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Scientific note

A scientific note on the identification of honey bee semen using a mitochondrial DNA marker

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There has been discussion in the United States about the need for importation of honey bee (*Apis mellifera* L.) stock to offset the relative homogeneity of commercial breeding populations [5, 6] and to provide additional genetic variation for the development of stocks resistant to parasitic mites or diseases. Importation of bees through quarantine is necessary to prevent the introduction of new parasites or diseases. Importing germplasm without the larval, pupal or adult life stages significantly reduces the risks. Honey bee semen has been shipped successfully in the mail [10, 11]. Recent work has shown that the viability of honey bee spermatozoa stored at room temperatures is high for at least six weeks [1], adequate time to ship semen and use it for artificial insemination.

Undesirable genotypes, such as defensiveness, could also cause problems if imported. Screening procedures for Africanized honey bees in several states (CA, AZ) now rely on characterization of mitochondrial DNA [2, 9] to assess African heritage. Reports from other species when genotypes were determined from sperm [4] used microsatellite (nuclear) DNA. Because many mitochondria fuse during spermatogenesis [3], we had some concern that mitochondrial DNA would not be a usable alternative. We report here that a standard method for identification of honey bee mitochondrial haplotypes is also effective with semen from individual drones.

Ten drones from each of seven European colonies in Washington D.C., USA, and seven Africanized colonies near Weslaco, TX, USA, were induced to ejaculate, causing the endophallus to evert with semen and mucus on its tip. Semen was immediately collected either by removing the semen and adhering mucus using a sterile micropipette (D.C. only), or removing the tip of the everted endophallus with semen and mucus using a forceps (D.C. & TX). Drones from one colony (D.C.) were collected using both techniques. In all cases, the semen and remaining drone body were placed in separate sterile microcentrifuge tubes and kept frozen until DNA extraction.

We used a total nucleic acid extraction protocol developed for use with honey bees [7]. The drone thorax was macerated in 250 µl of cold buffer A (10 mM Tris-HCl [pH 8.0], 60 mM NaCl, 5% sucrose, 10 mM Na_2 EDTA) in a 1.5 ml microcentrifuge tube. An equal volume of cold buffer B (300 mM Tris-HCl [pH 8.0], 1.25% SDS, 5% sucrose, 20 mM Na_2 EDTA; plus 12 µl of diethyl pyrocarbonate per 1 ml solution) was added and the mix incubated on ice for 15 m. The semen/mucus was mixed using only 100 µl of the same solutions. The endophallus was removed with a sterile wooden applicator.

An equal volume of buffer-equilibrated phenol was added, the sample vortexed, held on ice for 3 m, then centrifuged for 5 m at 3 000 *g* at 4 °C. The upper aqueous layer was removed to a clean tube. This extraction was repeated twice more, first with phenol:chloroform:isoamyl alcohol (25:24:1) and second with chloroform: isoamyl alcohol (24:1). Thorax (semen) DNA was precipitated from the final aqueous layer by adding 50 (20) µl 3 M sodium acetate and 1(.4) ml of cold, absolute ethanol (2 hours at –20 °C), and then pelleted by centrifugation (12 000 *g* for 30 m at 4 °C). The pellet was resuspended in 100 (20) µl of modified, 1 X TE buffer (10 mM Tris-HCl, 0.1 mM Na_2 EDTA, pH 8.0), reprecipitated in 10 (2) µl 3 M sodium acetate and 500 (200) µl ethanol, dried in air overnight and dissolved in a final volume of 100 µl 1X TE.

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For amplification, PCR (35 cycles: 94 °C for 1 min, 46 °C for 1 min 15 s, 64 °C for 2 min) was done using primers for a portion of the cytochrome b region of the honey bee mitochondrial DNA [2, 8]. One (1) µl of extracted thorax DNA solution was used as template, and 2 µl of the semen DNA sample. Equal volumes of the PCR products were digested using *Bgl* II endonuclease (Boehringer Mannheim, GmbH, Germany), gel electrophoresed, stained and visualized under UV light (Fig. 1).

The figure is a descriptive run with pairs of thorax/semen samples in lanes 1, 3, 6, and 8 that show the two fragments (291 and 194 bp) expected with European honey bee DNA (worker in lane 5); samples in lanes 2, 7, and 9 show the uncut 485 bp fragment typical of AHB (worker in lane 4). All of the semen samples give clear bands identical to the thorax material from the same (a) or brother [b] drone. The two semen collection methods (forceps or micropipette) also gave the same results. For ease of collection in the field, removal of the endophallus tip plus semen with a forceps is preferred.

Our results clearly show that with only minor modification of existing techniques semen samples from individual drones can be used to characterize the mtDNA of the drones and therefore of the mother queen. Nuclear-DNA-based identification systems work with semen extractions as well [4]. This would be very useful to obtain genetic information about a queen without sacrificing her. Similarly, semen samples from a population of drones, or pooled semen, could be used to verify that specific traits were or were not present. As molecular genetic markers for traits of economic importance, like mite resistance, become available, such confirmation of the exact biological type of the semen becomes increasingly valuable, even when importation is legal.

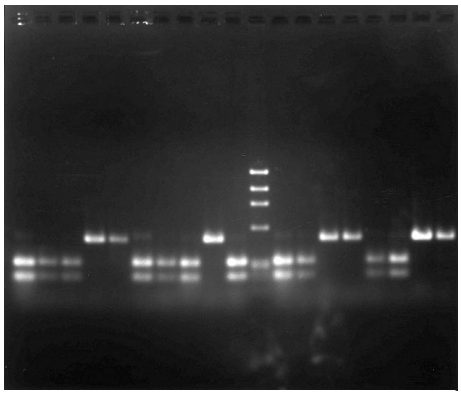

Figure 1. DNA amplified from drone thorax or semen extractions, digested (*Bgl* II endonuclease), electrophoresed on 1.5% agarose gel and stained with ethidium bromide. Lanes 1, 2, 3, 6, 7, 8, and 9 are thorax samples, the adjacent lanes are (a) the semen collected from that drone using a micropipette, and (b) forceps-collected semen from brother drones. Lanes 4 and 5 are an Africanized and European worker thorax respectively. The standard (center) is phage ϕX-174 DNA digested with *Hae* III.

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Note scientifique sur l'identification du sperme de l'abeille domestique à l'aide d'un marqueur d'ADN mitochondrial.

Wissenschaftliche Notiz über die Indentifizierung von Bienensperma mit mitochondrialen DNA Markern.

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