How genetic integrity in honey bees, addressed in a regional law, can be monitored using mitochondrial DNA

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Animal and Food Genomics Group

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INSECT-IMP

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Conservation of *Apis mellifera*

Across Europe

- protected conservation areas
- mating stations
- mantaining of pure-bred lines

In Italy:

Law n.2 of March art.7

Law n.17 October 2022 art.3

Approaches capable to discriminate this subspecies from other hybrid population

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Apis mellifera – Italy

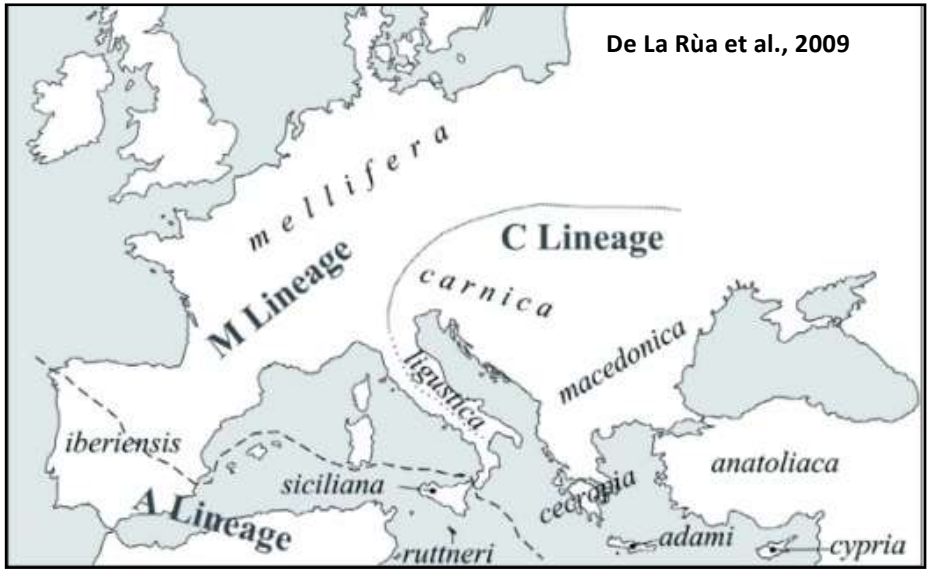
Italian honeybee (*A. m. ligustica*)

Carniolan honeybee (*A. m. carnica*)

} C

Black honeybee (*A. m. mellifera*) M →

Sicilian honeybee (*A. m. siciliana*) A →



carnica

mellifera

ligustica
C1/M7

siciliana

4 subspecies

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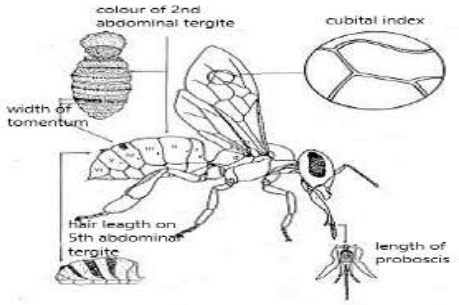
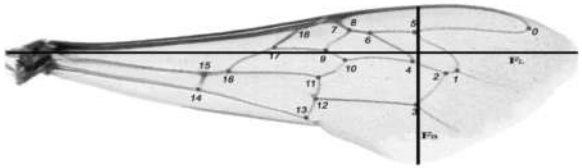
Methods for the identification

