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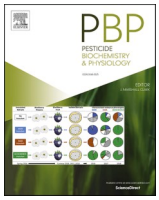

Aberrant splicing of a nicotinic acetylcholine receptor alpha 6 subunit is associated with spinosad tolerance in the thrips predator *Orius laevigatus*

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ABSTRACT

Susceptibility to insecticides is one of the limiting factors preventing wider adoption of natural enemies to control insect pest populations. Identification and selective breeding of insecticide tolerant strains of commercially used biological control agents (BCAs) is one of the approaches to overcome this constraint. Although a number of beneficial insects have been selected for increased tolerance to insecticides the molecular mechanisms underpinning these shifts in tolerance are not well characterised. Here we investigated the molecular mechanisms of enhanced tolerance of a lab selected strain of *Orius laevigatus* (Fieber) to the commonly used biopesticide spinosad. Transcriptomic analysis showed that spinosad tolerance is not a result of overexpressed detoxification genes. Molecular analysis of the target site for spinosyns, the nicotinic acetylcholine receptor (nAChR), revealed increased expression of truncated transcripts of the nAChR α6 subunit in the spinosad selected strain, a mechanism of resistance which was described previously in insect pest species. Collectively, our results demonstrate the mechanisms by which some beneficial biological control agents can evolve insecticide tolerance and will inform the development and deployment of insecticide-tolerant natural enemies in integrated pest management strategies.

1. Introduction

Natural enemies play an important role in successful integrated pest management (IPM), providing an effective alternative to traditional chemical control. Many IPM programs rely on a combination of various biological control agents (BCAs) to suppress pest populations. Thanks to decades of improvements in insect husbandry most BCAs are now reared commercially (van Lenteren et al., 2018; van Lenteren, 2012), and are most widely used in greenhouse crops in Europe and North America (van Lenteren, 2012). However, the widespread use of insecticides in agricultural systems can severely limit the deployment efficacy of BCAs. For example, exposure to insecticides often leads to deleterious sub-lethal effects on beneficial insects (Siviter and Muth, 2020; Sánchez-Bayo, 2021; Bielza et al., 2009). The intensive use of insecticides can lead to the evolution of insecticide resistance. This has been documented in both pests and beneficial insects (Bielza, 2016), however, knowledge of the molecular mechanisms underlying resistance primarily comes from work on pest species (Sparks and Nauen, 2015). In the context of predatory insects, insecticide resistance may be seen as a beneficial trait and the isolation of more tolerant strains by direct selection of laboratory populations or further selection of field tolerant strains has been attempted (Abbas et al., 2014; Bonafos et al., 2007).

Members of the *Orius* genus are used worldwide in IPM programs to control populations of thrips and other damaging pests such as aphids and whiteflies (Chambers et al., 1993). Like other beneficial insects, the effectiveness of *Orius* spp. as biological control agents (BCAs) can be negatively impacted by exposure to insecticides. In fact, members of the *Orius* genus have been reported to show high susceptibility to commonly used insecticides, including some which are considered lower risk (Siviter and Muth, 2020; Lin et al., 2021). *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) is used extensively in Europe, Africa and Asia to control common pests of protected crops such as sweet peppers, cucumbers or chysamtemums (Bouagga et al., 2018). Although a generalist predator, *O. laevigatus* can complete development and survive feeding on plant material such as pollen and sap, contributing to population survival and increased length of crop protection (Bouagga et al.,

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2018). Its commercial success as a BCA has led to selective breeding efforts to improve its biological and ecological characteristics (Bielza et al., 2020). Strains of *O. laevigatus* have been artificially selected for a larger body size (Mendoza et al., 2020), better survival on pollen feed (Mendoza et al., 2021) and improved tolerance to synthetic insecticides belonging to the neonicotinoid (Balanza et al., 2019) and pyrethroid classes (Balanza et al., 2021a), as well as emamectin benzoate (Balanza et al., 2022) and the biopesticides spinosyns (Balanza et al., 2021b). The latter are natural compounds produced by fermentation of the Actinomycete bacterium, *Saccharopolyspora spinosa (*Thompson et al., *2000)* and are used for control of pests in the Lepidoptera, Diptera and Thysanoptera orders in conventional and organic agriculture. Since their introduction, the impact of spinosyns on BCAs has been investigated, and although acute toxicity was usually not reported, deleterious sublethal effects were observed, particularly in parasitoid wasps (Biondi et al., 2012). However, the widespread use of spinosyns has led to the development of significant resistance levels in many pest species, which were usually attributed to modifications of spinosyn target site, the α6 subunit of the nicotinic acetylcholine receptor (Bao et al., 2014; Guillem-Amat et al., 2020; Berger et al., 2016; Hsu et al., 2012).

