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Phylogeography of the Recent Expansion of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in South America and the Caribbean Basin

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Abstract

The Old World bollworm, *Helicoverpa armigera* (Hübner), is one of the most destructive agricultural pests worldwide. It was first recorded in Brazil in 2013, yet despite this recent introduction, *H. armigera* has spread throughout much of Latin America. Where *H. armigera* has become established, it is displacing or hybridizing with the congeneric New World pest *Helicoverpa zea*. In addition to the adaptive qualities that make *H. armigera* a megapest, such as broad range pesticide resistance, the spread of *H. armigera* in the New World may have been hastened by multiple introductions into South America and/or the Caribbean. The recent expansion of the range of *H. armigera* into the New World is analyzed herein using mtDNA of samples from South America, the Caribbean Basin, and the Florida Peninsula. Phylogeographic analyses reveal that several haplotypes are nearly ubiquitous throughout the New World and native range of *H. armigera*, but several haplotypes have limited geographic distribution from which a secondary introduction with Euro-African origins into the New World is inferred. In addition, host–haplotype correlations were analyzed to see whether haplotypes might be restricted to certain crops. No specialization was found; however, some haplotypes had a broader host range than others. These results suggest that the dispersal of *H. armigera* in the New World is occurring from both natural migration and human-mediated introductions. As such, both means of introduction should be monitored to prevent the spread of *H. armigera* into areas such as the United States, Mexico, and Canada, where it is not yet established.

Key words: agricultural pest, invasive species, long-distance dispersal, Old World bollworm, polyphagy

The Old World bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is one of the most damaging pests to agriculture worldwide. Larvae have been recorded feeding on hosts in 68 different plant families, including a wide range of crops such as corn, soybean, sorghum, cotton, peppers, and tomatoes as well as an assortment of ornamentals (Cunningham and Zalucki 2014). Females lay an average of over 700 eggs (Liu et al. 2004), contributing to explosive population growth. Because *H. armigera* can enter facultative diapause, they can tolerate a wide range of temperatures and drought (Hackett and Gatehouse 1982, Liu et al. 2010). Furthermore, adults can avoid poor climate conditions because they are able to migrate more than 40 km in a single night (Jones et al. 2015) and up to 1,000 km in a lifetime (Zhou et al. 2000, Feng et al. 2004). This mobility is likely to have contributed to the establishment of this species throughout most of the world.

The mobility of *H. armigera* has probably been an important factor in the nearly worldwide establishment of the species. *Helicoverpa armigera* is widespread in Europe, Asia, Africa, and Oceania (Hardwick 1965). It was confirmed to be present in Brazil in 2013 (Czepack et al. 2013, Tay et al. 2013), though, it is likely that *H. armigera* populations were established between 2006 and 2008 (Tay et al. 2013, Sosa-Gomez et al. 2016). Mitochondrial DNA (mtDNA) analysis has shown that the Brazilian populations had Eurasian and African origins through multiple introductions, probably as a result of human-mediated dispersal (Tay et al. 2017). Since its introduction, *H. armigera* has spread throughout much of Brazil (Mastrangelo et al. 2014, Sosa-Gomez et al. 2016), and its presence has also been confirmed in Paraguay (Senave 2013), Argentina (Murua et al. 2016), Bolivia (Kriticos et al. 2015), and Uruguay (Arnemann et al. 2016). Larvae intercepted at U.S. and European ports have confirmed its presence in Colombia, the Dominican Republic, Peru, and Surinam (Gilligan et al. 2015, 2019). Given the dispersal rate, the abundance of available host plants, and the high fecundity of this species, *H. armigera* was eventually detected

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in U.S. territories, with the first find in Puerto Rico in 2014 (Smith 2014). Less than a year later, three individual *H. armigera* were captured near Bradenton, Manatee County, FL, on 3 June 2015, 17 June 2015, and 9 July 2015 (El-Lissy 2015, Hayden and Brambila 2015). Although no other individuals were detected after subsequent surveys, it is likely that incursions will continue as the range of *H. armigera* expands in the New World.

The *Helicoverpa zea* (Boddie) lineage is estimated to have diverged from the *H. armigera* lineage about 1.4 mya with the *H. armigera* lineage having a greater host range, contributing to its preadaptation to a range of synthetic pesticides (Pearce et al. 2017). The broad host range and extensive, and in some cases, excessive use of pesticides in the course of modern agricultural practices strongly selected for pesticide resistance in *H. armigera* (McCaffery 1998). In areas where *H. armigera* co-occurs with the closely related *H. zea*, hybridization is likely to spread insecticide resistance among *H. zea*, potentially compounding insect damage and challenge control practices (Anderson et al. 2018). It is crucial that biosecurity measures are in place to prevent the establishment of a population in the United States. Current measures include monitoring for its presence in areas to which natural dispersion from South and Central America is possible, and inspections at U.S. ports of entry to prevent introduction through trade commodities. These measures may be improved by knowledge of the incursion pathways of the Puerto Rico and Florida invasions to prevent its reintroduction by similar pathways and calculate risk analysis more accurately.

