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Scientific note

A scientific note on the preparation of high molecular weight DNA from honeybee *Apis mellifera* L. pupae for PFGE analysis

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Although the honeybee is not a basic genetic model organism so far, the complex behavioural repertoire, the male haploidy, the extremely high rate of recombination and recent QTL and mapping studies have shown that the honeybee is becoming a model organism for genomic studies of naturally occurring traits [5, 7] and sex determination [1, 6]. Physical mapping of linked DNA markers is therefore a basic strategy for further molecular analysis of genomic regions of interest. The strategy is based on long-range restriction maps. Large restriction fragments are generated by restriction of high molecular weight DNA with rare cutting restriction enzymes. Large fragments are subsequently separated in a pulsed-field gel electrophoresis (PFGE). Various methods have been described for the isolation of DNA suitable for PFGE analysis in *Drosophila* [10] and mammals [3, 9] but we were not able to isolate high molecular weight DNA following these procedures. More recently a simple and fast protocol has been described to isolate high molecular weight DNA from dissected honeybee queen-ovaries [4]. Here we describe a procedure which did not rely on dissection of specific tissues from insects. Since very large amounts of high molecular weight DNA is needed as a basic tool for different cloning [2] and physical mapping strategies we used workers instead of queens. Further nuclei were isolated and purified following a density gradient approach using most of the insect body. High molecular weight DNA could be obtained from larvae and pupae but not from adult worker bees. However, DNA obtained from larvae was less restrictable than DNA isolated from pupae (data not shown).

One hundred worker pupae of the mature white stage were frozen in liquid nitrogen. Heads and thoraces were homogenized in 20 mL of buffer 1 (40 mM KCl, 10 mM NaCl, 10 mM Tris, 0.1 mM spermine, 0.3 mM spermidine, 250 mM sucrose, 1.5 % Triton X-100, pH 7.4) by 10–20 strokes with a Dounce homogenator. The homogenates were filtered through cheesecloth (about 20 µm mesh), collected in centrifuge tubes (50 mL Oak Ridge tubes, Nalgene™, New York) and centrifuged at 1 000 *g* for 2 min. The relatively high speed is crucial to remove most debris and to obtain restrictable DNA. The supernatants were carefully removed and combined in a new centrifuge tube. The pellet and a large proportion of the liquid at the bottom of the tube (about 10 mL) were discarded. The suspension of nuclei was underlayered with 15 mL of buffer 2 (30 mM KCl, 7.5 mM NaCl, 7.5 mM Tris, 0.1 mM spermine, 0.25 mM spermidine, 0.5 M sucrose, 0.5 % Triton X-100, pH 7.4) and centrifuged at 8 000 *g* (4 °C) for 10 min in a SS34 rotor (Sorvall™). The supernatant and the fat at the top were carefully removed while the tube was directly cooled on ice. The remaining fat was wiped immediately from the inner surface of the tube using a paper tissue. The pellet containing the nuclei was resuspended in an equal volume of 125 mM EDTA, pH 7.5 (of about 300 µL) and mixed with an equal volume of prewarmed (45 °C) 1.5 % low melting agarose (in 125 mM EDTA, pH 7.5). The suspension was pipetted into insert moulds and cooled at 4 °C for gelling. Agarose inserts were treated with Proteinase K (2 mg·mL^{-1}) in NDS-buffer (0.5 M EDTA, 10 mM Tris, pH 9, 1 % Sarkosyl) for a minimum of 24 h at 50 °C.

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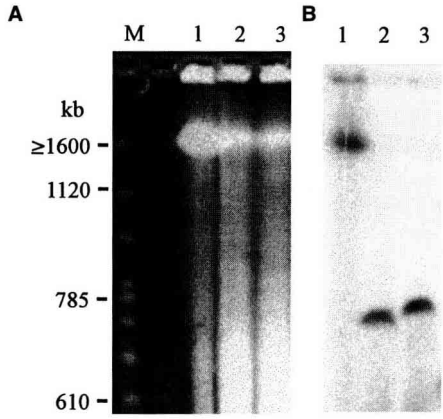

Figure 1. PFGE Gel and Southern hybridization of bee DNA. **A.** Ethidium bromide stain of pulsed-field gel with M = *Saccharomyces cerevisiae* chromosomes. Lane 1: bee DNA undigested; lane 2: DNA digested with *Sfi* I (Promega, Madison, WI); lane 3: bee DNA digested with *Asc* I (New England Biolabs, MA). One percent agarose gels were run in a CHEF DR II (Bio-Rad) apparatus at 6 V/cm with pulse times of 60–100 s for 24 h in 0.5 × TBE (50 mM Tris-HCl, 50 mM boric acid, 1 mM EDTA) at 14 °C. **B.** Hybridization of the Southern membrane (Hybond-NX, Amersham Life Science) to a single copy cosmid probe (Z-clone [1, 2]). The Southern blot and hybridization were performed according to the manufacturer's recommendations overnight following stringent washes. Film (Hyperfilm, Amersham Life Science) was exposed for 2 days at –80 °C using an intensifying screen. The exact size of the hybridization signal in lane 1 could not be determined, because it was located in the compression region where DNA of different sizes was concentrated. The largest fragment which could be resolved using these running conditions were 1.6 Mb fragments (as observed by size of the marker standard). We therefore concluded that undigested DNA is equal or even larger than 1.6 Mb (≥ 1.6 Mb using the described technique).

The proteinase K was inactivated by 0.2 mM PMSF treatment for 2 h followed by several washes in TE-buffer (10 mM Tris, 1 mM EDTA, pH 7.5) (see [8]).

About 200–250 µg of DNA were obtained per 100 individuals. The quality of DNA was assessed by endonuclease restriction and Southern blot analysis of PFGE gels (*figure 1*). A ^{32}P-labelled cosmid clone [2] (MPM Gc72E11247Q2) containing a single copy DNA marker [1] was hybridized to Southern blotted PFGE DNA (see *figure 1*). Single bands as large as about 750 kb were obtained. Honeybee larvae and pupae have a high polysaccharide and fat content due to the nutritious provisioning by adult workers. The method presented here might be suitable for the isolation of high molecular weight DNA in other insects that tend to have high nuclease activity and high polysaccharide contents.

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Note scientifique sur l'obtention d'ADN de masse moléculaire élevée à partir de nymphes d'abeilles (*Apis mellifera* L.) pour l'analyse par PFGE

Eine wissenschaftliche Notiz über die Isolierung hochmolekularer DNA aus Puppen der Honigbiene *Apis mellifera* für die PFGE Analyse

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