In this study we investigated the molecular mechanisms of insecticide resistance in a recently reported artificially-selected strain of *O. laevigatus* which exhibits significant tolerance (approximately 20-fold) to spinosad and spinetoram (Balanza et al., 2021b). We also utilized genomic and transcriptomic resources to fully annotate nicotinic acetylcholine receptors (nAChR) which are molecular targets for both spinosyns and neonicotinoids in this species.

2. Methods

2.1. Insects and chemicals

All standalone chemicals used were purchased from Merck (Germany). The *O. laevigatus* spinosad susceptible agrobio (SUS) and resistant SPI38 (RES) strains have been described previously (Balanza et al., 2021b). Additionally, a cross of SUS and RES and survivors of F4 500 ppm spinosad treatment were used in this study (CRS). Briefly, two hundred individuals in the last nymphal stage of the SUS and RES strains were isolated in 5 ml vials with *Ephestia kuehniella* eggs (hereafter *Ephestia* eggs) as food. Upon adulthood they were sexed and to obtain the F1, virgin SUS females and males were mated with virgin RES males and females, respectively. After 4 days, all the females were placed together and reared in the laboratory by using 1l plastic containers with filter paper on the lid, with ad libitum access to frozen *Ephestia* eggs as food, pieces of green bean pods as moisture source and egg-laying substrate, and black wheat husk as hideout to avoid cannibalism. This colony were maintained under controlled conditions at 26 ± 1 °C, 65 ± 5% rh, and L16:D8 light regime, and were allowed to inbreed to F4. Then, 650 adults (1–3-days old) were tested with a discriminating dose of spinosad (500 ppm), expecting a mortality around 50–75%. This dose is much higher than the recommended field rate for spinosad (120 ppm). The bioassay was carried out using 60 ml cups with ventilated lids. Bean pods were cut and sealed by using paraffin wax. These beans were dipped into the insecticide solution and agitated for 60 s, then air dried and put into the containers. *Ephestia* eggs ad libitum as source of food and buckwheat husk as refuge were added into the containers. Mortality was assessed after 72 h. Individuals were considered dead if no movement could be observed. The survivors were used in this study.

2.2. Nucleic acid extractions and cDNA synthesis

Total RNA was extracted from a pool of 10 individuals using the Isolate II RNA mini kit (Meridian Bioscience, USA) following the manufacturer's protocol. The quality of RNA was checked using Nanodrop, Qubit™ Broad range RNA kit (ThermoFisher Scientific, USA), and agarose gel electrophoresis. A total of 1 μg of total RNA was used for cDNA synthesis using Maxima H Minus Reverse Transcriptase (ThermoFisher Scientific, USA) following the manufacturer's protocol. Random hexamers were used for cDNA synthesis for use in qPCR reactions and Oligo $(dT)_{15}$ for use in high fidelity RT-PCR to verify all nAChR open reading frames (ORFs).

2.3. RNAseq and in silico data mining

Four biological replicates were used for both SUS and RES populations for the generation of barcoded libraries (TrueSeq RNA library preparation, Illumina), which were then sequenced on an Illumina NovaSeq using a 150 bp paired-end reads (PE) (Novogene, China) generating over <40 M reads per replicate.

The *O. laevigatus* genome assembly GCA_018703685.1 was obtained from NCBI and the resistant and susceptible RNAseq libraries were aligned using HISAT2 v2.1.0 (Kim et al., 2015). Protein coding genes were predicted using the Braker2 v2.1.6 pipeline incorporating the RNA-seq alignments as evidence (Bruna et al., 2021). The resulting output was filtered using AGAT v0.6.0 (*NBISweden/AGAT: AGAT-v1.2.0 (v1.2.0) v. v1.2.*0, 2023) to remove short (< 200 bp) and incomplete gene models. The translated gene models were searched against the NCBI nr and Interpro databases and these results were loaded into OmicsBox v2.0.36 (Conesa et al., 2005) to complete functional annotation using BLAST2GO mapping and annotation features. The completeness of the set of gene models was assessed using BUSCO 5.2.2 (Simao et al., 2015) in protein mode against the Insecta dataset.

Differential expression analysis was performed using HISAT2 and Stringtie v1.3.4 (Pertea et al., 2015) to align and quantify expression, followed by identification of differentially expressed genes in DESeq2 1.36.0 (Love et al., 2014). A threshold of absolute (logFC) ≥ 1 and adjusted *p*-value <0.05 was used to identify significantly differentially expressed genes. GO enrichment of significantly upregulated/down-regulated genes was performed in OmicsBox using Fisher's Exact Test All sequence data has been deposited with NCBI under BioProject PRJNA1046017, while annotation data was deposited with Zenodo DOI: https://doi.org/10.5281/zenodo.10160568.

