Central Lancashire Online Knowledge (CLoK)

Title	Genetic structure and insecticide resistance characteristics of fall armyworm populations invading China
Type	Article
URL	https://clok.uclan.ac.uk/34014/
DOI	https://doi.org/10.1111/1755-0998.13219
Date	2020
Citation	Zhang, Lei, Liu, Bo, Zheng, Weigang, Liu, Conghui, Zhang, Dandan, Zhao, Shengyuan, Li, Zaiyuan, Xu, Pengjun, Wilson, Kenneth et al (2020) Genetic structure and insecticide resistance characteristics of fall armyworm populations invading China. Molecular Ecology Resources, 20 (6). pp. 1682-1696. ISSN 1755-098X
Creators	Zhang, Lei, Liu, Bo, Zheng, Weigang, Liu, Conghui, Zhang, Dandan, Zhao, Shengyuan, Li, Zaiyuan, Xu, Pengjun, Wilson, Kenneth, Withers, Amy, Jones, Christopher M., Smith, Judith Alexis, Chipabika, Gilson, Kachigamba, Donald L., Nam, Kiwoong, d'Alençon, Emmanuelle, Liu, Bei, Liang, Xinyue, Jin, Minghui, Wu, Chao, Chakrabarty, Swapan, Yang, Xianming, Jiang, Yuying, Liu, Jie, Liu, Xiaolin, Quan, Weipeng, Wang, Guirong, Fan, Wei, Qian, Wanqiang, Wu, Kongming and Xiao, Yutao

It is advisable to refer to the publisher's version if you intend to cite from the work.
https://doi.org/10.1111/1755-0998.13219

For information about Research at UCLan please go to http://www.uclan.ac.uk/research/

MOLECULAR ECOLOGY
RESOURCES WILEY

Genetic structure and insecticide resistance characteristics of fall armyworm populations invading China

Lei Zhang[1] | Bo Liu[1] | Weigang Zheng[1] | Conghui Liu[1] | Dandan Zhang[2] |
Shengyuan Zhao[2] | Zaiyuan Li[1] | Pengjun Xu[3,4] | Kenneth Wilson[4,1] |
Amy Withers[4] | Christopher M. Jones[5] | Judith A. Smith[6] | Gilson Chipabika[7] |
Donald L. Kachigamba[8] | Kiwoong Nam[9] | Emmanuelle d'Alençon[9] | Bei Liu[1] |
Xinyue Liang[1] | Minghui Jin[1] | Chao Wu[1] | Swapan Chakrabarty[1] | Xianming Yang[2] |
Yuying Jiang[10] | Jie Liu[10] | Xiaolin Liu[11] | Weipeng Quan[12] | Guirong Wang[1] |
Wei Fan[1] | Wanqiang Qian[1] | Kongming Wu[2] | Yutao Xiao[1]

[1]Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China

[2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

[3]Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao, China

[4]Lancaster Environment Centre, Lancaster University, Lancaster, UK

[5]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi

[6]School of Forensic and Applied Sciences, University of Central Lancashire, Preston, UK

[7]Zambia Agriculture Research Institute (ZARI), Lusaka, Zambia

[8]Department of Agricultural Research Services (DARS), Bvumbwe Research Station, Limbe, Malawi

[9]DGIMI, Univ. Montpellier, INRA, Montpellier, France

[10]National Agro-Tech Extension and Service Center, Beijing, China

[11]Novogene Bioinformatics Institute, Beijing, China

[12]Grandomics Biosciences, Co., Ltd, Beijing, China

Correspondence
Yutao Xiao, Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China.
Email: xiaoyutao@caas.cn

Kongming Wu, State Key Laboratory for Biology of Plant Diseases and Insect PestsInstitute of Plant ProtectionChinese, Academy of Agricultural Sciences, Beijing,China.
Email: wukongming@caas.cn

Wanqiang Qian, Shenzhen Branch, Guangdong Laboratory for Lingnan Modern

Abstract

The rapid wide-scale spread of fall armyworm (*Spodoptera frugiperda*) has caused serious crop losses globally. However, differences in the genetic background of sub-populations and the mechanisms of rapid adaptation behind the invasion are still not well understood. Here we report the assembly of a 390.38-Mb chromosome-level genome of fall armyworm derived from south-central Africa using Pacific Bioscience (PacBio) and Hi-C sequencing technologies, with scaffold N50 of 12.9 Mb and containing 22,260 annotated protein-coding genes. Genome-wide resequencing of 103 samples and strain identification were conducted to reveal the genetic background of fall armyworm populations in China. Analysis of genes related to pesticide- and *Bacillus thuringiensis* (Bt) resistance showed that the risk of fall armyworm developing

Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China.
Email: qianwanqiang@caas.cn

Wei Fan, Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China.
Email: fanwei@caas.cn

resistance to conventional pesticides is very high. Laboratory bioassay results showed that insects invading China carry resistance to organophosphate and pyrethroid pesticides, but are sensitive to genetically modified maize expressing the Bt toxin Cry1Ab in field experiments. Additionally, two mitochondrial fragments were found to be inserted into the nuclear genome, with the insertion event occurring after the differentiation of the two strains. This study represents a valuable advance toward improving management strategies for fall armyworm.

KEYWORDS
gene insertion, population structure, resistance risk, *Spodoptera frugiperda*, subpopulations

1 | INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a polyphagous pest that is native to tropical and subtropical America, with a strong capacity for migration and reproduction (Johnson, 1987; Mitchell et al., 1991; Westbrook, Nagoshi, Meagher, Fleischer, & Jairam, 2016). It was first detected in Africa in 2016 (Goergen, Kumar, Sankung, Togola, & Tamò, 2016) and spread to 44 African countries within 2 years. It was detected in India in 2018, and has now spread to several southeastern Asian countries (Nagoshi et al., 2020). Such rapid spread poses a global threat to food production. The strong environmental adaptability of fall armyworm is not only reflected in its polyphagy for a wide range of host plants (Luginbill, 1928), but also in its evolution of resistance to chemical pesticides and genetically modified crops expressing *Bacillus thuringiensis* (Bt) toxins (Bernardi et al., 2015; Leibee & Capinera, 1995; Monnerat et al., 2015; Signorini et al., 2018; Storer et al., 2010). Studies have shown that gene families related to detoxification and metabolic processes in the fall armyworm have clearly expanded (Gouin et al., 2017; Liu et al., 2019). In addition, there are two morphologically identical, but genetically distinct, subpopulations or strains of fall armyworm, the rice-strain (R-strain) and the corn-strain (C-strain), which differ in their host plant selection and sex pheromone composition (Lima & McNeil, 2009; Pashley, 1986; Pashley, Hammond, & Hardy, 1992; Pashley & Martin, 1987). However, there is no absolute mating barrier between the two strains and productive hybridization has been

confirmed in both laboratory and field studies (Dumas et al., 2015; Nagoshi, Meagher, Nuessly, & Hall, 2006).

To date, several field-evolved resistant populations of fall armyworm have been detected, including those displaying resistance to a variety of chemical pesticides and Bt crops (Chandrasena et al., 2018; Gutiérrez-Moreno et al., 2019; Zhu et al., 2015). The reported mechanisms of resistance to pesticides are mainly due to variation in receptor genes, such as amino acid changes in the ryanodine receptor (RyR) (diamide), acetylcholinesterase (AChE) (organophosphate) and voltage-gated sodium channel (VGSC) (pyrethroids) (Boaventura et al., 2020; Carvalho, Omoto, Field, Williamson, & Bass, 2013; Yu, Nguyen, & Abo-Elghar, 2003). In addition, the frameshift mutation resulting in early termination of the ATP-dependent Binding Cassette subfamily C2 gene (*ABCC2*) gene, caused by a 2-bp insertion, is linked to resistance to Bt toxin Cry1Fa (Banerjee et al., 2017). Field-evolved strains resistant to Bt toxin Vip3Aa20 were obtained by screening homozygous resistance loci in F_2 generations in the laboratory (Yang et al., 2018). Clarifying the development of pesticide- and Bt-resistance in fall armyworm would be helpful in providing scientific support for the commercialization of genetically modified crops and Bt biopesticides.

Recent studies have indicated that molecular identification of the C- and R-strains of fall armyworm is dependent on which markers are used (Meagher & Gallo-Meagher, 2003; Nagoshi, 2012). Early molecular markers based on mitochondrial *Cytochrome Oxidase Subunit I* (*COI*) and Z-chromosome-linked *Tpi* genes failed to

accurately assign the strain genetic background (Juárez et al., 2014; Nagoshi, 2019; Nagoshi, Goergen, Goergen, Du Plessis, van den Berg, & Meagher, 2019; Nagoshi et al., 2017). The dominant populations of fall armyworm invading Africa and Asia were speculated to be hybrid populations based on these two molecular markers (Zhang et al., 2019). In addition, an Africa-specific haplotype, different from those native to the Americas, was also reported in African and Chinese samples based on the *Tpi* gene (Liu et al., 2019; Nagoshi et al., 2019), which makes strain identification and studies of population genetic structure more complicated. Therefore, a genome-wide analysis of the genetic characteristics of invasive fall armyworm is becoming imperative. Although several versions of the fall armyworm genome have now been published (Gouin et al., 2017; Kakumani, Malhotra, Mukherjee, & Bhatnagar, 2014; Liu et al., 2019; Nam et al., 2019; Nandakumar, Ma, & Khan, 2017), a high-quality genome assembly from a different geographical source is a valuable addition to the genomic resources for this species. Moreover, the different biological properties of the C- and R-strains and the debate regarding strain identification will benefit from further genomic support and explanation. Here we report a chromosome-level genome sequence of a male moth from an inbred fall armyworm strain, which derived from field populations collected in Zambia in 2017 and would be classed as C-strain based on *COI* genotype but possessed an Africa-specific *Tpi* haplotype which differs from the Western Hemisphere (henceforth American) R- and C-strain. We also resequenced 103 fall armyworm samples from 16 Provinces in China, as well as four samples collected from two African countries (Zambia and Malawi). The genome-wide genetic backgrounds of the invading fall armyworm samples were compared, and insecticide-resistance risk was assessed based on analysis of potential resistance-related genes. Comparative genomic analyses of these data will help to reveal the resistance-related mechanisms and the population genetic characteristics of fall armyworm, which may facilitate its future management.

