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1 Global FAW population genomic signature supports complex introduction events across the 2 Old World

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15

16 Abstract

17 Accurate genomic knowledge can elucidate the global spread patterns of invasive pests. The high-
18 profile invasive agricultural pest *Spodoptera frugiperda* (fall armyworm; FAW) is a case in point. Native to the
19 Americas, the FAW was first reported in West Africa in 2016 and has rapidly spread to over 64 countries across
20 the Old World, resulting in significant economic losses. The chronological order of reported detections has led
21 to the hypothesis that the FAW moved eastwards across Africa and then Asia, however genomic evidence
22 remains lacking to test this hypothesis and to identify the potential origin of invasive populations. Using a whole
23 genome sequencing approach, we explored the population genomic signatures of FAW populations from the
24 Americas and the Old World. Analyses of complete mitochondrial DNA genomes identified 12 maternal lineages
25 across the invasive range, while genomic signatures from 870 high-quality nuclear genome-wide single
26 nucleotide polymorphic (SNP) DNA markers identified five distinct New World populations that broadly reflected
27 their native geographical ranges and absence of host-plant preference status. Unique FAW populations in the
28 Old World were also identified that suggested multiple introductions underpinned their rapid global spread. We
29 identified in Asian FAW individuals, genomes lacking evidence of admixture; while analysis of identified complex
30 substructure revealed significant directional geneflow from Asia into East Africa, in contrast to a simple east-to-
31 west spread. Our study highlights the need for population genomics approaches in analysing complex pest
32 invasions, and the importance of international partnership to address global biosecurity challenges presented
33 by emerging high priority insect pests.

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40 **Running title:** FAW global spread by multiple introductions

41

42 **Key words:** Fall armyworm, global agricultural biosecurity, invasion biology, draft mitochondrial DNA genomes,
43 single nucleotide polymorphic markers

44

1 Introduction

2 Global agriculture is increasingly affected by the spread of invasive pests and diseases, which is often
3 assisted by global trade. A well-documented global invasion by an insect pest is that of the hemipteran sap-
4 sucking *Bemisia tabaci* cryptic MEAM1 and MED species¹ that spread from the species' endemic population
5 origins in the Middle East-Asia Minor and the Mediterranean region, respectively, to at least 54 countries².
6 Global agricultural trade has also been linked with the introductions of the highly polyphagous lepidopteran pest
7 *Helicoverpa armigera* from the Old World³ to South America^{4,5} and the Caribbean islands^{3,6-8}. This noctuid
8 moth's wide host range, flight ability⁹ and ability to develop resistance to insecticides¹⁰ has enabled
9 establishment across the New World with significant economic^{11,12}, and ecological consequences. Importantly,
10 the availability of samples from early stages in the invasion has made it possible to distinguish demographic from
11 adaptive evolutionary processes, as well as to study hybridization upon second contact with a sister species<sup>13-
12 16</sup>.

13 Long a significant pest of agriculture in its native New World range, the noctuid fall armyworm (FAW)
14 *Spodoptera frugiperda* was first reported in West Africa (Nigeria and São Tomé and Príncipe) in early 2016¹⁷,
15 followed by confirmation across central (Congo¹⁸; Togo¹⁹, Southern²⁰ and Eastern²¹) sub-Saharan Africa
16 between early 2017 to 2018²². The Middle East (Yemen²³) followed by India^{24,25} and surrounding nations (e.g.,
17 Bangladesh, Sri Lanka). Myanmar²⁶ and Thailand²⁷ also reported detection of the pest between August and
18 December 2018, followed by Southern China (Yunnan Province) in early January 2019²⁸⁻³⁰. Detections of FAW
19 since January 2019 have gathered speed: south-ward to Malaysia (March 2019) and Indonesia (Sumatra, April
20 2019; Java, July 2019; Kalimantan July 2019); Hong Kong (April 2019), Taiwan (May/June 2019); Laos and
21 Vietnam (April 2019³¹), the Philippines (June 2019^{32; 33}), South Korea (June 2019), and Japan (June 2019)³⁴.
22 Within China, the FAW has been reported in a northward expansion pattern from Yunnan to 18 provinces by
23 July 2019³⁵⁻³⁷. As of August 2019, a total of 64 African and Asian nations have reported FAW³⁸. In January 2020,
24 FAW was trapped in Australia's special biosecurity zone in the Torres Strait islands of Saibai and Erub, and
25 confirmed on 3 February 2020, and on mainland Australia in Bamaga on 18 February 2020^{39,40}.

26 This chronologically ordered eastward spread of detections led to a widely adopted assumption⁴¹ that the
27 FAW was actually spreading west-to-east across and then from Africa. Based on the detection timeline,
28 predictive simulations that assumed human-assisted spread, in particular agricultural trade, have modelled this
29 very vagile pest's movement from the east coast of America/the Greater Antilles to West Africa (e.g., Togo¹⁹)
30 between Central and Southern America and Africa, and between Africa and Asia (e.g., India, China; South East
31 Asia⁴²). The human-assisted spread model⁴² was also used to warn China and South East Asian nations of
32 imminent impact by FAW following confirmation of the pest in India⁴³. This model further forms the basis of
33 international research efforts to track the movement, including using molecular tools to examine invasion
34 biology (e.g.,^{19,44,45}), and simulations to model long distance dispersal (e.g.,^{42,46,47}). Indeed a meteorological
35 data-based simulation study (i.e., wind currents, monsoon wind patterns) concluded the Yunnan FAW
36 populations originated from Myanmar, consistent with FAW being officially reported earlier in Myanmar
37 (December 2018^{48,49}) than in China (January 2019³⁵). Other work has examined the impact and implications for
38 global plant health and agricultural biosecurity (e.g.,^{28,50}), integrated pest management (IPM) and bioeconomics
39⁵¹⁻⁵³, and insecticide resistance^{37,45,54}.

40 Genetic studies on the spread of FAW have focussed on single genes on the mitochondrial genome,
41 occasionally supported by a single partial nuclear gene marker. These markers have been widely used because,
42 throughout much of the native range, FAW populations consist of two morphologically identical host races, the
43 rice-preferred and corn-preferred *S. frugiperda* ('*Sfr*' and '*Sfc*', respectively), that have also been considered as
44 potential sister species (e.g.,^{21,55,56}). These two host races are supported by phylogenetic analyses based on
45 nuclear and mitochondrial DNA genomes⁴⁴, and partial mitochondrial DNA genes (e.g.,^{17,21,25,29,55}). The
46 distribution of these *Sfr* and *Sfc* populations in their New World range has only recently been investigated based
47 on partial mitochondrial and nuclear genes⁵⁷, while at the whole genome level they are less well-understood.
48 Genotypes from both host races/sister species are present in the invasive populations (e.g.,^{19,58-60}). Since 2010
49 (e.g.,^{61,62}) and especially in recent times during the FAW range expansion (e.g.,^{29,45,59,63}), the partial *Triose
50 Phosphate Isomerase (Tpi)* gene on the Z-chromosome has been adopted to aid in the clarification of the *Sfc* and
51 *Sfr* host race status. The *Tpi* marker relies on the presence of a single nucleotide polymorphic (SNP) marker at
52 position 183 (e.g.,^{62,63}) to differentiate between corn- or rice-preferred FAW. Similarly, inconclusive host
53 preferences based on the mtCOI gene marker also detected both *Sfc* and *Sfr* on corn-host plants (e.g.,²¹).
54 Contrary to the introduction patterns of the related and invasive noctuid *H. armigera* in South America⁴ which
55 showed significant genetic diversity (e.g.,^{3,5,15}) similar to that reported for global populations of *H. armigera*⁶⁴⁻

1 ⁶⁶, the current global partial mtCOI signatures of both *Sfc* and *Sfr* have been consistent with a single introduction,
2 which, when considered together with the *Tpi* locus, was suggested to likely have a Florida/Greater Antilles
3 source population ¹⁹.

4 What is missing from current research into the spread of FAW is analysis of broader genomic evidence.
5 Genome-wide single nucleotide polymorphic (SNP) markers aligned to well-annotated genomes can provide
6 powerful genomic evidence for understanding introduction pathways (e.g., ¹) and eliminate candidate
7 populations ¹⁴ as well as elucidate hybrid signatures ¹³. The genomes of both *Sfr* and *Sfc* have been sequenced
8 and annotated ⁴⁴, allowing higher resolution analysis of genetic structure, migration patterns and sub-species
9 status based on a high number of genome-wide SNPs to enable identification of the potential New World origins,
10 and the species and admixture status of the invasive *Sfc* and *Sfr* populations.

11 In this study, we provide an assessment of global FAW movement history based on genomic data that
12 incorporates populations from both Northern, Central, and Southern Americas, and the Caribbean (i.e.,
13 representing the original population range), Western and Eastern Africa, and Western and Eastern Asia,
14 representing the pest's Old World expansion. Here we reveal a multi-locus invasion that is likely independent of
15 the reported detection patterns and their timelines, and provide genomic-based evidence to support multiple
16 introductions of the FAW into the Old World, with significant movements of FAW detected between Asia and
17 Africa. We also re-evaluated the pest's global spread directionality to highlight implications in future
18 management of FAW, and the need for on-going global agricultural biosecurity research and cooperation to
19 improve preparedness for emerging invasive agricultural pest issues.

20

21 **Material and Methods**

22 *Spodoptera frugiperda* populations sampled and analysed in this study were sourced from Florida (n=24)
23 ⁶⁷, Mississippi (n=18) ⁴⁴, Puerto Rico (n=15) ⁶⁸, Peru (n=16), Brazil (n=12; IBAMA Permit number:
24 18BRO28445/DF), Mexico (n=10), Guadeloupe (n=4), French Guiana (n=3), Benin (n=4), India (n=12) ⁶⁹; Tanzania
25 (n=1), Uganda (n=15), Malawi (n=16), and three populations from Yunnan Province, China (CC=19; CY=12;
26 CX=15) ⁵⁴, and one individual (CH06) from Australia's pre-border interception program overseen by the
27 Department of Agriculture, Water and the Environment (DAWE), also from Yunnan, China (Suppl. Table 1). The
28 initial differentiation of these individuals as 'corn-preferred' or 'rice-preferred' was based on the partial mtDNA
29 COI gene region (see ⁵⁵) and a polymorphism within the Triose Phosphate Isomerase (*Tpi*) gene (see ⁶²).

30 Extraction of total genomic DNA was carried out at the CSIRO Black Mountain Laboratories site in Canberra
31 Australia for the Brazil, Peru, Tanzania, Malawi and Uganda populations, as well as the pre-border intercepted
32 FAW sample from China, using the Qiagen Blood and Tissue DNA extraction kit following instructions as
33 provided, with genomic DNA eluted in 200µL EB. Total genomic DNA for the other three Chinese populations
34 were extracted at Nanjing Agricultural University as detailed in Guan et al. ⁵⁴. Total genomic DNA from
35 Mississippi, Florida, Puerto Rico, Guadeloupe, Mexico, and French Guiana, and Indian populations was carried
36 out at INRAE DGIMI (Univ. Montpellier, INRAE, France) as described in Yainna et al. ⁶⁹.

