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Differential detoxification enzyme profiles in C-corn strain and R-rice strain of *Spodoptera frugiperda* by comparative genomic analysis: insights into host adaptation

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Abstract

Background The fall armyworm (FAW) *Spodoptera frugiperda*, a highly invasive, polyphagous pest, poses a global agricultural threat. It has two strains, the C-corn and R-rice strains, each with distinct host preferences. This study compares detoxification enzyme gene families across these strains and related *Spodoptera* species to explore their adaptation to diverse host plant metabolites.

Results A total of 1,995 detoxification-related genes, including cytochrome P450 monooxygenases (CYPs), carboxylesterases (COEs), glutathione S-transferases (GSTs), UDP-glucuronosyltransferases (UGTs), and ATP-binding cassette transporters (ABCs), were identified across the genus *Spodoptera*, including *S. littoralis*, *S. litura*, *S. picta*, *S. exigua*, and both FAW strains. A higher abundance of phase I detoxification enzymes (CYPs and COEs) and GSTs was observed in *Spodoptera* species, while FAW strains exhibited fewer detoxification genes, with notable differences in copy numbers between the C and R strains. Analyses at the subfamily level revealed significant variation in gene distribution and expression, particularly within phase I and II detoxification enzymes. Expansions in *CYP6AE* were detected in the C strain, while contractions in *GST-ε*, *CYP9A*, *CYP4M*, *UGT33B*, and *UGT33F* occurred in both strains. In contrast, no substantial variation was observed in phase III ABC enzymes. Functional predictions and protein interaction networks suggest a broader expansion of metabolism-related genes in the R strain compared to the C strain.

Conclusions These findings emphasize the pivotal role of phase I and II detoxification enzymes in host adaptation, providing molecular insights into FAW's capacity for host range expansion, which are crucial for devising targeted and sustainable pest management strategies.

Keywords *Spodoptera frugiperda*, Detoxification enzymes, Host plant adaptation, Polyphagy, Evolutionary adaptation

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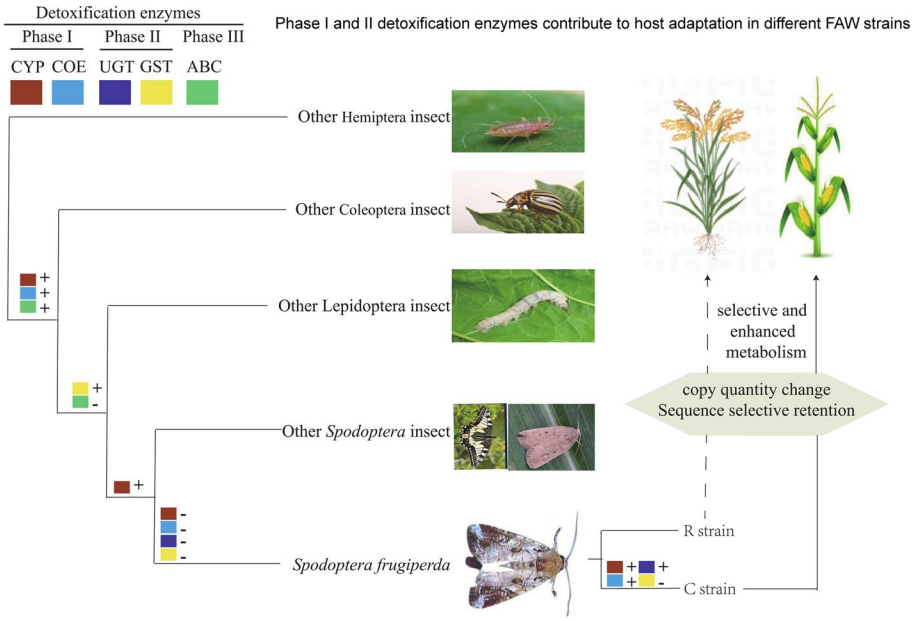
Graphical Abstract

The differential expression of detoxification enzyme gene families in different strains of *Spodoptera frugiperda*.



Background

The order Lepidoptera encompasses a vast diversity of plant pests, including industrial and ornamental insects. One of the superfamilies within this order, Bombycoidea, diverged from the Noctuoidea superfamily approximately 94 million years ago [1]. The Noctuoidea superfamily, which constitutes over one-third of all known Lepidoptera species, includes many significant pests that affect both agriculture and forestry. While *Bombyx mori* from the Bombycoidea superfamily is strictly monophagous, members of the Noctuoidea display a wider range of feeding habits, including omnivorous, oligophagous, and monophagous species [2]. Among these, the fall armyworm (*Spodoptera frugiperda*, FAW) stands out as a particularly damaging omnivorous pest that impacts agricultural production, particularly in the Americas and the Caribbean, feeding on grasses such as rice, wheat, sorghum, maize, and various other crops like cotton and soybeans [3, 4]. In recent years, FAW has spread to production areas across Africa and Asia, presenting a substantial threat to global food security [5].

Host plant adaptation plays a critical role in the lifecycle of FAW, providing both mating and oviposition sites, as well as nutritional resources [6]. Currently, FAW comprises two strains that are phenotypically similar but selectively differentiated. The first strain, commonly referred to as the "rice strain" (R strain), feeds mainly on rice and various forages, and is found in a limited number of regions. The second strain, which is more widespread, especially during invasions, is the "maize strain" (C strain), feeding primarily on maize, cotton, and sorghum [4, 7, 8]. These two strains are believed to have diverged around 2 million years ago and display partial reproductive segregation both before and after mating [9]. Gouin et al. [10] sequenced and compared the genomes of the two strains, revealing significant genomic differentiation driven by positive selection and variations in gene copy number. These differences were observed in genes associated with chemosensation, detoxification, and digestion, indicating that these processes play a key role in the divergence of the two strains [10]. However, these genetic insights are still insufficient for a full understanding of the molecular mechanisms underlying FAW's host plant preferences [6].

FAW strains, represented by their feeding on maize or rice, encounter various plant metabolites that differ between their respective host plants. For instance, tebufenpyrad-like metabolites are found predominantly in maize, whereas coixol is a characteristic metabolite of rice. Additionally, because of their different feeding

preferences, each strain must also deal with varying concentrations of toxic metabolites common to both plants. For example, maize contains a higher concentration of lignin than rice, which might have influenced the detoxification mechanisms of the two strains over time [11]. The detoxification enzyme systems in the FAW strains likely adjust in both quantity and activity to accommodate the specific chemical landscapes of their host plants. This suggests that detoxification enzyme efficiency, particularly concerning host-specific metabolites, may play a significant role in the dietary adaptation of FAW [12].

Detoxification is a critical process through which insects overcome toxic secondary metabolites produced by plants, and variations in detoxification capabilities may ultimately limit host plant suitability [13]. This is particularly relevant to FAW's resistance to insecticides, which are often based on mechanisms involving these enzymes [8]. Specifically, enzymes such as cytochrome P450s (CYPs), glutathione S-transferases (GSTs), and ATP-binding cassette (ABC) transporters help FAW metabolize toxic compounds in plants and insecticides [14–17]. Variations in the efficiency and diversity of these enzymes may not only influence host suitability but also contribute to insecticide resistance in FAW populations [15]. For example, CYP enzymes are involved in the oxidative metabolism of plant toxins and insecticides, and differences in their expression and activity between FAW strains may underlie their ability to tolerate different plant defenses [8, 14, 15].

Insects employ several classes of detoxification enzymes in their metabolism of harmful plant chemicals, and FAW is no exception [8]. Five main categories of detoxification enzymes are involved in FAW's ability to cope with the chemical defenses of its host plants: Phase I enzymes, which are responsible for oxidation, hydrolysis, and/or reduction reactions (Cytochrome P450s, known as CYPs, and Carboxyl/Cholinesterases, known as COEs), Phase II enzymes, which engage in conjugation reactions (UDP-Glucuronosyltransferases, or UGTs, and Glutathione S-Transferases, or GSTs), and Phase III enzymes, which are involved in transporting metabolites (ATP-Binding Cassette transporters, or ABC transporters) [14]. These enzymes exhibit unique structures and can be further subdivided into various subfamilies with distinct functions. For instance, CYP enzymes are divided into four major evolutionary branches: CYP2, CYP3, CYP4, and mitochondrial CYPs [15], while Carboxyl/Cholinesterases (CCEs) are categorized into three primary phylogenetic groups: intracellular catalytic, secreted catalytic, and neurogenic developmental classes [16]. Additionally, ABC transporter genes are classified into eight subfamilies (A-H) [17]. These detoxification enzymes are essential for metabolizing insecticides, allowing FAW to neutralize toxic compounds. This ability to break down insecticides is a critical part of its survival strategy, helping it withstand chemical controls and thrive in agricultural environments.

In the present study, we conducted an in-depth analysis of the five major families of detoxification enzymes in *Spodoptera* species. Specifically, we focused on the two strains of FAW, separating them for a detailed analysis of the copy number characteristics of their detoxification enzyme families. Furthermore, we explored the phylogenetic patterns of local expansion and contraction within these enzyme families, providing insights into how these processes may have contributed to the detoxification abilities of the different strains. Based on this phylogenetic analysis, we tentatively identified potential molecular networks that could explain the differences in feeding behavior between the two FAW strains. Our findings offer a comprehensive comparison of the selective pressures imposed by host plant metabolites on FAW and reveal the potential molecular mechanisms underlying its detoxification processes. Understanding these molecular networks is crucial for developing strategies to mitigate the impact of FAW on global agriculture, especially in areas where the pest continues to invade new territories. The co-evolutionary relationships between FAW and its host plants, mediated by detoxification enzymes, highlight the evolutionary complexity of plant–insect interactions and underscore the importance of continued research in this area.

