



Predicting the insecticide-driven mutations in a crop pest insect: Evidence for multiple polymorphisms of acetylcholinesterase gene with potential relevance for resistance to chemicals

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1 **Predicting the insecticide-driven mutations in a crop pest insect: evidence for**
2 **multiple polymorphisms of acetylcholinesterase gene with potential relevance**
3 **for resistance to chemicals**

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Abstract

The silverleaf whitefly *Bemisia tabaci* (Gennadius, 1889) (Homoptera: Aleyrodidae) is a serious invasive herbivorous insect pest worldwide. The ~~misuse and~~ excessive use of pesticides ~~has~~ progressively selected *B. tabaci* specimens, ~~progressively~~ reducing the effectiveness of the treatments, and ultimately ending in the selection of pesticide-resistant ~~ce~~ strains. The management of this crop pest has thus become challenging owing to the ~~high~~ levels of resistance to all major classes of recommended insecticides, ~~including organophosphates (OPs)~~. Here, we used *in silico* techniques for detecting molecular mutations in *ace1* gene from naturally occurring *B. tabaci* variants, and monitor the presence and frequency of the detected mutations from 30 populations of the silver whitefly from Egypt and Pakistan. We found several point mutations in *ace1*-type acetylcholinesterase (*ace1*) in the studied *B. tabaci*-~~B~~ variants (~~MEAM1~~) naturally occurring in the field. By comparing *ace1* sequence data ~~and point mutations with those reported from~~ ~~on an~~ organophosphate-susceptible ~~and an organophosphate-resistant~~ strains of *B. tabaci* (~~SUD-S~~), ~~and to~~ *ace1* sequence data retrieved from GenBank for that species ~~and to~~ nucleotide polymorphisms from other arthropods, we identified novel mutations that could potentially influence insecticide resistance. ~~Seven Egyptian field strains had 100% homology with the fragment of the Israeli B. tabaci susceptible allele (SUD-SUD), revealing a high conservation of the sequence/nucleotide, and/or possible missense mutations. We also report novel amino acid substitutions (S369W, C316W, P317G, and P317F) as likely candidates conferring resistance to organophosphates. Indeed, the location of these mutations, which we visualized on the 3D crystal structure of acetylcholinesterase, occur close to the access of the active site gorge of the enzyme. The phylogenetic analysis revealed that these novel gene sequences appeared at the top of the clade in the tree, suggesting that the reported novel point mutations have evolved recently.~~ Homology modeling and molecular docking analyses were performed to determine if the ~~mutation-induced changes in form 1 acetylcholinesterase (AChE1) structure could confer resistance to carbamate and organophosphate insecticides. Mutations had small effects on binding energy (ΔG_b) interactions between mutant AChE1 and insecticides; they altered the conformation of the peripheral anionic site of AChE1, and modified the enzyme surface, and these changes have potential effects on the target-site sensitivity.~~ Altogether, the results from this study provides information on genic variants of *B. tabaci ace1* for future monitoring insecticide resistance development and report a potential case of environmentally driven gene variations.

Keywords: Acetylcholinesterase, environmental toxicants, resistance, putative mutations, ~~target-site insensitivity~~ ~~homology modeling, molecular docking~~

Introduction

Significant changes in farming procedures have become a vital necessity when human population numbers started to grow significantly, and this has favored the development and spread of land-extensive and intensive agriculture methods (Raven and Wagner 2021). Nowadays, about 11% of land surface is planted with crops (Raven and Wagner 2021) whose productivity is being continuously improved by novel technologies. For instance, genetic engineering has been promoted in the past decades to ensure subsistence and sufficiency in food production, and maintain competitiveness and economic reliability in the long term (Jordan 2002; Babar et al. 2020). To maximize agricultural productivity, application of soil amendments and synthetic fertilizers are additional techniques commonly employed for maintaining soil fertility in association with the spray of pesticides that limit crop losses (Hepperly et al. 2009; Gianessi 2013; Popp et al. 2013; Bonanomi et al. 2020). Yet, in the long-term, the frequent application of fertilizers and pesticides have impacts on the environment biodiversity and ecosystems (Katayama et al. 2015; Arslan 2018; Tibbett et al. 2020), including in the surrounding fields (Tscharntke et al. 2005), and the long-lasting effects after agricultural abandonment are alarming (Isbell et al. 2019). Worst, the misuse and excessive use of pesticides have progressively selected specimens of the chemically targeted species, progressively reducing the effectiveness of the treatments, and ultimately ending in the selection of pesticide-resistant strains (Vontas et al. 2011; Voudouris et al. 2011; Engell Dahl et al. 2021; Renault and Colinet 2021). The phenomenon has been so important and widespread for insects that a committee dedicated to the management of insecticide resistance has been created in 1984: Insecticide Resistance Action Committee (IRAC, <https://irac-online.org/>) (Sparks et al. 2021).

The development of mechanisms of insecticide resistance occurs at any stage of the mode of action of the chemical insect, and can belong to one out of four categories: (i) behavioral resistance, whereby the insect avoids the insecticide behaviorally (Zalucki and Furlong 2017), (ii) increased resistance to penetration due to modifications in cuticle structure which reduce (or slow down) the insecticide's absorption (Lilly et al. 2016), (iii) increased metabolic resistance implying improvement in the detoxification capacities of the insect (Ahmad et al. 2006), and (iv) target site insensitivity and/or alteration of pharmacokinetic processes, whereby the sensitivity to the chemical is reduced by mutation-derived changes in the structure of the site(s) targeted by the insecticide (Soderlund and Knipple 2003). Importantly, different combinations of these categories have been reported from resistant populations of several insect species, offering a multi-layered protection against chemicals and an extremely high level of insecticide resistance (Ahmad et al. 2006).

Nowadays, increased metabolic resistance, target site insensitivity and/or modifications of pharmacokinetic processes represent the main known mechanisms of insecticide resistance

(Soderlund and Knipple, 2003; Hemingway et al. 2004; Li et al. 2007; Panini et al. 2016). Metabolic resistance mainly relies on the action of the following enzymes: glutathione-S-transferases (Pavlidis et al. 2018), cytochrome P450 monooxygenases (Liu et al. 2015), and non-specific esterases (Li et al. 2007). Target site insensitivity, developed as the resistance mechanism to carbamate and organophosphate insecticides, is associated with point mutations of the key enzyme in neurotransmission encoded by *ace* genes, acetylcholinesterase (AChE, acetylcholine acetylhydrolase, E.C. 3.1.1.7) (AChE, EC 3.1.1.7) (Hemingway et al. 2004). With the exception of some species of Diptera, insects possess two distinct acetylcholinesterase (*ace*) gene clusters (Huchard et al. 2006). Meanwhile, it is assumed that among one of these two genes, only *ace1* which encodes acetylcholinesterase 1 (AChE1), plays a role in insecticide-resistance (Bourguet et al. 1996; Benting and Nauen 2004; Kim et al. 2006). Mutations making AChE1 less sensitive to the organophosphate- and carbamate-synthesized insecticides have been reported from multiple insect species, including the silverleaf whitefly *Bemisia tabaci* (Gennadius, 1889) (Homoptera: Aleyrodidae) (Alon et al. 2008; Jiang et al. 2009; Zhang et al. 2012; Hopkinson et al. 2020).

Historically, mutations and/or genic variations in insecticide resistance-related genes, as for instance *ace1*, have long been assumed to be somehow limited (Ffrench-Constant et al. 1998). However, this view has been refuted by several studies who reported that adaptation to man-made contaminants can occur rapidly, as found with the mutations at the *Ace* locus which occurred during three independent mutations, producing multiple simple and complex resistant-*ace* alleles in the fruit fly *Drosophila melanogaster* (Karasov et al. 2010). Yet, mutations and/or variation in nucleotides coding for amino acids that may confer resistance to organophosphates are now well-characterized for *ace* (Menozzi et al. 2004; Temeyer et al. 2013; Bendele et al. 2015; Aguilar et al. 2018; Nagar et al. 2018). For the nicotinic acetylcholine receptor (nAChR: the neonicotinoid target site), Wang et al. (2017) identified 23 single-nucleotide polymorphisms leading to the replacement of 19 amino acids in insecticide resistant strains of *B. tabaci*.

The silverleaf whitefly *B. tabaci* is a key severe agricultural crop pest, causing considerable damage worldwide by sucking the phloem sap of more than 60 crop plants, in addition to transmitting hundreds of economically important plant viruses (Ex.: *Geminiviridae*) (Navas-Castillo et al. 2011; Leke et al. 2015). When feeding, the sugar droplets made by *B. tabaci* contaminate the cotton plant, severely affecting the cotton industry by making cotton fibers sticky (van der Sluijs 2019), and by favoring the development of microorganisms (Oliveira et al. 2011). Control of *B. tabaci* populations has been long depended on conventional insecticides such as organophosphates, carbamates and pyrethroids (Palumbo et al. 2001). By the late 1980s, these broad-spectrum insecticides have progressively failed in controlling this crop pest (Ahmad et al. 2010; Horowitz et al. 2020; Shaurub et

al. 2021), and management of *B. tabaci* populations became highly challenging as they developed high levels of resistance to recommended insecticides (Denholm et al. 1998; Horowitz et al. 2020).

Control strategies aiming at containing populations of the silverleaf whitefly are also further complicated by the fact that *B. tabaci* encompasses several cryptic species (at least 39 genetically divergent variants), and this complex of different genetic groups (populations) is distributed worldwide in tropical and sub-tropical regions (de Moya et al. 2019). Among these variants, the B and Q variants, also known as Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) genotypes (de Barro et al. 2011), respectively, have established outside their endemic range (de Moya et al. 2019), and have developed strong resistance to all major insecticide classes (Horowitz et al. 2020). The B variant (MEAM1) causes extreme damages to field crops, in particular in irrigated agroecosystems in Egypt (Shaurub et al. 2021), Pakistan (Shah et al. 2020; Paredes-Montero et al. 2021), and more largely in the Indian subcontinent, Sudan, and in Central and South America (Azab et al. 1971; Brown and Bird 1992; Horowitz et al. 2020).

~~In Egypt and Pakistan, the~~ management of *B. tabaci* in Egypt and Pakistan has largely relied on the application of organophosphates (Abdallah 1991; Sobeiha et al. 1997; Ahmad et al. 2002, 2008, 2010; El Kady and Devine 2003; Shaurub et al. 2021), and farmers are increasingly complaining about the reduced effectiveness of the recommended chemicals. Hence, the extensive use of ~~these chemicals~~ organophosphates in addition to carbamates over the past decades has certainly contributed to the selection of naturally occurring genetic variants whose insecticide resistance is driven by distinct mutations in *ace1*-type AChE. In this work, we analyzed molecular sequence data from resistant strains of *B. tabaci* to determine if changes in the sites targeted by organophosphate insecticides have occurred and could confer insecticide resistance. In this study, we used *in silico* techniques for detecting molecular mutations in *ace1* gene from naturally occurring *B. tabaci* variants, and monitor the presence and frequency of the detected mutations from 30 populations of the silver whitefly from Egypt and Pakistan. We first examined if similar point mutations as those reported by Alon et al. (2008) from ~~an~~ organophosphate-resistant strain of *B. tabaci* (B-ref) could be observed. Second, to assess newly identified mutations that would potentially influence insecticide resistance, we detailed amino acid substitutions/indels and their positions in the protein sequence to infer the potential changes this would have on structural and chemical properties of the enzyme. The relatedness and differences of *ace1* gene among *B. tabaci* populations, alongside with other insect species, was examined; this phylogenetic analysis helped in determining if *B. tabaci* variants resistant to organophosphates have a common ancestor, and if some point mutations in *ace1* gene could be shared among species. Finally, computational methods were used to predict protein structure and protein-ligand interactions. A 3D structure of the susceptible and resistant *B. tabaci* AChE1 deduced

amino acid sequence was built through homology modeling. Then, molecular docking was performed to determine if the structural modifications in form 1 acetylcholinesterase (AChE1) due to point mutations could alter the protein-ligand binding affinity, and confer resistance to carbamate and organophosphate insecticides.

Material and Methods

Entrez searches and sequence alignment

The silverleaf whitefly *Bemisia tabaci* sequence genes encoding acetylcholinesterases (*ace1*) were downloaded from the National Center for Biotechnology Information nucleotide database (NCBI's GenBank, <https://www.ncbi.nlm.nih.gov/nucleotide/>) by using the Entrez search and retrieval system EDirect (Kans, 2022). Two mRNA sequence sets (LC199300–LC199317 [Egypt] and KU307116.1–KU307130.1 [Pakistan]) of the *B. tabaci* B variant (MEAM1) were retrieved. As the mutations in *ace1* mRNA sequences from *B. tabaci* populations from Australia, China and Israel were already analyzed (Hopkinson et al. 2020; Jiang et al. 2009; Zhang et al. 2012; Alon et al. 2008, respectively), these datasets were not considered in our study.

The *esearch* function was used to scrutinize the NCBI database with the following queries: 'acetylcholinesterase or AChE', 'ace1, ace 1 or ace-1', '*Bemisia tabaci*', 'B biotype', 'B variant', 'MEAM1', 'Egypt', 'Pakistan', and 'Middle East Asia Minor'. The *ace1* sequences were sorted out by length with the function *efilter* so fragments in the range 450–650 bp could be retained. The filtered nucleotide sequences were downloaded in FASTA format using the *efetch* function. Sequences were aligned using MUSCLE v3.8.31 (Edgar 2004) implemented with the "Align Multiple Sequences" tool available in Mesquite v3.70 (Maddison and Maddison 2021). They were additionally trimmed to remove poorly aligned sequence ends using trimAl v2.0 (Capella-Gutiérrez et al. 2009), based on automatically selected thresholds. These steps yielded a total of 16 *ace-1* sequences for Egyptian variants (Supplementary Information S1 Table 1), and 14 *ace-1* sequences for Pakistani variants.

Finally, the dataset was enriched by adding two reference sequences described by Alon et al. (2008) from an organophosphate-susceptible strain of *B. tabaci* (SUD-S)₁ and from one organophosphate-resistant strain (B-ref) from Israel. The verified sequences and documented mutations were obtained from GenBank. The *B. tabaci* resistant alleles (accession no. ABV45412) are characterized by the substitution of F392 with W392 (F392W), while the F392 is conserved in the alleles (accession no. ABV45413) from the susceptible variant (accession no. ABV45413). These documented sequences were used as reference sequences for performing comparisons with the 16 *ace-1* sequences we downloaded/uploaded from GenBank.

Sequence comparisons of *ace1*-type acetylcholinesterase

Egyptian *Bemisia tabaci* variants

Sequence comparisons of the *ace1*-type acetylcholinesterase were realized in order to characterize and predict those point mutations inducing amino acid substitutions that could be associated with insecticide resistance. Multiple sequence alignment was realized with MultAlin (Corpet 1988; available at: <http://multalin.toulouse.inra.fr/multalin/>), and CLC Genomics (Qiagen, Netherlands), to compare alleles from reference (susceptible, resistant) *B. tabaci* variants with those from supposedly resistant Egyptian strains. The lengths of the nucleotide sequences, and their positions on the sequenced *ace1* fragments, and thus deduced protein sequences (AChE1), markedly differed between the Egyptian and Pakistan *B. tabaci* variants. Hence the screening for mutations, either nucleotide- or protein-based, had to be performed separately.

