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Scientific note

A scientific note on a simple method for karyotyping honey bee (*Apis mellifera*) eggs*

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In the honey bee (*Apis*) sex is determined by the *complementary sex determining* locus or *csd* (Beye et al., 2003), located on linkage group 3 of the honey bee genome (Solignac et al., 2007). Diploid individuals that are heterozygous at the *csd* are female, whereas individuals that are haploid, and therefore hemizygous at the *csd* are male (Cook and Crozier, 1995). Rarely, a diploid individual will be homozygous at the *csd*, usually due to inbreeding. These individuals develop as diploid males, but are killed by workers at the first larval instar (Woyke, 1963). Diploid males can be reared artificially, but doing so is tedious (Polaczek et al., 2000).

Sometimes it is desirable to be able to determine the ploidy of honey bee eggs. For example, it may be useful to determine if low egg viability is due to the presence of haploid eggs in worker cells, or to other causes such as policing of worker-laid eggs or disease. Although there are effective protocols for examining metaphase chromosomes from the gonads of adult queens and drones (Hoshiba and Kusanagi, 1978; Hoshiba and Okada, 1986) or from the brain ganglia of pre-pupae (Stanimirovic et al., 2005) we have been unable to find a procedure suitable for examining metaphase chromosomes in eggs. Here we report a simple protocol for determining the ploidy of honey bee eggs based on Imai et al. (1988), who provide elaborate details of the fixation procedure.

To obtain biological material we caged a queen on an empty comb comprising worker and drone sized cells overnight. We then collected 10 eggs from worker cells on three consecutive days after oviposition and 12 three day-old eggs from drone cells. We cut freshly harvested eggs in half and incubated them in a hypotonic colchicine solution (0.005% colchicine in 1% sodium citrate solution) on a clean glass slide for 40 minutes. We then drained the solution off the slide and saturated the tissue with freshly-prepared fixative solution I (60% 1:1 acetic-ethanol: glacial acetic acid 3 mL/ethanol (99.5%) 3 mL/distilled water 4 mL). We then dissociated the tissue with two needles and quickly added two drops of fixative II (1:1 acetic-ethanol: glacial acetic acid 2 mL/ethanol (99.5%) 2 mL; freshly prepared) over the spread, draining off the fixative I and blotting it from the edge of the slide using strips of filter paper. Fixative III (100% glacial acetic acid) was then dripped over the preparation while draining off the remaining fixative II, blotting it from the edge of the slide. We then air dried the slides overnight and stained them the following day with freshly prepared Giemsa solution (3% in Sorensen's phosphate buffer pH 6.8) for 15 minutes at room temperature.

Slides were examined under light microscope and photographed using a Zeiss Axiophot photomicroscope coupled with Olympus DP71 colour camera.

Preparations of day-old eggs did not reveal any cells suitable for cytogenetic analysis. Only a few nuclei could be seen around the egg's micropile and these showed features of prophase cells such as loose chromatin and an absence of nucleoli (Fig. 1A). Two- day old eggs showed typical interphase cells with compact chromatin and the presence of nucleoli (Fig. 1B). Only the preparations from three-day old eggs showed cells in metaphase,

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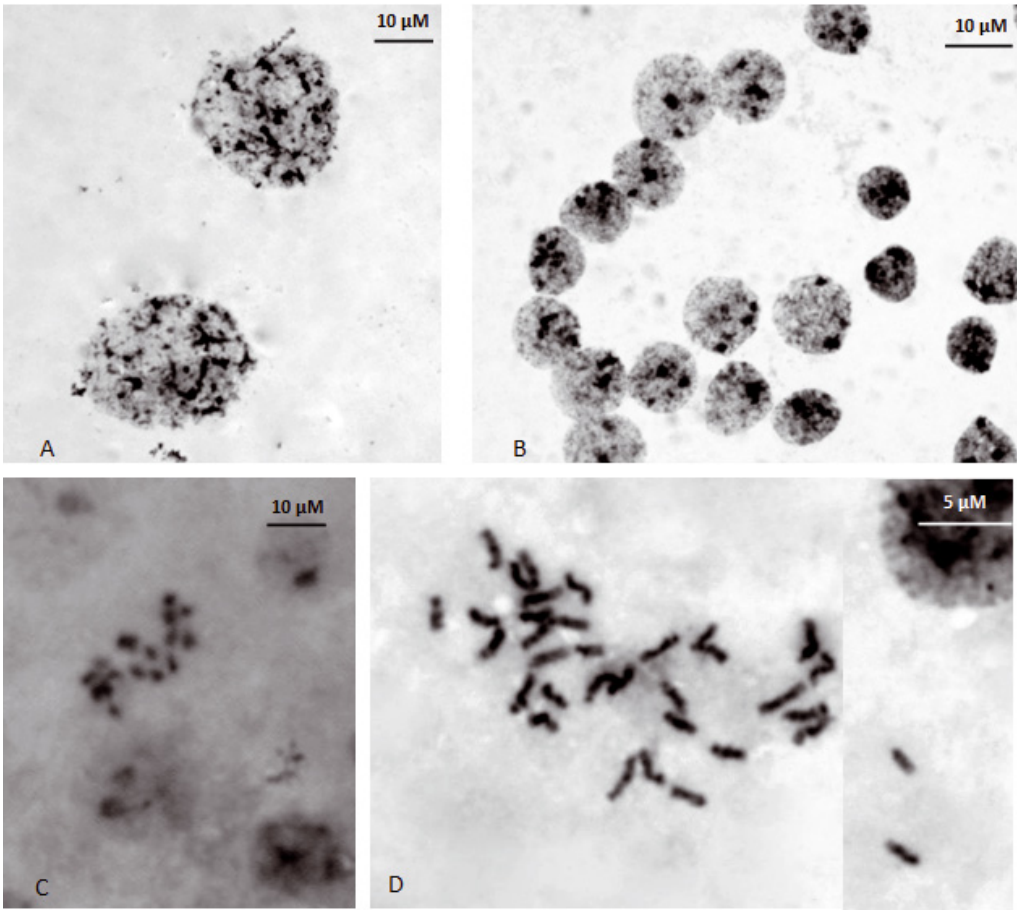

Figure 1. Nuclei and metaphase obtained from *Apis mellifera* eggs. A. One day-old; B: two day-old; C: three day-old male metaphase (n = 16); D: three day-old worker metaphase (2n = 32).

6 per slide on average. These cells showed the expected chromosome number for *A. mellifera*, n = 16 or 2n = 32 (Fig. 1C and D).

Although our technique yields a relatively small number of metaphase cells per slide, it allows a rapid assessment of ploidy level in eggs. The technique is potentially useful for the detection of worker-laid (haploid) eggs in worker-cells and queen cells or determining if individuals homozygous at multiple loci are diploid or haploid. Hybridization techniques such as GISH or FISH with specific probes for known genes and subsequent counting of positive hybridization spots could be applied in two-day old eggs, as preparations from eggs of this age showed nuclei suitable for this purpose.

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Note scientifique sur une méthode simple pour établir le caryotype des œufs de l'abeille domestique (*Apis mellifera*).

Eine wissenschaftliche Notiz über eine einfache Methode zur Karyotyp-Bestimmung von Eiern der Honigbiene (*Apis mellifera*).

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