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## Chapter 1

### A Comprehensive Characterization of the Honeybees in Siberia (Russia)

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Additional information is available at the end of the chapter

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#### Abstract

A comprehensive study of some populations of honeybee (332 colonies) in Siberia (Tomsk region, Krasnoyarsk Krai (Yenisei population), Altai) using morphometric and molecular genetic methods was conducted. Infestation of bees (132 colonies) by *Nosema* has also been studied. Three variants of the COI-COII mtDNA locus were registered: PQQ, PQQQ (typical for *Apis m. mellifera*), and Q (specific for southern races). It was established that 64% of bee colonies from the Tomsk region and all colonies studied from the Krasnoyarsk and the Altai territories originate from *Apis m. mellifera* on the maternal line. According to the morphometric study, the majority of bee colonies of the Tomsk region are hybrids; in some colonies the mismatch of morphometric and mtDNA data was observed. Moreover, the majority of bee colonies infected by *Nosema* were hybrids. Yenisei population may be considered as a unique *Apis m. mellifera* population. Microsatellite analysis (loci A008, Ap049, AC117, AC216, Ap243, H110, A024, A113) showed the specific distribution of genotypes and alleles for some loci in the bees, which differ by geographical location. Loci A024 and Ap049 are of considerable interest for further study as candidate markers for differentiation of subspecies; locus A008 can be considered informative for determining of different ecotypes of *Apis m. mellifera*.

Keywords: honeybee, COI-COII locus, microsatellites, *Nosema*, Siberia

#### 1. Introduction

In Siberia, the honeybee was introduced about 230 years ago. It was the dark-colored forest bee *Apis mellifera mellifera* L., or the Middle Russian race (a term adopted in Russia), that was cultivated

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#### 2.5. Microsatellite analysis

Variability of eight microsatellite loci was studied: A008 (=A8), Ap049, AC117, AC216, Ap243, H110, A024, and A113. PCR was performed using specific primers and reaction conditions according to Solignac et al. [7]. Amplification products were analyzed with ABI Prism 3730 Genetic Analyser (Applied Biosystems, Inc., Foster City, CA) and GeneMapper Software (Applied Biosystems, Inc.). Two microliters of PCR products were mixed with GeneScan500-ROX size standards (Applied Biosystems, Inc.) and deionized formamide. Samples were run according to the manufacturer's recommendations. These genetic parameters were calculated: allelic frequencies and standard error.

#### 2.6. Infestation of honeybees by *Nosema*

From 10 to 70 bees were randomly selected from each bee colony and were examined for the presence of *Nosema*. Bee samples were stored in 70% (v/v) ethanol at room temperature prior to testing. The analysis was performed separately for each bee. The midgut of each sample was isolated, and one part of the midgut was used for the detection of *Nosema* spores under a light microscope, while the other part was used for DNA extraction. The midgut was suspended in 200 µL of distilled water and examined by dark-field microscopy for the presence of *Nosema* spores [8]. DNA was extracted from the midgut using a DNA purification kit, PureLink™ Mini (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol.

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