Specific changes in the amino acid sequences of *ace1* resulting in the alteration of the targeted site are commonly numbered according to the modeled protein structure. For consistency with former works, the conventional numbering system based on *Torpedo (=Tetronarce) californica* (Pacific electric ray) for AChE ~~proteingenes~~ (CAA27169.1) was used (Mutero et al. 1994), and this also aligns with the recommendations made by Hopkinson (et al. 2020) (Supplementary Information S2 Fig. 1A). ~~Then, we used the three-dimensional (3D) crystal structure of AChE obtained from *T. californica* AChE (Dvir et al. 2010) to make inference on the substrate binding domain, the amino acid residues forming the catalytic triad (active site), and putative binding of AChE in the active site in *B. tabaci* AChE (Fig. 1B,C).~~

A molecular phylogenetic tree of *B. tabaci* AChE1 alongside with other arthropod species was constructed by neighbor-joining criteria using Archaeopteryx v.0.9920 beta (Han and Zmasek 2009) (based on forester 1.045), and by considering the JTT matrix-based model (Jones et al. 1992). This procedure conveniently describes the evolutionary distances among species (Roy et al. 2014). The 24 species considered in the tree, and accession numbers, were: *Pediculus humanus corporis* (Body louse, ncbi:BAF46104), *Rhopalosiphum padi* (Bird cherry-oat aphid, ncbi:AAT76530), *Aphis citricidus* (Brown citrus aphid, ncbi:ALS35348), *Nilaparvata lugens* (Brown planthopper, ncbi:AFC88123), *Sogatella furcifera* (White-backed planthopper, ncbi:AGN31550), *Liposcelis paeta* (Grain psocid, ncbi:ADA60183), *Locusta migratoria manilensis* (Oriental migratory locust, ncbi:ABY75631), *Periplaneta americana* (American cockroach, ncbi:ALJ10969), *Blattella germanica* (German cockroach, ncbi:ABB89946), *Bombyx mori* (Silk moth, refseq:NP_001037380), *Spodoptera exigua* (Beet armyworm, ncbi:AGO59308), *Helicoverpa armigera* (Cotton bollworm, ncbi:AAY59530), *Cydia pomonella* (Codling moth) ncbi:ABB76667, *Chilo suppressalis* (Asiatic rice borer moth, ncbi:ABO38111), *Plutella xylostella* (Diamondback moth, ncbi:AAY34743), *Leptinotarsa decemlineata* (Colorado potato beetle, ncbi:AEI70751), *Apis mellifera* (Honeybee, ncbi:ANT80563), *Harpegnathos*

226 *saltator* (Jerdon's jumping ant, ncbi:EFN89510, *Tremex fuscicornis* (Tremex wasp, ncbi:AKM70918),
 227 *Culex quinquefasciatus* (Southern house mosquito, ncbi:AFR68411), *Culex pipiens pallens* (Mosquito)
 228 ncbi:AAV28503), *Aedes aegypti* (Yellow fever mosquito, ncbi:ABN09910), *Anopheles gambiae* (African
 229 malaria mosquito, ncbi:AJZ73939), and *Ctenocephalides felis* (Cat flea, ncbi:CBI832507). Bootstrap
 230 (10000 replicates) values are indicated for each root. The tick *R. microplus* AChE1 (JAC59086) was used
 231 as the out-group. The tree was drawn to scale, with branch lengths measured in the number of
 232 substitutions per site. The analysis involved 45 amino acid sequences (226 total sites, 29 conserved
 233 sites). All positions containing gaps and missing data were removed.

234

235 Pakistani *Bemisia tabaci* variants

236 The analysis of the different Pakistani *B. tabaci* variants characterized by *ace1* mutations was
 237 performed by collecting available information from GenBank from 14 Pakistan *B. tabaci* variants, *i.e.*,
 238 GenBank nucleotide sequences KU307116.1–KU307130.1; the corresponding deduced proteins are
 239 AMO25775.1–AMO25789.1. Note that the sequences KU307116–KU307121 belong to the B variant of
 240 *B. tabaci* while the nucleotide sequence KU307122–KU307130 belong to the Asia II I variant. The
 241 sequences were then compared with those of the Israeli resistant and susceptible variants of *B. tabaci*
 242 AChE1 (Alon et al. 2008) in order to examine if similar patterns of mutations were shared among *B.*
 243 *tabaci* strains. Malik et al. (2016) generated the sequence distance matrix for this dataset with SDT
 244 v1.2. They showed that *ace1* sequences were 100% conserved among the Pakistan-endemic *B. tabaci*
 245 strains.

246

247 Predicting the nature of mutations through ESTHER database

248 To examine if point mutations are shared among species, or have resulted in different classes of
 249 AChE1, [BLAST-based searches against the ESTHER Database \(ESTerases and alpha/beta-Hydrolase](#)
 250 [Enzymes and Relatives\)](#) were conducted. This analysis further supported our predictions of the
 251 potential links between point mutations and insecticide resistance in the *B. tabaci* variants considered
 252 in the present work. To that aim, protein sequences were aligned, and compared to experimentally
 253 verified insecticide resistance-associated mutations in the AChE1-type acetylcholinesterase
 254 sequences from different insects, ticks, and mites (information obtained from ESTHER database,
 255 <https://www.re3data.org/repository/r3d100010542>). Specifically, we included the sequences from
 256 *Anopheles gambiae* (CAD32684), *Camponotus floridanus* (EFN61766), *Chilo suppressalis* (AKL78859),
 257 *Cyrtorhinus lividipennis* (AHW29553), *Lygus hesperus* (JAG37539), *Nasonia vitripennis* (K71U37),
 258 *Pediculus h. capitis* (BAF46106), *Phlebotomus papatasi* (AFP20868), *Rhipicephalus microplus*
 259 (AJA71270), and *Tetranychus urticae* (ADK12685). Sequence homology was analyzed with the program

SeqMan v.7.0.0 (DNASTAR Lasergene, Madison, WI, USA), and the translated AChE1 protein sequences were run against the ESTHER database. The sixteen deduced *B. tabaci* AChE1 amino acid sequences obtained from GenBank for Egypt (BAW19578-BAW19595; **Table 1**), the two reference sequences of *B. tabaci* AChE1 from Israel (ABV45412 and ABV45413, for resistant and susceptible alleles, respectively; **Supplementary Information S1Table 1**), and forty-two AChE1 sequences from various insect species and orders (the species and the GenBank accessions numbers are displayed on the phylogenetic tree) were aligned with MUSCLE.

Three-dimensional structure modeling and molecular docking

All-atom three-dimensional (3D) structure prediction of the sequence ABV45413.1 of the *B. tabaci* AChE1 protein was modeled with the SWISS-MODEL webserver (Biasini et al. 2014). The model quality assessment of the protein structure was evaluated with the SWISS-MODEL and Molprobiy webserver (Williams et al. 2018). Then, molecular dynamics simulations were carried out for 100 ns with the GROMACS software (Van Der Spoel et al. 2005). For the simulations, the protein was solvated using the TIP4P water model, and ionized with 0.154 M NaCl at 37 °C and 1 atm pressure (Harrach and Drossel 2014). CHARMM27, a popular force field for biomolecular simulations, was run using periodic boundary conditions at normal volume and temperature (NVT) ensemble (Huang and MacKerell 2013). Trajectory clustering was carried out with the University of California San Francisco (UCSF) Chimera 1.14 software (Pettersen et al. 2004), and allowed to identify four groups of trajectories (clusters) whose representative conformations (four representative structural models, i.e., one 3D representative structure for each group of trajectory) were further analyzed.

Next, the mutations (Egyptian strains: F392W, S369W, L370V, C316W, P317G, and P317F; Pakistani strains: G251D, N252R, P253S, C329S, and L330T) were introduced to each representative structural model, and subsequently minimized with the WinCoot 0.9.2 software (Emsley and Cowtan 2004). The structure of acetylcholine (ACh), and of 20 organophosphate and 20 carbamate pesticides, were retrieved from PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) in the 3D structure data file (SDF) format (Kimet al. 2020). Insecticide selection was based on information available at <https://irac-online.org/pests/bemisia-tabaci/>. For organophosphates, the list included: azinphos-methyl, bromophos ethyl, chlorfenvinphos, chlorpyrifos, cyanofenphos, dialifos, diazinon, EPN, isoxathion, leptophos, phosalone, phosmet, phoxim, pirimiphos-methyl, profenofos, prothiofos, quinalphos, sulprofos, tetrachlorvinphos, and triazophos. For carbamates, the list included: aldicarb, aminocarb, bendiocarb, butocarboxim, carbaryl, carbofuran, carbosulfan, ethiofencarb, fenobucarb, fenoxycarb, formetanate, formparanate, methiocarb, methomyl, metolcarb, mexacarbate, pirimicarb, promecarb, propoxur, and thiofanox.

Code de champ modifié

Molecular docking was performed on the four different conformations for each mutated protein (eleven) and the wild type. AutoDock Vina software was used with a flexible ligand in a flexible active site protocol (Trott and Olson 2010). The active site was retrieved by superimposing the *B. tabaci* AChE1 to the solved structure (PDB ID: 6ARX) (Cheung et al. 2018). Molecular dynamics simulations were run for 877 ns, as it allowed to reach one of the equilibration stages of the protein. A cartoon representation of the superimposition of the 3D structures was subsequently drawn after 877 ns of molecular dynamics simulation.

A comparison of the four representative structures of each mutated protein to the original 3D AChE1 conformation highlighted one active site triad (S262, E388 and H501; see results below). The effect of the mutations on the AChE1 pKa values was predicted with the PropKa online webserver (Li et al. 2005). Changes in active site pKa values (a lower pKa would reveal a greater ability in the release of the proton) in mutant AChE1 of *B. tabaci* in comparison to its wild type relative could yield an efficient mutant enzyme.

Results

AChE1 homology and molecular phylogenetic tree

The homology matrix of amino acid sequences of AChE of *B. tabaci* from Egyptian field strains (see Supplementary Information S1Table 1 for strain designations) is presented in Figure 1. The partial predicted amino acid sequences of the eleven *B. tabaci* field strains (Mbs, Mas, Qbn, Mym, Myi, Gkt, Fas, Fib, Dbq, Beb, and Res (B variant [MEAM1])) had 96-100% homology; these sequences were homologue at 97.6-100% with the fragment of the *B. tabaci* resistant allele (B-ref variant) (Alon et al. 2008). The analysis revealed that variations in specific amino acid residues did exist, suggesting the occurrence of nonsynonymous mutations. Seven field strains (HS-S, Dmg, Bwn, Fsn, Gzf, Qqb, and Mys) had 100% homology, including with the fragment of the *B. tabaci* susceptible allele (SUD-S4D), revealing a high conservation of the sequence/nucleotide, and/or possible missense mutations.

Phylogenetic analysis based on *B. tabaci* AChE1 sequences showed the existence of two groups: (i) with some few exceptions, one cluster grouped susceptible strains, and (ii) the second group encompassed resistant strains (Supplementary Information S3Fig-2). *Bemisia tabaci* formed a cluster with AChE1 from different insect species, including planthoppers, locusts, and cockroaches, suggesting the existence of similar forces driving evolutionary changes in AChE1s among species.

Sequence comparisons of ace1-type acetylcholinesterase from Egyptian *B. tabaci* variants

Detection of ace1-type AChE mutations

Eighteen cDNAs containing putatively partial open reading frames (ORFs) of *B. tabaci ace1* genes were obtained from GenBank under the accession numbers LC199300-LC199317 (Egypt) (Supplementary Information S1 Table 1). Those sequences had a total length of approximately 500 bp. Point mutations that occurred in the coding region of *ace1* gene were investigated by comparing nucleic acid and deduced amino acid sequences (Table 12 and Fig. 2A,B). The data revealed the existence of:

- (a) four silent (synonymous; a mutation of a single DNA nucleotide that is not altering the amino acid sequence of the encoded protein but may alter the splicing process) mutations: (i) I298I (ATC→ATT) in the Dbq variant, alongside with three other silent mutations, (ii) G307G (GGC→GGT), (iii) V354V (GTC→GTG), and (iv) G389G (GGC→GGG) occurred in the eleven strains Egy-B Lab, Beb, Gkt, Qbn, Dbq, Mym, Mas, Fas, Myi, Mbs, and Fib.
- (b) six non-silent (non-synonymous, includes missense mutations corresponding to the replacement of one amino acid by another amino acid in the protein; nonsense mutations corresponding to the substitution of an amino acid by a stop codon; mutations in splice sites; or finally insertions or deletions) mutations:
 - i) substitution of the amino acid F392W which was found in the 10 Egyptian field strains (Beb, Dbq, Fas, Fib, Gkt, Qbn, Mym, Myi, Mbs, and Mas) and in the laboratory strain Egy-B). This substitution has been reported to confer resistance to OPs in populations of the B variant of *B. tabaci* (Alon et al. 2008).
 - (II, III) two point mutations, the substitution of the amino acids S369W (TCC→TGG) and Leu370Val (CTG→GTG) were reported from four variants (Fas, Myi, Mbs, Fib).
 - (IV, V) two point mutations, the substitution of the amino acids C316W (TGC→TGG) and P317G (CCT→GGT) observed in two variants (Mym and Mas)
 - (VI) one point mutation, with the replacement of the amino acid P317F (CCT→TTT) in one variant (Fib) (Table 12).

Conformational changes induced by the detected ace1-type AChE mutations

Mutations are likely to confer conformational changes to the encoded protein structure, thereby reducing, or even preventing, the action of the insecticide. The detected substitutions of the amino acids S369W, C316W, and P317F reported above are located at the acyl pocket of the protein, which is the cavity corresponding to the active site of the enzyme and where the ligands bind (Fig. 1B,C). In the four strains (Fas, Myi, Mbs, Fib) characterized by the substitution of the amino acid S369W, the mutation resulted in a narrower and more hydrophobic acyl pocket, reducing its accessibility to alkoxyl group or inhibitors.

Sequence comparisons of *ace1*-type acetylcholinesterase from Egyptian-Pakistani *B. tabaci* variants

Our analysis of the data ~~yet~~ revealed the occurrence of five missense mutations among the fourteen *B. tabaci* strains (*ace1* sequence variants) from Pakistan (Fig. 3A,B). As exemplified with AMO25775.1, the N-terminal sequence of the Pakistan strain is characterized by the replacement of three amino acids: G251D (GGG→GAT), N252R (AAC→CGA), and P253S (CCG→ICG). Moreover, our analysis also revealed the substitution of two additional amino acids at the C-terminal sequence, namely C329S (TGC→TCG) and L330T (CTC→ACC) (Fig. 3A).

Searching for candidate mutations potentially linked to insecticide resistance

To search for common residues involved in insecticides' resistance and evaluate the frequency of mutations and variations in amino acid residues in two specific segments of our *B. tabaci* AChE1 dataset from Egypt, the sequences were compared with other AChE1-type acetylcholinesterases from different arthropods (insects, ticks, and mites) using the ESTHER database (Marchot and Chatonnet 2012; Lenfant et al. 2013) (Supplementary Information S4Fig-5). The sites where point mutations have occurred and conferred resistance to organophosphates and/or to carbamates show a high level of similarity. Specifically, AChE1 sequences from the different analyzed insect species exhibited 65-97% identity (79-98% similarity). Two different point mutations, P317G (BAW19591.1) and P317F (BAW19586.1), seem to be involved in the resistance of *B. tabaci* to organophosphates. By combining the available information, the analysis also suggested that the other mutations may not be related to insecticide resistance.

Three-dimensional structure models

The 3D protein structure prediction_s of *B. tabaci* AChE1 were plotted using the SWISS-MODEL webserver. The global model quality estimation (GMQE), and the qualitative model energy analysis (QMEAN) scores were 0.77 and 0.87±0.05, respectively. The protein RMSD trajectory reached equilibrium after five ns, with an RMSD value of 2.1 Å, before it increased to 2.7 Å after 70 ns (Figure 4A). The radius of gyration (RoG) and solvent accessible surface area (SASA) values were stable during the entire simulation, with values of 23 Å and 22500 Å², respectively. The total number of formed H-bonds (780) was highly stable. The Ramachandran plot of the models, which was run to ensure the structure reliability, revealed that the proportions of residues in most favored, allowed, and disallowed regions were 99.4, 94.4 %, and 0.6 %, respectively (up-right panel of Figure 4B).

The root-mean-square fluctuations per residue (in Å) averaged over the duration of the simulation (100 ns) is presented in Figure 4B. Protein structure is shown in a cartoon mode, with red (L400-D450), magenta (S490-W496), and yellow (L545-S553) coloring indicating regions with high RMSF values (>

2.5 Å). The random coil-like conformation revealed the presence of regions of disorders which may potentially be involved in the binding of a ligand. Residues of active sites are labeled in black (S262, E388, and H501), and have RMSF values < 2 Å. AChE1 mutations detected in Egyptian and Pakistani populations of *B. tabaci* lie apart from the three highly fluctuating regions (RMSF < 2 Å).

