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Honey bees (*Apis mellifera*) are exposed to natural and synthetic xenobiotics, requiring genetic adaptations for survival. Several gene families have been implicated in insect pesticide resistance, including cytochrome P450s, glutathione-S-transferases (GSTs), esterases, and uridine diphosphate (UDP)-glycosyltransferases. This study investigates genetic variation in these detoxification gene families and predicts the structural and functional effects of non-synonymous SNPs (single nucleotide polymorphisms) on protein structure and function. We analyzed SNPs mapped to these detoxification genes extracted from over 1,500 whole genomes representing 15 subspecies and the four main honey bee lineages: M, C, A, and O. Functional annotation and variant effects were predicted using SnpEff. Allele frequencies and each SNP's fixation index (FST) were calculated per population and evolutionary lineage. Bioinformatics and molecular modeling techniques were employed to evaluate non-synonymous SNPs' structural and functional consequences. Protein structures were generated from FASTA files using AlphaFold3, converted from mmCIF to PDB format, and visualized in PyMOL. Functional site predictions were performed using Proteins Plus, and molecular dynamics simulations were conducted in YASARA to assess stability and conformational changes in proteins. Our results indicate many non-synonymous SNPs in some subspecies, such as *A. m. jemenitica* and *A. m. intermissa*. The genes with the highest number of non-synonymous mutations belong to the CYP family, particularly Probable cytochrome P450 6a14 and CYP9Q1. Conversely, genes such as Cytochrome P450 6k1 and Methyl farnesoate epoxidase exhibit no non-synonymous SNPs. By understanding intraspecific genetic variation, we move closer to reliably predicting how honey bee populations will respond to pesticide exposure.

This work was conducted in the framework of the projects MEDIBEES - Monitoring the Mediterranean Honey Bee Subspecies and their Resilience to Climate Change for the Improvement of Sustainable Agro-Ecosystems funded by PRIMA, Better-B, funded by the European Union, the Swiss State Secretariat for Education, Research and Innovation, and UK Research and Innovation, under the UK government's Horizon Europe funding guarantee (grant number 10068544) and Bee3Pomics: Omics insights into molecular effects of plant protection products in honey bees (*Apis mellifera*) funded by the RESTART-FCT

Genetic variation of detoxification genes: from genes to proteins

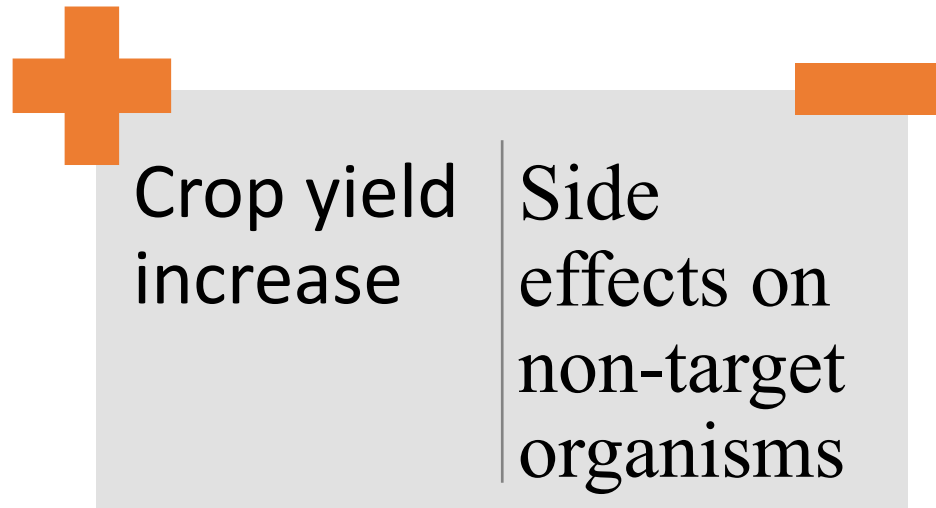
Dora Henriques; Fernanda Li; Sana Bashir; Andreia Quaresma; Ana R. Lopes; Demetris Taliadoros; Matthew T. Webster; Carlos S. H. Shiraishi; Carlos A. Yadro Garcia; Rui M. V. Abreu; José Rufino; Annelise Rosa-Fontana; Gilles Verbinnen; Dirk C. de Graaf; Lina De Smet; M. Alice Pinto



APIMONDIA 2025
Copenhagen
Denmark



Effects of pesticides on honey bees



Memory deficit

Learning deficit

Abnormal behaviors

Mortality



Impaired foraging

Honey bee and pesticide risk assessment

- Variation in median lethal dose (LD₅₀) values and other toxicity indices for honey bees exposed to the same pesticide
- Differences among subspecies and genetic stocks in their sensitivity to identical compounds

Comparative Toxicities of Four Topically Applied Insecticides to Africanized and European Honey Bees (Hymenoptera: Apidae)

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RESEARCH ARTICLE

Genetics, Synergists, and Age Affect Insecticide Sensitivity of the Honey Bee, *Apis mellifera*

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CHARACTERISTICS OF IMIDACLOPRID TOXICITY IN TWO *APIS MELLIFERA* SUBSPECIES

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(Received 2 June 1999; Accepted 10 December 1999)

Differences in larval pesticide tolerance and esterase activity across honey bee (*Apis mellifera*) stocks

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Imidacloprid was approximately 10 times more toxic to *A. m. mellifera* than to *A. m. carnica*, and imidacloprid ranks