2.4. RT-PCR, cloning and sanger sequencing

All primers used in this study are listed in supplementary table 2. Characterisation of full-length ORFs of genes of interest and SNP verification was done by PCR using Q5 taq polymerase (New England Biolabs, USA) in 25 μl reactions containing 1 μl of cDNA and 12.5 pmol of each primer. Primer annealing temperatures were calculated using the NEB Tm calculator (https://tmcalculator.neb.com/#!/main). PCRs were run on a T100 Thermal cycler (Bio-rad, USA) using cycling conditions as follows: initial denaturation at 98 °C for 30 s, 35 cycles of 98 °C for 10 s, primer pair specific annealing Tm for 20 s, 72 °C for 2 min and final extension of 72 °C for 5 min. PCR products were then run on a 1% agarose gel and bands of the expected size were purified using the Qiaquick gel extraction kit (Qiagen, Germany) following the manufacturer's instructions. PCR products were then ligated in pJet 1.2 vector using CloneJET PCR Cloning kit (ThermoFisher Scientific, USA) following manufacturer's protocol and transformed into DH5α competent cells (ThermoFisher Scientific, USA). Positive clones were identified via colony PCR using vector specific primers and MyTaq™ Mix (Meridian Bioscience, USA). Pure plasmid DNA was obtained using GeneJET plasmid miniprep kit (ThermoFisher Scientific, USA) following the manufacturer's protocol. Sanger sequencing was done using the SupremeSeq Service from EuroFins (Germany). Predicted protein sequence alignments were done in Geneious Software v. 10.2.6 (Biomatters, NZ) using MUSCLE. Annotated insect nicotinic acetylcholine receptors from *Acyrthosiphon pisum*, *Anopheles gambiae*, *Apis mellifera*, *Bombyx mori*, *Drosophila melanogaster*, *Nilaparvata lugens* and *Tribolium castaneum* together with *Orius laevigatus* sequences were pulled from NCBI and used to construct a phylogenetic tree (accession numbers in

supplementary table 4). The best fit protein substitution model was elucidated using MEGAX. The final tree was constructed in Geneious using the PhyML plugin running Le and Gascuel with a discrete Gamma distribution model (LG + G) (Le and Gascuel, 2008).

2.5. qPCR

All qPCR reactions (15 μl) contained, 7.5 μl of SYBR® Green JumpStart™ Taq ReadyMix (Merck, Germany), 4 μl of cDNA (5 ng) and 0.25 μM of each primer (Supplementary table 2). Cycling conditions comprised: 3 min at 95 °C, followed by 40 cycles of 95 °C for 15 s, 58 °C for 15 s and 72 °C for 15 s. A final melt-curve step was included post-PCR (ramping from 65 to 95 °C by 0.5 °C every 5 s) to confirm the absence of any non-specific amplification. The efficiency of PCR for each primer pair was assessed using a serial dilution of 100 ng to 0.01 ng of cDNA. Each qPCR experiment consisted of at least 8 biological replicates with two technical replicates for each. Data were analysed according to the $\Delta\Delta C_T$ method (Pfaffl, 2001). The expression level was normalized to two reference genes, VGSC (voltage-gated sodium channel) and Actin. Data was visualized using GraphPad Prism v10 (GraphPad Software, USA).

3. Results and discussion

3.1. Spinosad resistance is not mediated by overexpression of metabolic enzymes

To investigate the molecular mechanisms underlying the high tolerance of *O. laevigatus* to spinosyns we generated transcriptomes for both the SUS and RES strains. In order to leverage the data generated for reference genome-guided identification of differentially expressed gene we conducted annotation of the recently published genome of *O. laevigatus* (Bailey et al., *2022*) using BRAKER2 (Bruna et al., 2021; Buchfink et al., 2015; Hoff et al., 2019; Lomsadze et al., 2014; Stanke et al., 2008; Stanke et al., 2006). We identified 18,784 protein-coding genes, resulting in a BUSCO score of 88.5%. We used this annotation for comparison between SUS and RES strains and identified 198 differentially expressed genes (DEGs), out of which 62 were upregulated and 136 downregulated (sup. Table 1). GO enrichment analysis only identified GO terms in the downregulated gene subset associated with endopeptidase activity (sup. Table 3). Alterations of endopeptidase activity have been reported previously in various insects exposed to a range of different insecticides (Wilkins, 2017). In the cases relating to spinosad resistance, general upregulation of protease activities was observed in *Musca domestica* (Saleem et al., *2009*) and *Frankliniella occidentalis* (Zhang et al., *2013*), and upregulation of serine protease transcripts in *Bactrocera oleae* (Sagri et al., *2014*). We cannot completely rule out an indirect effect of these genes on insecticide tolerance, however, this phenomenon was never shown to have a mechanistic effect on insecticide metabolism and could simply be attributed to an overall stress response (Wilkins, 2017). Manual curation of DEGs did not identify any genes commonly involved in insecticide detoxification or cuticle formation. The lack of clear change in expression of the detoxification enzymes corroborates bioassay results of the RES strain which showed no significant synergistic effects when exposed to common inhibitors of the main detoxification gene families (Balanza et al., 2021b). Together these results suggest that metabolic mechanisms are unlikely to play a role in the resistance of the RES strain, suggesting target site modifications are the most likely primary mechanism of resistance as originally proposed by Balanza et al (Balanza et al., 2021b).