37 Genomic libraries prepared by CSIRO were constructed using an Illumina Nextera Flex DNA Library Prep Kit
38 following manufacturer's instructions and sequenced by Illumina NovaSeq6000 S4 300 sequencing system at the
39 Australian Genome Research Facility (AGRF). Sequencing efforts were shared between three research
40 institutions: 61 samples were prepared at CSIRO (populations from Brazil, Peru, Uganda, Tanzania, and Malawi),
41 46 samples were prepared by NJAU for populations from China Yunnan Province (CC, CY and CX counties), and
42 89 samples were prepared by DGIMI, France (populations from Florida, Mississippi, Puerto Rico, Guadeloupe,
43 French Guiana, Mexico, Benin and India). The Peru FAW samples and the single FAW sample CH06 from Yunnan
44 China were intercepted at Australia's pre-border inspections of imported agricultural and horticultural
45 commodities by the Department of Agriculture, Water and the Environment (DAWE) on fresh vegetables and
46 cut flowers, respectively. The FAW CH06 was sequenced using the Illumina MiSeq high throughput sequencing
47 (HTS) platform following the methods as described by Tay et al. ⁷⁰.

48

49 Mitochondrial genomes assembly and haplotypes characterisation

50 The mitochondrial DNA genome for all samples were assembled using Geneious 11.1.5 based on strategies
51 previously used for assembly of *Helicoverpa* species as outlined in Walsh et al. ⁷¹. Assembled mitogenomes were
52 annotated using MITOS ⁷² selecting invertebrate mitochondrial genetic code. All annotated protein coding

1 genes/coding sequences (PCGs/CDS) were re-annotated visually to identify putative stop codons and to align
2 start codon positions. Four regions of low complexity (corresponding to BC55 nt6065-6092; nt9544-9580;
3 nt12807-12838; nt15047-15276) were trimmed due to alignment difficulties and low genome assembly
4 confidence associated with simple repeat units, resulting in all samples having final mitochondrial DNA genome
5 length of 15,059 bp. We identified unique mitogenome haplotypes using the DNACollapser in FaBox (1.5)
6 <<https://users-birc.au.dk/~palle/php/fabox/dnacollapser.php>> ⁷³ after alignment using MAFFT Alignment
7 v7.450 ^{74,75} within Geneious 11.1.5 and selecting the Auto option for Algorithm and 200PAM / K=2 for Scoring
8 matrix, Gap open penalty of 1.53, and offset value of 0.123.

9

10 SNPs selection

11 The reference genomes ⁴⁴ are from native populations, *Sfc* from Guadeloupe and *SfR* from Florida (see also
12 Yainna et al. ⁶⁹, and Nam et al. ⁶⁸ for high quality assemblies of native population genomes for *SfR*, and Nam et
13 al. ⁷⁶ for high quality genome assemblies of native *Sfc*). The reference genomes published recently by three
14 Chinese groups may differ since they are from invasive populations, from Yunnan in China ⁷⁷, from the Zhejiang
15 province of China ⁷⁸, and from 2017 sampled FAW individuals collected from maize fields in Lusaka, Zambia ⁴⁵.
16 In this study, we used the original assembled genome of Gouin et al. ⁴⁴ for our raw data processing. Genomic
17 raw data was cleaned and trimmed using Trimmomatic and aligned to the *S. frugiperda* (rice v1) genome ⁴⁴ using
18 BWA-MEM ⁷⁹. Variants were predicted using BMAP ⁸⁰ and indel's normalised using BCFtools ⁸¹ to obtain a whole
19 genome SNP panel. Variants were filtered to remove SNP's with minimum allele frequency of 0.01 and linkage
20 disequilibrium (LD) pruned to obtain 870 unlinked SNP's across all individuals.

21

22 Phylogeny analyses

23 Phylogenies based on trimmed partial mitochondrial DNA genomes of 15,059 bp and from genome-wide
24 SPSs were individually inferred using IQ-Tree <<http://iqtree.cibiv.univie.ac.at>> ⁸². For the nuclear SNPs, the panel
25 of 870 SNPs from each individual in fasta format was up-loaded to the IQ-Tree web server and selecting the
26 automatic substitution model option. For the mitochondrial DNA genome Maximum Likelihood (ML) phylogeny
27 was inferred with edge-linked partition for the 13 protein coding genes and excluding all four regions of low
28 complexity. We used the Ultrafast bootstrap analysis ⁸³ with 1,000 bootstrap alignments to assess branch
29 support for both mitochondrial DNA genome and nuclear SNPs phylogenies. Output consensus tree files in
30 Newick format were visualised and manipulated using Dendroscope version 3.5.7 ⁸⁴.

31

32 Genetic diversity and neutrality tests

33 Observed (H_{obs}) and expected (H_{exp}) heterozygosity were calculated for each population using the
34 populations program in Stacks ⁸⁵ and the ADEGENET package in R ^{86,87}. The number of loci departing significantly
35 from Hardy-Weinberg equilibrium (HWE) in the global population and individual populations was assessed using
36 PLINK 2.0 ⁸⁸ and VCFtools ⁸⁹. To test for neutrality, Tajima's D ⁹⁰ and Fu and Li's D* ⁹¹ were calculated for each
37 population using the PopGenome package in R ⁹². Nucleotide diversity (π) and Wright's inbreeding coefficient,
38 F_{IS} ⁹³, were calculated using the populations program in Stacks. Pairwise comparisons of weighted F_{ST} values
39 between populations were calculated using Genepop (v4.7.5 ⁹⁴) and differentiation between populations tested
40 for significance using the exact G test.

41

42 Population structure and migration

43 Principal component analysis (PCA) was performed using PLINK v1.9 ⁹⁵. Admixture was estimated using
44 Admixture v1.3.0 ⁹⁶. For plotting of networks, the R package NetView ^{97,98} was used. The network drawn using
45 the plotAdmixture function in this package is based on a Maximum Likelihood (ML) distance matrix calculated
46 from the IQ-Tree phylogeny shown in Fig. 3, using the R package 'ape' ⁹⁹.

47 To estimate directional gene flow between the populations, as well as the relative magnitudes of these
48 flows, the function divMigrate in the R package diveRsity ¹⁰⁰ online version was used
49 <<https://popgen.shinyapps.io/divMigrate-online/>> ¹⁰¹. Gene flows between all sites were calculated and then
50 normalized to obtain relative migration rates (between 0 and 1). The program divMigrate searches for gene flow
51 directionality between each pair of populations by identifying significant asymmetry using a hypothetically

1 defined pool of migrants for each pair. This pool is then compared to pairs in the overall population to calculate
 2 directional genetic differentiation and relative migration. To evaluate the significance of asymmetric migration,
 3 1,000 bootstraps were performed. Resulting migration matrices were then plotted using Gephi
 4 <<https://gephi.org/>> to generate network graphs. These show directional gene flows between populations
 5 (located at the nodes), with the thickness of the lines showing relative strength of gene flow.
 6

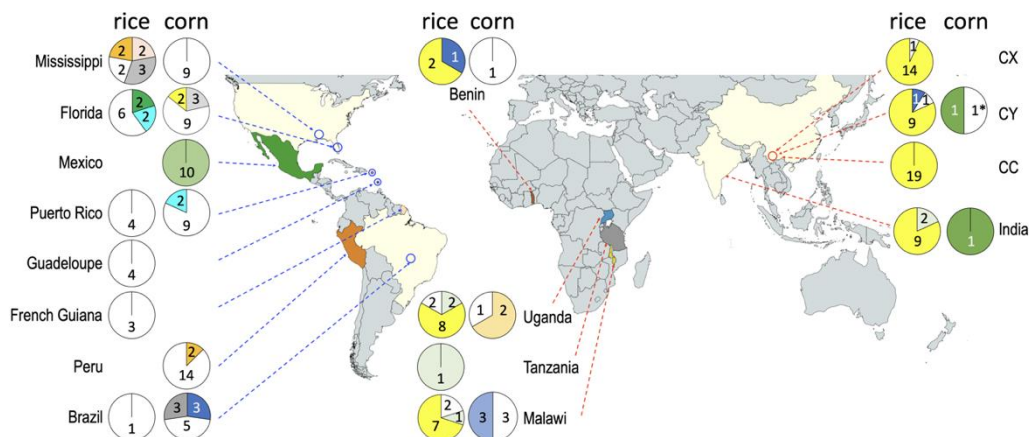
7 Results

8 A total of 197 FAW individuals were sequenced, 102 from the native New World range and 95 from the
 9 invasive Old World range (Fig. 1). From the pest's native range, we detected 25 'rice' mitochondrial genome (i.e.,
 10 mitogenome) haplotypes, and 51 'corn' mitogenome haplotypes. All FAW from Mexico and Peru had the 'corn'
 11 mitogenome while FAW from Guadeloupe and French Guiana were all 'rice' mitogenomes. Of the FAW from the
 12 invasive range nine 'corn' and 'rice' mitogenome haplotypes were identified; one of the 'corn' mitogenome
 13 haplotype (represented by green colour, Fig. 1) was shared between CY and Indian individuals. No African corn
 14 mitogenome haplotypes were shared with Asian FAW populations. In contrast, 83% (i.e., 68/82) of African and
 15 Asian FAW with 'rice' mitogenomes shared a common haplotype (represented by the yellow colour, Fig. 1). FAW
 16 individuals from China and Benin shared a rare rice mitogenome haplotype (blue colour haplotype), and
 17 individuals from Uganda, Tanzania, Malawi and India shared one unique (i.e., light green colour) haplotype (Fig.
 18 1). In general, the high diversity of haplotypes in both 'rice' and 'corn' in the native range and the lack of diversity
 19 in the invasive range is consistent with patterns observed in invasive populations that have a relatively small
 20 number of founders. GenBank accession numbers for full mitochondrial genomes from all individuals are listed
 21 in Suppl. Table 1.