2 | MATERIALS AND METHODS

2.1 | Samples and sequencing for genome assembly

The fall armyworm samples were collected from maize fields in Lusaka, Zambia, in 2017 and reared to produce an inbred strain. One male moth, derived from seven successive generations of single-pair sib mating, was selected for genomic sequencing for the primary assembly data set and all other individuals used in the Hi-C and RNAseq experiments were from the same inbred strain. DNA was extracted using the Qiagen Genomic DNA kit (Cat. no. 13323, Qiagen) followed by purity assessment and quantification with a NanoDrop One UV-Vis spectrophotometer (Thermo Fisher Scientific) and Qubit 3.0 Fluorometer (Invitrogen), respectively. About 0.5 μg genomic DNA (gDNA) was used as input to generate a PCR-free Illumina genomic library using the Truseq Nano DNA HT Sample preparation Kit (Illumina), with 350-bp insert size and this

library was sequenced in 2 × 150-bp format on the Illumina NovaSeq 6000 platform. Five micrograms of gDNA from the same individual was used as an input for ~20-kb insert libraries (SMRTbell Template Prep Kit 1.0, Cat. no. 100-259-100, PacBio) sequenced on the PacBio Sequel (Pacific Biosciences). Two third-instar larvae were selected for Hi-C library construction, and nuclear DNA was cross-linked in situ, extracted and then digested with the restriction enzyme *Dpn*II. Hi-C libraries were amplified by 12–14 cycles of PCR and sequenced on the Illumina NovaSeq 6000 platform with 2 × 150-bp reads. In addition, three fifth-instar larvae, three pupae, three female moths and three male moths were used for RNA sequencing. Total RNA was extracted using the RNeasy Mini extraction kit (Qiagen), and a NanoPhotometer spectrophotometer (Implen) and Qubit 2.0 Flurometer (Life Technologies) were used to check the purity and concentration of RNA, respectively. One microgram total RNA per sample was used to make indexed cDNA libraries using the NEBNext Ultra RNA Library Prep Kit for Illumina (NEB) following the manufacturer's recommendations. The libraries had insert sizes of 250–300 bp and were sequenced on the Illumina NovaSeq 6000 platform with 150-bp paired-end output.

2.2 | Genome assembly and correction

The raw PacBio reads longer than 5 kb were assembled into contigs using the software WTDBG2 version 2.4 with the parameters "-p 0 -k 15 -AS 2 -s 0.05 -L 5000" (Ruan & Li, 2019). ARROW version 2.1.0 (https://github.com/PacificBiosciences/GenomicConsensus) was used to correct assembly errors after comparing contigs with PacBio reads using PBALIGN version 0.4.1 (https://github.com/PacificBiosciences/pbalign). The Illumina raw reads were filtered by trimming the adapter and low-quality regions using CLEAN _ ADAPTER version 1.1 with the parameter "-a Both-adapter -r 75 -s 12" and CLEAN _ LOWQUAL version 1.0 with the parameter "-e 0.001 -r 75" (https://github.com/fanagislab/ assembly_2ndGeneration/tree/master/clean_illumina). The filtered Illumina reads were aligned to the assembled contigs by BWA MEM version 0.7.17 (Li & Durbin, 2009), and single base errors in the contigs were corrected by PILON version 1.21 (Walker et al., 2014).

2.3 | Genome estimation and evaluation

A distribution analysis of 17 k-mer frequencies was performed to estimate the genome size of fall armyworm. The filtered Illumina reads were used as input to construct k-mer frequencies by JELLYFISH (https://github.com/gmarcais/Jellyfish). Genome size was estimated using $G = K\_num/K\_depth$, where the K_num is the total number of K-mers, and K_depth is the frequency occurring more frequently than the others (Li et al., 2010). We used the arthropoda gene set (odb9) to assess the integrity of the genome by Benchmarking Universal Single-Copy Ortholog (BUSCO) version 3.0.2 (Simao, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015).

2.4 | Chromsome assembly based on Hi-C data

The Hi-C sequencing raw reads were filtered to remove reads containing <5 bases of adaptor sequence; >50% of bases with phred quality value of <19; and <5% of unknown bases (N). Filtered reads were then aligned to the assembled contigs using BOWTIE2 (version 2.2.3; http://bowtie-bio.sourceforge.net/bowtie2/index.shtml) (Langmead & Salzberg, 2012). Invalid read pairs were filtered using default settings by HIC-PRO (version 2.7.8; https://github.com/nservant/HiC-Pro) (Servant et al., 2015). LACHESIS (https://github.com/shendurelab/LACHESIS) (Burton et al., 2013) was applied to cluster, order and orient contigs based on the agglomerative hierarchical clustering algorithm. For each chromosome cluster, the ordered contigs were oriented by building a weighted, directed acyclic graph (WDAG). The orientation of each contig in each chromosomal group was predicted according to the maximum-likelihood path through WDAG. Finally, we cut the chromosomes predicted by LACHESIS into bins of equal length (100 kb) and constructed a heatmap based on the interaction signals revealed by valid mapped read pairs between bins using HIC-PRO.

2.5 | Gene prediction and annotation

A de novo repeat library of fall armyworm was constructed by REPEATMODELER version 1.0.4 (http://www.repeatmasker.org/RepeatModeler.html). Transposable elements (TEs) were identified by REPEATMASKER version 4.0.6 (http://www.repeatmasker.org/) using both the de novo library and Repbase library (Repbase-20150923), and tandem repeats were predicted using TANDEM REPEATS FINDER (Benson, 1999) version 4.07b. We used a combination of ab initio prediction, homology searches and RNA-seq annotation to predict genes in the *Spodoptera frugiperda* genome. We performed ab initio prediction using AUGUSTUS 2.5.5 with default parameters (Stanke & Waack, 2003). For homology-based annotation, we queried the *S. frugiperda* genome sequences against a database containing nonoverlapping protein sequences from closely related species (*Bombyx mori*, *Helicoverpa armigera*, *Spodoptera litura*) by GENBLASTA with default parameters (She, Chu, Wang, Pei, & Chen, 2009). GENEWISE (Birney, Clamp, & Durbin, 2004) was used to refine the GENBLASTA mappings to the genome. For the RNA-seq annotation, the RNA-seq data were mapped to the assembled genome of *S. frugiperda* using TOPHAT version 2.0.12 and alignments were processed by CUFFLINKS version 2.2.1 with default parameters to generate transcript predictions (Trapnell et al., 2012). EVIDENCE MODELER (Haas et al., 2008) version 1.1.1 was used to combine ab initio predictions, homology-based searches and RNA-seq alignments. Predicted gene models supported by at least one of the annotations using the UniProt database, NR database and RNA-seq data were retained. Gene functional annotation was performed by aligning the predicted protein sequences to the NCBI NR, UniProt, eggNOG, and KEGG databases with BLASTP version 2.3.0+, applying an *E*-value cut-off < 10^{-5}.

2.6 | Phylogenetic tree construction and genomic comparison

Orthologous and paralogous gene families identified in a set of 10 species (*Drosophila melanogaster*, *Plutella xylostella*, *Bombyx mori*, *Manduca sexta*, *Danaus plexippus*, *Heliconius melpomene*, *Operophtera brumata*, *Helicoverpa armigera*, *Spodoptera frugiperda*, *Spodoptera litura*) with published genomes were analysed by ORTHOFINDER version 2.3.1 with default parameters. Orthologous groups that contain single-copy genes for each species were selected to construct the phylogenetic tree. The multisequence alignment of proteins was accomplished by MUSCLE (Edgar, 2004) version 3.8.31. A neighbour-joining (NJ) phylogenetic tree was constructed using MEGA version 7.0.14. The current assembled genome was aligned with two published versions of fall armyworm genomes using the MUMMER3.23 (Kurtz et al., 2004) package with cutoff of identity >80% and coverage >80%. Alignments were filtered to generate a multi-alignment data set using the delta-filter utility with 85% minimum identity (-i 85) and minimum alignment length 10 (-l 10). A set of unique alignments was created using the same filter criteria but with the addition of the -r and -q flags.

2.7 | Sampling for resequencing and population genetic study

A total of 103 Chinese fall armyworm samples were used for resequencing. All samples were collected as larvae on maize or sugarcane from 50 cities of 16 provinces (autonomous regions or municipalities) of China. The larvae were fed with fresh maize leaves and brought back to the laboratory under ambient conditions during transportation. Larval bodies were cleaned and then stored in a freezer at −80°C. Detailed sample information is presented in Table S1 and the sample distribution in China is shown in Figure S1. In addition, four fall armyworm samples from Africa were also used for resequencing, including two samples (AFR4 and 5) from the same inbred strain (AFR2017) as the genome sequencing in this study, and another two samples (AFR14 and 15) which were collected from maize fields in Bvumbwe, Malawi, in January 2019, which is also an inbred strain (AFR2019) reared in the laboratory. A total of 1.5 µg gDNA of each sample was used to construct a 350-bp insert library using the Truseq Nano DNA HT Sample preparation Kit (Illumina) sequenced in 150-bp paired-end mode as described in section 2.1. Raw reads were aligned to the NCBI NT database using BLASTN, and reads with significant matches (identity > 95% and coverage > 80%) to microbes or host plants were removed.

A further 173 fall armyworm samples from 21 provinces in China were used for strain identification and molecular detection using PCR amplification and Sanger sequencing. Genomic DNA was extracted using the Multisource Genomic DNA Miniprep Kit (Axygen) according to product instructions. The 50-µl PCR mixture contained 25 µl of 2 × Easytaq mix, a mixture of 2 µl forward and reverse primers (10 µM), 2 µl DNA, and 21 µl diethy1 pyrocarbonate (DEPC) H_2O.

PCR was performed at 94°C for 5 min, 34 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, and finally 72°C for 5 min. A total of 10 μl of the PCR products containing the target fragment were sequenced by Life Technology. These samples were collected from the field as larvae or adult moths. Detailed sample information is presented in Table S2 and the sample distribution in China is shown in Figure S1. Mitochondrial *COI* and *Tpi* markers were used for strain identification. ABCC2 and AChE genes were detected based on primers designed according to published mutation sites (Banerjee et al., 2017; Carvalho et al., 2013). Inserted mitochondrial fragments in the nuclear genome were detected using primers designed in this study. All primer sequence information in this study is shown in Table S3.

2.8 | Read mapping and SNP calling

The Illumina raw reads from resequenced samples were filtered using CLEAN_ADAPTER and CLEAN_LOWQUAL software as described in section 2.1, resulting in high-quality reads with an average error rate of <0.01. The high-quality reads were then aligned to the fall armyworm reference genome (American C-strain) and mitochondrial genome sequences using BWA MEM software (Li & Durbin, 2009) version 0.7.5a with default parameters. Alignments for each sample were processed by removing duplicate reads using the SAMTOOLS (Li et al., 2009) software package version 1.3. The mpileup function in SAMTOOLS was used to generate mpileup files for each sample. VCFTOOLS (Li, 2011) was used to identify SNPs and small indels. Several criteria were considered in SNP filtering: (a) a read mapping score higher than 40; (b) minimum coverage greater than 10; and (c) SNP genotypes called in >90% of samples. We also conducted principal component analysis (PCA) to evaluate genetic structure using the software Genome-wide Complex Trait Analysis (GCTA) version 1.04 (Yang, Lee, Goddard, & Visscher, 2011).