Methods

Data sources

The genomes of two FAW strains were retrieved from the LepidoDB database (http://bipaa.genouest.org/is/lepidodb/spodoptera_frugiperda/) [10]. Additionally, genomic data for other *Spodoptera* species, including *S. littoralis*, *S. litura*, *S. picta*, and *S. exigua*, were sourced from the NCBI database (GCF_002706865.2, GCA_902850265.1, GCA_902829305.4) and DRYAD (https://datadryad.org/stash/dataset/doi:10.5061/dryad.6wwpzgn5w). Phylogenetic relationships for 22 insect species were extracted from the TimeTree database (http://www.timetree.org/), and the numbers of detoxification enzyme genes in non-*Spodoptera* species were based on findings from previous studies [18].

De novo identification of detoxification enzyme gene families

To identify potential detoxification enzyme families (CYP, COE, UGT, GST, ABC) in *Spodoptera* species, HMMER (v.3.01) was utilized [19]. This tool was used to analyze

predicted protein datasets derived from the *Spodoptera* species genomes. Protein sequences were scanned using the Pfam Hidden Markov Model (HMM) profiles: COE—PF00135, GST—PF00043, PF02798, PF01124, CYP—PF00067, UGT—PF00201, and ABC—PF00664, PF00005, or PF00950 [14, 20–22]. Identified detoxification enzyme families were further validated by confirming the structural domains through the NCBI-CD database (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) and the SMART database (http://smart.embl.de) [23].

Renaming and classification of detoxification enzyme gene families

The classification and naming of detoxification enzyme family genes in *Spodoptera* species were performed using blastp comparisons [4]. For CYP genes, sequences were compared against the Cytochrome P450 nomenclature database (https://drnelson.uthsc.edu/nomenclature/), with the naming based on a sequence identity threshold of > 55% for subfamily assignment [8, 24]. UGT gene naming followed comparisons against published UGT gene nomenclature, with subfamily assignment determined based on sequence identity > 60% [25]. Similarly, GST, COE, and ABC genes were named by blastp comparison with previously reported sequences, and the highest percentage identity was used as a reference for gene naming [18].

Phylogenetic analysis of detoxification enzyme gene families

For systematic classification of detoxification enzyme family genes in *Spodoptera* species, we constructed phylogenetic trees based on the amino acid sequences of each enzyme family. MUSCLE was used to align all selected sequences, and unaligned regions and gaps were excluded. The optimal evolutionary models were calculated using IQ-TREE [26]. The selection of these models was guided by the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC) to ensure the most accurate fit to the data. IQ-TREE calculates the optimal evolutionary model for each dataset by evaluating multiple model candidates based on model selection criteria, ensuring the reliability and robustness of the phylogenetic tree construction. Phylogenetic trees were generated using the maximum likelihood (ML) method with pairwise deletion, uniform substitution rates, and 1000 bootstrap replicates. The models for each detoxification enzyme family were as follows: PMB + R6 for ABC transporters, WAG + F + R6 for COEs, JTT + F + R6 for CYPs, LG + R4 for GSTs, LG + F + R5 for MGSTs, and LG + R5 for UGTs. These models were selected based on their ability to balance model complexity and data fitting, as supported by model comparison results from IQ-TREE.

Comparative analysis of homologous genes

To conduct a comparative analysis of detoxification enzyme gene families across *Spodoptera* species, we utilized OrthoVenn3 and CAFE5 (https://orthovenn2.bioinfotoolkits.net/) [27]. These tools enabled the identification and visualization of orthologous gene groups within and between species. For CAFE5, we employed the default statistical thresholds to identify significant gene family expansion or contraction, with an E-value threshold of < 1E-2 and an inflation value > 1.5. The E-value threshold of < 1E-2 was chosen to filter out low-confidence hits, ensuring that only highly reliable gene pairs were analyzed. The inflation value > 1.5 was selected to allow for the detection of both gene family expansion and contraction, reflecting evolutionary patterns that may indicate adaptive changes or shifts in metabolic needs. These statistical thresholds were empirically validated in previous studies of gene family evolution and were deemed appropriate for our dataset.

Protein–protein interaction analysis

We investigated the protein–protein interaction (PPI) networks of detoxification enzyme genes in *Spodoptera* species using the STRING database [28]. The analysis focused on the Noctuoidea family, applying a confidence threshold of > 40 and predicting up to 20 interactions for each protein. The resulting PPI networks were imported into Cytoscape 3.9.1 [29] for visualization and refinement. The layout of the networks was optimized to highlight interactome hubs and functional modules. Additionally, we incorporated Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations from STRING to enhance our understanding of the functional roles of detoxification enzymes in *Spodoptera* species.

Results

Gene copy number comparison of detoxification enzyme families in *Spodoptera* species

To explore the differences in gene copy numbers across detoxification enzyme families (CYP, COE, UGT, GST, ABC) in various strains of FAW, we conducted a comprehensive analysis using the Timetree system alongside gene family distribution data. In total, 1,995 detoxification enzyme genes were identified across multiple *Spodoptera* species genomes (*S. littoralis*, *S. litura*, *S. picta*, *S. exigua*) and two FAW strains (rice strain and maize strain). From a phylogenetic standpoint, the Lepidoptera and Coleoptera orders showed a higher number of Phase

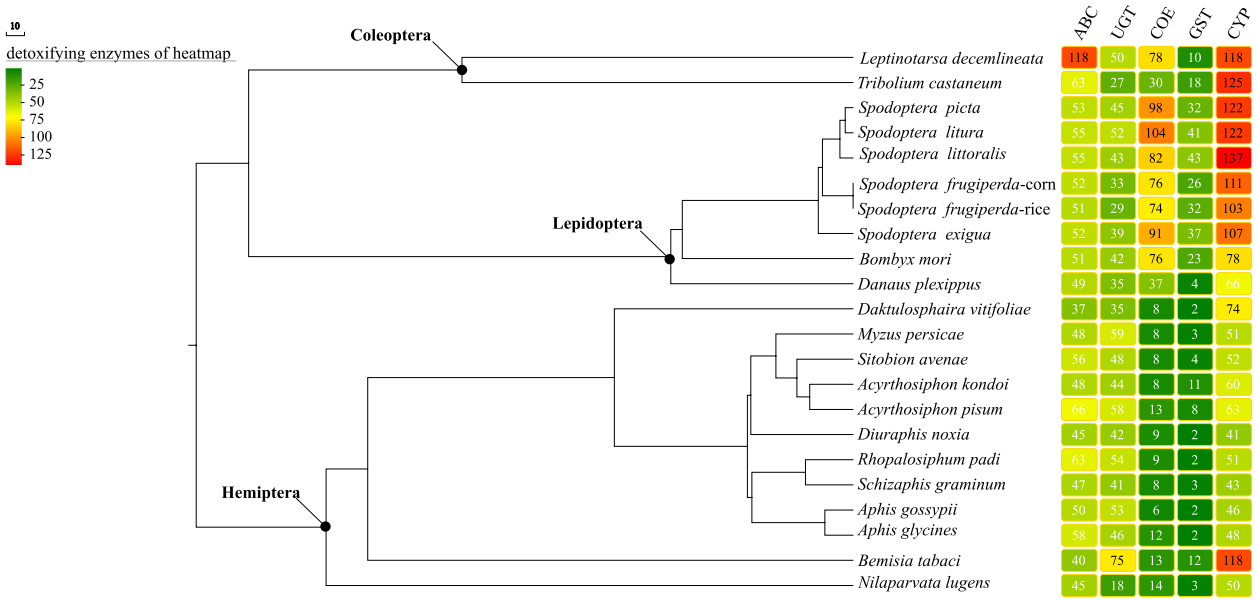


Fig. 1 The distribution of detoxifying enzyme genes in *Spodoptera* and other insects. ABC, ABC transporter; UGT, uridine diphosphate glycosyltransferase; COE, carboxylesterase; GST, glutathione S-transferase; CYP, cytochrome P450

I detoxification enzymes (CYP, COE). Within Lepidoptera, Phase II detoxification enzymes (GST) were more prevalent, while Coleoptera had higher frequencies of Phase III detoxification enzymes (ABC). Phase II detoxification enzymes (UGT) were broadly distributed across all examined insect groups (Fig. 1).

Within the order Lepidoptera, *Spodoptera* species exhibited a higher number of Phase I detoxification enzymes (CYPs) compared to more distantly related species such as *Bombyx mori* and *Danaus plexippus*, both of which had consistently fewer detoxification genes. Within *Spodoptera*, FAW possessed a relatively lower number of Phase I and Phase II detoxification genes than other species. Nevertheless, the number of transporter genes remained relatively stable. In the comparison of the two FAW strains, the maize strain (C strain) demonstrated a higher genomic presence of Phase I and II (UGT) detoxification enzymes and transporter genes compared to the rice strain (R strain), which exhibited a lower number of Phase II (GST) detoxification enzymes (Fig. 1).

