ENBE 2025

XXI International Meeting of the Portuguese Association for Evolutionary Biology

BOOK OF ABSTRACTS

18th-19th December 2025

Bragança

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Edited by

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Instituto Politécnico de Bragança, Portugal

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POSTER 17| WHY SINGLE SNP ANALYSES FAIL: EPISTATIC STRUCTURAL EFFECTS IN HONEY BEE CYP336A1

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Abstract

Cytochrome P450 enzymes are central to pesticide metabolism and resistance, yet how these proteins diversify substrate specificity while maintaining catalytic function remains poorly understood. A genome-wide analysis of CYP336A1 (a nicotine-metabolizing P450) across 1467 *Apis mellifera* males from 25 countries spanning the Mediterranean, Middle East, Europe, and Cuba revealed an intricate haplotype architecture. Despite the detection of only 28 single-nucleotide variants (SNPs), 45 distinct haplotypes were detected for CYP336A1. Among these, 23 haplotypes carried at least four SNPs, and four harboured more than 10. A five-SNP haplotype (D202G; M207I; I222V; V226I; Q238K) dominated at 36% frequency, far exceeding the next most common single-SNP haplotype (D262N, 9%). Interestingly, this dominant haplotype was completely absent from the Iberian Peninsula, North Africa, and Oman and, consequently, from five *A. mellifera* subspecies: *iberiensis, intermissa, jemenitica, mellifera and sahariensis*. To investigate the functional impact of the identified variants, individually and in combination, we used *in sillico* protein structural approaches. Protein models were generated with trRosetta, validated with MolProbity, and evaluated using TM-score and RMSD via TM-Align. Structural modelling revealed remarkable fold congruency: the enzyme encoded by the five-SNP haplotype retained a near-identical fold as compared to the wild-type enzyme (TM-score = 0.998, RMSD = 0.34 Å), as did a rarer 13-SNP haplotype (2%) (TM-score = 0.998, RMSD = 0.38 Å). Individual SNPs also produced minimal backbone displacement (0.32–0.54 Å), suggesting that P450 diversification proceeds through subtle structural adjustments rather than major disruption. Moreover, most SNPs clustered within substrate-recognition regions, whereas catalytic residues remained invariant across haplotypes, demonstrating a partitioning between substrate-recognition/binding evolution and preservation of catalytic machinery. Importantly, single-variant effects cannot predict multi-variant haplotype outcomes. As such, heavy reliance on individual SNPs for pesticide risk assessment may misestimate real metabolic capacity.

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