2.9 | Bioassays of insecticides and Bt maize in the field

Bioassays were conducted by a topical application procedure (Armes, Jadhav, Bond, & King, 1992). Two inbred strains (cdcc and cdyc) collected from Yunnan Province and reared for multiple generations in the laboratory were tested using 14 types of pesticide commonly used in agricultural production (Table S4). Drops (1.0 μl) of a serial dilution of technical insecticides in acetone solution were applied with a micropipette to the thoracic dorsum of the third-instar larvae, with control larvae treated with 1.0 μl acetone. After treatment, the larvae were reared individually in 24-well plates containing ad libitum artificial diet without any Bt proteins or insecticides. Larvae were retained in an insect chamber with a controlled environment of 26 ± 1°C, 60 ± 10% relative humidity and a photoperiod of 16 hr: 8 hr (light–dark). Mortality was assessed after 72 hr of treatment. Larvae were considered dead if they were unable to move in

a coordinated manner when prodded with a small soft brush. We used median lethal doses (LC_{50}) to evaluate the resistance level of different fall armyworm populations. The LC_{50} and 95% fiducial limit (FL) for each insecticide were estimated by probit analysis using the software package POLO-PC (Russell, Robertson, & Savin, 1977) (LeOra Software).

The Bt toxin field bioassays were conducted at a genetically modified (GM) test base in Yunnan Province, China. Test seeds of GM maize (expressing Cry1Ab) and control maize were provided by the DBN Biotech Center, Beijing DBN Technology Group Co., Ltd. Both maize types were planted in ~180 m^2, with each type being replicated three times. Larval density and maize damage rates were investigated at different growth stages of maize at seven different dates during June and July. The investigation was performed in a five-spot sampling method with 20 maize plants per point. Fall armyworm damage assessment was performed according to standard procedures (Davis, Ng, & Williams, 1992; Williams, Buckley, & Daves, 2006; Wiseman & Widstrom, 1984).

3 | RESULTS

3.1 | High-quality genome assembly of fall armyworm

A total of 25.89 Gb raw PacBio long reads and 162.4 Gb Illumina raw reads were generated. After filtering low-quality and duplicated reads, 24.72 Gb PacBio long reads and 95.4 Gb high-quality Illumina reads were used for genome assembly, together representing an ~300× coverage of the fall armyworm genome. Using WTDBG2 (Ruan & Li, 2019), the final genome was assembled into 776 contigs with size of 390.38 Mb and contig N50 length of 5.6 Mb (longest, 18.5 Mb), including a complete mitochondrial sequence (Table 1). The assembled genome size was close to the estimated size of 395 Mb based on k-mer depth distribution analysis, which was also similar to that determined by flow cytometry (396 ± 3 Mb) (Gouin et al., 2017). After interaction analysis based on a total of 78 Gb data obtained through Hi-C sequencing, 143 contigs were concatenated into 31 linkage groups with a scaffold N50 of 12.9 Mb, accounting for 96.3% of the total genome length (Figure 1). By aligning the Illumina data with the assembled fall armyworm genome, the mapping rate and coverage were 98.8% and 99.7% (≥5 reads) respectively, highlighting the accuracy and high integrity of the genome assembly. The genome size reported in this study is intermediate between those of previously published fall armyworm versions, but the genome is nearly 140 Mb smaller than that recently published by Liu et al. (2019). Genome collinearity analysis showed that more than 98% of the current assembled genome was consistent with previously published versions (Gouin et al., 2017; Liu et al., 2019) (Table S5), and regions within the assembly presented in this study align to multiple regions of Liu's assembly, indicating the previous assembled genome with larger size was mainly caused by high heterozygosity of sequenced samples.

TABLE 1 Summary of assembly results of *Spodoptera frugiperda*

Assembly feature	FAW (this study)	FAW (corn strain)	FAW (rice strain)
Assembled sequences (Mb)	390	438	371
Longest scaffold size (kb)	21,916.7	943.2	314.1
N50 size of scaffold (kb)	12,966.7	52.8	28.5
N90 size of scaffold (kb)	7,574.2	3.5	6.4
Longest contig size (kb)	18,555.4	362.9	191.4
N50 size of contig (kb)	5,606.9	16.9	24.3
N90 size of contig (kb)	991.8	2.9	5.6
GC content in genome (%)	36.4	36.0	36.1
Number of gene models	22,260	21,700	26,329
BUSCO complete gene (%)	98.4	88.1	93.5
BUSCO duplicated gene (%)	2	11.3	2
BUSCO missing gene (%)	1.4	4.2	2.3

By combining homology-based and de novo approaches, we identified ~27.2% of repetitive elements in the assembled fall armyworm genome. Among the known repeat families, long interspersed nuclear elements (LINE) constituted the most abundant repeat family, representing 8.7% of the repetitive sequences, while long terminal repeats (LTR) comprised only 1.4% (Table S6). To annotate the fall armyworm genome, we performed deep transcriptome sequencing of larvae, pupae, and male and female moths, including three different developmental stages, which generated 98.4 Gb of RNA-seq data. By combining homologue-based, ab initio and transcriptome-based approaches, we predicted 22,260 protein-coding genes (gene models) in the fall armyworm genome, which is greater than the number of predicted genes in other lepidopteran genomes that have so far been published (Dasmahapatra et al., 2012; Kanost et al., 2016; Pearce et al., 2017; Wan et al., 2019; Xia et al., 2008; You et al., 2013; Zhan, Merlin, Boore, & Reppert, 2011). More than 85.5% of the predicted coding sequences (CDS) were supported by transcriptome sequencing data (defined as when ≥ 70% of the predicted CDS of a gene was covered by transcriptome reads). Further assessment of assembly integrity based on BUSCO analysis showed that the current genome contained 98.4% complete BUSCO genes.

Comparative analysis of orthogroups of nine Lepidoptera species and *Drosophila melanogaster* (Diptera) was performed (Table S7). Among them, 17,180 genes in 10,755 orthogroups were found in the current genome of fall armyworm, and the remaining 5,080 lineage-specific genes were identified as unassigned genes. Compared with *Spodoptera litura*, *S. frugiperda* has more species-specific genes, and the number of unassigned genes is much greater than that of *S. litura* (Figure 2a). Phylogenomic analyses of the 10 species were conducted using 1,571 single-copy genes. As shown in Figure 2a, the taxonomic relationship and phylogenetic status of current species was similar to phylogenetic analyses based on 13 mitochondrial protein-coding genes (Lämmermann, Vogel, & Traut, 2016). Three

species of Noctuidae, including *S. frugiperda*, formed one group, which then clustered with *Bombyx mori* (Bombycidae) and *Manduca sexta* (Sphingidae). Two butterflies, *Danaus plexippus* and *Heliconius melpomene* (both Nymphalidae), clustered together as an outer branch, while *Plutella xylostella* (Plutellidae) is the outermost branch of Lepidoptera (Figure 2a).

3.2 | Genetic background of fall armyworm populations in China

A total of 103 fall armyworm samples from China were resequenced, as well as four samples from two countries in Africa (Zambia and Malawi). The generated Illumina data ranged from 8.6 to 18.9 Gb for each sample, with a median genome coverage of 32.5×. First, we analysed the whole mitochondrial genome sequences of all samples. A total of 208 SNP loci were selected for analysis, based on comparison of the published mitochondrial sequences of both the American R-strain (AXE) and C-strain (ASW) (Gouin et al., 2017). Genotypes were obtained at these 208 sites for each individual after mapping the filtered sequence reads to the assembled mitochondrial genome. We found that most of the samples were assigned to the R-strain, and all four samples from Africa were C-strain, while only four out of 103 samples in China were assigned to the C-strain based on the mitochondrial genome (Figure 3a). Note that most R-strain samples surprisingly contain heterozygous mitochondrial SNPs, which could be caused by inserted C-strain fragments or existing standing variation of low frequency. The proportion of the C-strain in this sample set was ~10% and was similar to that of the 173 Chinese fall armyworm samples identified by PCR based on the *COI* gene in this study (Table S2).