Table 1
List of identified nAChR subunits.

Subunit	ORF length (bp)	scaffold	Accession no.	Exon no.
Alpha 1	1605	JAGWEN010000050.1	PP078866	7
Alpha 2	1614	JAGWEN010000050.1	PP078867	7
Alpha 3	1833	JAGWEN010000140.1	PP078868	11
Alpha 4	1653	JAGWEN010000959.1	PP078869	11
Alpha 6	1494	JAGWEN010000221.1 and JAGWEN010000667.1	PP078870	10
Alpha 7	1488	JAGWEN010001260.1 and JAGWEN010001305.1	PP078871	10*
Alpha 8	1596	JAGWEN010000050.1	PP078872	11
Alpha 9	1266	JAGWEN010000050.1	PP078873	9
Alpha10	1554	JAGWEN010000785.1	PP078874	8*
Beta 1	1512	JAGWEN010000292.1	PP078875	7
Beta 3	1243	JAGWEN010000298.1	PP078876	9

* Partial sequence missing from genomic scaffolds.

3.2. Manual curation of O. laevigatus nAChR subunits

Previous research has identified insect nicotinic acetylcholine receptors as molecular targets of spinosyn insecticides (Thompson et al., 2000), specifically the α6 subunit appears to be critical for binding and insecticidal mode of action of these compounds. Using a combination of genomic and transcriptomic data we identified and manually curated 11 distinct genes encoding nAChR subunits in *O. laevigatus* (Table 1). The predicted ORFs were then used as a reference for in silico mapping of RNAseq reads from both strains to verify the completeness of computational gene models and identify any potential single nucleotide polymorphisms (SNPs). Finally, we experimentally validated the coding sequence of all 11 nAChR subunits in the SUS and RES strains via PCR and Sanger sequencing and deposited the verified sequences with NCBI (Table 1). Phylogenetic analysis and protein BLAST searches categorized these genes as 9 alpha and 2 beta subunits. This is in line with other insect species, which show a subset of well-conserved α subunits, at least one β subunit and a subset of poorly conserved species-specific divergent subunits (Fig. 1). Comparison of PCR and RNAseq mapping between the SUS and RES strains indicated missense SNPs were only found in the *Olα6* subunit gene, thus subsequent analysis focused on this subunit.

3.3. Point mutations in the nAChR α6 subunit is not linked with spinosad tolerance

Due to the apparent very poor expression of the *Olα6* gene in *O. laevigatus* (only ~200 reads successfully mapped to the predicted ORF), additional analysis was required to fully resolve both its relative expression and the presence of SNPs. RT-PCR, cloning, and Sanger sequencing of the full-length *Olα6* from both strains identified seven missense SNPs in exon 5 of *Olα6* (Fig. 3A) in both strains. These lead to the following amino acid substitutions N134S, N135G, N155S, K147R, K147E, I152M, T155A. In the case of N135 and K147 two potential substitutions were detected in sequenced populations. The identified cluster of mutations found in exon 5 does not appear to be a product of a mutually exclusive splice site but rather a combination of different allelic forms of the receptor. Modifications of α6 subunit genes have been commonly associated with resistance to spinosad in multiple species. These include amino acid (AA) substitutions such as G275E found in thrips *Frankliniella occidentalis* (Puinean et al., *2013*) and *Thrips palmi* (Bao et al., *2014*), G275V in *Frankliniella intonsa* (Hiruta et al., *2018*), P146S in *Drosophila melanogaster* (Somers et al., *2015*), and the AA deletion F238del in tomato pinworm *Tuta absoluta* (Grant et al., *2019*). No AA substitutions at the equivalent positions were found in *O. laevigatus Olα6*, although all 8 SNPs were identified near P146, in a functionally important part of the protein within and immediately downstream of loop E (Fig. 2A). Multiple alignments with other α6 subunit genes showed that out of 8 substitutions, only K147 is well conserved across different species. RT-PCR, cloning, and sequencing of the region from pools of 10 individuals was conducted to establish the frequency of each polymorphism in the RES and SUS strains. Additionally, spinosad-selected (CRS) insects were included in this analysis to examine if any of the mutations increased in frequency in survivors of