22

23 **Fig. 1:** New and Old Worlds' FAW populations and proportions of mitochondrial DNA haplotypes based on 15,059
 24 bp of the mitochondrial DNA genomes and excluding four regions of low complexity. For the New World 'rice'
 25 FAW, a total of 20 unique mitogenome haplotypes (represented by white colour proportion of each pie chart),
 26 and 11 non-unique mitogenome haplotypes were detected (i.e., a total of 25 mitochondrial haplotypes in rice
 27 FAW in the New World). For the 'corn' mitogenomes, 46 unique haplotypes were detected from the native
 28 range, while 25 corn FAW individuals shared a total of seven haplotypes (i.e., a total of 46+7=53 mitochondrial
 29 haplotypes). In the invasive range, six unique 'rice' mitogenomes (i.e., white portion of the pie charts,
 30 representing two individuals from Uganda, two individuals from Malawi, and two individuals from China (CY,
 31 n=1; CX, n=1) and three shared mitogenomes (i.e., dark blue, yellow, pale green) were detected from 76
 32 individuals from Africa (n=22), India (n=11) and China (n=43). For the 'corn' FAW from the invasive range,
 33 six unique mitogenome haplotypes (i.e., white portions of pie charts) and three non-unique mitogenome
 34 haplotypes (pale orange, pale blue and dark green) were detected, although only one individual each from China
 35 and India shared a common mitogenome (represented by dark green). With the exception of white colour
 36 representing unique mitogenomes, colour schemes are otherwise independent between 'corn' and 'rice'
 37 mitogenome haplotypes. China FAW populations from Yunnan Province of Cangyuan (CC), Yuanjiang (CY), and
 38 Xinping (CX) are indicated. One pre-border FAW intercepted on December 2016 from cut flowers that originated
 39 from Yunnan China (CH06) with a unique corn mitogenome is indicated with '*' (placed together with the CY
 40 corn pie-chart). Numbers within pie-charts indicate individuals for each mitogenome haplotype.

41



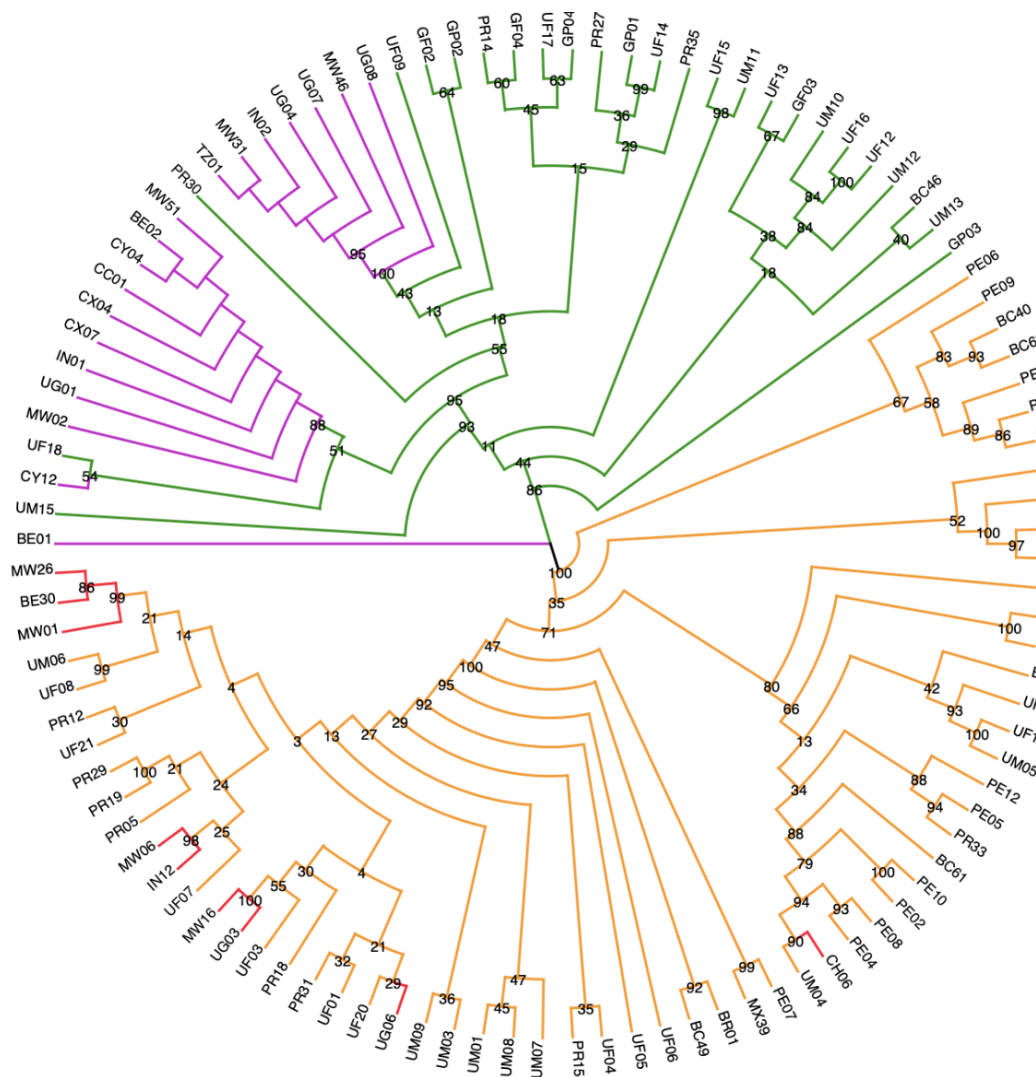
1 Mitochondrial DNA genome phylogeny

2 The trimmed (15,059bp) mitochondrial DNA genome phylogeny of all individuals in our study identified
3 two distinct clades that corresponded to the 'rice-preferred' and 'corn-preferred' clusters (Fig. 2). Based on the
4 near complete mitogenome phylogeny, a minimum of four and five introduction events were likely associated
5 with the 'rice' and 'corn' maternal lineages, respectively (Fig. 2). Except for the 'corn' specimen (CH06) from
6 Yunnan that clustered strongly with an individual from Mississippi (UM04), all 'corn' individuals from the invasive
7 range (i.e., MW26, BE30, MW01, MW06, IN12, MW16, UG03, UG06) clustered weakly with individuals from
8 Florida. Similarly, apart from the Benin individual (i.e., BE01), all remaining 'rice' FAW from the invasive range
9 also clustered weakly with individuals from Florida. Therefore, the likely origins of the invasive 'corn' and 'rice'
10 FAW in the Old World is inconclusive based on the near complete draft mitogenome phylogeny.

11

12 **Fig. 2:** FAW Maximum Likelihood phylogeny constructed using IQ-Tree based on 15,059 bp partial mitochondrial
13 genome with edge-linked partition for the 13 protein coding genes and excluding four regions of low complexity.
14 Node support estimated from 1,000 bootstrap replications. 'Rice' clade is indicated by branches in green (native
15 range) and purple (invasive range), and 'corn' clade is indicated by branches in orange (native) and red (invasive
16 range). Unique haplotypes from all populations are included. Country codes are: UF (USA-Florida), UM (USA-
17 Mississippi), PR (Puerto Rico), GP (Guadeloupe), GF (French Guiana), PE (Peru), MX (Mexico), BC (Brazil-CC), BR
18 (Brazil-rCC), BE (Benin), UG (Uganda), TZ (Tanzania), MW (Malawi), IN (India), and four populations from China
19 Yunnan Province (Australia pre-border interception (CH06); Cangyuan (CC), Yuanjing (CY), and Xiping (CX)).

20



21

22

1 Nuclear SNP Phylogeny

2 The ML phylogeny based on 870 unlinked and neutral SNPs revealed four distinct clades (clades I, II, III,
3 IV; see Fig. 3) across the sampled populations. Native and invasive individuals were a component of each clade
4 which enabled a side-by-side comparison of population structure. Members within each clade were grouped
5 with high (90-96%) bootstrap branch node support values. Clade I included the majority of the invasive FAW
6 individuals from China (CX, CY, CC populations), India (IN), Uganda (UG), and Benin (BE) as well as individuals
7 from Brazil. Overall, subclades within Clade I indicated unique genomic signatures between the CC and CY/CX
8 populations. Indian and African populations (i.e., Uganda, Benin) were scattered among the CC and CY/CX
9 populations. This interspersed clustering of subclades from Chinese, African and Indian populations suggests a
10 complex FAW spread across the Old World, with some of the China CY individuals potentially sharing a New
11 World origin similar to the Brazil rCC (i.e., 'BR' code, Fig. 3 Clade I) individuals.

12 Clade II, which is phylogenetically most closely related to Clade I, is dominated by individuals from
13 Mississippi. Within this clade, individuals from China (i.e., CX), Uganda, Benin and India are also present,
14 indicative of likely separate introductions of FAW from population(s) with genetic similarity to the Mississippi
15 population into the Old World. Clade III is represented by a separate Brazilian (i.e., 'BC') FAW population and the
16 Peru FAW individuals. Invasive populations clustered within clade III were the Malawi FAW population, a single
17 Tanzania and three Ugandan individuals, suggesting that these African FAW shared a similar origin that is
18 different from other African (e.g., Benin, rest of Uganda) and Asian populations. The Ugandan population in
19 particular appears genetically highly heterogeneous, indicating it too have mixed introduction backgrounds.

20 Clade IV is dominated by the Florida population and other Caribbean islands/Greater Antilles (e.g.,
21 Puerto Rico)/Lesser Antilles (e.g., Guadeloupe)/ Central American (e.g., Mexico), and parts of the northern
22 region of South America (e.g., French Guiana) FAW populations. Clade IV contained a single invasive Chinese
23 FAW (i.e., CH06; intercepted at Australia's pre-border inspection program). Taken as a whole, the nuclear SNP
24 phylogeny provides clear evidence for multiple introductions of FAW to the Old World (African and Asian
25 continents), while identifying populations associated with the Mississippi and the Brazilian 'BR' populations as
26 likely sources of invasive populations into the Old World. The source population for Malawi's FAW was likely
27 population(s) from South America, currently represented by Peru/Brazil (BC) populations. Based on interception
28 data, with the exception of a single unique FAW, Florida and the Greater Antilles do not appear to be likely
29 sources for the current invasive populations in the Old World.

30 Our nuclear SNP phylogeny therefore clearly showed that the native range FAW populations could be
31 classified based on their geographic origins. The unexpected direct phylogenetic relationship between the US
32 Mississippi and Brazil 'BR' population, suggested potential movements of populations within North America (i.e.,
33 Mississippi is not the wintering ground for FAW and represents the melting-pot for migrating individuals from
34 Texas and Florida that are only present in the summer seasonal migration¹⁰²) and between North and South
35 America. Finally, a significant overall finding was that our panel of neutral SNPs selected from whole genome
36 sequencing did not separate individuals based on 'corn' or 'rice' mitochondrial DNA genome signatures, nor did
37 they support the host strain characterisation based on the *Tpi* partial gene marker (see below).

