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The genetic adaptations of fall armyworm *Spodoptera frugiperda* facilitated its rapid global dispersal and invasion

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Abstract

The fall armyworm (*Spodoptera frugiperda*) is a lepidopteran insect pest that causes huge economic losses. This notorious insect pest has rapidly spread over the world in the past few years. However, the mechanisms of rapid dispersal are not well understood. Here, we report a chromosome-level assembled genome of the fall armyworm, named the ZJ-version, using PacBio and Hi-C technology. The sequenced individual was a female collected from the Zhejiang province of China and had high heterozygosity. The assembled genome size of ZJ-version was 486 Mb, containing 361 contigs with an N50 of 1.13 Mb. Hi-C scaffolding further assembled the genome into 31 chromosomes and a portion of W chromosome, representing 97.4% of all contigs and resulted in a chromosome-level genome with scaffold N50 of 16.3 Mb. The sex chromosomes were identified by genome resequencing of a single male pupa and a single female pupa. About 28% of the genome was annotated as repeat sequences, and 22,623 protein-coding genes were identified. Comparative genomics revealed the expansion of the detoxification-associated gene families, chemoreception-associated gene families, nutrition metabolism and transport system gene families in the fall armyworm. Transcriptomic and phylogenetic analyses focused on these gene families revealed the potential roles of the genes in polyphagia and invasion of fall armyworm. The high-quality of the fall armyworm genome provides an important genomic resource for further explorations of the mechanisms of polyphagia and insecticide resistance, as well as for pest management of fall armyworm.

KEYWORDS

chromosome-level genome, comparative genomics, fall armyworm, insecticide resistance, polyphagia

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1 | INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (Lepidoptera, Noctuidae), is a highly invasive noctuid insect pest that causes huge agricultural production and economic losses (Martinelli, Barata, Zucchi, Silva-Filho Mde, & Omoto, 2006). According to the report of Centre for Agriculture and Biosciences International, the fall armyworm has the potential to cause maize losses of approximately 8.3–20.6 million tons per year in Africa (Abrahams et al., 2017). Several features of fall armyworm biology contribute to the outbreak of population growth in newly established regions. These include high fecundity, long adult life span and high spawning rate, with an adult female estimated to produce an average of 1,500 eggs over her typically 10 days lifespan (Prasanna, Huesing, Eddy, & Peschke, 2018). In addition, due to a strong ability to fly, the fall armyworm has the capacity to migrate long distances with air currents, e.g., at least 500 km per generation across Africa (Westbrook, Nagoshi, Meagher, Fleischer, & Jairam, 2016). With a suitable air current, moths have reportedly dispersed to a record distance of 1,600 km in 30 hr (Martinelli et al., 2006).

The fall armyworm survives year-round in the tropical and subtropical areas of the Americas and was also widespread in the northern areas of the United States and as far north as southern Canada (Todd & Poole, 1980) before 2016. In January 2016, an outbreak of this pest occurred in the rainforest zone of South-Western Nigeria of Africa (Goergen, Kumar, Sankung, Togola, & Tamo, 2016). A total of 44 countries had officially reported the invasion of this pest by 2018 (Feldmann, Rieckmann, & Winter, 2019). In May 2018, Sharanabasappa et al. (2018) reported invasions of fall armyworm in various districts of Karnataka state in India for the first time. Subsequent invasions were reported in Yemen, Myanmar, Thailand, Bangladesh, Sri Lanka, and other Asian countries (Ganiger et al., 2018; Li et al., 2019).

By January 2019, the presence of fall armyworm was confirmed in southwest Yunnan province of China (China National Agricultural Technology Extension and Service Center,NATESC, 2019a; Wu, Jiang, & Wu, 2019). Due to fast dispersal rates and available suitable habitats (central, southwest and northern regions in China), by 17 August 2019, the fall armyworm had spread to 1,366 counties (cities and districts) across 24 provinces of China (NATESC, 2019b). Such a rapid spread of fall armyworm poses a serious threat to maize and wheat production in China.

The polyphagous fall armyworm feeds on more than 350 plants in such families as Poaceae, Asteraceae and Fabaceae (Montezano et al., 2018). For many years, the fall armyworm has been known to have two haplotypes, the "rice strain" (R strain) preferring to feed on rice and grasses and "corn strain" (C strain) preferring to feed on corn and sorghum (Pashley, 1988; Pashley, Johnson, & Sparks, 1985). The two strains are morphologically identical, but they significantly differ in composition of sex pheromones, susceptibility to chemical insecticides and transgenic *Bacillus thuringiensis* (Bt) crops, and reproductive behaviours (Adamczyk, Holloway, Leonard, & Graves, 2013; Cruz-Esteban, Rojas, Sanchez-Guillen, Cruz-Lopez, & Malo, 2018; Lima & McNeil, 2009; Schofl, Heckel, & Groot, 2009). A molecular method used to distinguish the two strains is based on the sequence of mitochondrial *Cytochrome Oxidase Subunit I* (*COI*) and strain-specific sites in the fourth exon of the Z-chromosome-linked gene *Triosephosphate isomerase* (*Tpi*) (Juarez et al., 2014; Nagoshi, Goergen, Du Plessis, van den Berg, & Meagher, 2019; Nagoshi et al., 2017).

Application of chemical insecticides and planting transgenic Bt corn have been the main strategies used to control this pest (Carvalho, Omoto, Field, Williamson, & Bass, 2013; Yu, Nguyen, & Abo-Elghar, 2003). Unfortunately, the widespread and indiscriminate use of insecticides and transgenic Bt corn has led to the development of high levels of resistance to these control methods. In the Americas, the fall armyworm has developed resistance to at least 29 insecticidal active ingredients in six mode-of-action groups (Mota-Sanchez & Wise, 2017). In Puerto Rico and Mexico, the fall armyworm has developed field-evolved resistance to chlorpyriphos, permethrin, flubendiamide, chlorantraniliprole, methomyl, and thiodicarb (Gutierrez-Moreno et al., 2019). Furthermore, the fall armyworm has developed resistance to different Bt proteins, such as Cry1F, Cry1Ac and Cry1Ab in Puerto Rico (Storer et al., 2010), Cry1A.105 and Cry1F in the United States (Jakka et al., 2016), Cry1F and Cry1Ab in Brazil (Omoto et al., 2016) and Cry1F Argentina (Chandrasena et al., 2018).

In developing new pest control strategies, it is necessary to understand the genetic information of the fall armyworm. Though several versions of the fall armyworm genome have been reported before (Gouin et al., 2017; Kakumani, Malhotra, Mukherjee, & Bhatnagar, 2014; Nandakumar, Ma, & Khan, 2017), these versions of genome assemblies are of low quality with a scaffold N50 of <700 kb. Recently, three new versions of chromosome-level genome assemblies have been reported in the pre-print journal bioRxiv, but the sequences are not fully released to date (Liu, Lan, et al., 2019; Nam et al., 2019; Zhang et al., 2019). In addition, all of these versions of chromosome-level genomes lack the information of the W chromosome.

To uncover the genetic background of the fall armyworm found in Zhejiang Province of China, we sequenced and assembled a chromosome-level genome of a female pupa collected from a corn field in Zhejiang Province. Henceforth, we refer to this genome assembly as the ZJ-version. The scaffold N50 of this genome is ~16 Mb, making it a high quality and potentially the best quality fall armyworm genome available to date. Furthermore, we assembled a portion of the W chromosome. To the best of our knowledge, this is the first report of W chromosome sequence from the fall armyworm. We also analysed the haplotype of the fall armyworm invading Zhejiang Province and the expanded gene families associated with invasiveness, such as cytochrome P450, gustatory receptor and β-fructofuranosidase. This high-quality fall armyworm genome and the comparative genomic analysis provide new insights into the mechanism of fall armyworm invasion, polyphagia and insecticide resistance.

2 | MATERIALS AND METHODS

2.1 | Insects

Fall armyworms were collected in Dongyang (29.27°N, 120.23°E, Zhejiang province, China), in June 2019 and reared on artificial diets under laboratory conditions of 25°C, 16:8 light/dark photoperiod and relative humidity of 70%–80%.

2.2 | Genome sequencing and *de novo* assembly

We applied the PacBio SMRT platform (Pacific Biosciences) to sequence the genome of the fall armyworm. High-quality genomic DNA was extracted from a female pupa (Table S1) using a DNeasy Blood & Tissue Kit (QIAGEN). The integrity of the DNA was determined with the Agilent 4,200 Bioanalyser (Agilent Technologies). Genomic DNA was sheared using g-Tubes (Covaris), and concentrated with AMPure PB magnetic beads (Pacific Biosciences). The SMRT bell library was constructed using the PacBio SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences). Finally, one SMRT cell was run for genome sequencing. PacBio subreads were initially cleaned using CANU v1.8 (Koren et al., 2017) (https://github.com/marbl/canu) for sequence error correction. The PacBio corrected reads were then assembled by SMARTdenovo (https://github.com/ruanjue/smartdenovo) as described by Istace et al. (2017). The redundans pipeline (https://github.com/lpryszcz/redundans) (Pryszcz & Gabaldón, 2016) was used to remove the redundant contigs from the initial *de novo* assembly genome with the parameters "--identity 0.5, --overlap 0.75", yielding the final assembled genome of the fall armyworm that we named the ZJ-version.

2.3 | Hi-C library preparation

A sixth instar larva was used for Hi-C library preparation (Table S1). The Hi-C library preparation was performed following the protocol published by Shi et al. (2019). After washing diced larval tissue in cooled phosphate buffered saline, crosslinking was performed by incubation at room temperature in a 2% formaldehyde solution for 10 min. The reaction was quenched by 5 min incubation with 2.5 M glycine solution.