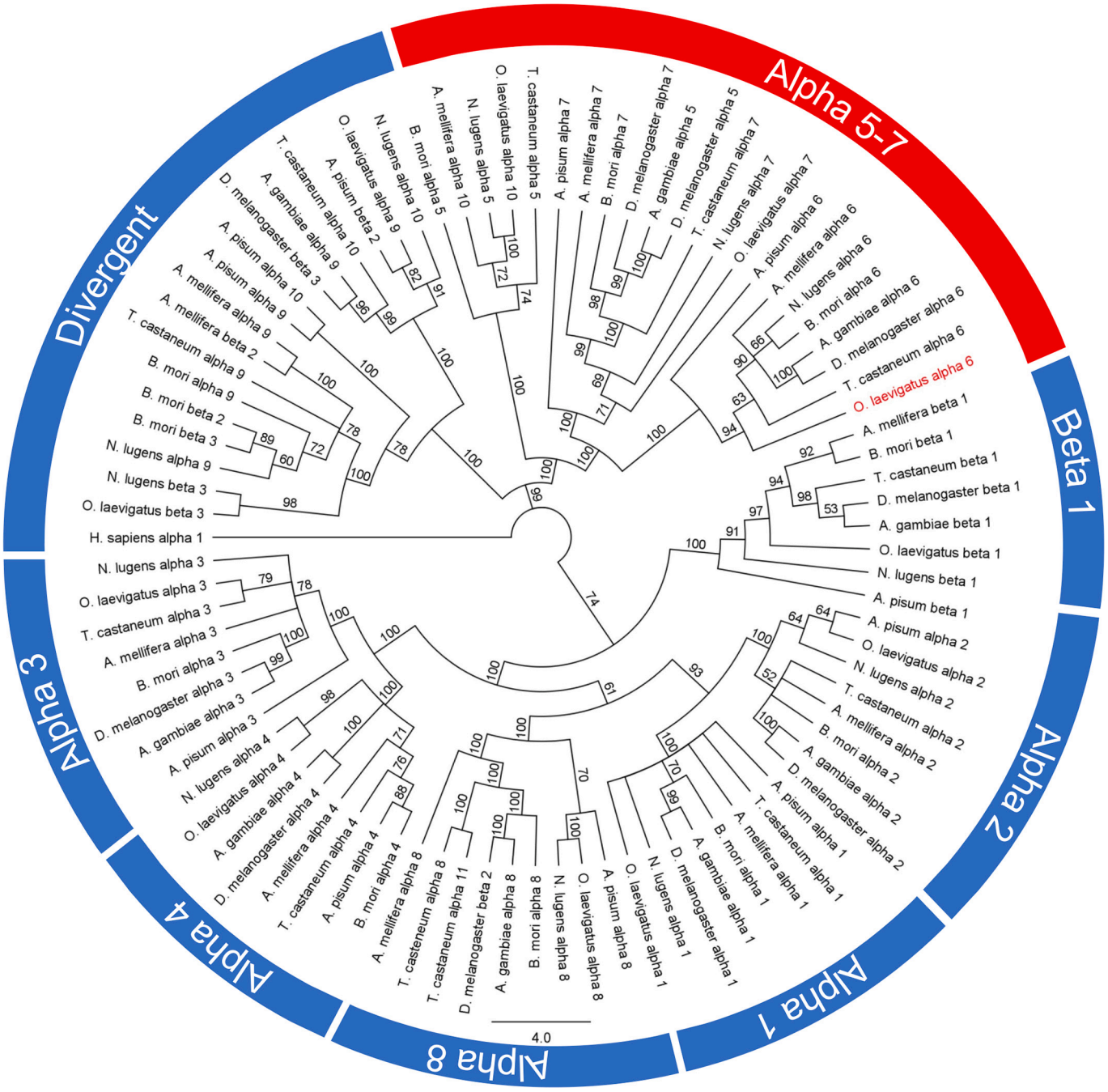

Fig. 1. Phylogenetic tree with boostraps values characterising 11 nAChR subunit genes identified in *O. laevigatus* genome and transcriptomes, compared to annotated representatives from each major insect order using the human nAChR *α1 subunit* as an outgroup. Distribution of *O. laevigatus* genes closely follows the pattern of other insects, the Ola α6 subunit is highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

exposure to a discriminating dose of spinosad (500 ppm) well above a field recommended rate of 120 ppm. In total, 37 susceptible, 39 resistant, and 40 selected individual colonies were sequenced, and the frequency of each mutation was calculated. Although N135S and T155A do appear at a higher frequency in the resistant insects, their relative abundance in the susceptible population indicates they are unlikely to be responsible for the resistant phenotype (Fig. 2B). A similar phenomenon was observed in the spinosad resistance study of the olive fly, *Bactrocera oleae*, which identified 3 missense SNPs in the same region of the receptor including the equivalent mutation to K147R reported here. However, in the case of *B. oleae* these point mutations had no effect on computationally predicted receptor structure (Sagri et al., 2014). Moreover, as in the case of *B. oleae* the levels of resistance reported in the *O. laevigatus* strains used in our study (~20 fold) (Balanza et al., 2021b) are not consistent with those typically seen in other insects with nAChR α6 subunit point mutations, which can reach well over ~1000 fold (Puinean et al., 2013; Perry et al., 2007).

