

Nucleotide sequence differences in the mitochondrial CO-I gene in the honey-bee subspecies *Apis mellifera mellifera* and *Apis m. ligustica*.

Nukleotid sekvens forskelle i det mitokondrielle CO-I gen hos honningbi underarterne *Apis mellifera mellifera* og *Apis m. ligustica*.

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Summary

Sequencing of a 371 bp PCR amplified mitochondrial nucleotide sequence from the CO-I (cytochrome oxidase) gene shows nine base differences between typical individuals of

Apis mellifera ligustica and *Apis mellifera mellifera* from the island of Læsø in Denmark. The comparison of the sequences exhibits 8 transitions and 1 transversion, one transition resulting in an amino-acid change.

Key words: *Apis*, cytochrome oxidase subunit I, honey bee, mitochondrial DNA sequences, PCR.

Resumé

Sekventering af en 371 bp PCR amplificeret mitokondrie nukleotidsekvens fra CO-I (cytochrom oxidase) genet, viser ni basers forskel imellem typiske individer af *Apis mellifera ligustica* og

Apis mellifera mellifera fra Læsø. Sammenligningen af sekvenserne fremviser 8 transitioner og 1 transversion. En af transitionerne resulterer i en aminosyreforskel.

Key words: *Apis*, cytochrom oxidase subunit I, honningbi, mitokondriel DNA sekvens, PCR.

Results

Læsø is supposed to have an almost pure population of the domesticated brown honey bee *Apis*

mellifera mellifera. A slight mixing with yellow Italian honey bees has been found (3,5).

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(a) 701 tcatatatcaggaatttcctcaattataggatcattaaacttaatagtta
(b) 711 GGAATTTCCCTCAATTATAGG-----
(c) 711 GGAATTTCCCTCAATTATAGG-----

(a) 751 caattataataataaaaaattttctataaattatgaccaaatttcatta
(b) 751 -----
(c) 751 -----

(a) 801 tttccatgatcagtttttattacagcaattttattaattatattacc
(b) 801 -----
(c) 801 -----

(a) 851 tgtattagctggagcaattactatactattatttgatcgaaattttaata
(b) 851 -----
(c) 851 -----

(a) 901 catcatttttcgatcctataggaggtggagatccaattctttatcaacat
(b) 901 ----ATTTTTCGATCCTATAGGAGGTGGAGATCCAATTCITTTATCAACAT
(c) 901 ----.....T.....T.....

(a) 951 ttattttgattttttgggtcatccagaagtttatattttaattttacctgg
(b) 951 TTATTTTGATTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCTGG
(c) 951

(a) 1001 atttggattaatctctcatattgtaataaatgaaagaggaaaaaaagaaa
(b) 1001 ATTTGGATTAATCTCTCATATTGTAATAAATGAAAGAGGAAAAAAGAAA
(c) 1001

(a) 1051 tttttggtaatttaagaataatttatgcaatattaggaattggatttcta
(b) 1051 TTTTTGGTAATTTAAGAATAATTATGCAATATTAGGAATTGGATTTCTA
(c) 1051A...C.....

(a) 1101 ggttttattgtttgagcacatcacatatttacagtcggattagatgttga
(b) 1101 GGTTTTATGTTTGAGCACATCACATATTTACAGTCGGATTAGATGTTGA
(c) 1101T.....

(a) 1151 tactcgagcatattttacttcagcaacaataatcattgctgtaccaacag
(b) 1151 TACTCGAGCATATTTTACTTCAGCAACAATAATCATTGCTGTACCAACAG
(c) 1151 C.....T.....

(a) 1201 gaattaaagttttttagatgattagcaacttatcatggttcaaaattaa
(b) 1201 GAATTAAGTTTTTAGATGATTAGCAACTTATCATGGTTCAAATTA
(c) 1201C.....

(a) 1251 ttaaataatttcaattttatgatcactaggttttattatactatttactat
(b) 1251 TTAAATATTTCAATTTTATGATCAC-----T
(c) 1251 ..G.....-----T

(a) 1301 tgggtgattaacaggaattatattatcaaatcttctattgatattattc
(b) 1301 TGGTGGATTAACAGGAATTATTA
(c) 1301 TGGTGGATTAACAGGAATTATTA

Description of differences between subspecies of *Apis mellifera* has been made by analysing mitochondrial DNA by RFLP (4). *A. m. mellifera* from Læsø was a.o. used in this study. Sequencing of the mitochondrial CO-I & II gene from Italian honey bees *A.m.l.* from a local hive in Australia has been performed (1).

In this investigation PCR amplification has been performed on mt-DNA from typical Italian honeybees, *A.m.l.* of known breed (Roskilde 202) and from typical brown bees *A.m.mellifera* from Læsø (Læsø 2,6).

The procedures for DNA extractions from muscle tissue, DNA amplification by PCR and the sequencing follow with minor modifications Hillis and Moritz (2) and local protocols at Institute of Population Biology (IP). There were used mt CO-I primers from a bumble bee project in progress at IP.

Sequencing of a 371 bp region (Fig. 1) from position 905 to 1275 showed 9 base differences in the positions 911, 939, 1058, 1062, 1124, 1151, 1184, 1238, and 1253. The base change in position 1058 is a transversion, the others are transitions. The change in position 939 should result in an amino acid change in cytochrome oxidase subunit I from leucine in *A.m.l.* to phenylalanine in *A.m.m.*

The appearance of two base differences between the 3-end of the primer and the known

A.m.l. sequence in position 1322 and 1323 has no influence on the PCR amplification.

The relatively high mutation rate in the mt CO-I gene is supposed to make this region usable in calculation of the genetic distance between subspecies of *Apis mellifera*.

Literature

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Fig. 1. Nucleotide sequence of part of the mitochondria CO-I from:

(a) *A.m.ligustica* as sequenced by Crozier et al. (1) shown in small letters.

(b) *A.m.ligustica* from Roskilde (Roskilde 202).

(c) *A.m.mellifera* from Læsø (Læsø 2.6).

Deduced sequences and used PCR-primers shown in capitals. PCR-primers shown by underlining, dots indicate agreements in sequence of *A.m.l.* and *A.m.m.*, hyphens represent unresolved regions in the DNA-sequencing. Numbering follows Crozier et al. (1).

Nukleotid sekvens af del af mitokondrie CO-I genet fra:

(a) *A.m. ligustica* sekventeret af Crozier et al. (1) vist med små bogstaver.

(b) *A.m. ligustica* fra Roskilde (Roskilde 202)

(c) *A.m. mellifera* fra Læsø (Læsø 2.6)

De udledte sekvenser og de benyttede PCR-primere er vist med store bogstaver. PCR-primere vist ved understregning, punktum indikerer overensstemmelse i sekvensen imellem *A.m.l.* og *A.m.m.*, bindestreger repræsenterer regioner, som ikke er adskilt ved DNA sekventeringen. Nummerering følger Crozier et al. (1).