Phase I – CYP gene family in *Spodoptera* species

Phase I detoxification enzymes primarily function to oxidize, reduce, or hydrolyze toxic substances. Among these enzymes, CYPs are crucial for the oxidation of various exogenous compounds [15]. To clarify the variability of CYP gene families in FAW, we combined the distribution of gene families across species. Four CYP clans were identified in *Spodoptera* species (CYP2, CYP3, CYP4, and mitochondrial), with two clans (CYP2 and mitochondrial) exhibiting low, stable copy levels, while the other two (CYP3 and CYP4) showed higher, less stable copy numbers (Table S1, Fig. S1, Treefile S1). A comparison of FAW strains revealed that three of the clans (CYP2, CYP4, and mitochondrial) had consistent copy numbers, whereas the CYP3 clan was significantly reduced in the R strain (Fig. 2A).

These four CYP clans were subdivided into 28 families and 54 subfamilies. The variability in gene copy numbers primarily arose in the CYP3 and CYP4 subfamilies, including five subfamilies of CYP3 (CYP6AB, CYP6AE, CYP6B, CYP6BG, CYP9A) and five of CYP4 (CYP340A, CYP340AA, CYP4C, CYP4M, CYP304V). Among these, the C strain exhibited expansions (C+) in most subfamilies (CYP6AB, CYP6AE, CYP6B, CYP9A, CYP340A, CYP340AA, CYP4M), while a few subfamilies (CYP4C, CYP304V, CYP6BG) were contracted (C-) (Fig. 2B). These variations, particularly in CYP6AE, likely play a crucial role in the detoxification of both plant allelochemicals and insecticides, supporting the strains' adaptations to different host plants and their ability to metabolize harmful compounds.

Homologous gene assays and CAFE5 analysis helped identify key gene amplifications and reductions. OrthoVenn3 classified 702 CYP genes into 94 clusters, with 64% of the core clusters shared across *Spodoptera* species. Six clusters (18-CYP6B, 21-CYP367A, 76-CYP6B,

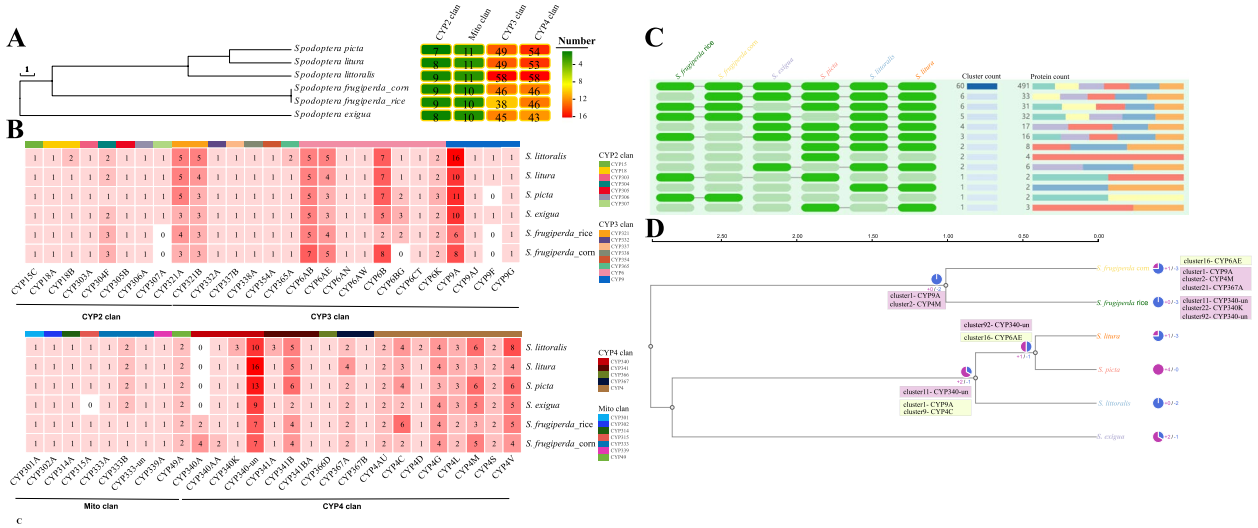


Fig. 2 The distribution of *CYP* genes in *Spodoptera*. **A** Comparison of copy number of *Spodoptera CYP* genes at clan level, (**B**) at subfamily level; (**C**) Cluster distribution of homologous genes in the *Spodoptera CYP* family and protein composition of each cluster as determined by OrthoVenn3; (**D**) Expansion and contraction analysis of clusters in the *Spodoptera* CYP family as determined by CAFE5

79-CYP4L, 81-CYP6B, 82-CYP4V) were absent only in the R strain, while three clusters (22-CYP340K, 69-CYP4C, 78-CYP4L) were missing in the C strain (Fig. 2C). CAFE5 revealed that cluster11 (CYP340-un) experienced significant contraction in the ancestor of closely related species (*S. litura*, *S. picta*, *S. littoralis*), while cluster1 (CYP9A) and cluster9 (CYP4C) were expanded. Further, in the ancestor of *S. litura* and *S. picta*, cluster92 (CYP340-un) contracted significantly, whereas cluster16 (CYP6AE) was significantly expanded (Fig. 2D). Several genes in the CYP gene families underwent copy number variations, suggesting that CYP expansions and contractions (CYP6AE, CYP9A, CYP4M) likely mediate secondary metabolic adaptations to host plants and insecticide detoxification in both FAW strains.

Phase I – COE gene family in *Spodoptera* species

Carboxyl/cholinesterases (COEs), another group of Phase I detoxification enzymes, play an essential role in the hydrolysis of ester-containing xenobiotics [16]. To investigate the variability in COE genes in FAW, we examined the distribution of gene families across *Spodoptera* species. We identified 10 COE subfamilies across three major classes (Table S2, Fig. S2, Treefile S2). The

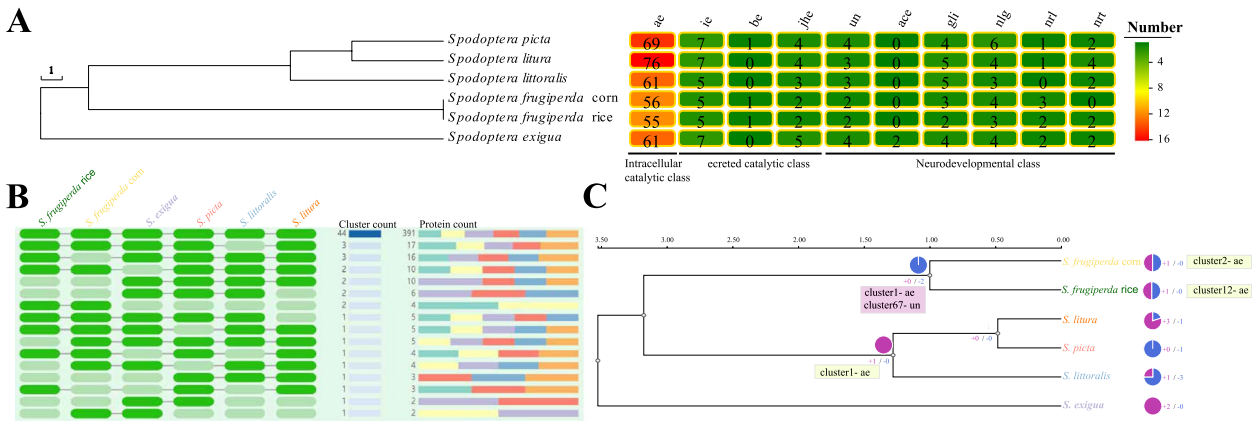


Fig. 3 The distribution of *COE* genes in *Spodoptera*. **A** Comparison of copy number of *Spodoptera COE* genes at subfamily level; (**B**) Cluster distribution of homologous genes in the *Spodoptera* COE family and protein composition of each cluster as determined by OrthoVenn3; (**C**) Expansion and contraction analysis of clusters in the *Spodoptera* COE family as determined by CAFE5; ae, α-esterase; ie, integument esterase; be, β-esterase; jhe, juvenile hormone esterase; un, uncharacterized; ace, acetylcholinesterase; gli, gliotactin; nlg and nrl, neuroligin; nrt, neurotactin

intracellular catalytic class of COEs exhibited lower copy numbers in FAW compared to closely related species, with the R strain having even fewer copies. In the secreted catalytic class, COE gene copy numbers were consistent across FAW strains. However, in the neurodevelopmental class, the C strain exhibited higher copy numbers in three subfamilies (gli, nlg, nrl), while the nrt subfamily was lost (Fig. 3A).

Using homologous gene analysis and CAFE5, we identified key gene amplifications and reductions. OrthoVenn3 classified 525 COE genes into 67 clusters, with core clusters present in 66% of *Spodoptera* species. Three clusters (14-nrt, 50-ae, and 54-nrt) were absent only in the C strain (Fig. 3B). CAFE5 indicated that cluster1 (ae) significantly expanded in the ancestor of *S. litura*, *S. picta*, and *S. littoralis*, while it had contracted before the divergence of FAW strains. Both types of ae were expanded in different FAW strains (C+: cluster2, R+: cluster12; Fig. 3C). These findings suggest that differential expansion of ae subfamilies may have driven secondary metabolic adaptations in FAW strains.