For extracting the chromatin, the supernatant was removed and the tissues were grounded in liquid nitrogen. The tissues were resuspended in 25 ml of extraction buffer I (0.4 M sucrose, 10 mM Tris HCl, pH 8.0, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride [PMSF], and 1 μl protease inhibitor, Sigma) and then was filtered through miracloth (Calbiochem). The filtrate was centrifuged at 4,000 rpm at 4°C for 20 min. Next, the supernatant was removed and the pellet was resuspended in 1 ml extraction II buffer (0.25 M sucrose, 10 mM Tris HCl, pH 8.0, 10 mM $MgCl_2$, 1% Triton X-100, 5 mM β-mercaptoethanol, 0.1 mM PMSF, and 1 μl protease inhibitor, Sigma), followed by centrifuging at 18,400 *g* at 4°C for 10 min. Again, the supernatant was removed and the pellet was resuspended in 300 μl extraction buffer III (1.7 M sucrose, 10 mM Tris HCl, pH 8.0, 0.15% Triton X-100, 2 mM $MgCl_2$, 5 mM β-mercaptoethanol, 0.1 mM PMSF, and 1 μl protease inhibitor, Sigma). The solution was centrifuged at 18,400 *g* at 4°C for 10 min.

The chromatin was washed with the following steps. First, removing the supernatant and resuspending the pellet in 500 μl precooling 1× CutSmart buffer, washing it for twice, and then centrifuged at 2,500 *g* for 5 min. Second, the nuclei were washed with 0.5 ml restriction enzyme buffer and transferred to a safe lock tube. Next, the chromatin was solubilized with dilute SDS by incubating at 65°C for 10 min. After quenching the SDS by Triton X-100, the chromatin was digested with 400 units MboI at 37°C on a rocking platform overnight.

Next, the DNA was labelled with biotin-14-dCTP (Invitrogen) and blunt-end ligated with crosslinked fragments. Then, the proximal chromatin DNA was religated by ligation enzyme at 16°C for overnight. The nuclear complexes were reversed crosslinked by incubating with proteinase K (Invitrogen) at 65°C. DNA was purified with phenol chloroform extraction method. Biotin-C was removed from nonligated fragment ends using T4 DNA polymerase (NEB). Fragments was sheared to a size of 100–500 bp by sonication. The fragment ends were repaired by the mixture of T4 DNA polymerase (NEB), T4 polynucleotide kinase (NEB) and Klenow DNA polymerase (NEB). Biotin labelled Hi-C sample were specifically enriched using streptavidin magnetic beads. The fragment ends were subjected to A-tailing by exo-Klenow and Illumina paired-end sequencing adapter were added by ligation. At last, the Hi-C libraries were amplified by 10–12 cycles of PCR amplification and sequenced using Illumina HiSeq platform with 2 × 150 bp reads. Hi-C library preparation and sequencing was performed by Annoraod Gene Technology Co. Ltd.

2.4 | Scaffolding with Hi-C

Hi-C scaffolding was performed according to the pipeline reported in Servant et al. (2015) and Burton et al. (2013). The HIC-PRO v2.7.8 (Servant et al., 2015) pipeline (https://github.com/nservant/HiC-Pro) was used to identify valid read pairs. In this pipeline, each read in the pair is mapped independently and, where ligation sites are detected by exact matching, the 3′ sequence is trimmed from the read and the 5′ portion remapped. The sequence alignments were made using BOWTIE2 v2.2.3 (Langmead & Salzberg, 2012) with the parameters "--very-sensitive -L 20 --score-min L,-0.6,-0.2 --end-to-end --reorder --rg-id BMG --phred33-quals -p 5". The processed mappings were then merged into a single alignment file with valid interaction pairs expected to involve two different restriction fragments. Then the valid interaction pairs were used to build the interaction matrices and we scaled up the primary genome assembly contigs into chromosome-scale scaffolds (hereafter pseudo-chromosomes) with LACHESIS (https://github.com/shendurelab/LACHESIS) (Burton et al., 2013). To access the accuracy of the scaled-up genome assembly, we cut the pseudo-chromosomes

predicted by LACHESIS into bins with 100 kb lengths. Then we constructed a heatmap based on the interaction signals that were revealed by valid mapped read pairs between bins. The matrix was produced by HiC-Pro and then visualized as a heatmap to show the diagonal patches of strong linkages.

2.5 | Transcriptome sequencing and analysis

The transcriptomes of the larva (from first instar to six instar), female pupa, female adult and male adult of fall armyworm (Table S1) were sequenced using the Illumina HiSeq 2000 platform with paired-end libraries. Three biological replicates were obtained for each RNA-Seq sample type. Low-quality bases in the RNA-Seq raw reads were first filtered using TRIMMOMATIC v0.38 (Bolger, Lohse, & Usadel, 2014). The clean reads were then mapped to the genome assembly using HISAT2 v2.1.0 (Kim, Landmead, & Salzberg, 2015) and STRINGTIE v2.0 (Pertea et al., 2015) to obtain putative transcripts. To determine gene expression levels, the RNA-Seq clean reads were mapped to the genome assembly using BOWTIE2 v2.3.5 (Langmead & Salzberg, 2012), and transcript abundances were estimated by RSEM v1.3.1 (Li & Dewey, 2011).

2.6 | Assessment of genome assembly

We used the BUSCO v3.0 (Waterhouse et al., 2018) (Benchmarking Universal Single-Copy Orthologues) software to scan 1,658 universal single-copy orthologous genes selected from insecta_db 9 data sets in genome assembly with default parameters.

2.7 | Genome annotation

We identified repeat sequences and transposable elements (TEs) by both homology-based and *de novo* prediction methods. For *de novo* predictions, REPEATMODELER v1.0.7 was used to construct a *de novo* repeat library with default parameters. For homology-based predictions, REPEATMASKER v4.0.5 (Tarailo-Graovac & Chen, 2009) was used with Repbase library (Bao, Kojima, & Kohany, 2015).

We annotated the protein coding genes by integrating the evidence of *de novo*, homology-based and RNA-Seq-based annotations. First, AUGUSTUS v2.5.5 (Stanke, Diekhans, Baertsch, & Haussler, 2008) and SNAP v2013-11-29 (Korf, 2004) were used to generate the *de novo* annotation with internal gene models. Then, EXONERATE v2.2.0 (Slater & Birney, 2005) and GENOMETHREADER v1.7.1 (Gremme, Brendel, Sparks, & Kurtz, 2005) were used to align the proteins obtained from NCBI invertebrate RefSeq (https://www.ncbi.nlm.nih.gov/refseq/) to the genome assembly with default parameters. The transcripts of the fall armyworm were obtained by HISAT2 v2.1.0 (Kim et al., 2015) and STRINGTIE v2.0 (Pertea et al., 2015) pipeline with default parameters. We next integrated these three types of evidences with different weights (the weight for *de novo* annotation is "1", for homology-based annotation is "5", for RNA-Seq-based annotation is "10") for each by EVidenceModeler (EVM) (Haas et al., 2008) to obtain the official gene set (OGS). Gene Ontology (GO) analysis was carried out using the software BLAST2GO v5.2 (Conesa et al., 2005). We further mapped these genes to data from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using the BLASTKOALA v2.2 (Kanehisa, Sato, & Morishima, 2016) online service.

2.8 | Phylogenetic reconstruction

Proteins sequences of 22 insect species were clustered using the ORTHOMCL v2.0.9 pipeline with default parameters (Li, Stoeckert, & Roos, 2003). These accessions were: *S. frugiperda* (this study), *Spodoptera litura* (GCA_002706865.2, from NCBI), *Trichoplusia ni* (GCF_003590095.1, from NCBI), *Helicoverpa armigera* (GCF_002156985.1, from NCBI), *Heliothis virescens* (GCA_002382865.1, from NCBI), *Bombyx mori* (GCF_000151625.1, from NCBI), *Antheraea yamamai* (from GigaDB, http://gigadb.org/dataset/100382), *Manduca sexta* (GCF_000262585.1, from NCBI), *Operophtera brumata* (GCA_001266575.1, from NCBI), *Cydia pomonella* (from InsectBase; Yin et al., 2016), *Danaus plexippus* (GCA_000235995.2, from NCBI), *Papilio xuthus* (GCF_000836235.1, from NCBI), *Plutella xylostella* (GCF_000330985.1, from NCBI), *Stenopsyche tienmushanensis* (from GigaDB, http://gigadb.org/dataset/100538), *Drosophila melanogaster* (GCF_000001215.4, from NCBI), *Anopheles gambiae* (from VectorBase, https://www.vectorbase.org/organisms/anopheles-gambiae), *Tribolium castaneum* (GCF_000002335.3, from NCBI), *Leptinotarsa decemlineata* (GCF_000500325.1, from NCBI), *Apis mellifera* (GCF_003254395.2, from NCBI), *Nasonia vitripennis* (OGS2, from Ensemble Database, ftp://ftp.ensemblgenomes.org/pub/metazoa/release-38/fasta/nasonia_vitripennis/dna/), *Melanaphis sacchari* (GCF_002803265.2, from NCBI) and *Rhodnius prolixus* (from VectorBase, https://www.vectorbase.org/organisms/rhodnius-prolixus).