Morphometry

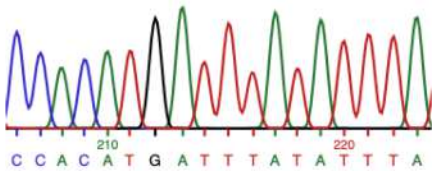




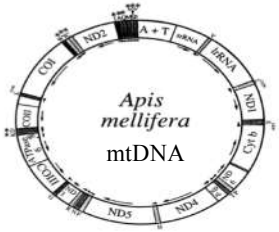
Molecular

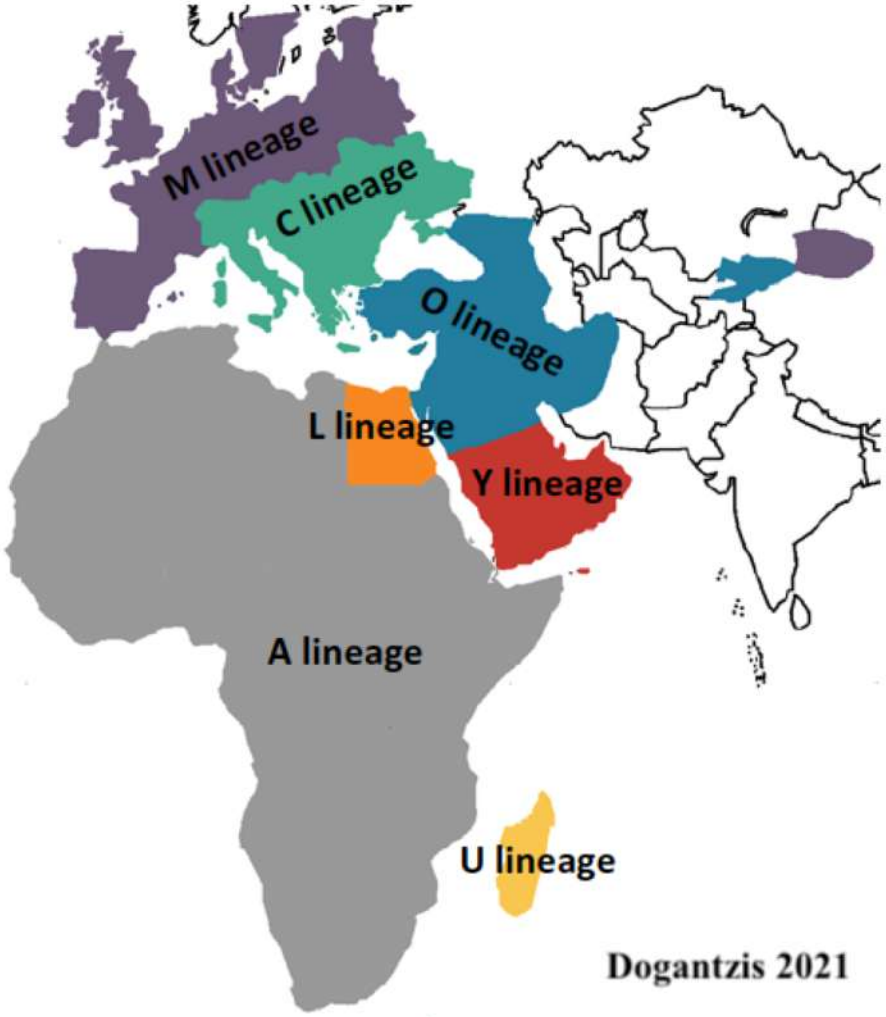
Mitochondrial DNA

