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Ploidy Determination in Insects

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Parthenogenesis is often associated with polyploidy. A single population may contain several ploidy levels (7). To estimate the distribution of ploidy levels allozymes or microsatellites can be used, but homozygosity and null alleles can lead to underestimates (6). These estimates can be more or less accurate, but the ploidy level of single individuals can rarely be determined.

In most cases, it is desirable that the estimate of ploidy level is accurate or that the ploidy level of every individual is known. Flow cytometry has previously been used for this purpose (3,5). This method has the disadvantage of requiring expensive equipment. Direct counting of the chromosomes from every individual is accurate but has the disadvantage of being extremely time consuming.

Here, I present an inexpensive and easy method to determine the ploidy level of a single individual if the different chromosome numbers within the species have been previously established. Chromosome numbers can be determined by paraffin sectioning of oocytes or by staining chromosomes in mitosis. The method described below relies on the fact that nuclei with different number of chromosomes are of different size (2). In most species in which sexual forms exist, males can be used as a diploid nuclear size standard if the chromosome number has not been established. A similar approach has been used previously in vertebrates where the erythrocyte size is correlated with ploidy level (1).

Weevils (*Otiorhynchus scaber*) frozen at -70°C were placed in 1× Citrate-PBS (120 mM NaCl, 2.7 mM KCl, 10 mM NaPO₄, pH 7.2, 20 mM sodium citrate). Malpighian tubules were dissected and placed in Citrate-PBS containing 1/8 volume of propidium iodide staining solution [0.05 mg/mL propidium iodide, 0.1% sodium citrate, 0.03% Nonidet® P-40 (4)]. The Malpighian tubules were stained for 30 min at room temperature. The nuclei were then photographed with a digital camera at 200× magnification. To avoid drying and flat-

Table 1. Average Area and Standard Deviation of the Nuclei from the Three Ploidy Levels

	2n	3n	4n
Average Area	868.3	1337.6	1686.3
SD	127.94	64.86	82.08
t-test comparing 2n and 3n	$P = 9.58 \times 10^{-8}$		
t-test comparing 3n and 4n	$P = 6.76 \times 10^{-9}$		
Two-sided t-tests assuming unequal variance were performed in Microsoft® Excel® 2000.			

tening of nuclei, the Malpighian tubules are stained and photographed on slides with concave wells covered by cover glass. At least 10 nuclei from every individual are photographed and compared.

The nuclei from the weevil individuals form three distinct size classes with no overlap (Figure 1). Only sphere-shaped nuclei were photographed. Most nuclei were of the same size, but some larger nuclei were also present. Only the smaller nuclei were photographed. The larger size is probably due to endopolyploidy. A flow cytometric study of *Heliothis virescens* (Lepidoptera) showed that, although there may be high endopolyploid chromosome numbers in the Malpighian tubules, most of the nu-

clei had the diploid amount of DNA (8).

Using Adobe® Photoshop® 5.5 and dot-blot analysis in Gel-Pro® Analyzer 3.0 (Media Cybernetics, Silver Spring, MD, USA), the area of 10 nuclei were measured from three individuals of different ploidy levels. The nuclear area differed significantly among the three forms (Table 1).

I also calculated the volume of the nuclei based on the average area of the three forms. The volume seems to increase linearly with ploidy level (2n: 19247, 3n: 36799, and 4n: 52090; volumes were calculated from the measured areas in Table 1).

To test how applicable the method is to determine ploidy levels in different insects, I dissected Malpighian tubules from *Apis mellifera* (honeybee), *Euura mucronata* (bud-galling sawfly), and *Coccotrypes dactyliperda* (the date pit beetle, Scolytidae) males and females. The males are haploid, and the females diploid in these species. Their nuclei formed two non-overlapping size classes. Figure 2 shows the difference in *Apis mellifera*.

It is possible to measure the nuclei in the microscope if a camera is not available. Since only the Malpighian tubules are used, the rest of the individual can be used for DNA preparation. It

is important that the nuclei of the individuals are intact. Therefore, live material or material that has been frozen alive should be used. Using this method, the ploidy level is determined by comparing the nucleus area from individuals of unknown ploidy level to the nuclei of known ploidy level.

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Figure 1. Nuclei from diploid, triploid, and tetraploid *Otiorhynchus scaber* Malpighian tubules stained with propidium iodide. Photographed at 200× magnification.

Figure 2. Nuclei from *Apis mellifera* male and female. Photographed at 200× magnification.