In total, 328 single-copy genes were obtained from OrthoMCL results and were used for phylogeny reconstruction. First, the protein sequences of each gene family were independently aligned by MAFFT v7 (Katoh & Standley, 2013). Then, TRIMAL v1.2 (Capella-Gutierrez, Silla-Martinez, & Gabaldon, 2009) was used to clean each alignment and extract the conserved block. Next, we concatenated all single-copy genes to create one super gene for each species. We used ModelFinder (Kalyaanamoorthy, Minh, Wong, von Haeseler, & Jermiin, 2017) to select the best model. IQ-TREE v1.5.5 (Nguyen, Schmidt, von Haeseler, & Minh, 2015) was used to construct the phylogenetic tree using the LG + F + I + G4 model and 1,000 bootstrap replicates. To estimate the divergence time of the fall armyworm, we applied three calibration points based on fossil records in Paleobiology Database (www.paleobiology.org): (a) stem Trichoptera (*Phryganea solitaria*) at 311.45–314.6 mya; (b) stem Lepidoptera (fossil unnamed) at 201.3–208.5 mya; and (c) stem Noctuoidea (*Noctuites incertissima*) at 28.1–33.9 mya. The divergence time was estimated by using MCMCtree in PAML v4.9e (Yang, 2007) with the topology of

these insects we built above. The tree was visualized using FIGTREE v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

2.9 | Whole-genome synteny

Whole-genome synteny between *S. frugiperda*, *S. litura*, and *B. mori* were estimated using SATSUMA v3.1.0 (Grabherr et al., 2010), a package of SPINES with default parameters (https://www.broadinstitute.org/genome-sequencing-and-analysis/spines). Synteny blocks were plotted across chromosomes using CIRCOS v0.69-9 (Krzywinski et al., 2009).

2.10 | Gene family expansion and contraction

We used CAFÉ v4.2.1 (De Bie, Cristianini, Demuth, & Hahn, 2006) to perform a gene family expansion and contraction analysis. The protein sequences from twenty-two insects were aligned to the TREEFAM v9 (Schreiber, Patricio, Muffato, Pignatelli, & Bateman, 2014) database to obtain the TreeFam ID for each protein. The TREEFAM v9 results and a tree with estimated divergence time were used as inputs of CAFÉ. We used a criterion of $p < 0.05$ for significantly changed gene families.

2.11 | Gene family analysis

For the P450 gene family, we first downloaded reference protein sequences of Lepidoptera P450s from NCBI GenBank and manually confirmed these sequences to obtain a clean reference sequences for Lepidoptera P450s. TBLASTN (BLAST v2.9.0) was used to search P450 candidate sequences in the fall armyworm genome assembly (*E*-value < 1E−5). GENEWISE v2.4.1 (Madeira et al., 2019) and EXONERATE v2.2.0 (Slater & Birney, 2005) were used to define the gene structure. And we also confirmed the P450 candidate sequences using HMMER v3.2.1 (Potter et al., 2018) against sequences from the Pfam database (Pfam domain PF00067, *E*-value < 1E−5) (Finn et al., 2014). The fall armyworm P450 sequences were compared to P450 genes of *S. litura* and *B. mori* by phylogenetic studies for name assignment. RSEM v1.3.0 (Li & Dewey, 2011) was used for gene expression level (FPKM) calculation. In this study, if the expression level of a given gene with FPKM > 0.4 in all three RNA-Seq repetitions of a given development stage, this gene was regarded as expressed in this development stage.

For the gustatory receptor (GR) gene family, we searched GR candidate sequences in the fall armyworm genome assembly using TBLASTN (*E*-value < 1E−5) (BLAST v2.9.0) with a set of GR reference sequences obtained from NCBI GenBank. Then, GENEWISE v2.4.1 (Madeira et al., 2019) and EXONERATE v2.2.0 (Slater & Birney, 2005) were used to define the gene structure. For GR subfamily annotation, we compared the fall armyworm GR sequences with GRs from *S. litura* and *B. mori* by phylogenetic studies. RSEM v1.3.0 (Li & Dewey, 2011) was used for GR gene expression level (FPKM) calculation.

For other gene families, including glutathione-S-transferases (GSTs), carboxylesterases (COEs), ATP-binding cassette transporters (ABC transporters), olfactory receptors (ORs), ionotropic receptors (IRs), odorant-binding proteins (OBPs), chemosensory proteins (CSPs), and β-fructofuranosidase (β-FFase), we identified each gene family's genes using a two-step method in OGS. First, we collected the reference protein sequences of each gene family from NCBI GenBank. And the reference protein sequences were further manually confirmed. Then, we used BLASTP to determine candidate sequences from OGS of each insect (*E*-value < 1E−5). Next, HMMER was used to align the candidate sequences to the Pfam database (*E*-value < 1E−5) (Finn et al., 2014).

For the phylogenetic analysis of gene families, we aligned protein sequences of each gene family using MAFFT v7 (Katoh & Standley, 2013) and filtered sequences with TRIMAL v1.2 (Capella-Gutierrez et al., 2009) to obtain the conserved blocks. IQ-TREE v1.5.5 (Nguyen et al., 2015) was used to construct the phylogenetic tree with the best model estimated by ModelFinder (1,000 ultrafast bootstrap approximation replicates) (Kalyaanamoorthy et al., 2017). The tree was visualized using FIGTREE v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). An R package RIdeogram v.0.1.1 was used to map and visualize genes in chromosomes (Hao et al., 2019).

2.12 | Determination of the fall armyworm strain in Zhejiang Province

The *Tpi* gene was used as a marker to identify the strain of fall armyworm that had invaded the Zhejiang province of China. We identified the *Tpi* gene and determined the strain from the Zhejiang population using sites in the fourth exon of *Tpi* (TpiE4-165, TpiE4-168 and TpiE4-183).

2.13 | Sex chromosomes

To identify the sex chromosomes (Z and W chromosomes) in fall armyworm, one female pupa and one male pupa were resequenced using Illumina HiSeq platforms to obtain an approximate 40× coverage. The paired-end sequencing data of the female pupa was used as an input to JELLYFISH v2.2.0 (Marcais & Kingsford, 2011) with k-mer length = 17 and genomescope (https://github.com/schatzlab/genomescope; Vurture et al., 2017) for assessment of genomic heterozygosity and genomic size. Normalized coverage levels of sequence reads from the Z chromosome in males should be twice that of females. In contrast, males do not have any DNA contribution from the W chromosome, while the autosomes should have equal coverage between males and females. Thus, a difference in sequencing coverage ratio is expected for both Z and W chromosomes between sexes, but not autosomes and this difference can be used to identify sex-linked scaffolds. After filtering with FQTOOLS v0.1.8 (Droop, 2016), genome

TABLE 1 Comparison of fall armyworm genome assemblies of this and previous studies

	ZJ-version (this study)	Nam et al. (2019)	Zhang et al. (2019)	Liu, Lan, et al. (2019)		Gouin et al. (2017)		Nandakumar et al. (2017)	Kakumani et al. (2014)
		C strain		SFynMstLFR	SFynFMstLFR	C strain	R strain	*Sf*9	*Sf*21
Sequencing info									
DNA source	Single female pupa	Fourth instar male larvae	Single male moth	Single male adult	Single female adult	Fourth instar male larvae	Single male larva	*Sf*9 cell line	*Sf*21 cell line
Assembly approach	PacBio + Hi-C	PacBio + Illumina +Hi-C	PacBio + Illumina +Hi-C	MGISEQ + Hi-C	MGISEQ + Hi-C	Illumina	Illumina	PacBio	Illumina
Genome assembly									
Assembly level	Chromosomes (30A + Z + W)	Chromosomes (30A + Z)	Chromosomes (30A + Z)	Chromosomes (30A + Z)	Chromosomes (30A + Z)	Scaffolds	Scaffolds	Scaffolds	Scaffolds
Genome size (Mb)	486.3	384.46	393.25	542.4	530.8	437.9	371.0	514.2	358.0
Number of contigs	618	-	777	–	–	–	–	2,844	97,607
Number of scaffolds	93	125	311	–	–	41,562	29,127	2,396	37,235
Gaps number	525	–	–	–	–	13,694	3,818	2,635	95,454
Gap length (kb)	53	346	–	37,947	35,693	11,378	131	891	27,685
Quality assessment									
Contig N50 (kb)	1,130.0	–	5,606.9	92.0	125.0	21.6	25.4	516.1	7.8
Scaffold N50 (kb)	16,346.9	13,151.2	13,317.1	14,162.8	14,883.7	52.7	28.5	601.1	53.7
BUSCO genes (%)	93.1	96.6	98.2	95.0	94.5	88.6	89.3	90.8	–
Genomic features									
Protein-coding genes	22,623	–	23,281	22,201	–	21,700	26,329	25,699	11,595
Repeat (%)	28.0	–	27.2	28.2	–	29.2	29.1	28.1	20.3
SINEs (%)	0.7	–	1.0	–	–	12.5	12.9	0.7	–
LINEs (%)	9.1	–	8.7	–	–	1.9	1.7	10.7	0.1
LTR elements (%)	0.3	–	1.4	–	–	0.08	0.07	1.1	0.09
DNA elements (%)	1.7	–	2.7	–	–	0.3	0.3	1.7	0.03
G + C (%)	36.4	–	36.4	36.5	36.6	35.1	36.1	36.5	24.3
Annotation									
Genes with GO terms	13,044	–	–	–	–	13,369	9,261	15,623	5,713
Genes with KEGG annotations	7,818	–	–	16,072	–	–	–	9,213	4,220

resequencing reads were aligned to the fall armyworm genome assembly using BOWTIE2 v2.3.5 (Langmead & Salzberg, 2012) with default parameters. Analysis and visualization of the log2 of the male: female (M:F) coverage ratio were performed using the R package changepoint v2.2.2 (https://CRAN.R-project.org/package=changepoint).