Molecular docking

The four 3D models were superimposed on each other, and the residues of active sites were represented by black dots (Figure 5A). This procedure allowed us to identify the active site gorge of AChE1 and to characterize residues that may be part of gorge lining (Fig. 5B,C).

Mutations detected in Egyptian and Pakistani populations (F392W, S369W, L370V, C316W, P317G, P317F, G251D, N252R, P253S, C329S, and L330T) were then introduced to the four conformations of the protein, and the mutated proteins were subjected to geometry optimization using the universal force field (UFF) (Emsley and Cowtan, 2004; Rappé et al. 1992). In all mutants but one, F392W whose mutation which is was located in the choline-binding domain of AChE1, mutations were located outside the active site gorge. By focusing on the structural motifs of the active site gorge, it appeared that the three disulfide bonds (C130-C157, C316-C329, and C463-C585; red colored sticks in Fig. 6A) which are typical from wild type *B. tabaci* AChE1 were likely disrupted by point mutations C316W and C329S. These mutations are located at the peripheral anionic binding site (black sticks in the dotted region of Figure 6A) of AChE1. Three other mutation points, P317G, P317F, and L330T, are also located at this site, and altered the aromatic content at the gorge entrance. Point mutations S369W and L370V are located at the surface of the enzyme (Fig. 6A, top-left panel; mutations are represented by black sticks), and should thus affect binding with neighboring residues, and the structure of the protein at the bottom of the gorge (Figure 6B). Last, point mutations G251D, N252R, and P253S (Fig. 6C; mutations are represented by black sticks) are surface-accessible residues, with potential effects on protein recognition by other cellular factors.

The average binding energies (in kcal/mol) of the 20 organophosphate and the 20 carbamate insecticides against the active site residues WT, F392W, S369W, L370V, C316W, P317G, P317F, G251D, N252R, P253S, C329S, and L330T of AChE1 protein are shown in Supplementary Information Material S5A and S5B. The docking study showed that the average binding energies of the 40 insecticides ranged from -4.8 kcal/mol to -8.55 kcal/mol, revealing weak to moderately weak interaction energies (Figure 6A,B). In general, the natural substrate molecule ACh had higher values, around -4.6 kcal/mol, thus characterizing relatively weak interaction energies (see Supplementary Information S5A and S5B for the detailed average scores for each model). No significant change was reported in the binding affinity with ACh or with the pesticides between the WT and the mutated

protein isoforms (Figure 6A,B). Three insecticide organophosphates, methomyl, aldicarb, and butocarbaxim, and methomyl were characterized by average binding energies in the range of those reported for AChE1 (Figure 6A; Supplementary Information S5A).

pKa predictions of wild-type and mutant AChE1

As pKa values of amino acid side chains play a crucial role in determining the pH-dependent characteristics of an enzyme, pKa values of wild-type and mutant AChE1 models of *B. tabaci* were predicted with the PropKa (<https://www.ddl.unimi.it/vegaol/propka.htm>). E388 was the only ionizable residue from the active site triad, and calculations of averaged pKa values were thus restricted to E388 for each AChE1 variant (WT and 11 mutated AChE1), and for the four 3D models of the protein. One point mutation, F392W, altered average pKa value (Mutant: 5.605 ± 1.568 ; Wild type: $pKa = 5.575 \pm 1.568$). This change echoed the slightly higher binding energy (-4.5 kcal/mol) of mutant F392W AChE1 as compared with the one reported for the wild type (-4.6 kcal/mol) (Supplementary Information 5A,B).

Discussion

The misuse and repeated application of insecticides has contributed to the resurgence of several insect pests, and to the selection of insecticide-resistant strains (Binu 2019; Li et al. 2019; Umina et al. 2019). The silverleaf whitefly *B. tabaci* is no exception (Horowitz et al. 2019) and has developed high resistance to several recommended insecticides (Elbert and Nauen, 2000; Zhang et al. 2015; Naveen et al. 2017), in particular to organophosphates that target AChE (Elbert and Nauen, 2000; Zhang et al. 2015; Naveen et al. 2017; Horowitz et al. 2019,20). This serine hydrolase is widely known for its role in hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses, in addition to being involved in the development, mutation, and maintenance of vertebrate and invertebrate nervous system (Grisaru et al. 1999; Ranson et al. 2002). In the resistant insects, the alteration of AChE structure prevents the action of OPs (Siegfried and Scharf, 2001). In this work, we analyzed molecular sequence data that could be candidates of the changes of the sites targeted by organophosphate insecticides in resistant strains of *B. tabaci*.

Site mutations of AChE conferring insensitivity to organophosphates have been identified in insects as the primary mechanism of resistance to these insecticides (Walsh et al. 2001; Vontas et al. 2002; Nabeshima et al. 2003; Cassanelli et al. 2006). In *B. tabaci*, the F392W mutation of the *ace1* gene confers resistance to organophosphates, and this mutation has been reported from several strains (Alon et al. 2008; Jiang et al. 2009; Vassiliou et al. 2011; Gauthier et al. 2014; Hopkinson et al. 2020). In the original AChE model established from *Torpedo-T. californica*, this mutation corresponds

463 to F331W. We also report here the existence of three new novel putative point mutations C316W,
464 P317F, and S369W from *B. tabaci ace* gene, and likely representing novel paralogous genes. The
465 existence of *ace*-paralogous genes is frequent in insect pests, as found by Gao et al. (2002) in the green
466 bug *Schizaphis graminum*, or by Weill et al. (2002, 2004) who formerly reported *ace*-paralogous gene
467 from fifteen mosquito species. In the mosquitoes *Culex pipiens* and *Anopheles gambiae*, the
468 insensitivity of AChE to insecticides was also resulting from the substitution of a single amino acid
469 G119S (Weill et al. 2002, 2004).

470 A single amino acid substitution is sufficient for conferring insecticide resistance as reported by
471 Nabeshima et al. (2003, 2004) in a pirimicarb-resistant strain of *Myzus persicae* (S431F) and in an
472 organophosphate-resistant strain of *Culex tritaeniorhynchus* (F455W). In addition to the mutation
473 point, the nature of the substitution, i.e., the type of amino acid, has a strong incidence on the
474 subsequent activity of the enzyme. Specifically, the replacement of the amino acid C418 by tryptophan
475 in the α subunit M4 domain of the enzyme in *T. californica* nAChR resulted in a 26-fold increase in the
476 ion channel response to acetylcholine. In contrast, a phenylalanine substitution in that position only
477 produced a 3-fold increase in the open time constant (Ortiz-Miranda et al. 1997). Finally, it is worth
478 noting that in some cases, more than one mutation in the target protein can act harmoniously
479 together to result in much stronger resistance as seen in *Drosophila* and houseflies (Menozzi et al.
480 2004; Wang et al. 2012).

481 Exposure to anthropogenic-related activities, in particular to repeated application of insecticides,
482 greatly contribute to the selection of resistance in insects, as demonstrated in mosquitoes (Vonbas et
483 al. 2018), in the diamondback moth *Plutella xylostella* (Trocza et al. 2017). The silverleaf whitefly *B.*
484 *tabaci* is no exception, with median lethal concentrations of pymetrozine being increased
485 approximately 11 times after 18 generations (Wang et al. 2021). The recent results provided by Khalid
486 et al. (2021) are even more striking, as these authors reported a very high increase of the resistance
487 level to buprofezin (127-fold) and imidacloprid (86-fold) after five generations only. A masterpiece
488 case of rapid adaptation was conducted by Karasov et al. (2010) in *Drosophila* demonstrating that
489 organophosphate treatments quickly induced *ace* point mutations, resulting in multiple simple and
490 complex resistant-*ace* alleles in the investigated fly subpopulations. In addition to being rapid, the
491 selection of insecticide resistant specimens can quickly spread throughout population and strains. For
492 instance, we found that four strains of *B. tabaci* shared the same non-silent mutations. In *D.*
493 *melanogaster*, several strains can similarly share the same mutations in *ace1* (I161V, G265A, D330W
494 and G368A) supporting the idea that mutations conferring insecticide resistance can be selected
495 rapidly and become widespread in a given species (Menozzi et al. 2004).

496 When studying point mutations, gene sequence comparisons are most often performed by the
 497 analysis of single nucleotide polymorphisms (SNPs), as SNPs are often characterized by a high level of
 498 genetic variation (Zwick et al. 2003; Cingolani et al. 2012; Aguilar et al. 2018). Mutations occurring on
 499 SNPs are synonymous, meaning that they change the nucleotide sequence without altering the amino
 500 acid sequence – or change one single amino acid due to wobble rules in the genetic code (Zwick et al.
 501 2003). We found four synonymous mutations in the studied *B. tabaci* strains, and silent mutations
 502 have long been assumed to have no effect on insecticide resistance. However, the strong correlation
 503 in the distribution of non-synonymous and synonymous mutations in mosquitoes owing to their
 504 resistance to permethrin suggest that both mutation types could be major drivers of insecticide
 505 resistance and mutation inheritance (Xu et al. 2012, and also see the discussion in Liu 2015). The
 506 effects of co-occurring synonymous and non-synonymous mutations on insecticide resistance have
 507 never been investigated in *B. tabaci*, even though ~~this~~-these co-occurring mutations have been also
 508 seen in~~from~~ other variants (Hopkinson et al. 2020). We thus suggest that this could represent an
 509 interesting research avenue for future works. Also, even if synonymous mutations on SNPs are not
 510 resulting in divergence between genomes, multi-nucleotide polymorphisms of 2 or 3 nucleotides
 511 (signaled as double and triple nucleotide polymorphisms) can be potential sources of genetic
 512 variation. These nucleotide changes would affect multiple positions in a codon that can change the
 513 amino acid residue(s) at two adjacent positions (Rosenfeld et al. 2010). Similar changes were observed
 514 in Egyptian and Pakistani *B. tabaci* variants. Specifically, the Egyptian strains Fas, Myi, Mbs, and Fib
 515 were characterized by the same two adjacent point mutations (S369W (TCC-TGG) and L370V (CTG-
 516 >GTG)) corresponding to double nucleotide polymorphisms. Similarly, the strains Mym and Mas
 517 exhibited replacements of two adjacent amino acids (C316W (TGC->TGG) and P317G (CCT->GGT)). For
 518 Pakistan, the analyzed sequences revealed triple nucleotide polymorphism (G251D (GGG->GAT),
 519 N252R (AAC->CGA), and P253S (CCG->ICG)). Moreover, our analysis also revealed the substitution of
 520 two additional amino acids (double nucleotide polymorphism) at the C-terminal sequence (C329S
 521 (TGC->TCG) and L330T (CTC->ACC).

522 The phylogenetic relationship of the AChE1 protein sequences from Egypt with other AChE1 from
 523 different insect orders was inferred using the Neighbor-neighbor-joining method to determine the
 524 closest associations among these AChE1. Interestingly, sequences from *B. tabaci* appeared at the top
 525 of the clad in the phylogenetic tree, suggesting that the reported point mutations have evolved
 526 recently as compared with the other analyzed insect species. AChE1 from higher insect orders, in
 527 particular Hymenoptera, Diptera (mainly mosquitoes), and Lepidoptera were grouped together
 528 forming a large clade, excluding homopterans (including aphids and whiteflies) and orthopteroids,
 529 which are continuously in contact with agriculturalle insecticides, group together under different

branches in another successive clade. This analysis reveals that the most frequent mutations of acetylcholinesterase associated with insecticide resistance in insects are heterogeneous, as formerly reported by Menozzi et al. (2004) from several populations of *D. melanogaster*. Yet even if mutations can occur in combination, or isolated, it seems that frequent exposures to organophosphates ~~has~~ have a tendency to group insect variants with mutated AChE proteins.

When some amino acid substitutions disturb the space and hydrophobicity of the acyl pocket of the protein, the enzymatic properties of AChE can be modified, as a consequence of altered specificity with the ligand. More generally, and as mentioned above, a single amino acid substitution is sufficient for conferring insecticide resistance (Nabeshima et al. 2003, 2004). To predict the functional effects of amino acid mutations on AChE1-insecticide interactions, homology models were built. Mutations detected in Egyptian and Pakistani populations (F392W, S369W, L370V, C316W, P317G, P317F, G251D, N252R, P253S, C329S, and L330T) were introduced to the four conformations of the protein, and the mutated proteins were subjected to geometry optimization using the universal force field (UFF). By constructing 3D model of the AChE1 enzyme, we have been able to visualize the locations of amino acid mutations detected in Egyptian and Pakistani populations of *B. tabaci*. The highest RMSF values were found in three regions of the enzyme, and subsequent docking calculations revealed that those mutations have slightly affected AChE1-insecticide binding affinities (values ranging from -6.0 to -8.5 kcal/mol). However, some insecticides, in particular aldicarb, butocarboxim, and methomyl were characterized by binding affinities (> -5.5 kcal/mol) to AChE1 in the range of those reported for the positive control ACh (-4.6 kcal/mol). These results suggest that mutations may have little effects on the protein-pesticide interaction. They may rather induce inadequate steric interaction that prevents the completion of the phosphorylation of the enzyme, as formerly proposed by Reyes-Espinosa et al. (2019). This assumption was further confirmed by calculations of pKa values of the active site of the wild-type and mutant AChE1 of *B. tabaci*, and evidenced the alteration of the AChE1-Acetylcholine interaction induced by the mutation F392W. The mutation of the protein altered the acidity of the active site, and lowered its affinity with the ligand (acetylcholine). This result suggested that the slight increase in the pKa value of the active site E388 may contribute to explain the slight modification of the binding energy (lower binding affinity).

By analyzing the models and the active site gorge of AChE1, it has been possible to make inferences on the effects of each point mutation on the structure and function of the AChE1 enzyme. Point mutations C316W and C329S most probably disrupted the disulfide bonds of the enzyme. As these bonds are essential for the molecular architecture of proteins (Siadat et al. 2006; Feige et al. 2018; Betz 1993; Wedemeyer et al. 2000), these mutations may have resulted in structural modifications at the entrance of the active site gorge. In previous studies, two out of the three regions

containing disulfide bonds (C313-326 and C332-340) were suggested to be part of the peripheral anionic binding site of AChE1 (Dvir et al. 2010). Here, we provided supporting evidence that mutations C316W and C329S, as well as P317G, P317F, and L330T affected the peripheral anionic binding site of the protein by changing the aromatic content at the gorge entrance. Point mutations S369W and L370V may confer structural modifications near the bottom of the gorge, and these neighboring residues of the binding site should affect ligand binding owing to the changes of electrostatic interactions they induce. Consistently, point mutation S369W added new bonds with the residue E364 (red lines in Fig. 6B) which lost the interaction with the neighboring residue P468. As a consequence, the distance between residues P468 and L370 was 3.7 Å in the wild type enzyme, and has been increased by 1 Å in the mutated enzyme (interaction with the residue V370). Mutations G251D, N252R, and P253S will change the surface charge of the protein. Mutation G251D will add a -1 charge to the enzyme surface, while the mutation N252R will add a +1 charge. The P253S mutation will affect hinge flexibility as proline will be replaced by serine, which is a flexible and water-soluble residue as compared with proline which confers an exceptional conformational rigidity.

Future work should investigate the dose-responses and resistance ratios to organophosphates in the Egyptian and Pakistani variants we studied to get a better understanding of the incidence of the AChE protein mutations we found. If the resistance is confirmed by bioassay data, the methodology developed in this study could be valuably used for getting predictions of the level of resistance of different *B. tabaci* variants, and assess the frequency of this resistance among field populations. More generally, we encourage the implementation of more studies on the genetic structure of *B. tabaci* population, and on the identification of resistance genes, as it would allow a better understanding of the evolution of resistance. Finally, the target insensitivity or inheritance patterns of organophosphate resistance in *B. tabaci* will also require additional research to confirm the resistance and to establish more efficient management strategies for *B. tabaci* worldwide.

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Authorship contribution

AM and DR conceived and designed the study. AM, AE, and DR analyzed the data. AM, AE, and DR interpreted the results and prepared the figures and the tables. AM and DR wrote the first version of the manuscript. AM, and DR reviewed the drafts of the manuscript.

Availability of data and materials

All data analyzed during this study are included in this published article.