Nuclear genome



NCBI





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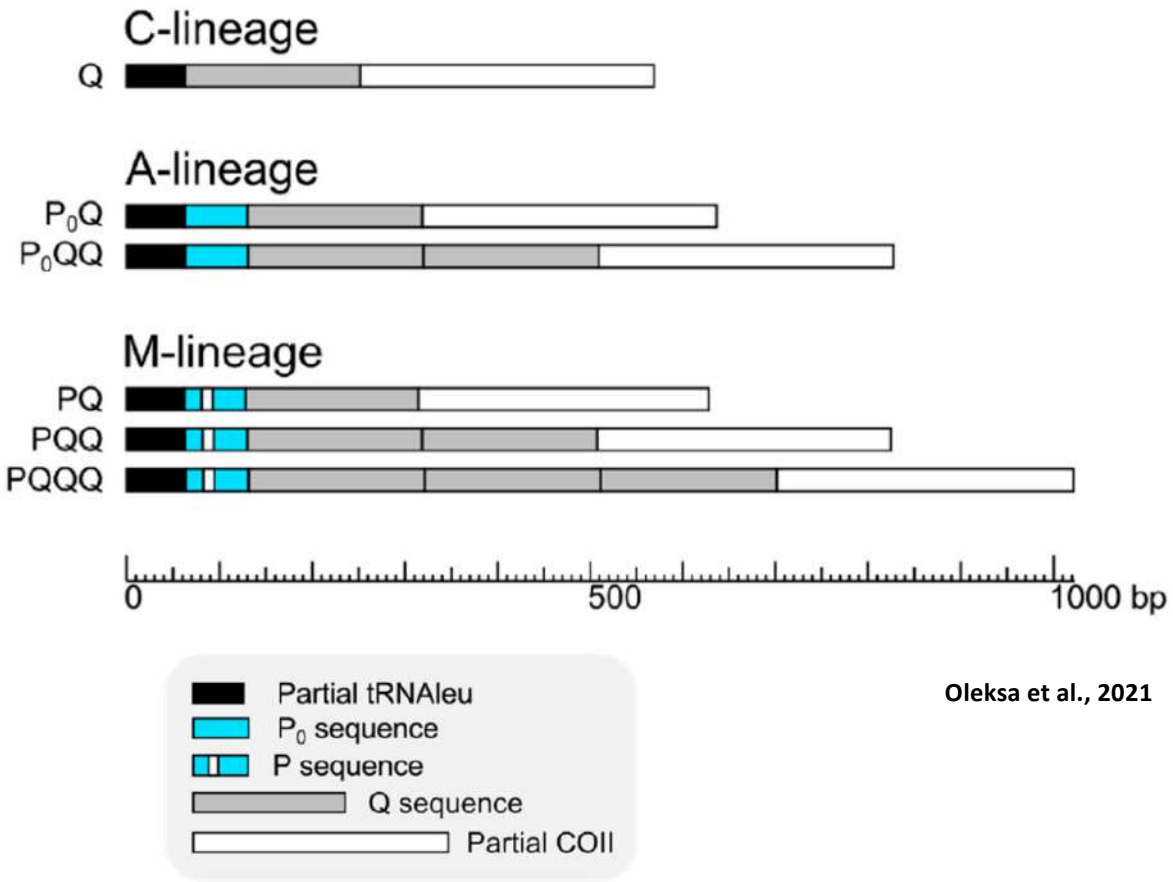
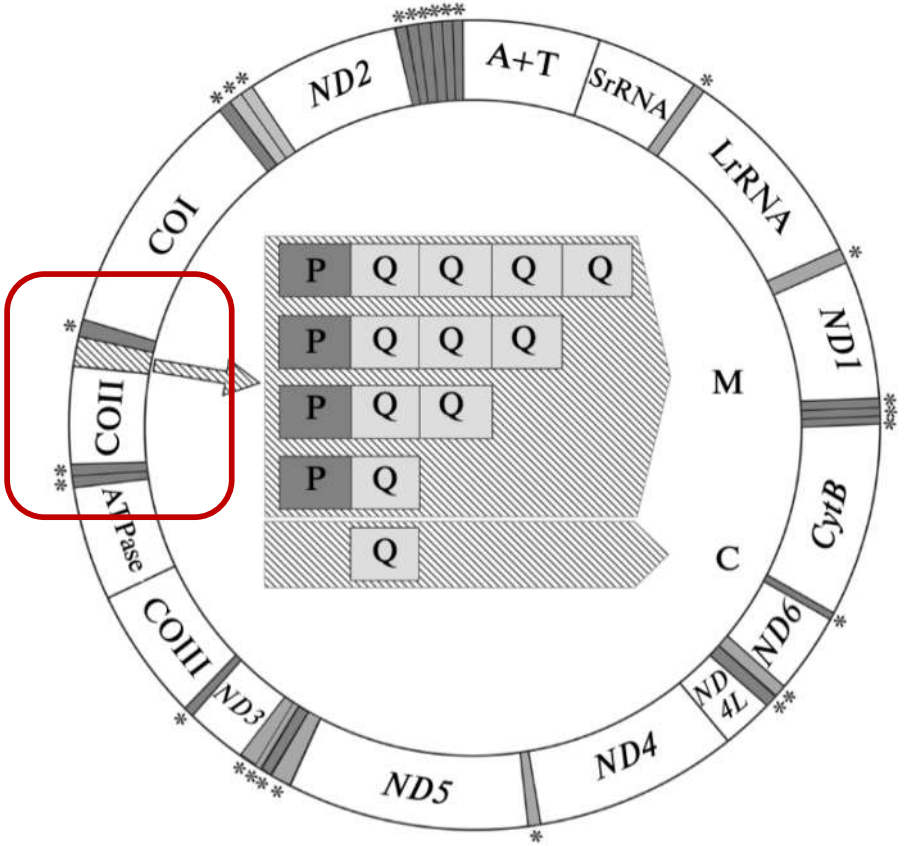
Molecular methods