Phase II – GST gene family in *Spodoptera* species

Phase II detoxification enzymes, including glutathione S-transferases (GSTs), are responsible for conjugating hydrophilic compounds with xenobiotics or Phase I products, enhancing their solubility [30]. Seven GST subfamilies were identified across *Spodoptera* species, with one unclassified subfamily showing single-copy stability and six subfamilies exhibiting copy number variability (Table S3, Figs. S3 and S4, Treefile S3 and S4). FAW strains exhibited copy number differences, with one subfamily (GST-δ) expanded in the C strain and two subfamilies (GST-ε, GST-θ) contracted. In particular, the GST-ε subfamily was reduced by 33% to 59% in the C strain compared to other species (Fig. 4A).

Further gene amplification and reduction analyses, using homologous gene detection and CAFE5, revealed key driver molecules in GST gene families. OrthoVenn3 classified 210 GST genes into 43 clusters, with distinct clusters identified for different subfamilies, such as GST-σ, which was subdivided into three clusters (8, 24, 34; Fig. 4B). CAFE5 indicated significant reductions in GST-σ and GST-ε subfamilies in the C strain (Fig. 4C),

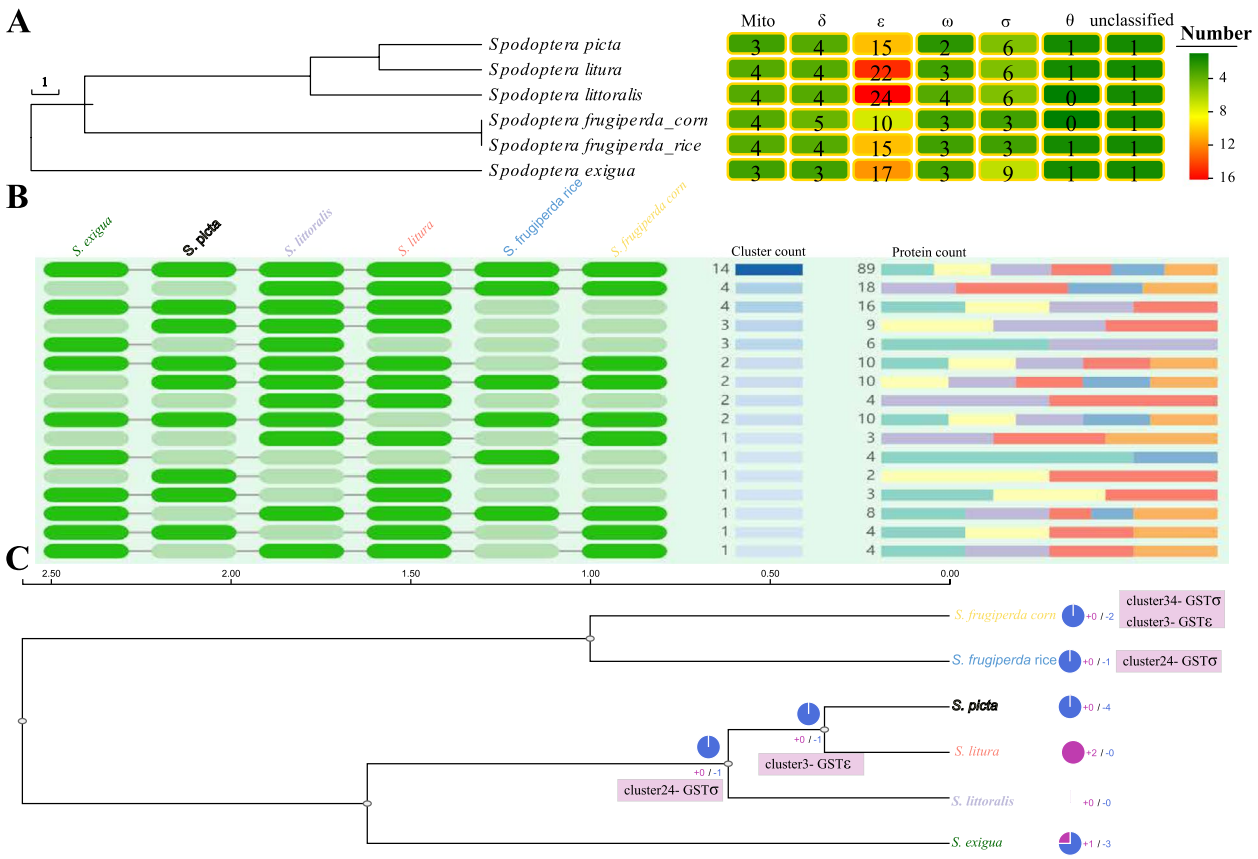


Fig. 4 The distribution of *GST* genes in *Spodoptera*. **A** Comparison of copy number of *Spodoptera GST* genes at subfamily level; **B** Cluster distribution of homologous genes in the *Spodoptera* UGT family and protein composition of each cluster as determined by OrthoVenn3; **C** Expansion and contraction analysis of clusters in the *Spodoptera* UGT family as determined by CAFE5

suggesting that copy variants in these GST subfamilies may mediate FAW strains' adaptations to different host plants.

Phase II – UGT gene family in *Spodoptera* species

UDP-glucuronosyltransferases (UGTs), another Phase II detoxification enzyme family, are involved in conjugating sugar groups to xenobiotics or Phase I products to enhance solubility [31]. In *Spodoptera* species, we identified 12 known UGT families and one newly discovered family, tentatively named UGT334 (Table S4, Fig. S5, Treefile S5). This newly identified family had single-copy stability in *Spodoptera* species and showed a 38.08% sequence identity (E-value = 5.64E-75) to UGT33B, justifying its provisional classification as a new family. Five established UGT families (UGT34, UGT39, UGT41, UGT43, UGT44, UGT48, UGT50) were stable across species. Among FAW strains, three UGT families exhibited copy number variations: UGT33 was expanded (C+), while UGT41 and UGT46 were contracted (C-; Fig. 5A).

Subfamily-level analysis of the three UGT families (%identity > 60) revealed that the C+UGT33 family consisted of five subfamilies (B, F, J, T, V), with the B and F subfamilies exhibiting the most prominent expansions (Fig. 5B). The contractions in UGT41 and UGT46 were primarily due to reductions in the UGT41B and UGT46A subfamilies (Fig. 5B). Homologous gene analysis classified 241 UGT genes into 24 clusters, with most core clusters (63%) shared across *Spodoptera* species. Notably, cluster24 was only present in the C strain and *S. litura*. CAFE5 analysis indicated significant expansions and contractions in UGT families during FAW evolution, with significant contraction observed in two UGT clusters of the R strain (Fig. 5D). These copy variants likely reflect secondary metabolic adaptations in FAW.

Phase III – ABC gene family in *Spodoptera* species

ABC transporters, Phase III detoxification enzymes, are primarily responsible for the efflux and transport of toxic compounds [32]. We identified eight ABC subfamilies (ABCA-H) in *Spodoptera* species (Table S5, Fig. S6, Treefile S6). Across FAW strains, seven ABC subfamilies exhibited copy number differences, with C+expansions in subfamilies ABCB, ABCC, and ABCH, and C- contractions in subfamilies ABCA, ABCD, ABCE, and ABCG. The ABCG subfamily was found to be reduced in FAW relative to other *Spodoptera* species, with further reductions observed in the C strain (Fig. 6A).

Homologous gene analysis and CAFE5 expansion and reduction analysis identified no significant changes in ABC subfamilies at any evolutionary node (Fig. 6C). This indicates that Phase III detoxification enzymes likely do not play a major role in the detoxification and evolutionary adaptation of FAW strains to different feeding habits.