38

39 Genetic diversity and neutrality tests

40 Basic population diversity statistics for each population are listed in Table 1. Nucleotide diversity (π)
41 varied across a narrow range (0.287-0.329), for the limited number of variable and independent SNPs analysed,
42 that included no invariant loci. No significant overall difference was observed between the native and invasive
43 range populations. All populations showed higher average observed heterozygosity (H_{obs}) than the average
44 expected heterozygosities (H_{exp}), both in the native and invasive ranges, with the highest H_{obs} seen in the Malawi
45 population. Negative F_{IS} values for all populations were consistent with H_{obs} being higher than H_{exp} , and
46 suggested systematic avoidance of consanguineous mating¹⁰³ within FAW subpopulations as a whole. The
47 heterozygosity excess observed in all these populations is most likely indicative of the recent mixing of previously
48 distinct populations. These are likely the result of multiple introductions into the invasive range, as already
49 suggested by the nuclear SNP phylogeny (Fig. 3) and PCA (Fig. 4). The heterozygosity excess detected for the
50 native range populations may similarly be due to high levels of migration among populations in the native range.
51 Consistent with these observations, a number of the populations including most from the invasive range also
52 contained significant numbers of loci not in Hardy-Weinberg equilibrium (HWE). This was especially the case for
53 the two largest populations from China (i.e., CY and CX), Malawi and Uganda, as well as for several native range

1 populations; many of the populations studied therefore appear to result from recent mixing of previously
 2 separated populations. Approximately half the number of loci departed significantly from HWE in the global
 3 population (i.e., 437 of the total 870) and highlighted the complex global population structure.

4 The statistical tests for neutrality were also highest for those populations with highest H_{obs} and lowest
 5 numbers of loci in HWE. For the Tajima's D estimates, all populations showed positive D values. Some
 6 populations showed lower Tajima's D values (below 0.4), suggesting that these populations were evolving at
 7 close to mutation-drift equilibrium. The high Tajima's D calculated for most of the populations (particularly
 8 values > 1.40) supports them having structure that likely reflects each comprising multiple recent introductions
 9 from different source populations. The other test for neutrality, Fu & Li's D*, gave consistent results with
 10 Tajima's D, further supporting the finding of genomic signatures in these populations due to recent introduction
 11 of different populations. These demographic factors are the likely reason for the elevated neutrality test results,
 12 since these were averages across the complete SNP set derived from the whole genome, rather than results for
 13 individual loci that might be under balancing selection⁶⁹. Interestingly, this may also apply to populations in the
 14 native range. Another potential explanation for the high D and D* values, that of rapid population collapse, is
 15 unlikely since it would require much longer evolutionary time frames than in the current populations sampled.
 16

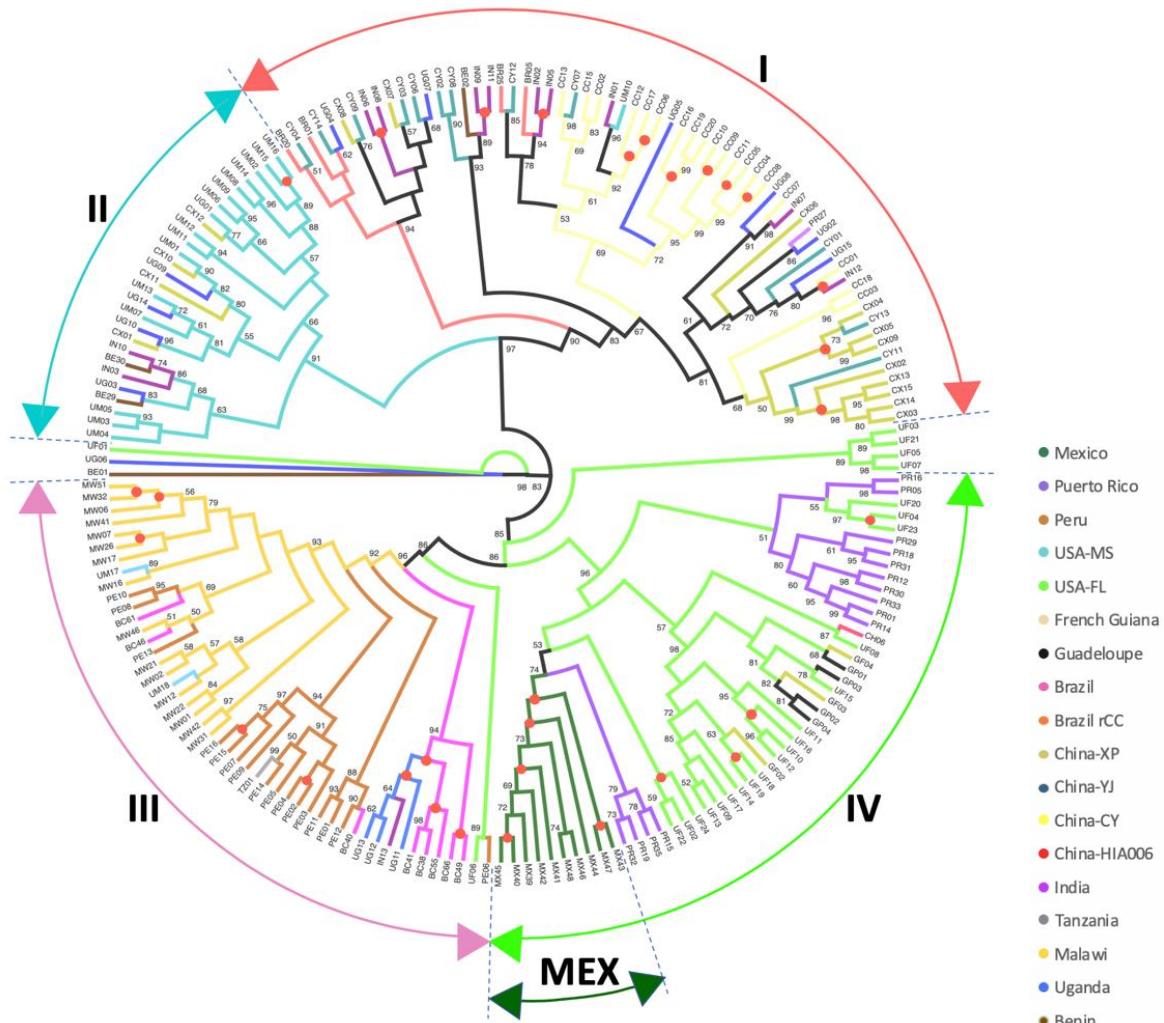
17 **Table 1:** Population statistics for native and invasive range FAW populations.

Pop. Code	Pop.	No. Samples	Avg. H_{exp}	Avg. H_{obs}	HWE, $P > 0.001$	Tajima's D	Fu & Li's D*	F_{IS}	Nt diversity (π)
BC	Brazil-CC	8	0.289	0.420	870	0.693	0.671	-0.241	0.309
BE	Benin	4	0.274	0.408	870	0.331	0.328	-0.179	0.313
BR	Brazil-rCC	4	0.263	0.396	870	0.130	0.130	-0.178	0.301
CC	China-CY	19	0.282	0.400	796	1.418	1.286	-0.262	0.289
CH	China-H	1							
CX	China-XP	15	0.293	0.416	837	1.249	1	-0.263	0.303
CY	China-YJ	12	0.284	0.405	870	1.013	1	-0.248	0.296
GF	French Guiana	3	0.247	0.375	870			-0.138	0.296
GP	Guadeloupe	4	0.245	0.359	870	0.293	0	-0.152	0.279
IN	India	12	0.289	0.403	870	1.137	1	-0.239	0.301
MW	Malawi	16	0.319	0.461	838	1.411	1.30	-0.303	0.329
MX	Mexico	10	0.265	0.403	870	0.717	0.610	-0.263	0.279
PE	Peru	16	0.319	0.456	848	1.554	1.293	-0.295	0.329
PR	Puerto Rico	15	0.288	0.404	845	1.241	1.188	-0.251	0.298
TZ	Tanzania	1							
UF	USA-FL	24	0.281	0.383	810	1.470	1.699	-0.242	0.287
UG	Uganda	15	0.305	0.428	843	1.795	1.298	-0.266	0.315
UM	USA-MS	18	0.320	0.453	820	1.717	1.452	-0.293	0.329

18 **Note:** The native range FAW populations are: USA-Florida (UF), USA-Mississippi (UM), Brazil-rCC (BR), Brazil-CC
 19 (BC), Puerto Rico (PR), Guadeloupe (GP), French Guiana (FG), Peru (PE), Mexico (MX) and the invasive range FAW
 20 populations are Benin (BE), Uganda (UG), Tanzania (TZ), Malawi (MW), India (IN), and China (CH, CC, CY, CX). See
 21 Suppl. Table 1 for sample and population details, and see Materials and Methods for details of how the statistics
 22 were calculated. Neutrality tests (Tajima's D and Fu & Li's D*) were only calculated for populations with at least
 23 4 samples. Nucleotide diversity was calculated using Stacks only for the variant loci analysed and no window size
 24 specified.

25
 26
 27

1 **Fig. 3:** IQ-Tree with 1,000 bootstraps replications to estimate node support for *Spodoptera frugiperda*
 2 populations from Northern America (Mississippi, Florida), Caribbean (Puerto Rico, Guadeloupe, French Guiana),
 3 and South America (Peru, Brazil), as well as *S. frugiperda* populations representing the Old World invasive range
 4 from Western Africa (Benin), Eastern Africa (Uganda, Tanzania, Malawi), and Asia (India, China). A total of 870
 5 independent SNPs (i.e., unlinked) from non-coding regions distributed across the genome with no missing data
 6 were used. Populations are represented by unique colour schemes as indicated. Three populations of *S.*
 7 *frugiperda* from China Yunnan Province are Cangyuan (CC), Yuanjiang (CY), and Xiping (CX), and two
 8 populations of *S. frugiperda* from Brazil are Brazil-CC (BC) and Brazil-rCC (BR). Branch nodes with 100% bootstrap
 9 support are indicated by red dots. Bootstrap values of <50% are not shown.



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14 Population structure and migration

15 Multivariate Principal Component Analysis (PCA) of the 197 individuals in the native and invasive
 16 populations was also based on the 870 neutral and unlinked SNP loci and showed the individuals to largely
 17 cluster according to their populations, as previously observed in the phylogenetic analyses (above). The native
 18 FAW populations formed five clusters as shown in Fig. 4A. More detailed analysis of the native range samples
 19 showed those from Peru to clustered overall with the Brazil-CC population (code 'BC') while also overlapping
 20 with those from Florida (Fig. 4B), and the samples from Puerto Rico, Guadeloupe and French Guiana tended to
 21 cluster with Floridian population with 96% confidence (Fig. 4C). This panel also showed the invasive FAW
 22 population from Malawi clustering with Brazil-CC and Peru in Clade III, with 96% confidence. The Ugandan
 23 population was scattered across Clades I, II and III (Fig. 4D) while the Benin individuals fell within clades I and II

1 and that from Tanzania fell just outside of 96% confidence of Clade III. Indian FAW individuals showed similar
2 clustering patterns to those of Ugandan individuals, being found in Clades I, II, and III (Fig. 4E). The Chinese FAW
3 populations were predominantly clustered within Clade I, with a few XP individuals also found within Clade II
4 (Fig. 4F). No individual from China was found in Clade III, while one individual (CH06) was clustered with Florida
5 population (Clade IV) at 96% confidence. We did not identify any invasive population to cluster with the Mexican
6 population.

