UNIVERSIDADE ESTADUAL DE CAMPINAS
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GENÔMICA POPULACIONAL DO INSETO PRAGA *Spodoptera frugiperda* NO BRASIL E ARGENTINA

POPULATION GENOMICS OF THE INSECT PEST *Spodoptera frugiperda* IN BRAZIL AND ARGENTINA

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Orientadora: MARIA IMACULADA ZUCCHI

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RESUMO

A *Spodoptera frugiperda* é um inseto praga que apresenta grande importância econômica para diversas culturas devido ao seu hábito polífago. Apesar de ser uma praga conhecida no continente americano, nos últimos anos ela foi introduzida em novos continentes e ganhou muita notoriedade pelo seu potencial de dano. No Brasil, populações resistentes a diversos inseticidas e proteínas *Bt* já foram reportadas, e o manejo desta praga é uma questão sempre atual. A espécie compreende duas raças que são morfologicamente idênticas, porém apresentam diferenças genéticas e ecológicas. Apesar de haver muitos estudos sobre essa espécie, ainda há dúvidas sobre a estruturação genética dessas populações na América do Sul. Devido à importância de se conhecer e monitorar a variabilidade genética de populações de insetos que são pragas agrícolas, o objetivo principal desse estudo foi esclarecer o nível de estruturação genética das populações dentro do Brasil e como essas populações se diferenciam das populações da Argentina. Primeiramente, utilizei sequências parciais do gene mitocondrial *COI* para caracterização da variabilidade genética, da estruturação de populações e da história demográfica. Além do *COI*, uma porção do éxon 4 do gene *Tpi* foi sequenciado para melhor identificação das raças a que pertenciam os indivíduos. Em seguida, através da metodologia de Genotyping-By-Sequencing (GBS), obtive milhares de marcadores SNPs do genoma nuclear para aumentar a resolução na caracterização da diversidade genética das populações. De forma geral, a análise de haplótipos com o *COI* não indicaram presença de clusters geográficos, enquanto o GBS indicou estruturação entre populações da Argentina e as do Brasil. Análises de F_{ST} par a par, tanto através do marcador mitocondrial quanto dos marcadores nucleares, indicaram estruturação genética entre as populações da Argentina. Os dois tipos de marcadores também evidenciaram fluxo gênico entre as populações do Brasil, e que estas estão estruturadas somente pela presença das duas raças. Este é o estudo mais abrangente no território nacional em termos de genética e genômica de populações de *S. frugiperda*, e as implicações dos resultados em termos práticos são discutidos.

Palavras-chave: lagarta-do-cartucho, diversidade genética, genômica de populações, insetos-pragas, mtDNA, *COI*, *Tpi*, SNP, GBS.

ABSTRACT

Spodoptera frugiperda is an insect pest that has great economic importance in several crops due to its polyphagous nature. Despite being a known pest in the American continent, in recent years it has been introduced into new continents and has gained much notoriety for its ability to cause damage. In Brazil, populations resistant to several insecticides and Bt proteins have been reported, and the management of this pest is an ever-present issue. The species comprises two strains that are morphologically identical, however feature genetic and ecological differences. Although there are many studies on this species, genetic structure of these populations is still unclear in South America. Due to the importance of knowing and monitoring the genetic variability of insect populations that are agricultural pests, the main objective of this study was to clarify the level of genetic structure of populations within Brazil and how these populations differ from populations in Argentina. First, I used partial sequences of the mitochondrial *COI* gene to characterize genetic variability, population structure and demographic history. In addition to the *COI* marker, a portion of exon 4 of the *Tpi* gene was sequenced to better identify the strains of each individual. Then, through the Genotyping-By-Sequencing (GBS) methodology, I obtained thousands of SNP markers from the nuclear genome to increase resolution in the characterization of the genetic diversity of populations. In general, the analysis of *COI* haplotypes did not indicate the presence of geographic clusters, while the GBS indicated structuring between populations from Argentina and Brazil. Pairwise F_{ST} analyses, generated either by the mitochondrial or the nuclear markers, indicated genetic structure among populations within Argentina. The two types of markers also indicated presence of gene flow among the Brazilian populations, which are structured only by the presence of the two strains. This is the most comprehensive study in the national territory in terms of genetics and genomics of *S. frugiperda* populations, and the implications of the results in practical terms are discussed.

Keywords: fall armyworm, genetic diversity, population genomics, insect pests, mtDNA, *COI*, *Tpi*, SNP, GBS.

Abbreviations

- AFLP: Amplified Fragment Length Polymorphism
- AMOVA: Analysis of molecular variance
- BLAST: Basic Local Alignment Search Tool
- *Bt*: *Bacillus thuringiensis*
- *COI*: Cytochrome c oxidase subunit I
- DAPC: Discriminant Analysis Principal Components
- ddRAD: Double digest restriction-site associated DNA
- DNA: Deoxyribonucleic acid
- FAW: Fall armyworm (*Spodoptera frugiperda*)
- F_{ST}: Fixation index
- GBS: Genotyping-By-Sequencing
- GO: gene ontology
- IPM: Integrated pest management
- IRM: Integrated resistance management
- JH: Juvenile hormone
- LAMP: loop-mediated isothermal amplification
- mtDNA: mitochondrial DNA
- PCA: Principal components analysis
- PCR: Polymerase chain reaction
- RAPD: Random Amplified Polymorphic DNA
- RFLP: Restriction Fragment Length Polymorphism
- RNA: Ribonucleic acid
- SfC: *Spodoptera frugiperda* corn-strain
- SfR: *Spodoptera frugiperda* rice-strain
- SNP: single nucleotide polymorphism
- *Tpi*: Triosephosphate isomerase

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Introduction

The term “Integrated Pest Management” (IPM) was first used in 1967 to describe the concurrent application of multiple control measures to reduce the damage caused by insects in agricultural systems. Fundamentally, an IPM strategy involves a holistic approach, being essential to have an in-depth understanding of how management practices will influence insect biology and other environmental interactions, which requires monitoring pest populations to apply the most effective control measures [1].

In this context, the Genomic Revolution enabled to understand an organism or a population at the genomic level. For this reason, modern IPM strategies also include understanding of the genetic diversity of populations and gene flow patterns [2].

Knowledge of how *Spodoptera frugiperda* populations are genetically structured can be used to understand risks of spread of resistance alleles [3]. Moreover, incorporating population genomics also allows us to understand the genetic components that lead to evolution and adaptation in different geographies. Although the use of molecular markers has proven efficient to characterize populations, there are relatively few genomic studies of *Spodoptera frugiperda* in Brazil [4,5].

In this study, we aimed to fill this knowledge gap by assessing FAW populations from Brazil and Argentina regarding their genetic diversity, population structure, demographic history, and loci putatively under selection.

This thesis is organized in three chapters. Chapter 1 is a review of molecular markers developed for characterization of population genetics and genomics of FAW. Chapter 2 shows the assessment of genetic diversity, population structure and demographic history of FAW populations using *COI* as molecular marker. Chapter 3 presents FAW population genomics using the GBS methodology. The thesis ends with future directions for FAW research on population genomics.

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Objective

Characterize genetic diversity and genomic of populations of *Spodoptera frugiperda* from Brazil and Argentina.

Specific objectives:

1. Characterize genetic diversity and infer demographic history through *COI* haplotypes;
2. Understand genetic structure of populations:
    - among geographic locations,
    - between countries,
    - between strains;
3. Identify loci putatively under selection.

Chapter 1

Overview on *Spodoptera frugiperda* Population Genetics and Genomics

Abstract

The fall armyworm (FAW), *Spodoptera frugiperda* is one of the most important Lepidopteran pests in agriculture. The research with genetic and genomic diversity can greatly add value for the adequate management of the pest in infested crops, and therefore this area has received considerable attention. Thus, methods to characterize FAW diversity is bringing increasingly resolution to our understanding of FAW populations. Here we review the available genetic and genomic studies on FAW structure in the native range, in introduced regions and other applications of molecular studies for this species. Lastly, we explore the methods used for host strain identification, an important feature of FAW.

Keywords: Fall armyworm, invasive pest, population structure, host strains, pest management

1. Introduction

1.1. Fall Armyworm: A Major Global Pest

The fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), is a polyphagous and multivoltine migratory species with a recent history of invasion success. The native range of FAW comprehends the tropical regions of the American continent [1]. FAW was first described in 1797 in Georgia, USA, and sorghum was considered the preferred host plant [2]. The species started to call more attention after showing occasional outbreaks in US since 1856 [3]. In South America, FAW damage in maize was first reported in Colombia in 1914 [4]. The species arrived in West Africa in 2016 and it is now present in over 130 nations worldwide, except for Europe where the pest is on the quarantine list and it is present only in the Canary Islands [5].

The biology and ecology characteristics of FAW greatly favor aggressiveness and make it one of the most important pests to control in its range of occurrence. Some aspects of biology and ecology of FAW that made this species such a successful pest are discussed below and illustrated in Figure 1.

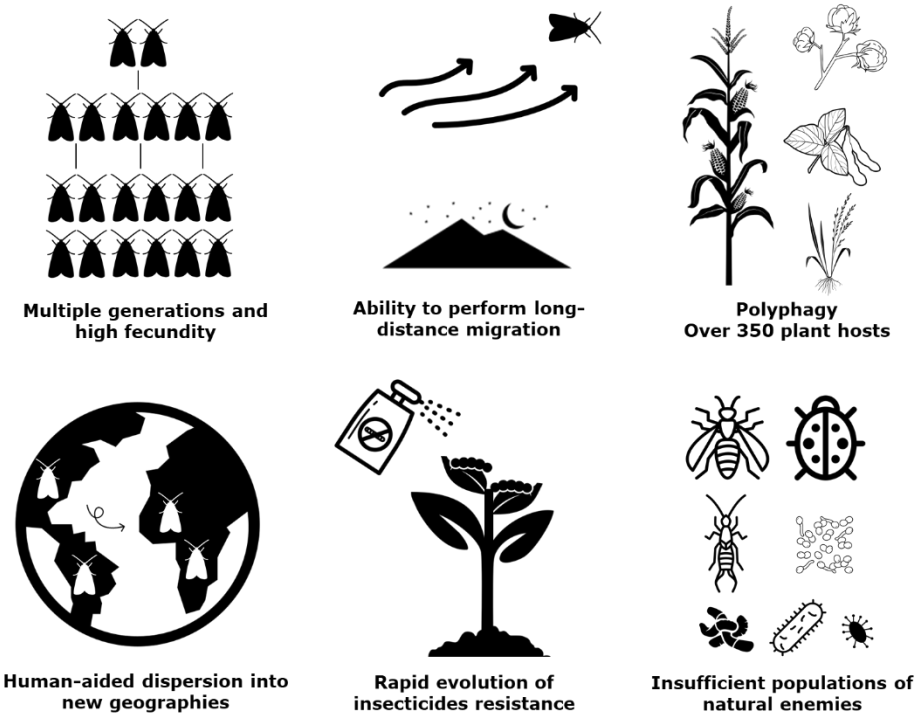

Figure 1. Biological and external factors affecting fall armyworm populations.

1.1.1. High reproduction rate

FAW adult females are relatively short lived but highly fecund, with average lifetime fecundity of over 2,300 eggs per female [6,7]. The life cycle is completed in 30 days under optimal conditions, and may extend up to 90 days at lower temperatures [8]. The number of generations over the year is principally dependent on temperature, and it is capable of producing 12 generations per year in laboratory conditions, and over eight generations in the field [9]. In field conditions, rapid generation turnover and multiple overlapping generations are facilitated by the current cropping system, which consists of consecutive crops throughout the year. This green bridges favors high populations of FAW and presence of all stages (egg, larvae, pupae and adults) [10].

1.1.2. Dispersion and Migration

Likewise other moths belonging to Noctuidae family, long-distance migration is greatly influenced by winds and occurs over multiple generations through nocturnal flights. Migration enables populations to travel from Southern Texas and Florida way up to Canada after one to three months, in a distance of nearly 2,500 km [11,12]. Laboratory studies have

shown that moths have the best flight performance in the second night, and can fly over 24h and up to 46h, although no more than 12h is expected per night [11,13]. FAW moths can travel over hundreds of kilometers per night under favorable synoptic weather conditions, such flight capability was documented when a distance of 1,600 km from US to Canada was covered in only 30h [6,11]. Long distance migration in other continents has not been described at the same extent as North America, mainly due to the challenging nature of studying migratory pathways of large populations of insects over large geographic areas [14].