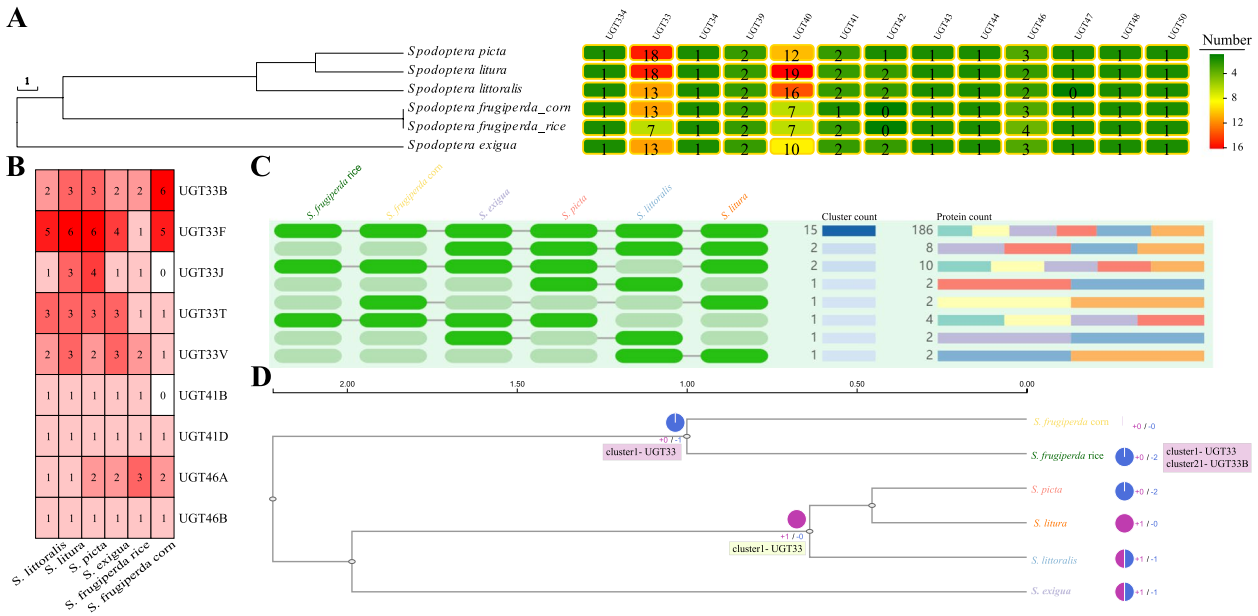


Fig. 5 The distribution of *UGT* genes in *Spodoptera*. **A** Comparison of copy number of *Spodoptera UGT* genes at family level, (**B**) at subfamily level; (**C**) Cluster distribution of homologous genes in the *Spodoptera UGT* family and protein composition of each cluster as determined by OrthoVenn3; (**D**) Expansion and contraction analysis of clusters in the *Spodoptera UGT* family as determined by CAFE5

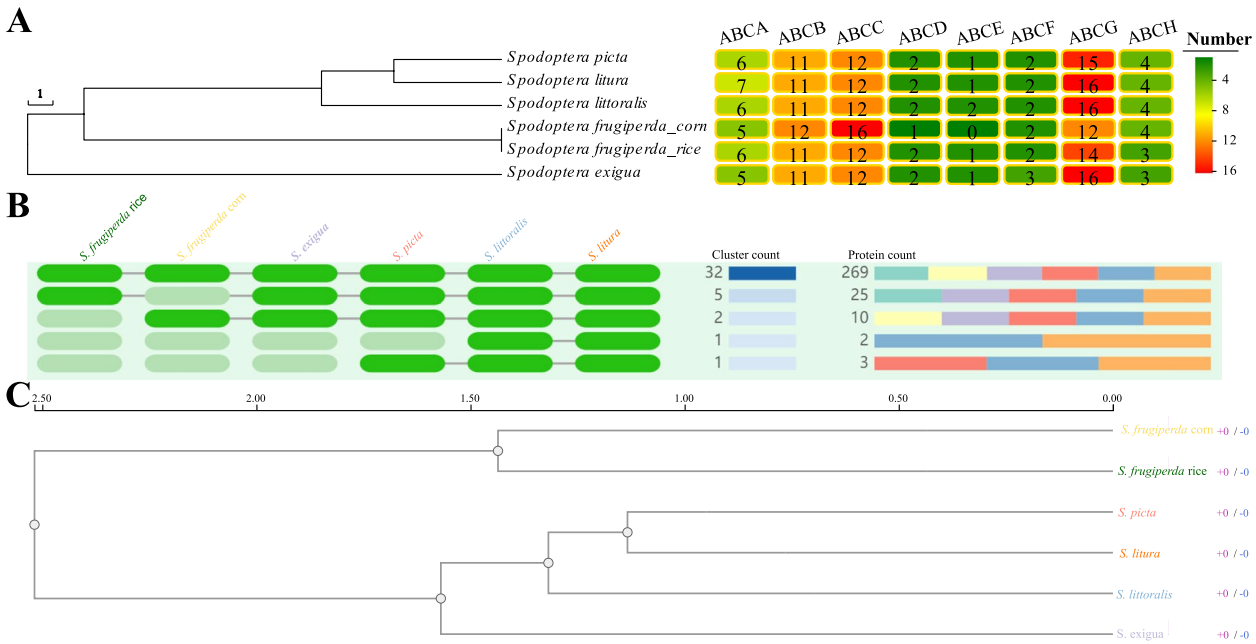


Fig. 6 The distribution of *ABC* genes in *Spodoptera*. **A** Comparison of copy number of *Spodoptera ABC* genes at subfamily level; (**B**) Cluster distribution of homologous genes in the *Spodoptera ABC* family and protein composition of each cluster as determined by OrthoVenn3; (**C**) Expansion and contraction analysis of clusters in the *Spodoptera ABC* family as determined by CAFE5

Protein–protein interaction network of copy number variants

Figures 2, 3, 4, 5 and 6 revealed substantial copy number variations in detoxification enzyme subfamilies, especially in CYP and UGT genes. To further assess the functional impact of these variations, we constructed protein–protein interaction networks and performed functional enrichment analyses for the C- subfamilies (dominant in the R strain) and the C + and R- subfamilies (dominant in the C strain). The networks constructed for UGT33B, UGT33F, and CYP6AE subfamilies comprised 225 associations formed by 23 proteins (Fig. 7A, Table S6). Gene Ontology (GO) functional enrichment indicated that these networks were primarily associated with transcriptional regulation, Wnt pathway regulation, and muscle development. Reactome pathway analysis indicated involvement in transcriptional processes and adipocytosis (Fig. 7B). These interactions, particularly involving CYP6AE, suggest that the network is integral to detoxification processes and cellular defense mechanisms, highlighting its potential as a target for pest control interventions.

The protein interaction network constructed for GST-ε, CYP9A, and CYP4M subfamilies comprised 211 associations formed by 31 proteins (Fig. 7C). GO enrichment indicated that these networks were related to the metabolism of sugars, sterols, and other metabolites, while KEGG pathway analysis suggested associations with sugar and amino acid biosynthesis, as well as vitamin metabolism. Reactome pathway analysis further confirmed these networks' involvement in sterol metabolism and adipocyte formation (Fig. 7D, Table S7). These findings underscore the importance of these networks in supporting the adaptive strategies of the FAW strains, with GST-ε and CYP9A playing key roles in detoxifying both host plant defenses and pesticides. These insights open avenues for targeted pest control strategies, particularly aimed at disrupting metabolic networks involved in resistance.

Selective sequence variations in key subfamilies

Figures S2 and S3 revealed expansions in certain branches of the ae and GST-σ subfamilies in FAW strains, including R + expansions (GST-σ cluster34, ae cluster12; SfrGSTs1, Sfrae50, Sfrae51, Sfcae53) and C + expansions (GST-σ cluster24, ae cluster2; SfcGSTs7, Sfrae5, Sfrae12, Sfrae13, Sfrae14, Sfrae15, Sfcae12, Sfcae14, Sfcae15, Sfcae16, Sfcae22, Sfcae23). To better understand the functions of these branches, we constructed protein interaction networks and conducted functional enrichment analyses.

The R + -related protein networks consisted of 100 associations formed by 23 proteins, including subnetworks formed by GST-σ and ae proteins (Fig. 8A). GO functional enrichment suggested associations with transcriptional regulation, compound transport, and