Given that there is a considerable transcriptomic diversity of the *Olα6* coding sequence in *O. laevigatus*, the AA changes identified may be a result of a different mechanism unrelated to insecticide exposure. The α6 subunit is known to undergo RNA editing in several different species and extensive alternative splicing (Jin et al., 2007). Interestingly all identified AA changes are a result of A to G transitions within exon 5. The reason for this is unclear, patterns of A to G transitions have been

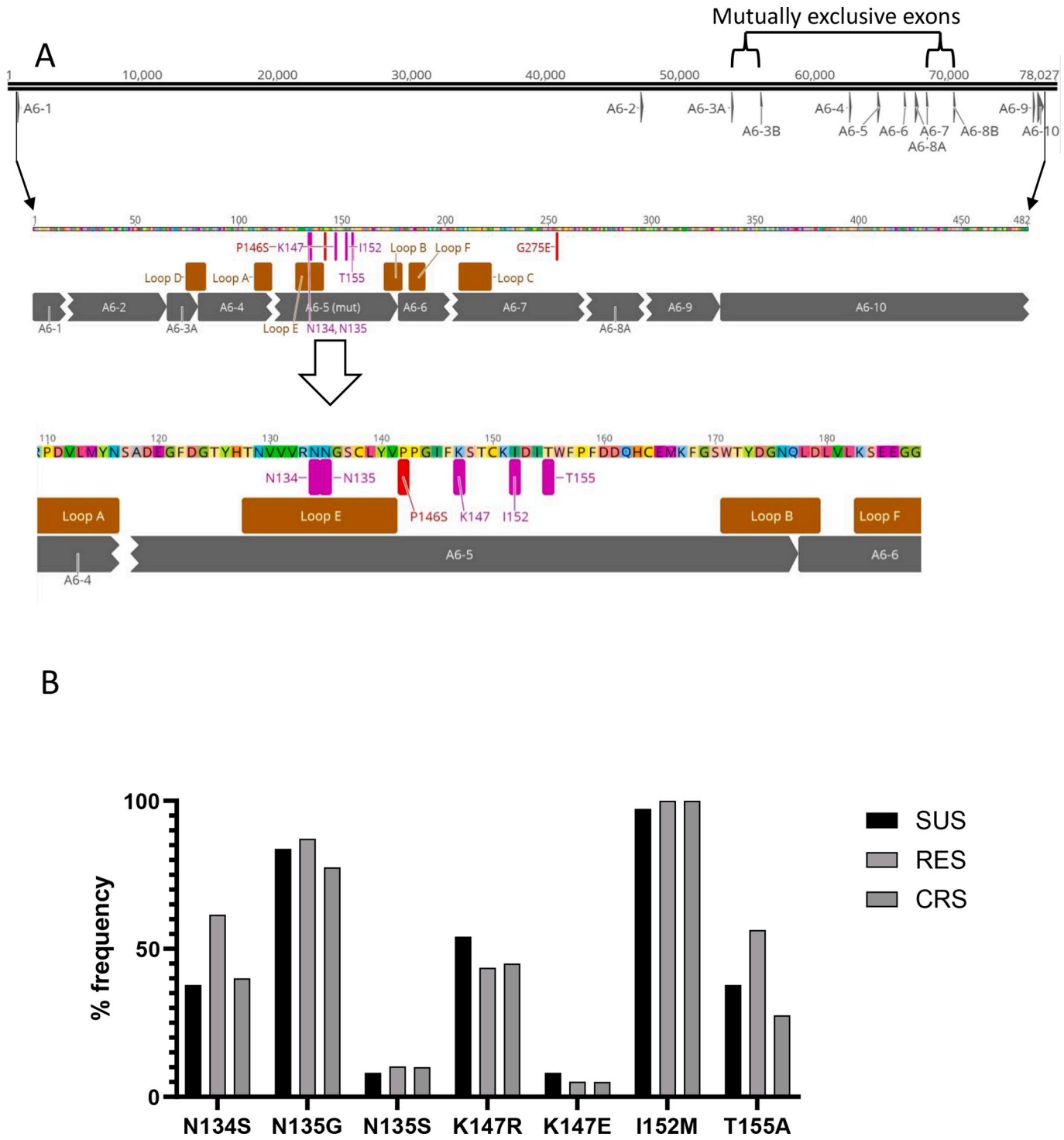

Fig. 2. Graphical representation of the *O. laevigatus* α6 subunit genomic structure (top **A**), predicted protein sequence (middle) with corresponding exons (grey), functional loop domains (brown), previously identified point mutations (red), and novel point mutations (purple). The bottom panel **B** shows a close-up of exon 5 which contains all the identified point mutations with *D. melanogaster* P146S highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

linked with the regulation of CpG methylation in mammals (Waters and Swann, 2000). In silico analysis of the *Olα6* ORF revealed that a large portion of exon 5 sits in a putative CpG island (232-464 bp) as predicted by GC-profile 2.0 (Lai and Gao, 2022). However, gene methylation levels and patterns in insects are very different in comparison to mammals and there is still considerable debate on the specific function of methylation in invertebrates (Duncan et al., 2022).