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References

- Abdallah MD (1991) A general view of the resistance problem of cotton pests in Egypt. Resist Pest Manag (USA) 3:22–25
- Ahmad M, Arif MI, Ahmad Z, Denholm I (2002) Cotton whitefly (*Bemisia tabaci*) resistance to organophosphate and pyrethroid insecticides in Pakistan. Pest Manag Sci 58:203–208. <https://doi.org/10.1002/ps.440>
- Ahmad M, Arif MI, Naveed M (2010) Dynamics of resistance to organophosphate and carbamate insecticides in the cotton whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Pakistan. J Pest Sci 83:409–420. <https://doi.org/10.1007/s10340-010-0311-8>
- Ahmad M, Denholm I, Bromilow RH (2006) Delayed cuticular penetration and enhanced metabolism of deltamethrin in pyrethroid-resistant strains of *Helicoverpa armigera* from China and Pakistan. Pest Manag Sci 62:805–810. <https://doi.org/10.1002/ps.1225>
- Aguilar G, Olvera AM, Carvajal BI, Mosqueda J (2018) SNPs and other polymorphisms associated with acaricide resistance in *Rhipicephalus microplus*. Front Biosci (Landmark Ed) 23:65–82. <https://doi.org/10.2741/4582>
- Alon M, Alon F, Nauen R, Morin S (2008) Organophosphates' resistance in the B-biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) is associated with a point mutation in an *ace1*-type acetylcholinesterase and overexpression of carboxylesterase. Insect Biochem Mol Biol 38:940–949. <https://doi.org/10.1016/j.ibmb.2008.07.007>
- Arslan ZF (2018) Decrease in biodiversity in wheat fields due to changing agricultural practices in five decades. Biodivers Conserv 27:3267–3286. <https://doi.org/10.1007/s10531-018-1608-9>
- Azab, AK, Megahed, MM, El-Mirsawi, DH (1971) On the biology of *Bemisia tabaci* (Genn.) [Hemiptera—Homoptera: Aleyrodidae]. Bull Entomol Soc Egypt 4: 305–315.

638 Babar U, Nawaz MA, Arshad U, Azhar MT, Atif RM, Golokhvast KS, Tsatsakis AM, Shcherbakova K,
 639 Chung G, Rana IA (2020) Transgenic crops for the agricultural improvement in Pakistan: a
 640 perspective of environmental stresses and the current status of genetically modified crops.
 641 GM Crops Food 11:1–29. <https://doi.org/10.1080/21645698.2019.1680078>
 642 Bendele KG, Guerrero FD, Miller RJ, Li AY, Barrero RA, Moolhuijzen PM, Black M, McCooke JK, Meyer
 643 J, Hill CA, Bellgard MI (2015) Acetylcholinesterase 1 in populations of organophosphate-
 644 resistant North American strains of the cattle tick, *Rhipicephalus microplus* (Acari: Ixodidae).
 645 Parasitol Res 114:3027–3040. <https://doi.org/10.1007/s00436-015-4505-0>
 646 Benting J, Nauen R (2004) Biochemical evidence that an S431F mutation in acetylcholinesterase-1 of
 647 *Aphis gossypii* mediates resistance to primicarb and omethoate. Pest Manag Sci 60:1051–
 648 1055. <https://doi.org/10.1002/ps.971>
 649 ~~Betz SF (1993) Disulfide bonds and the stability of globular proteins. Protein Science 2: 1551–1558.~~
 650 ~~<https://doi.org/10.1002/pro.5560021002>~~
 651 Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T, Kiefer F, Gallo Cassarino T,
 652 Bertoni M, Bordoli L, Schwede T (2014) SWISS-MODEL: modelling protein tertiary and
 653 quaternary structure using evolutionary information. Nucleic Acids Res 42(Web Server
 654 issue):W252–W258. <https://doi.org/10.1093/nar/gku340>
 655 Bonanomi G, De Filippis F, Zotti M, Idbella M, Cesarano G, Al-Rowaily S, Abd-ElGawad A (2020)
 656 Repeated applications of organic amendments promote beneficial microbiota, improve soil
 657 fertility and increase crop yield. Appl Soil Ecol 156:103714.
 658 <https://doi.org/10.1016/j.apsoil.2020.103714>
 659 Bourguet D, Raymond M, Fournier D, Malcolm CA, Toutant JP, Arpagus M (1996) Existence of two
 660 acetylcholinesterases in the mosquito *Culex pipiens* (Diptera: Culicidae). J Neurochem
 661 67:2115–2123. <https://doi.org/10.1046/j.1471-4159.1996.67052115.x>
 662 Brown JK, Bird J (1992) Whitefly-transmitted geminiviruses and associated disorders in the Americas
 663 and the Caribbean Basin. Plant Dis 76:220–225. <https://doi.org/10.1094/PD-76-0220>
 664 Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: a tool for automated alignment
 665 trimming in large-scale phylogenetic analyses. Bioinformatics 25:1972–1973.
 666 <https://doi.org/10.1093/bioinformatics/btp348>
 667 Cassanelli S, Reyes M, Rault M, Manicardi C, Sauphanor B (2006) Acetylcholinesterase mutation in an
 668 insecticide-resistant population of the codling moth *Cydia pomonella* (L.). Insect Biochem Mol
 669 Biol 36:642–653. <https://doi.org/10.1016/j.ibmb.2006.05.007>
 670 Cheung J, Mahmood A, Kalathur R, Liu L, Carlier PR (2018) Structure of the G119S mutant
 671 acetylcholinesterase of the malaria vector *Anopheles gambiae* reveals basis of insecticide
 672 resistance. Structure 26:130–136.e2. <https://doi.org/10.1016/j.str.2017.11.021>
 673 Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Lu X, Ruden, DM (2012) A program
 674 for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in
 675 the genome of *Drosophila melanogaster* strain w¹¹¹⁸; iso-2; iso-3. Fly 6:80–92.
 676 <https://doi.org/10.4161/fly.19695>
 677 De Barro PJ, Liu SS, Boykin LM, Dinsdale B (2011) *Bemisia tabaci*: a statement of species status. Ann
 678 Rev Entomol 56:1–19. <https://doi.org/10.1146/annurev-ento-112408-085504>
 679 de Moya RS, Brown JK, Sweet AD, Walden KKO, Paredes-Montero JR, Waterhouse RM, Johnson, KP
 680 (2019) Nuclear orthologs derived from whole genome sequencing indicate cryptic diversity in
 681 the *Bemisia tabaci* (Insecta: Aleyrodidae) complex of whiteflies. Diversity 11:151.
 682 <https://doi.org/10.3390/d11090151>
 683 Denholm I, Cahill M, Dennehy TJ, Horowitz AR (1998) Challenges with managing insecticide resistance
 684 in agricultural pests exemplified by the whitefly, *Bemisia tabaci*. Philos Trans R Soc Lond, B,
 685 Biol Sci 353:1757–1767. <https://doi.org/10.1098/rstb.1998.0328>
 686 Dvir H, Silman I, Harel M, Rosenberry TL, Sussman JL (2010) Acetylcholinesterase: from 3D structure
 687 to function. Chem Biol Interact 187:10–22. <https://doi.org/10.1016/j.cbi.2010.01.042>

688 Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput.
689 Nucleic Acids Res 32:1792–1797. <https://doi.org/10.1093/nar/gkh340>

690 El Kady HE, Devine GJ (2003) Insecticide resistance in Egyptian populations of the cotton whitefly,
691 *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Manag Sci 59:865–871.
692 <https://doi.org/10.1002/ps.687>

693 Elbert A, Nauen R (2000) Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in
694 southern Spain with special reference to neonicotinoids. Pest Manag Sci 56:60–64.
695 [https://doi.org/10.1002/\(SICI\)1526-4998\(200001\)56:1%3C60::AID-PS88%3E3.0.CO;2-K](https://doi.org/10.1002/(SICI)1526-4998(200001)56:1%3C60::AID-PS88%3E3.0.CO;2-K)

696 Emsley P, Cowtan K. Coot: model-building tools for molecular graphics (2004) Acta Crystallogr D Biol
697 Crystallogr 60(Pt 12 Pt 1):2126–2132. <https://doi.org/10.1107/s0907444904019158>

698 [Engell Dahl J, Marti S-L, Colinet H, Wiegand C, Holmstrup M, Renault D. 2021. Thermal plasticity](#)
699 [and sensitivity to insecticides in populations of an invasive beetle: Cyfluthrin increases](#)
700 [vulnerability to extreme temperature. Chemosphere 274:129905.](#)

701 [Feige MJ, Braakman I, Hendershot LM \(2018\) Disulfide bonds in protein folding and stability. In: Feige](#)
702 [MJ \(ed\) Oxidative Folding of Proteins: Basic Principles, Cellular Regulation and Engineering,](#)
703 [The Royal Society of Chemistry, London, pp 1–33. https://doi.org/10.1039/9781788013253-](#)
704 [00001. eISBN: 978-1-78801-325-3](#)

705 Ffrench-Constant RH, Pittendrigh B, Vaughan A, Anthony N (1998) Why are there so few resistance-
706 associated mutations in insecticide target genes? Philos Trans R Soc Lond, B, Biol Sci
707 353:1685–1693. <https://doi.org/10.1098/rstb.1998.0319>

708 Gao JR, Kambhampati S, Zhu KY (2002) Molecular cloning and characterization of a greenbug
709 (*Schizaphis graminum*) cDNA encoding acetylcholinesterase possibly evolved from a duplicate
710 gene lineage. Insect Biochem Mol Biol 32:765–775. [https://doi.org/10.1016/s0965-](https://doi.org/10.1016/s0965-1748(01)00159-x)
711 [1748\(01\)00159-x](#)

712 Gauthier N, Clouet C, Perrakis A, Kapantaidaki D, Peterschmitt M, Tsagkarakou A- (2014) Genetic
713 structure of *Bemisia tabaci* Med populations from home-range countries, inferred by nuclear
714 and cytoplasmic markers: impact on the distribution of the insecticide resistance genes. Pest
715 Manag Sci 70:1477–1491. <https://doi.org/10.1002/ps.3733>

716 Gianessi LP (2013) The increasing importance of herbicides in worldwide crop production. Pest
717 Manag Sci 69:1099–1105. <https://doi.org/10.1002/ps.3598>

718 Grisaru D, Sternfeld M, Eldor A, Glick D, Soreq H (1999) Structural roles of acetylcholinesterase
719 variations in biology and pathology. Eur J Biochem 264:672–686.
720 <https://doi.org/10.1046/j.1432-1327.1999.00693.x>

721 Han MV, Zmasek CM (2009) phyloXML: XML for evolutionary biology and comparative genomics. BMC
722 Bioinform 10:356. <https://doi.org/10.1186/1471-2105-10-356>

723 [Harrach MF, Drossel B \(2014\) Structure and dynamics of TIP3P, TIP4P, and TIP5P water near smooth](#)
724 [and atomistic walls of different hydroaffinity. J Chem Phys 140:174501.](#)
725 <https://doi.org/10.1063/1.4872239>

726 Hemingway J, Hawkes NJ, McCarroll L, Ranson H (2004) The molecular basis of insecticide resistance
727 in mosquitoes. Insect Biochem Mol Biol 34:653–665.
728 <https://doi.org/10.1016/j.ibmb.2004.03.018>

729 Hepperly P, Lotter D, Ulsh CZ, Seidel R, Reider C (2009) Compost, manure and synthetic fertilizer
730 influences crop yields, soil properties, nitrate leaching and crop nutrient content. Compost
731 Sci Util 17:117–126. <https://doi.org/10.1080/1065657X.2009.10702410>

732 Hopkinson J, Puma S, van Brunschot S, Fang C, Frese M, Tay WT, Walsh T (2020) Insecticide resistance
733 status of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) in Australian cotton production
734 valleys. Austral Entomol 59:202–214. <https://doi.org/10.1111/aen.12436>

735 Hopkinson J, Puma S, van Brunschot S, Fang C, Frese M, Tay WT, Walsh T (2020) Insecticide
736 resistance status of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) in Australian cotton
737 production valleys. Austral Entomol 59:202–214. <https://doi.org/10.1111/aen.12436>

738 Horowitz AR, Ghanim M, Roditakis E, Nauen R, Ishaaya I (2020) Insecticide resistance and its
 739 management in *Bemisia tabaci* species. J Pest Sci 93:893–910.
 740 <https://doi.org/10.1007/s10340-020-01210-0>
 741 Huang J, MacKerell AD Jr (2013) CHARMM36 all-atom additive protein force field: validation based
 742 on comparison to NMR data. J Comput Chem 34:2135–2145.
 743 <https://doi.org/10.1002/jcc.23354>
 744 Huchard E, Martinez M, Alout H, Douzery E J, Lutfalla G, Berthomieu A, Berticat C, Raymond M, Weill
 745 M (2006) Acetylcholinesterase genes within the Diptera: takeover and loss in true flies. Proc
 746 Royal Soc B 273:2595–2607. <https://doi.org/10.1098/rspb.2006.3621>
 747 Isbell F, Tilman D, Reich PB, Clark AT (2019) Deficits of biodiversity and productivity linger a century
 748 after agricultural abandonment. Nat Ecol Evol 3:1533–1538.
 749 <https://doi.org/10.1038/s41559-019-1012-1>
 750 Jiang X, Qu M, Denholm I, Fang J, Jiang W, Han Z (2009) Mutation in acetylcholinesterase1 associated
 751 with triazophos resistance in rice stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae).
 752 Biochem Biophys Res Commun 378:269–272. <https://doi.org/10.1016/j.bbrc.2008.11.046>
 753 Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein
 754 sequences. Comput Appl Biosci 8:275–282. <https://doi.org/10.1093/bioinformatics/8.3.275>
 755 Jordan CF (2002) Genetic engineering, the farm crisis, and world hunger. BioScience 52: 523–529.
 756 [https://doi.org/10.1641/0006-3568\(2002\)052\[0523:GETFCA\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2002)052[0523:GETFCA]2.0.CO;2)
 757 Kans J (2022) Entrez Direct: E-utilities on the Unix Command Line. In: Entrez Programming Utilities
 758 Help [Internet]. Bethesda (MD): National Center for Biotechnology Information (US) National
 759 Library of Medicine; 2010-. <https://www.ncbi.nlm.nih.gov/books/NBK179288/>
 760 Karasov T, Messer PW, Petrov DA (2010) Evidence that adaptation in *Drosophila* is not limited by
 761 mutation at single sites. PLoS Genet 6:e1000924.
 762 <https://doi.org/10.1371/journal.pgen.1000924>
 763 Katayama N, Baba YG, Kusumoto Y, Tanaka K (2015) A review of post-war changes in rice farming
 764 and biodiversity in Japan. Agric Syst 132:73–84. <https://doi.org/10.1016/j.agsy.2014.09.001>
 765 Khalid MZ, Ahmed S, Al-Ashkar I, El Sabagh A, Liu L, Zhong G (2021) Evaluation of resistance
 766 development in *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) in cotton against different
 767 insecticides. Insects 12:996. <https://doi.org/10.3390/insects12110996>
 768 Kim JI, Jung CS, Koh YH, Lee SH (2006) Molecular, biochemical and histochemical characterization of
 769 two acetylcholinesterase cDNAs from the German cockroach *Blattella germanica*. Insect Mol
 770 Biol 15:513–522. <https://doi.org/10.1111/j.1365-2583.2006.00666.x>
 771 Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L,
 772 Zhang J, Bolton EE (2021) PubChem in 2021: new data content and improved web interfaces.
 773 Nucleic Acids Res 49(D1):D1388–D1395. <https://doi.org/10.1093/nar/gkaa971>
 774 Leke WN, Mignouna DB, Brown JK, Kvarnheden A (2015) Begomovirus disease complex: emerging
 775 threat to vegetable production systems of West and Central Africa. Agric Food Secur 4:1.
 776 <https://doi.org/10.1186/s40066-014-0020-2>
 777 Lenfant N, Hotelier T, Velluet E, Bourne Y, Marchot P, Chatonnet A (2013) ESTHER, the database of
 778 the α/β -hydrolase fold superfamily of proteins: tools to explore diversity of functions.
 779 Nucleic Acids Res 41(Database issue):D423–429. <https://doi.org/10.1093/nar/gks1154>
 780 Li H, Robertson AD, Jensen JH (2005) Very fast empirical prediction and rationalization of protein pKa
 781 values. Proteins 61:704–721. <https://doi.org/10.1002/prot.20660>
 782 Li X, Schuler MA, Berenbaum MR (2007) Molecular mechanisms of metabolic resistance to synthetic
 783 and natural xenobiotics. Annu Rev Entomol 52:231–253.
 784 <https://doi.org/10.1146/annurev.ento.51.110104.151104>