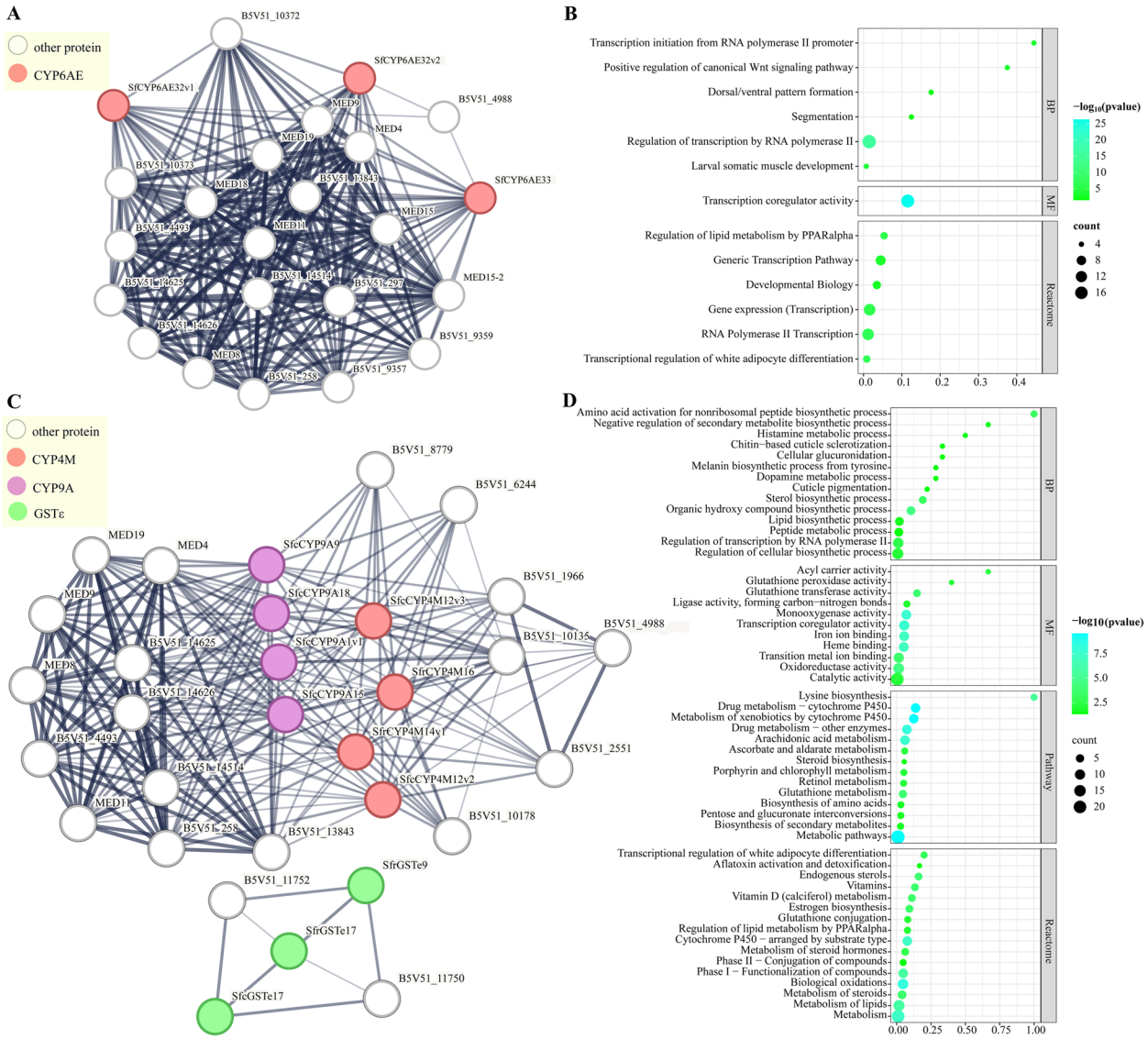


Fig. 7 Significant copy altered protein interaction network in FAW. **A** Interaction network and (**B**) functional enrichment for the C + subfamily; (**C**) Interaction network and (**D**) functional enrichment for the R + subfamily; nodes that did not match or did not produce an enriched network were removed, and the thickness of the connecting lines in the interaction network indicates its score. MED4/8/9/11/15/15–2/18/19: Mediator of RNA polymerase II transcription subunit 4/8/9/11/15/15–2/18/19, B5V51_9357: Med13_N domain-containing protein, B5V51_10373: Mediator of RNA polymerase II transcription subunit 13, B5V51_10135: Cytochrome b5 heme-binding domain-containing protein, B5V51_11750: Microsomal glutathione transferase, B5V51_2551: Fatty acid hydroxylase domain-containing protein, B5V51_258/297/1966/4493/4988/6244/8779/9359/10178/10372/11752/13843/14514/14625/14626: Uncharacterized protein

presynaptic membrane formation, while KEGG and Reactome pathway enrichment indicated involvement in the metabolism of multiple compounds (Fig. 8B). In contrast, C + -related networks comprised 228 associations formed by 24 proteins, with no interactions observed for GST-σ proteins (Fig. 8C). GO enrichment suggested associations with RNA binding and terpene transport, while KEGG pathway analysis indicated involvement in protein processing, and Reactome pathway analysis linked these networks to rRNA processing (Fig. 8D). These findings suggest that, while the C strain is more involved in RNA and protein synthesis, the R strain may exhibit greater metabolic adaptation in relation to compound detoxification.

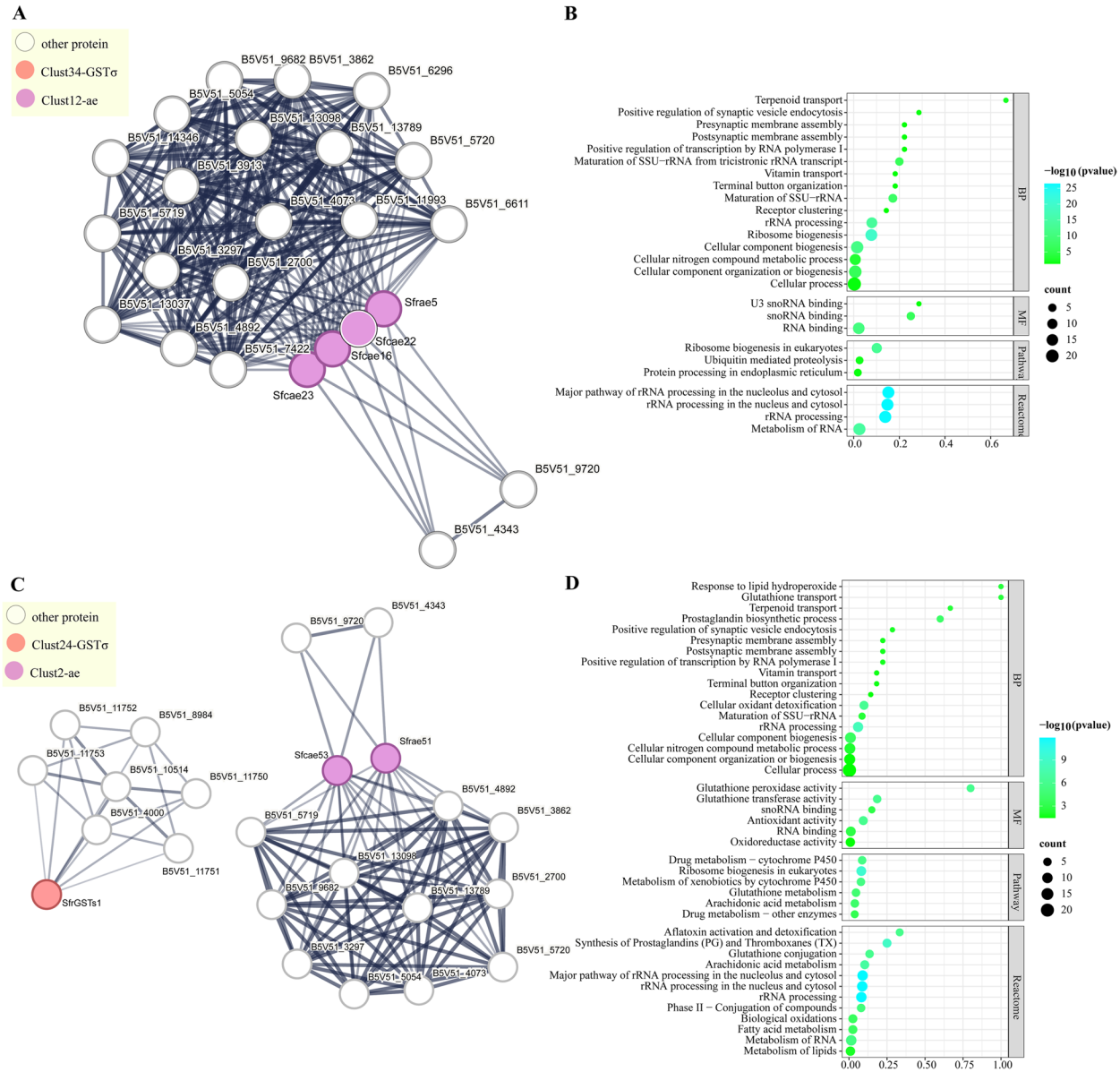


Fig. 8 Selective significant copy changes for sequence differences altered protein interaction network in FAW. **A** Interaction network and (**B**)` functional enrichment for the cluster24 (GST-σ) and cluster2 (ae); (**C**) Interaction network and (**D**) functional enrichment for the cluster34 (GST-σ) and cluster12 (ae); nodes that did not match or did not produce an enriched network were removed, and the thickness of the connecting lines in the interaction network indicates its score. B5V51_3862/3913/4892/5054/7422/9682: WD_REPEATS_REGION domain-containing protein, B5V51_5720: U3 small nucleolar ribonucleoprotein protein MPP10, B5V51_13098: U3 small nucleolar RNA-associated protein 11, B5V51_10514: Glutaredoxin domain-containing protein, B5V51_9720: LAM_G_DOMAIN domain-containing protein, B5V51_11750: Microsomal glutathione transferase, B5V51_2700: PINc domain-containing protein, B5V51_3297/4000/4073/4343/5719/6296/6611/8984/11751/11752/11753/11993/13037/13789: Uncharacterized protein

Discussion

In this study, we conducted a comprehensive analysis of detoxification enzyme-related gene families (Phase I: CYP, COE; Phase II: GST, UGT; Phase III: ABC) across multiple species in the genus *Spodoptera*, with particular emphasis on the comparison of gene diversity, gene number, and functional enrichment across different species and strains of the FAW. Specifically, we explored the expansion and contraction rates of subfamilies of genes potentially involved in FAW strain differentiation. The results revealed significant differences in the number and diversity of detoxification enzyme genes among FAW

strains, providing insights into the molecular mechanisms of host adaptation and insecticide resistance [33]. These findings have important implications for developing strain-specific pest control strategies [34]. By understanding the molecular differences in detoxification pathways, we can identify potential vulnerabilities in each strain, particularly in how they metabolize plant toxins and insecticides [35]. In particular, the differential detoxification abilities across strains may be linked to their ability to metabolize specific plant defense metabolites. For example, the interaction between plant metabolites like lignin in maize and coixol in rice with detoxification enzymes could explain the differences in strain-specific host range adaptations [11]. Targeting these specific detoxification pathways could offer opportunities for more environmentally sustainable pest management strategies, minimizing reliance on broad-spectrum insecticides and reducing potential resistance development.