Imidacloprid *Apis mellifera* Honeybees Lethal dose Insecticide

Toxicity of neonicotinoid insecticides on different honey bee genotypes

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Abstract

Toxicity effects of the neonicotinoid insecticides clothianidin, imidacloprid, and thiamethoxam were tested in the laboratory on different honey bee (*Apis mellifera* L.) genotypes belonging to the following subspecies: *Apis mellifera mellifera* L., *Apis mellifera ligustica* Spinola, and *Apis mellifera carnica* Pollmann. Oral and indirect contact trials were carried out on adult worker honey bees for each pesticide, using commercial formulations. The acute oral toxicity (AOT) LD₅₀ and the acute indirect contact toxicity (ICT) LC₅₀ were calculated. Mean AOT LD₅₀ values at 24 hours (clothianidin 3.53 ng/honey bee, imidacloprid 118.74 ng/honey bee, thiamethoxam 4.40 ng/honey bee), 48 hours (clothianidin 3.35 ng/honey bee, imidacloprid 90.09 ng/honey bee, thiamethoxam 4.27 ng/honey bee), and 72 hours (clothianidin 3.28 ng/honey bee, imidacloprid 69.68 ng/honey bee, thiamethoxam 4.16 ng/honey bee) from test start were of the same order of magnitude of those reported in the literature for all three neonicotinoids. Statistically significant differences emerged in a few instances between groups of honey bees coming from the different hives tested for clothianidin, between the groups of honey bees coming from the single *A. m. mellifera* hive and the four *A. m. ligustica* hives tested for imidacloprid, and more extensively between the two *A. m. carnica*, the single *A. m. mellifera*, and the six *A. m. ligustica* groups of honey bees tested for thiamethoxam. ICT LC₅₀ values were obtained for a reduced number of hives: the single *A. m. mellifera* and two *A. m. ligustica* hives for clothianidin, the single *A. m. mellifera* and one *A. m. ligustica* hive for imidacloprid, the single *A. m. mellifera*, three *A. m. ligustica* hives, and one *A. m. carnica* hive for thiamethoxam. Nevertheless statistically significant differences were observed for clothianidin and thiamethoxam, but not for imidacloprid. The results confirm that genetic differences in the response to pesticide toxic action exist in the honey bee, but they do not constitute the key factor involved in the uneven results observed in toxicity tests. In any case, the LD₅₀ or other similar toxicity indexes should not be determined on a single colony.

Tested honey bee subspecies

A. m. ligustica

A. m. mellifera

Russian

A. m. carnica

A. m. caucasia

Africanized



31

Subspecies

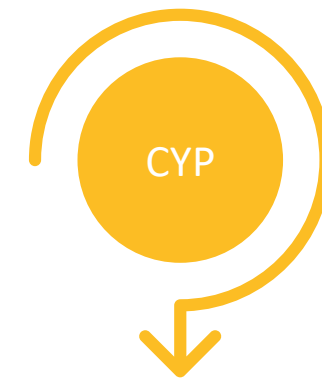
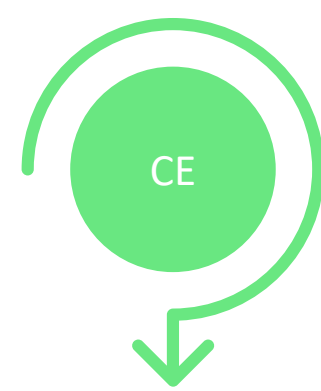
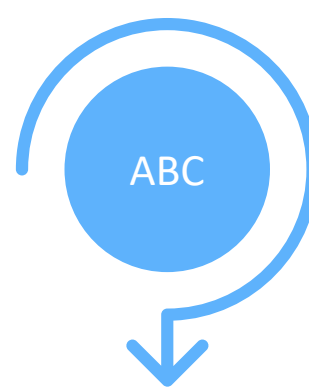
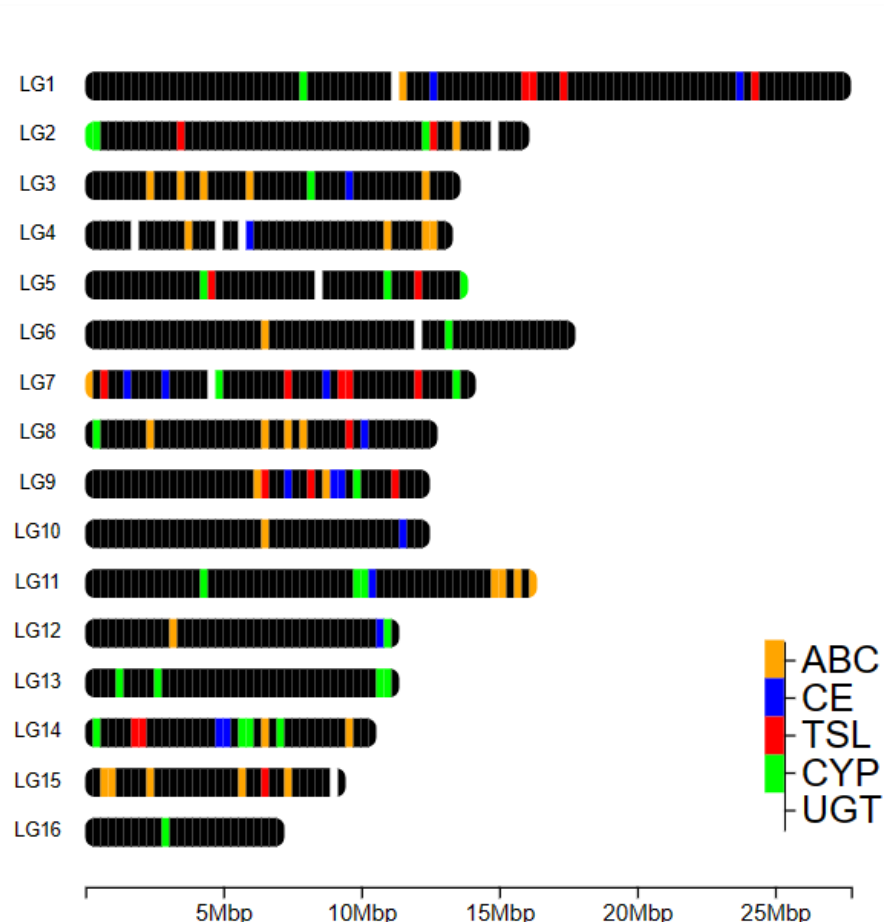


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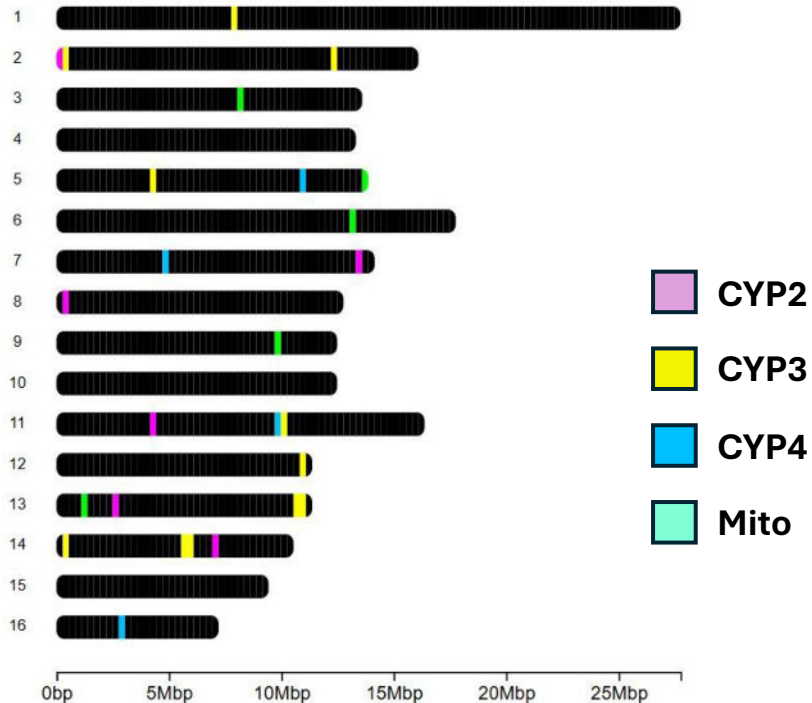