The origin of an invasive species may be determined by comparing haplotypes in its new territory with haplotypes found in theoretical source populations. Using this method, the origin of *H. armigera* in the New World has been evaluated several times, with the primary focus being on the source of the Brazil invasions (Tay et al. 2013, 2017; Anderson et al. 2016). These genetic studies unanimously demonstrate that the Brazilian population consists of multiple haplotypes originating from Europe, Asia, and Africa. However, the origins of the Puerto Rican and Floridian incursions have yet to be determined. The present study determined the haplotype identity and ancestry of New World *H. armigera*. This was accomplished by sequencing New World *H. armigera* mtDNA as well as mtDNA from theoretical source populations of *H. armigera*. Given the biology of *H. armigera*, several phylogeographic patterns should be expected including 1) genetic structuring and divergence should be weakly correlated with geographic origin as dispersal through natural means and trade homogenizes insect populations (Roderick 1996) and 2) genetic structuring and divergence should not be driven by host preference given the polyphagous nature of this species (Mopper 1996). As such, understanding how these patterns persist or change with time can be important for understanding biological invasion, tracking and preventing future invasions, and generating additional research questions.

In its ancestral range, *H. armigera* is highly polyphagous (Cunningham and Zalucki 2014); however, during the early stages of invasion into novel environments, it is unknown whether polyphagy will persist at similar levels (Janz and Nylin 2008). Initial work in South America has shown that *H. armigera* is utilizing common crops found in the historic range such as cotton, soybean, maize, and tomatoes; however, larvae and eggs have been found on novel host plant species such as pequi (*Caryocar brasiliense* A.St.-Hil., Caryocaraceae) (Cunningham and Zalucki 2014, Pinto et al. 2015). This suggests that the haplotypes introduced into the New World are polyphagous much like elsewhere in the world and possibly expanding in host range breadth. However, it is unknown whether the observed polyphagy in the New World is the result of divergent haplotypes living sympatrically and using different host plants or whether divergent haplotypes are using the same host plants resulting in panmictic populations. Host preference could also be influenced by geographic location wherein a newly introduced haplotype is found with only a single host plant species because host choice is limited in that area. To test these alternative scenarios, association tests were applied that analyzed whether significant correlations could be found between these factors. Determining which of these scenarios underlies *H. armigera* invasion and which host plants are involved could be a useful tool in helping prevent future spread of this species.

Materials and Methods

Collection and Identification of Specimens Used in This Study

The specimens used in this study are summarized in Table 1 and Supp Table S1 [online only]. The 171 specimens for this study came from port interceptions, domestic survey trapping efforts, and donations made by collaborators. The sample set comprised 149 larvae and 22 adults. Samples were obtained from Australia, Brazil, China, Colombia, Dominican Republic, Spain, India, Israel, Italy, Jordan, Japan, Kenya, South Korea, Morocco, Macedonia, the Netherlands, Pakistan, Peru, the Philippines, Puerto Rico, Portugal, Palestine, Thailand, Uganda, South Africa, and Zimbabwe. The three specimens that were captured in Florida were also included to determine their origins. Specimens included in the analysis were associated with several host crops (Table 1; Supp Table S1 [online only]). Larvae that were intercepted with a commodity were assumed to have used the commodity as a host plant. A broad geographic sampling of New World samples was included to test whether host associations were the result of locally evolved haplotype–host associations. Inferences from the association tests were as follows: 1) if haplotype and host were strongly associated across multiple geographic sites, then a strong haplotype–host interaction would be inferred; 2) if a haplotype was strongly associated with a given host at one location but strongly associated with a different host at different location, then local adaptation would be inferred; and 3) if neither pattern is found, then a generalist behavior would be inferred. Most specimens used in this study were intercepted at U.S. ports of entry where port inspectors identified the host plant species. The host plant species identified through port interceptions included pea (*Pisum sativum* L., Fabaceae), basil (*Ocimum basilicum* L., Lamiaceae), pepper (*Capsicum annuum* L., Solanaceae), sage (*Salvia officinalis* L., Lamiaceae), bean (*Phaseolus* L. sp. Fabaceae), oregano (*Origanum vulgare* L., Lamiaceae), waxflower (*Chamelaucium* Desf. sp., Myrtaceae), and cucumber (*Cucumis sativus* L., Cucurbitaceae). In addition, adult specimens associated with sorghum [*Sorghum bicolor* (L.) Moench, Poaceae] and pigeon pea [*Cajanus cajan* (L.) Millsp., Fabaceae] were acquired using pheromone traps. All specimens used in this study have been preserved in 100% ethanol and are archived at the USDA-APHIS-PPQ-S&T laboratory in Fort Collins, CO.