Copy number variations in detoxification enzyme gene families

The evolutionary expansion and contraction of gene families are commonly associated with key biological innovations or adaptations to ecological traits, such as feeding habits and exposure to environmental stressors [34, 35]. Notably, detoxification-related gene families tend to expand more rapidly in the Noctuidae family, which includes the genus *Spodoptera* [2]. This family exhibits a broad range of nutritional traits, and our analysis suggests that the expansion of detoxification genes plays a critical role in enabling these species to adapt to various diets. We found that *Spodoptera* species have a higher number of cytochrome P450 (CYP) genes compared to more distantly related lepidopteran species such as *Bombyx mori* and *Danaus plexippus*. CYP genes are crucial for the biosynthesis of endogenous metabolites and the detoxification of exogenous substances [8, 36, 37]. Their expansion in *Spodoptera* likely facilitates the species' adaptation to diverse host plants, which is particularly important for agricultural pests like FAW. This ability to adapt to diverse host plants highlights a critical consideration for pest management: strain-specific targeting of detoxification pathways [38]. For instance, strains with a broader host range, like FAW, may require more complex management strategies that account for the specific detoxification mechanisms in different strains, such as how certain plant-derived toxins may interact with specific enzyme subfamilies [39]. The identification of these strain-specific detoxification pathways could enable the development of tailored pest management strategies that target specific enzymes, potentially improving control efficacy across different agricultural environments.

FAW is recognized as a highly omnivorous pest with a broader host range compared to other closely related species such as *S. littoralis*, *S. litura*, *S. picta*, and *S. exigua* [12, 40]. This broader host range may be explained by the evolutionary expansion of detoxification enzyme-related gene families, which contribute to the insect's ability to metabolize a wide variety of plant defense compounds, enhancing host adaptation [2, 37, 41, 42]. Despite this, FAW has a lower number of genes associated with Phase I and Phase II detoxification enzymes compared to other species within the genus *Spodoptera* [43]. Interestingly, the number of Phase III detoxification enzyme genes, particularly the ABC transporters, remains relatively constant across FAW strains, suggesting that Phase III detoxification may be more conserved [44]. These findings suggest that pest control strategies could focus on Phase I and II detoxification pathways, as these are more variable across strains. For example, identifying compounds that target the specific detoxification enzymes in each strain could lead to more effective and strain-specific insecticide development [40, 43].

FAW is divided into two phenotypically similar strains: the R strain, which feeds on rice, forages, and tall grasses, and the C strain, which prefers maize, cotton, and sorghum [7]. A comparative analysis between these strains revealed significant differences in detoxification gene numbers. The C strain exhibits a higher number of Phase I (CYP, COE) and Phase III (ABC) detoxification enzymes, as well as UDP-glucosyltransferase (UGT) genes, which are crucial for the initial steps of metabolite processing and the glucuronidation of toxins. On the other hand, the C strain shows a reduction in glutathione S-transferase (GST) genes, which play a key role in conjugating glutathione to metabolites, thereby enhancing their solubility and facilitating detoxification [30]. This reduction suggests that the C strain may rely less on glutathione conjugation and more on alternative detoxification mechanisms for adaptation to its host plants. The strain-specific differences in detoxification pathways could be leveraged in the development of targeted pest control methods [45]. For instance, pest control approaches targeting the Phase I and III detoxification enzymes in the C strain could be considered more effective, whereas strategies for the R strain might focus on enhancing the expression of GST enzymes, which are less abundant in this strain.

Building on Breeschoten et al. [2] findings of a significant positive correlation between the size of the GST detoxification gene family and the degree of omnivory

in Lepidoptera, it is worth exploring whether the R strain's potentially broader host range is linked to this correlation. The relationship between GST family size and host adaptation in FAW remains an area that warrants further research, especially in the context of strain-specific detoxification capabilities. Further investigation into the connection between GST family size and host range could provide insights into the development of strain-specific strategies [45]. By understanding these correlations, pest management strategies could incorporate compounds that specifically disrupt detoxification pathways in strains with broader host ranges [46]. Investigating these relationships in greater depth could help develop targeted insecticide strategies based on the distinct detoxification profiles of the strains, thereby enhancing pest control efforts while reducing non-target effects.

Phase I detoxification enzymes

Phase I detoxification enzymes, particularly CYP and COE genes, play a crucial role in the early stages of metabolizing toxic substances, primarily through oxidation or reduction reactions that reduce toxicity [15, 16]. CYP enzymes, which function as mixed-function oxidases or monooxygenases, are distributed across various insect tissues and are involved in numerous life processes, including the synthesis and degradation of hormones like ecdysteroids and juvenile hormones, as well as the detoxification of xenobiotics from natural and synthetic sources [38]. The functional diversity of CYP genes, driven by their structural diversity, is essential for enabling insect species to adapt to a wide range of chemical environments, particularly plant chemical defenses.

The CYP gene family is systematically classified into four major categories: clan (phylogenetic identity), family (>40% sequence identity), subfamily (>55% sequence identity), and individual gene (>95% sequence identity) [24]. Insects typically possess mitochondrial CYP clans as well as cytoplasmic CYP clans, including CYP2, CYP3, and CYP4 [47]. Our results show that these clans are conserved across all species of *Spodoptera*, with key genes like CYP306A (CYP2 clan) and others in the Mito CYP clan being involved in hormone biosynthesis, which remains conserved across multiple insect orders [48]. These CYP genes are associated with basal life activities and remain conserved, irrespective of species divergence or strain differences within FAW.

The CYP3 and CYP4 clans, in contrast, are highly species-specific and tend to undergo expansion in response to environmental factors, such as the availability of host plants or insecticide exposure [38]. We identified eight CYP3 gene families (CYP321, CYP332, CYP337, CYP338, CYP354, CYP365, CYP6, CYP9) in *Spodoptera*, three of which (CYP321, CYP6, CYP9) exhibited multiple subfamilies and copy number variations [49]. The C strain of FAW showed significant expansion in certain CYP genes, particularly CYP6 and CYP9, which are known to detoxify plant defense compounds and insecticides. However, a more detailed explanation of the functional impact of these copy number variations in detoxification genes is required. For example, the expansion of specific gene families like CYP6 and CYP9 in the C strain may enhance its ability to metabolize specific plant toxins or insecticides, thus providing a functional advantage over the R strain in certain host plants. A more in-depth analysis of how these changes influence the detoxification abilities of the FAW in response to different host plants could help clarify the practical implications of these genetic variations [50].

Carboxylesterases (COEs) are another important group of Phase I detoxification enzymes that hydrolyze insecticides, such as organophosphates, carbamates, and pyrethroids [51]. Our analysis revealed significant differences in COE gene copy numbers among *Spodoptera* species, with certain subfamilies, such as neurotactin (M class), being absent in the C strain but present in other species like *S. litura* [52]. In addition, FAW exhibits a reduction in acetylcholinesterase (Ache) genes and other COE subfamilies compared to other species, which may indicate differences in detoxification capabilities related to host use [52, 53].

Phase II detoxification enzymes

Phase II detoxification enzymes, including GSTs and UGTs, conjugate toxic metabolites with small molecules such as glutathione or sugars, enhancing their solubility and facilitating their excretion from the body [54]. GSTs are particularly important for providing resistance to insecticides and oxidative stress [55–57]. In insects, GSTs are divided into seven major classes: δ, ε, ω, θ, σ, ζ, and unclassified [30]. Six of these classes were identified in *Spodoptera* species, with significant variation in gene number across strains [58]. The C strain showed a notable reduction in GST-ε genes, which are involved in the metabolism of insecticides like organophosphates, organochlorines, and pyrethroids [21]. This reduction suggests that the C strain may rely less on GST-mediated detoxification, potentially using alternative detoxification pathways [59].

UDP-glucosyltransferases (UGTs) catalyze the transfer of sugar moieties to various substrates, enhancing their solubility and enabling detoxification [31]. Thirteen UGT families were identified in *Spodoptera*, with the C strain

showing significant expansions in UGT40 and UGT42, which are involved in insecticide metabolism [60, 61]. By contrast, the R strain exhibited a contraction in UGT33 genes, which are involved in metabolizing maize-derived toxins like DIMBOA [12]. This reduction may explain the R strain's preference for non-maize hosts, as the reduced detoxification capacity may limit its ability to feed on maize.

Phase III detoxification enzymes

Phase III detoxification involves the excretion of soluble toxic metabolites from the body, a process mediated by ATP-binding cassette (ABC) transporter proteins [62]. Insects possess eight subfamilies of ABC transporters (ABCA-H), and all of these subfamilies were found to be conserved across *Spodoptera* species [63]. Unlike Phase I and II detoxification enzymes, the number of ABC transporter genes remained relatively stable between FAW strains, suggesting that these genes may be under more stabilizing selection [64]. However, some variation was observed in specific ABC subfamilies, such as ABCA and ABCG, which may contribute to differences in lipid transport and insecticide detoxification between strains [65].