2.14 | Positive selection analysis

All 5,410 single-copy genes shared by four Noctuidae insects, *S. frugiperda*, *S. litura*, *T. ni* and *H. armigera* were used for positive selection analysis. Protein sequences of each single-copy gene family were aligned using MAFFT v7 (Katoh & Standley, 2013), and then the protein alignments were converted to their corresponding nucleotide alignments by the Perl script PAL2NAL v14 (Suyama, Torrents, & Bork, 2006). The dN/dS ratio was estimated for each homologous cluster using the CodeML program in the PAML v4.9e package (branch-site model) (Yang, 2007). We calculated the significances of obtained positive-selected genes using the Chi-square test with a false discovery rate (FDR) cutoff of 0.05.

2.15 | Enrichment analysis

The GO and KEGG enrichment analyses were conducted using OMICSHARE CLOUDTOOLS under this tool's default instructions (http://www.omicshare.com/).

3 | RESULTS AND DISCUSSION

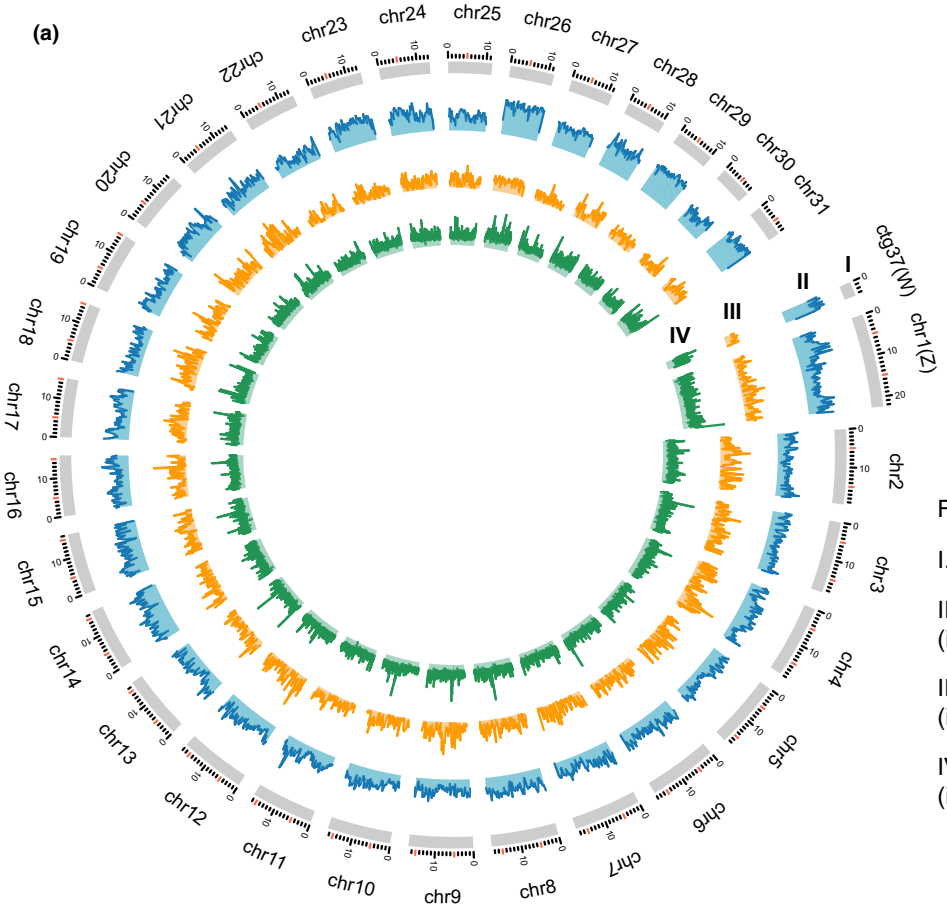
3.1 | Chromosome-level genome assembly of fall armyworm

A female pupa of the fall armyworm was used for genome sequencing by PacBio long-read technology, yielding ~126 Gb PacBio subreads. The PacBio reads were self-corrected using CANU v1.8 (Koren et al., 2017) and finally assembled into 361 contigs using SMARTdenovo (Tables S1–S3, see Methods) (Istace et al., 2017). There were 194 contigs (126 Mb) identified as representing allelic variants of sequence already present in the assembly and these were removed. We named this genome assembly the ZJ-version. The assembled genome size was 486 Mb with a contig N50 of 1.13 Mb. Surprisingly, the assembled genome size was 155 Mb larger than the genome size estimated by 17-mer analysis. The k-mer analysis showed that the fall armyworm has high heterozygosity of 3.45% (Figure S1). An inflated assembly size relative to k-mer based estimates has also been observed in other highly heterozygous species assemblies such as *Anchorythroculter nigrocauda*, *Pyrocoelia pectoralis*, and *Oncopeltus fasciatus* (Fu et al., 2017; Panfilio et al., 2019; Zhang et al., 2020). Previously released genomes indicated the genome size of the fall armyworm ranges from 358 Mb to 542 Mb (Table 1). There is also a big difference between the genome sizes of two cell lines derived from pupal ovaries of the fall armyworm (Table 1) (Kakumani et al., 2014; Nandakumar et al., 2017). The genome size variation might be due to the following reasons: (a) different sequencing technologies, sequencing depth, and assembly approaches; and (b) fall armyworm samples are from different areas/habitats. It has been reported that variable genome size of different strains within the same species may be a result of the amplification, deletion and divergence of repetitive sequences; colonization of new environments; variation of environmentally-dependent life history traits (Ellis et al., 2014; Nardon et al., 2005). Further study is needed to determine the reason of the genome size variation in the different strains of fall armyworm.

According to our Hi-C interaction information, we cut the primary assembly into 618 contigs, then anchored 556 (97.4% in length) contigs to 31 chromosomes (30 autosomes and Z chromosome) (Figure 1a, Tables S4–S7, and Figure S2). Hi-C correction and scaffolding did not change the genome size or contig N50, but increased the scaffold N50 to 16.3 Mb, which was much higher than any other published genome of the fall armyworm (Table 1). The length of N50 for both contigs and scaffolds were much longer than those of other published Noctuidae insects including *T. ni* (Chen et al., 2019), *H. armigera* (Pearce et al., 2017) and *S. litura* (Cheng et al., 2017) (Table S8).

The ZJ-version genome assembly had only 525 gaps and the gap lengths were estimated to be 53 kb, suggesting that the ZJ-version genome assembly was highly complete (Table 1). BUSCO analysis indicated that 93.1% complete genes (1,658 universal single-copy orthologous genes of insects) exist in the ZJ-version genome assembly (Table 1), and the fragmented BUSCOs (1.0%) was apparently less than that in Liu, Lan, et al. (2019) (2.8% in male and 3.1% in female) (Table S5). The complete and duplicated BUSCO component of the ZJ-version genome was 20.3%, which was higher than that in Liu, Lan, et al. (2019) (9.8% in male and 7.8% in female), and also higher than that in Nam et al. (2019) (1.7%), suggesting that the potential allelic duplication might be present in the ZJ-version genome. Taken together, these results suggest that we obtained a robust fall armyworm genome assembly will provide a solid foundation for future analyses.

Using the genome resequencing data of a single male pupa and a single female pupa, we calculated the sequencing coverage of each scaffold and identified the Z chromosome and a portion of the W chromosome (Figure 1b, Table S9, see Methods). The largest super-scaffold (Chr1) yielded two-fold greater male coverage, as expected for the Z chromosome. Although we failed to obtain an intact W chromosome using Hi-C scaffolding, we have identified 4.7 Mb W-linked sequences in the unanchored contigs, including a long W-linked contig (ctg37, contig 37) of a length of 3.5 Mb (Figure 1b, Table S9). Because the Lepidopteran W chromosome is enriched in repeat sequences, it is difficult to assemble a complete W chromosome with present sequencing and assembly methods (Sahara, Yoshido, & Traut, 2012). Although a number of chromosome-level genomes of Lepidoptera insects have been released, the W chromosome has only been reported from *C. pomonella* with a length of about 5 Mb (Wan et al., 2019), as well as from



From outer to inner circles:

I. 32 chromosomes at the Mb scale

II. repeat density across the genome (in 0.1 Mb non-overlapping windows)

III. gene density across the genome (in 0.1 Mb non-overlapping windows)

IV. GC content across the genome (in 0.1 Mb non-overlapping windows)

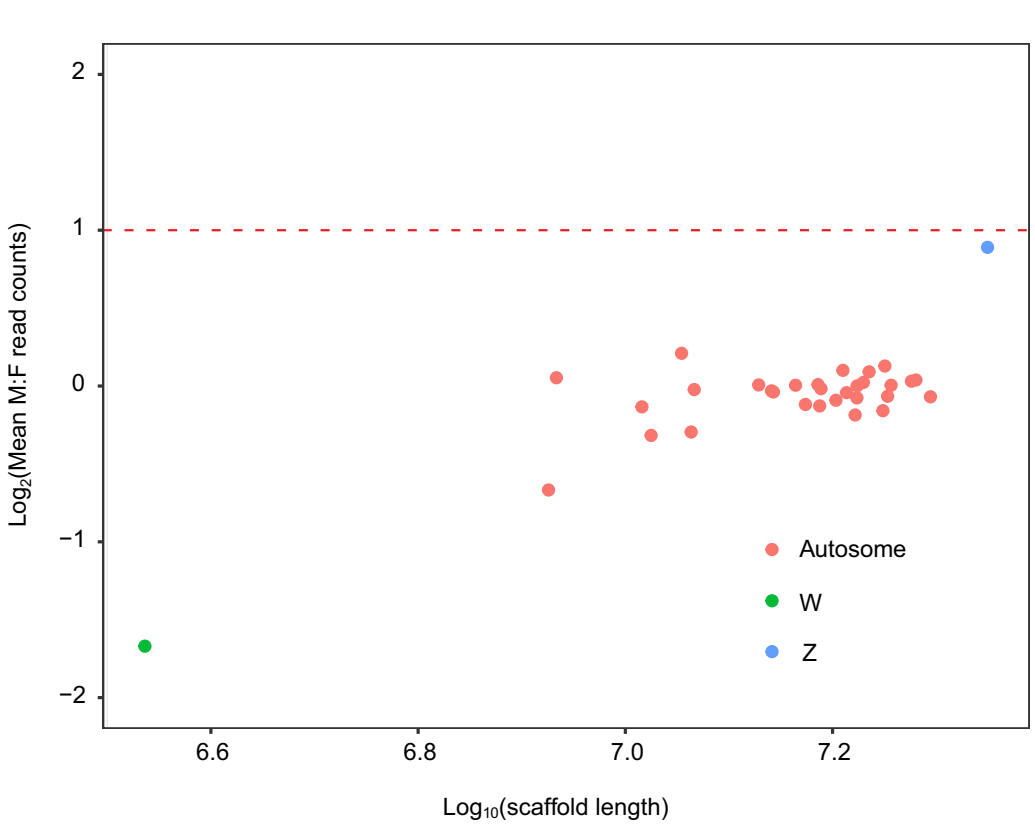


FIGURE 1 Chromosome-level genome assembly of the ZJ-version fall armyworm. (a) Circos plot showing the genomic landscape of the 32 fall armyworm chromosomes. From outer to inner circles: I, 32 chromosomes at the Mb scale; II and III, repeat density (blue) and gene density (yellow) across the genome, respectively, drawn in 0.1 Mb nonoverlapping windows; IV, GC contents (green) across the genome, drawn in 0.1 Mb nonoverlapping windows. (b) Male: female coverage ratios for each chromosome. Each point represents a single chromosome. The dotted red line shows the expectation for the Z chromosome [Colour figure can be viewed at wileyonlinelibrary.com]

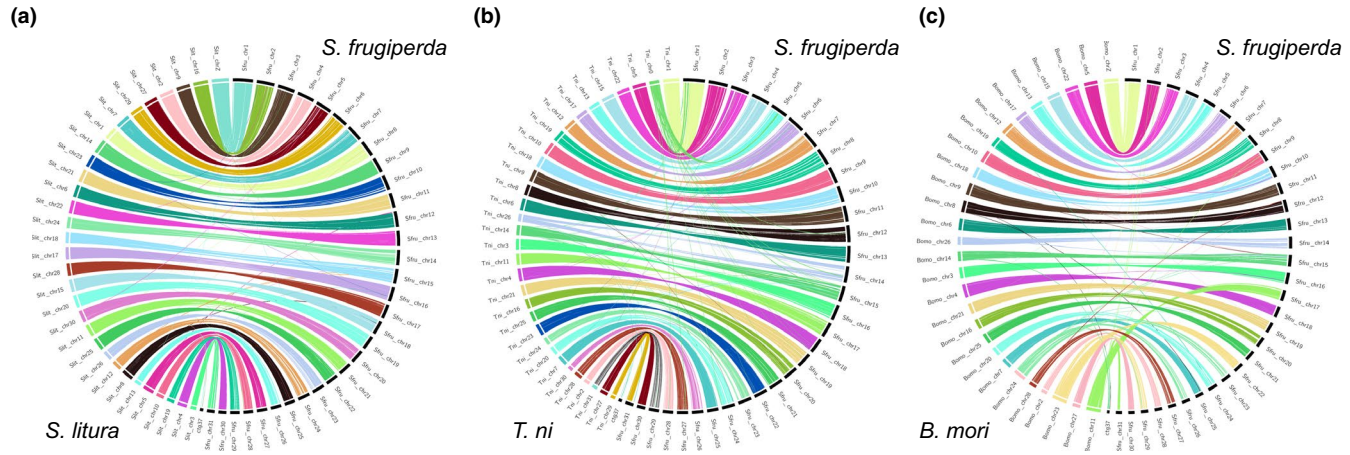


FIGURE 2 Whole-genome synteny between *S. frugiperda* and *S. litura*, *T. ni* and *B. mori*. (a) The fall armyworm genome shares high synteny with *S. litura*, (b) *T. ni*. (c) Three fusion events were founded in autosomes between the fall armyworm and silkworm, Chr17 and Chr31, Chr22 and Chr29, and Chr23 and Chr26 in the fall armyworm were fused to Chr11, Chr23, and Chr24 in the silkworm, respectively. Synteny analysis was carried out using Satsuma and viewed with Circos v 0.69 [Colour figure can be viewed at wileyonlinelibrary.com]

the *T. ni* Hi5 germ cell line (Fu et al., 2018). Here, we report the first partial W chromosome for Noctuidae insects.

The fall armyworm genome shares high synteny with other Lepidopteran insect genomes showing a strong evidence for genome conservation at the chromosome level in Noctuidae insects (Figure 2a,b, Table S10). The syntenic relationship between the silkworm and fall armyworm revealed three fusion events in autosomes: Chr17 and Chr31, Chr22 and Chr29, and Chr23 and Chr26 in the fall armyworm were fused to Chr11, Chr23, and Chr24 in the silkworm, respectively (Figure 2c, Table S10).

3.2 | Genome annotation

In total, 28% of the ZJ-version fall armyworm genome was annotated as repeat sequences (Table 1). Although the total genome size varies between different *S. frugiperda* genome assemblies, the proportions of repeat sequences were similar (Table 1). The content of repeat sequences in the fall armyworm were larger than that in *T. ni* (Fu et al., 2018) and in *H. armigera* (Pearce et al., 2017), but less than that in *S. litura* (Cheng et al., 2017) (Table S8). After masking these repeat sequences, the EVidenceModeler (EVM) pipeline (Haas et al., 2008) was used to predict protein-coding genes by integrating the evidence of protein homology, *de novo* predictions, and RNA-Seq transcripts (first instar to six instar larvae, female pupae, male and female adult) (see Methods). In total, 22,623 protein-coding genes were annotated in the ZJ-version fall armyworm genome (Table 1), 14,123 (62.4%) of which were detected in at least one sample of RNA-Seq data. The number of protein-coding genes in the fall armyworm is the largest set of genes in the published Noctuidae insect genomes (Table S8). Of these annotated genes, 13,044 (57.7%) genes have GO terms and 7,818 (34.6%) genes have homology in the KEGG database (Table 1). The number of annotated protein-coding genes in the ZJ-version fall armyworm genome was similar to the genes identified in five previously published versions of the genome, but more than that in the genome of *Sf*21 cell line. Furthermore, we manually annotated several gene families associated with insect adaptation, including 169 P450s, 59 GSTs, 98 COEs, 79 ABC transporters, 70 ORs, 221 GRs, 41 IRs, 35 OBPs, and 29 CSPs (able S11).

3.3 | The Zhejiang Province fall armyworm is the C strain

The Z chromosome-linked gene *Tpi* is commonly used to identify the strain of fall armyworm (Nagoshi, 2010). The strain-specific sites in the fourth exon of *Tpi* include the sites E4-165, E4-168 and E4-183 (Nagoshi et al., 2019). Based on these sites, we determined that the strain of fall armyworm indicated by the ZJ-version genome is the C strain, which is the same strain found in the Yunnan population (Figure S3) (Liu, Lan, et al., 2019; Liu, Xiao, et al., 2019; Zhang et al., 2019).

3.4 | Gene orthologues and comparative genomic analysis

Comparative genomics analysis was carried out using 22 insect genomes covering six insect orders (Lepidoptera, Trichoptera, Diptera,

Coleoptera, Hymenoptera, and Hemiptera). A phylogenetic tree was constructed using 238 single-copy genes (Figure 3). In addition, 3,076 N:N:N genes, 6,160 Lepidoptera-specific genes, and 2,608 Noctuidae-specific genes were identified (Figure 3, Table S12). Compared to that of four other moth species in Noctuidae, the fall armyworm has the largest number of Noctuidae orthologous genes, species-specific genes and species-specific duplicated genes, 1,646 species-specific duplicated genes were identified (Table S12) and 494 were found to be tandemly duplicated on the chromosomes (Figure S4), suggesting that gene expansion might have occurred in the fall armyworm genome. Based on our phylogenetic tree, the fall armyworm and *S. litura* may have diverged from their common ancestor approximately 9.8 Mya ago (Figure 3).

The gene family evolution analysis indicated that the fall armyworm genome displayed 774 expanded and 1,048 contracted gene families compared with gene families of the common ancestor of fall armyworm and *S. litura* (Figure 3). The common ancestor of Noctuidae species showed 449 expanded and 288 contracted gene families compared to that of the common ancestor of Noctuidae species and *O. brumata*. Notably, Noctuidae expanded gene families were enriched in nutrition metabolism pathways, including protein digestion and absorption (ko04974, $p = 1.269365 \times 10^{-19}$, Hypergeometric test, FDR-adjusted), glycerolipid metabolism (ko00561, $p = 2.63951 \times 10^{-18}$), and fructose and mannose metabolism (ko00051, $p = 3.279737 \times 10^{-9}$) (Tables S13–S14). In addition, the fall armyworm expanded gene families were enriched not only in nutrition metabolism but also in transport system, such as ABC transporters (ko02010, $p = 3.012781 \times 10^{-3}$) (Tables S15–S16). Noctuidae diverged from the Bombycoidae superfamily *ca.* 94 million years ago (Wahlberg, Wheat, & Pena, 2013), and most of the pests in Noctuidae are polyphagous, while the silkworm in Bombycoidae is a monophagous species. For the polyphagous fall armyworm, the number of host plants is as much as 353 species among 76 families (Montezano et al., 2018). The expansion of nutrition metabolism and transport system genes might facilitate the absorption of nutrients from different plant hosts and the detoxification of natural xenobiotics from plants. We suspect that the expansion of these genes may have facilitated the high invasion of fall armyworm.