The identification of specimens for this study was carried out using morphological characters, genitalic dissections, and/or sequencing of cytochrome oxidase I (COI) DNA barcodes. For specimens identified using polymerase chain reaction (PCR), reactions for COI were conducted using primers LepF1/LepR1 (Hebert et al. 2003, 2004). PCR and sequencing methods are described in the following section. Sequences of all DNA barcodes were identified using the 'Species Level Barcode Records' database in the 'BOLD Identification System' of www.boldsystems.org (BOLD; Ratnasingham and Hebert 2007). In all cases, the DNA barcode

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Table 1. General specimen collection information for individuals newly sequenced and included in analyses for this study

Country of collection	Number of specimens	Host species	Life stage(s)
Australia	2	*Capsicum* sp.	Larva
Brazil	2	NA	Larva
China	1	*Capsicum* sp.	Larva
Colombia	4	NA	Larva
Dominican Republic	7	*Capsicum* sp.	Larva
Spain	10	*Leucospermum* sp.	Adult
India	3	*Chrysanthemum* sp.	Larva
Israel	6	*Ocimum* sp., *Salvia* sp., *Grevillea* sp., *Rosmarinus officinalis*, *Leucospermum* sp., *Origanum* sp.	Larva
Italy	1	*Genista* sp.	Larva
Jordan	1	*Cicer arietinum*	Larva
Japan	2	*Eustoma* sp., *Dianthus* sp.	Larva
Kenya	5	*Gypsophila paniculata*, *Rosa* sp., *Ornithogalum* sp., *Veronica* sp.	Larva
South Korea	1	Piperaceae	Larva
Morocco	1	NA	Larva
Macedonia	1	*Capsicum* sp.	Larva
The Netherlands	5	*Angiozanthus* sp., *Ageratum* sp., *Leucospermum* sp., *Ornithogalum arabicum*	Larva
Pakistan	1	*Helianthus* sp.	Larva
Peru	85	*Chamelaucium* sp., *Origanum vulgare*, *Pisum sativa*, *Salvia officinalis*	Larva
Philippines	1	*Rosa* sp.	Larva
Puerto Rico	14	*Sorghum* sp. (pheromone trap), *Cajanus cajan* (pheromone trap), *Cucumis sativus*	Adult and larva
Portugal	4	NA	Larva
Palestinian Territory	1	*Leucospermum* sp.	Larva
Thailand	1	*Veronica* sp.	Larva
Uganda	2	*Rosa* sp.	Larva
United States, FL, Bradenton	3	NA	Adult
South Africa	5	NA	Adult
Zimbabwe	2	*Leucospermum* sp., *Anigozanthos* sp.	Larva

Host data were not always available for specimens obtained during port interceptions. See Supp Table S1 [online only] for a detailed listing of specimens. NA (not applicable).

identifications corroborated the morphological identifications. All DNA sequences generated for this study were uploaded to GenBank under accession numbers MK645053-MK645220.

DNA Extraction, PCR, Sequencing, and Sequence Analyses

The DNA extracted for this study was taken from the best available tissue depending on the developmental stage of the specimen. For adult specimens, one to three legs or a portion of the thorax was used for DNA extractions. For larval specimens, one or two segments of the abdomen were used. Qiagen DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA) were used for DNA extractions, generally following the manufacturer's recommended protocol. Tissue samples were pulverized using 2.3-mm zirconia/silica beads placed in 1.5-ml microcentrifuge tubes along with the tissue and then agitated on high for 1 min in a mini-beadbeater (Biospec Products, Bartlesville, OK). Immediately after grinding, 180-µl buffer ATL and 20-µl Proteinase K were added to the tube and the tissue/buffer slurry was incubated overnight at 56°C in an Eppendorf ThermoMixer (Eppendorf AG, Hamburg, Germany). Thereafter, samples were lysed with buffer ATL, column purified using buffers AW1 and AW2, and eluted with a final volume of 50-µl buffer AE. Cross-contamination was prevented by sanitizing all equipment and materials between specimen dissections, and filter tips were used to handle all liquids containing DNA. No-tissue extraction controls (i.e., reactions performed without addition of tissue) were used for each extraction batch/plate to control for contamination throughout all steps. DNA concentration and purity values were estimated with the 260/230 nm and 260/280 nm wavelength ratios for all samples using a NanoDrop 2000 Ver. 1.6 spectrophotometer (Thermo Scientific/NanoDrop Wilmington, DE) from 2 µl of DNA extract per sample. Two readings were taken for each sample to ensure instrument consistency.