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Detoxification gene families



P450 CYP genes



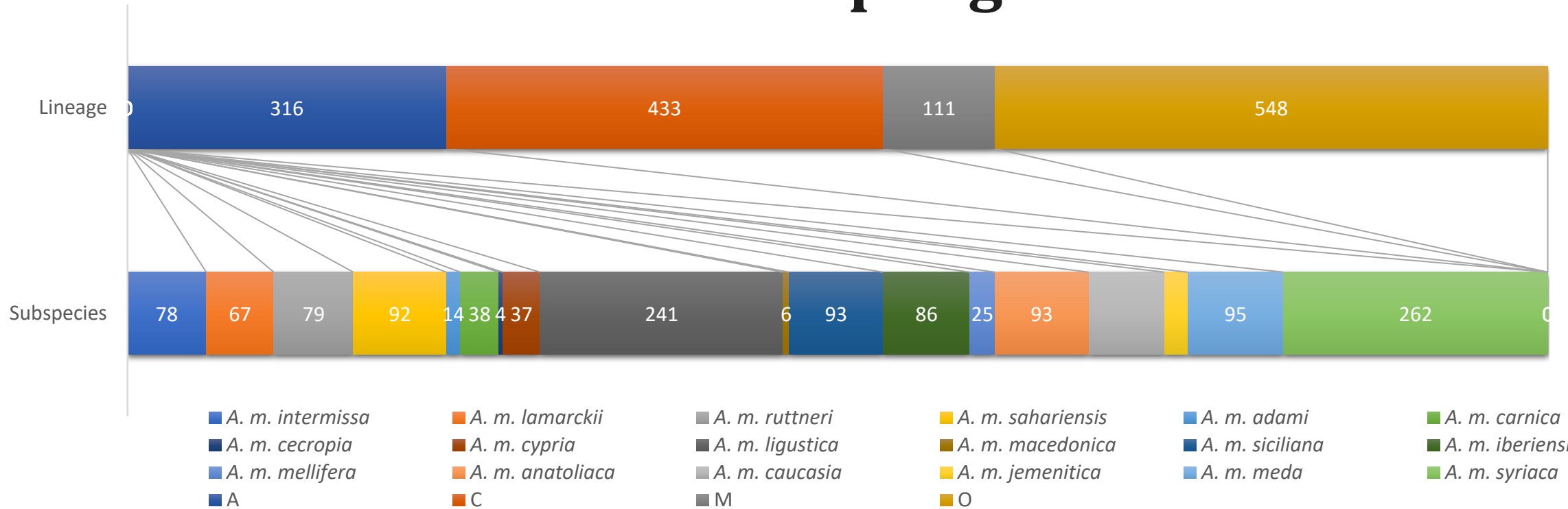
- **CYP2:** Physiological functions (e.g., ecdysteroid, juvenile hormone biosynthesis)
- **CYP3:** Xenobiotic detoxification, especially families CYP6 and CYP9
- **CYP4:** Insecticide metabolism and pheromone processing
- **Mitochondrial CYP:** Fatty acid, sterol, and hormone metabolism