Based on orthologous gene annotation by OrthoMCL across four Noctuidae insects (*S. frugiperda*, *S. litura*, *T. ni* and *H. armigera*), 5,410 single-copy genes were used for positive selection analyses. As a result, we identified 835 positive selected genes in the fall armyworm using the Branch-site model in PAML, including

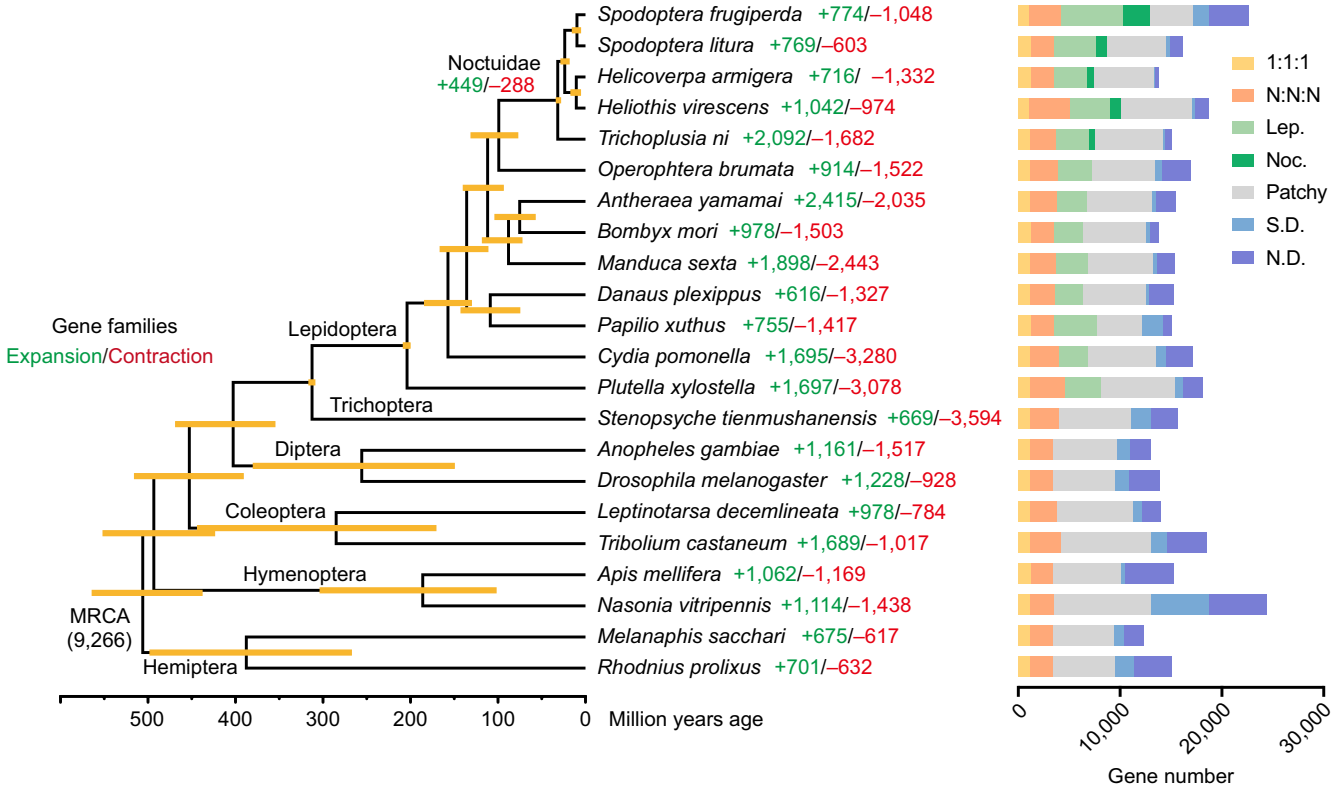


FIGURE 3 Genome evolution of fall armyworm. The phylogeny tree of 22 insects covering six insect orders was calculated by maximum-likelihood analyses using 238 single-copy proteins. Bootstrap values based on 1,000 replicates are equal to 100 for each node. Divergence times calculated by MCMCtree are indicated by yellow bars at the internodes, and the bars indicate the 95% confidence intervals of the divergence time. The number of expanded gene families (green) and contracted gene families (red) obtained from TREEFAM and CAFÉ software are shown on the branches. Bars are subdivided to represent different types of orthology across 22 insects: "1:1:1" indicates universal single-copy genes present in all species, absence and/or duplication in, at most, one genome is included; "N:N:N" indicates other universal genes; "Lep." indicates common unique genes in Lepidoptera; "Noc." indicates common unique genes in the family Noctuidae; "S.D." indicates species-specific duplication; "N.D." indicates species-specific genes; "Patchy" includes all remaining genes [Colour figure can be viewed at wileyonlinelibrary.com]

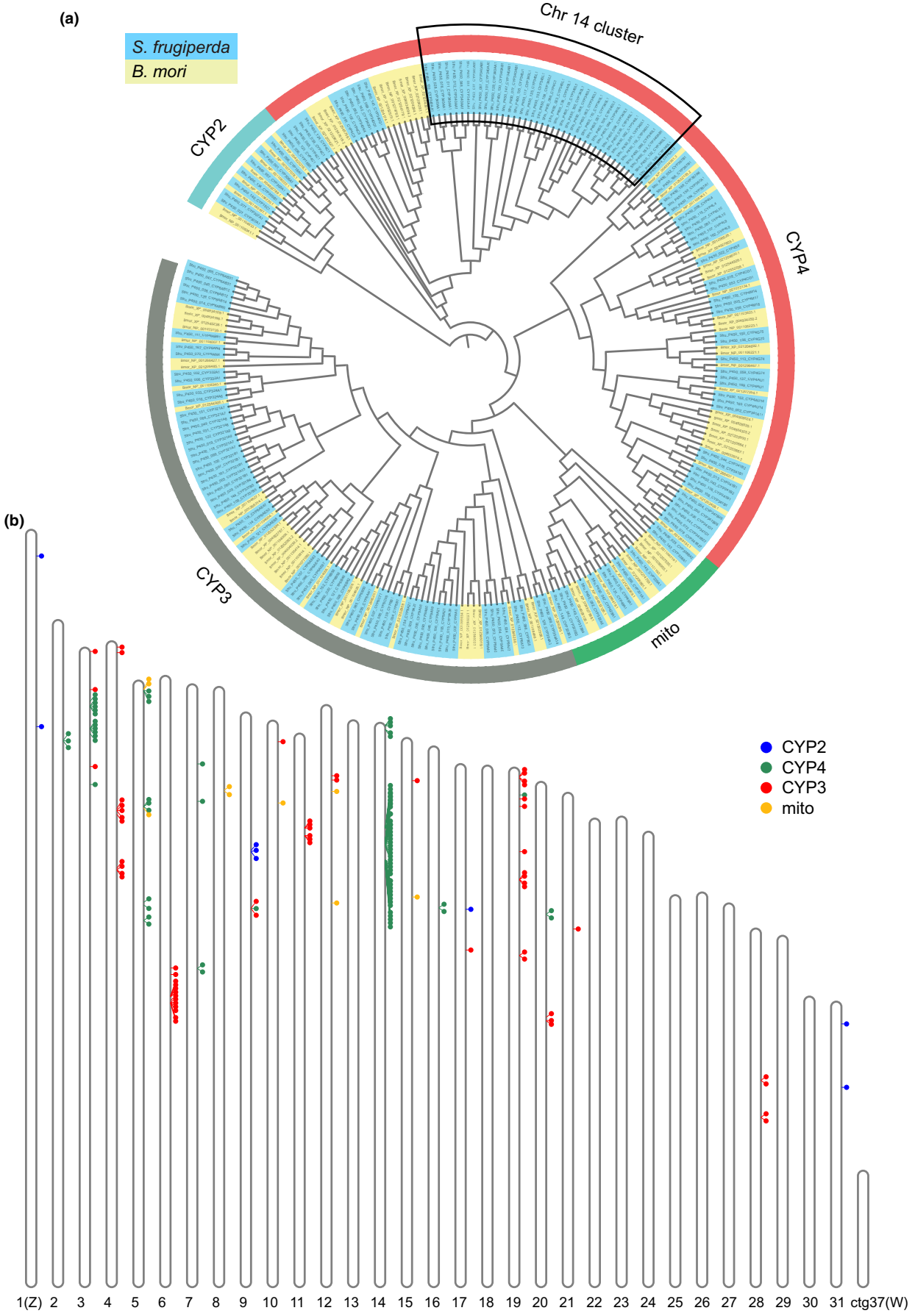


FIGURE 4 Cytochrome P450s in fall armyworm. (a) Maximum-likelihood phylogenetic analysis of P450 genes in fall armyworm and silkworm. The largest *CYP340* cluster on Chr14 is shown. (b) Distribution of 166 P450 genes in the fall armyworm chromosomes [Colour figure can be viewed at wileyonlinelibrary.com]