Conventional PCR was performed on a Bio-Rad C1000 Touch (Bio-Rad Laboratories, Inc., Hercules, CA) thermocycler to generate amplicons for downstream Sanger sequencing. The PCR reactions for generation of amplicons used in Sanger sequencing were performed using TaKaRa Ex Taq HS polymerase (Takara Bio, Shiga, Japan) in total volumes of 50 µl using the manufacturer's recommended volumes of 10× Ex Taq buffer, dNTP mixture, and water. We employed the two mitochondrial regions from Behere et al. (2007) and Tay et al. (2017) such that the newly generated data could be aligned to previously published haplotypes. The COI gene was selected based on high levels of polymorphism and its utility in phylogeographic studies and was amplified using the primer set COI-F01 and COI-R01 (Behere et al. 2007). This COI primer set is often used in concert with the cytochrome oxidase B gene with the primer set CytB-F01 and CytB-R01 (Behere et al. 2007, Tay et al. 2017). Amplification with this primer set had a high failure rate, with almost one third of samples failing to amplify. Due to this high failure rate and the fact that polymorphisms were often found in low-quality portions of the read (the 5′ and 3′ ends), CytB sequences were excluded from our study. In addition, all COI sequences used in this study was trimmed to 510 bp and not the 512 bp used by

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Behere et al. (2007) and Tay et al. (2017) due to the presence of low-quality calls in the 5′ end of the read. Thermocycling conditions included an initial denaturation step of 94°C (3 min), 32 cycles of 94°C (20 s)/50°C (20 s)/72°C (30 s), and an extension step of 72°C (5 min). Amplification products were visualized on 1% agarose gels, and if bands were present, purified with a Qiagen QIAquick PCR Purification Kit (Qiagen). Following purification, PCR products were sequenced by the University of Chicago Cancer Research Center DNA Sequencing Facility using an Applied Biosystems 3730XL DNA sequencer (Applied Biosystems, Foster City, CA). Primers used for PCR (Table 2) were also used for sequencing. The electropherograms from the sequencing runs were inspected for quality and then assembled, trimmed, and aligned using Geneious Pro 8.1.8 (Biomatters, Auckland, New Zealand; Kearse et al. 2012).

The *H. armigera* COI sequences generated by Behere et al. (2007) and Tay et al. (2017; GenBank EF116226.1–EF116258.1 and KX494879.1–KX494899.1) were used in combination with our sequences to produce a global COI data set. The 54 haplotypes (Harm01–Harm54) identified by Tay et al. (2017) were aligned with the 171 edited sequences generated from our study in Geneious Pro 8.1.8 using the MUSCLE (multiple sequence comparison by log-expectation; Edgar 2004) algorithm. The alignments were manually examined and all sequences were trimmed to 510 bp such that the newly generated sequences were matched to the previously published data without gaps or the inclusion of ambiguously coded nucleotides. The alignment was used to assign previously published haplotype names (Harm01–Harm54) to our sequences or to discover new haplotypes. After haplotype names were assigned, the data set was reduced, so that each country/haplotype combination was present without duplication. The resulting 156 individual × 510 bp matrix was then employed in subsequent analytical procedures described in the following sections. For the Bayesian analyses, monomorphic loci were removed before proceeding, which resulted in a matrix with 53 polymorphic loci.

Table 2. *Helicoverpa armigera* haplotypes by country

	New World						Europe						Africa											Asia										Aust.	
COI Haplotype	BRA	**COL**	**DOM**	**PER**	**PRI**	**USA**	ESP	FRA	**ITA**	**MKD**	**NLD**	**PRT**	BFA	CMR	GHA	**KEN**	**MAR**	MDG	SEN	TCD	UGA	**ZAF**	**ZWE**	CHN	IND	**ISR**	**JOR**	**JPN**	**KOR**	PAK	**PHL**	**PSE**	**THA**	AUS	NZL
Harm01																																			
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Unique haplotypes for a region are color coded. Countries not included in previous analyses are marked in bold (20 total), as are newly discovered COI haplotypes (5 total). Country codes not included in Supp Table S1 [Online only] are as follows: FRA (France), BFA (Burkina Faso), CMR (Cameroon), GHA (Ghana), MDG (Madagascar), SEN (Senegal), TCD (Chad), NZL (New Zealand).

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Population Genetic and Phylogeographic Analyses

The COI data set was partitioned into continental and country source populations to assess the origins of *H. armigera* to the Americas and within the Americas as well as patterns of diversity, population structure, and any possible correlation between haplotype and host. The program GenAlex 6.5 (Peakall and Smouse 2006, 2012) was used to assess measures of genetic diversity and distance metrics for the data sets. The TCS statistical parsimony algorithm (Clement et al. 2000) as implemented in PopArt 1.7 (Leigh and Bryant 2015) was employed to assess and visualize similarities and differences among and between the individuals sampled. Nodes on the network were labeled with country of origin to assess geographic patterns in the data set. Data sets were also analyzed using Geneland 4.0.0 (Guillot et al. 2005a,b, 2008, Guillot 2008, Guillot and Santos 2010, Guedj and Guillot 2011) such that genetic, spatial, and phenotypic data could be analyzed together. Phenotypic data for the model was a matrix of hosts from which individuals were collected. In this way, patterns about host plant specificity, geographic location, and haplotype could be analyzed in a single model. The Markov chain Monte Carlo runs in Geneland were also run without phenotypic data and/or spatial data to see how each component of the model influenced the overall pattern of assignment and clustering. Parameters for the Geneland runs were as follows: 100 independent runs, 100,000 iterations per run, every 100th iteration held in memory, 200 postprocessing burnin, correlated allele frequencies, and a value of 360 for uncertainty on coordinates when using the spatial model to allow for migration between populations, and so that individuals from the same location did not bias the spatial model.