Expansion/reduction of subcategories

CYP321A is related to allelochemicals such as cinnamic acid, purine toxin or coumarin and pyrethroid insecticides [66, 67]. CYP4V is involved in the transformation of polycyclic aromatic hydrocarbons [68], and CYP4C is involved in the detoxification of pyrethroid insecticides [68]. Nrt plays an important role in cell adhesion [69]. Avermectin can inhibit the expression of UGT33V, while UGT33J is slightly induced and up-regulated, but both are induced and up-regulated by insecticides such as indoxacarb, metaflumizone, chlorantraniliprole and cypermethrin [15]. UGT46A is crucial for maintaining insect olfaction and also limits its feeding range [70]. In the R strain, 12 detoxification enzyme subfamilies (CYP321A, CYP6BG, CYP4C\V, GSTθ, nrt, UGT33J\V, UGT41B, UGT46A, UGT340-un\K) have significant homolog expansions; at the same time, the R strain also shows functional enrichments in transcriptional regulation, compound transport, presynaptic membrane formation, and metabolism of multiple compounds. This may support the long-term adaptation of the R strain to exogenous compounds and plant allelochemicals, and due to the enhanced olfaction, FAW concentrates its damage on relatively narrow hosts such as rice.

GST-δ is involved in the metabolism of multiple insecticides [55], and UGT33F is specialized for the reglucosylation of DIMBOA in the FAW gut [71]. The overexpression of Gli triggers the expression of miR-184, thereby activating the BMP signaling pathway and affecting key processes such as cell proliferation, differentiation and tissue morphogenesis [72], neuroligin (nlg and nrl) can regulate the sensitivity of the peripheral olfactory system of insects [73]. In the C strain, 13 detoxification enzyme subfamilies (CYP6B\AB\AE, CYP9A, CYP340A\AA, CYP367A, CYP4M, gli, nlg, nrl, GST-δ, UGT33F) have significant homolog expansions; at the same time, the C strain also shows functional enrichments related to rRNA, protein processing and terpene transport. This may support its adaptation to the allelochemicals of the maize host and promote its olfaction and wing formation, thereby affecting its host range and migratory ability.

CYP6AE is involved in the detoxification of multiple plant toxins and pyrethroid insecticides, but it cannot metabolize gossypol, and slight variations in its amino acids can change its metabolism of plant allelochemicals [74, 75]. The ae is very important for the metabolic detoxification of xenobiotics, and different ae enzymes produce different metabolic choices for poisons [76]. The GST-σ has DDT dehydrochlorinase activity [77], and the overexpression of GST-ε contributes to the metabolic detoxification of xenobiotics [78]. In the R strain and C strain, there are differences in copy numbers and enzyme sub-classifications in the CYP6AE, ae, GST-σ\ε, UGT33B subfamilies, indicating that there is a selective accumulation of metabolites in the two FAW strains, thereby promoting their adaptation to the host or the external environment.

Furthermore, considering restricting these expanded subfamilies related to olfaction or cell differentiation may help limit the host selection of FAW and may also restrict its development and migration. Meanwhile, the development of molecular diagnostic tools and the screening and development of control agents based on the expression patterns of detoxification enzyme genes or single nucleotide polymorphisms (SNPs) of different strains can quickly and accurately identify and control the C and R strains in the field. Additionally, based on the detoxification gene differences obtained from the above results, it is helpful to timely discover the potential hosts of the C and R strains. According to their host ranges, planting trap crops or intercropping with non-host plants can effectively disrupt the host adaptation, olfactory recognition and host location ability of FAW. Moreover, by taking advantage of their certain preferences for different crops, attracting them to lay eggs. Regularly harvesting the trap crops and conducting centralized treatment can thus reduce the harm to the main planted crops.

Conclusions

This study provides a comprehensive analysis of detoxification enzyme-encoding genes (CYP, COE, GST, UGT, ABC) across the *Spodoptera* genus, with a focus on *S. frugiperda* strains. Our analysis identified 1,995 genes involved in detoxification, revealing significant differences in gene diversity and copy number among FAW strains. While FAW exhibits a lower overall number of Phase I and II detoxification genes compared to other *Spodoptera* species, strain-specific expansions and contractions were observed. The C strain showed expansions in CYP and UGT genes, which may enhance its ability to detoxify host plant toxins and insecticides, while the R strain showed contractions in GST and UGT genes, possibly reflecting its more specialized diet. These findings highlight the critical role of detoxification genes in host adaptation and provide a foundation for further research aimed at developing targeted pest control strategies that minimize environmental impact. Based on these findings, future research should focus on characterizing the specific functions of expanded detoxification gene families in both FAW strains, particularly through functional genomics and transcriptomics approaches, to understand the precise role of these enzymes in insecticide resistance and host plant adaptation. Furthermore, strain-specific pest control strategies could be developed by targeting the key detoxification enzymes identified in this study. For example, interventions that disrupt the function of CYP and UGT enzymes in the C strain or enhance GST-mediated detoxification in the R strain could provide more effective and environmentally sustainable pest management approaches. Additionally, integrating this molecular knowledge with ecological factors, such as pesticide use and host plant availability, would help refine pest control methods and improve their long-term efficacy.

Abbreviations

FAW	*Spodoptera frugiperda*
ABC	*ABC transporter*
UGT	Uridine diphosphate glycosyltransferase
COE	Carboxylesterase
GST	Glutathione S-transferase
CYP	Cytochrome P450
Ae	*α*-Esterase
ee	Integument esterase
be	*β*-Esterase
jhe	Juvenile hormone esterase
un	Uncharacterized
ace	Acetylcholinesterase
gli	Gliotactin
nlg and nrl	Neuroligin
nrt	Neurotactin
C+	C strain expanded
C-	C strain contracted
R+	R strain expanded
R-	R strain contracted

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-11185-2.

Supplementary Material 1: Fig. S1. Phylogenetic tree of phase I detoxification enzymes (CYP) of the genus *Spodoptera* constructed using ML.

Supplementary Material 2: Fig. S2. Phylogenetic tree of phase I detoxification enzymes (COE) of the genus *Spodoptera* constructed using ML.

Supplementary Material 3: Fig. S3. Phylogenetic tree of phase II detoxification enzymes (GST) of the genus *Spodoptera* constructed using ML.

Supplementary Material 4: Fig. S4. Phylogenetic tree of phase II detoxification enzymes (MGST) of the genus *Spodoptera* constructed using ML.

Supplementary Material 5: Fig. S5. Phylogenetic tree of phase II detoxification enzymes (UGT) of the genus *Spodoptera* constructed using ML.

Supplementary Material 6: Fig. S6. Phylogenetic tree of phase III detoxification enzymes (ABC) of the genus *Spodoptera* constructed using ML.

Supplementary Material 7: Table S1. Phase I detoxification enzymes (CYP) identified in the genus *Spodoptera* and their nomenclature.

Supplementary Material 8: Table S2. Phase I detoxification enzymes (COE) identified in the genus *Spodoptera* and their nomenclature.

Supplementary Material 9: Table S3. Phase II detoxification enzymes (GST) identified in the genus *Spodoptera* and their nomenclature.

Supplementary Material 10: Table S4. Phase II detoxification enzymes (UGT) identified in the genus *Spodoptera* and their nomenclature.

Supplementary Material 11: Table S5. Phase III detoxification enzymes (ABC) identified in the genus *Spodoptera* and their nomenclature.

Supplementary Material 12: Table S6. Names, structural domains and descriptions of proteins in protein interaction networks.

Supplementary Material 13: Table S7. GO function, KEGG and reactome pathway enrichment in protein interaction networks.

Supplementary Material 14: Table S8. Comparison of amino acid identity between UGT334 and EGT.

Supplementary Material 15: Treefile S1. Phylogenetic tree of phase I detoxification enzymes (CYP) of the genus *Spodoptera* constructed using ML.

Supplementary Material 16: Treefile S2. Phylogenetic tree of phase I detoxification enzymes (COE) of the genus *Spodoptera* constructed using ML.

Supplementary Material 17: Treefile S3. Phylogenetic tree of phase II detoxification enzymes (GST) of the genus *Spodoptera* constructed using ML.

Supplementary Material 18: Treefile S4. Phylogenetic tree of phase II detoxification enzymes (MGST) of the genus *Spodoptera* constructed using ML.

Supplementary Material 19: Treefile S5. Phylogenetic tree of phase II detoxification enzymes (UGT) of the genus *Spodoptera* constructed using ML.

Supplementary Material 20: Treefile S6. Phylogenetic tree of phase III detoxification enzymes (ABC) of the genus *Spodoptera* constructed using ML.

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Authors' contributions

P-HY and SHM: Data curation, Formal analysis, Methodology, Validation, Visualization, Software, Writing—original draft, Writing—review & editing. M-FY: Conceptualization, Visualization, Writing—review & editing. C-XH: Conceptualization, Formal analysis, Methodology, Supervision, Validation, Visualization,

Writing—review & editing. All authors revised the manuscript and approved the final version.

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Data availability
The datasets generated and/or analysed supporting the conclusions of the current study are available in the following repositories:
• Genomic data for *Spodoptera frugiperda*: LepidoDB (http://bipaa.genouest.org/is/lepidodb/spodoptera_frugiperda/).
• Additional genomic datasets: DRYAD (https://datadryad.org/stash/dataset/doi:10.5061/dryad.6wwpzgn5w).
• Phylogenetic relationship data is available from the TimeTree database (http://www.timetree.org/).
All datasets are publicly accessible and detailed in the Methods section of this manuscript."

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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