The dispersion and migration characteristics of FAW moths can be generally described as:

(i) Flight performance is influenced by temperature, relative humidity (RH) and wind patterns. The most suitable conditions for FAW flight are 20–25°C and 60–90% RH [13];

(ii) Nocturnal moths fly during the night, starting their flight at dusk and resting at dawn the next day [15].

(iii) Nocturnal moths can disperse following seasonal orientation patterns [16] and can perform long distance migration according to wind velocity (speed and direction) [17]

(iv) Whereas temperature threshold for FAW development is 13.8ºC during egg, larval and pupal phases [18], low temperature threshold for moths flight is 13.1ºC [19].

(v) Mark-release-recapture studies in Brazil indicated that males flew distances up to 800 m distance, which determined the distance of the refuge area adopted for Bt corn in Brazil [20].

1.1.3. Polyphagy and Damage in Maize

A total of 353 hosts were reported for FAW larva, and most hosts belong to the Poaceae family [21]. Of all potential hosts cultivated in large scale, maize is the most affected crop and can suffer from FAW attack at virtually all growth stages and parts of the plants [22]. In Latin America, the species is commonly known as the “whorl caterpillar”, referring to larval habit to feed mostly on maize whorls, and “military caterpillar” because when food is less available, there is a mass larval movement towards new potential hosts. FAW has been documented to inflict significant maize damage in the Americas, with yield losses up to 34%

in Brazil and 72% Argentina [23,24]. In Africa, the estimated mean loss of maize in Ghana was 45% and in Zambia 40% [25]. Yield losses caused by FAW in other crop hosts were discussed elsewhere [26]. The high potential damage caused by FAW larvae in the maize crop is due to aggressive feeding habits. Despite of direct defoliation, larvae feeding can affect the growing point, destroying the growth potential of plants. Big larvae can also feed on newly emerged plantlets, reducing the plant stand considerably. Ears can also be attacked from the tip and through the husk, causing damage to the kernels [1]. Therefore FAW is a devastating agriculture pest for its ability to feed on different plant parts and have a wide range of potential hosts.

1.1.4. Human aided dispersion

Invasive insects of agricultural importance may introduce novel alleles, thereby increasing the population genomic diversity [27]. Therefore phytosanitary measures are important for effective pest management, to minimize or avoid exchange of resistance and adaptive alleles. Since the pest can be transported in contaminated commodities, it is difficult to estimate how much trade activities are leading to dispersion of FAW. In Galapagos island, air transport modeling indicated that FAW presence was unlikely due to natural migrations from the mainland locations of Ecuador, suggesting that introgressions of FAW into Galapagos islands occurred via contaminated cargo [28]. Multiple introduction events also characterize the invasion of FAW into Africa, Asia and Oceania [27]. FAW populations thus have been affected by human-aided dispersion events.

1.1.5. Resistant Populations

Fall armyworm has the ability to rapidly evolve resistance to insecticides and transgenic plants in the native range, where management of this pest has relied substantially on the use of insecticides, since the 1970’s. The high adoption of Bt-crops also contributed to rapid selection of resistant populations against Bt proteins [8]. It was reported FAW resistance against 42 active ingredients, including resistance to pyrethroids, carbamates, organophosphates, spinosyns, and diamides, and the *Bacillus thuringiensis* insecticidal proteins Cry1F, Cry1Ab and Cry1A.105+Cry2Ab2 [8,29–31]. The intense insecticides exposure has been selecting resistant populations in the field, and this can be attributed to

FAW increased metabolism, target-site insensitivity, cuticular alteration and copy number variation of detoxification genes [32,33].

1.1.6. Natural enemies

The FAW is attacked by a large number of natural enemies, which include parasitoids (Diptera and Hymenoptera), microorganisms (entomopathogenic fungi, bacteria, viruses and nematodes), and predators (Coleoptera, Dermaptera, Hemiptera, Hymenoptera) [31,34]. Egg parasitoids are the most common natural enemies employed in augmentative biological control in the native area for their easier to rear in large scale [31]. In Argentina, larval parasitism ranged from 6.6% to 21.9% [35], and in Ethiopia from 39.5% to 49.9% [36]. In Mexico, 18.2% of the surveyed larva were attacked by natural enemies, including both parasitoids and entomopathogens [37].

Variation in parasitism rates across regions occur due to natural and cultural practices affecting the natural enemies populations [38]. Insecticides spraying can have side effects on natural enemies, such as lethal and sublethal effects, therefore it is important to have assessments of non-target effect of pesticides to protect beneficial insects [8,34]. The vegetation surrounding the fields also affect populations of natural enemies for being a natural reservoir and refuge area for many species, therefore lack of diverse hosts can negatively affect the diversity of natural enemies [35]. Even though FAW larva populations are usually not controlled by the natural enemies alone, it is important to preserve the natural enemies in the system. Besides, biological control projects can be implemented for higher parasitism rates, and the success depends on appropriate environmental conditions and cultural practices [38].

1.2. Strains of Fall Armyworm

The species *Spodoptera frugiperda* comprises two recognized strains named after their host of preference: the ‘rice strain’ (SfR) and the ‘corn strain’ (SfC) [39,40]. Analyses of molecular dating suggest that the two strains may have diverged more than 2 million years ago [41]. The origin of the species is not clear and it is not possible to infer geography divergence of stains due to lack of isolated, pure populations [42]. The SfC and SfR strains occur in sympatry in most places worldwide and cannot be distinguished morphologically. However, there is some level of reproductive isolation including pre and pos zygotic barriers

in interstrain mating, including different female pheromone compositions [43–45], and temporal partitioning of mating activities throughout the night [46,47]. Besides, strains feature physiological differences, such as increased tolerance to insecticides and Bt plants in SfC strain compared to SfR strain [48].

2. Relevance of Genomic Research for Fall Armyworm

Population genetic studies can provide insights about ecological and evolutionary aspects of an insect pest life history, such as past movement and colonization history [49], presence of divergent lineages, biotypes and strains [14,50,51], species hybridization [52], genes related to host-plant adaptation [53], and invasive dynamics of insect pests [12,54–56]. Molecular markers are therefore an indispensable tool for assessing the geographic structure of populations and inferring patterns of gene flow [57]. More examples of genomic approaches developed for insect studies are explored in other studies [58].

2.1.Genetic structure patterns

Identifying population structure and patterns of gene flow featured by insect pest populations is critical for understanding outbreak patterns and for predicting the spread of resistance alleles [57,59]. Gene flow is a result of effective dispersal, and it can be indirectly estimated with molecular markers using population genetic models to compare genotype frequencies [60,61]. Gene flow can be affected by both geographic distances and environmental factors [62]. In the case of insects, the dispersion can be greatly biased by human-aided movements, when specimens are passively transported by contaminated cargo [28,49].

FAW population genetic structure is underlined by the existence of two strains engaged in a speciation process, which makes this an interesting organism for speciation studies [42,45,53,63]. SfR is likely the ancestral strain [64–66]. There are two drivers that explain the divergence of strains: allochrony and host-plant specialization. On the one side, FAW is referred as “timing strains” or “allochronic strains” rather than “host strains”, because moths of each strain feature differential timing of reproduction, therefore genetic isolation between strains is maintained by a reproductive barrier [47,63,66,67]. On the other side, the FAW strains are also referred as “host strains”. In this hypothesis, ancestral strains with differential host usage experienced divergent selection on specific genome regions leading to

reduced gene flow, and thus host-plant adaptation was one the main drivers of the incipient speciation in the FAW [53,65,68]. Nevertheless, the factors underlying the strains divergence should gain clarification with improved analysis power and development of new methodologies.

Regarding genetic structure patterns, geographic structure in USA was characterized using polymorphisms in the mitochondrial *COI* gene, subdividing corn individuals into four haplotype categories: CSh1, CSh2, CSh3, and CSh4 [69]. Populations from Texas and Florida separated by the Gulf of Mexico differed in the proportions of CSh2 and CSh4 haplotypes, with an overlapping area where mixed haplotypes can be found [69]. Individuals of the SfC from Puerto Rico e from the Caribbean resembled the Florida (FL) haplotype, while populations from South America and Mexico belonged to the Texas (TX) haplotype [28,70]. By relying on *COI* haplotypes, thus, migratory pathways in the Americas were simplified as two routes with an intermixing zone in USA.

Specifically to Brazil, existing population studies on FAW focused on geographic differentiation and relationship with host plants using the molecular markers RAPD, AFLP, the mitochondrial gene *COI*, AFLP, microsatellites and SNPs [71–76]. However, most of these studies have focused on differentiating races associated with host plant preference (corn, rice or cotton), and many studies had sampling issues [77].

Previous research with ALFP markers suggested that the populations of Bahia were more resident than the rest of the locations sampled in Brazil [71]. Moreover, AFLP markers did not reveal genetic structure in Brazilian populations in comparison to the USA, Panama and Puerto Rico, but identified that there were subpopulations in Argentina with significant isolation by distance and genetic structure [77]. However, it is not possible to confirm whether this variation within Argentina was in fact due to population genetic structure or to the occurrence of the two host strains since the study lacked stains identification.

Based on analyzes of SNPs markers obtained by the ddRAD method, Brazilian populations collected from maize plants were characterized as genetically similar to each other, and therefore these populations are not genetically structured. Some individuals collected in the rice crop were grouped with individuals collected from corn, raising the hypothesis that these individuals were hybrids, but the study lacked *Tpi* sequencing, which would confirm the presence of hybridization. An interesting fact of this study was that part of the population of Castro, in Paraná State, collected from corn was genetically differentiated

from the other populations (F_{ST} > 0.11205), even in comparison with Cascavel population, in the same state, whose distance between the collection points was less than 400 km, which could be explained by adaptive selection acting in the genetic differentiation [76].

Nuclear markers obtained with nine microsatellite loci, in turn, failed to distinguish populations from Brazil and Paraguay as SfC and SfR, leading to the conclusion that the host strains were having enough interbreeding to the point of no differentiation in the nuclear genome [75]. The microsatellites markers likely did not cover important regions of the FAW genome that were related to strains differentiation, and studies with SNPs markers indicated that divergent loci between host strains were mainly located in the nuclear chromosomes Z, 12, 16, and 24 [47].

2.2.FAW as an Introduced Pest

Another application of genomics for FAW research is the characterization of invasive populations. FAW was first reported in Africa in 2016 and it was probably introduced as stowaways on commercial aircraft, before subsequent widespread dispersal [78]. Comparisons in polymorphisms in the gene *COI* indicated that the first introduction to Africa was probably from populations from Florida and Caribbean [25,79,80]. Moreover, it was found that SfR is rare or absent in Africa, although most invasive populations feature the discordant genotype between *COI* and *Tpi* markers: *COI*-RS and *Tpi*C, suggesting that invasive FAW could be a interstrain hybrid [81]. To confirm strain identity, a more recent study with genome-wide SNPs showed that invasive populations belong to the SfC strain [56].

Low mitochondrial haplotypic diversity in invasive populations indicates that a relatively small number of founders stablished the population in Africa that colonized the invasive range [82], however, studies with both nuclear SNPs and mitochondrial regions evidenced multiple incursions of FAW from different origin sources, indicating that human-assisted activities resulted in the current pest distribution [12,27,56,82].

As an innovation from FAW being invasive to new continents, molecular identification methods were developed for accurate species identification, such as the loop-mediated isothermal amplification (LAMP) [83–85]. New FAW introductions from genetically different populations can certainly change the genetic composition of the invasive populations and greatly impact pest management. As a recent historic of invasions, much is

yet to be revealed and learnt from the FAW population genomics and dynamics in the non-endemic continents. It is noteworthy to mention that knowledge regarding invasive populations is subject to sampling bias and methodology issues, and more research should confirm or clarify our current understanding of this pest in the invasive range.