7 Pairwise gene flow estimates (F_{ST}) between the populations varied significantly (Table 2). The Mexico
8 and Brazil-rCC (BR) populations overall showed very limited gene flow with all other populations, while the Brazil
9 population showed a low level of gene flow with both Peru and US Mississippi (UM) populations. There was a
10 lack of population substructure especially between invasive range populations which suggests varying levels of
11 gene flow. Significant population substructure was detected between Peru and invasive FAW populations from
12 China-CY, China-XP and China-YJ, and India, while F_{ST} estimates indicated gene flow occurring between Peru and
13 African populations (Benin, Tanzania, Uganda, and Malawi), suggesting some level of movements within African
14 populations.

15

16 Admixture analysis

17 Analysis of populations using Admixture showed structure evident at K values from 3 to 5 (Fig. 5). At
18 K=3, a total of six Chinese individuals from the CY and YJ populations appeared to be non-admixed (grey dots).
19 Similarly, at K=4, three of these six FAW individuals remained non-admixed as indicated by grey dots. However,
20 at K=5, the number of non-admixed individuals nearly doubled compared with K=3, with individuals from CC
21 (indicated by grey dots) and CX (indicated by red dots) being most different overall, although there was also one
22 individual from CX showing a non-admixed genome signature similar to those detected in the CC population,
23 and one CY individual with a non-admix genome pattern similar to those detected in CX. No other FAW
24 individuals from the invasive range otherwise showed non-admixed genomic signatures irrespective of the K-
25 values of 3, 4 or 5. As expected based on PCA and nuclear SNP phylogeny findings, the Malawi FAW individuals
26 share very similar admixture patterns as FAW individuals from Peru and Brazil-CC (i.e., 'BC') populations. This
27 shared admixed profile between Malawi and Peru/BC populations is especially clear at K=5, which also enable
28 clearer visualisation of the Tanzanian individual and selected Ugandan individuals (e.g., UG11, UG12, UG13) as
29 also having similar admixture profiles as Malawi individuals.

30 Admixture analysis of native populations of FAW showed that majority of individuals have admixed
31 genomic signatures. The exceptions being individuals from Florida (e.g., UF19, UF09, UF12, UF16), and
32 Guadeloupe (GP02, GP04) at predominantly K=4 and K=5. Interestingly, these individuals with non-admixed
33 genomic signatures (at either K=3, 4 and/or 5) also possessed the rice mitogenome haplotypes (Fig. 5). This
34 observation is similar to that observed for the non-admixed Chinese individuals that have mitogenomes which
35 also exhibited the rice haplotypes. Admixture analysis also revealed most Mexican individuals as having non-
36 admixed genome patterns and with the corn mitogenome haplotypes (Suppl. Figs. 1A and 1B). As found in the
37 phylogenetic analyses (above), comparison of the admixture patterns to mitogenomes and the *Tpi* locus of
38 native and invasive FAW populations failed to identify any evidence to support the characterisation of FAW, and
39 especially in the invasive range, as either 'corn-' or 'rice-' preferred *S. frugiperda*. The genome admixture
40 signatures of FAW across its African and Asian invasive range again suggest a complex pattern for FAW
41 introduction into the Old World. For example, given the highly admixed genomic patterns detected in African
42 and Indian FAW individuals, it is unlikely that matings between these admixed populations would lead to
43 individuals with non-admixed genomic signatures in China unless there was some very strong selection pressure
44 acting across these selected CY, CC, and CX individuals' genome as a whole.

45

1 **Table 2:** Population pairwise gene flow estimates (F_{ST}) between native and invasive range FAW populations.

2

	BE	BC	BR	CC	CH	CX	CY	GF	GP	IN	MW	MX	PE	PR	TZ	UF	UM	UG
BE	N/A											***						
BC	0.027	N/A		***		***	***			***	^	***		***		***		***
BR	0.071	0.039	N/A	***		***	***			***	+	***		+		***		***
CC	0.012	0.05	0.09	N/A		***			***		***	***	***	***		***	***	***
CH	0.029	0.02	0.124	0.057	N/A													
CX	0.019	0.05	0.08	0.02	0.049	N/A			***		***	***	***	***		***	***	
CY	0.006	0.04	0.08	0.008	0.052	0.012	N/A		^		***	***	***	***		***	***	
GF	0.036	0.039	0.088	0.048	0.035	0.053	0.052	N/A				***						
GP	0.041	0.048	0.086	0.06	0.046	0.06	0.05	0.002	N/A		+	***	^					***
IN	0.004	0.04	0.07	0.008	0.032	0.012	0.006	0.037	0.043	N/A	^	***	***	***		***	***	
MW	0.007	0.02	0.05	0.02	0.024	0.03	0.02	0.039	0.05	0.02	N/A	***		***		***	***	
MX	0.07	0.07	0.1	0.08	0.088	0.08	0.08	0.09	0.09	0.07	0.07	N/A	***	***		***	***	***
PE	0.02	0.011	0.042	0.05	0.018	0.05	0.04	0.037	0.04	0.04	0.014	0.06	N/A	***		***		***
PR	0.018	0.03	0.06	0.03	0.024	0.03	0.03	0.031	0.039	0.02	0.028	0.05	0.03	N/A		***	***	***
TZ	0.041	0.051	0.14	0.071	Nan	0.076	0.069	0.081	0.098	0.047	0.007	0.132	0.019	0.066	N/A			
UF	0.016	0.03	0.06	0.03	0.016	0.03	0.03	0.008	0.014	0.02	0.03	0.06	0.03	0.01	0.062	N/A	***	***
UM	0.034	0.018	0.042	0.05	0.026	0.05	0.05	0.041	0.05	0.04	0.02	0.06	0.01	0.03	0.021	0.03	N/A	***
UG	0.003	0.03	0.06	0.02	0.025	0.021	0.013	0.032	0.043	0.011	0.001	0.07	0.02	0.02	0.011	0.03	0.03	N/A

3

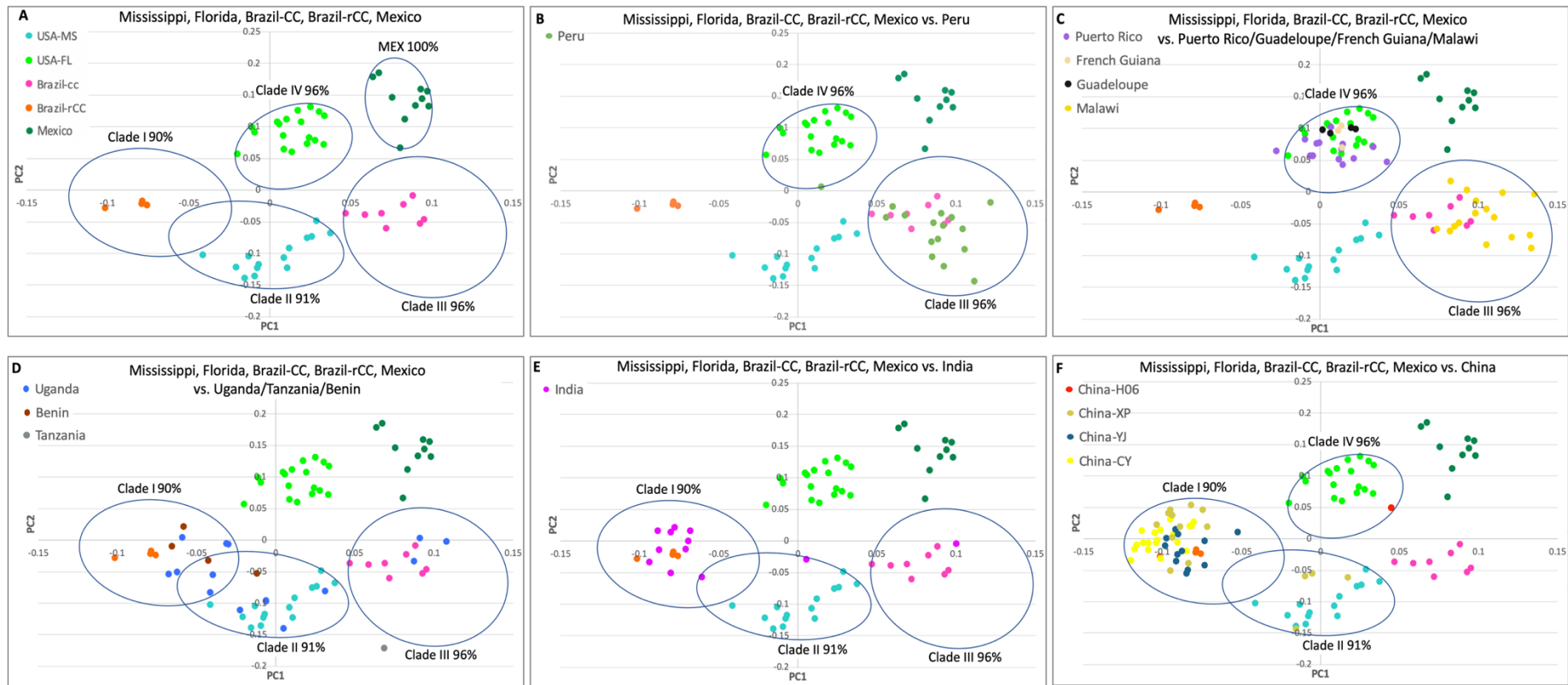
4 **Note:** The populations are denoted as in Table 1. The F_{ST} values are given in the lower left half of the table, and the p-values (***) $p < 0.001$; + $p \leq 0.01$; ^ $p \leq 0.05$) in the
 5 upper right. Both Tanzania and China-H06 populations consisted of one individual each and their pairwise F_{ST} was therefore not estimated.