Mitochondrial DNA analysis (mtDNA) - tRNAleu - COX2



Oleksa et al., 2021



Ilyasov et al., 2015

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Application

scientific reports

Distribution of honey bee mitochondrial DNA haplotypes in an Italian region where a legislative act is protecting the *Apis mellifera ligustica* subspecies

Valeria Taurisano, Anisa Ribani, Dalal Sami, Kate Elise Nelson Johnson, Giuseppina Schiavo, Valerio Joe Utzeri, Samuele Bovo & Luca Fontanesi✉

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Aim

To investigate the distribution and diffusion of mtDNA haplotypes of the *Apis mellifera* population managed in Emilia Romagna region

↓

Baseline for monitoring the trend in the frequency of *Apis mellifera ligustica* mtDNA haplotypes after the application of the regional law

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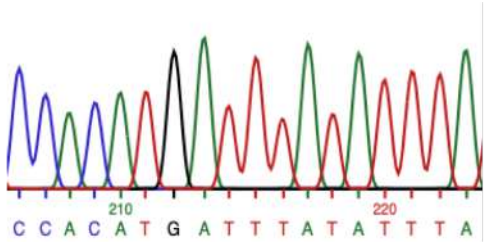
Material and Methods

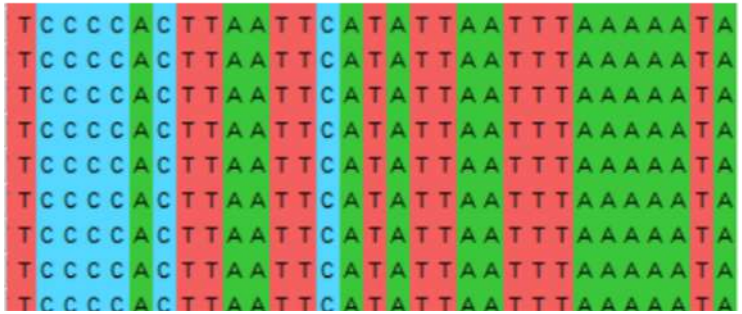
DNA extraction → PCR → Sanger Sequencing → Sequencing Analysis → Multiple Alignment

Sequence data analyses and phylogenetic analyses

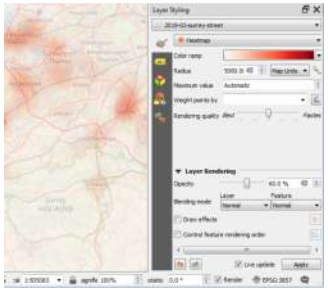
MEGA Molecular Evolutionary Genetics Analysis