2.3.Other applications of genomics in FAW research

Besides studies related to speciation and invasive process, the species is widely studied regarding genetic aspects of resistance to chemical pesticides [33,86–89] and Bt crops [90–94]. Moreover, FAW pupal ovarian tissue was used to isolate the famous Sf21 cell line and its Sf9 clone [95], very popular for insect cell culture and studies with baculovirus (reviewed in [96]). To put it succinctly, FAW is becoming a model organism in genomic research [97]. Taking all these facts into consideration, several institutions have sequenced the genome and mitogenome of the fall armyworm (Table 1). The haploid genome comprises 31 chromosomes, with expected size of 396 ± 3 Mb, and GC around 36% [98,99]. Females are heterogametic (ZW system), whereas males are the homogametic sex and has two Z chromosomes. The mitogenome has around 15,365 bp and includes 13 protein-coding genes (PCGs), two rRNA genes, 22 tRNA genes and a AT-rich region [100].

2.4.DNA Markers Used for Host Strain Identification

Rice and corn strains of FAW are morphologically indistinguishable and can only be identified by molecular techniques. The host strains were first characterized by electrophoretic allozymes profiles using starch gels [14,40,101], and since then a number of molecular markers to diagnose host strains were described, such as RFLP - Restriction Fragment Length Polymorphisms [102] and AFLP – Amplified Fragment Length Polymorphisms [103]. Tandemly repeated DNA sequences called FR (Fall armyworm Rice Strain) enable detection of the SfR strain through observation of large PCR products, present in females 120-fold more than in males [104]. Individuals from SfC strain can also have the FR sequences, however at low copy number and not in large tandem arrays [105]. Less used method of PCR-RFLP targeting the ND1, a mitochondrial gene coding for NADH dehydrogenase 1 protein, has also been employed for strains diagnosis [42] and characterization of population from corn in cotton [106]. These markers were directly visualized as discrete bands revealed by agarose or polyacrylamide gel electrophoresis.

Nevertheless, their use in both population genetic research and strain diagnose were replaced by the advent of affordable and efficient DNA sequencing methodologies.

Table 1. Fall armyworm genome assemblies

Description	Accession Number / Bioproject	Reference
Draft genome of Sf21 cell line	PRJNA257248 (NCBI)	[107]
Genome for male SfC and for male SfR strain	PRJEB13110 (NCBI)	[99]
Draft genome of Sf9 cell line, derived from pupal ovarian tissue	PRJNA380964 (NCBI)	[108]
Genome of Sf-RVN cell line	PRJNA344686 (NCBI)	[109]
Genome with 31 chromosomes of a single male adult originally from Zambia	PRJNA591441 (NCBI)	[110]
Genome of SfR strain male pupae	PRJNA663441 (NCBI)	[111]
Genome with 31 chromosomes of a SfC male pupa	PRJNA662887 (NCBI)	[33]
Chromosome-level genome of field adults from China: one male and one female	CNP0000513 (CNSA)	[98]
Genome of a single female pupa with 31 chromosomes and a portion of W chromosome	PRJNA590312 (NCBI)	[112]
Draft genome of maize associated FAW from Kenya	PRJNA863575 (NCBI)	Unpublished
Chromosome-level genome of larvae	PRJNA809428 (NCBI)	Unpublished

There are currently two genes whose sequences are more often used, either alone or combined: *COI* and *Tpi* [113,114]. *COI* is a mitochondrial gene coding for cytochrome oxidase subunit I protein and it is maternally inherited. A PCR-RFLP method based on the restriction enzyme *Msp*I was first developed to identify FAW strains [115]. Sequencing of the *COI* fragment enables further population genetic studies based on haplotype groups classified according to the presence of two polymorphic sites [116]. Although *COI* sequence is employed as a global barcode for DNA-based identification system in animal species [117], its utility for strain diagnose is partially compromised due to maternal inheritance, and because strains can intermate and produce fertile hybrids, individuals resulted from interstrain crossing are classified based on their maternal strain. Nevertheless, *COI* continues to be used due to simplicity, the PCR-RFLP methodology enables fast strain identification without the need of sequencing and the informative mitochondrial sequences can be employed in population genetic and phylogeny studies.

The triosephosphate isomerase (*Tpi*) gene is located in the sex chromosome Z and it is a useful marker for identifying Noctuidae subpopulations in the initial stages of speciation [118]. One single polymorphism at site 183 (g*Tpi*183) in the exon 4 can reliably discriminate FAW strains [113]. Because females possess only one chromosome Z, interstrain hybrids can be determined only in male individuals, which inherit one Z chromosome from each parent [119]. Additionally, intron sequences from the *Tpi* gene are highly variable and are also employed to understand levels of genetic variation in FAW populations [120].

More recently, four SNPs showed to be reliable to differentiate between strains and interstrain hybrids using real-time PCR based TaqMan assays, including three SNPs in the Z-chromosome and one SNP in the chromosome 16. The autosomal SNP can be useful in detecting interstrain hybridization in both males and females, which is not possible with Z-linked *Tpi* and maternally inherited *COI* [121].

3. Conclusions

Effective integrated pest management programs for FAW can be greatly favored by information regarding genetic and genomic features of FAW populations in its current geographic distribution area, native or invasive. Many insights on genetic variability, population structure, and even migration patterns have been obtained through the use of molecular markers, but, except for the North America continent, migration patterns in other geographies are not clear. There are many indicatives that populations may be structured, and these hypotheses should be further explored. Therefore, the use of genomic approaches has been of foremost importance to advance in our understanding of FAW biodiversity.

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Chapter 2

Diversity and distribution of *COI* haplotypes of fall armyworm in Brazil and Argentina

Abstract

The fall armyworm (FAW) is a major lepidopteran pest throughout the world, posing serious economic losses upon feeding. Knowledge on genetic diversity, geographic structure and demography is relevant in the context of managing this important pest. Using the sequences of mitochondrial DNA cytochrome c oxidase subunit I (*COI*) as genetic marker, we analyzed the population genetic diversity and structure of 250 individuals collected from 15 FAW locations across Brazil and Argentina. In addition, we estimated the demographic history using neutrality test and mismatch distribution analysis. Analysis of molecular variance revealed that most of genetic variation (76.1%) was explained by the presence of two strains. Low pairwise F_{ST} values between Brazilian populations indicated presence of gene flow in the entire sampled area, explaining the sharing of haplotypes between distant populations. Populations from Brazil also featured higher haplotypic diversity values, ranging from 0.667 to 0.928. On the opposite, Argentinian populations were more differentiated among each other and presented low values for haplotype (0 – 0.350) and nucleotide diversity (0 – 0.308). Overall, the 40 haplotypes were linked in a complex mutational network that did not reveal clusters based on geography. Shared haplotypes in Brazil and Argentina population suggested existence of gene flow between countries. The demographic analyses showed that FAW had experienced population expansion. Our data is consistent with the current understanding of FAW populations in South America, with additional information on population history and structure.

Keywords: *Spodoptera frugiperda*, insect pest, molecular entomology, gene flow, population structure, mtDNA, mitochondrial gene

Introduction

The characterization of genetic variation within a species allows the inference of the evolutionary origin and history of populations [1]. Populations with high levels of genetic diversity may be able to respond to environmental changes and therefore to adaptive pressures more effectively [2]. Understanding the distribution of genetic variability among and within populations enables the characterization of genetic structure in these populations, including estimations of gene flow, and risks of resistance alleles spreading [3]. In the case of insect pests, understanding genetic diversity and structure of populations therefore have practical implications for pest management [4].

Amongst the molecular markers employed to the study of genetic relationships and biogeography of populations, the cytochrome oxidase subunit I (*COI*) gene has been widely used due its moderate evolutionary rate and clear pattern of evolution, making it robust for determining intra and interspecific variation [5]. In the case of insect pests, besides genetic variation studies [6,7], *COI* has been employed as a barcode for molecular identification of species [8], inference on the origin of invasive species [9,10], characterization of migration patterns [11], geographic structure [12], and demographic history [13].

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is an important and cosmopolitan pest that attacks numerous cultivated crops, including maize, cotton, soybeans, and sorghum [14]. Economic losses resulted from FAW larval feeding can reach up to 72% [15]. Although FAW is a highly polyphagous pest, grasses are the preferred hosts. Due to host preference during feeding, FAW has been subdivided into two distinct genetic groups called corn strain (SfC) and rice strain (SfR) [16]. Molecular dating based on mitochondrial and nuclear genes suggest that FAW strains have diverged more than 2 million years ago, long before the domestication of maize (estimated around 10,000 years ago), and the introduction of rice, sorghum and sugarcane in the Americas [17,18]. Therefore, the strains of FAW are examples of herbivorous insects that were able to adapt to cultivated plants and have since been evolving in response to the agricultural production landscape [19].

Studies on population structure and genetic diversity of FAW relying on *COI* haplotypes showed little genetic diversity in the native region, indicating that host strains are sympatric and Argentina haplotype ratios are similar to Brazil's [20,21]. Thus, the present investigation was designed to examine the genetic structure of FAW in Brazil and Argentina and analyze the demographic history in the native range.

Materials and Methods

Insect sampling

Larvae of FAW were collected from the field between 2018 and 2021 and reared in artificial diet until become adults. All populations were collected from corn fields, except for RS population from a rice field. Collections from Brazil locations were reported under SISBIO license number 58435. The total dataset included a combination of 22 individuals unique to this study and 228 sequences obtained from Genbank (Accession numbers ON704174 - ON704401), providing a total of 250 sequences of FAW from Brazil and Argentina. Genbank sequences were obtained from the same populations used in this current study.

DNA isolation, amplification and sequencing

DNA was extracted from moths legs using the CTAB method with modifications [22]. DNA concentration was estimated based on agarose gel stained with SYBR Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, USA) and diluted to 30 ng/µL.

A 581-bp sequence of the mitochondrial gene Cytochrome C oxidase subunit I (*COI*) was amplified by PCR using primers 891F (5′-TACACGAGCATATTTTACATC-3′) and 1472R (5′-GCTGGTGGTAAATTTTGATATC-3′) following procedure described elsewhere [23]. PCR products were sequenced using Sanger sequencing method. The sequences were checked and trimmed in BioEdit.7.2.5 [24]. *COI* sequences were submitted to BLASTn search from NCBI database to confirm the species identity. Sequences were aligned with Clustal W [25] using MEGA 7 [26]. Sequences were deposited with the NCBI GenBank database to obtain accession numbers (Table 1).

Haplotype distribution and neutrality test

Populations were analyzed for descriptive statistics such as nucleotide diversity (π), number of haplotypes (H), and haplotype diversity (Hd) using DnaSP 6 [27]. Popart software was used to generate the median-joining (MJ) haplotype network and the haplotype distribution map [28].

Further, to ascertain the demography of the population and evolutionary neutrality of FAW populations, neutrality test Tajima's D [29] and pairwise mismatch distribution were

performed in DnaSP 6 [27]. Pairwise mismatch distributions were implemented to assess whether strains experienced expansion events or have been facing neutral evolution.

Analysis of molecular variance (AMOVA)

Analysis of molecular variance (AMOVA) was used to investigate the genetic structure of two groups. We first investigated whether populations were structured by geographical location, by comparing populations from Argentina with those from Brazil. Then we performed another analysis grouping individuals as their host strain according to diagnostic polymorphism in the *COI* region. We additionally calculated pairwise F_{ST} distances among the 15 populations. Both AMOVA and F_{ST} statistics were calculated using Arlequin 3.1 with 1000 permutations [30].

Table 1. Details of samples used to study population genetics of fall armyworm in Brazil and Argentina. N refers to the number of specimens of each population.