6

7

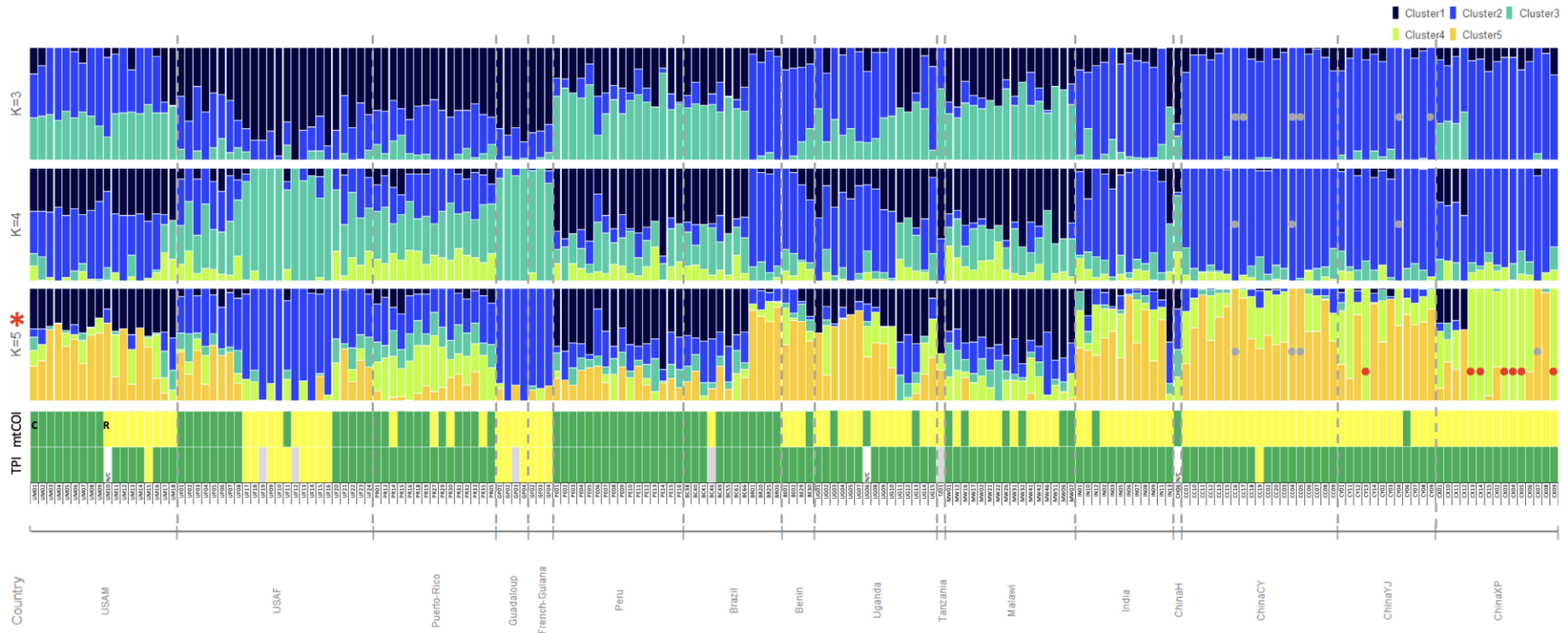
8

1 **Fig. 4:** Principal Component Analyses of native and invasive FAW populations based on 870 neutral and unlinked SNP loci. **Panel A** shows the five clusters of native FAW
 2 populations (identified also from the genome-wide SNP phylogeny in Fig. 3). Circles indicate confidence as shown in Fig. 3. **Panel B:** Peru individuals clustered overall with
 3 Brazil-CC population (Clade III; pink colour) but also overlapped Florida population (Clade IV, light green colour). **Panel C:** Puerto Rico (purple colour), Guadeloupe (black
 4 colour) and French Guiana (wheat) overall clustered with Florida population with 96% confidence, while the invasive FAW population from Malawi (yellow colour) clustered
 5 in Clade III with Brazil-CC and Peru with 96% confidence. **Panel D:** PCA of Uganda population (blue colour) indicated the population were scattered across Clades I, II and III,
 6 Benin individuals (Saddlebrown colour) fell within clades I and II, while Tanzania (Azure 4 colour) fell just outside of 96% confidence of Clade III. **Panel E:** Indian FAW individuals
 7 showed similar clustering patterns as the Ugandan individuals, being found in Clades I, II, and III. **Panel F:** Chinese FAW populations were predominantly clustered within
 8 Clade I, with few CX individuals also found within Clade II. No individual from China was found in Clade III, while one individual originating from Australia's pre-border
 9 inspection program was clustered with Florida population (Clade IV) at 96% confidence. No invasive populations were clustered with the Mexican population. Colour codes
 10 for populations as provided in Fig. 3.



11
 12

1 **Fig. 5:** Admixture analysis based on K=3 (Cross-Validation Error (CVE): 0.375), K=4 (CVE: 0.377), and K=5 (CVE: 0.380). Populations 'USAM' and 'USAF' are from Mississippi
 2 and Florida, respectively. Populations from China were from Cangyuan (CY), Yuanjiang (YJ), Xiping (XP) in Yunnan Province. Corn- ('C') or rice- ('R') preferred plant hosts are
 3 identified based on mtCOI as per Dumas et al.⁵⁵, and by *Tpi* SNP approach as per Nagoshi⁶² are indicated by green or yellow bars, respectively. Specimen ID's and sampling
 4 countries are as labelled. 'N/C' for *Tpi* indicates no coverage. Grey bars indicate individuals with heterozygous *Tpi* SNPs.



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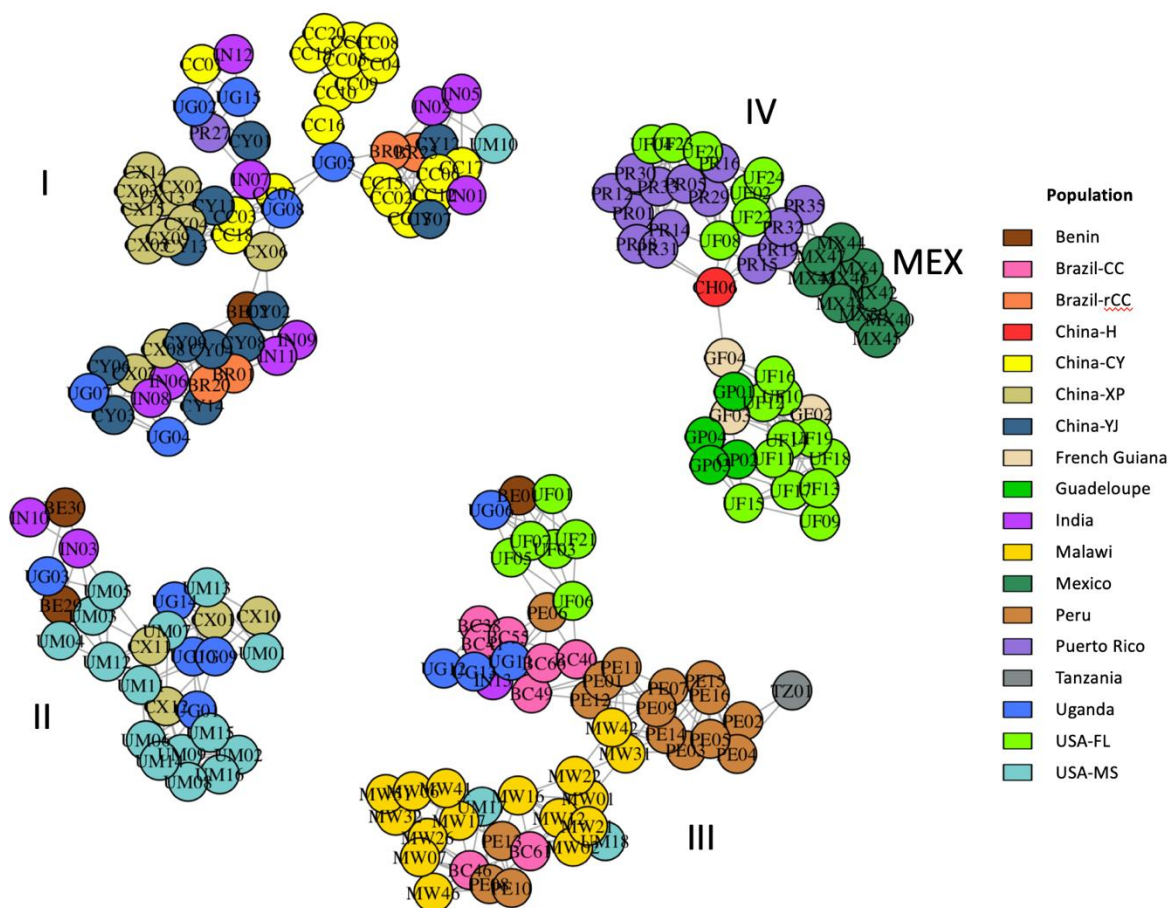
1 Admixture networks

2 To explore the population substructure revealed by the admixture analysis in relation to the ML clusters
 3 obtained from phylogeny and PCA, we performed network analysis using the plotAdmixture function in the
 4 NetView R package. The ML network of individuals belonging to each of the specified populations is shown in
 5 Fig. 6A. The four major clusters, I - IV, correspond to those shown in the ML tree (Fig. 3). Individuals from some
 6 populations were shown to be spread across multiple clades, e.g., PR, UF and UM from the native range and IN,
 7 BE and CX from the invasive populations. Of the populations in the invasive range, those from China were found
 8 predominantly in cluster I, with some CX individuals in cluster II and the single CH06 individual in cluster IV.

9

10 **Fig. 6a:** Maximum Likelihood (ML) network showing individuals belonging to the populations as given in the
 11 legend. The network was drawn using the plotAdmixture function in the R package NetView^{97,98}, and is based
 12 on a ML distance matrix calculated from the IQ-Tree shown in Fig. 3. using the R package ape⁹⁹. The four major
 13 clusters, I – IV, correspond to those shown in the tree. Individuals are identified by country codes as follows:
 14 China XP (CX), China YJ (CY), China CY (CC), China HIA006 (CH), India (IN), Uganda (UG), Tanzania (TZ), Malawi
 15 (MW), Benin (BE), Brazil CC (BC), Brazil rCC (BR), Peru (PE), French Guiana (GF), Mexico (MX), Guadeloupe (GP),
 16 Puerto Rico (PR), USA-Florida (UF), and USA-Mississippi (UM). See Supplementary Table 1 for complete
 17 information about the individuals.