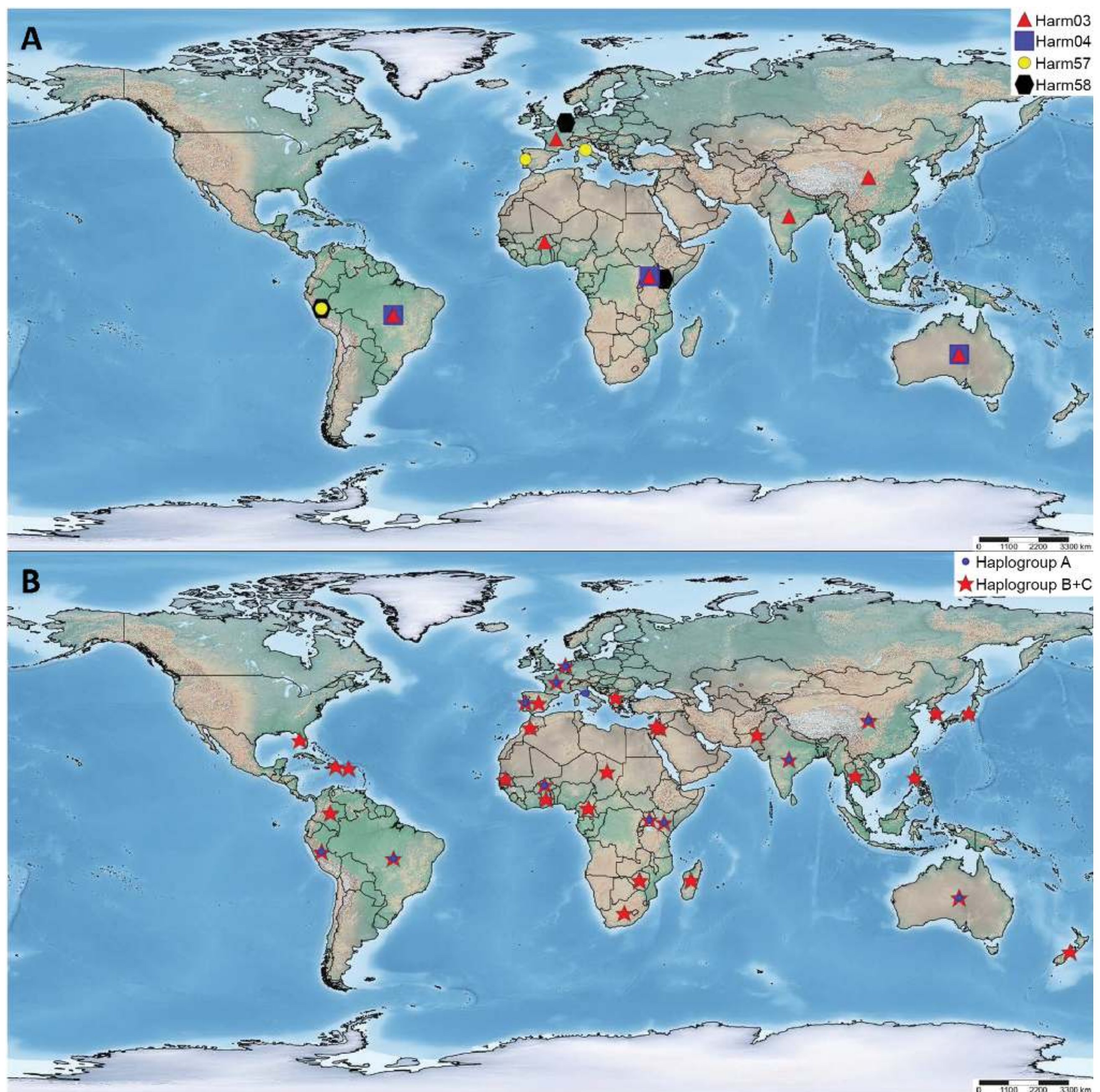

Fig. 1. Distribution of *Helicoverpa armigera* haplotypes. (A) Countries in which haplotypes from PCoA group A were found. (B) Comparison of the countries in which PCoA group A haplotypes were found and haplotypes in groups B and C.