Population	N	State	Country	Collection date	Accession No
AR01	12	Metán - Salta	Argentina	22-Feb-18	ON704174 - ON704184
AR02	7	America - Buenos Aires	Argentina	24-Jan-18	ON704185 - ON704187
AR03	16	San Justo - Santa Fe	Argentina	27-Feb-18	ON704188 - ON704202
BA02	19	São Desidério - Bahia	Brazil	26-Jun-18	ON704203 - ON704221
BA03	18	Barreiras - Bahia	Brazil	19-Jul-18	ON704222 - ON704239
DF	20	Planaltina - Distrito Federal	Brazil	8-Jun-18	ON704240 - ON704259
GO	24	Rio Verde - Goiás	Brazil	22-Jun-18	ON704260 - ON704283
MA01	10	São Luís - Maranhão	Brazil	12-Dec-18	ON704284 - ON704293
MA02	14	São Luís - Maranhão	Brazil	19-Dec-18	ON704294 - ON704306
MT01	24	Campo Novo do Parecis - Mato Grosso	Brazil	25-Jun-18	ON704307 - ON704330
MT02	21	Campo Novo do Parecis - Mato Grosso	Brazil	1-Jul-18	ON704331 - ON704351
PR	10	Toledo - Paraná	Brazil	20-Jun-18	ON704352 - ON704360
RS	16	Alegrete - Rio grande do Sul	Brazil	13-Jan-21	ON704361 - ON704363
SC	16	Chapecó - Santa Catarina	Brazil	14-Dec-20	ON704364 - ON704378
SP	23	Taquarituba - São Paulo	Brazil	14-Jul-18	ON704379 - ON704401

Results

mtDNA *COI* sequencing and host strains

We assessed 250 sequences of 537 bp from 12 FAW populations from Brazil and 3 FAW populations from Argentina, totaling 15 populations. The accesses are located within the main corn and rice producing regions of Brazil and Argentina, and are distributed in seven ecoregions (Figure 1). A total of 22 sequences were generated for this study to increase

sample size and were deposited in Genbank (accession numbers OQ849779-OQ849800). All 16 samples from the rice field collected in Rio Grande do Sul State were assigned as SfR. Most samples of AR03 and MA02 locations collected from corn fields were also assigned as SfR. Few individuals from other locations had the diagnostic polymorphism for SfR, for this reason these populations were classified as SfC.

Genetic diversity and haplotype distribution

The values of the haplotypic diversity parameter vary between 0 and 1, with higher values meaning a greater diversity among the analyzed individuals in each population. Here, haplotype diversity analysis indicated 37 polymorphic sites. The number of individuals (N), number of haplotypes (H), and haplotype diversity (Hd) and nucleotide diversity (π) for each population are shown in Table 2. Haplotype H1 was both the most widespread, shared by 101 samples from 12 populations (BA02, BA03, DF, GO, MA01, MA02, MT01, MT02, PR, SC, and SP). There were 22 unique haplotypes (Table 3), six of them were present only in GO population (H17, H18, H21, H22, H24, H25), followed by four unique haplotypes in SP population (H37, H38, H39, H40).

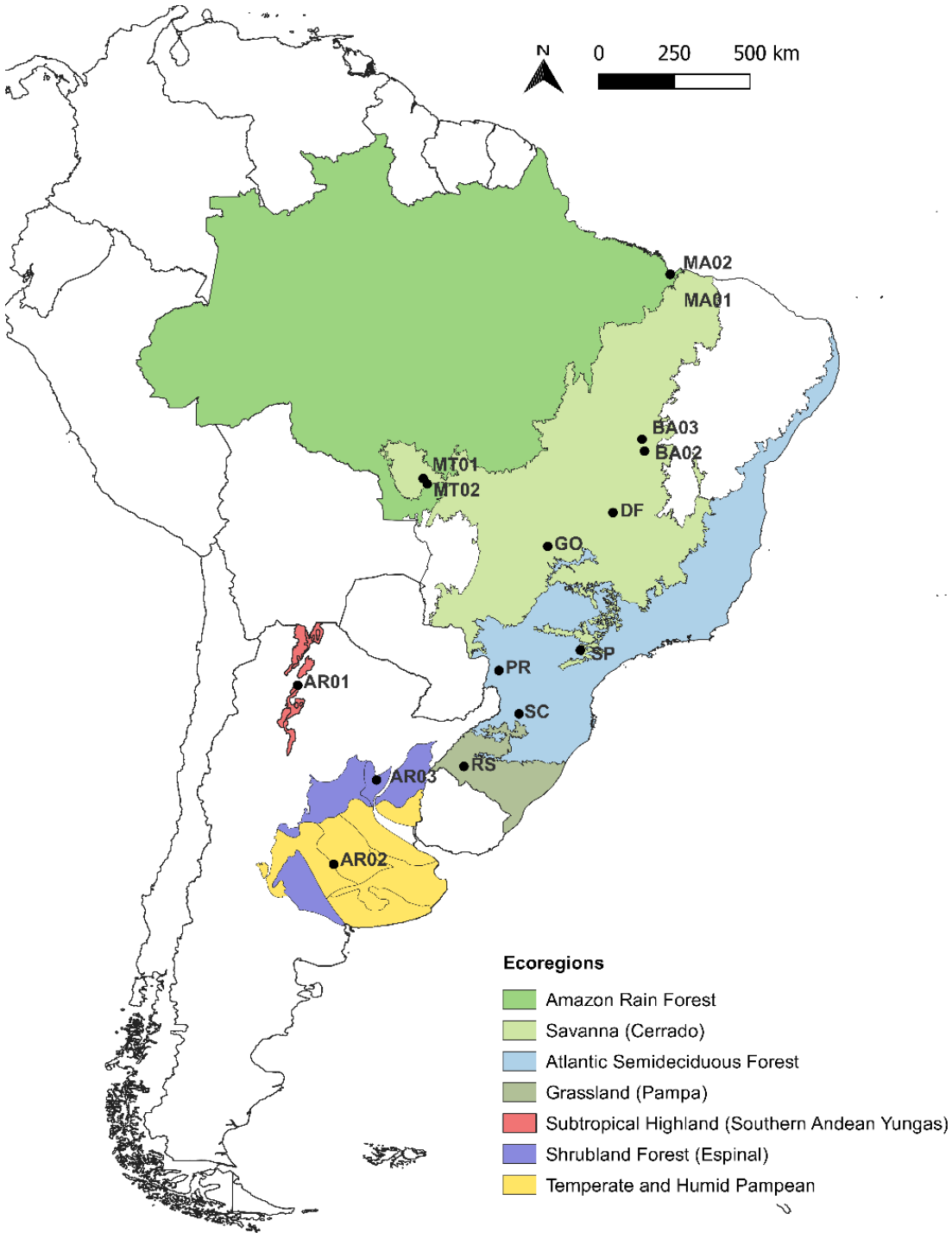

Figure 1. Fall armyworm populations from Brazil and Argentina. Map was generated using the software QGIS v3.28.3-Firenze, using shapefiles of Brazil and Argentina ecoregions from public domain provided by IBGE and by Geoportal Idesa.

Table 2. Number of samples (N) and haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), standard deviation (SD), and neutrality test (Tajima's D) in each geographic region. A negative Tajima's D denotes a higher number of low frequency polymorphisms than anticipated. *Statistical significance: P < 0.10.

Locality	N	H	Hd ± SD	π ± SD	Tajima's D
AR01	12	1	0	0	-
AR02	7	1	0	0	-
AR03	16	3	0.350 ± 0.148	0.00308 ± 0.00187	-2.25695*
BA02	19	6	0.760 ± 0.090	0.00481 ± 0.00181	-1.31569
BA03	18	7	0.752 ± 0.103	0.00415 ± 0.00175	-1.98870*
DF	20	8	0.732 ± 0.094	0.00224 ± 0.00049	-1.29703
GO	24	15	0.928 ± 0.039	0.00531 ± 0.00125	-2.0182*
MA01	10	5	0.756 ± 0.130	0.00245 ± 0.00070	-1.03527
MA02	14	6	0.747 ± 0.111	0.01017 ± 0.00180	0.9744
MT01	24	8	0.703 ± 0.098	0.00250 ± 0.00060	-1.85123*
MT02	21	10	0.886 ± 0.049	0.00450 ± 0.00143	-1.67893*
PR	10	5	0.667 ± 0.163	0.00207 ± 0.00067	-0.82229
RS	16	6	0.767 ± 0.084	0.00199 ± 0.00038	-0.96266
SC	16	6	0.742 ± 0.105	0.00793 ± 0.00244	-0.22717
SP	23	10	0.798 ± 0.078	0.00537 ± 0.00175	-1.48715
Total	250	40	0.802 ± 0.022	0.00863 ± 0.00059	-0.99688

Small values of Hd and π (Hd < 0.5 and π < 0.005) were observed in all three populations of Argentina, which may be due to population bottleneck or founder event by single or a few mtDNA lineages. In contrast, 10 Brazilian populations presented high Hd and low π (Hd > 0.5 and π < 0.005), indicating these populations had undergone population bottleneck followed by rapid population growth and accumulation of mutations. Haplotype network (Figure 2) showed a complex mutational network with clear separation between SfR and SfC, caused by six mutations. where haplotypes could be better explained by their host strain, and populations located further South such as AR02, AR03 and RS, lacked the most common haplotype H1 presented in the rest of the locations, indicating restricted gene flow among these regions (Figure 3).

Table 3. Haplotypes from *COI* sequences of 250 fall armyworm individuals from Brazil and Argentina populations.

Haplotype number	Frequency	Locality
H1	101	AR01, BA02, BA03, DF, GO, MA01, MA02, MT01, MT02, PR, SC, SP
H2	29	AR02, AR03, DF, GO, MA01, MT01, MT02, PR, SP
H3	33	AR03, BA03, MA02, MT02, RS, SC, SP
H4*	1	AR03
H5	2	MA02, MA02
H6	13	BA02, BA03, DF, GO, MA01, MT02, SC, SP
H7	4	BA02, GO, MT01
H8	9	BA02, MA02, RS, SC
H9	7	BA02, DF, MT01, MT02, PR, SC
H10	2	BA03, GO
H11*	1	BA03, GO
H12	5	BA03, GO
H13*	1	DF
H14	2	DF, PR
H15	3	DF, MT02, SP
H16*	1	DF
H17*	1	GO
H18*	1	GO
H19	7	GO, MA01, MT02, SC
H20	2	GO, SP
H21*	1	GO
H22*	1	GO
H23	3	GO, MA01, MA02
H24*	1	GO
H25*	1	GO
H26	2	MA02, MT02
H27*	1	MT01
H28*	1	MT01
H29	2	MT01
H30*	1	MT02
H31*	1	MT02
H32*	1	PR
H33*	1	RS
H34	2	RS
H35*	1	RS
H36*	1	RS
H37*	1	SP
H38*	1	SP
H39*	1	SP
H40*	1	SP

*Unique haplotypes

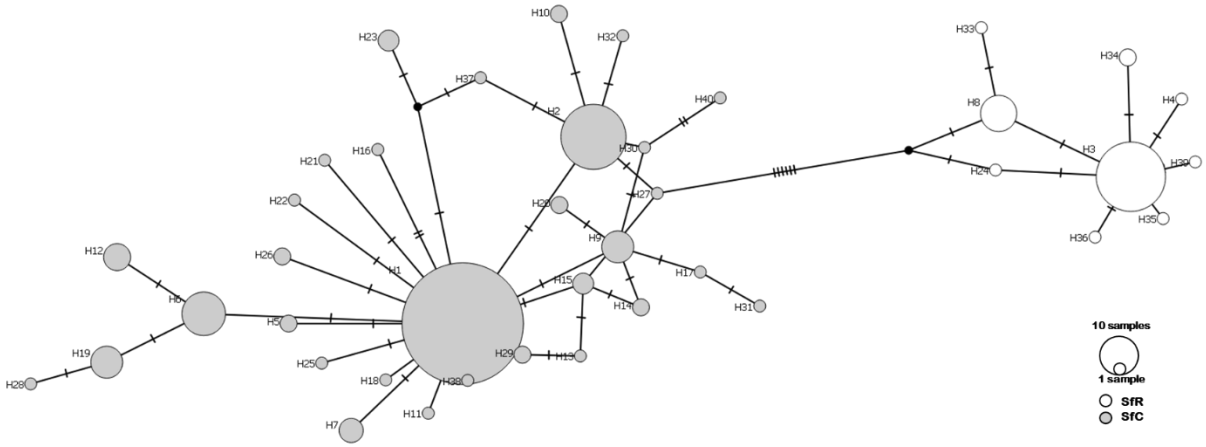

Figure 2. Median-joining haplotype network of FAW *COI* gene partial sequences from 15 populations in Brazil and Argentina. Each circle represents a unique haplotype, the circle size is proportional to the number of sequences. The lines between linked haplotypes correspond to the number of mutations that separate the haplotypes. A total of 40 haplotypes are indicated according to the two FAW strains, shown with different colors.