NCBI





Geographical representation and density map



QGIS

x 1143

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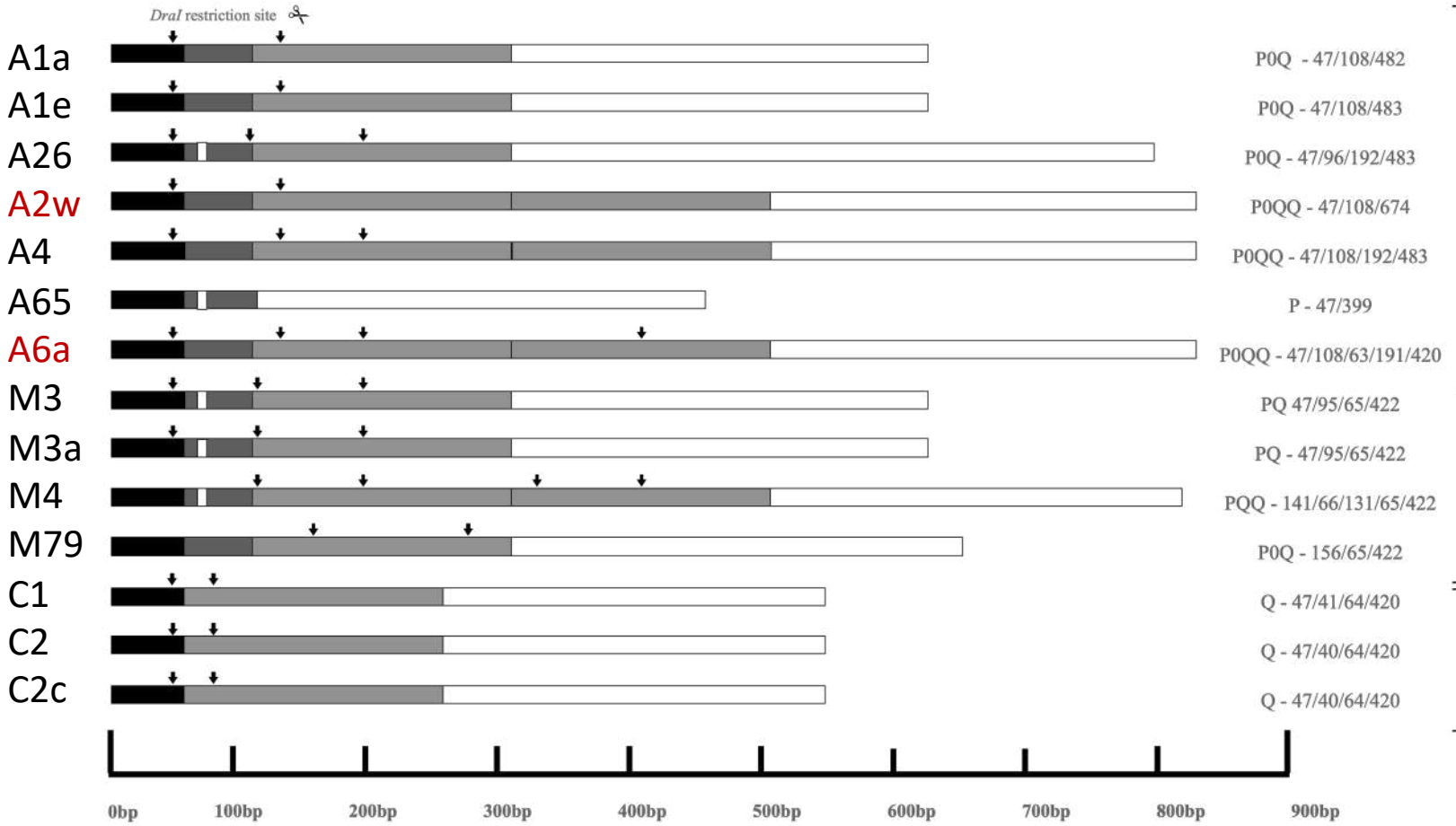
Results -1

- 500-900bp fragments
- 14 different mitochondrial haplotypes
- 2 novel haplotypes



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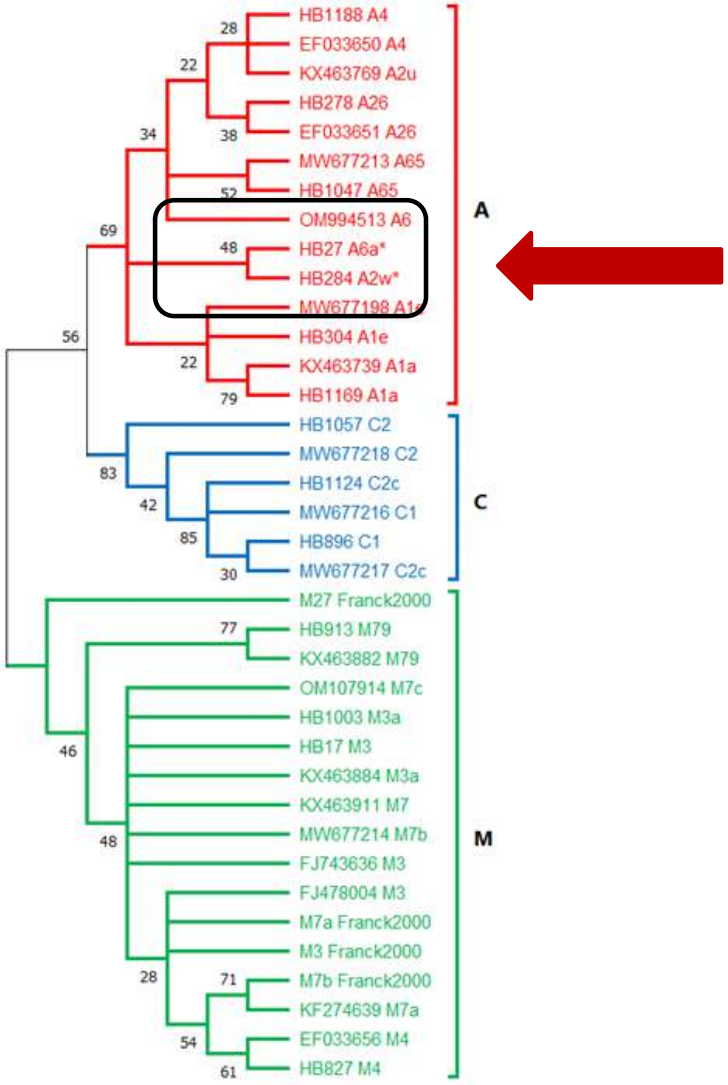
Results -2