Li Y, Xu J, Zhong D, Zhang H, Yang W, Zhou G, Su X, Wu Y, Wu K, Cai S, Yan G, Chen XG (2018) Evidence for multiple-insecticide resistance in urban *Aedes albopictus* populations in southern China. *Parasit Vectors* 11:4. <https://doi.org/10.1186/s13071-017-2581-y>

Lilly DG, Latham SL, Webb CE, Doggett SL (2016) Cuticle thickening in a pyrethroid-resistant strain of the common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae). *PLoS One* 11:e0153302. <https://doi.org/10.1371/journal.pone.0153302>

Liu N (2015) Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. *Annu Rev Entomol* 60:537–559. <https://doi.org/10.1146/annurev-ento-010814-020828>

Liu N, Li M, Gong Y, Liu F, Li T (2015) Cytochrome P450s – Their expression, regulation, and role in insecticide resistance. *Pestic Biochem Physiol* 120:77–81. <https://doi.org/10.1016/j.pestbp.2015.01.006>

Maddison WP, Maddison DR (2021) Mesquite: a modular system for evolutionary analysis. Version 3.70. <http://www.mesquiteproject.org>

Malik HJ, Raza A, Amin I, Scheffler JA, Scheffler BE, Brown JK, Mansoor S (2016) RNAi-mediated mortality of the whitefly through transgenic expression of double-stranded RNA homologous to acetylcholinesterase and ecdysone receptor in tobacco plants. *Sci Rep* 6:38469. <https://doi.org/10.1038/srep38469>

Marchot P, Chatonnet A (2012) Hydrolase versus other functions of members of the alpha/beta-hydrolase fold superfamily of proteins. *Protein Pept Lett* 19:130–131. <https://doi.org/10.2174/092986612799080239>

Menozi P, Shi MA, Lougarre A, Tang ZH, Fournier D (2004) Mutations of acetylcholinesterase which confer insecticide resistance in *Drosophila melanogaster* populations. *BMC Evol Biol* 4:4. <https://doi.org/10.1186/1471-2148-4-4>

Mutero A, Pralavorio M, Bride JM, Fournier D (1994) Resistance-associated point mutations in insecticide-insensitive acetylcholinesterase. *Proc Nat Acad Sci USA* 91:5922–5926. <https://doi.org/10.1073/pnas.91.13.5922>

Nabeshima T, Kozaki T, Tomita T, Kono Y (2003) An amino acid substitution on the second acetylcholinesterase in the pirimicarb-resistant strains of the peach potato aphid, *Myzus persicae*. *Biochem Biophys Res Commun* 307:15–22. [https://doi.org/10.1016/S0006-291X\(03\)01101-X](https://doi.org/10.1016/S0006-291X(03)01101-X)

Nabeshima T, Mori A, Kozaki T, Iwata Y, Hidoh O, Harada S, Kasai S, Severson D W, Kono Y, Tomita T (2004) An amino acid substitution attributed to insecticide-insensitivity of acetylcholinesterase in a Japanese encephalitis vector mosquito, *Culex tritaeniorhynchus*. *Biochem Biophys Res Commun* 313:794–801. <https://doi.org/10.1016/j.bbrc.2003.11.141>

Nagar G, Sharma AK, Kumar S, Saravanan BC, Kumar R, Gupta S, Kumar S, Ghosh S (2018) Molecular mechanism of synthetic pyrethroid and organophosphate resistance in field isolates of *Rhipicephalus microplus* tick collected from a northern state of India. *Exp Appl Acarol* 75:319–331. <https://doi.org/10.1007/s10493-018-0265-8>

Navas-Castillo J, Fiallo-Olivé E, Sánchez-Campos S (2011) Emerging virus diseases transmitted by whiteflies. *Annu Rev Phytopathol* 49:219–248. <https://doi.org/10.1146/annurev-phyto-072910-095235>

Naveen NC, Chaubey R, Kumar, D, Rebijith KB, Rajagopal R, Subramanyam B, Subramanian S (2017) Insecticide resistance status in the whitefly, *Bemisia tabaci* genetic groups Asia-I, Asia-II-1 and Asia-II-7 on the Indian subcontinent. *Sci Rep* 7:40634. <https://doi.org/10.1038/srep40634>

Oliveira MRV, Henneberry TJ, Anderson P (2001) History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Prot* 20:709–723. [https://doi.org/10.1016/S0261-2194\(01\)00108-9](https://doi.org/10.1016/S0261-2194(01)00108-9)

Ortiz-Miranda SI, Lasalde JA, Pappone PA, McNamee MG (1997) Mutations in the M4 domain of the *Torpedo californica* nicotinic acetylcholine receptor alter channel opening and closing. *J Membr Biol* 158:17–30. <https://doi.org/10.1007/s002329900240>

835 Palumbo J.C, Horowitz A.R, Prabhaker N Insecticidal control and resistance management for *Bemisia*
836 *tabaci*. Crop Prot 20:739–765. [https://doi.org/10.1016/S0261-2194\(01\)00117-X](https://doi.org/10.1016/S0261-2194(01)00117-X)
837 Panini M, Manicardi GC, Moores GD, Mazzoni E (2016). An overview of the main pathways of
838 metabolic resistance in insects. Invertebr Surviv J 13:326–335.
839 <https://doi.org/10.25431/1824-307X/isj.v13i1.326-335>
840 Paredes-Montero JR, Haq QMI, Mohamed AA, Brown JK (2021) Phylogeographic and SNPs analyses
841 of *Bemisia tabaci* B variant populations reveal only two of eight haplotypes are
842 invasive. Biology 10:1048. <https://doi.org/10.3390/biology10101048>
843 Pavlidi N, Vontas J, Van Leeuwen T (2018) The role of glutathione S-transferases (GSTs) in insecticide
844 resistance in crop pests and disease vectors. Curr Opin Insect Sci 27:97–102.
845 <https://doi.org/10.1016/j.cois.2018.04.007>
846 Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF
847 Chimera—a visualization system for exploratory research and analysis. J Comput Chem
848 25:1605–1612. <https://doi.org/10.1002/jcc.20084>
849 Popp J, Pető K, Nagy J (2013) Pesticide productivity and food security. A review. Agron Sustain Dev
850 33:243–255 <https://doi.org/10.1007/s13593-012-0105-x>
851 Ranson H, Claudianos C, Ortel F, Abgrall C, Hemingway J, Sharakhova M V, Unger MF, Collins FH,
852 Feyereisen R (2002) Evolution of supergene families associated with insecticide resistance.
853 Science 298:179–181. <https://doi.org/10.1126/science.1076781>
854 Rappé AK, Casewit CJ, Colwell K, Goddard III WA, Skiff WM (1992) UFF, a full periodic table force field
855 for molecular mechanics and molecular dynamics simulations. J Am Chem Soc 114:10024–
856 10035. <https://doi.org/10.1021/ja00051a040>
857 Raven PH, Wagner DL (2021) Agricultural intensification and climate change are rapidly decreasing
858 insect biodiversity. Proc Natl Acad Sci U S A 118:e2002548117.
859 <https://doi.org/10.1073/pnas.2002548117>
860 Renault D, Colinet H (2021) Differences in the susceptibility to commercial insecticides among
861 populations of the lesser mealworm *Alphitobius diaperinus* collected from poultry houses in
862 France. Insects 12:309. <https://doi.org/10.3390/insects12040309>
863 Reyes-Espinosa F, Méndez-Álvarez D, Pérez-Rodríguez MA, Herrera-Mayorga V, Juárez-Saldivar A,
864 Cruz-Hernández MA, Rivera G (2019) In silico study of the resistance to organophosphorus
865 pesticides associated with point mutations in acetylcholinesterase of
866 *Lepidoptera: B. mandarina, B. mori, C. auricilius, C. suppressalis, C. pomonella, H. armigera,*
867 *P. xylostella, S. frugiperda, and S. litura*. Int J Mol Sci 20(10): 2404.
868 <https://doi.org/10.3390/ijms20102404>
869 Rosenfeld, JA, Malhotra, AK, Lencz T (2010) Novel multi-nucleotide polymorphisms in the human
870 genome characterized by whole genome and exome sequencing. Nucleic Acids Res 38: 6102–
871 6111. <https://doi.org/10.1093/nar/gkq408>
872 Roy S, Dasgupta R, Bagchi A (2014) A Review on phylogenetic analysis: A journey through modern
873 era. Comput Mol Biosci, 4:39–45. <http://dx.doi.org/10.4236/cmb.2014.43005>
874 Shah, SHJ, Paredes-Montero, JR, Malik, AH, Brown JK, Qazi J (2020) Distribution of *Bemisia tabaci*
875 (Gennadius) (Hemiptera: Aleyrodidae) variants in commercial cotton fields in the Punjab
876 province of Pakistan. Fla Entomol 103:41–47. <https://doi.org/10.1653/024.103.0407>
877 Shaurub, EH, Paredes-Montero, JR, Brown, JK, Zein HS, Mohamed AA (2021) Metabolic resistance to
878 organophosphate insecticides in natural populations of the whitefly *Bemisia*
879 *tabaci* (Hemiptera: Aleyrodidae) in Egypt and molecular identification of
880 variants. Phytoparasitica 49:443–457. <https://doi.org/10.1007/s12600-020-00858-9>
881 Siadat OR, Lougarre A, Lamouroux L, Ladurantie C, Fournier D (2006) The effect of engineered
882 disulfide bonds on the stability of *Drosophila melanogaster* acetylcholinesterase. BMC
883 Biochem 7:12. <https://doi.org/10.1002/j.1460-2075.1992.tb05223.x>

884 Siegfried BD, Scharf ME (2001) Mechanisms of organophosphate resistance in insects: In Ishaaya, I.
885 (ed.). *Biochemical Sites of Insecticide Action and Resistance*. Springer-Verlag, Heidelberg, pp.
886 269–291. https://doi.org/10.1007/978-3-642-59549-3_13

887 Sobeiha AK, Zidan ZH, Mowafy MM, Marzouk AS (1997) Effect of certain insecticide regimes on some
888 sucking insects and natural enemies inhabiting cotton plants. *Ann Agric Sci* 42:665–674.
889 <https://agris.fao.org/agris-search/search.do?recordID=EG1998001313>

890 Soderlund DM, Knipple DC (2003) The molecular biology of knockdown resistance to pyrethroid
891 insecticides. *Insect Biochem Mol Biol* 33:563–577. [https://doi.org/10.1016/s0965-](https://doi.org/10.1016/s0965-1748(03)00023-7)
892 [1748\(03\)00023-7](https://doi.org/10.1016/s0965-1748(03)00023-7)

893 [Sparks TC, Storer N, Porter A, Slater R, Nauen R \(2021\) Insecticide resistance management and](#)
894 [industry: the origins and evolution of the Insecticide Resistance Action Committee \(IRAC\)](#)
895 [and the mode of action classification scheme. *Pest Manag Sci* 77:2609–2619.](#)
896 <https://doi.org/10.1002/ps.6254>

897 Suhag A, Yadav H, Chaudhary D, Subramanian S, Jaiwal R, Jaiwal PK. Biotechnological interventions
898 for the sustainable management of a global pest, whitefly (*Bemisia tabaci*). *Insect Sci.* 2021
899 Oct;28(5):1228–1252. <https://doi.org/10.1111/1744-7917.12853>

900 Temeyer KB, Olafson PU, Brake DK, Tuckow AP, Li AY, Pérez de León AA (2013) Acetylcholinesterase
901 of *Rhipicephalus (Boophilus) microplus* and *Phlebotomus papatasi*: Gene identification,
902 expression, and biochemical properties of recombinant proteins. *Pestic Biochem Physiol*
903 106:118–123. <https://doi.org/10.1016/j.pestbp.2013.01.005>

904 Tibbett M, Fraser TD, Duddigan S (2020) Identifying potential threats to soil
905 biodiversity. *PeerJ* 8:e9271. <https://doi.org/10.7717/peerj.9271>

906 Troczka BJ, Williamson MS, Field LM, Davies TGE (2017) Rapid selection for resistance to diamide
907 insecticides in *Plutella xylostella* via specific amino acid polymorphisms in the ryanodine
908 receptor. *Neurotoxicology* 60:224–233. <https://doi.org/10.1016/j.neuro.2016.05.012>

909 Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new
910 scoring function, efficient optimization, and multithreading. *J Comput Chem* 31:455–461.
911 <https://doi.org/10.1002/jcc.21334>

912 Tscharnkte T, Klein AM, Kruess A, Steffan-Dewenter I, Thies C (2005) Landscape perspectives on
913 agricultural intensification and biodiversity – ecosystem service management. *Ecol Lett*
914 8:857–874. <https://doi.org/10.1111/j.1461-0248.2005.00782.x>

915 Umina PA, McDonald G, Maino J, Edwards O, Hoffmann AA (2019) Escalating insecticide resistance in
916 Australian grain pests: contributing factors, industry trends and management opportunities.
917 *Pest Manag Sci* 75:1494–1506. <https://doi.org/10.1002/ps.5285>

918 van der Sluijs R (2019) Beyond the farm gate, Ginning. In: Australian Cotton Production Manual (eds
919 A Twine & R Redfern), pp. 131–135. CottonInfo, Narrabri

920 [Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJ \(2005\) GROMACS: fast,](#)
921 [flexible, and free. *J Comput Chem* 26:1701–1718.](#) <https://doi.org/10.1002/jcc.20291>

922 Vontas J, Grigoraki L, Morgan J, Tsakireli D, Fuseini G, Segura L, Niemczura de Carvalho J, Nguema R,
923 Weetman D, Slotman MA, Hemingway J (2018) Rapid selection of a pyrethroid metabolic
924 enzyme CYP9K1 by operational malaria control activities. *Proc Natl Acad Sci U S A* 115:4619–
925 4624. <https://doi.org/10.1073/pnas.1719663115>

926 Vontas J, Hernández-Crespo P, Margaritopoulos JT, Ortego F, Feng H-T, Mathiopoulos KD, Hsu J-C
927 (2011) Insecticide resistance in tephritid flies. *Pestic Biochem Physiol* 100:199–205.
928 <https://doi.org/10.1016/j.pestbp.2011.04.004>

929 Vontas JG, Hejazi MJ, Hawkes NJ, Cosmidis N, Loukas M, Hemmingway J (2002) Resistance-associated
930 point mutations of organophosphate insensitive acetylcholinesterase in the olive fruit fly,
931 *Bactrocera oleae*. *Insect Mol Biol* 11:329–336. [https://doi.org/10.1046/j.1365-](https://doi.org/10.1046/j.1365-2583.2002.00343.x)
932 [2583.2002.00343.x](https://doi.org/10.1046/j.1365-2583.2002.00343.x)

933 Voudouris CC, Sauphanor B, Franck P, Reyes M, Mamuris Z, Tsitsipis JA, Vontas J, Margaritopoulos JT
934 (2011) Insecticide resistance status of the codling moth *Cydia pomonella* (Lepidoptera:
935 Tortricidae) from Greece. *Pestic Biochem Physiol* 100:229–238.
936 <https://doi.org/10.1016/j.pestbp.2011.04.006>

937 Walsh SB, Dolden TA, Moores GD, Kristensen M, Lewis T, Devonshire AL, Williamson MS (2001)
938 Identification and characterization of mutations in housefly (*Musca domestica*)
939 acetylcholinesterase involved in insecticide resistance. *Biochem J* 359:175–181.
940 <https://doi.org/10.1042/0264-6021:3590175>