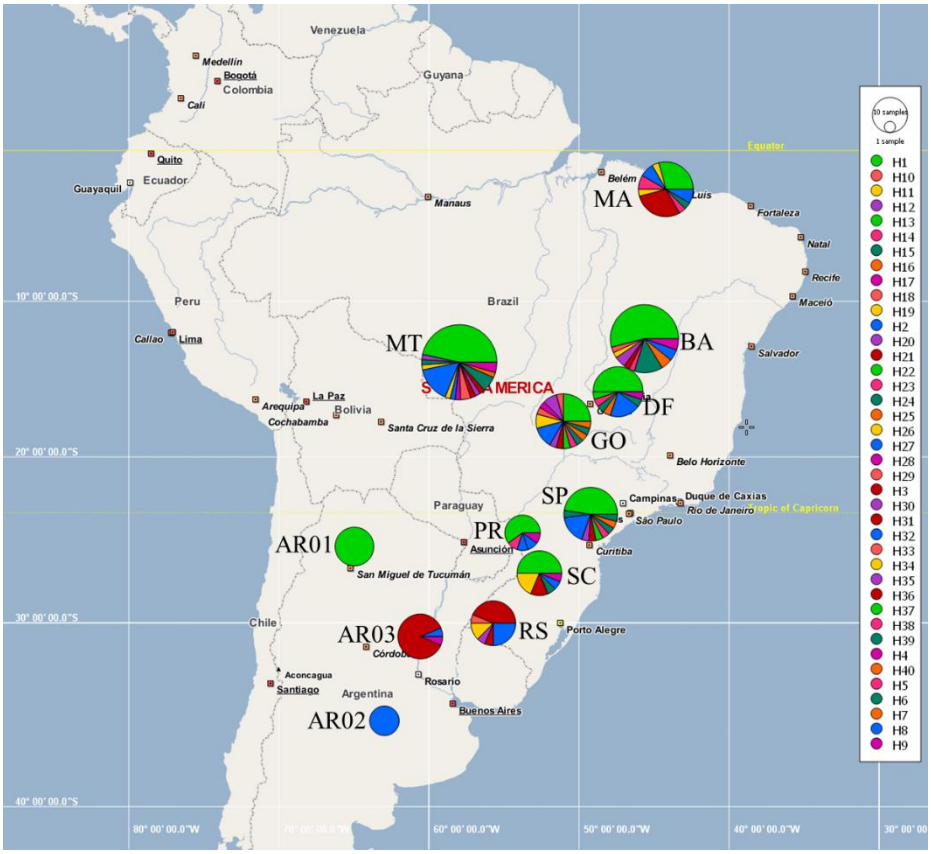

Figure 3. Haplotype distribution map. A total of 40 haplotypes are indicated by different colors and positioned in the geographical area of insect sampling. Populations within the same federal unit were combined to improve visualization of haplotypes frequency.

Population genetic differentiation

The genetic structure of Brazilian populations of FAW estimated by AMOVA (Table 4) indicated no significant differentiation between populations from Argentina with those from Brazil (P-value = 0.10166, F_{ST} = 0.04451). When grouping individuals according to their host strain, significant F_{ST} value of 0.76095 (P-value < 0.0000) confirms that genetic structure among the populations studied is more related to the strains SfR and SfC.

Table 4. Analysis of Molecular Variance (AMOVA) results for the fall armyworm groups

Group	Source of variation	Df	Sum of Squares	Variance components	Total variance (%)	P-value
Argentina and Brazil	Among groups	1	24.098	0.13473	4.45	0.10166
	Among populations within groups	13	244.509	1.01056	33.38	
	Within populations	235	442.222	1.88179	62.17	
	Total	249	710.828	3.02708		
SfR and SfC	Among groups	1	359.503	4.53933	76.09	0.00000
	Among populations within groups	21	42.65	0.06393	1.07	
	Within populations	226	307.839	1.36212	22.83	
	Total	248	709.992	5.96538		

Pairwise F_{ST} values (Table 5) varied from 0.000 to 0.937 when comparing the 15 populations, showing the same pattern of genetic differentiation according to host strains, except for AR02 population, which had high significant differentiation when compared to most populations belonging to either strain. There was no significant differentiation between most populations from SfC in Brazil, except for MT01 and SP populations which showed significant difference of F_{ST} = 0.049.

Table 5. Pairwise F_{ST} values among populations of fall armyworm based on *COI* region. Significant F_{ST} values are shown in bold (P-value ≤ 0.05). The populations AR03, MA02 and RS were considered SfR strain based on *COI* diagnostic polymorphism.

	AR01	AR02	**AR03**	BA02	BA03	DF	GO	MA01	**MA02**	MT01	MT02	PR	**RS**	SC	SP
AR01															
AR02	**0.697**														
AR03	**0.775**	**0.697**													
BA02	0.015	**0.197**	**0.664**												
BA03	0.008	**0.215**	**0.701**	0.000											
DF	0.021	**0.214**	**0.745**	0.015	0.007										
GO	0.004	0.017	**0.520**	0.011	0.003	0.014									
MA01	**0.061**	**0.199**	**0.715**	0.000	0.000	0.000	0.000								
MA02	**0.551**	**0.450**	0.102	**0.407**	**0.464**	**0.529**	**0.311**	**0.474**							
MT01	0.010	0.024	**0.504**	0.039	0.032	0.032	0.000	0.000	**0.310**						
MT02	0.043	**0.118**	**0.687**	0.000	0.000	0.000	0.009	0.000	**0.448**	0.041					
PR	**0.086**	**0.289**	**0.726**	0.000	0.000	0.000	0.000	0.006	**0.483**	0.002	0.000				
RS	**0.937**	**0.894**	0.037	**0.798**	**0.832**	**0.871**	**0.612**	**0.875**	**0.243**	**0.586**	**0.812**	**0.892**			
SC	0.088	**0.145**	**0.558**	0.000	0.009	0.072	0.019	0.027	**0.277**	0.051	0.003	0.048	**0.701**		
SP	0.046	0.068	**0.646**	0.000	0.002	0.006	0.021	0.001	**0.393**	**0.049**	0.000	0.000	**0.771**	0.000	

Demographic history

Neutrality tests evaluate the range of historical population expansion, with negative values associated to demographic expansion, and positive values to equilibrium. Tajima's D statistic test of neutrality index indicates whether the mean number of differences between pairs of sequences is compatible with the observed number of segregating sites [29]. Here, significant negative values for this test (Table 2) were observed in populations AR03, BA03, GO, MT01, and MT02, indicating that they have been undergoing population expansion throughout its evolutionary history. Thus, the results obtained in this study reject the hypothesis of neutral evolution for at least part of the FAW populations investigated in Brazil and Argentina.

Likewise, the mismatch distribution pattern of pairwise nucleotide differences demonstrated that the observed curve had a similar pattern with the expected curve. The unimodal curves therefore indicated population expansion of FAW in the large spatial scales (Figure 4).

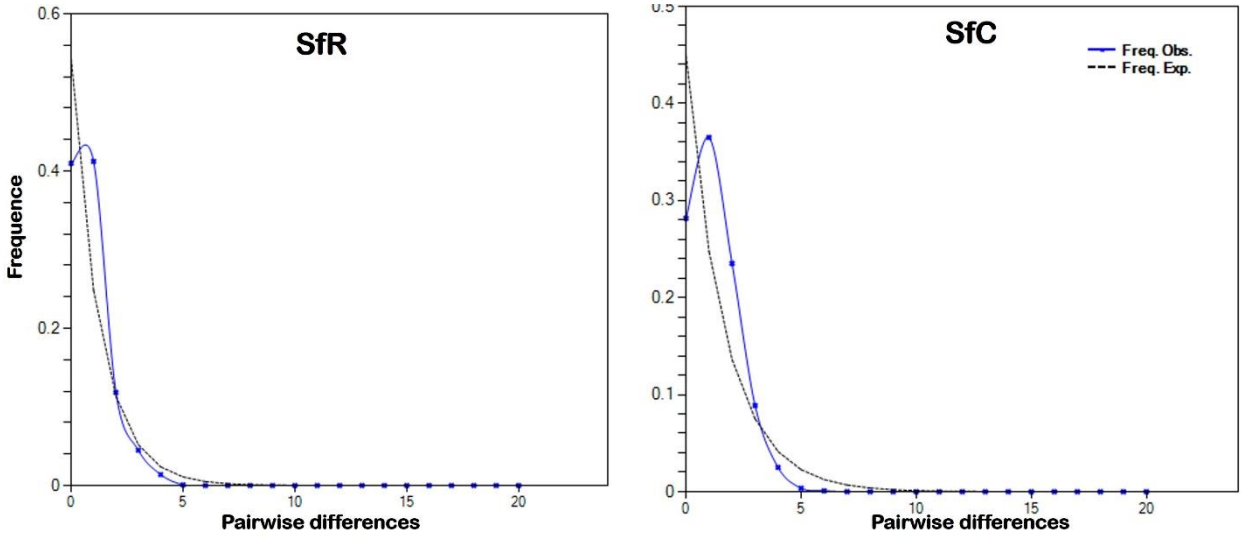

Figure 4. *COI* mismatch distribution unimodal curves. Frequencies of pairwise differences for the observed (solid blue line) and expected (dotted black line) pairwise nucleotide site divergences computed with DnaSP 3.1 for each strain of FAW. SfR = *Spodoptera frugiperda* rice-strain. SfC = *Spodoptera frugiperda* corn-strain.

Discussion

We investigated FAW genetic diversity, structure and demographic history in Brazil and Argentina by assessing polymorphisms in a 537 pb region of the mitochondrial gene *COI*. First, we screened for the diagnostic polymorphism to assign each individual to a host strain: rice-strain (SfR) or corn-strain (SfC). We confirmed that the host strains are sympatric, due the presence of few SfR individuals in populations collected in corn fields. The discordant genotype SfR was observed in MA02 and AR03 locations, which were expected to be mostly composed by SfC individuals. The collection from the rice field, in turn, presented only SfR individuals.

AMOVA analysis did not indicate that populations were structured by country (only 4.4% of total variance), but rather by host strains SfR and SfC (76.1% of total variance). However, Argentinian populations collected in Salta (AR01), Buenos Aires (AR02) and Santa Fe (AR03) were more differentiated among each other, with significant pairwise F_{ST} values. Overall, all three populations from Argentina had low values for haplotype (0 – 0.350) and nucleotide diversity (0 – 0.308), suggesting that these populations had undergone bottleneck or founder event by single or a few mtDNA lineages from an ancestral population [31].

Argentina features a unique landscape in South America, and compared to Brazil it has more diversified ecoregions. Evidence of semi-arid conditions in Argentina in the late

Pleistocene (12,100 -13,400 years before present) [32] must have hindered establishment of FAW in this country for humidity being an important factor for larvae and adults survival [33,34]. Even to this date, most part of Argentina is not suitable for the establishment of permanent FAW populations [35], however the genetically structured populations in this study indicates that FAW populations of Argentina are not the result of year-round migrating populations from a common source. Altogether, we can hypothesize that once landscape became favorable for FAW survival in Argentina, populations have established from few ancestral SfC and SfR lineages different from those that colonized Brazil.

Brazilian populations within each host strain had low pairwise F_{ST} distances, indicating high levels of gene flow among regions, and presented high haplotype diversity and low nucleotide diversity, which is indicative of population bottleneck followed by rapid population growth and accumulation of mutations. Despite of low genetic structure observed in SfC from Brazil locations, there was significant F_{ST} distance (0.049) between SP and MT01. Cases of single populations which seem to be more differentiated than others have been reported for the States of Bahia [36] and Paraná [37] in Brazil.

Nevertheless, shared haplotypes were observed between countries and localities, indicating that gene flow occurred among these populations in recent history. The lack of the most common haplotype H1 in AR02, AR03 and RS should be better investigated though, since it may be evidence that restricted gene flow is leading to genetic isolation in the south of the continent.

Concerning demographic history of FAW, both the neutrality test and the mismatch distribution analysis indicated that populations are experiencing expansion. Our results corroborate with other studies that analyzed the native area of FAW occurrence [38,39]. Recent population expansion was also described for another crop pest native from Argentina and Brazil, the sugarcane borer, where the demographic events coincided with agricultural expansion of host crops [40]. Agriculture in Argentina has expanded substantially for row crops production, with a 45% increase in the cultivated area between 1990 and 2006, changing from cattle grazing activities to row crops system of agriculture [41]. This expansion has been more evident in the ecoregions of Chaco, Pampas and Espinal [41], which coincide with the sampling locations of this study. Similarly, agricultural frontier in Brazil expanded twice the area across Cerrado ecoregion between 2003 and 2013 [42]. Therefore

population expansion is supported by intensification and expansion of agriculture both in Argentina and Brazil.