Phylogenetic analyses



Results -3

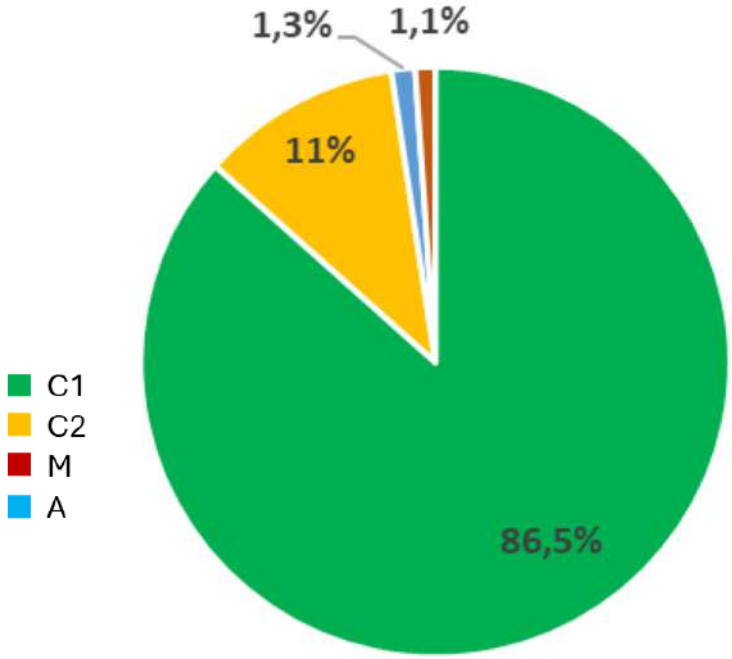
		mtDNA haplotypes			
Year	N. of honey bees	C1	C2	M	A
2020	94	80 (0.860)	9 (0.090)	3 (0.040)	2 (0.010)
2021	588	511 (0.869)	65 (0.111)	6 (0.010)	6 (0.010)
2022	461	398 (0.863)	52 (0.113)	4 (0.009)	7 (0.015)
Total	1143	989 (0.866)	126 (0.110)	13 (0.011)	15 (0.013)