Goal

Characterizing variation in CYP genes



Sampling

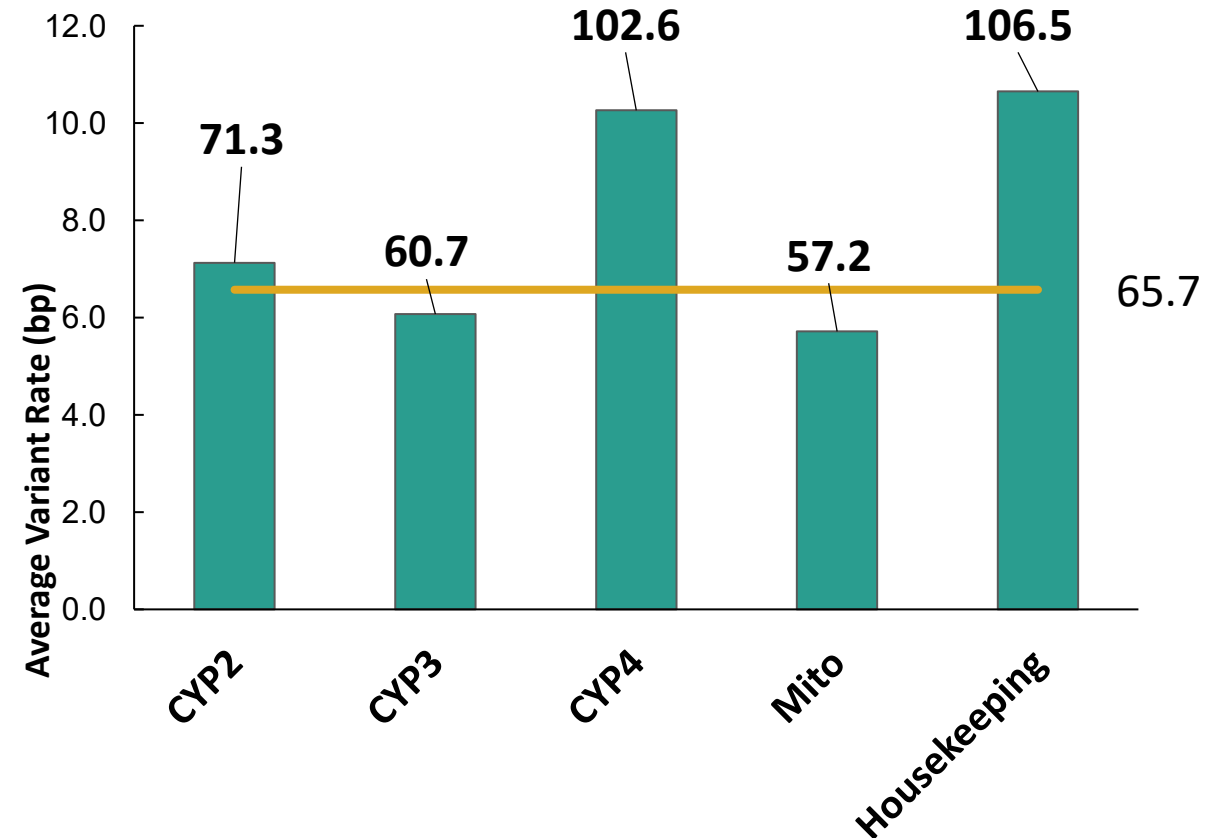
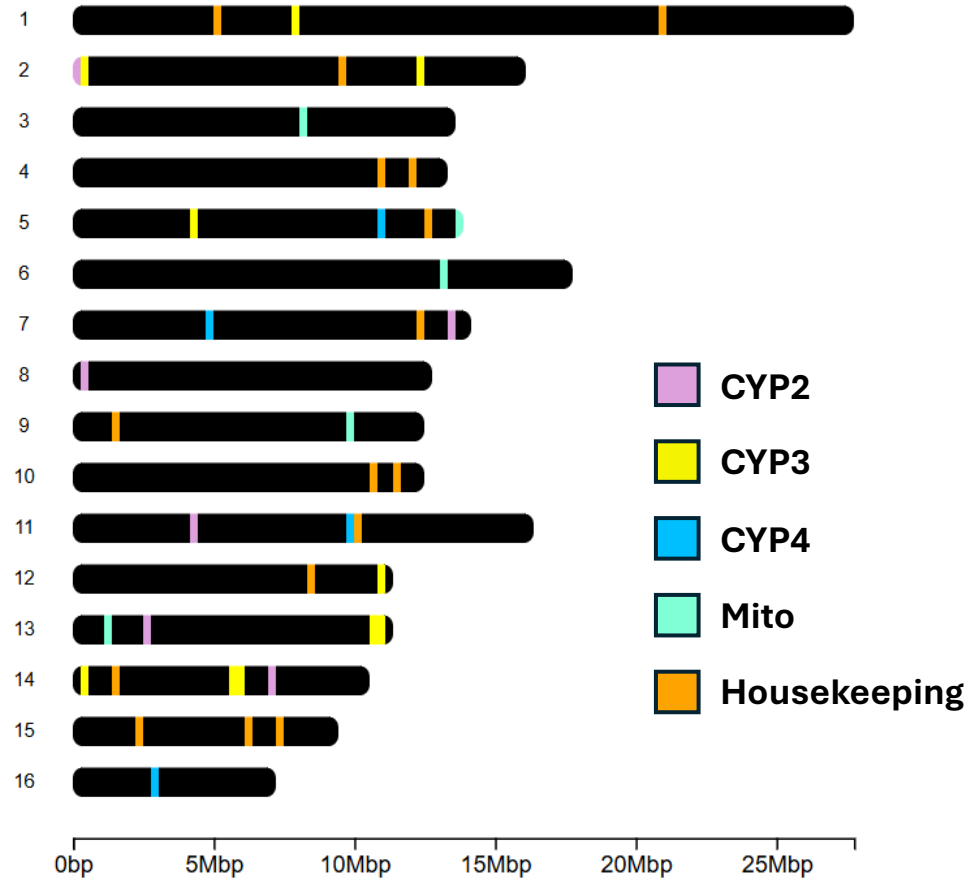


- 1467
- 25 countries
- 18 subspecies
- 4 lineages (A, C, M, and O)