As agriculture landscape may be hugely affecting evolutionary aspects of crop pests, so are the control strategies. While one is favoring populations expansion, the other is acting as population reduction at local scale, with selection pressures favoring individuals with specific resistance alleles. Here we found that populations from Brazil presented high haplotype diversity, are not genetically structured, and have experienced rapid population growth and accumulation of mutations. This information can alert for risks of resistance evolution and spread of resistance alleles through gene flow.

The haplotypes obtained from *COI* sequences indicated shared haplotypes between countries and therefore recent gene flow among populations from Argentina and Brazil. More studies are needed to confirm to whether extent populations further south are geographically isolated and becoming more differentiated than the rest of the continent. This information is relevant for insect management programs, since the populations from Argentina presented low values of haplotypic and nucleotide diversity, and therefore may feature a decreased ability to respond to control tools, such as insecticides and transgenic plants.

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Chapter 3

PLOS ONE

RESEARCH ARTICLE

Population genomics of fall armyworm by genotyping-by-sequencing: Implications for pest management

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Abstract

The fall armyworm (FAW), *Spodoptera frugiperda*, is a significant pest of many crops in the world and it is native to the Americas, where the species has shown the ability to rapidly evolve resistance to insecticides and transgenic plants. Despite the importance of this species, there is a gap in the knowledge regarding the genetic structure of FAW in South America. Here, we examined the genetic diversity of FAW populations across a wide agricultural area of Brazil and Argentina using a Genotyping-by-Sequencing (GBS) approach. We also characterized samples by their host strain based on mitochondrial and Z-linked genetic markers. The GBS methodology enabled us to discover 3309 SNPs, including neutral and outlier markers. Data showed significant genetic structure between Brazil and Argentina populations, and also among the Argentinian ecoregions. Populations inside Brazil showed little genetic differentiation indicating high gene flow among locations and confirming that structure is related to the presence of corn and rice strains. Outlier analysis indicated 456 loci putatively under selection, including genes possibly related to resistance evolution. This study provides clarification of the population genetic structure of FAW in South America and highlights the importance of genomic research to understand the risks of spread of resistance genes.

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a major agricultural pest that can feed on several different hosts [1]. FAW have the ability to

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evolve rapid resistance to insecticides and transgenic crops, which can impact the effectiveness of control strategies [2]. The spread of such resistance traits is dependent on the migratory behavior of FAW and it is therefore important for effective pest management to delineate the pattern and magnitude of population movement across national boundaries.

In North America, FAW populations from Texas and Florida make annual migrations northwards to recolonize areas in the north of USA and Canada [3]. This behavior reflects the inability of FAW to survive freezing temperatures, which limits winter populations to the most southern locations in the states of Texas and Florida [4]. Less is known about migration patterns in South America, which experiences a significantly different climate than North America. Climate suitability modeling shows that South America features suitable conditions for persistent FAW populations in this continent, except for most of Argentina [5]. This suggests an annual migration towards Argentina from more suitable locations, with the neighboring countries being the prime candidates for the migratory sources [6].

A complicating factor for FAW is that the species can be subdivided into two groups called host strains that differ in their distribution on plant hosts in the field, with the C-strain preferentially found in corn and the R-strain in pasture grasses and, to a lesser extent, rice [7, 8]. The host strains are morphologically indistinguishable and so can only be identified through the use of molecular markers, with the most commonly used being polymorphisms in the mitochondrial cytochrome oxidase subunit I (COI) and the Z-chromosome linked triosephosphate isomerase (Tpi) genes [9, 10].

At this time, the most consistent evidence of population structure in South America is that indicative of the two host strains, although some level of genetic structure has been observed for populations within Paraguay and within Brazil [11–13]. Overall, it remains unclear whether and to what extent geographically separated FAW in South America exhibit genetic differentiation. To better address this issue, we used Genotyping-by-Sequencing (GBS), a variation of the commonly used restriction-site-associated DNA sequencing methodology to identify single nucleotide polymorphisms (SNPs). GBS data allows us to resolve patterns of genetic diversity and spatial structure at very fine scale [14], and it was utilized in this study to evaluate patterns of gene flow and genetic structure of FAW populations across Brazil and Argentina. We additionally examined the information of host strains to discover informative loci putatively under selection. According to the results, we discuss some practical implications of our findings to the FAW management in South America.

Material and methods

Sampling and DNA extraction

Fall armyworm caterpillars were manually collected in fields from 12 locations in Brazil under the SISBIO license number 58435, and from 3 locations of Argentina, between June of 2018 and January of 2021 (Fig 1, Table 1). Larvae were transferred to trays containing artificial diet and reared in laboratory conditions until become moths. Moths were placed in 1.5 mL polypropylene microcentrifuge tubes with 98% ethanol and stored at -20°C. Species identification was confirmed by morphology and sequencing of the COI gene (see below). We extracted DNA from the legs of random adults using CTAB-based method [15]. DNA quality and quantification were assayed by agarose electrophoresis gel (1% w/v) stained with SYBR Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, USA). DNA concentrations were adjusted to approximately 30 ng/μL.

Distribution map of the populations was drawn using the software QGIS v3.28.3-Firenze. (Open Access Geographic Information System, https://qgis.org/en/site/. Accessed on February 26th, 2023). Publicly available shapefile of South American country boundaries was downloaded

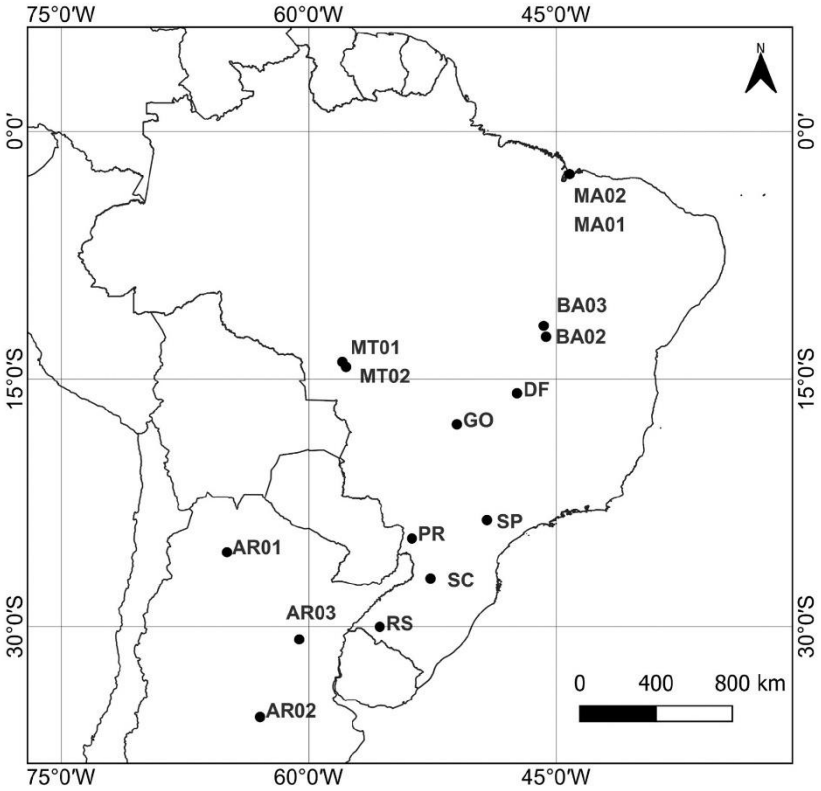

Fig 1. Fall armyworm sampling locations in Brazil and Argentina according to ecoregions. Shapefile of limits of countries in South America: IBGE-Mapas (IBGE—Brazilian Institute of Geography and Statistics; Available at: <https://geoftp.ibge.gov.br/cartas_e_mapas/bases_cartograficas_continuas/bc250/versao2021/shapefile/bc250_shapefile_2021_11_18.zip>; Acessed on February 26, 2023) QGIS v3.28.3 (Available at: <https://qgis.org/en/site/>; Acessed on: February 26, 2023). DATUM: SIRGAS 2000.

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from IBGE-Mapas (IBGE–Brazilian Institute of Geography and Statistics, https://geoftp.ibge.gov.br/cartas_e_mapas/bases_cartograficas_continuas/bc250/versao2021/shapefile/bc250_shapefile_2021_11_18.zip. Accessed on February 26th, 2023).

Host strains identification

For the 228 specimens whose reads were successfully retained after GBS processing, we did polymerase chain reaction (PCR) amplifications of the COI and TpiEI4 regions to characterize host strains based on diagnostic polymorphisms at mCOI1164, mCOI1287 and gTpi183 positions using the same primer sequences and procedures as described elsewhere [4]. Samples featuring both C and T nucleotides at the gTpi183 position were identified as hybrids (*Tpi*H). The COI sequences were used to confirm species identification as well. Sequences were aligned

Table 1. Fall armyworm (*Spodoptera frugiperda*) sampling information. N refers to the number of samples successfully sequenced after GBS processing, totaling 228 individuals.

Location Code	Country	Locality (City, State)	Collection Date	Latitude	Longitude	Host	N
AR01	Argentina	Metán—Salta	22-Feb-18	-25.5	-64.97	Corn	11
AR02	Argentina	America—Buenos Aires	24-Jan-18	-35.48	-62.97	Corn	3
AR03	Argentina	San Justo—Santa Fe	27-Feb-18	-30.78	-60.58	Corn	15
BA02	Brazil	São Desidério—Bahia	26-Jun-18	-12.44	-45.64	Corn	19
BA03	Brazil	Barreiras—Bahia	19-Jul-18	-11.78	-45.78	Corn	18
DF	Brazil	Planaltina—Distrito Federal	8-Jun-18	-15.87	-47.4	Corn	20
GO	Brazil	Rio Verde—Goiás	22-Jun-18	-17.75	-51.04	Corn	24
MA01	Brazil	São Luís—Maranhão	12-Dec-18	-2.59	-44.21	Corn	10
MA02	Brazil	São Luís—Maranhão	19-Dec-18	-2.58	-44.21	Corn	13
MT01	Brazil	Campo Novo do Parecis—Mato Grosso	25-Jun-18	-13.97	-57.98	Corn	24
MT02	Brazil	Campo Novo do Parecis—Mato Grosso	1-Jul-18	-14.27	-57.76	Corn	21
PR	Brazil	Toledo—Paraná	20-Jun-18	-24.67	-53.76	Corn	9
RS	Brazil	Alegrete—Rio Grande do Sul	13-Jan-21	-30.02	-55.71	Rice	3
SC	Brazil	Chapecó—Santa Catarina	14-Dec-20	-27.09	-52.64	Corn	15
SP	Brazil	Taquarituba—São Paulo	14-Jul-18	-23.54	-49.22	Corn	23

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with Clustal W [16] using MEGA 7 [17]. The genetic data presented in this study are publicly available on GenBank (BioProject PRJNA847933, and accession numbers ON704174—ON704629).

GBS library sequencing and data processing

The steps of the GBS library preparation were done according to methodology described elsewhere [18] with the following modifications. Genomic DNA was digested with restriction enzymes *Nsi*I-*Mse*I (New England Biolabs—NEB, Ipswich, MA, USA). The barcoded *Nsi*I adapters and a common *Mse*I adapter were ligated to the digested DNA of each sample. Barcoded DNA fragments from all samples were pooled in a single tube and amplified by PCR. The libraries were single-end sequenced to 150 nucleotides on a single lane using the Illumina NextSeq500/550 sequencing kit v2 (Illumina, Inc. San Diego, CA, USA) at the Genome Investigation and Analysis Laboratory of University of São Paulo.