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Table 3. Listing of haplotypes, country where collected and assignments from distance-based PCoA, best-supported Bayesian partition using a nonspatial model (ns), and the best-supported Bayesian partition applying a spatial prior

Country	Haplotype	PCoA group	Pop1 (ns)	Pop2 (ns)	Pop3 (ns)	Pop4 (ns)	Assign (ns)	Pop1	Pop2	Pop3	Assign
Australia	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Australia	Harm02	B	0.240	0.018	0.615	0.127	3	0.609	0.236	0.155	1
Australia	Harm03	A	0.027	0.953	0.000	0.019	2	0.609	0.236	0.155	1
Australia	Harm04	A	0.027	0.953	0.000	0.019	2	0.609	0.236	0.155	1
Australia	Harm05	C	0.298	0.021	0.390	0.290	3	0.609	0.236	0.155	1
Australia	Harm08	B	0.276	0.021	0.481	0.223	3	0.609	0.236	0.155	1
Australia	Harm10	C	0.298	0.013	0.408	0.281	3	0.609	0.236	0.155	1
Australia	Harm13	C	0.365	0.013	0.252	0.371	4	0.609	0.236	0.155	1
Australia	Harm18	B	0.255	0.016	0.547	0.182	3	0.609	0.236	0.155	1
Australia	Harm20	C	0.273	0.018	0.447	0.263	3	0.609	0.236	0.155	1
Australia	Harm28	C	0.302	0.006	0.282	0.410	4	0.609	0.236	0.155	1
Australia	Harm29	C	0.329	0.018	0.279	0.374	4	0.609	0.236	0.155	1
Australia	Harm30	C	0.458	0.018	0.160	0.365	1	0.609	0.236	0.155	1
Australia	Harm31	C	0.332	0.011	0.281	0.376	4	0.609	0.236	0.155	1
Burkina Faso	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Burkina Faso	Harm02	B	0.235	0.013	0.629	0.123	3	0.375	0.348	0.278	1
Burkina Faso	Harm03	A	0.027	0.953	0.000	0.019	2	0.375	0.348	0.278	1
Burkina Faso	Harm10	C	0.319	0.011	0.400	0.269	3	0.375	0.348	0.278	1
Burkina Faso	Harm14	C	0.379	0.011	0.305	0.305	1	0.375	0.348	0.278	1
Burkina Faso	Harm16	C	0.226	0.010	0.195	0.569	4	0.375	0.348	0.278	1
Burkina Faso	Harm17	C	0.324	0.013	0.245	0.418	4	0.375	0.348	0.278	1
Brazil	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Brazil	Harm02	B	0.255	0.021	0.606	0.118	3	0.381	0.125	0.494	3
Brazil	Harm03	A	0.027	0.953	0.000	0.019	2	0.381	0.125	0.494	3
Brazil	Harm04	A	0.027	0.953	0.000	0.019	2	0.381	0.125	0.494	3
Brazil	Harm10	C	0.295	0.016	0.411	0.277	3	0.381	0.125	0.494	3
Brazil	Harm15	C	0.226	0.008	0.195	0.571	4	0.381	0.125	0.494	3
Brazil	Harm21	C	0.389	0.013	0.171	0.427	4	0.381	0.125	0.494	3
Brazil	Harm35	C	0.365	0.016	0.237	0.382	4	0.381	0.125	0.494	3
Brazil	Harm44	C	0.447	0.023	0.152	0.379	1	0.381	0.125	0.494	3
Brazil	Harm45	B	0.226	0.018	0.627	0.129	3	0.381	0.125	0.494	3
Brazil	Harm46	C	0.329	0.024	0.234	0.413	4	0.381	0.125	0.494	3
Brazil	Harm54	B	0.268	0.019	0.582	0.131	3	0.381	0.125	0.494	3
Brazil	Harm55	C	0.465	0.026	0.152	0.358	1	0.381	0.125	0.494	3
China	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
China	Harm02	B	0.250	0.021	0.615	0.115	3	0.570	0.183	0.248	1
China	Harm03	A	0.027	0.953	0.000	0.019	2	0.570	0.183	0.248	1
China	Harm06	B	0.189	0.015	0.742	0.055	3	0.570	0.183	0.248	1
China	Harm10	C	0.311	0.018	0.384	0.287	3	0.570	0.183	0.248	1
China	Harm15	C	0.239	0.013	0.182	0.566	4	0.570	0.183	0.248	1
China	Harm21	C	0.373	0.015	0.177	0.435	4	0.570	0.183	0.248	1
China	Harm22	C	0.282	0.019	0.265	0.434	4	0.570	0.183	0.248	1
Cameroon	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cameroon	Harm21	C	0.376	0.015	0.179	0.431	4	0.420	0.368	0.213	1
Cameroon	Harm41	C	0.448	0.021	0.147	0.384	1	0.420	0.368	0.213	1
Columbia	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Columbia	Harm55	C	0.484	0.015	0.137	0.365	1	0.343	0.220	0.438	3
Dominican Republic	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dominican Republic	Harm14	C	0.376	0.010	0.295	0.319	1	0.374	0.160	0.466	3
Dominican Republic	Harm44	C	0.453	0.023	0.148	0.376	1	0.374	0.160	0.466	3
Dominican Republic	Harm55	C	0.466	0.021	0.145	0.368	1	0.374	0.160	0.466	3
Spain	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Spain	Harm09	C	0.361	0.015	0.202	0.423	4	0.378	0.338	0.285	1
Spain	Harm10	C	0.276	0.019	0.415	0.290	3	0.378	0.338	0.285	1
Spain	Harm15	C	0.235	0.013	0.185	0.566	4	0.378	0.338	0.285	1
Spain	Harm52	C	0.358	0.008	0.229	0.405	4	0.378	0.338	0.285	1
Spain	Harm53	B	0.282	0.011	0.574	0.132	3	0.378	0.338	0.285	1
Spain	Harm56	C	0.384	0.016	0.261	0.339	1	0.378	0.338	0.285	1
France	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
France	Harm03	A	0.027	0.953	0.000	0.019	2	0.438	0.326	0.236	1
France	Harm33	C	0.276	0.011	0.221	0.492	4	0.438	0.326	0.236	1
France	Harm48	C	0.366	0.006	0.261	0.366	4	0.438	0.326	0.236	1
France	Harm50	C	0.395	0.011	0.229	0.365	1	0.438	0.326	0.236	1