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P450 CYP genes

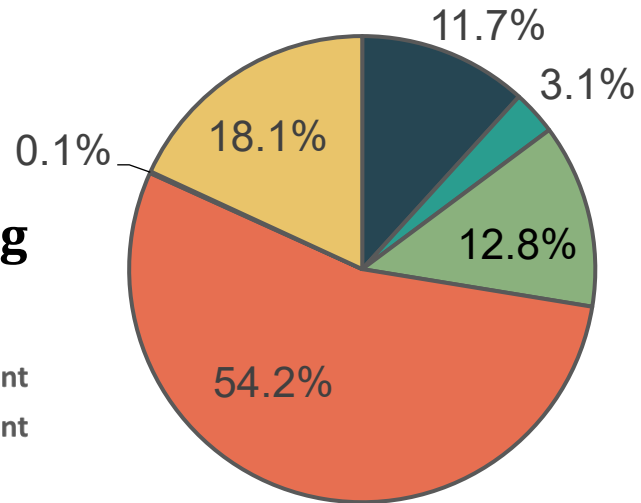


CYP 5,756 SNPs

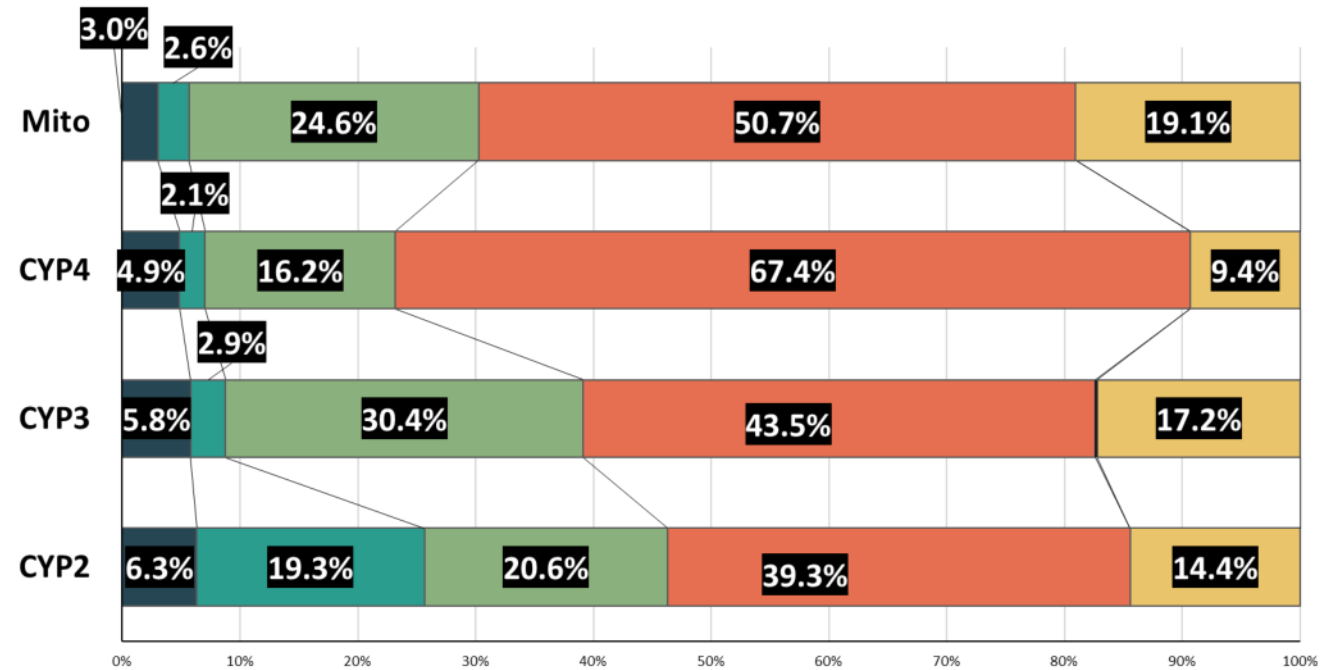
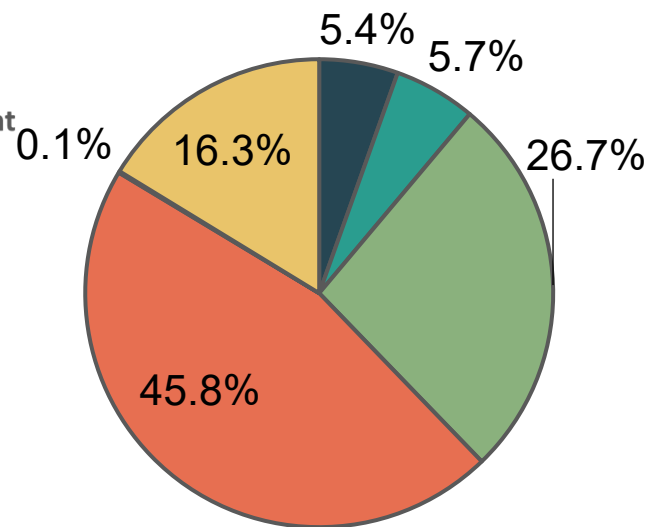
Housekeeping 1,728 SNPs

Variant distribution

Housekeeping



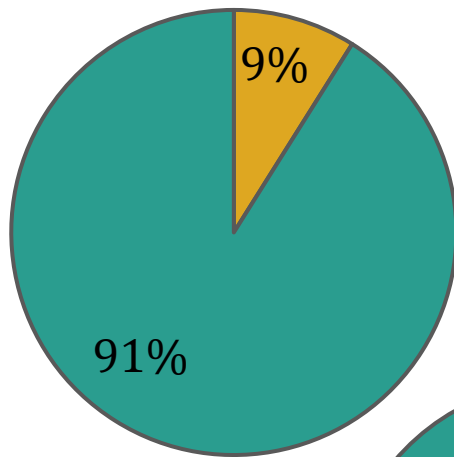
CYP



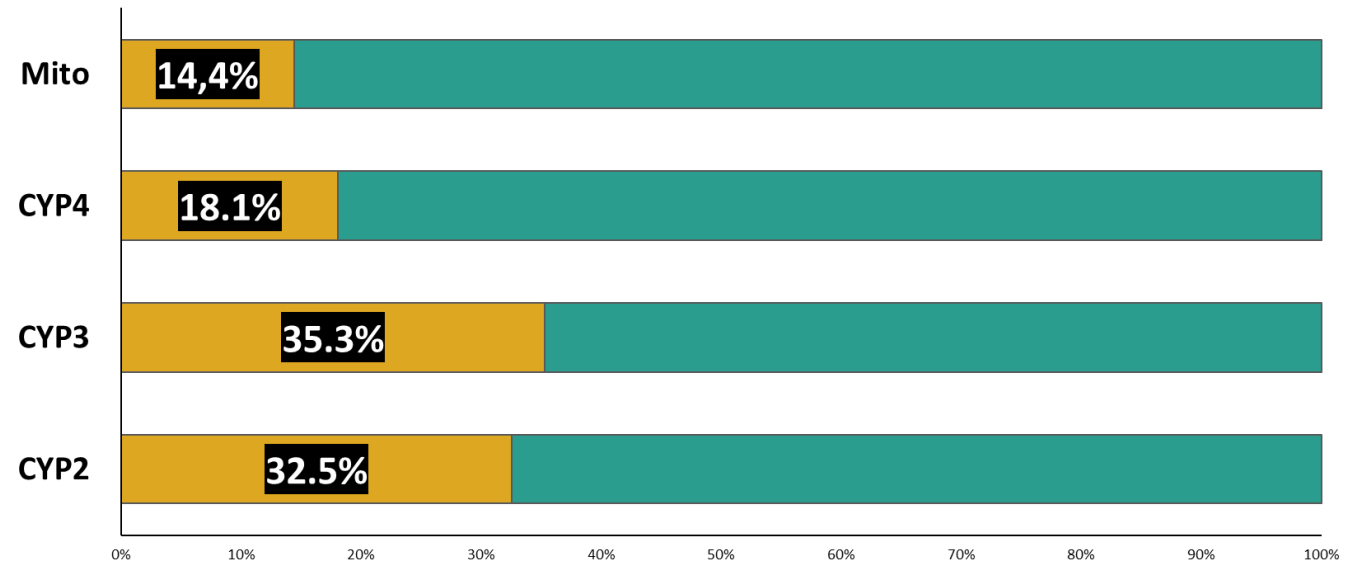
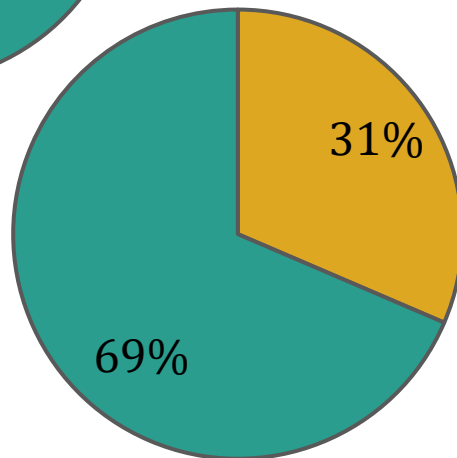
Variant distribution