Next, we analysed the *Tpi* gene, which is commonly used in strain identification of fall armyworm (Nagoshi, 2012). By comparing the full-length *Tpi* gene of the American R-strain (AXE) and C-strain (ASW), 22 SNP loci were found. The genotype of each individual was analysed based on these 22 sites. The results showed that all fall armyworm samples collected from China contained more C-strain SNP loci, as did the Malawi samples (AFR14, AFR15), but not those from Zambia samples (AFR4, AFR5) which represents the Africa-specific haplotype and which contained ~50% of R-strain SNP loci. Genotypes of seven Chinese samples were identical to the American C-strain (ASW) and the remaining samples contained a small proportion of R-strain genotypes or heterozygous SNPs (Figure 3b). However, none of the samples was found to be identical to the American R-strain genotype (AXE). We further used PCR to analyse genotypes of 173 samples based on 10 strain-biased SNPs within the *Tpi* gene reported previously (Nagoshi, 2012). The results showed that almost all of the samples correspond to C-strain genotypes, although three samples (G-GXW11, G-GXW13, G-EP6) were identified as an Africa-specific haplotype, which was significantly different from known R- or C-strain genotypes (Figure 4; Table S2). In summary, our genotyping results show that there are obvious contradictions between strain identification using mitochondrial and *Tpi* gene markers.
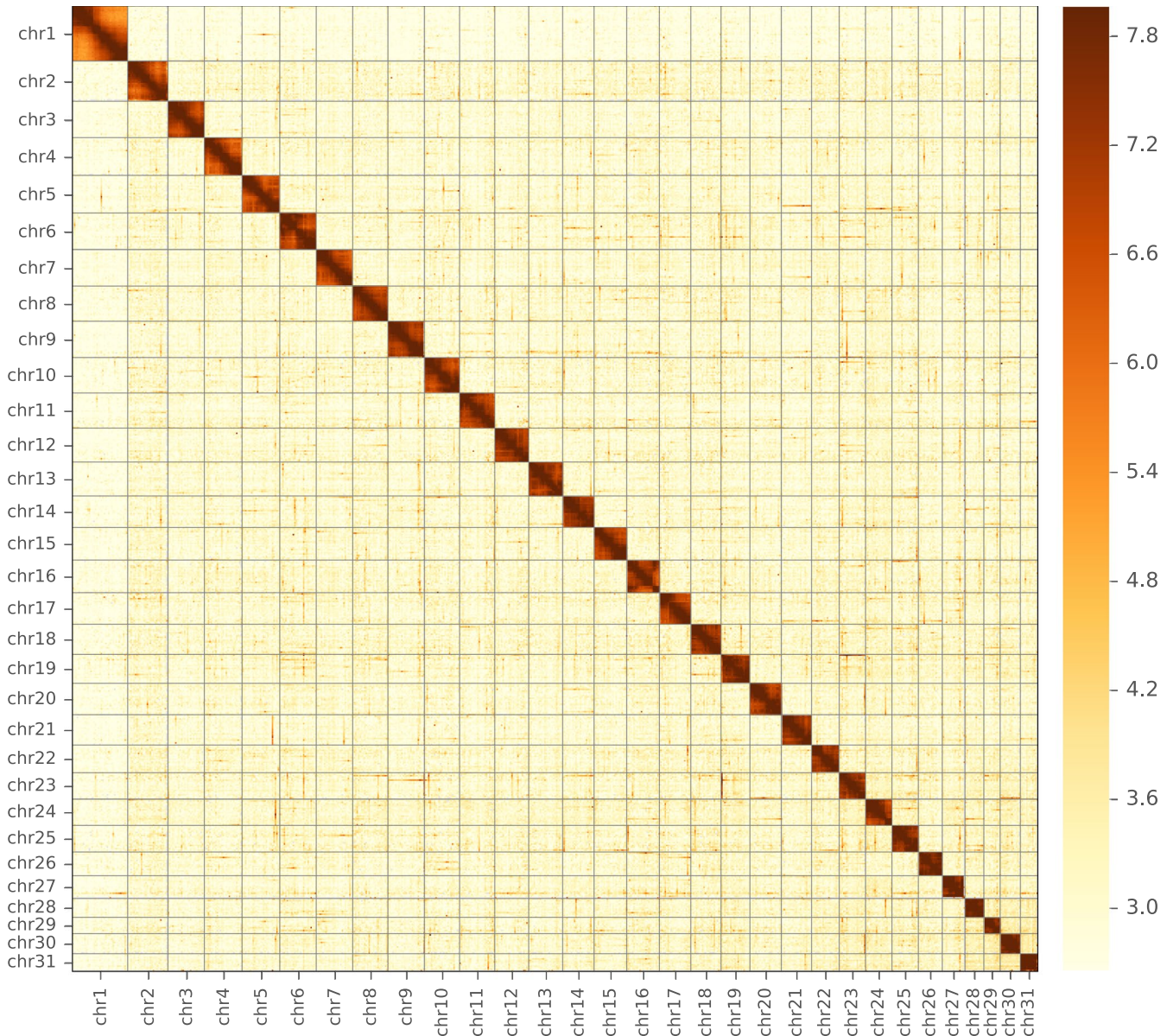
FIGURE 1 A genome-wide contact matrix from Hi-C data between each pair of the 31 chromosomes

To clarify the genetic background of fall armyworm populations invading China, we screened a total of 707,353 SNPs exhibiting homozygous differences between the reference American R-strain (AXE) and C-strain (ASW) in the 107 resequenced samples (Figure 3c). The results showed that all the samples, including the four from Africa, had more than 70% of the genetic background of the American C-strain (ASW) genotype. The proportion of R-strain SNPs was about 15%, and the remaining 12% were heterozygous. The results showed that fall armyworm invading China have a dominant percentage of the C-strain background. PCA based on 5,998,089 whole-genome SNPs also demonstrated that samples from China were much closer to the C-strain (ASW) than to the R-strain (AXE), in which PC1 explained 6.45% of the variation. African samples from Zambia (AFR4, AFR5) were separated on PC2, which explained 2.15% of the variation (Figure 3d). By comparing the results of the mitochondrial genome, *Tpi* gene and genome-wide identification, it becomes apparent that there

is no correlation between the mitochondrial and whole genome genotype. Although *Tpi* genotyping shows results more similar to those of the whole genome, the presence of the Africa-specific *Tpi* haplotype increases the complexity of using this marker for identification.

3.3 | Fall armyworm is developing a high risk of resistance to conventional pesticides

Insecticide resistance evolution is one of the most challenging problems in the control of fall armyworm. Identifying resistance-related genes is helpful for the monitoring and prevention of fall armyworm outbreaks. We selected 14 previously reported resistance-related genes of lepidopteran pests and scanned the resequenced samples to analyse variation in target genes. The results showed that all the target genes had multiple variation sites with a high frequency of SNPs in the CDS region (Table S8).
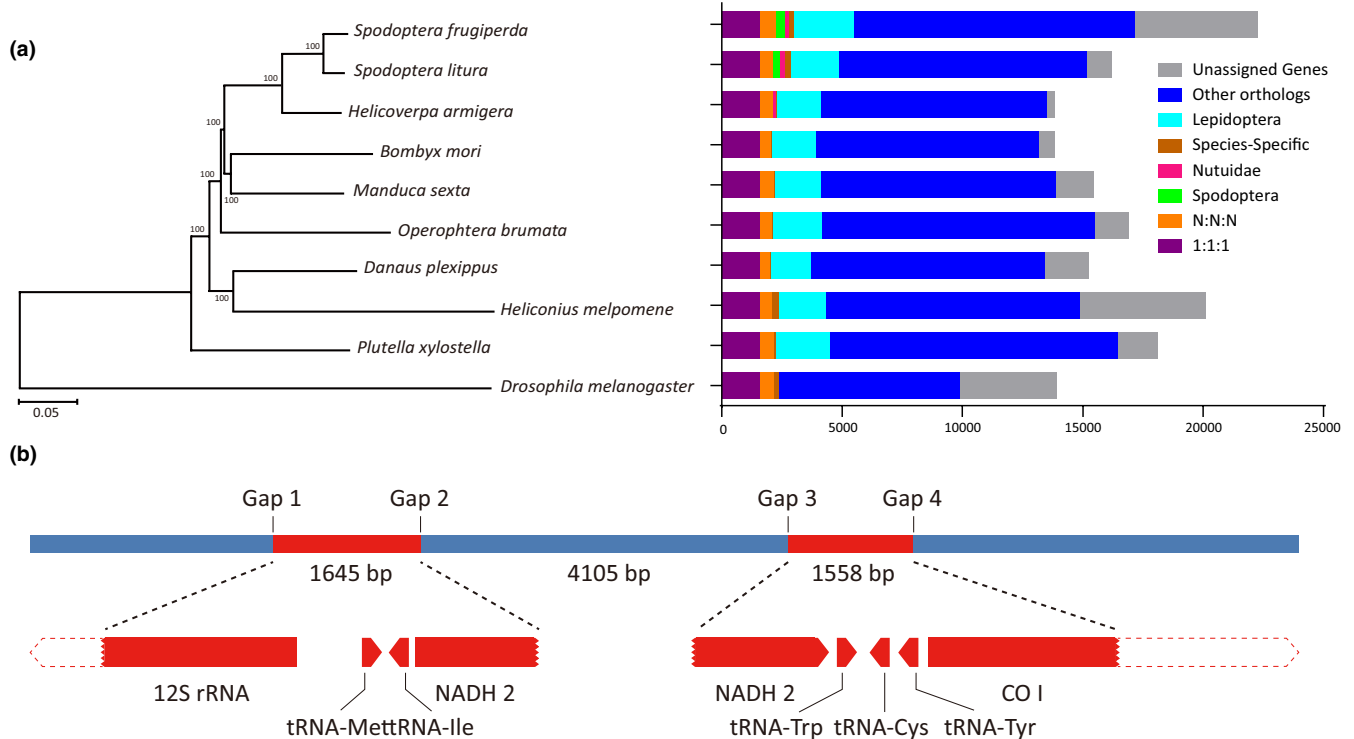
FIGURE 2 Phylogenetic relationships and schematic map of mitochondrial insertion. (a) Phylogenetic tree and genomic comparison of 10 species of Lepidoptera and Diptera. *Drosophila melanogaster* was used as an outgroup and bootstrap value was set as 1,000, 1:1:1 includes the common orthologues with the same number of copies in different species, N:N:N includes the common orthologues with different copy numbers in different species, other orthologues include the unclassified orthologues, and unassigned genes include the genes that cannot be clustered into known gene families. (b) A schematic map of two mitochondrial fragments inserted into the nuclear genome; the *NADH2* gene was separated by a 4,105-bp fragment, and both of two inserted mitochondrial fragments were identical with the C-strain genotype
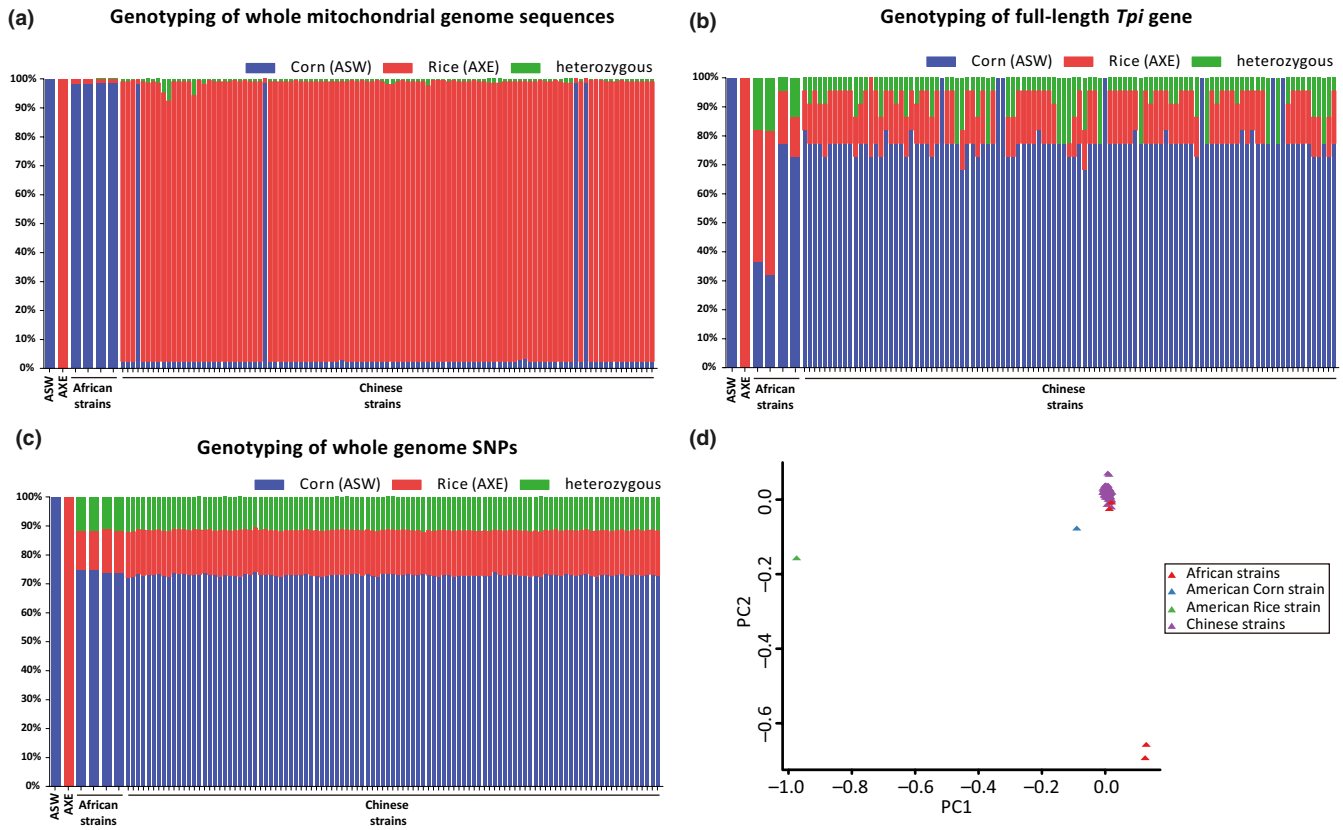
Studies have shown that the amino acid substitutions in AChE (A201S, G227A, F290V), VGSC (T929I, L932F, L1014F) and RyR (I4790M, G4946E) result in resistance to organophosphate, pyrethroid and diamide insecticides, respectively. The results of variation scanning of the 107 resequenced samples showed that resistance mutations were present in amino acids 201 and 290 of AChE (Figure 5a). Among them, the first locus had 17.1% heterozygous mutations, and the third locus had 29.7% homozygous resistance mutations and 58.2% heterozygous mutations. No resistance mutations were detected at the targeted sites of the VGSC and RyR gene in any samples. We also designed primers to detect the resistance mutation sites in *AChE* in 173 Chinese samples by PCR amplification and Sanger sequencing. The results were similar to the Illumina data, showing that ~75% of samples have homozygous or heterozygous variation at amino acid 290.