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Table 3. Continued

Country	Haplotype	PCoA group	Pop1 (ns)	Pop2 (ns)	Pop3 (ns)	Pop4 (ns)	Assign (ns)	Pop1	Pop2	Pop3	Assign
France	Harm51	B	0.215	0.031	0.629	0.126	3	0.438	0.326	0.236	1
Ghana	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ghana	Harm36	C	0.339	0.013	0.229	0.419	4	0.400	0.300	0.300	1
Ghana	Harm37	C	0.387	0.016	0.244	0.353	1	0.400	0.300	0.300	1
India	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
India	Harm02	B	0.218	0.013	0.639	0.131	3	0.511	0.259	0.230	1
India	Harm03	A	0.027	0.953	0.000	0.019	2	0.511	0.259	0.230	1
India	Harm06	B	0.190	0.015	0.744	0.052	3	0.511	0.259	0.230	1
India	Harm09	C	0.348	0.011	0.219	0.421	4	0.511	0.259	0.230	1
India	Harm10	C	0.305	0.015	0.387	0.294	3	0.511	0.259	0.230	1
India	Harm15	C	0.240	0.011	0.182	0.566	4	0.511	0.259	0.230	1
India	Harm17	C	0.332	0.015	0.250	0.403	4	0.511	0.259	0.230	1
India	Harm19	C	0.371	0.021	0.226	0.382	4	0.511	0.259	0.230	1
India	Harm21	C	0.379	0.015	0.173	0.434	4	0.511	0.259	0.230	1
India	Harm23	C	0.318	0.010	0.265	0.408	4	0.511	0.259	0.230	1
India	Harm24	C	0.361	0.018	0.240	0.381	4	0.511	0.259	0.230	1
India	Harm27	C	0.334	0.015	0.279	0.373	4	0.511	0.259	0.230	1
India	Harm32	C	0.321	0.019	0.247	0.413	4	0.511	0.259	0.230	1
India	Harm33	C	0.255	0.015	0.208	0.523	4	0.511	0.259	0.230	1
Israel	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Israel	Harm10	C	0.324	0.011	0.406	0.258	3	0.369	0.456	0.175	2
Italy	Harm57	A	0.027	0.953	0.000	0.019	2	0.440	0.379	0.181	1
Jordan	Harm10	C	0.295	0.023	0.405	0.277	3	0.371	0.455	0.174	2
Japan	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kenya	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kenya	Harm21	C	0.371	0.015	0.189	0.426	4	0.455	0.386	0.159	1
Kenya	Harm58	A	0.027	0.953	0.000	0.019	2	0.455	0.386	0.159	1
Korea	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Morocco	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Madagascar	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Madagascar	Harm02	B	0.245	0.013	0.642	0.100	3	0.510	0.289	0.201	1
Madagascar	Harm06	B	0.192	0.015	0.735	0.058	3	0.510	0.289	0.201	1
Madagascar	Harm33	C	0.269	0.013	0.208	0.510	4	0.510	0.289	0.201	1
Madagascar	Harm38	C	0.321	0.016	0.255	0.408	4	0.510	0.289	0.201	1
Madagascar	Harm39	C	0.332	0.021	0.208	0.439	4	0.510	0.289	0.201	1
Madagascar	Harm40	C	0.360	0.010	0.248	0.382	4	0.510	0.289	0.201	1
Macedonia	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
The Netherlands	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
The Netherlands	Harm02	B	0.232	0.018	0.627	0.123	3	0.471	0.320	0.209	1
The Netherlands	Harm58	A	0.027	0.953	0.000	0.019	2	0.471	0.320	0.209	1
New Zealand	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
New Zealand	Harm02	B	0.266	0.016	0.594	0.124	3	0.543	0.225	0.233	1
New Zealand	Harm05	C	0.294	0.018	0.371	0.318	3	0.543	0.225	0.233	1
New Zealand	Harm47	C	0.331	0.013	0.244	0.413	4	0.543	0.225	0.233	1
Pakistan	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pakistan	Harm06	B	0.200	0.015	0.737	0.048	3	0.459	0.326	0.215	1
Pakistan	Harm07	B	0.187	0.015	0.735	0.063	3	0.459	0.326	0.215	1
Pakistan	Harm21	C	0.376	0.010	0.179	0.435	4	0.459	0.326	0.215	1
Peru	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Peru	Harm02	B	0.250	0.018	0.603	0.129	3	0.335	0.246	0.419	3
Peru	Harm05	C	0.289	0.013	0.400	0.298	3	0.335	0.246	0.419	3
Peru	Harm10	C	0.306	0.021	0.382	0.290	3	0.335	0.246	0.419	3
Peru	Harm14	C	0.360	0.015	0.339	0.287	1	0.335	0.246	0.419	3
Peru	Harm35	C	0.369	0.011	0.239	0.381	4	0.335	0.246	0.419	3
Peru	Harm39	C	0.329	0.021	0.221	0.429	4	0.335	0.246	0.419	3
Peru	Harm44	C	0.448	0.023	0.155	0.374	1	0.335	0.246	0.419	3
Peru	Harm54	B	0.265	0.021	0.602	0.113	3	0.335	0.246	0.419	3
Peru	Harm55	C	0.456	0.027	0.158	0.358	1	0.335	0.246	0.419	3
Peru	Harm57	A	0.027	0.953	0.000	0.019	2	0.335	0.246	0.419	3
Peru	Harm58	A	0.027	0.953	0.000	0.019	2	0.335	0.246	0.419	3
Philippines	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Puerto Rico	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Puerto Rico	Harm39	C	0.323	0.021	0.239	0.418	4	0.373	0.149	0.479	3