CDS variants

Housekeeping



CYP



■ Non-synonymous
■ Synonymous



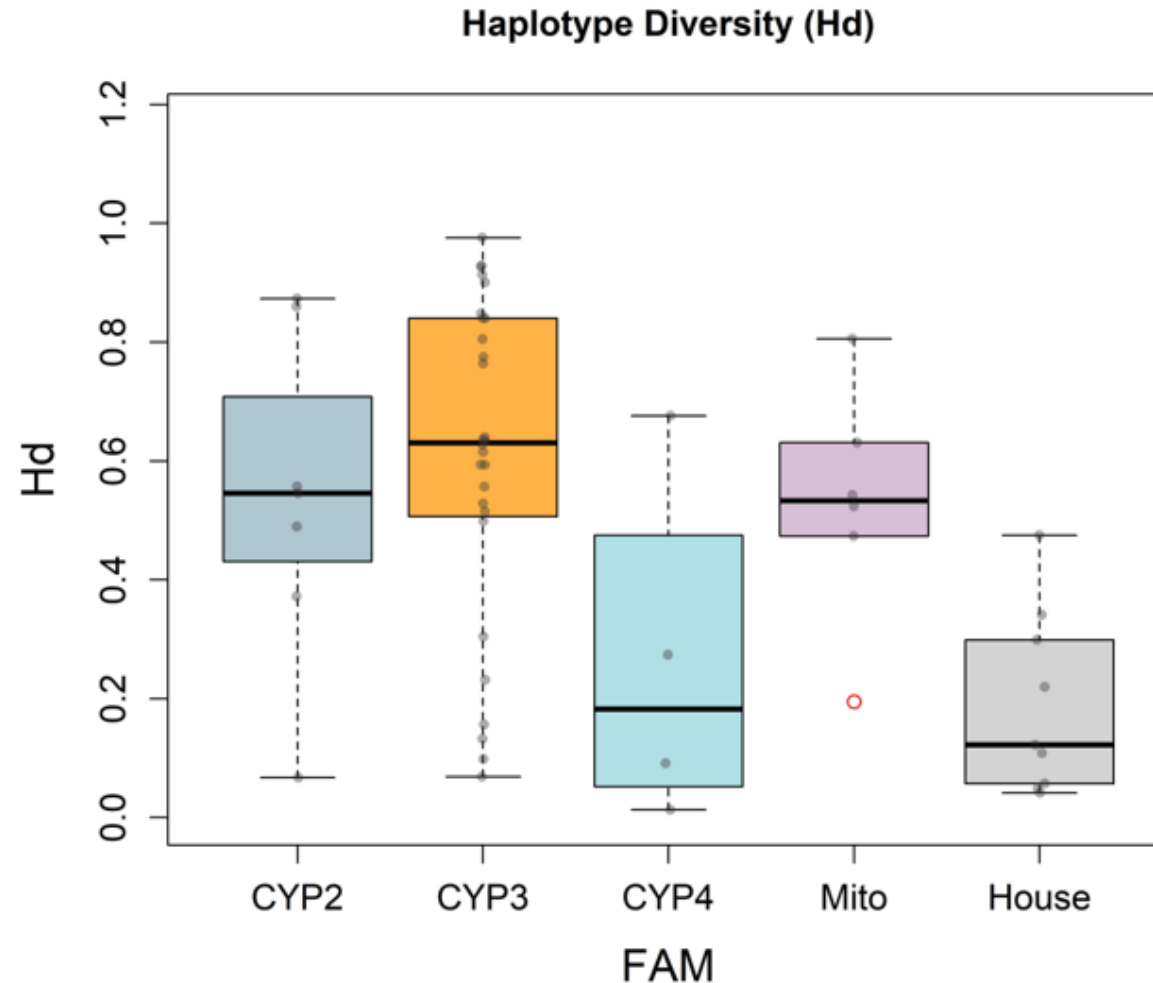
Haplotypes

- 1,306 haplotypes

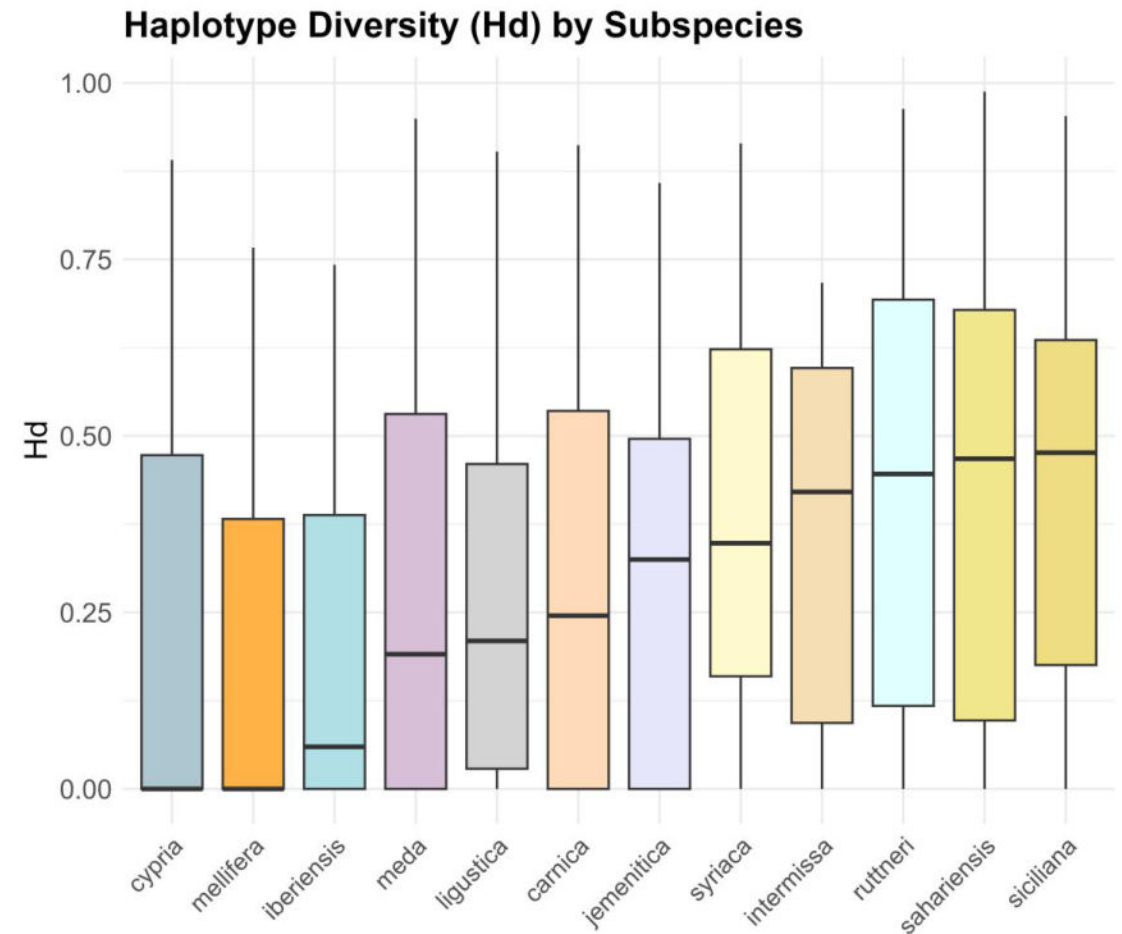
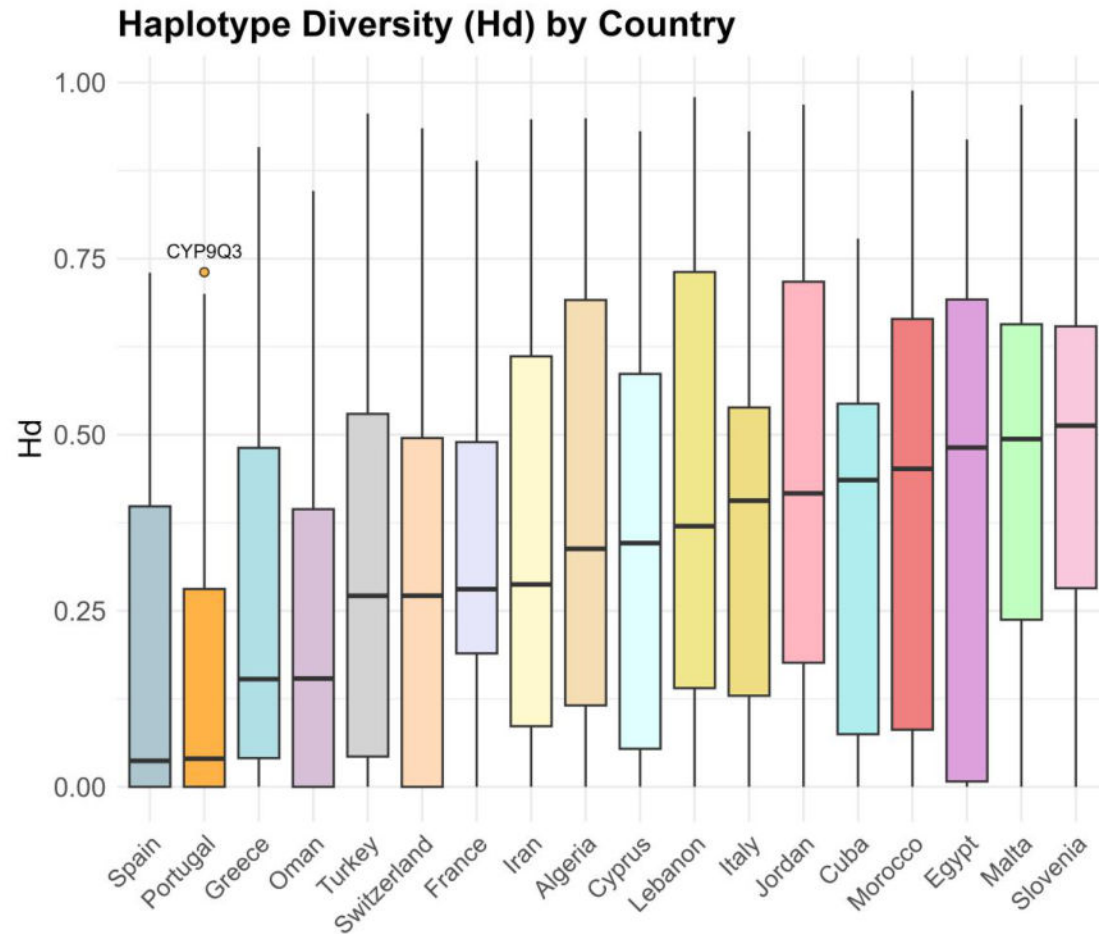
84% **CYP 3 clan**
Detoxification

Genes with $Hd \geq 0.9$

CYP9S1/CYP9R1**
CYP6AS3
CYP6AS12
CYP9P1
CYP9Q3



Haplotype diversity per Subspecies and Country



In silico analysis of nsSNPs



DynaMut2 Assessing changes in stability and flexibility upon missense mutations

Assess the functional impact of missense mutations