18



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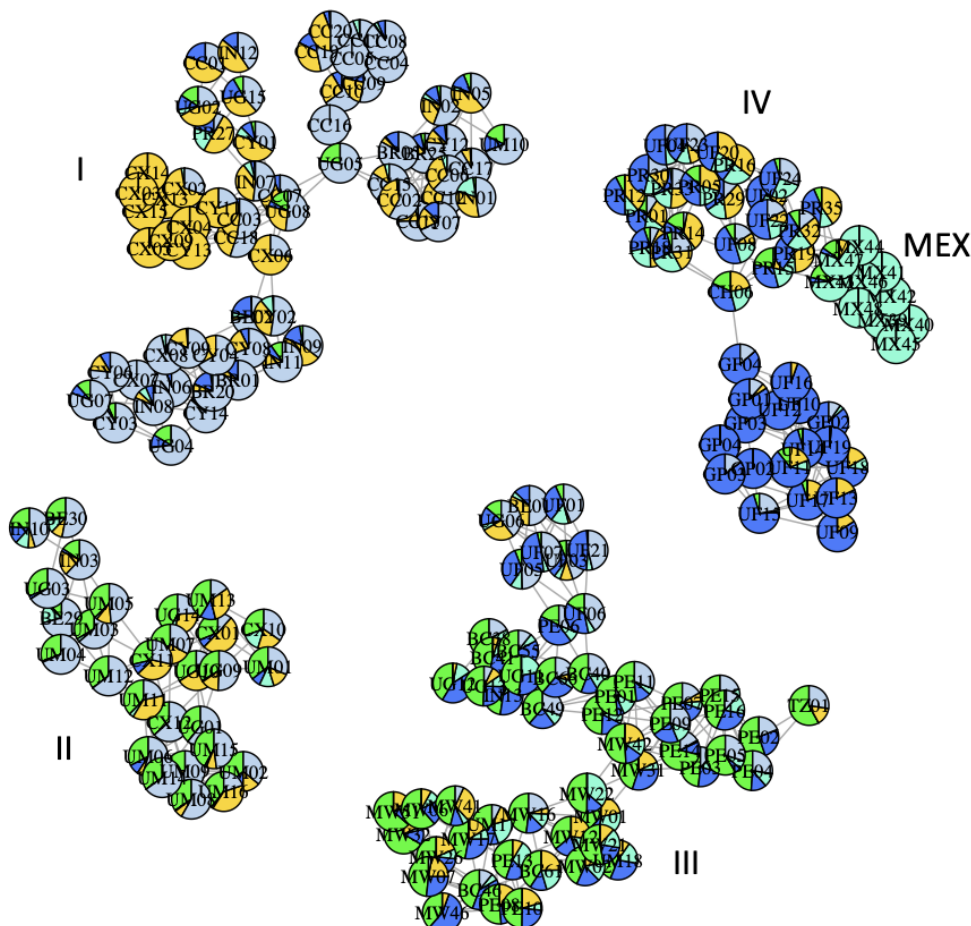
20

21 Plotting admixture proportions at K=5 on this network provided further insight into the complex
 22 relationships between these populations (Fig. 6B). The different populations from China that predominantly
 23 comprise Cluster I each have distinct admixture profiles that are significantly shared with those of individuals
 24 from Uganda and India. In cluster II, China-XP (CX), India (IN), Benin (BE), and Uganda (UG) formed network with

1 USA-Mississippi (UM) individuals. In Cluster III, all Malawi (MW) individuals and various Ugandan (UG) individuals
2 and the single Tanzanian (TZ) individual formed a network cluster with Peru (PE), Brazil-CC (BC), and some USA
3 Florida (UF) individuals. In cluster IV, only one Chinese FAW (CH) was found to group to this predominantly
4 Caribbean/Central America FAW group (consisting of UF, Puerto Rico (PR), French Guiana (GF), Guadeloupe (GP),
5 and Mexico (MX) FAW individuals).

6

7 **Fig. 6b:** Maximum Likelihood-distance network with admixture analysis at K=5 presented as pie charts for each
8 individual. The ML network is that shown in Figure 6b and individuals, identified by the same codes, and the
9 same four distinct clusters (I-IV) are indicated. Cluster I comprises predominantly different Chinese populations
10 each with distinct admixture profiles but included also individuals from Uganda, India, Brazil-rCC (BR) and Puerto
11 Rico. In cluster II, China-XP (CX), India, Benin, and Uganda formed network with USA-Mississippi individuals. In
12 Cluster III, all Malawi individuals and various Tanzania and Uganda individuals were grouped with Peru, Brazil-
13 CC (BC), and selected USA-FL individuals. In cluster IV, only one Chinese FAW (CH) was found to group to this
14 predominantly Caribbean/Central America FAW group (consisting of USA-FL, Puerto Rico, French Guiana,
15 Guadeloupe, and Mexico FAW individuals). Note that individuals sharing the same colour schemes do not
16 necessarily have the same genetic content, and that the MEX group consisted only of individuals from Mexico
17 showing little admixture with any other population.



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20 Directionality of gene flow analysis using divMigrate

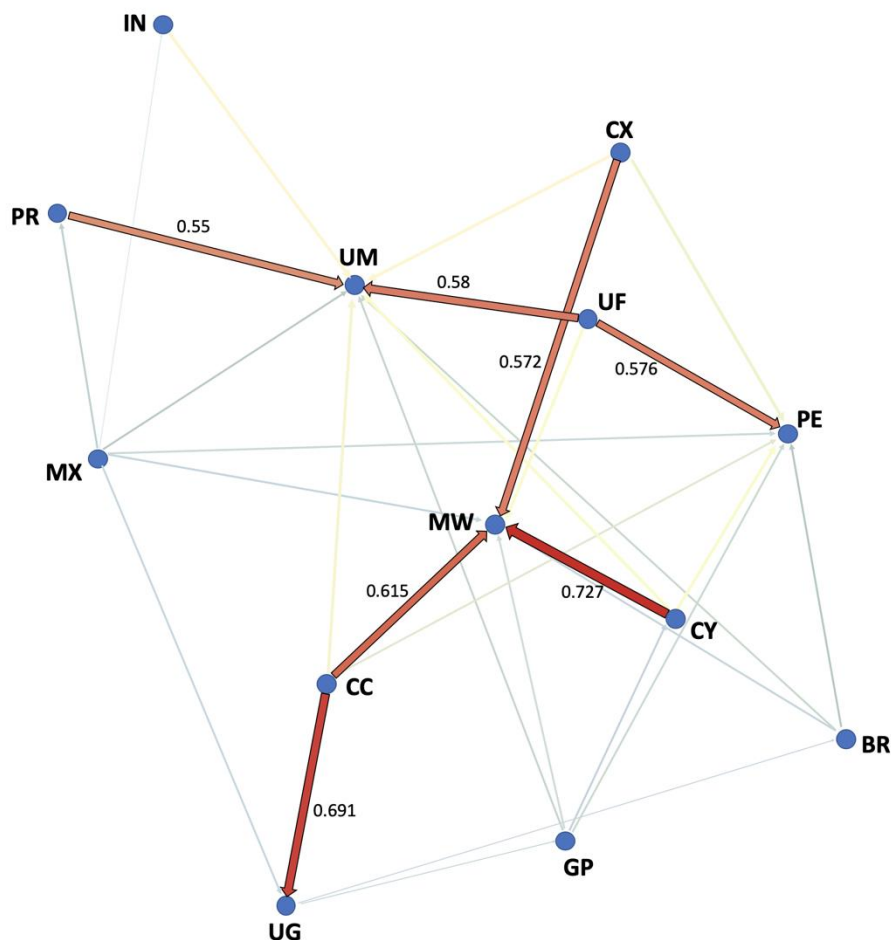
21 Analysis of the directionality of gene flow (i.e., relative directional migration) between populations
22 using divMigrate enabled investigation of possible introduction pathways leading to the complex population
23 substructure patterns seen in the above analyses. As shown in Fig. 7, the most significant directional gene flow
24 signatures seen were from all three Chinese populations (i.e., CX, CY, CC) into Malawi and from the Cangyuan

1 (CC) population into Uganda. Significant gene flow from Florida (UF) and from Puerto Rico (PR) into the
2 Mississippi (UM) FAW population, which the above (e.g., Figs. 3, 4A, 5, 6a) had shown to be distinct was also
3 detected. No evidence was found for directional gene flow from any of the populations studied into China, nor
4 any from or into India. Together with the Admixture results (Fig. 5), these results indicate the East African FAW
5 populations likely originated from China, with some independent 'non-China' introductions also detected in
6 Malawi. The Admixture signatures within the Ugandan FAW population suggested the presence of two
7 genetically distinct FAW populations (Figs. 5, 6B), one of which originated from Asia and involved genetic
8 contribution from the Yunnan Cangyuan (CC) population (Fig. 7), as well as gene flow from Malawi (Fig. 5). While
9 the Malawi population overall showed admixture patterns similar to Peru (Fig. 5) with the PCA showing the
10 Malawi, Peru and Brazil-CC (BC) populations clustered together (Figs. 4B, 4C), directionality analysis indicated
11 genetic contributions from all three Chinese FAW populations (Fig. 7).

12

13 **Fig 7:** Analysis using divMigrate to infer directionality of gene flow (i.e., relative directional migration) between
14 New World native and Old World invasive *Spodoptera frugiperda* populations. The divMigrate analysis was run
15 using the online server <<https://popgen.shinyapps.io/divMigrate-online/>>⁹⁹. The analysis was performed with
16 the G_{ST} migration statistic of 10^4 and 10^5 at filter threshold = 3.0 and 1,000 bootstrap replications to assess
17 confidence with alpha value set at 0.05 (i.e., 95% confidence). Weighted values above 0.50 are indicated.
18 Population codes are IN (India), PR (Puerto Rico), MX (Mexico), UG (Uganda), CC (China Cangyuan), CY (China
19 Yuanjiang), CX (China Xinping), MW (Malawi), PE (Peru), GP (Guadeloupe), BR (Brazil-rCC), UM (USA Mississippi),
20 and UF (USA Florida). Significant gene flow is seen from all three Chinese populations into Malawi and from
21 Cangyuan (CC) to Uganda (UG). Significant gene flow from Florida and from Puerto Rico into the Mississippi FAW
22 population is also detected.

23



24

25

1 Discussion

2 The genomic analysis of FAW from native and invasive ranges in this work contradicts recent published
3 theories on the pathway, origin, and direction of spread of this pest across the Old World. Using neutral and
4 unlinked genome-wide SNPs obtained from material available at early stages of the invasion, we showed,
5 through population admixture analysis, ML distance network, and gene flow directionality analyses, that there
6 were likely multiple introductions to both Africa and Asia. Studies to date have relied on analyses of limited
7 partial mitochondrial DNA (e.g., partial COI and CYTB; ^{21,60}) and the nuclear *Tpi* partial gene (e.g., Nagoshi et al.⁵⁹)
8 of various African, Asian and South East Asian invasive *S. frugiperda* populations, with comparisons to native
9 New World *S. frugiperda* populations. These studies inferred the directionality of spread from the timing of
10 official reporting to the FAO, and described a single introduction of FAW to the Old World from an eastern
11 American/Greater Antilles population, that spread rapidly across the sub-Saharan African nations, before
12 moving to the Indian sub-continent via the Middle East, and then to South East Asia, and China ⁵⁹.

