarian follicles

| | DNA content of sperms carrying the X chromosome (pg) | Size of the X chromosome (pg) | Size in basepairs ($\times 10^7$) | % of haploid genome | Follicle type |
|---|---|---|---|---|---|
| *Gryllus*[11] | 2.30 | 0.39 | 36.9 | 17 | Panoistic |
| *Dysdercus* | 0.81 | 0.17 | 16.1 | 21 | Telotrophic-meroistic |
| *Drosophila*[9,10] | 0.18 | 0.032 | 3.0 | 18 | Polytrophic-meroistic |

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