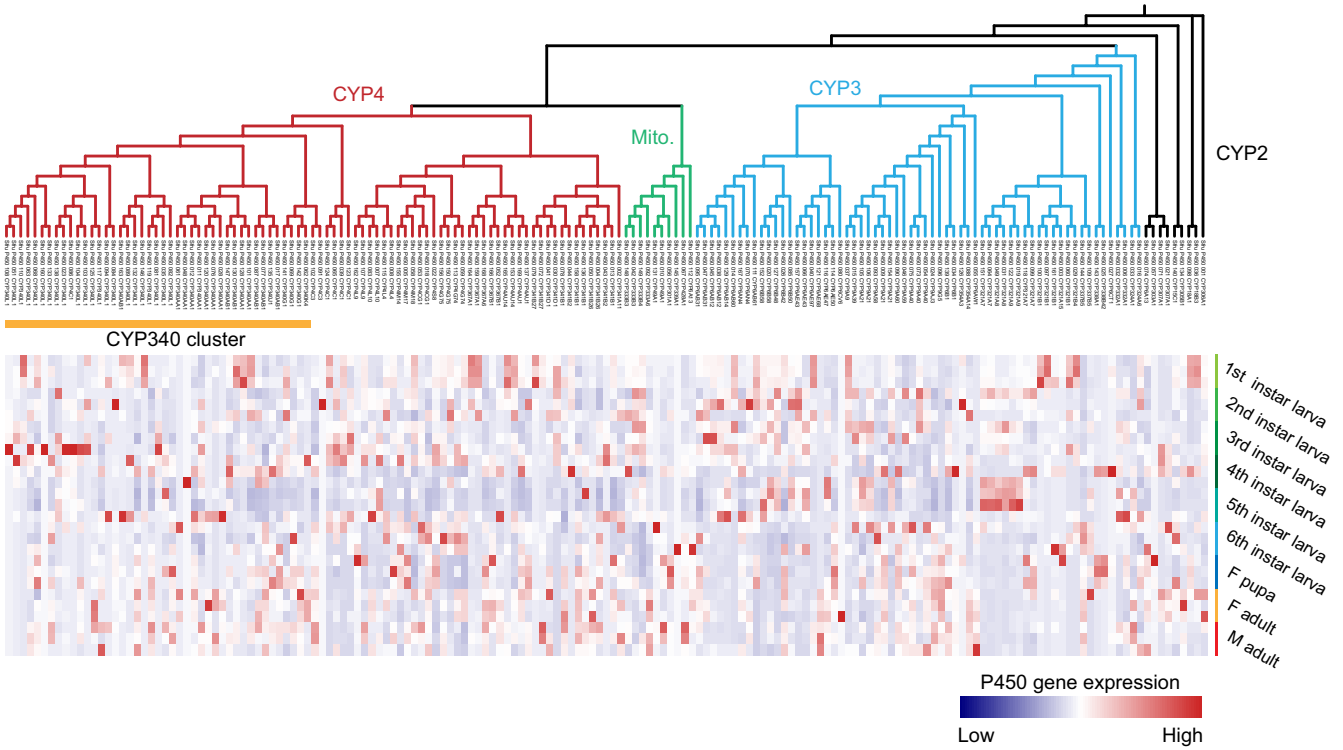


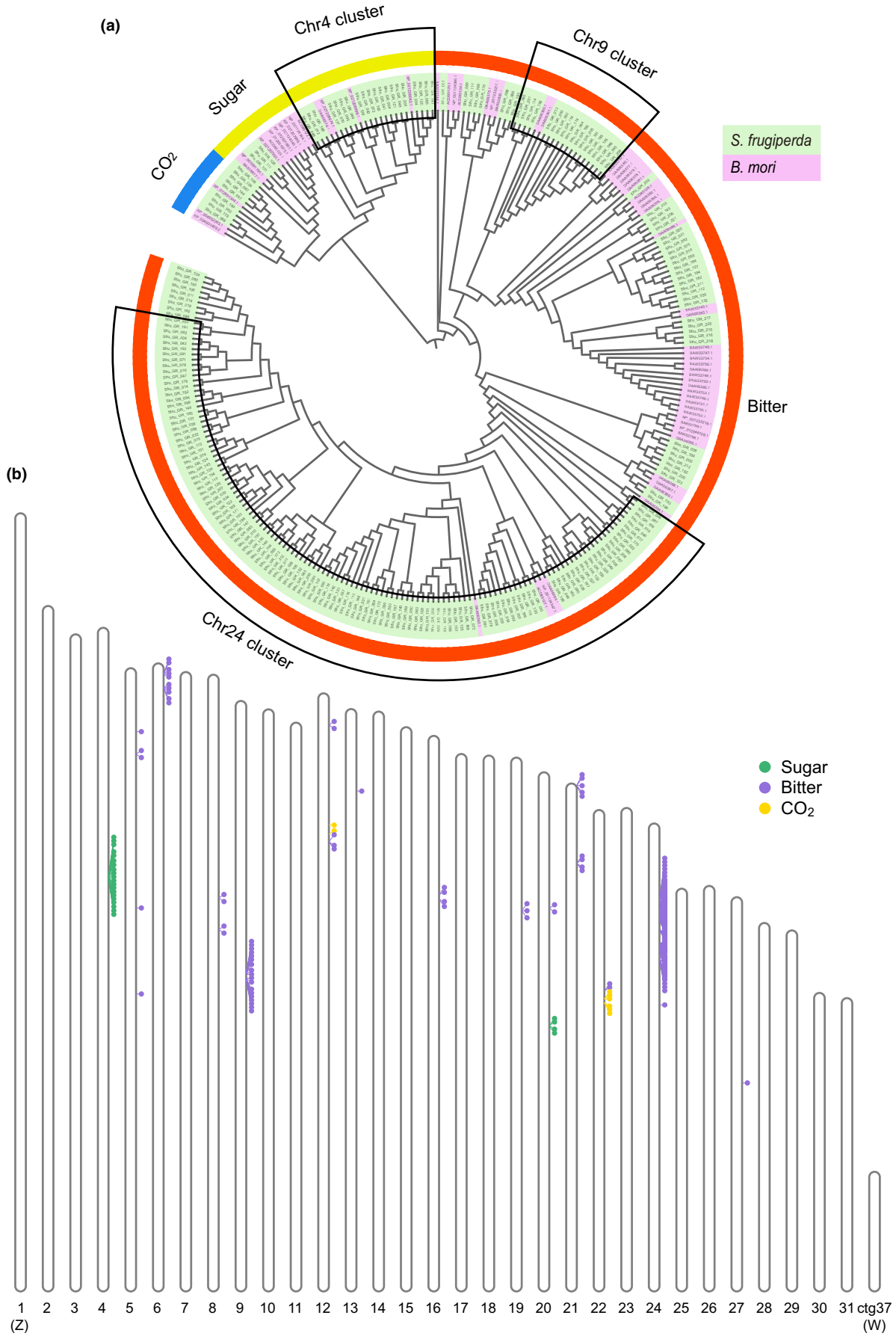
FIGURE 5 Expression profiles of fall armyworm P450 genes from individuals at different developmental stages. F, female; M, male [Colour figure can be viewed at wileyonlinelibrary.com]

the GRs (p < .05, FDR-adjusted, Table S17). The GO and KEGG enrichment analyses indicated that the significant terms and pathways were involved in metamorphosis (GO: 0,007,552, $p = 1.813556 \times 10^{-4}$), instar larval or pupae development (GO: 0,002,165, $p = 1.824223 \times 10^{-3}$), glycerophospholipid metabolism (ko00564, p = .01394475) and sphingolipid metabolism (ko00600, p = .01753068) (Tables S18–S19).

3.5 | The expansion and widespread expression of cytochrome P450 gene family in fall armyworm

Insect pests, especially the polyphagous insects, can adapt to tolerate the plant toxic defense chemicals induced after the insect feeds on the host (Gatehouse, 2002). These insects have evolved a strong detoxification system in response to the plant defense system, such as overproducing detoxification enzymes to metabolize the toxins (Despres, David, & Gallet, 2007) and enhancing the excretion activity (Dermauw & Van Leeuwen, 2014). Using comparative genomics and a BAC library, Giraudo et al. (2015) identified 42 P450 genes in the fall armyworm. Their allelochemicals and xenobiotics inducing experiment indicated 29 P450 genes were induced by plant secondary metabolites, insecticides and model inducers. We also have reported the expansion of the P450 gene family using the genome of the *Sf*9 cell line (Mei, Yang, Ye, Xiao, & Li, 2019). Here, with the ZJ-version chromosome-level genome, we predicted 169 cytochrome P450 genes by TBLASTN and Genewise (Table S11). This number is more than previously reported numbers, suggesting the ZJ-version genome contains a more complete gene set of higher quality. The number of P450 genes is almost twice that of the silkworm. Phylogenetic analysis indicated P450 clans 3 and 4 show a large expansion in the fall armyworm comparing with that in the model insect of Lepidoptera, the silkworm (Figure 4a, Table S20). However, P450 clans Mito and 2 were strongly conserved between the fall armyworm and silkworm (Figure 4a, Table S20). A total of 163 P450 genes were mapped to the 23 chromosomes of fall armyworm. Distribution analysis showed at least 19 P450s clusters exist in the fall armyworm genome (Figure 4b). The largest P450 cluster was located on Chr14 and consisted of 39 *CYP340* genes (Figure 4a,b).

