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Genetic factors potentially reducing fitness cost of organophosphate-insensitive acetylcholinesterase(s) in *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae)*

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Abstract

Acaricidal activity of organophosphate (OP) and carbamate acaricides is believed to result from inhibition of acetylcholinesterase (AChE). Previous studies in *Rhipicephalus* (*Boophilus*) *microplus* demonstrated the presence of three presumptive AChE genes (*BmAChE*s). Biochemical characterization of recombinant BmAChE proteins expressed in the baculovirus system demonstrated that each of the three *R.* (*B.*) *microplus* rBmAChEs have enzymatic properties consistent with designation as functional acetylcholinesterases. Complementary DNAs (cDNAs) for each of the three *BmAChE*s were cloned and sequenced from individual adult tick synganglia excised from OP-susceptible and OP-resistant strains. The data revealed the presence of multiple transcript sequences within individual ticks for each of the *BmAChE*s, suggesting alternative mRNA splicing or expression of multiple alleles for each of the *BmAChE* genes. Quantitative real-time PCR provided evidence of possible gene duplication or amplification for each of the *BmAChE* genes, and direct sequencing of genomic DNA provided evidence of structural *BmAChE* gene diversity with respect to presence or absence of introns, as well as the presence or absence of sequence polymorphisms. Baculovirus expression of rBmAChE1 and rBmAChE3 proteins containing some of the predicted amino acid sequence polymorphisms resulted in production of OP-insensitive AChE, demonstrating that at least some OP-resistant individuals contain mutations that reduce OP-inhibition for at least two of the three known BmAChEs. RNA interference was utilized to silence *in vivo* expression of the *BmAChE* genes in adult ticks, resulting in tick mortality if all three *BmAChEs* were silenced simultaneously, strongly suggesting that the BmAChE proteins functionally complement one another *in vivo*. Together, the results presented provide strong evidence that OP-resistance in *R. microplus* is at least partially mediated by a combination of the expression of multiple genes encoding acetylcholinesterase, mutations in *BmAChE*s resulting in OP-insensitivity, gene duplication, and maintenance of allelic diversity, including both OP-sensitive and OP-insensitive alleles within individual ticks. The authors propose that these factors may mitigate fitness costs that might otherwise result from *BmAChE* mutations, and demonstrate the extreme complexity of OP-resistance in *R.* (*B.*) *microplus*. It is hoped that elucidation of the complex interactions among the multiple BmAChEs and their physiological roles may enable development of new opportunities for tick control.

Key words: Acaricide, resistance, gene amplification, complementation, fitness.

Introduction

The Southern cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini), was discovered to vector bovine babesiosis and anaplasmosis in 1893, which led to an intensive effort over many decades culminating in its eradication from the United States (Graham & Hourrigan, 1977). Since

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