Sequencing quality of GBS libraries was evaluated using FastQC [19]. The 3'end of raw reads were trimmed to 90 bp and were inspected for adaptor sequences removal. We performed demultiplex using *process-radtags* in STACKS v.1.42 [20]. Reads could be assigned to each individual based on the sequence of the barcodes. Samples with less than 200,000 sequences and/or unexpected GC contents were removed for further analysis. Reads were aligned to the *Spodoptera frugiperda* genome *ZJU_Sfru_1.0*, under Bioproject PRJNA590312 [21], using the algorithm BWA-MEM of the software BWA 0.7.17 [22]. Alignment files were processed with SAMtools [23] and Picard (http://broadinstitute.github.io/picard). We identified SNPs using freebayes 1.3.4 [24] with - -standard_filters option. Filtering was performed using VCFtools v0.1.12a [25] and BCFtools 0.1.12 [22]. We retained bi-allelic SNPs that passed the following criteria: minor allele frequency $\geq$ 0.01, read depth $\geq$ 5X, mapping quality $\geq$ 20, maximal amount of missing data per locus = 10%. Variants were separated by a minimum distance of 150 bp and r^2 threshold of 0.6. Results were stored in variant call format (VCF) after an additional filter to remove six samples with more than 25% of missing data, and SNPs identified at sexual chromosome W present only in females, mitochondrial genome or in unanchored contigs of the reference genome.

Genetic differentiation analysis

Genetic diversity was estimated by calculating the observed heterozygosity H_O, expected heterozygosity H_E, nucleotide diversity π, the inbreeding coefficients (F_{IS}), and the pairwise Fixation Index (F_{ST}) using *hierfstat* package [26, 27]. We tested for significant differences in heterozygosities and F_{IS} using confidence intervals calculated based on 1000 bootstrap resamples. F_{ST} relations were illustrated by heatmaps generated by the *RColorBrewer* R package. Genetic structure was additionally explored through the principal component analysis (PCA) and the discriminant analysis of principal components (DAPC) using *ade4* [28] and *adegenet* [29, 30] for R software [31]. Specimens were grouped by the ADMIXTURE v1.3.0 software, and the best value of inferred genetic groups (K) was implemented by the cross-validation method [32].

Outlier SNPs detection and annotation

We searched for loci putatively under selection in the set of 15 populations and in the set of strains, where samples were identified as R- or C- strains using the *Tpi* marker as previously described [4]. *Tpi*H individuals were not included in this analysis. Outlier SNPs were identified using three methods. The *fsthet* package [33] generates smoothed quantiles for the F_{ST}–heterozygosity relationship from the empirical distribution, and outliers were selected with a 95% confidence level threshold. The *pcadapt* package [34] considers population structure determined by PCA, and outliers were identified using a false discovery rate (FDR) of 0.05. The program FLK [35] assumes that SNPs were polymorphic in an ancestral population, and uses a population tree to build a null model of the behavior of F_{ST}. We retained candidate markers identified by at least two methods and the remaining loci were considered as neutral. We compared the sequences containing outliers with the annotation files of the reference genome to identify loci present in encoding genes. We generated a fasta file containing the protein sequences to run Blast2GO [36] software using blastp with Insecta Taxa, InterPro Scan, GO mapping and functional annotation. The GO terms for each gene were submitted to Web Gene Ontology Annotation Plot (WEGO) for summarization [37].

Results

Fall armyworm host strains

Utilizing diagnostic polymorphisms in the COI and Tpi regions, we were able to determine the strain composition of the fall armyworm populations. A higher percentage of the samples collected from corn fields were identified as C-strain using the *Tpi* marker (95%) than COI (86%), consistent with other studies suggesting that *Tpi* is a better strain marker than COI [10]. The AR03 population in Argentina (Santa Fe) had the lowest agreement between markers (7%). *Tpi*-hybrids (*Tpi*H) were found only in Brazilian locations, mainly in MA02 population, which also had many R-strain samples (Fig 2).

SNPs discovery

The GBS library generated a total of 187,842,557 reads that were retained after demultiplexing and quality checking. A total of 3309 SNP loci were retained in 228 individuals from 15 locations across Brazil and Argentina. Mean sequencing depth per SNP was 23.3 x (min = 8.7x, max = 45.1x). Estimates of genetic diversity were similar across locations and are summarized in S1 Table.

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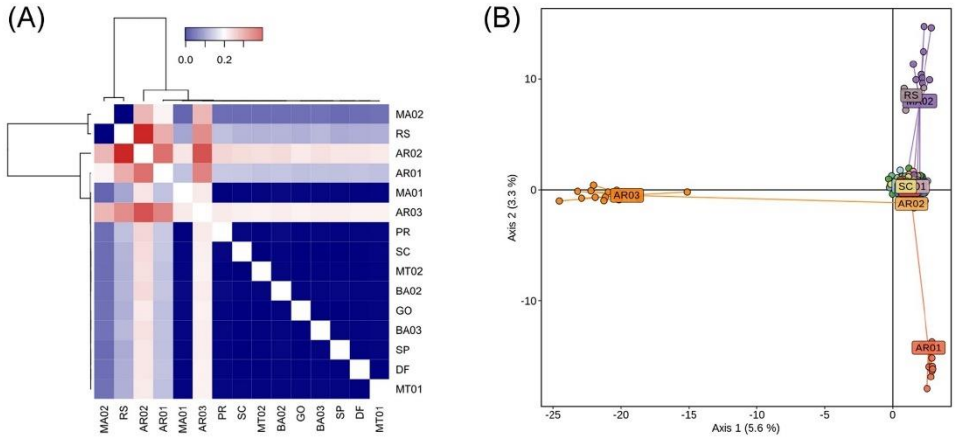

Fig 3. Genetic structure of FAW populations from Brazil and Argentina based on 3309 SNP loci. (A) Heatmap and dendrogram based on F_{ST} pairwise distances among 15 locations. Red color represents a greater degree of differentiation. (B) Principal Component Analysis (PCA) showing the first two components. Geographic locations are represented by different colors, and dots represent different individuals.

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three clusters, grouping the AR02 (Buenos Aires) closest to the Brazilian populations (Fig 4A). Analysis of populations using Admixture showed that four individuals from MA02 featured similar admixture patterns as FAW individuals from AR02 and AR03 (Fig 5A). The outlier loci in turn resulted in four clusters: C-strain-Argentina, AR03, C-strain-Brazil, and R-strain-Brazil (Fig 4B). When considering loci under positive selection, an increased number of

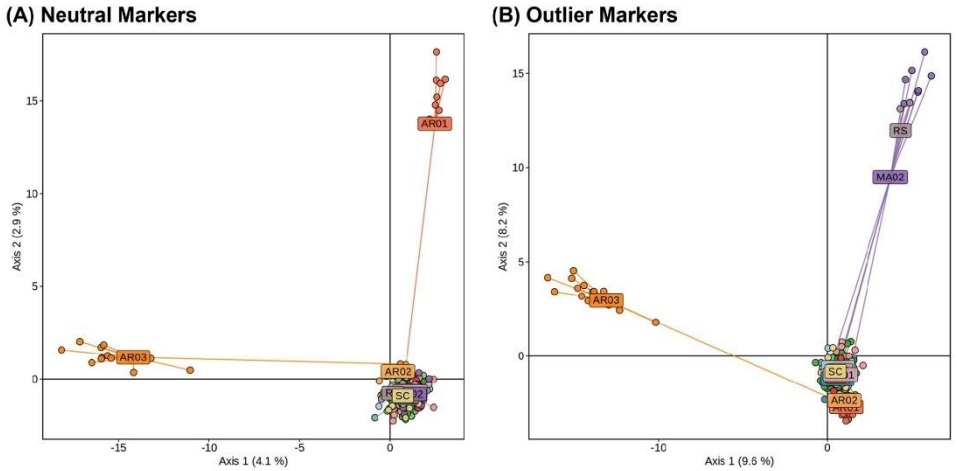

Fig 4. PCA analysis generated with (A) 2,853 neutral SNPs, (B) 456 outliers. Dots represent different individuals of each location.

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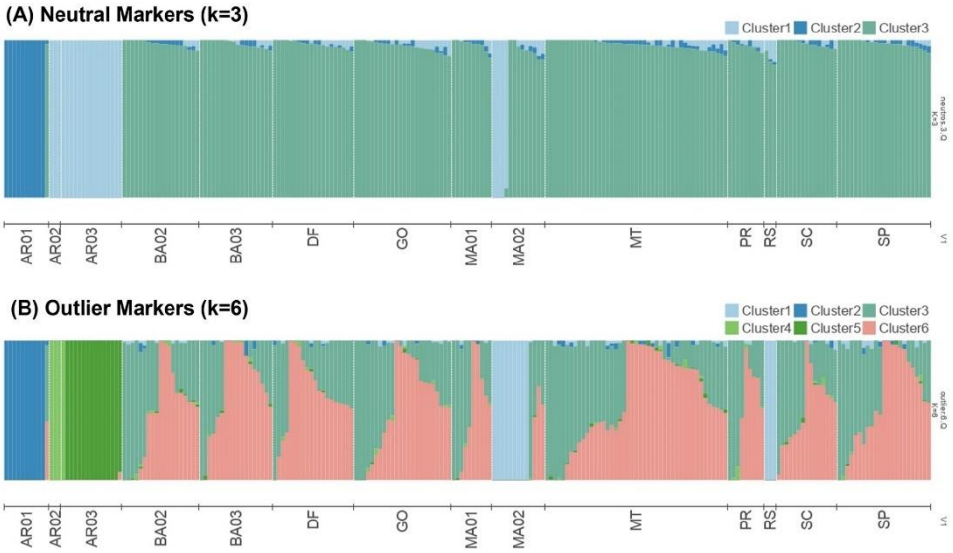

Fig 5. Admixture analysis of fall armyworm populations based on (A) 2,853 neutral SNP loci and (B) 456 outlier SNP loci. Bar plot colors indicate estimated proportions of ancestry for each individual shown as a vertical line.

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distinct genetic pools was obtained in Admixture analysis (K = 6), and more individuals appeared to feature non-admixed pattern (Fig 5B).

Annotation of outlier loci

For outlier analyses, 456 SNP loci putatively under selection were compared to the annotations of the genome of a specimen collected in China (*ZJU_Sfru_1.0*) and 306 of these were within predicted protein coding genes of FAW. After Blast2GO analysis, 220 proteins were successfully mapped, and 94 were annotated (S2 Table). We found many outlier loci within genes possibly involved in binding functions and associated with the cell membrane (Fig 6A). Among the 94 loci with GO IDs, SNP_1055 was annotated as an ABC transporter C subfamily member 13, and the mutation was putatively under selection when comparing different locations, rather than related to the presence of two host strains. Regarding detoxifying activity, the locus SNP_3077 was in a gene similar to cytochrome P450 CYP314A1 (this locus was detected as outlier when comparing host strains), and the locus SNP_1559 was in a gene similar to an annotated esterase FE4-like. Another mechanism of insect defense involves chitin processing, and here we had five loci related to cuticle proteins or chitin binding (SNP_574, SNP_1209, SNP_1853, SNP_2799, SNP_3139).

Analysis of outliers comparing host strains resulted in 68 outlier SNPs (23% of 293) in the sex chromosome Z, which concentrated over twice the number of outliers compared to the autosomal chromosomes (Fig 6B). The locus SNP_421 located at chromosome 3 was within a gene similar to an odorant receptor and could be related to sensorial stimulus to either host perception or male mating preferences. The locus SNP_3298 was in a gene that had 100%

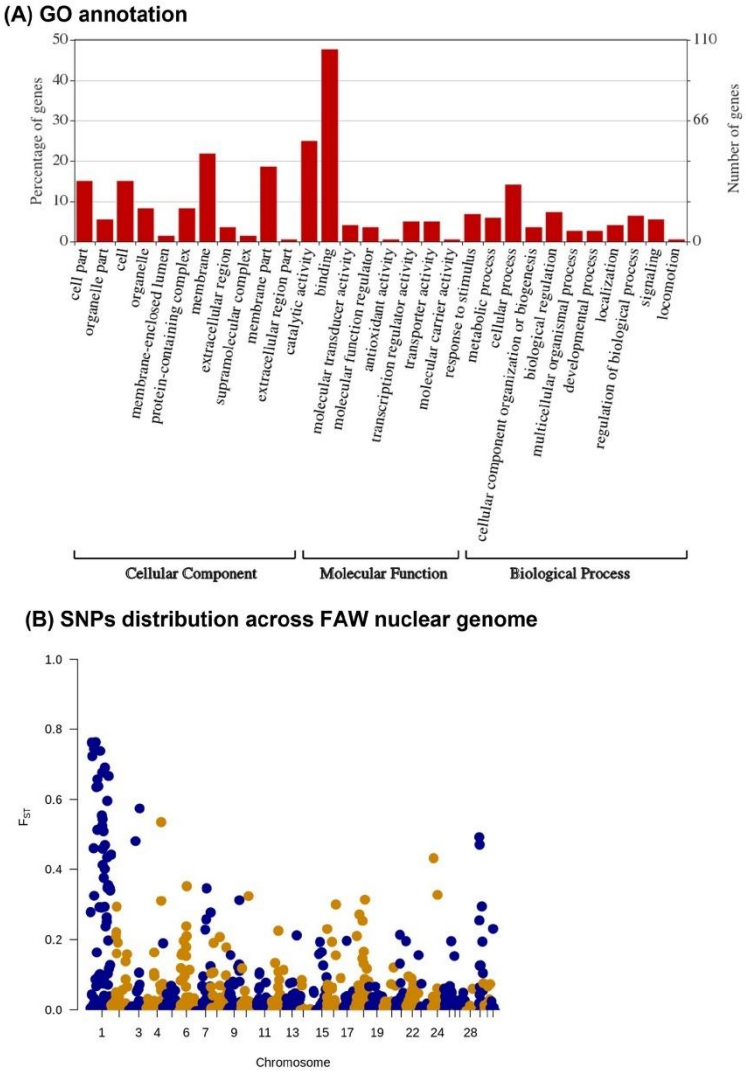

Fig 6. Outlier SNPs under positive selection using Pcadapt, FstHet, and FLK. (A) GO categories of putative loci under selection generated from WEGO. The results are presented in three main categories: biological process, cellular component, and molecular function. The left y-axis indicates the percentage of a specific category of genes in the main category. The right y-axis indicates the number of genes in each category. (B) Manhattan plot of F_{ST} analysis comparing C-strain and R-strain against the position of SNPs on each of the 31 chromosomes. Chromosome 1 corresponds to the sex chromosome Z. Each SNP is represented by a dot. Chromosomes are represented by blue (odd) and orange (even).