To understand the baseline susceptibility of fall armyworm invading China, we determined the LC_{50} values to 14 insecticides for two Chinese fall armyworm populations collected from Yunnan Province. The results showed that the LC_{50} for both fall armyworm populations to chlorpyrifos, a fenvalerate, were at relatively high level, and well above those of the laboratory-susceptible *Helicoverpa armigera* strain (Bird & Downes, 2014). The LC_{50} to chlorantraniliprole was low, as were those to emamectin benzoate and indoxacarb, which were similar to results of a previous study on *H. armigera* and could

be considered as the susceptible baseline (Bird, 2015) (Figure 6). Resistance levels of the two populations to pyrethroids and organophosphate pesticides were very high; in particular, resistance ratios to chlorpyrifos of the two populations were more than 300-fold compared to a laboratory susceptible fall armyworm population that was sampled in 1975 (Yu, 1991) (Figure 5b). These results provide a susceptible baseline for fall armyworm populations invading China to different pesticides, which can provide guidance for resistance monitoring and pesticide management strategies.

3.4 | Fall armyworm invading China are currently sensitive to Bt toxin in a field-evolved experiment

The insertion of 2 bp in the *ABCC2* gene of fall armyworm was reported to cause a frame-shift mutation and results in resistance to Cry1Fa (Banerjee et al., 2017). We did not detect the same insertion mutation in 107 resequenced samples nor in 173 samples screened by using PCR and Sanger sequencing. Although the percentage of SNPs in the CDS region of other Bt receptors such as *SR-C* (scavenger receptor class C gene, a specific receptor for Vip3Aa in Sf9 cells), *TSPAN1* and other ABC gene families related to Cry toxin were also very high (Table S8), no reported resistant mutation was found in any target resistance genes.

(a)

Genotyping of whole mitochondrial genome sequences

(b)

Genotyping of full-length *Tpi* gene

(c)

**Genotyping of whole genome SNPs**

(d)

FIGURE 3 Genetic background of 107 fall armyworm samples. (a) Genotyping based on 208 mitochondrial SNP loci. From left to right, the leftmost two samples are ASW (the American corn strain) and AXE (the American rice strain), four African strains (AFR4 and 5 from Zambia, then AFR14 and 15 from Malawi), and 103 strains from China; the order of each sample is consistent with Table S1. (b) Genotyping based on 22 SNP loci in the *Tpi* gene. (c) Genotyping based on 707,353 genome SNP loci. (d) Principal component analysis (PCA) based on 5,998,089 whole-genome SNPs. Colour codes indicate samples from different sources; the two samples at the bottom in red are African samples AFR4 and AFR5
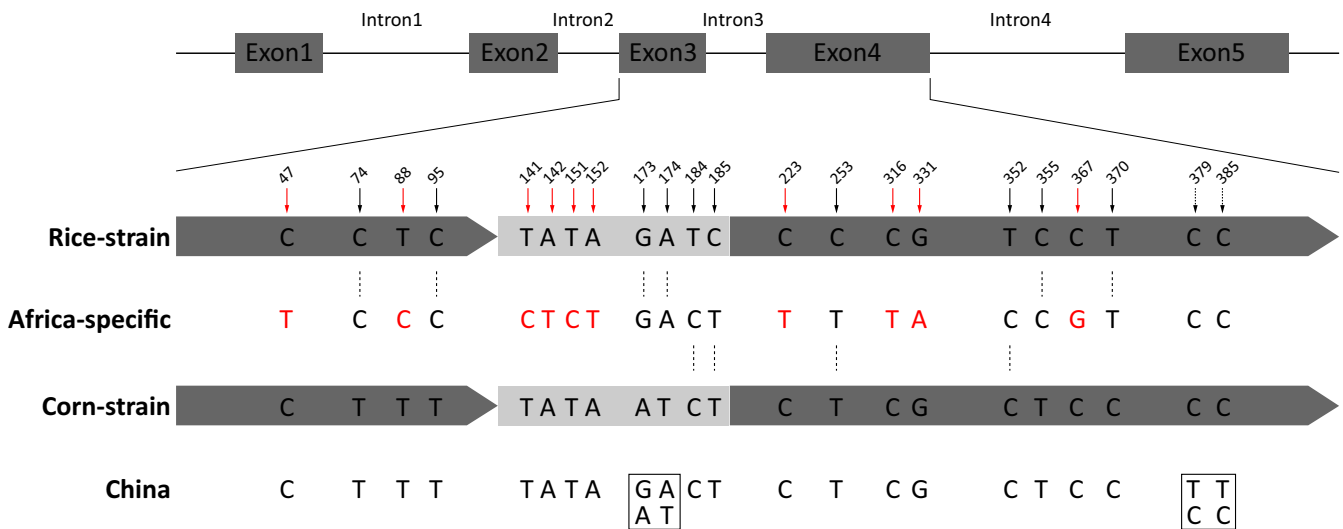
FIGURE 4 Diagram of the *Tpi* gene segments with respect to consensus Western Hemisphere sequences and the haplotypes observed in samples collected from Africa and China. Black solid arrows indicate 10 SNPs used to identify the American R-strain and C-strain fall armyworm, in which P370 was considered to be an effective diagnostic marker. Red solid arrows indicate 10 SNPs specific to the Africa-specific strain. The boxes represent two variable loci in some Chinese samples, including homozygous or heterozygous genotypes
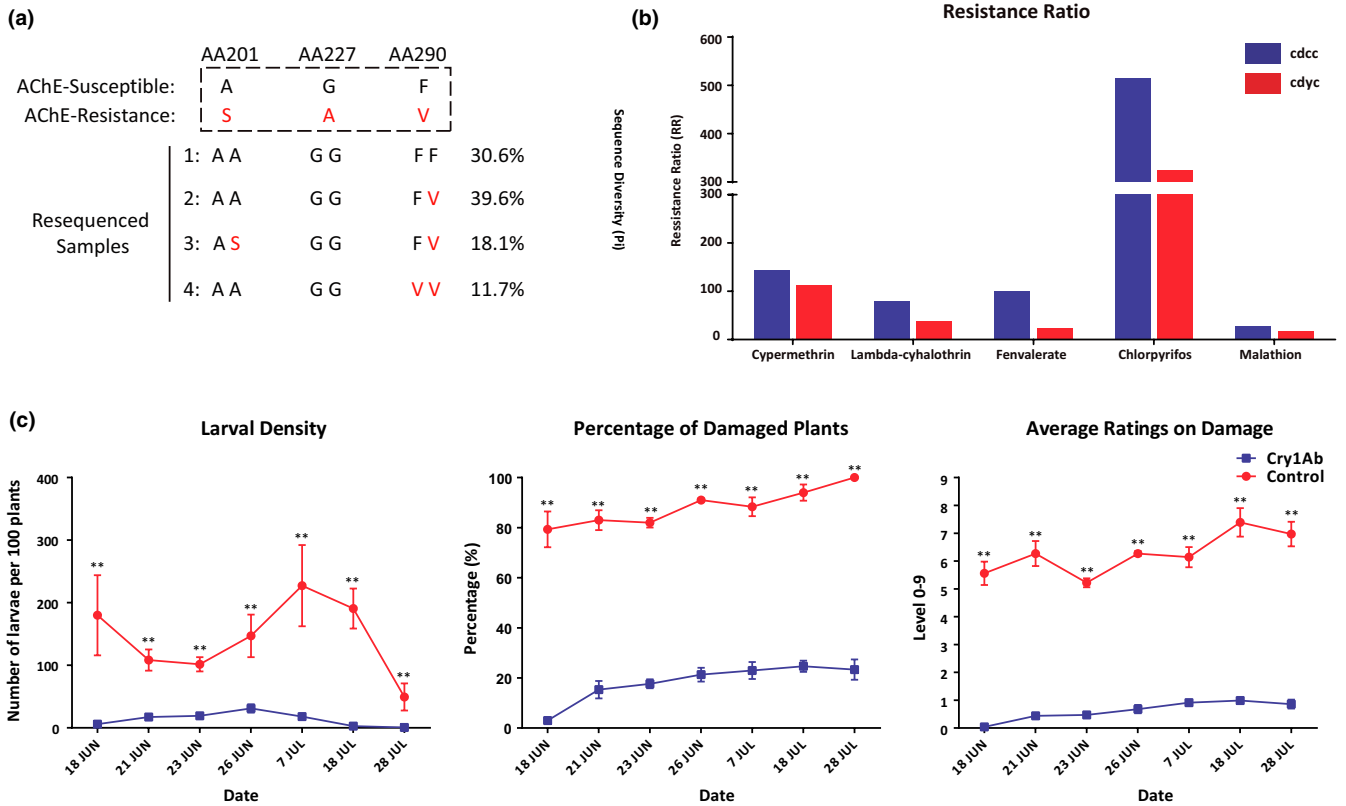
FIGURE 5 Genome scans and bioassays of fall armyworm for insecticide resistance. (a) Genotype and resistance mutation sites of the AChE gene in fall armyworm populations in China. (b) The resistance ratios (RRs) of two Chinese fall armyworm populations to pyrethroid (cypermethrin, lambda-cyhalothrin, fenvalerate) and organophosphate (chlorpyrifos, malathion) insecticides; cdcc and cdyc represent two inbred strains collected from Yunnan Province in China. RRs were calculated from the LD_{50} (μg/g) of a field population over the LD_{50} of a susceptible population as in Yu (1991). (c) Resistance tests of GM maize and non-GM maize to fall armyworm in field experiments. Error bars are the SD ($n = 15$), and asterisks indicate significant differences based on Student's t test (**$p < .01$)
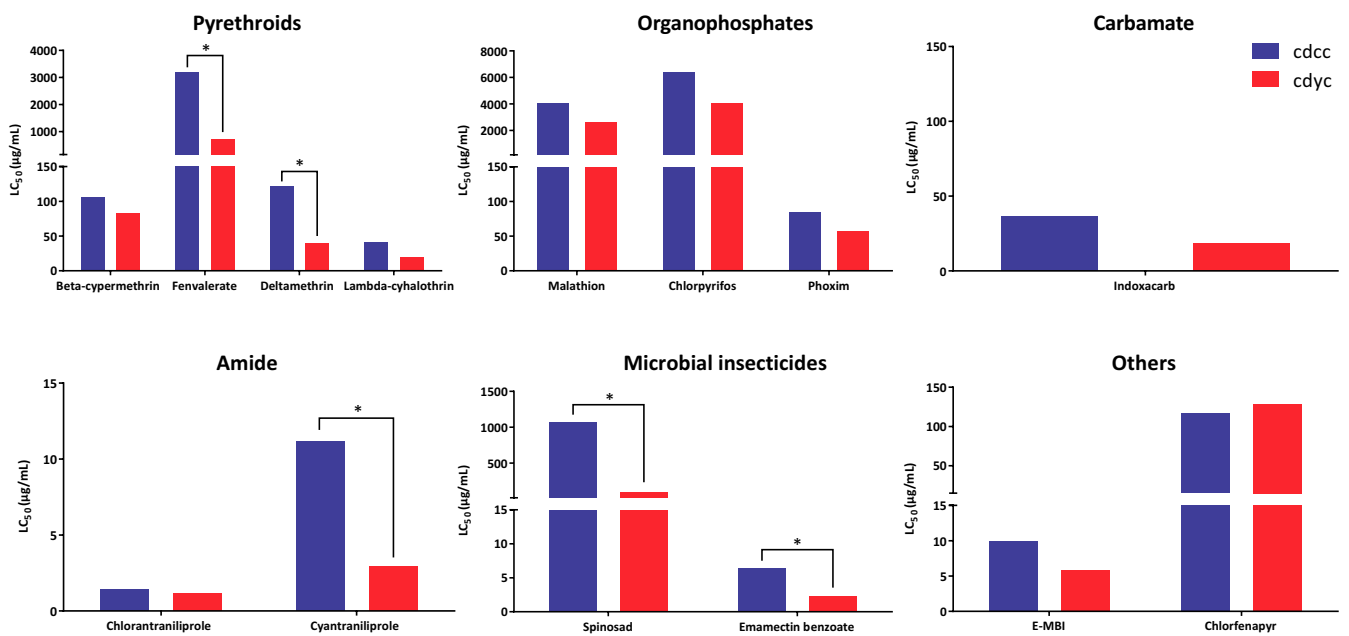
FIGURE 6 The LC_{50} values of two Chinese fall armyworm populations to different kinds of insecticides. cdcc and cdyc represent two inbred strains collected from Yunnan Province in China. Statistical significance of the difference was assessed by whether the 95% fiducial limits overlap (*$p < .05$)