941 Wang F, Liu J, Shuai S, Miao C, Chi B, Chen P, Wang K, Li H, Liu Y. (2021) Resistance of *Bemisia tabaci*
942 Mediterranean (Q-biotype) to pymetrozine: resistance risk assessment, cross-resistance to
943 six other insecticides and detoxification enzyme assay. *Pest Manag Sci* 77:2114–2121.
944 <https://doi.org/10.1002/ps.6240>

945 Wang Q, Li M, Pan J, Di M, Liu Q, Meng F, Scott JG, Qiu X (2012) Diversity and frequencies of genetic
946 mutations involved in insecticide resistance in field populations of the house fly (*Musca*
947 *domestica* L.) from China. *Pestic Biochem Physiol* 102:153–159.
948 <https://doi.org/10.1016/j.pestbp.2011.12.007>

949 [Wang W, Wang S, Han G, Du Y, Wang J \(2017\) Lack of cross-resistance between neonicotinoids and](#)
950 [sulfoxaflor in field strains of Q-biotype of whitefly, *Bemisia tabaci*, from Eastern China. *Pestic*](#)
951 [Biochem Physiol](#) 136:46–51. <https://doi.org/10.1016/j.pestbp.2016.08.005>

952 Wedemeyer WJ, Welker E, Narayan M, Scheraga HA (2000) Disulfide bond and protein folding.
953 *Biochemistry* 39: 4207–4216. DOI: 10.1021/bi992922o

954 Weill M, Fort P, Berthomieu A, Dubois MP, Pasteur N, Raymond M (2002) A novel acetylcholinesterase
955 gene in mosquitoes codes for the insecticide target and is non-homologous to the *ace* in
956 *Drosophila*. *Proc Royal Soc B* 269:2007–2016. <https://doi.org/10.1098/rspb.2002.2122>

957 Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, Marquine M, Raymond M (2004) The
958 unique mutation in *ace-1* giving high insecticide resistance is easily detectable in mosquito
959 vectors. *Insect Mol Biol* 13:1–7. <https://doi.org/10.1111/j.1365-2583.2004.00452.x>

960 Williams CJ, Headd JJ, Moriarty NW, Prisant MG, Videau LL, Deis LN, Verma V, Keedy DA, Hintze BJ,
961 Chen VB, Jain S, Lewis SM, Arendall WB 3rd, Snoeyink J, Adams PD, Lovell SC, Richardson JS,
962 Richardson DC (2018) MolProbity: More and better reference data for improved all-atom
963 structure validation. *Protein Sci* 27:293–315. <https://doi.org/10.1002/pro.3330>

964 Xu Q, Zhang L, Li T, Zhang L, He L, Dong K, Liu N (2012) Evolutionary adaptation of the amino acid and
965 codon usage of the mosquito sodium channel following insecticide selection in the field
966 mosquitoes. *PLoS One* 7:e47609. <https://doi.org/10.1371/journal.pone.0047609>

967 Zalucki MP, Furlong MJ (2017) Behavior as a mechanism of insecticide resistance: evaluation of the
968 evidence. *Curr Opin Insect Sci* 21:19–25. <https://doi.org/10.1016/j.cois.2017.05.006>

969 Zhang B-z, Kong F-c, Zeng X-n (2015) Detoxification enzymes activity and gene expression in
970 diafenthiuron-resistant whitefly, *Bemisia tabaci*. *J Agric Sci* 7:66–76.
971 <https://doi.org/10.5539/jas.v7n9p66>

972 [Zhang N, Liu C, Yang F, Dong S, Han Z \(2012\) Resistance mechanisms to chlorpyrifos and F392W](#)
973 [mutation frequencies in the acetylcholine esterase *ace1* allele of field populations of the](#)
974 [tobacco whitefly, *Bemisia tabaci* in China. *J Insect Sci* 12:41.](#)
975 <https://doi.org/10.1673/031.012.4101>

976 Zwick ME, Cutler DJ, Chakravarti A (2003) Patterns of genetic variation in Mendelian and complex
977 traits. *Annu Rev Genomics Hum Genet* 1:387–407.
978 <https://doi.org/10.1146/annurev.genom.1.1.387>

Figure legends

Fig 1. The amino acid sequence homology matrix of *B. tabaci* AChE from Egyptian field strains (see Table 1 for strain designations), and from the Israeli resistant (R, ABV45412) and susceptible (SUD, ABV45413) *B. tabaci* variants from Alon et al. (2008).

Fig 2. Sequence alignment using ClustalX. **A.** Alignment of amino acid sequences of the different partial AChEs from Egyptian field-strains of *B. tabaci* and resistant (accession no. ABV45412.1) and susceptible (accession no. ABV45413.1) AChE1 strains from Alon et al. (2008). The numbers above the sequences denote the position of the amino acids. Dots represent identity in amino acid residues. **B.** Multiple sequence alignments of the partial *ace1* nucleotide sequences of the Egyptian field-strains of *B. tabaci* and susceptible strain (accession no. EF675188.1) from Alon et al. (2008). Nucleotides' position numbers indicated above sequences, while dots indicate the identical nucleotide.

Fig 3. A. Visualization of putative spread of *ace1* mutation in *B. tabaci* variants from different geographic regions (Egypt and Pakistan), based on deduced AChE1 sequences, in comparison with the resistant and sensitive strains described by Alon et al. (2008). The amino acid residues identical to ABV45413 are indicated by dots. The numbers above the lines indicate the amino acid residues according to accession no. ABV45413.1 and ABV45412.1 (Alon et al. 2008). Shadowed amino acids represent five missense mutations (nonsynonymous substitution or polymorphisms) in form of DNPs and TNPs. **B.** Visualization of *ace1* mutation occurrence in *B. tabaci* strains from different localities of Pakistan. The nucleotide positions identical to accession EF675187.1 (S allele) EF675188.1 (R allele) are indicated by dots.

In both figures, the differences in amino acid or nucleotide are shown by the appropriate letters, and '-' indicate unidentified gaps.

Fig. 4. Molecular dynamics simulation of *Bemisia tabaci* AChE1. **(A)** Root-mean-square deviation (RMSD) in Å (blue), radius of gyration (RoG) in Å (green), surface accessible surface area (SASA) in Å² (gray), and total number of formed H-bonds (yellow) as a function of the simulation time (in nanoseconds). **(B)** Root-mean-square fluctuations (RMSF) per residue (in Å) over the duration of the simulation. The three regions with high RMSF values (high flexibility of amino acid residues, large fluctuations of amino acid residues during the simulation) are highlighted in red, magenta, and yellow, and the cartoon diagram of the protein structure was constructed with the same color codes. The three amino acids forming the active site triad, Glutamic acid in position 388 (E388), Serine in position 262 (S262) and Histidine in position 501 (H501) are presented. The Ramachandran plot of the generated model is presented at the top right of the figure.

Fig. 5. (A) Superimposition of the representative structures of the four different clusters of wild type *Bemisia tabaci* AChE1. **(B)** Cartoon representation of the active site gorge of *Bemisia tabaci* AChE1 after 877 ns of simulation, and showing the locations of the acyl pocket, peripheral anionic site, acetylcholine, choline binding site, residues of active site triad, and oxyanion hole. The residues lining the active site gorge of wild type AChE1 enzyme are depicted in colored cartoons according to the color code presented at the bottom of the figure. **(C)** Zoom-in on the active site gorge of *Bemisia tabaci* AChE1 after 877 ns of simulation, alongside with residues that may be part of the gorge lining.

Fig. 6. The structural motifs of the active site gorge of *Bemisia tabaci* AChE1. (A) Cartoon representation of AChE1 active site gorge; the position of each mutation is represented in black. Disulfide bonds are red-colored; Peripheral anionic site (PAS) are represented in cyan and the region reported to be part of the peripheral anionic site is represented by several dark dots. The blue surface highlights the location of the active site triad (S262, E388, and H501), and green sticks show the ligand

1029 ACh. (B) Superposition of the wild type (WT, black), and variants S369W (magenta) and L370V (yellow)
1030 of *Bemisia tabaci* AChE1 showing the structural modifications of the mutated enzymes. For each
1031 mutation, the interacting residues are shown by red lines. (C) Superposition of the wild type (WT, black
1032 sticks), and variants G251D (purple lines), N252R (gray lines), and P253S (red lines) of *Bemisia tabaci*
1033 AChE1. The enzyme surface charges are modified by the mutation for the variants G251D and N252R.
1034

1035 **Fig. 7.** The average binding energies of the different organophosphate **(A)** and carbamate **(B)**
1036 insecticides in the wild type (WT) and mutated (F392W, S369W, L370V, C316W, P317G, P317F, G251D,
1037 N252R, P253S, C329S, and L330T) AChE1 enzyme. Acetylcholine (ACh) is used as a positive control.



Fig. 2A


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841                                     966
EF675188.1 TTC AGC CAG GCG ATC ATG GAG TCC GGG TCG GCG ACG GCG CCT TGG GCC ATC ATC TCC CGC CAG GAG TCC ATC ATC CCG GGC CTC CGC CTC GCC GAG GGT GTC GGC TGC CCT CAC ACG CGC GCG CAG
LC199315.1 .....
LC199311.1 .....
LC199301.1 .....
LC199309.1 .....
LC199303.1 .....
LC199304.1 .....
LC199306.1 .....
EF675187.1 .....
LC199300.1 .....
LC199310.1 .....
LC199302.1 .....
LC199312.1 .....
LC199305.1 .....
LC199317.1 .....
LC199313.1 .....
LC199316.1 .....
LC199314.1 .....
LC199307.1 .....
LC199308.1 .....

967                                     1092
EF675188.1 ATC CCT GAG GCT ATC GAG TCC CTC CGA AAG GTC AAC GCG AGC GTG CTC GTG GAG AAC GAG AGC GGG ACC CTA GGS ATC TGC GAC TTC CCG TTC GTC CCG GTC GTG GAC GGC TCC TTC CTG GAC GAG
LC199315.1 .....
LC199311.1 .....
LC199301.1 .....
LC199309.1 .....
LC199303.1 .....
LC199304.1 .....
LC199306.1 .....
EF675187.1 .....
LC199300.1 .....
LC199310.1 .....
LC199302.1 .....
LC199312.1 .....
LC199305.1 .....
LC199317.1 .....
LC199313.1 .....
LC199316.1 .....
LC199314.1 .....
LC199307.1 .....
LC199308.1 .....