13 Genome wide SNP analyses in this present study showed the populations in China and Africa to be
14 genetically diverse and demonstrates strong evidence for a more complex pattern of spread in the Old World,
15 including a significant proportion of east-to-west movement, with populations from Asia (e.g., China Yunnan
16 Province) as a source of invasive FAW populations in Africa (e.g., Malawi, Uganda). The confirmation of FAW
17 after reports by farmers of crop damage, i.e., in Nigeria and São Tomé and Príncipe in early 2016 ¹⁷ and in
18 northern and eastern Uganda since 2014 ³⁸, suggested that *S. frugiperda* was present in the African continent
19 earlier, and given the genomic evidence reported here would suggest that the FAW was present in Asia and/or
20 Southeast Asia prior to 2016. Incidences of *S. frugiperda* attacking stem and leaf parts were reported from a
21 farm producing turf grass for parks in Hà Nội, Viet Nam, between March and June (Spring/Summer seasons) of
22 2008, with heavy infestations reported between the months of April and May in 2008 (see Table 4.2 and Fig. 9
23 of Vu ¹⁰⁶; see also Nguyen and Vu ¹⁰⁷; Pham ¹⁰⁸), as well as being reported as an agriculture insect pest in the
24 production areas around Hà Nội since 2008 ¹⁰⁹. We also provided clear evidence for multiple introductions of
25 this agricultural pest into Africa, demonstrating conclusively that the Malawian FAW populations have a distinct
26 genomic signature representing a New World population different to the potential New World source
27 populations of the Chinese populations. The early detections and report of FAW in Asia/S.E. Asia (e.g., CH06
28 from this study from 2016, see GenBank record MT897262; 2008 FAW outbreaks on turf grass in Hà Nội ¹⁰⁶), and
29 the complex pattern of multiple introductions as evident from genomic signatures (e.g., this study; Yainna et al.
30 ⁶⁹), are consistent with the perceived rapid spread experienced across the African ¹¹⁰ and Asian continents ¹¹¹.

31 Despite being one of the worst agricultural pests in the New World, there has been limited work, at the
32 population genomic level, on the FAW pest complex. Through our genome-wide SNP analyses, we have
33 identified unexpected complexity in the FAW population structure in the New World. While the mitochondrial
34 genome analysis confirmed the two canonical clades that have long been suggested to define two strains with
35 different host preferences, i.e., corn (*Sfc*) and rice (*Sfr*), the neutral nuclear SNP analyses showed a more complex
36 population structure. FAW populations in the New World could be differentiated into at least five distinct groups
37 that broadly followed the species' geographic distributions, and with no obvious pattern related to host race
38 determination by mitochondrial or *Tpi* markers, providing the first genome-wide support for suggestions that
39 these mitochondrial genomes (and the often associated *Tpi* marker) do not define any real population structure
40 across the native range of FAW ^{57,112}, while Groot et al. ¹¹³ observed a lack of consistent correlation between
41 host plant and mitochondrial genome in native range populations. Frequent hybridisation has been known to
42 occur in the field (e.g., Nagoshi et al. ¹¹⁴), and would also account for the observed pattern. Furthermore,
43 Haenniger et al. ¹¹⁵ observed that African populations contained hybrids that were F2 or even later generations,
44 and mating time differences within the African populations were likely related to the differences in circadian
45 gene expression previously identified in *Sfc* or *Sfr* populations in their native range ¹¹⁶. Orsucci et al. ⁶⁷ suggested
46 that differences in mitochondria function could be directly related to host preferences, which could explain the
47 absence of a correlation between the mitochondrial and nuclear genotypes, but this lack of genomic correlation
48 points clearly to the need of genome-wide studies in field populations.

49 In the North American continent, we detected directional migration from Florida and the Puerto Rican
50 populations to the genetically distinct Mississippi one. This is consistent with findings based on mtCOI sequences
51 that the Mississippi populations were established through seasonal migration from Texas and Florida ¹⁰². There
52 also seems to be evidence for a wider Caribbean population including Florida, Puerto Rico, Mexico, the Lesser
53 Antilles (e.g., Guadeloupe) and the north-eastern region of South America (e.g., French Guiana). Mexican FAW
54 formed a separate sub-clade within the Florida/Greater Antilles/Lesser Antilles FAW group. Significant pairwise
55 F_{ST} estimates between Mexico and all New World FAW populations indicated very limited gene flow occurred

1 between the Mexican population and other New World (and invasive) populations. Northern Mexican
2 populations have been shown to be similar to the Southern Texas overwintering population so it is interesting
3 that in this study the Mexican population sits within the broader Caribbean clade that includes Florida ¹⁰².
4 Previous studies ^{117,118}, for example, have also identified Mexican FAW populations as potentially limited in
5 migratory interactions and biologically unique even between populations from different Mexican geographical
6 regions.

7 Our PCA on genome wide SNPs identified the Brazilian FAW as two genetically-distinct populations,
8 with one population ('BC') being phylogenetically more closely related to the Peruvian FAW population, and the
9 BR population which is phylogenetically more closely related to the Mississippi population. The Brazilian 'BR'
10 population included individuals that had been found to have a novel 12 bp deletion mutation in the ABC22 gene
11 ⁵⁴. The implications of the close phylogenetic relationship between the BR and Mississippi populations are
12 significant given that FAW is regarded as a major agricultural pest in Brazil ⁵⁴, and the possible movements of
13 alleles that could potentially underpin resistance, especially to Cry1F and Cry1A toxins, would add to the
14 challenge of managing this pest in the Americas.

15 The genomic analyses in the present study support multiple introductions from different sources into
16 Africa, rather than via a single introduction that first went into western Africa. Phylogenetic inference and PCA
17 clearly identified the South American FAW population, as represented by the Peru/Brazil (BC) samples, as the
18 likely source for the Malawi population. If this introduction was trade-related, it is more likely to have occurred
19 via neighbouring countries such as South Africa which has greater agricultural trade with South America than
20 does Malawi <<https://oec.world/en/>> (accessed 06-Dec-2019). A relationship between FAW populations in
21 Uganda, Tanzania and Malawi is evident, with the admixture analysis identifying the Tanzanian individual and
22 selected Ugandan individuals as sharing very similar genomic profiles with the Malawi population (e.g., Fig. 5,
23 K=5). Interestingly, one Indian FAW individual (i.e., individual IN13; Fig. 5) also shared very similar genomic
24 admixture profiles with the Malawi FAW population, and could indicate movements of East African FAW
25 population into the Asian continent, although it is currently not possible to rule out separate introductions
26 involving the South American Peru/Brazil population into the Indian sub-continent based on limited samples
27 from India.

28 The complex genetic relationships between populations in Africa and those elsewhere in the Old World
29 have implications for our understanding of pathways for biological invasions. The East African FAW populations
30 from Uganda and Malawi showed genetic relatedness to the Yunnan CC population, and the Yunnan CC, CY, and
31 CX populations, respectively, with directionality analyses showing gene flow from China to Africa, consistent
32 with admixture analyses, although we also note that our inference of FAW movements across the Old World is
33 significantly limited by the availability of Asian/East Asian/South East Asian populations. The ML network
34 analysis showed that the Benin FAW population also clustered within the same networks as the Yunnan CY, CX
35 and Ugandan populations (Clusters I and II), indicating that the spread of FAW from Yunnan into the African
36 continent potentially extended to west Africa. The detection of a shared rice mitogenome haplotype between
37 the two extremes of our invasive FAW sampling sites of Benin, and from the Yunnan CY population from China,
38 provided further evidence for this 'East to West' spread of FAW populations into Africa (Fig. 1). While it might
39 be logical to conclude, from the very limited data of shared mitogenome haplotypes between Benin and China,
40 that FAW spread was from west Africa to east Asia, applying bioinformatic analytical approaches enabled a re-
41 evaluation of the FAW global invasion patterns. For example, copy number variations (CNV) analysis from whole
42 genome sequence data ³⁸ also detected distinct signatures between the China/East African (Uganda, Malawi)
43 from the Benin/Indian FAW populations, thereby further supported multiple introductions of FAW across
44 different Old World regions. This and the study of Yainna et al. ⁶⁹ highlight the need for whole-genome derived
45 datasets to accurately trace population origins and global movements of highly mobile insect pests.

46 The invasive FAW in west Africa (i.e., Togo) was proposed to have originated from the Greater
47 Antilles/Eastern Florida based on inferences from a partial nuclear *Tpi* gene marker and two partial
48 mitochondrial DNA genes (i.e., COI, Cyt *b*) ¹⁹, and which was further linked to subsequent invasion into South-
49 eastern Asia and China ⁵⁹. This finding was not supported by our whole genome analysis, which identified at least
50 four distinct populations of FAW in Yunnan Province, involving at least five separate introduction events (Figs.
51 6a, 6b). While the Yunnan CH06 individual and the Yunnan CX, CY and CC populations all possessed their own
52 unique genetic signatures, only the various CX individuals could be linked to the USA Mississippi FAW population,
53 and the single CH06 FAW individual (Figs. 3 and 4F) to the USA Florida population. The Yunnan CY and CC
54 populations and the remaining CX individuals all clustered with the Brazil-rCC (i.e., 'BR') population (Figs. 3 and

1 4F), potentially indicating a shared origin, or the same source population and as a connected introduction event
2 across globally disparate locations.

3 While populations in the Yunnan Province and Africa have enabled inference of the overall
4 directionality of introductions, the lack of samples across much of the invasive range, in particular S.E. Asia (e.g.,
5 Myanmar, Thailand, Vietnam, Malaysia, Indonesia, etc.) meant that other candidate FAW invasive populations
6 could have been the 'source invasive populations' for the Asian/Old World invasions. One example is the
7 modelling of FAW spread via monsoon wind patterns from Myanmar into southern China ¹¹⁹, a hypothesis that
8 could be tested using genomic evidence. International trade pathways are increasingly being identified as
9 responsible for accidental introductions of invasive plant pests and pathogens (e.g., Lopes-da-Silva ¹²⁰).
10 Evaluation of introduction pathways will therefore need to also include trade data, as has been undertaken for
11 the invasion by *H. armigera* from the Old World into the New World ^{3,15} (reviewed also in Jones et al. ⁹), and for
12 the invasive *Bemisia tabaci* MED and MEAM1 species complex (reviewed by De Barro et al. ²; see also Elfekih et
13 al. ¹).

14 Indeed, global movements of invasive pests, exemplified by the spread of FAW, as well as the multiple
15 introductions of *H. armigera* into the South American continent ¹⁵ and various Caribbean nations ¹²¹ from Asia
16 ^{10,14} and various Old World regions ³, are timely reminders of the need for global coordination of enhanced
17 biosecurity preparedness strategies that build on advancement in genomic research. The potential negative
18 impacts of introductions of alien species include introgression of genetic traits to local species through
19 hybridisation ^{10,13,14,16,28}. Development of new trans-continental trade routes to increase economic growth
20 between trading partners must therefore recognise the significant risks and take into consideration the
21 biosecurity implications associated with the rapid spreading of highly invasive pests and pathogens of plants,
22 animals and humans ¹²² that could instead undermine the aim to grow the global economy.

23

24

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44

45 **Statement on Data Availability**

46 All assembled mitochondrial genomes have been submitted to GenBank (accession numbers MT897262 -
47 MT897458). The complete list of FAW population genome wide single nuclear polymorphic loci used is available
48 from CSIRO's public data access portal (doi: xxxxxxxxxx).

49

50 **Statement on Code Availability**

51 All programme codes used in this study have been cited. No custom program codes were used in this study.

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