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 31| BENCHMARKING LAMP PRIMER DESIGN PLATFORMS FOR PYRETHROID RESISTANCE SNP DETECTION IN *VARROA DESTRUCTOR*

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Keywords: *Varroa destructor;* pyrethroid resistance; voltage-gated sodium channel; LAMP diagnostics; evolutionary acaricide resistance monitoring

Abstract

Varroa destructor, an ectoparasitic mite of *Apis mellifera*, is a driver of colony and pollination service declines. Widely used pyrethroid acaricides targeting the mite voltage-gated sodium channel (VGSC) have selected for resistance mutations at codons 918 and 925 in Domain II. Genotyping 100 mites from 35 apiaries at the locus revealed ~40% pyrethroid-resistant haplotypes, with the double-resistant M918L/L925V variant at 43%, establishing the Portuguese baseline. PCR-based assays at this locus are robust but laboratory-bound, whereas loop-mediated amplification (LAMP) offers an isothermal, rapid, low-cost alternative for field resistance surveillance. Using this baseline, we benchmarked mutation-focused LAMP primer sets with PrimerExplorer V5, NEB LAMP Designer, and LAMP Designer v1.16 under shared, explicitly defined thermodynamic constraints. We constrained outer primers and inner-primer Tm to 55–70 °C, loops to ≈62–65 °C, GC to 40–70%, F2–B2 span to 120–180 bp, and filtered candidates using ΔG thresholds for hairpins and dimers. PrimerExplorer V5 provided flexible control over primer geometry around codons and loop placement, but required a loop-primer step and external specificity checks. NEB LAMP Designer rapidly generated optimised sets with conservative end-stability and self-dimer filtering, although limited parameter tuning and absence of BLAST restricted mutation-centred optimization. LAMP Designer v1.16 delivered an exhaustive thermodynamic assessment of hairpins, self-dimers, and cross-dimers together with BLAST-supported specificity and evaluation, at the cost of a steeper learning curve and reliance on proprietary software. In NEB LAMP Designer and LAMP Designer v1.16, targeted codons were embedded in distinct inner-primer segments (918 in F2, F1c; 925 in F1c, B1c, respectively), whereas PrimerExplorer V5 was used to design assays on codon 925 alone, given that M918L never occurred without L925V. Collectively, these comparisons show how software architecture and Tm/ΔG criteria shape LAMP primer solutions for resistance SNPs and inform the design of field-deployable diagnostics for evolutionary surveillance.

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