Field tests showed that fall armyworm samples invading China were sensitive to GM maize expressing Cry1Ab compared with the control group. Damage assessment on larval density, the percentage of damaged plants and average damage ratings of GM maize were significantly lower than those of the control group (Figure 5c), indicating that the GM maize expressing Cry1Ab currently has good control effects on the invading population of fall armyworm in China.

3.5 | Insertion of mitochondrial fragments into the nuclear genome in a recent evolution event

We found that two mitochondrial fragments, with sequence lengths of 1.5 kb (partial *COI* gene and *NADH2* gene) and 1.6 kb (partial *NADH2* gene and 12S rRNA gene), were inserted into the nuclear genome, separated by a 4.1-kb segment of the nuclear genome (Figure 2b). The total length of a ~ 7.3-kb fragment, including two inserted fragments, was supported by more than 28 raw reads of PacBio data. The lengths of all 28 reads were longer than 20 kb and completely covered the 7.3-kb fragment. However, the two insertions were not found in other published fall armyworm genomes. To verify the accuracy of this result, we designed four primers based on flanking sequences of four connection points (Gap1–4 in Figure 2b), and the results of PCR amplification confirmed the existence of the insertion. The same primers were used in PCR assays to detect the insertion in 173 fall armyworm samples and it was found that the insertion was present in only 26.0% of all samples (Table S2). At the same time, the resequencing data of 107 fall armyworm samples in this study also showed that there were varying numbers of reads covering the four junction points in 29 samples, and the percentage of samples with inserted reads was 27.1% (Table S9). Both the PCR and resequencing results showed that the insertion was not present in all samples, perhaps suggesting that it has a recent evolutionary origin.

Moreover, the genotype of the two inserted mitochondrial fragments was identical to that of the C-strain, indicating that the insertion occurred after differentiation of the R- and C-strains. Further analysis indicated that the two mitochondrial fragments were inserted into the intron region of the lysine-specific demethylase 3 B (*Kdm3B*) gene, which is not likely to affect expression of the gene. The inserted partial *COI* and *NADH2* gene fragments were also considered likely to be functionless.

4 | DISCUSSION

The rapid spread of the fall armyworm has attracted popular attention worldwide. Accurate identification of its genetic characteristics (strain and pesticide resistance properties) has a direct and practical importance in terms of risk assessment and control strategies. A genome-wide analysis can reveal more in-depth genetic information than conventional gene-level analyses. The results of this study show that the fall armyworm invading China has a genetic background dominated by American corn-strain genotypes. Most of the fall armyworm samples invading China were detected and collected from corn and sugarcane, which are more likely to show the characteristics of C-strain host plants. Along the invasion path of the migratory fall armyworm, there are large-scale rice planting areas in Southeast Asia and central China, although there are few reports of serious damage to rice caused by fall armyworm (http://www.fao.org/fall-armyworm). The established R-strain fall armyworm in the Americas mainly feeds on turf grass, and there were few reports of damage to rice in 1970s (Bowling, 1978; Gallego, 1967). In addition, the established R-strain *Tpi* genotype has not been detected in any of the samples collected from Africa or Asia. We therefore speculate that the American R-strain fall armyworm did not invade Africa or Asia, including China.

In our study, 103 resequenced Chinese samples were collected from different regions of 50 cities distributed across 16 provinces (Figure S1). The collection time and sites coincided almost perfectly with the spreading invasion of fall armyworm in China. However, there was no obvious correlation between the time or site of collection and the genetic structure of the fall armyworm population (Figure 3). Almost all samples have similar genomic backgrounds, which suggests that the invading population may originate from a single genetic source and there is no evidence for genomic selection during the invasion.

According to our results, commonly used strain identification of fall armyworms by mitochondrial or *Tpi* markers is limited or even inaccurate. The nuclear insertion of two C-strain partial *COI* fragments in this study further underlines the need for caution in interpreting mitochondrial genotypes. We also found that the AT/GC SNP located at *Tpi*-intron3 (P173/174) was inadequate as a diagnostic marker. In addition, the TT/CC SNP located at *Tpi*-exon4 (P379/385) was associated with sequence variation in *Tpi*-intron4 (Figure 4; Figure S2), which could further be developed as a marker to subdivide C-strain samples. It is noteworthy that a particular (Africa-specific) haplotype of the *Tpi* gene originally identified in Africa was tentatively designated as R-strain based on the E4[183] site (equal to P370 in Figure 4 in this study) in previous studies (Nagoshi, 2012). Our genome-wide SNP analysis revealed that this haplotype contained more C-strain SNPs than R-strain SNPs.

The sample used for the genome sequencing in this study represents a combination of the particular *Tpi* haplotype and C-strain *COI*. We also found combinations of the R-strain *COI* and particular *Tpi* (sample G-XW13), as well as heterozygous forms of the particular *Tpi* and *Tpi*-C with the R-strain *COI* in two samples (G-GXW11, G-EP6). These combinations of different genotypes show that the genetic boundaries between two established (American) R- and C-strains are obscure. The insertion of two mitochondrial fragments into the nuclear genome might be caused by random hybridization between different genotypes, which would suggest that fall armyworm invading China might be descendants of an interstrain hybrid population. This is the first report of DNA fragments transferred from mitochondria into the nuclear genome in a *Spodoptera* lineage, and two such fragments could be used to develop markers to identify specific populations and to follow further evolutionary events of fall armyworm.

The rapid evolution of insecticide resistance and the increasing levels of resistance observed in fall armyworm populations needs attention. In this study, reported mutations related to insecticide resistance were detected in the AChE gene. Although some mutation sites were detected as heterozygous in most samples, the frequency of resistant mutation sites will increase greatly under the selection pressure caused by application of related pesticides in the field. The bioassay results showed that armyworms invading China have evolved high levels of resistance to organophosphate pesticides, which was consistent with the results of molecular scanning of resistance-related genes, yet the resistance to pyrethroid pesticides cannot be explained by any reported mechanism. However, the fall armyworms invading China are currently sensitive to GM maize expressing Cry1Ab in field experiments, and are also sensitive to other Bt toxins in the laboratory, according to previous studies (Li et al., 2019). At present, GM maize shows better application prospects in controlling fall armyworm in China, as larval density and damage rate of GM maize were significantly less than that of non-GM plants, although this crop is currently not registered for use in China.