1093                                     1212
EF675188.1 ATG CCG TCC AAG TCC CTG GCG ACG AAG AAC TTC AAG AAG ACC AAC ATC CTC ATG GGG AGC AAC ACG GAG GAG GGC AAC TAC TTC ATC ATG TAC TAC CTG ACG GAC CTC TTC CCG AAG GAG
LC199315.1 .....
LC199311.1 .....
LC199301.1 .....
LC199309.1 .....
LC199303.1 .....
LC199304.1 .....
LC199306.1 .....
EF675187.1 .....
LC199300.1 .....
LC199310.1 .....
LC199302.1 .....
LC199312.1 .....
LC199305.1 .....
LC199317.1 .....
LC199313.1 .....
LC199316.1 .....
LC199314.1 .....
LC199307.1 .....
LC199308.1 .....

```

Fig. 2B

| | | | |
|------------|---|---|-----|
| | 242 | | 321 |
| ABV45413.1 | IRNNIHAFG GNP HNITLFGESAGAVSVSMHLLSPLSRNLF | SQAIMESGSATAPWAIISRQESIIRGLRLAEAVG CP HTRA | |
| ABV45412.1 | | | |
| BAW19595.1 |WG | | |
| AMO25775.1 | ----- DRS | | |
| | | | |
| | 322 | | 401 |
| ABV45413.1 | QIPEAIE CL RKVNASVLVENESGTLGICDFPFVFPVVDGSFLDEMP | SKSLATKNFKKTNILMGSENTEEGNYFIMYYLTDLF | |
| ABV45412.1 |W | | |
| BAW19595.1 |W | | |
| AMO25775.1 | ST ----- | | |

Fig. 3A

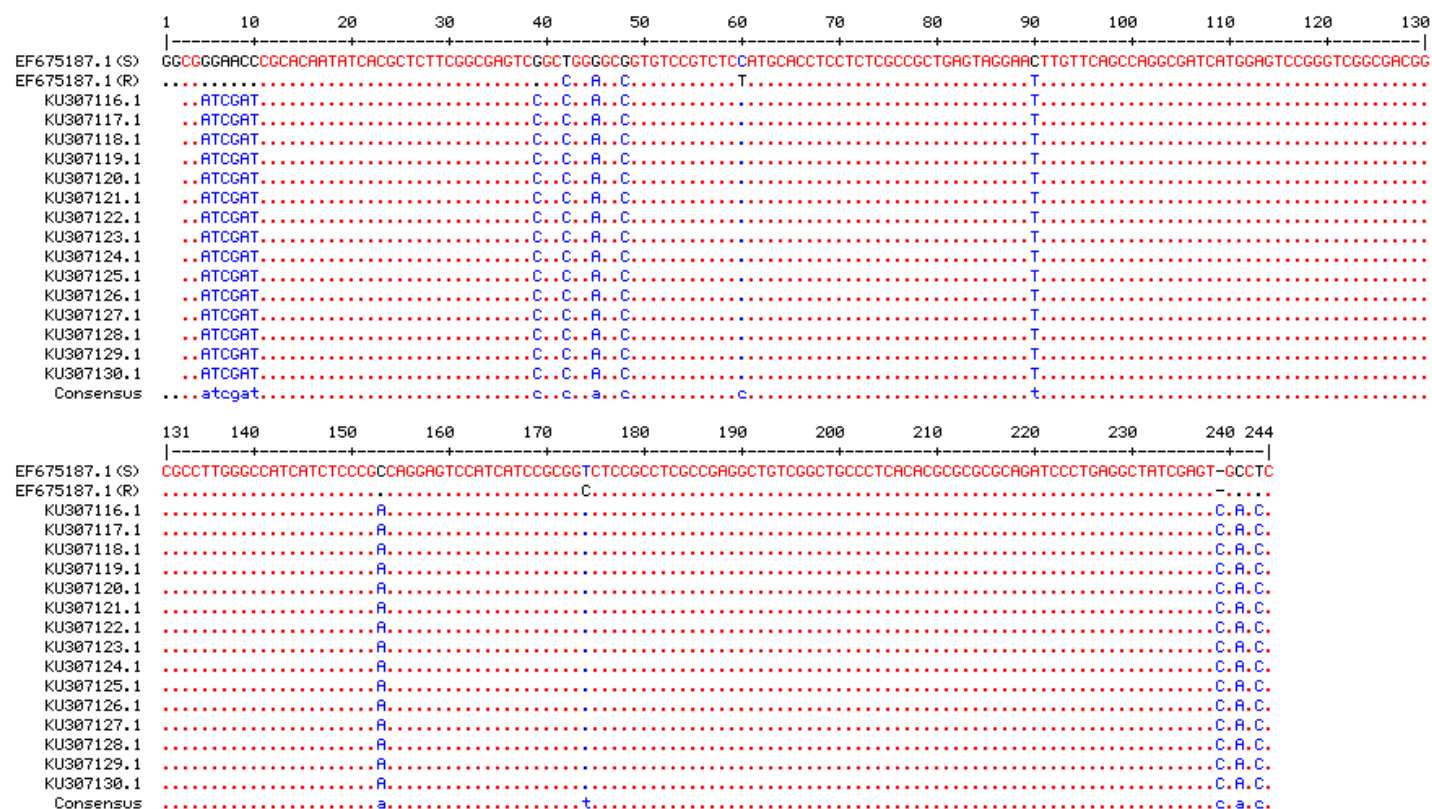


Fig. 3B

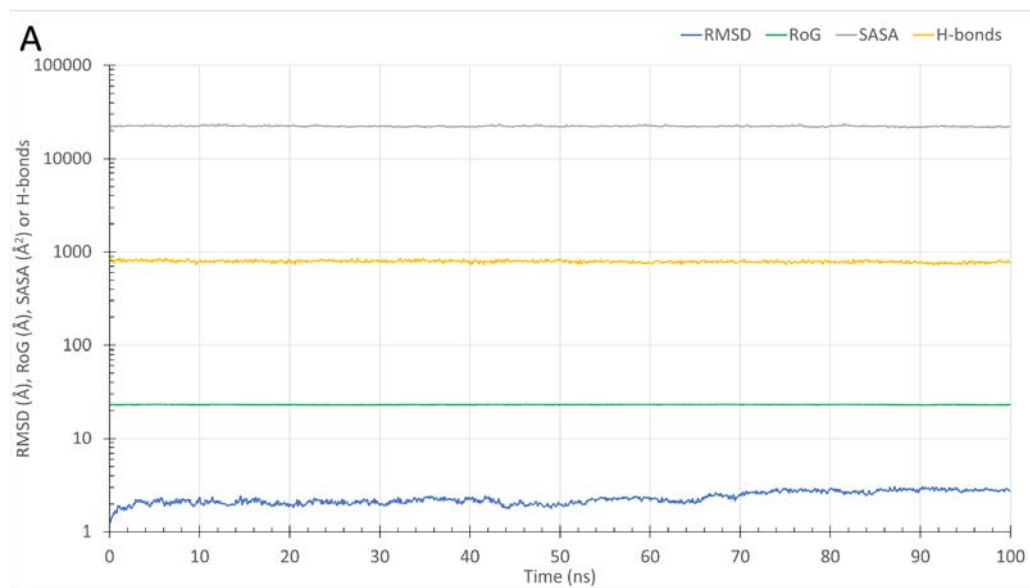


Fig. 4A

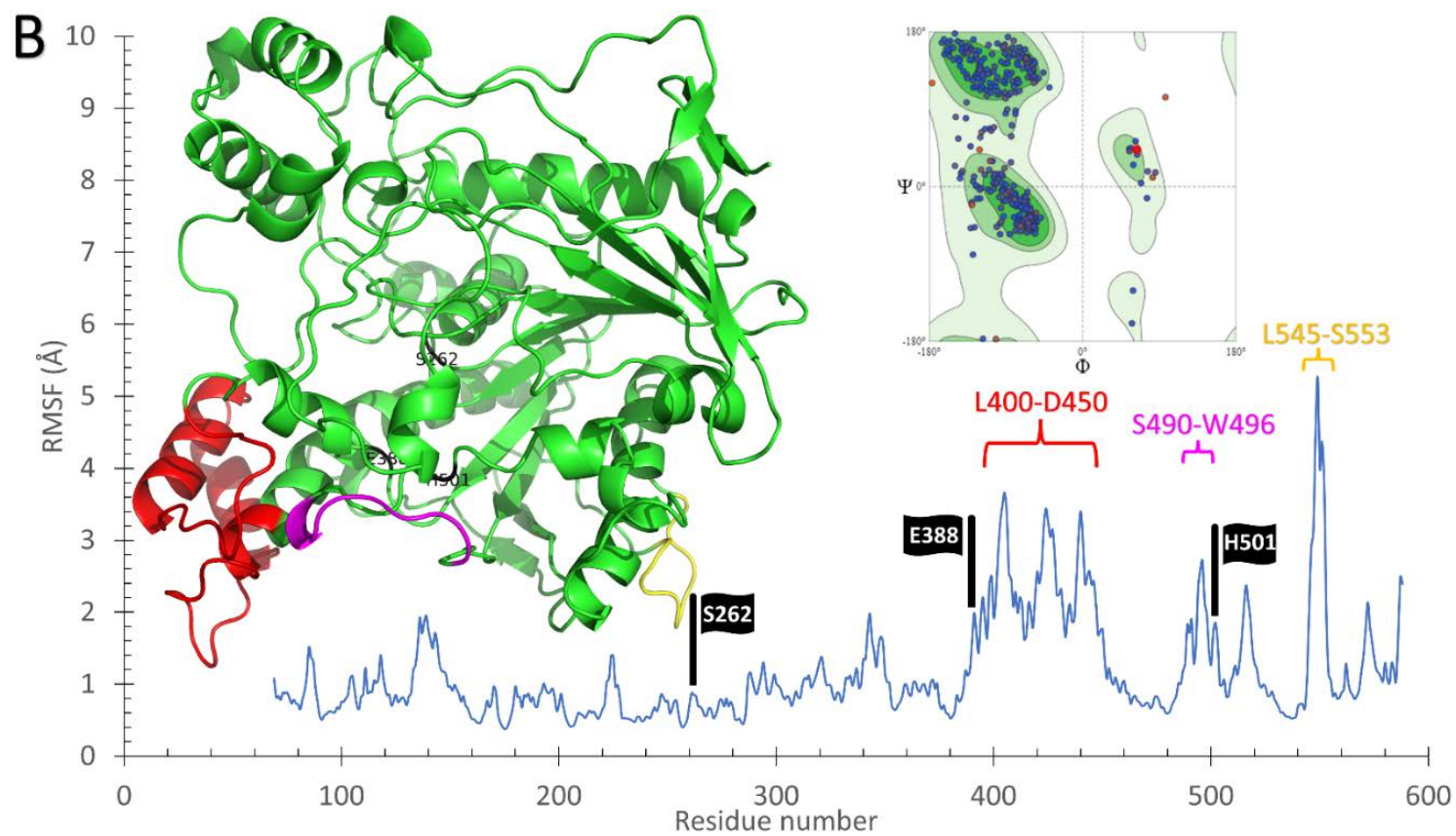


Fig. 4B

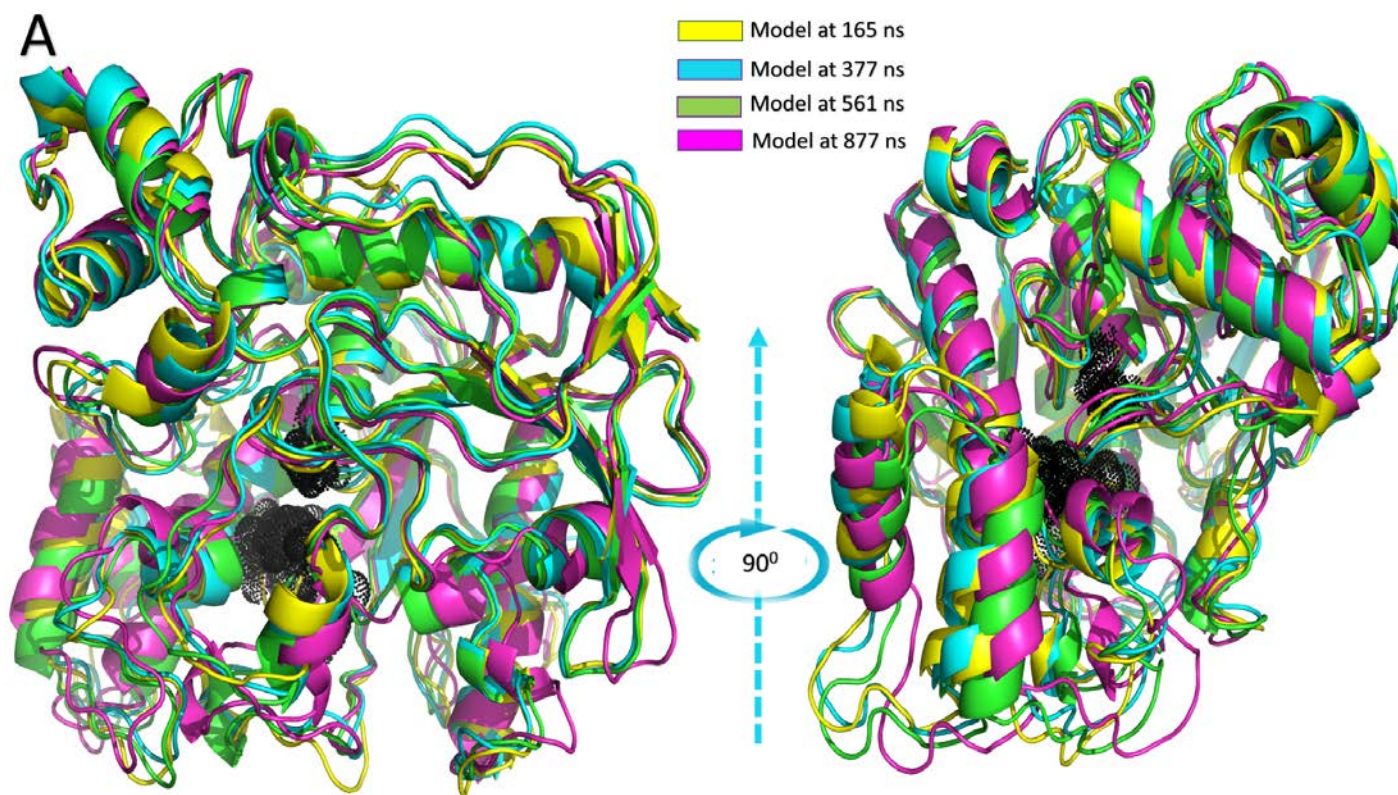


Fig. 5A

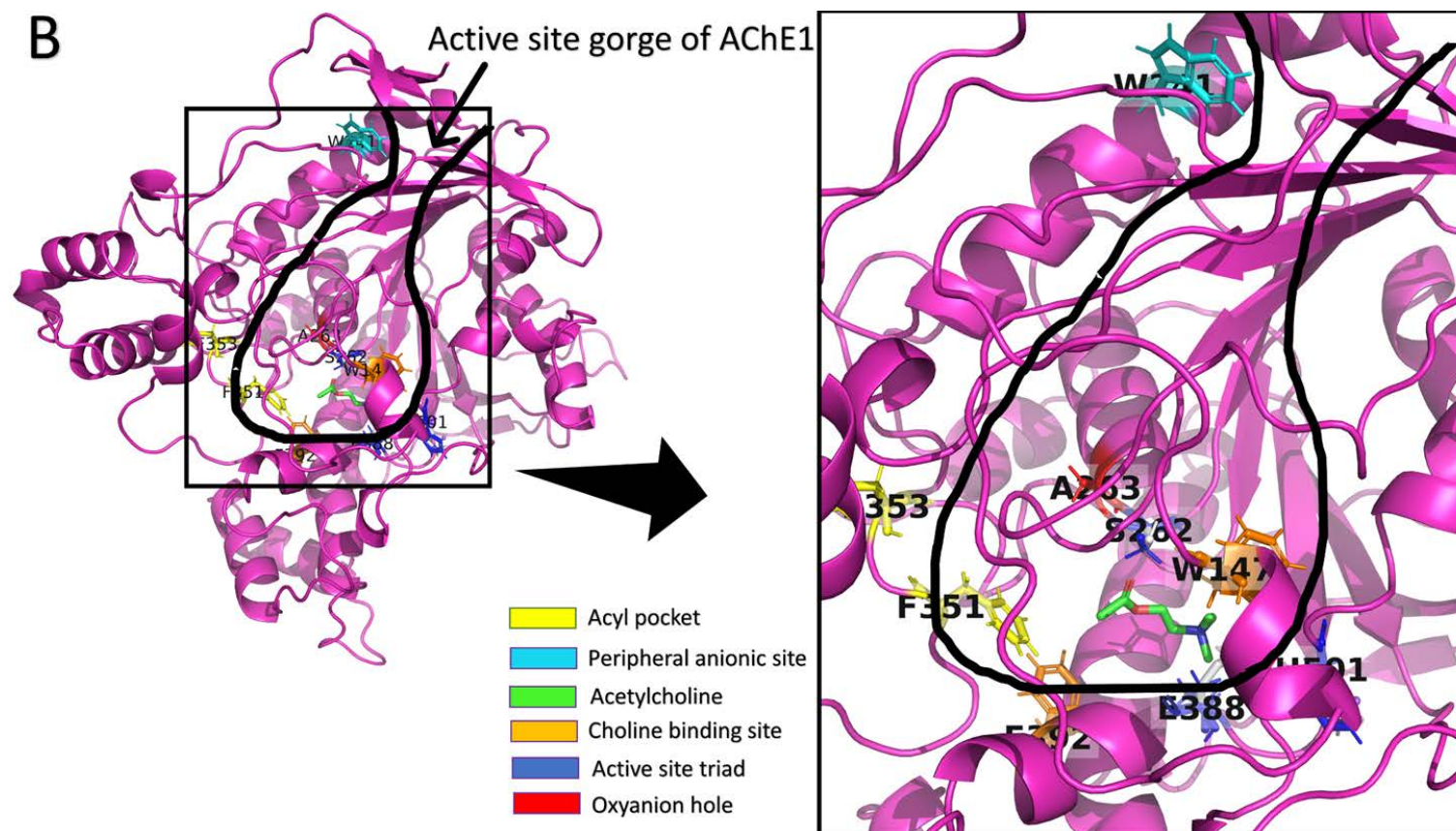
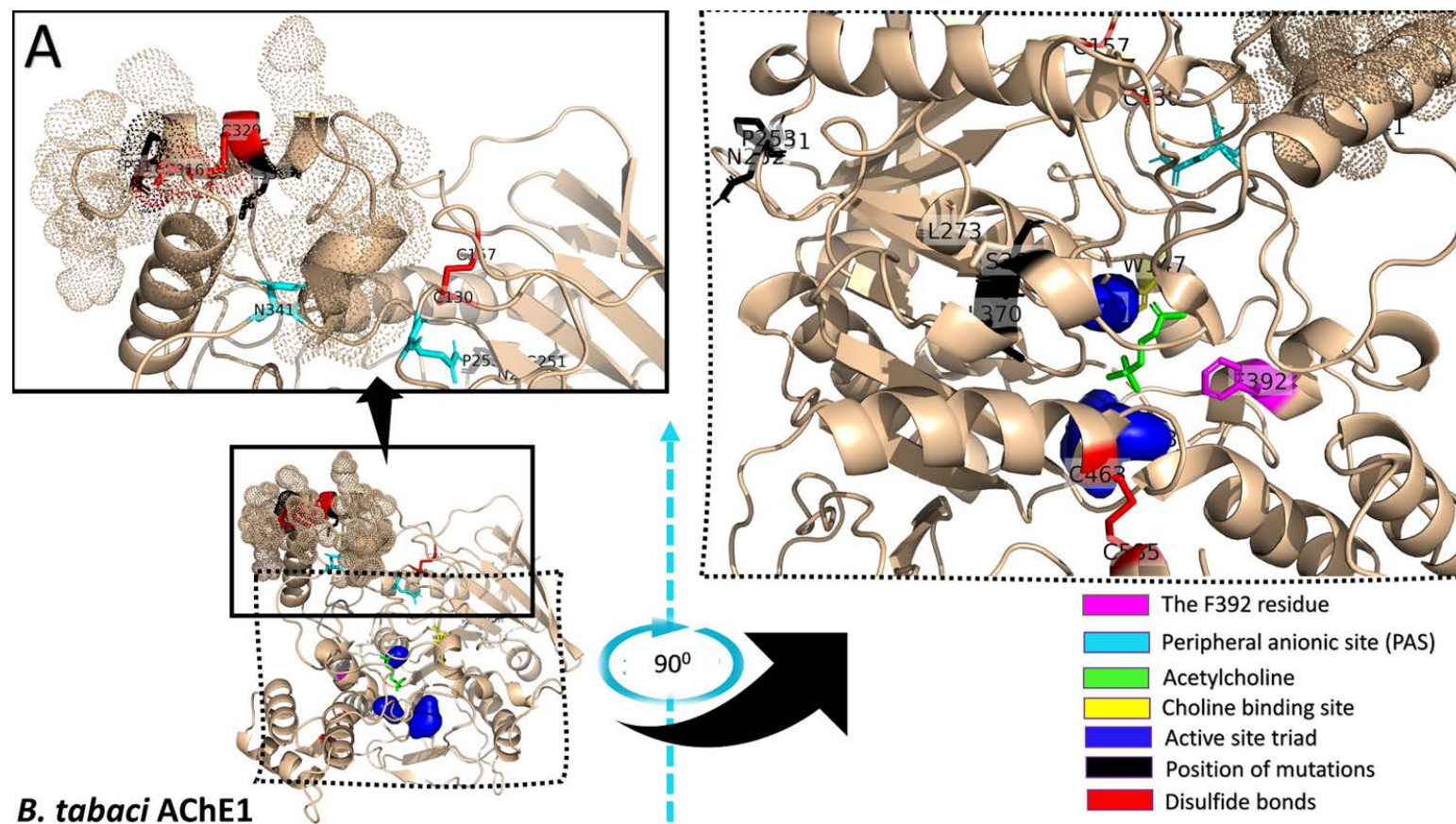


Fig. 5B



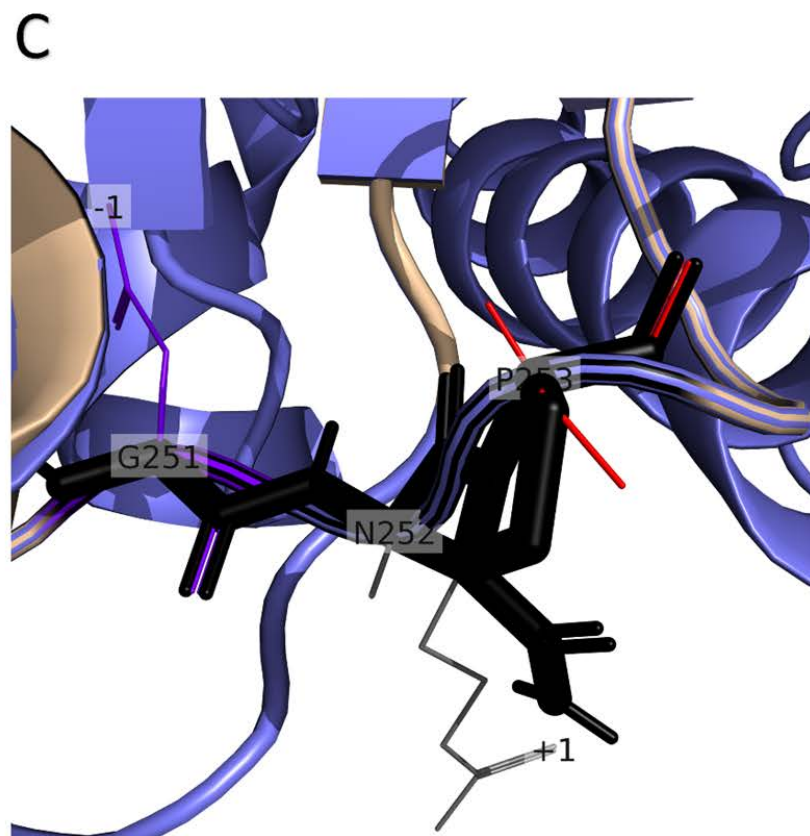
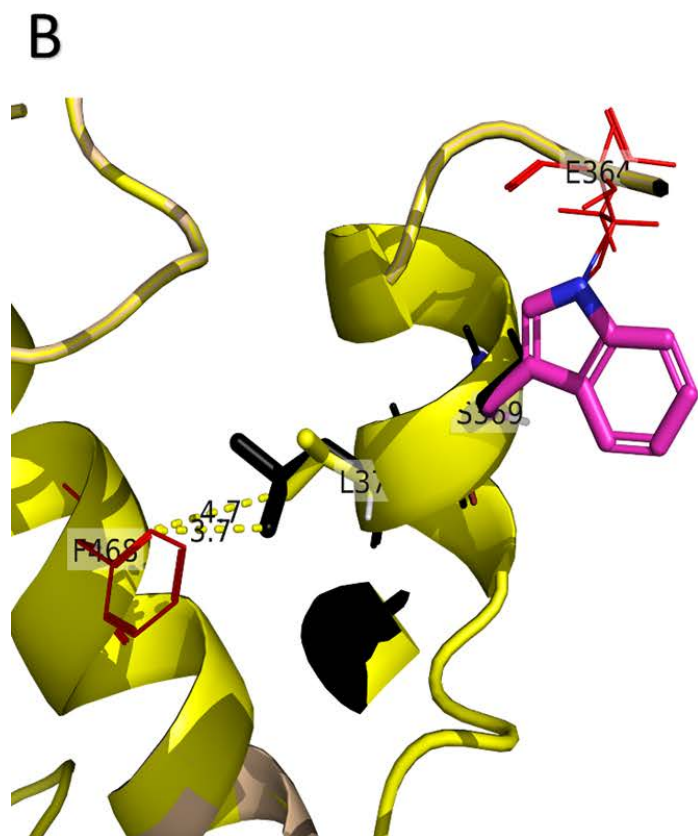


Fig. 6B, 6C

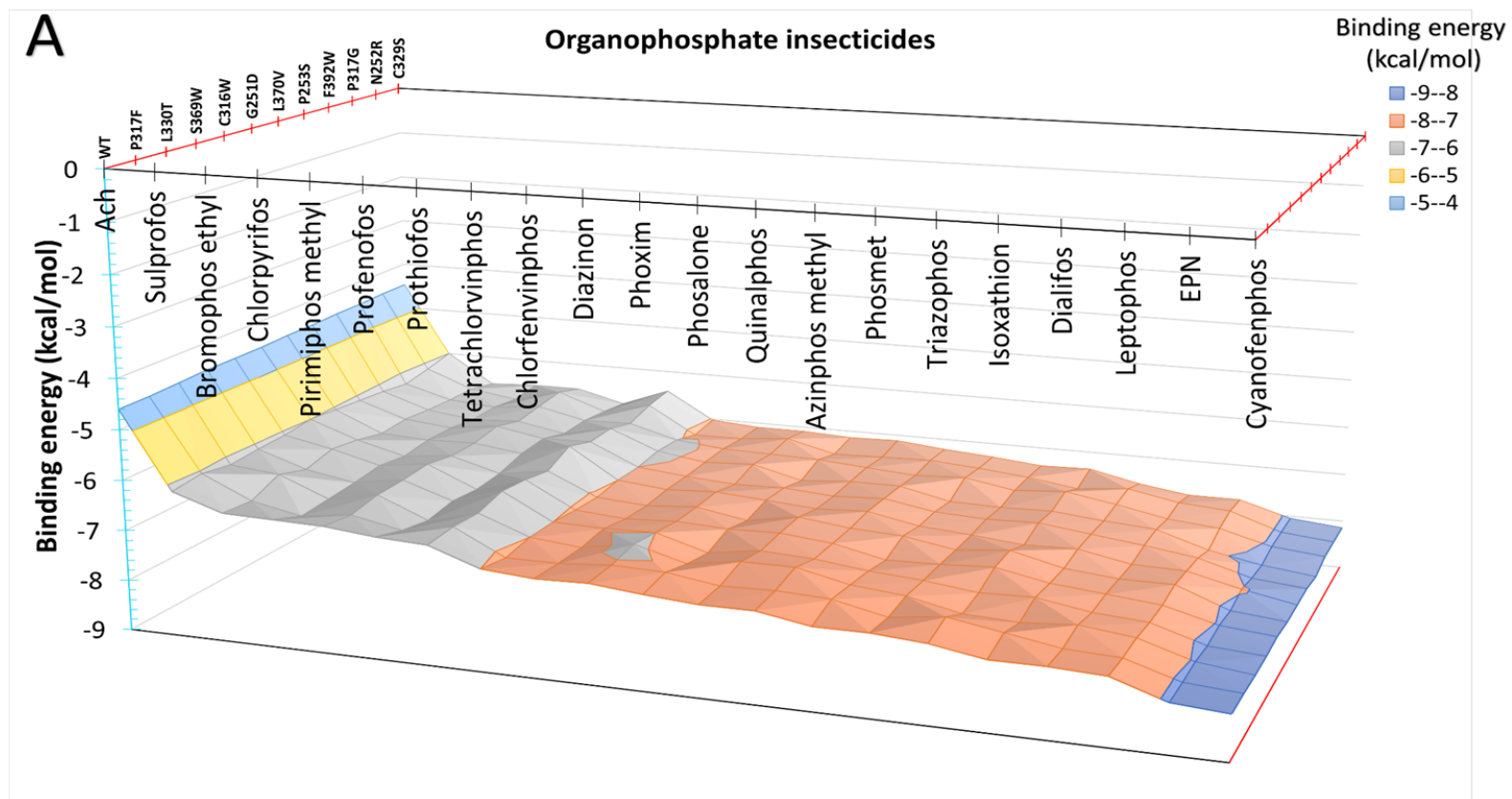


Fig. 7A

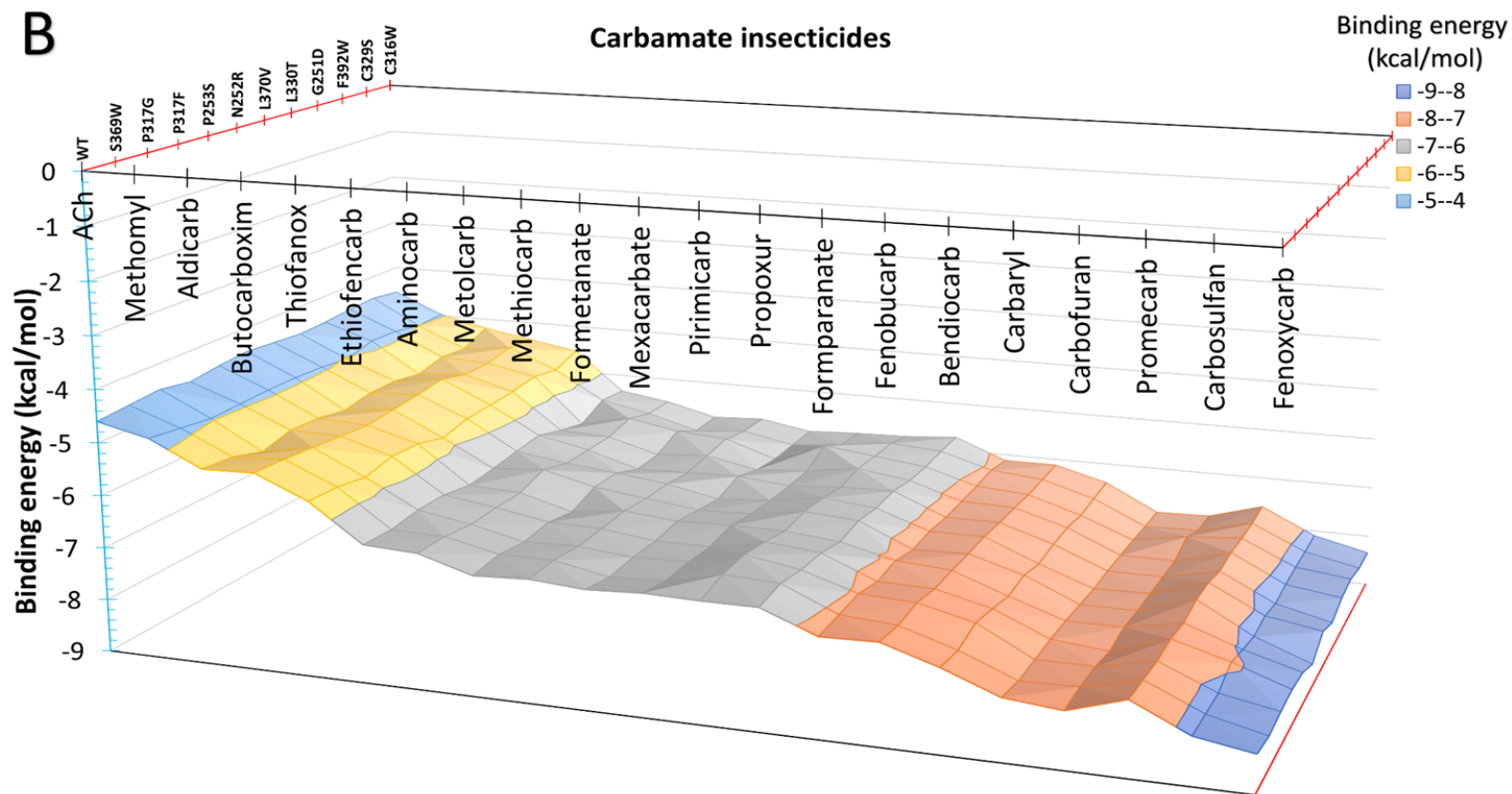


Fig. 7B

Table 1. Amino acid polymorphism and sequence variation (alleles) among the analyzed sequences of *B. tabaci ace1*-type acetylcholinesterase from different Egyptian populations

| Position n(aa)* | AA side chain chemistry change | Resistant group | | | | | | | | | | | Susceptible group | | | | | | |
|----------------------------|---|-----------------|-----|-----|-----|------------|------------|------------|------------|------------|------------|------------|-------------------|-----|-----|-----|-----|-----|-----|
| | | Egy-B Lab. | Beb | Gkt | Qbn | Dbq | Mym | Mas | Fas | Myi | Mbs | Fib | HS-S Lab. | Bwn | Dmg | Fsn | Mys | Gzf | Qqb |
| *[S]892(298) (ATC->ATT) | Neutral hydrophobic → None | I (ATC) | ... | ... | ... | I (ATT) | ... | ... | ... | ... | ... | ... | I (ATC) | ... | ... | ... | ... | ... | ... |
| [S]919(307) (GGC->GGT) | Neutral hydrophobic → None | G (GGT) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | G (GGC) | ... | ... | ... | ... | ... | ... |
| 946(316) (TGC->TGG) | Neutral polar → Aromatic hydrophobic | C (TGC) | ... | ... | ... | ... | W (TGG) | W (TGG) | ... | ... | ... | ... | C (TGC) | ... | ... | ... | ... | ... | ... |