Missense3D

Missense3D version 2 predicts structural changes caused by missense variants in protein tertiary structures and binary complexes. It supports both experimental and predicted models, including AlphaFold and GWYRE. Variants can be input using protein or genetic coordinates.

I-Mutant

Predictor of Protein Stability Changes upon Mutations



Binding pockets prediction



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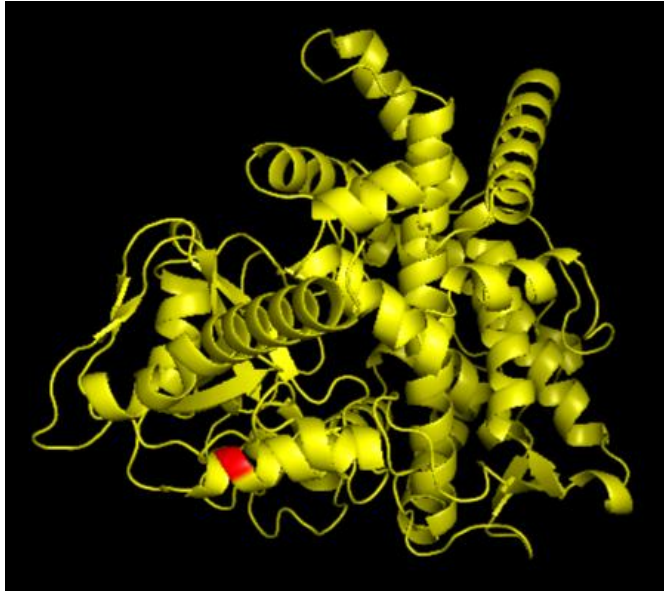
- 479 missense variants