This study provides a high-quality reference genome that demonstrates a genomic feature different from the established (American) C- or R-strain genotypes, as well as more comprehensive gene annotation. We also present resequencing data for 103 fall armyworm individuals invading China. The samples cover different regions and times during 2019, providing basic materials for analysing global population genetic and identifying patterns of invasiveness. Baseline resistance data for Chinese fall armyworm populations are shown to 14 common pesticides, providing guidance for the control and resistance monitoring of fall armyworm. Small-scale field experiments in this study suggest that fall armyworm in China are currently susceptible to GM maize, and these results could provide an important application reference for commercial planting of Bt maize in China. There are other important issues that remain for further exploitation using this whole genome approach, such as identifying the genes involved in polyphagy, migratory capability and olfaction, which could provide valuable tools for the future management of fall armyworms.

AUTHOR CONTRIBUTIONS

Y.X., K.W., W.Q. and W.F. conceived the project, designed content and managed the project; L.Z. and G.W. coordinated the project; B.L., Z.L. and X. Liu. performed read mapping, SNP calling and population analysis; W.Z. and C.L. performed genome assembly and annotation; L.Z. and B.L. performed Hi-C assembly; D.Z. performed the laboratory bioassay; S.Z. performed the field experiment; P.X. performed transcriptome analysis; K.N. and E.A. provided the raw data of American R- and C-strains; B. Liu., X.L., M.J., C.W. and X.Y. performed the DNA extraction, PCR and sequence variation analysis; W.Q. constructed DNA libraries, performed sequencing; Y.J. and J.L. collected and provided samples from China; L.Z. wrote the manuscript; K.W., A.W., C.M.J., J.A.S., G.C., D.L.K. and S.C. revised the manuscript. All authors commented on the manuscript.

ORCID

Lei Zhang https://orcid.org/0000-0003-0809-5836
Kenneth Wilson https://orcid.org/0000-0001-5264-6522
Christopher M. Jones https://orcid.org/0000-0002-6504-6224
Swapan Chakrabarty https://orcid.org/0000-0001-7096-5690
Wei Fan https://orcid.org/0000-0001-5036-8733

REFERENCES

Armes, N. J., Jadhav, D. R., Bond, G. S., & King, A. B. (1992). Insecticide resistance in *Helicoverpa armigera* in South India. *Pesticide Science*, *34*(4), 355–364.

Banerjee, R., Hasler, J., Meagher, R., Nagoshi, R., Hietala, L., Huang, F., … Jurat-Fuentes, J. L. (2017). Mechanism and DNA-based detection of field-evolved resistance to transgenic Bt corn in fall armyworm (*Spodoptera frugiperda*). *Scientific Reports*, *7*(1), 1–10. https://doi.org/10.1038/s41598-017-09866-y

Benson, G. (1999). Tandem repeats finder: A program to analyze DNA sequences. *Nucleic Acids Research*, *27*(2), 573–580. https://doi.org/10.1093/nar/27.2.573

Bernardi, O., Bernardi, D., Ribeiro, R. S., Okuma, D. M., Salmeron, E., Fatoretto, J., … Omoto, C. (2015). Frequency of resistance to Vip3Aa20 toxin from Bacillus thuringiensis in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations in Brazil. *Crop Protection*, *76*, 7–14. https://doi.org/10.1016/j.cropro.2015.06.006

Bird, L. J. (2015). Baseline Susceptibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to Indoxacarb, Emamectin Benzoate, and Chlorantraniliprole in Australia. *Journal of Economic Entomology*, *108*(1), 294–300. https://doi.org/10.1093/jee/tou042

Bird, L. J., & Downes, S. (2014). Toxicity and Cross-Resistance of Insecticides to Cry2Ab-Resistant and Cry2Ab-Susceptible *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, *107*(5), 1923–1930.

Birney, E., Clamp, M., & Durbin, R. (2004). GeneWise and genomewise. *Genome Research*, *14*, 988–995. https://doi.org/10.1101/gr.1865504

Boaventura, D., Bolzan, A., Padovez, F. E., Okuma, D. M., Omoto, C., & Nauen, R. (2020). Detection of a ryanodine receptor target-site mutation in diamide insecticide resistant fall armyworm, *Spodoptera frugiperda*. *Pest Management Science*, *76*(1), 47–54.

Bowling, C. C. (1978). Simulated insect damage to rice: Effects of leaf removal. *Journal of Economic Entomology*, *71*(2), 377–378. https://doi.org/10.1093/jee/71.2.377

Burton, J. N., Adey, A., Patwardhan, R. P., Qiu, R., Kitzman, J. O., & Shendure, J. (2013). Chromosome-scale scaffolding of de novo genome assemblies based on chromatin interactions. *Nature Biotechnology*, 31(12), 1119–1127. https://doi.org/10.1038/nbt.2727

Carvalho, R. A., Omoto, C., Field, L. M., Williamson, M. S., & Bass, C. (2013). Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*. *PLoS One*, 8(4), e62268. https://doi.org/10.1371/journal.pone.0062268

Chandrasena, D. I., Signorini, A. M., Abratti, G., Storer, N. P., Olaciregui, M. L., Alves, A. P., & Pilcher, C. D. (2018). Characterization of field-evolved resistance to Bacillus thuringiensis-derived Cry1F δ- endotoxin in *Spodoptera frugiperda* populations from Argentina. *Pest Management Science*, 74(3), 746–754.

Dasmahapatra, K. K., Walters, J. R., Briscoe, A. D., Davey, J. W., Whibley, A., Nadeau, N. J., ... Salazar, C. (2012). Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature*, 487(7405), 94–98.

Davis, F. M., Ng, S. S., & Williams, W. P. (1992). Visual rating scales for screening whorl-stage corn for resistance to fall armyworm. *Technical bulletin-Mississippi Agricultural and Forestry. Experiment Station(USA)*, 186, 1–9.

Dumas, P., Legeai, F., Lemaitre, C., Scaon, E., Orsucci, M., Labadie, K., ... Aury, J. M. (2015). *Spodoptera frugiperda* (Lepidoptera: Noctuidae) host-plant variants: Two host strains or two distinct species? *Genetica*, 143(3), 305–316. https://doi.org/10.1007/s10709-015-9829-2

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32(5), 1792–1797. https://doi.org/10.1093/nar/gkh340

Gallego, F. L. (1967). Lista preliminar de insectos de importancia económica y secundarios, que afectan los principales cultivos, animales domésticos y al hombre, en colombia. *Revista Facultad Nacional De Agronomía Medellín*, 26(65), 32–66.

Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A., & Tamò, M. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS One*, 11(10), e0165632.

Gouin, A., Bretaudeau, A., Nam, K., Gimenez, S., Aury, J. M., Duvic, B., ... Kuwar, S. (2017). Two genomes of highly polyphagous lepidopteran pests (*Spodoptera frugiperda*, Noctuidae) with different host-plant ranges. *Scientific Reports*, 7(1), 1–12. https://doi.org/10.1038/s41598-017-10461-4

Gutiérrez-Moreno, R., Mota-Sanchez, D., Blanco, C. A., Whalon, M. E., Terán-Santofimio, H., Rodriguez-Maciel, J. C., & DiFonzo, C. (2019). Field-evolved resistance of the fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. *Journal of Economic Entomology*, 112(2), 792–802. https://doi.org/10.1093/jee/toy372

Haas, B. J., Salzberg, S. L., Zhu, W., Pertea, M., Allen, J. E., Orvis, J., ... Wortman, J. R. (2008). Automated eukaryotic gene structure annotation using EVidenceModeler and the Program to Assemble Spliced Alignments. *Genome Biology*, 9(1), 1–22. https://doi.org/10.1186/gb-2008-9-1-r7

Johnson, S. J. (1987). Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda* in the Western Hemisphere. *International Journal of Tropical Insect Science*, 8(4-5-6), 543–549. https://doi.org/10.1017/S1742758400022591

Juárez, M. L., Schöfl, G., Vera, M. T., Vilardi, J. C., Múrua, M. G., Willink, E., ... Groot, A. T. (2014). Population structure of *Spodoptera frugiperda* maize and rice host forms in South America: Are they host strains? *Entomologia Experimentalis Et Applicata*, 152(3), 182–199.

Kakumani, P. K., Malhotra, P., Mukherjee, S. K., & Bhatnagar, R. K. (2014). A draft genome assembly of the army worm, *Spodoptera frugiperda*. *Genomics*, 104(2), 134–143. https://doi.org/10.1016/j.ygeno.2014.06.005

Kanost, M. R., Arrese, E. L., Cao, X., Chen, Y. R., Chellapilla, S., Goldsmith, M. R., ... Papanicolaou, A. (2016). Multifaceted biological insights from a draft genome sequence of the tobacco hornworm moth, *Manduca sexta*. *Insect Biochemistry and Molecular Biology*, 76, 118–147. https://doi.org/10.1016/j.ibmb.2016.07.005

Kurtz, S., Phillippy, A., Delcher, A. L., Smoot, M., Shumway, M., Antonescu, C., & Salzberg, S. L. (2004). Versatile and open software for comparing large genomes. *Genome Biology*, 5(2), 1–9.

Lämmermann, K., Vogel, H., & Traut, W. (2016). The mitochondrial genome of the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae), and identification of invading mitochondrial sequences (numts) in the W chromosome. *European Journal of Entomology*, 113, 482–488. https://doi.org/10.14411/eje.2016.063

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, 9(4), 357–359. https://doi.org/10.1038/nmeth.1923

Leibee, G. L., & Capinera, J. L. (1995). Pesticide resistance in Florida insects limits management options. *Florida Entomologist*, 78, 386–399. https://doi.org/10.2307/3495525

Li, G. P., Ji, T., Sun, X., Jiang, Y., Wu, K., & Feng, H. Q. (2019). Susceptibility evaluation of invaded *Spodoptera frugiperda* population in Yunnan province to five Bt proteins. *Plant Protection*, 45(3), 15–20.

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*, 27(21), 2987–2993. https://doi.org/10.1093/bioinformatics/btr509

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, 25(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., ... Durbin, R. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, 25(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Li, R., Fan, W., Tian, G., Zhu, H., He, L., Cai, J., ... Wang, J. (2010). The sequence and de novo assembly of the giant panda genome. *Nature*, 463(7279), 311–317.

Lima, E. R., & McNeil, J. N. (2009). Female sex pheromones in the host races and hybrids of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Chemoecology*, 19(1), 29–36. https://doi.org/10.1007/s00049-009-0005-y

Liu, H., Lan, T., Fang, D., Gui, F., Wang, H., Guo, W., ...Sahu, S. K. (2019). Chromosome level draft genomes of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), an alien invasive pest in China. *BioRxiv*, 671560. http://dx.doi.org/10.1101/671560

Luginbill, P. (1928). The fall armyworm. U.S. Dept. Agric. Tech. Bull, 34, 1–91.

