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Author(s): Piironen, Saija; Lindström, Leena; Lyytinen, Anne; Mappes, Johanna; Chen, Yolanda H; Izzo, Victor; Grapputo, Alessandro

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Online Supplementary material:

Piironen et al. "Pre-invasion history and demography shape the genetic variation in the insecticide resistance-related acetylcholinesterase 2 gene in the invasive Colorado potato beetle".

Additional file 5 Methodology used in the amplification of *AChE2*, *DP1* and putative *JHE* genes. To amplify *AChE2* gene, a forward primer as well as internal primers used in sequencing were designed from the Colorado potato beetle-*AChE2* gene available from Genbank [Genbank: L41180.1] while a previously described primer (Clark *et al.* 2001) was used as the reverse primer. To amplify *PD1* gene, primers were designed from the Colorado potato beetle *DP1* gene available in Genbank [Genbank: X86074.1] while previously described primers (Vermunt *et al.* 1998) were used to amplify *JHE* gene.

	<i>AChE2</i>	<i>DP1</i>	<i>JHE-b</i>
Primers			
Forward	5'-CGACGTTGTAAAACGACGGCCAGTACTCAACCCGGTGTTC-3' [*]	5'-CGAATTACCTTAAAAGGGAGCA-3'	5'-ATGGCATCCAATCAAAGATAC-3'
Reverse	5'-TTTCACACAGGAAAAGCTATGACACTGCTCTCATAACAGTCCATCA-3' [*]	5'-CAAATCTGCTCCAGCTCCAC-3'	5'-GATCATTTCAGGTGTCAATTG-3'
Internal forward used in sequencing	5'-AACCTTGGACGTTTACGACG-3'	5'-TGAAATCAAGCCCCACTATTT-3'	
Internal reverse used in sequencing	5'-CGTCGTAAACGTCCACCAAGGTT-3'	5'-TGGGTGTAGTTTGTCTGAATGG-3'	
PCR mix for amplification (total volume 25µl)	1.5 mM MgCl ₂ , 0.2 mM of each dNTP, 0.2 µM of each primer, 1 x buffer of Taq polymerase (Biotools), 1 unit of Taq DNA polymerase (Biotools) and 20-50 ng of genomic DNA	2 mM MgCl ₂ , 0.2 mM of each dNTP, 0.2 µM of each primer, 1 x buffer of Taq polymerase (Biotools), 1 unit of Taq DNA polymerase (Biotools) and 20-50 ng of genomic DNA	2 mM MgCl ₂ , 0.2 mM of each dNTP, 0.2 µM of each primer, 1 x buffer of GoTaq Flexi polymerase (Promega), 1 unit of GoTaq Flexi DNA polymerase (Promega) and 20-50 ng of genomic DNA
Thermal cycle program	(94°C for 3min) 1 cycle + (94°C for 30s, 60°C for 30s with 1 decrease in temperature per cycle, 72°C for 1min 30s) 10 cycles + (94°C for 30s, 50°C for 30s, 72°C for 1min 30s) 30 cycles + (72°C for 10min) 1 cycle	(94°C for 3min) 1 cycle + (94°C for 1min, 52°C for 1min, 72°C for 1min 30s) 30 cycles + (72°C for 5min) 1 cycle	(94°C for 2 min) 1 cycle + (94°C for 30s, 55°C for 30s, 72°C for 1min 30s) 36 cycles + (72°C for 5min) 1 cycle
Thermal cycle program for amplification of cloned inserts	(94°C for 3min) 1 cycle + (94°C for 30s, 45°C for 30s, 72°C for 1min 30s) 30 cycles + (72°C for 5min) 1 cycle	(95°C for 3min) 1 cycle + (94°C for 30s, 60°C for 30s, 72°C for 1min 30s) 30 cycles + (72°C for 2min) 1 cycle	

^{*}M13-tails in italics