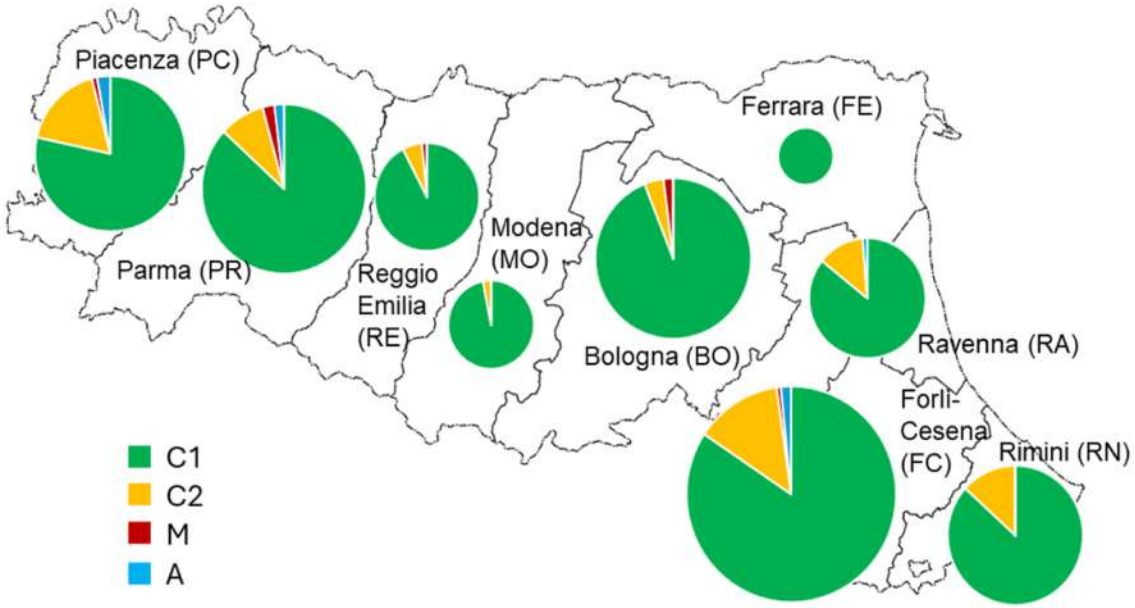
Haplotype Diversity (*H*) 0.239

Relatively low level -> high frequency of C1

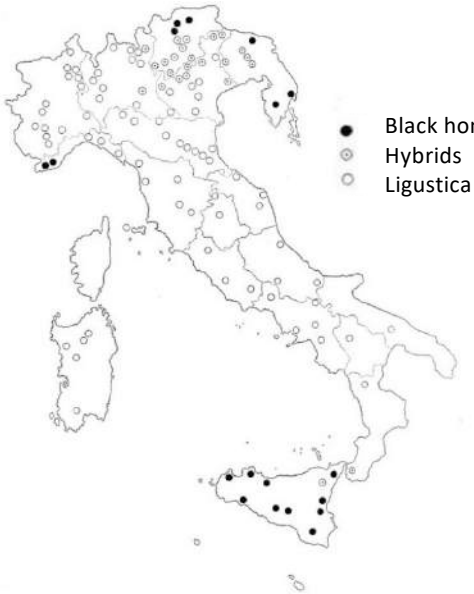
Results - 4

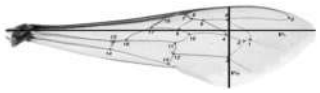
mtDNA haplotypes frequency





Sulla distribuzione geografica dell'*Apis mellifica ligustica* Spin. in Italia.- Bollettino del Laboratorio di zoologia generale e agraria della R. Scuola superiore d'agricoltura in Portici, 20: 150-168. VECCHI A., 1927.-





1927

Molecular Ecology (2000) 9, 907–921

Hybrid origins of honeybees from Italy (*Apis mellifera ligustica*) and Sicily (*A. m. sicula*)