Meagher, R. L. Jr, & Gallo-Meagher, M. (2003). Identifying host strains of fall armyworm (Lepidoptera: Noctuidae) in Florida using mitochondrial markers. *Florida Entomologist*, 86(4), 450–455. https://doi.org/10.1653/0015-4040(2003)086[0450:IHSOFA]2.0.CO;2

Mitchell, E. R., McNeil, J. N., Westbrook, J. K., Silvain, J. F., Lalanne-Cassou, B., Chalfant, R. B., ... Proshold, F. I. (1991). Seasonal periodicity of fall armyworm, (Lepidoptera: Noctuidae) in the Caribbean basin and northward to Canada. *Journal of Entomological Science*, 26(1), 39–50. https://doi.org/10.18474/0749-8004-26.1.39

Monnerat, R., Martins, E., Macedo, C., Queiroz, P., Praca, L., Soares, C. M., ... Bravo, A. (2015). Evidence of field-evolved resistance of *Spodoptera frugiperda* to Bt corn expressing Cry1F in Brazil that is still sensitive to modified Bt toxins. *PLoS One*, 10(4), e0119544. https://doi.org/10.1371/journal.pone.0119544

Nagoshi, R. N. (2012). Improvements in the identification of strains facilitate population studies of fall armyworm subgroups. *Annals of the Entomological Society of America*, 105(2), 351–358. https://doi.org/10.1603/AN11138

Nagoshi, R. N. (2019). Evidence that a major subpopulation of fall armyworm found in the Western Hemisphere is rare or absent in Africa, which may limit the range of crops at risk of infestation. *PLoS One*, *14*(4), e0208966. https://doi.org/10.1371/journal.pone.0208966

Nagoshi, R. N., Goergen, G., Du Plessis, H., van den Berg, J., & Meagher, R. (2019). Genetic comparisons of fall armyworm populations from 11 countries spanning sub-Saharan Africa provide insights into strain composition and migratory behaviors. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-44744-9

Nagoshi, R. N., Htain, N. N., Boughton, D., Zhang, L., Xiao, Y., Nagoshi, B. Y., & Motasanchez, D. (2020). Southeastern Asia fall armyworms are closely related to populations in Africa and India, consistent with common origin and recent migration. *Scientific Reports*, *10*(1), 1–10. https://doi.org/10.1038/s41598-020-58249-3

Nagoshi, R. N., Koffi, D., Agboka, K., Tounou, K. A., Banerjee, R., Jurat-Fuentes, J. L., & Meagher, R. L. (2017). Comparative molecular analyses of invasive fall armyworm in Togo reveal strong similarities to populations from the eastern United States and the Greater Antilles. *PLoS One*, *12*(7), e0181982. https://doi.org/10.1371/journal.pone.0181982

Nagoshi, R. N., Meagher, R. L., Nuessly, G., & Hall, D. G. (2006). Effects of fall armyworm (Lepidoptera: Noctuidae) interstrain mating in wild populations. *Environmental Entomology*, *35*(2), 561–568. https://doi.org/10.1603/0046-225X-35.2.561

Nam, K., Gimenez, S., Hilliou, F., Blanco, C. A., Hänniger, S., Bretaudeau, A., ...d'Alençon, E. (2019). Adaptation by copy number variation increases insecticide resistance in fall armyworms. *BioRxiv*. 812958. https://doi.org/10.1101/812958

Nandakumar, S., Ma, H., & Khan, A. S. (2017). Whole-genome sequence of the *Spodoptera frugiperda* Sf9 insect cell line. *Genome Announcements*, *5*(34), e00829–17. https://doi.org/10.1128/genomeA.00829-17

Pashley, D. P. (1986). Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): A sibling species complex? *Annals of the Entomological Society of America*, *79*(6), 898–904. https://doi.org/10.1093/aesa/79.6.898

Pashley, D. P., Hammond, A. M., & Hardy, T. N. (1992). Reproductive isolating mechanisms in fall armyworm host strains (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, *85*(4), 400–405. https://doi.org/10.1093/aesa/85.4.400

Pashley, D. P., & Martin, J. A. (1987). Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, *80*(6), 731–733. https://doi.org/10.1093/aesa/80.6.731

Pearce, S. L., Clarke, D. F., East, P. D., Elfekih, S., Gordon, K. H. J., Jermiin, L. S., ... Rane, R. V. (2017). Genomic innovations, transcriptional plasticity and gene loss underlying the evolution and divergence of two highly polyphagous and invasive *Helicoverpa* pest species. *BMC Biology*, *15*(1), 63. https://doi.org/10.1186/s12915-017-0402-6

Ruan, J., & Li, H. (2019). Fast and accurate long-read assembly with wtdbg2. *Nature Methods*, *17*, 1–4. https://doi.org/10.1038/s41592-019-0669-3

Russell, R. M., Robertson, J. L., & Savin, N. E. (1977). POLO: A new computer program for probit analysis. *Bulletin of the ESA*, *23*(3), 209–213. https://doi.org/10.1093/besa/23.3.209

Servant, N., Varoquaux, N., Lajoie, B. R., Viara, E., Chen, C., Vert, J., ... Barillot, E. (2015). HiC-Pro: An optimized and flexible pipeline for Hi-C data processing. *Genome Biology*, *16*(1), 259. https://doi.org/10.1186/s13059-015-0831-x

She, R., Chu, J. S. C., Wang, K., Pei, J., & Chen, N. S. (2009). genBlastA: Enabling BLAST to identify homologous gene sequences. *Genome Research*, *19*, 143–149. https://doi.org/10.1101/gr.082081.108

Signorini, A. M., Abratti, G., Grimi, D., Machado, M., Bunge, F. F., Parody, B., ... Araujo, M. P. (2018). Management of field-evolved resistance to Bt maize in Argentina: A multi-institutional approach. *Frontiers in Bioengineering and Biotechnology*, *6*(67), 1–5. https://doi.org/10.3389/fbioe.2018.00067

Simao, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annota- tion completeness with single-copy orthologs. *Bioinformatics*, *31*, 3210–3212. https://doi.org/10.1093/bioinformatics/btv351

Stanke, M., & Waack, S. (2003). Gene prediction with a hidden Markov model and a new intron submodel. *Bioinformatics*, *19*, 215–225. https://doi.org/10.1093/bioinformatics/btg1080

Storer, N. P., Babcock, J. M., Schlenz, M., Meade, T., Thompson, G. D., Bing, J. W., & Huckaba, R. M. (2010). Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. *Journal of Economic Entomology*, *103*(4), 1031–1038.

Trapnell, C., Roberts, A., Goff, L. A., Pertea, G., Kim, D., Kelley, D. R., ... Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature Protocols*, *7*(3), 562–578. https://doi.org/10.1038/nprot.2012.016

Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., ... Earl, A. M. (2014). Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly im- provement. *PLoS One*, *9*, e112963. https://doi.org/10.1371/journal.pone.0112963

Wan, F., Yin, C., Tang, R., Chen, M., Wu, Q., Huang, C., ... Wang, G. (2019). A chromosome-level genome assembly of *Cydia pomonella* provides insights into chemical ecology and insecticide resistance. *Nature Communications*, *10*(1), 1–14. https://doi.org/10.1038/s41467-019-12175-9

Westbrook, J. K., Nagoshi, R. N., Meagher, R. L., Fleischer, S. J., & Jairam, S. (2016). Modeling seasonal migration of fall armyworm moths. *International Journal of Biometeorology*, *60*(2), 255–267. https://doi.org/10.1007/s00484-015-1022-x

Williams, W. P., Buckley, P. M., & Daves, C. A. (2006). Identifying resistance in corn to southwestern corn borer (Lepidoptera: Crambidae), fall armyworm (Lepidoptera: Noctuidae), and corn earworm (Lepidoptera: Noctuidae). *Journal of Agricultural and Urban Entomology*, *23*, 87–95.

Wiseman, B. R., & Widstrom, N. W. (1984). Fall armyworm damage ratings on corn at various infestation levels and plant development stages. *Journal of Agricultural Entomology*, *1*(2), 115–119.

Xia, Q. Y., Wang, J., Zhou, Z. Y., Li, R. Q., Fan, W., Cheng, D. J., ... Xiang, Z. H. (2008). The genome of a lepidopteran model insect, the silkworm *Bombyx mori*. *Insect Biochemistry and Molecular Biology*, *38*(12), 1036–1045. https://doi.org/10.1016/j.ibmb.2008.11.004

Yang, F., Morsello, S., Head, G. P., Sansone, C., Huang, F., Gilreath, R. T., & Kerns, D. L. (2018). F2 screen, inheritance and cross-resistance of field-derived Vip3A resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected from Louisiana, USA. *Pest Management Science*, *74*(8), 1769–1778.

Yang, J., Lee, S. H., Goddard, M. E., & Visscher, P. M. (2011). GCTA: A tool for genome-wide complex trait analysis. *The American Journal of Human Genetics*, *88*(1), 76–82.

You, M., Yue, Z., He, W., Yang, X., Yang, G., Xie, M., ... Douglas, C. J. (2013). A heterozygous moth genome provides insights into herbivory and detoxification. *Nature Genetics*, *45*(2), 220–227. https://doi.org/10.1038/ng.2524

Yu, S. J. (1991). Insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). *Pesticide Biochemistry and Physiology*, *39*(1), 84–91. https://doi.org/10.1016/0048-3575(91)90216-9

Yu, S. J., Nguyen, S. N., & Abo-Elghar, G. E. (2003). Biochemical characteristics of insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). *Pesticide Biochemistry and Physiology*, *77*(1), 1–11. https://doi.org/10.1016/S0048-3575(03)00079-8

Zhan, S., Merlin, C., Boore, J. L., & Reppert, S. M. (2011). The monarch butterfly genome yields insights into long-distance migration. *Cell*, *147*(5), 1171–1185. https://doi.org/10.1016/j.cell.2011.09.052

Zhang, L., Liu, B., Jiang, Y., Liu, J., Wu, K., & Xiao, Y. (2019). Molecular characterization analysis of fall armyworm populations in China. *Plant Protection*, *45*, 10–27.

Zhu, Y. C., Blanco, C. A., Portilla, M., Adamczyk, J., Luttrell, R., & Huang, F. (2015). Evidence of multiple/cross resistance to Bt and organophosphate insecticides in Puerto Rico population of the fall armyworm, *Spodoptera frugiperda*. *Pesticide Biochemistry and Physiology*, *122*, 15–21. https://doi.org/10.1016/j.pestbp.2015.01.007

SUPPORTING INFORMATION

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