3.4. Aberrant splicing is more prevalent in the resistant strain

Apart from point mutations, spinosad resistance can evolve from aberrant splicing of the α6 subunit gene which results in premature stop codons and a truncated non-functional protein (Berger et al., 2016; Hsu et al., 2012). As described for other insects, we identified multiple splice forms of *Olα6* with 2 mutually exclusive splice sites in exons 3 and 8, and truncated ORFs with a deletion or partial deletion of either exon 3, 5 or

8. Out of these modifications only the deletion of exon 5 results in the formation of premature stop codon and a greatly truncated protein. Since exon 5 encodes sequences for both Loop E and a portion of loop B, its absence would result in a non-functional protein product (Fig. 3A). Exon 5 deletion transcripts were found in PCR products from all three tested strains and since all three also express the full-length receptor the resulting truncation is not a product of deletion in the genomic DNA. Although the overall amount of the *Olα6* subunit transcripts does not change between the tested strains (Fig. 3B), qPCR specific for the truncated ORF revealed an approximately 4-fold increase of the truncated version of *Olα6* in the RES strain in comparison to the SUS strain (Fig. 3C). Truncated receptors have been linked to a strong spinosad resistance in major pests such as *Plutella xylostella* (Baxter et al., *2010*), *Bactrocera dorsalis* (Hsu et al., *2012*) and *T. absoluta* (Berger et al., *2016*) and *Ceratitis capitata* (Guillem-Amat et al., *2020*). Lack of functional α6 subunit results in a loss of spinosad sensitivity, which has been experimentally confirmed in a transgenic *D. melanogaster Dmα6* null mutant (Perry et al., 2007). In the absence of other mechanisms, the relatively modest increase of the aberrant splicing in the RES strain appears to be the most likely driver of the spinosad-tolerant phenotype of *O. laevigatus*. The precise molecular mechanisms previously shown to drive the generation of aberrant splice forms differ from case to case. In the diamondback moth *P. xylostella* miss-splicing appears to occur as a result of a point mutation in the 5′ donor site of intron 9 in the α6 subunit gene (Baxter et al., 2010). While in *T. absoluta* the change in the methylation levels of one of the CpG sites located in intron 2, coupled with altered expression of splice factors was linked with the formation of truncated transcripts (Berger et al., 2016). In spinosad-resistant *B. dorsalis* deletion of exon 5 in the genomic DNA was the underlying mechanism (Hsu et al., 2012). The mechanism underlying aberrant splicing of *Olα6* requires further investigation and would benefit from selection of a strain that exclusively produces aberrant transcripts. We found no evidence of differential expression of splice factors in our RNAseq analysis, nor modification of genomic DNA (either mutation or exon deletion) as the WT full length transcripts of *α6 subunit* are still found in the RES strain.

4. Conclusions

Traditionally investigation of insecticide resistance predominately focuses on studying economically important pest species. The presence and nature of mechanisms of insecticide tolerance in beneficial insects (pollinators, natural enemies) are much less well characterised (Bielza, 2016). The development of spinosad resistance in beneficial insects has only been reported in the lacewing *Chrysoperla carnea,* where a field-resistant strain was made more tolerant to spinosad with selection (Abbas et al., 2014), although the molecular mechanisms involved were not investigated. In the case of *O. laevigatus* increase in the aberrant splicing of the *α6* subunit, a well-characterised mechanism of resistance in insect pests (Berger et al., 2016; Hsu et al., 2012; Baxter et al., 2010), appears to be the primary factor contributing to the resistant phenotype. It is tempting to hypothesise that the considerable spread of innate tolerance (up to 48-fold) to spinosad observed in the wild populations of *O. laevigatus* (Balanza et al., *2021b*) may be related to the natural extensive splicing diversity of the *α6* subunit gene. The development of stable increased tolerance to a pesticide in a beneficial insect, used commercially for biocontrol is a significant discovery and could be a