P. FRANCK,* L. GARNERY,† G. CELEBRANO,‡ M. SOLIGNAC† and J.-M. CORNUET*
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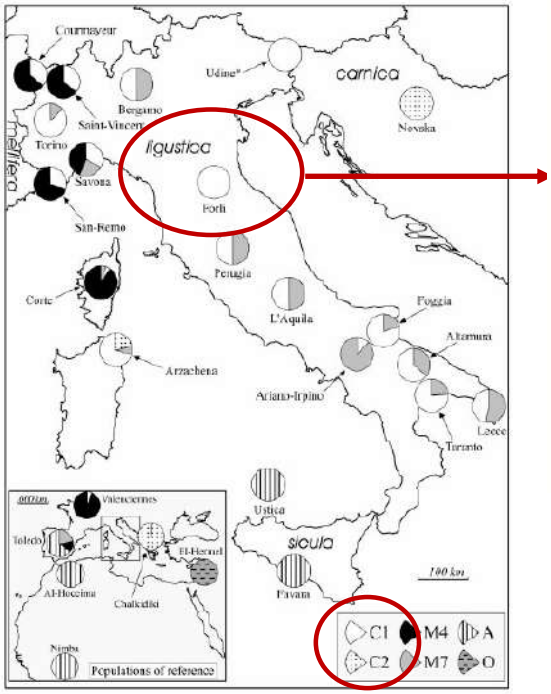
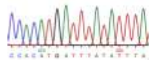
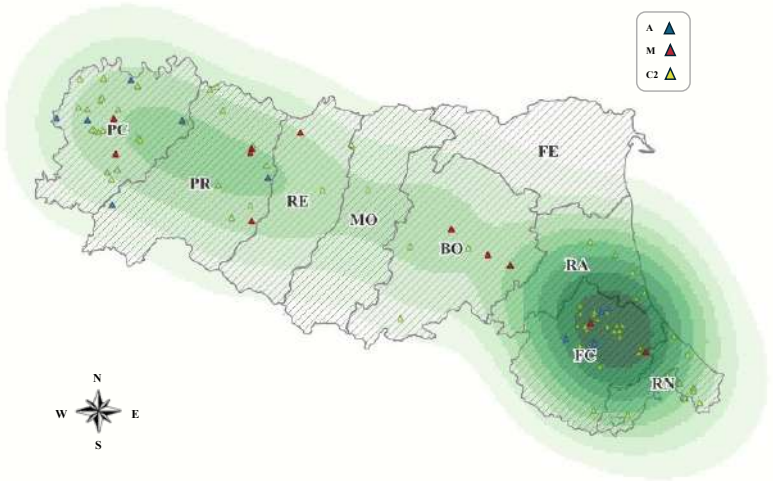




2000

18 sampling site
38 honey bee sampled in Emilia-Romagna region
1 Province Forlì Cesena



1143 sampling site
314 honey bees sampled in Forlì Cesena
9 Administrative Provinces

2024

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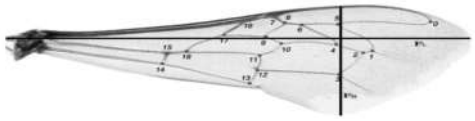
Another level

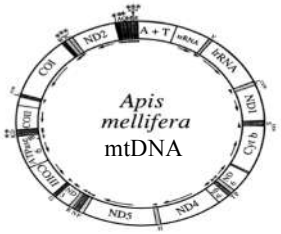
- 80 colonies
- 28 Apiaries

Morphometric Analysis

mtDNA honey bees

mtDNA honeycomb





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Conclusions

- comprehensive and unprecedented analysis of the genetic diversity of mtDNA haplotypes of the honey bee populations of Emilia-Romagna

- the analysed population includes honey bee carring mtDNA derived from the three evolutionary lineage A, M and C; C1 the most frequent haplotype

- identified and named two novel haplotypes of the A lineage

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Conclusions

- starting point for monitoring the effect of the regional law
- design and evaluate the potential effectiveness of conservation policies and action addressed to maintain the integrity of honey bee genetic resources in Italy
- based on the results we can recommend a more stringent policy to prevent diffusion of foreign mitotypes

Next steps ->

- complement the information obtained with nuclear genomic data

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Session 1. Genetic diversity, population structure and inbreeding management

Population genomics from a food matrix: estimating *csd* variability using next generation sequencing on *Apis mellifera* DNA present in honey

S. Bovo, A. Ribani, V. Taurisano, G. Schiavo, M. Bolner, F. Bertolini, L. Fontanesi

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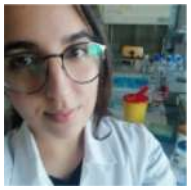

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(Columbia University)

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Acknowledgments

Thank you !

BEE-RER-1-2-3 RESEARCH PROJECT

mipaaf
ministero delle politiche agricole alimentari e forestali

Progetto realizzato con il contributo del Ministero delle Politiche Agricole Alimentari e Forestali, Regolamento UE 1308/2013, Programma 2020/2021, sottoprogramma ministeriale, L.R. 4 marzo 2019, n. 2, Regione Emilia-Romagna, Misura F (DELIBERAZIONE DELLA GIUNTA REGIONALE 28 LUGLIO 2020, N. 939).

RegioneEmilia-Romagna

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