We used the RNA-Seq data, which covered major developmental stages of the fall armyworm, to study the expression profile of all identified P450 genes. In total, 166 out of 169 P450 genes were detected as expressed genes (FPKM > 0.4 in all three repetitions). Moreover, the CYP321A (7-9) gene family tended to express in the fifth and sixth instar larva (Figure 5) and *CYP321A1* reportedly is induced to metabolize xanthotoxin in *Helicoverpa zea* (Rupasinghe, Wen, Chiu, & Schuler, 2007). We found that P450 genes tended to be widely expressed in all developmental stages (Table S21), suggesting the importance of P450 genes in all life stages of the fall armyworm. Particularly in the P450 clan 3 and clan 4, 51 P450 genes in clan 3 (including the family of CYP6AE, CYP6B, CYP6AB, and CYP9) were expressed in all of the nine stages (Tables S21 and S22). Meanwhile, 52 P450 genes (including CYP4, CYP340 and CYP341 gene family) in clan 4 were expressed in all nine stages (Tables S21 and S22). Most of the universally


(a)
Chr4 cluster
Chr9 cluster
Sugar
CO2
S. frugiperda
B. mori
Bitter
Chr24 cluster
(b)
Sugar
Bitter
CO2
1 (Z)
2
3
4
5
6
7
8
9
10
11
12
13
14
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31
ctg37 (W)

FIGURE 6 Gustatory receptor gene family expansion in fall armyworm. (a) Maximum-likelihood phylogenetic analysis of GR genes in fall armyworm and silkworm. Two large bitter GR clusters on Chr9 and Chr24 and one sugar GR cluster are shown. (b) Distribution of 221 GR genes in the fall armyworm chromosomes. The three main types of GR genes (sugar = green, bitter = purple, and CO_2 = yellow) are indicated in different colours [Colour figure can be viewed at wileyonlinelibrary.com]

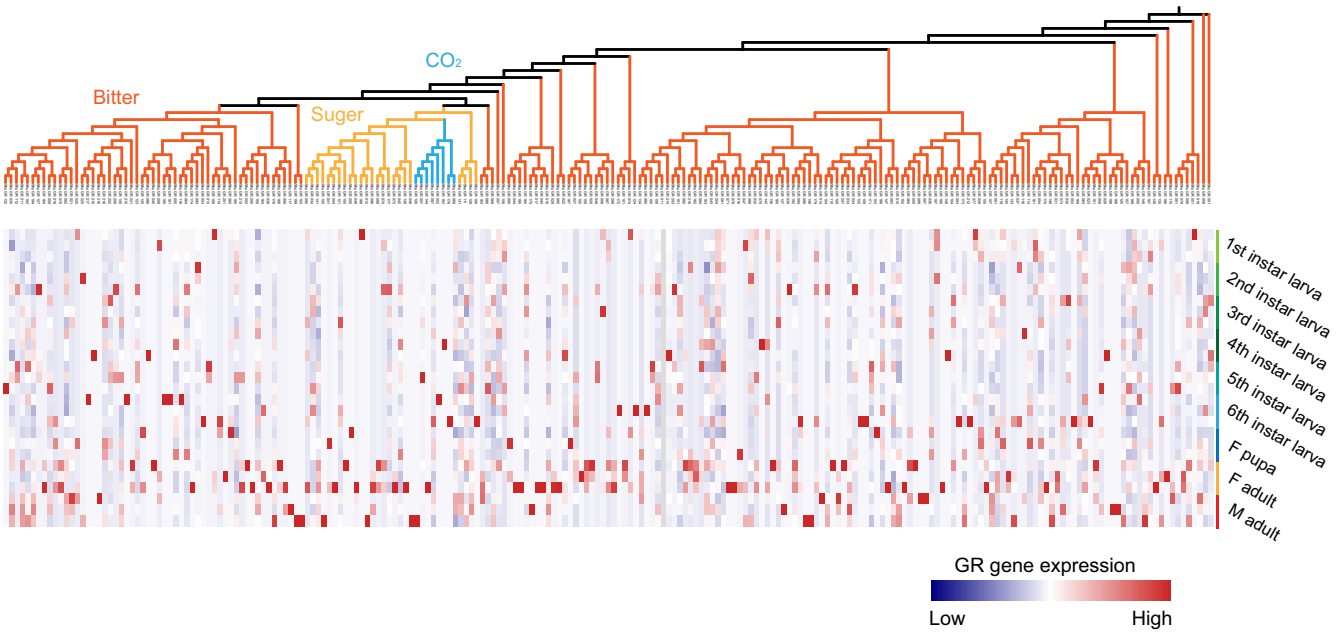

FIGURE 7 Expression profiles of fall armyworm GR genes from individuals at different developmental stages. The GR genes tended to express in the adult. F, female; M, male [Colour figure can be viewed at wileyonlinelibrary.com]

expressed P450 genes are important in metabolizing plant secondary metabolites in insects. *CYP6B1* can be induced to metabolize xanthotoxin in *Papilio polyxenes* (Petersen, Niamsup, Berenbaum, & Schuler, 2003). In *Cnaphalocrocis medinalis*, CYP6AE enzymes are implicated in the detoxification of rice phytochemicals (Liu, Chen, & Yang, 2010), and *CYP6AE14* is involved in gossypol detoxification in *H. armigera* (Krempl et al., 2016). Similarly, plant hosts of fall armyworm contain secondary metabolites that are toxic to the insects, such as cyclic hydroxamic acids (CHx) in the maize, wheat and rye; DIMBOA in maize (Kojima, Fujii, Suwa, Miyazawa, & Ishikawa, 2010); hemiterpene aldehyde in cotton (Stipanovic, Lopez, Dowd, Puckhaber, & Duke, 2006); and nicotine in tobacco (Booker et al., 2010). The expansion of P450 in clan 3 and clan 4, and the wide-spread expression of these P450 genes in almost all developmental stages are probably important for fall armyworm to detoxify the plant xenobiotics.

3.6 | Transcriptome and phylogenetic analysis of gustatory receptors in fall armyworm

Chemoreception is vital for insects to quickly find plant hosts, and is thus important for the spread and invasion of pest populations. We have reported chemoreception genes from a previous genome assembly from the *Sf*9 cell line (Liu, Xiao, et al., 2019). Here, with the ZJ-version genome assembly, we identified 221 gustatory receptors genes which includes 189 bitter receptors, 24 sugar receptors and eight CO_2 receptors using a manual annotation pipeline (Tables S11 and S23). These numbers were much more than what we identified from our previous studies. Phylogenetic analysis and gene distribution analysis indicated that bitter receptors were significantly expanded in the fall armyworm than in the silkworm. Two large GR clusters were found on Chr9 and 24 (Figure 6a,b). The sugar receptors were also tandemly duplicated on chromosome 4 of the fall armyworm (Figure 6a,b). Transcriptome analysis indicated that 152 out of 189 bitter GR genes were detected as expressed genes (FPKM > 0.4 in all three repetitions), the GR genes tended to express in the adult (Figure 7), which is similar to GR gene expression patterns in *H. armigera* (Xu, Papanicolaou, Zhang, & Anderson, 2016). Fast host-recognition is important to maintain the energy requirements for fall armyworm in long distance migration, the expansion of GR genes probably facilitates host-recognition.

3.7 | The expansion of β-fructofuranosidase genes

As a sucrase, β-FFase, is responsible for cleaving sucrose to maintain cell metabolism and growth in bacteria and plants and was assumed to have not existed in animals for many years (Koch, 2004; Liebl, Brem, & Gotschlich, 1998). Recent studies show that in insects, β-FFase genes were acquired via horizontal gene

transfer from bacteria and function in insect avoidance of plant secondary metabolites and glycometabolism modulation (Daimon et al., 2008; Gan et al., 2018; Zhao, Doucet, & Mittapalli, 2014). In the fall armyworm, five β-FFase genes (*SfruSuc1*, *SfruSuc2*, *SfruSuc3*, *SfruSuc4* and *SfruSuc5*) were identified (Figure S5) and they exhibited significant gene expansion compared with those of the silkworm. Only one β-FFase gene, *BmSuc1*, was identified in the silkworm to facilitate the avoidance of the toxic effects of alkaloidal sugar mimic glycosidases, such as 1, 4-dideoxy-1, 4-imino-D-arabinitol (D-AB1) and 1-deoxynojirimycin (DNJ) (Daimon et al., 2008). The β-FFase gene expansion might be an efficient solution for the fall armyworm to adapt and tolerate a high number of plant secondary metabolites, as well as maintain the balance in glucose metabolism.

Transcriptome analysis showed that all five β-FFase genes were expressed in different developmental stages (Figure S5). *SfruSuc1* (the orthologue gene of *BmSuc1*) was highly expressed in the six instar larvae and pupa, because the six instar larvae feeding on the maximum amount of food. This result is consistent with our result from *SfruSuc5* (Figure S5) and that from the study of Pedezzi et al. (2014) study of *Sl-β-fruct* in *Sphenophorus levis*. In contrast, *SfruSuc2* highly expressed from the first instar to the fifth instar larva, and *SfruSuc3* and *SfruSuc4* highly expressed in the adult (Figure S5).

In conclusion, we present data from a chromosome-level genome assembly of fall armyworm and validated the genome, ZJ-version, as a C strain. Furthermore, cytochrome P450 gene family, gustatory receptors, and β-fructofuranosidase genes were significantly expanded in the fall armyworm, revealing the genetic adaptations which may have facilitated the recent rapid invasion of this notorious insect pest worldwide.

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CONFLICT OF INTEREST

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

F.L. conceived and designed the whole project. X.H.Y., and Y.M. assembled and annotated the genome. X.H.Y., and Y.Y. performed the comparative genomics analysis. Y.M., and H.M.X. performed the gene family expression analysis and improved the figures. X.C. performed the sex chromosome analysis. H.X.X., Z.X.L., and Y.J.Y. collected and provided all insect samples. H.M.X., and X.H.Y. prepared the DNA and RNA samples for sequencing. T.L., Y.Y.Y., and W.F.Y. contributed to the genome sequencing. X.H.Y. drafted a part of the manuscript. Z.X.L contributed his efforts (discussion and editing the manuscript) in the first-round of manuscript revision. H.M.X., and F. L. improved the whole manuscript. All authors approved the final manuscript.

DATA AVAILABILITY STATEMENT

The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession WMCG00000000. The version described in this paper is version WMCG01000000. The raw genome and transcriptome data are publicly available at NCBI with the BioProject accession number PRJNA590312. All data mentioned in this paper can also be accessed at http://www.insect-genome.com/Sfru/.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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