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identity to a GPI-anchored glycoprotein. Besides this locus, another 188 loci were successfully blasted with more than 90% similarity, but had no GO ID associated with them (S3 Table), including one locus identified as a cytochrome P450 (SNP_423), one cadherin (SNP_1010), one zinc carboxypeptidase (SNP_1352), one GABA receptor (SNP_719), and one UDP-glucosyltransferase (SNP_2719). The frequencies of each polymorphism for some candidate genes under selection were also calculated for the Brazil locations (S5 Fig).

Discussion

GBS has been used successfully in insect pests to reveal insights about gene flow and coancestry [38], spatial and temporal genetic structure [39, 40], and incursions of invasive pests [41, 42]. Here we associated the informative data set provided by GBS with molecular markers used for host strain identification to better explain the patterns of FAW population structure in Brazil and Argentina and to identify candidate genes putatively under selection.

By assessing a large number of samples in Brazil, we confirmed that FAW collected in corn fields were predominantly C-strain, with less than 4% of samples featuring both the COI-RS and *Tpi*R diagnostic markers, and the few specimens featuring discordant genotypes likely represent vestiges of interbreeding events that occurred in the past. Based solely on diagnostic polymorphisms in COI and *Tpi* regions, MA02 and AR03 populations showed increased levels of strain hybridization, and we were able to describe the level of gene flow of these locations based on GBS data set.

GBS data revealed high levels of gene flow and low genetic differentiation between MA02 and RS population, which was composed by pure R-strain samples. Since these two populations were the most geographically distant locations sampled in Brazil, apart over 3200 km, we presumed that MA02 was composed by R-strain specimens with recent events of hybridization. Altogether, the genetic analysis based on pairwise F_{ST} distances and PCA plots confirmed that the Brazilian populations are structured by host strains, rather than by geographical ecoregions.

Long distance migration enables FAW populations to travel from Southern Texas and Florida up to Canada, a distance of nearly 2500 km, in less than three months [3, 43]. Therefore given its strong flight performance, we can hypothesize that FAW is also performing long distance migration within Brazil sufficient to keep populations homogeneous within each host strain.

Several studies comparing populations from Brazil and Argentina in the past showed strong similarities between the two countries [4, 44–46], which would be expected if the great majority of Argentina FAW are derived from seasonal migrations from Brazil. To some extent, Brazilian and Argentinian populations featured common ancestry, and the population from Buenos Aires (AR02) had the lowest genetic differentiation with Brazilian populations.

Nevertheless, F_{ST} analysis indicated significant genetic structure between countries and among provinces inside Argentina. This result is consistent with observations of mating incompatibility between populations collected in northern Argentina compared to those from the Pampas region, which indicated pre-reproductive isolating barriers between geographically separated populations [6]. GBS data for another important Noctuidae pest in the Americas, the sugarcane borer (SCB), showed a similar pattern of genetic structure between Argentina and Brazil populations, and among populations within Argentina [47, 48]. We hypothesize that Argentina likely contains one or more endemic FAW populations that exhibit significant geographical isolation and that additional studies are required to better investigate the fine scale genetic structure of FAW as well as identify locations capable of supporting permanent FAW populations in this country.

In order to explain how selection pressures might be affecting FAW populations in South America, we examined the putative annotations of genes containing outlier SNPs. Host strain outliers were mostly concentrated on the sex chromosome Z, suggesting that the selection pressures are acting upon specific regions of the FAW genome. This result corroborates with previous study where the preponderance of strain specific SNPs were Z-linked [49], and is consistent with the proposal that strain divergence is being driven primarily by Z-chromosome functions [50]. We believe that by reducing complexity of the genome, the GBS method was able to capture a fairly large number of polymorphisms in the Z-chromosome, and thereby discriminate between the R- and C-strains. It is possible that previous research based on nuclear SSR markers [12] that did not differentiate the host strains lacked sufficient coverage of the sex chromosome.

Other functional annotations revealed proteins that were likely involved in binding activities and that were present in or related to the cell membrane. Mutations in Cry receptor genes have been reported in numerous lepidopteran species to be the most common mechanism of resistance against *Bt* toxins [51], and here we found outlier SNPs in genes likely coding for Cry receptors such as GPI-anchored glycoprotein, cadherin and zinc carboxypeptidase. We also found outlier SNPs in genes possibly coding many important enzymes such as cytochrome P450 CYP314A1 [52], esterase FE4-like [53], JHAMT [54], and also proteins related to cuticle and chitin, which may be an indication of response to management with insecticides [55]. Two noteworthy outlier SNPs associated with host strains were in genes possibly encoding an odorant receptor and a UDP-glucuronosyltransferase. Odorant receptors function in insects olfaction process, which is indispensable for host selection for feeding and oviposition [54]. UDP-glucuronosyltransferase, in turn, appears to be associated with C-strain ability to detoxify DIMBOA [56], a toxic compound produced by corn plants but not rice. In conclusion, our work strongly suggest that positive selection is affecting allele frequencies at the level of populations and host strains.

From the Insect Resistance Management (IRM) perspective, resistance evolution is one of the most challenging problems in the sustainable control of FAW [57]. Therefore understanding patterns of gene flow and consequent risks for spread of field-evolved resistance alleles are crucial for effective management. Our GBS data set poses a challenging scenario in Brazil, where locations presented high levels of gene flow across all ecoregions and low genetic structure within host strains. Moreover, pairwise F_{ST} distances showed genetic structure between FAW populations of Brazil and Argentina, which has important IRM implications if resistant populations are reported in either country.

In conclusion, by combining classic molecular markers for FAW host strain identification, and genome-wide SNPs identified with GBS, we obtained more resolution of population structure than previously reported. The genetic structure and pattern of FAW in Argentina and Brazil reinforces the importance of phytosanitary barriers between countries for effective FAW management in each location. In agreement with this issue, outlier analysis suggested that positive selection is associated with field management and host strain divergences. Taking all this into consideration, current GBS data proved to be useful for population genomics research in South America and it may be applied to other geographies where the species has been introduced.

Supporting information

S1 Table. Genetic diversity estimates of fall armyworm (*Spodoptera frugiperda*) populations from 15 locations of Brazil and Argentina estimated from 3309 SNP loci. Most populations were not found at equilibrium. Brazilian populations featured lower observed

heterozygosity than the expected at $p < 0.05$. The coefficient FIS also indicated that the Brazilian populations collected in corn fields had significant inbreeding. On the other hand, the fall armyworm populations from Argentina featured more outbreeding and private alleles.
(DOCX)

S2 Table. Gene Ontology (GO) biological process description for outlier loci under positive selection. *associated with populations. **associated with host strains and populations. Loci with no mark are associated with host strains.
(DOCX)

S3 Table. Outlier loci under positive selection with blast hits in NCBI with > 90% similarity. *associated with populations. **associated with host strains and populations. Loci with no mark are associated with host strains.
(DOCX)

S4 Table. Geographic distance matrix showing the straight-line distances (Km) between locations. Darker orange indicates longer distances.
(DOCX)

S1 Fig. Pairwise FST for fall armyworm sampling locations. (A) FST calculated with all variant loci (3309 SNPs). (B) FST calculated using neutral markers (2853 SNPs). (C) FST was calculated using candidates putatively under positive selection (456 SNPs) obtained by three methods (FLK, PCAdapt, FstHet). Darker green color represents a higher degree of differentiation.
(TIF)

S2 Fig. Detection of outlier SNPs under positive selection using Pcadapt, FstHet, and FLK. The Venn diagrams shows the number of outlier SNPs associated to (A) FAW host strains and (B) populations.
(TIF)

S3 Fig. Cross-validation method implemented in ADMIXTURE v1.3.0 to estimate the number of ancestral populations (k) inferred from (A) neutral markers and (B) outlier markers.
(TIF)

S4 Fig. Discriminant Analysis of Principal Components (DAPC) scatterplot showing the first two linear discriminants. Geographic locations are represented by different colors, and dots represent different individuals. The inset shows BIC values for different number of k clusters. Analysis performed with (A) All SNP loci, (B) 2,853 neutral SNPs, (C) 456 outliers. Plots generated using adegenet package for R software. Sampling locations were considered as priori groupings.
(TIF)

S5 Fig. Polymorphisms frequencies in candidate genes under selection in Brazilian locations. Locations were represented mostly by C-strain moths, except for MA02 and RS locations where most samples were identified as R-strain.
(TIF)

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Future directions

The GBS methodology to obtain SNPs markers proved to be useful to study FAW populations. Because the method is reproducible, sequences can be generated independently and then pooled together for SNPs identification. In fact, my GBS libraries were generated with 3 years difference. Thus, the method can be extended to characterize more populations from South America at fine scale, such as those from our neighboring countries Paraguay and Uruguay. Besides, with advancements in genome quality and annotation, the current data can be revisited for higher amounts of SNP discovery and identification of loci putatively under selection.

In this study, while it is clear that Brazilian populations present strong gene flow country-wide, which is particularly concerning for pest management, it is also important to know to what extent these populations are doing long-distance migrations. Due to operational challenges for answering this question, one alternative may be screening for specific genes and monitoring mutations throughout the cropping years. While mutations can appear independently in nature, tracking them may be an option to infer whether they are following some dispersion pattern.

As for Argentinean populations, there is more to be explored in terms of population genomics. Argentina features more diverse ecoregions (forest, grasslands, and deserts), and likely poses some barriers for natural populations, including geographic barriers, such as the Andes Mountain. Some FAW populations may even experience decline or elimination at regional scale in severe winters. Therefore, although we sampled three different locations, it is not possible to estate if the observed genetic structure among all three populations is permanent. Therefore field populations collected from different crops in the same location, and also populations from more diverse ecoregions should also be characterized.

The screening of loci putatively under selection showed a number of genes related to resistance and response to insecticides chemicals and proteins. The incredible ability of FAW populations to adapt and become resistant to insecticides reinforces the need of an IPM strategy that relies on multiple control methods, including the use of biological control, mating disruption techniques, agroecological approaches and cultural control. FAW control has been relying mostly on chemical insecticides and/or Bt crops, and in order to increase the durability of the technologies, a more diversified and sustainable pest management strategy must take place.

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ANEXO I. Declaração de Bioética e/ou Biossegurança

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DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***Genômica populacional do inseto praga Spodoptera frugiperda no Brasil e Argentina***", desenvolvida no Programa de Pós-Graduação em Genética e Biologia Molecular do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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ANEXO II. Direitos autorais

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada - **Genômica populacional do inseto praga Spodoptera frugiperda no Brasil e Argentina**, não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 18/09/2023

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