| 949(317) (CCT->GGT) | Neutral hydrophobic → None | P (CCT) | ... | ... | ... | ... | G (GGT) | G (GGT) | ... | ... | ... | F (TTT) | P (CCT) | ... | ... | ... | ... | ... | ... |
| 958(320) (CGC->CCC) | Basic → Neutral hydrophobic | R (CGC) | ... | ... | ... | ... | ... | ... | P (CCC) | ... | ... | ... | R (CGC) | ... | ... | ... | ... | ... | ... |
| [S]1060(354) (GTC->GTG) | Neutral hydrophobic → None | V (GTG) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | V (GTC) | ... | ... | ... | ... | ... | ... |
| 1105(369) (TCC->TGG) | Neutral polar → Aromatic hydrophobic | S (TCC) | ... | ... | ... | ... | ... | ... | W (TGG) | W (TGG) | W (TGG) | W (TGG) | S (TCC) | ... | ... | ... | ... | ... | ... |
| 1108(370) (CTG->GTG) | Neutral hydrophobic → None | L (CTG) | ... | ... | ... | ... | ... | ... | V (GTG) | V (GTG) | V (GTG) | V (GTG) | L (CTG) | ... | ... | ... | ... | ... | ... |
| [S]1165(389) (GGC->GGG) | Neutral hydrophobic → None | G (GGG) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | G (GGC) | ... | ... | ... | ... | ... | ... |
| 1174(392) (TTC->TGG) | Aromatic hydrophobic → None | W (TGG) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | F (TTC) | ... | ... | ... | ... | ... | ... |

*Unbracketed numbers indicate the nucleotides position, while bracketed numbers denote amino acids position.

*Silent mutation; mutations that do not change the organism's phenotype. Although occur in nucleotides of a coding region but result in the same amino acid.

The conserved residues are shown with dots. The polymorphic codons between the resistant and susceptible strains are highlighted with gray color. The numerical designations indicating the positions of amino acids and nucleotides are based on the two *B. tabaci ace1*, obtained from GenBank, mentioned below.

Comparison of the nucleotide and deduced amino acid sequences of *B. tabaci ace1* (reference strains: S, susceptible; R, resistant, GenBank accession no. EF675188 and EF675187, respectively) and eighteen different strains from diverse Egyptian localities. The Egyptian strains were divided into two groups based on their sequence homology with the aforementioned S and R Alon et al. (2008) alleles (see Supplementary [Information Table S1](#)); the first group includes eleven strains (Egy-B, Beb, Gkt, Qbn, Dpq, Mym, Mas, Fas, Mai, Mbs, and Fib) which showed F392 substituted with W392 residue (F392W), based on the resistant *B. tabaci* AChE deduced amino acid sequence (GenBank accession no. ABV45412.1). The second group of sequences is expressed to be susceptible (HS-S, Bwn, Dmg, Fsn, Mys, Gzf, and Qqb), where the F392 is conserved among all the seven strains compared to the susceptible *B. tabaci* AChE deduced amino acid sequence (GenBank accession no. ABV45413.1).