69

SIFT

[illegible]

Analyzing high impact mutations



CYP9Q3 (T62M*)



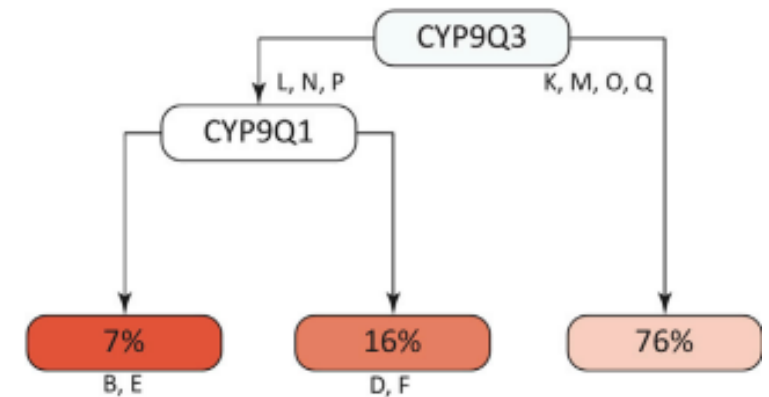
CYP9Q3 (T62M, M288T,V380A)

iScience

Article

Genetics of tolerance in honeybees to the neonicotinoid clothianidin

Nadejda Tsvetkov,^{1,3} Simran Bahia,¹ Bernarda Calla,^{2,4} May R. Berenbaum,² and Amro Zayed¹.

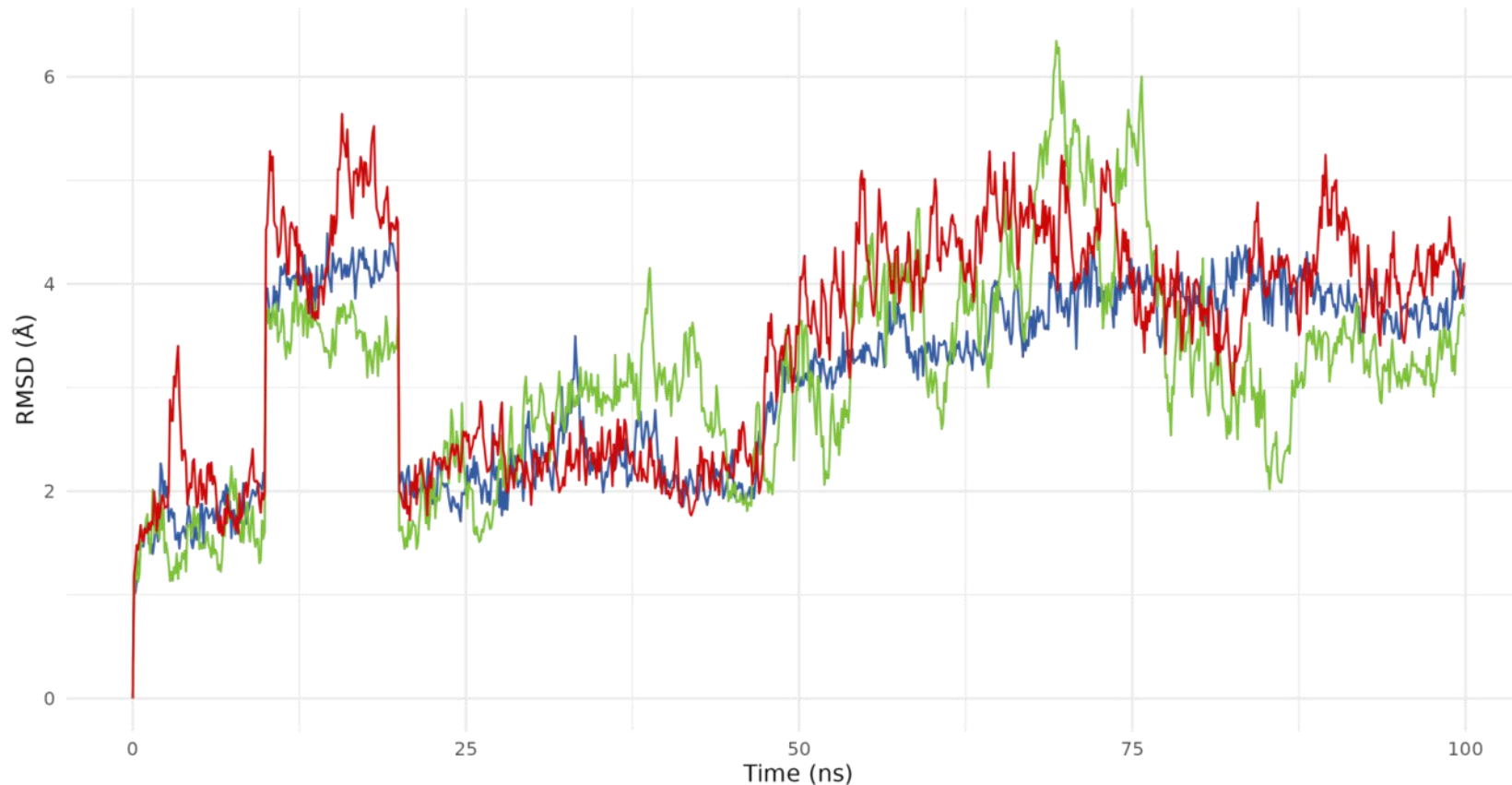


Molecular Dynamics simulation

- CYP9Q3

RMSD of Protein Backbone Over Time

Protein Variant — Reference — T62M — T62M, M288T, V380A



- T62M: 21 individuals
Italy, Jordan, Greece, Slovenia,
Turkey, Algeria
- T52M, M288T, V380A: 14
individuals
Cyprus, Jordan, Turkey
- Wild-type: 394 individuals
Not in Algeria, Cyprus, Morocco,
Oman, Portugal, Spain

To think about...

- What subspecies should be used in Environment Risk Assessment (ERA)?
- Should the most vulnerable subspecies be used in ERA or the native ones?
- Should populations for pollination services be selected based on their genetic background?



Bee3Pomics: Omics insights into molecular effects of plant protection products in honey bees (*Apis mellifera*) funded by the RESTART-FCT

Vilde Leipart



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Better-B at APIMONDIA 2025

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Better-B-project

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Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union, European Research Executive Agency (REA), SERI or UKRI. Neither the European Union nor the granting authorities can be held responsible for them.



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