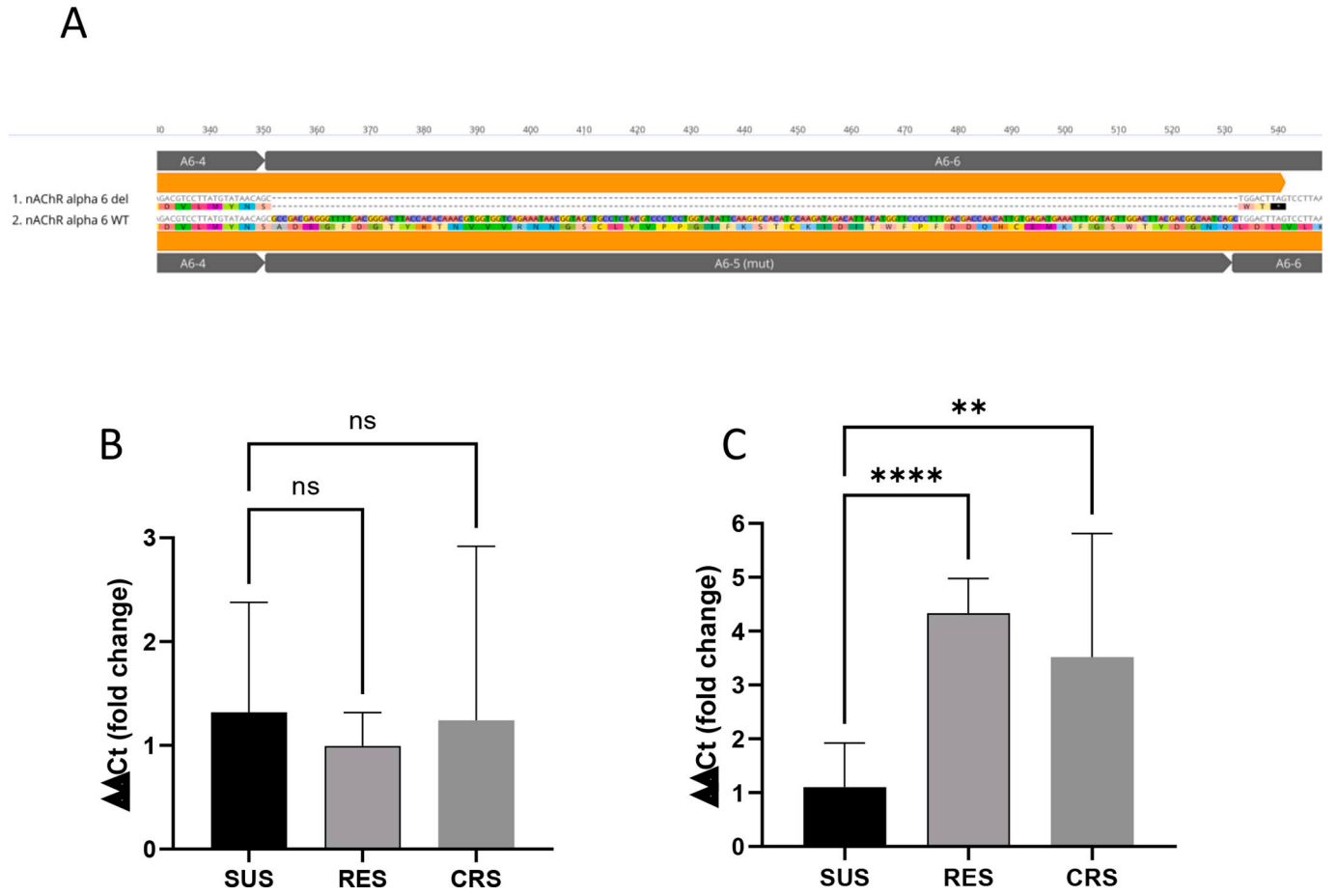

Fig. 3. Alignment of the WT and truncated α6 subunit DNA sequence with AA translation below. Predicted ORFs are labelled orange and exons are labelled grey. Deletion of exon 5 results in a frame shift and a premature stop codon (**A**). Relative expression of α6 subunit measured by qPCR in the SUS, RES and CRS strains (**B**), and the truncated transcript missing exon 5 (**C**). Error bars represent 95% confidence intervals, Significance was calculated using a two-tailed *t*-test, $N = 8$.

useful tool in improving existing IPM strategies.

CRediT authorship contribution statement

Bin Zeng: Methodology, Investigation, Formal analysis. **Benjamin J. Hunt:** Writing – review & editing, Software, Methodology, Investigation, Data curation. **Adam Pym:** Writing – review & editing, Formal analysis, Data curation. **Virginia Balanza:** Resources, Methodology, Investigation. **Chris Bass:** Writing – review & editing, Validation, Supervision, Resources, Project administration. **Pablo Bielza:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Bartlomiej J. Troczka:** Writing – original draft, Methodology, Formal analysis, Data curation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pestbp.2024.105837.

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