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Table 3. Continued

Country	Haplotype	PCoA group	Pop1 (ns)	Pop2 (ns)	Pop3 (ns)	Pop4 (ns)	Assign (ns)	Pop1	Pop2	Pop3	Assign
Puerto Rico	Harm44	C	0.456	0.023	0.144	0.377	1	0.373	0.149	0.479	3
Puerto Rico	Harm54	B	0.260	0.021	0.585	0.134	3	0.373	0.149	0.479	3
Puerto Rico	Harm55	C	0.461	0.021	0.134	0.384	1	0.373	0.149	0.479	3
Portugal	Harm14	C	0.363	0.013	0.318	0.306	1	0.384	0.311	0.305	1
Portugal	Harm44	C	0.450	0.023	0.152	0.376	1	0.384	0.311	0.305	1
Portugal	Harm57	A	0.027	0.953	0.000	0.019	2	0.384	0.311	0.305	1
Palestine	Harm15	C	0.235	0.010	0.181	0.574	4	0.371	0.455	0.174	2
Senegal	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Senegal	Harm02	B	0.248	0.010	0.619	0.123	3	0.356	0.298	0.346	1
Senegal	Harm34	C	0.319	0.013	0.373	0.295	3	0.356	0.298	0.346	1
Senegal	Harm35	C	0.365	0.011	0.252	0.373	4	0.356	0.298	0.346	1
Chad	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Chad	Harm42	C	0.366	0.011	0.244	0.379	4	0.390	0.439	0.171	2
Chad	Harm43	C	0.329	0.011	0.297	0.363	4	0.390	0.439	0.171	2
Chad	Harm49	C	0.240	0.013	0.197	0.550	4	0.390	0.439	0.171	2
Thailand	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Uganda	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
Uganda	Harm02	B	0.261	0.015	0.573	0.152	3	0.460	0.374	0.166	1
Uganda	Harm03	A	0.027	0.953	0.000	0.019	2	0.460	0.374	0.166	1
Uganda	Harm04	A	0.027	0.953	0.000	0.019	2	0.460	0.374	0.166	1
Uganda	Harm11	C	0.316	0.016	0.423	0.245	3	0.460	0.374	0.166	1
Uganda	Harm12	C	0.318	0.011	0.411	0.260	3	0.460	0.374	0.166	1
Uganda	Harm14	C	0.402	0.024	0.294	0.281	1	0.460	0.374	0.166	1
Uganda	Harm19	C	0.361	0.016	0.223	0.400	4	0.460	0.374	0.166	1
Uganda	Harm25	C	0.348	0.013	0.248	0.390	4	0.460	0.374	0.166	1
Uganda	Harm26	C	0.332	0.008	0.268	0.392	4	0.460	0.374	0.166	1
United States, Florida	Harm01	C	0.345	0.013	0.256	0.385	4	0.368	0.196	0.436	3
South Africa	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA
South Africa	Harm12	C	0.315	0.019	0.377	0.289	3	0.505	0.215	0.280	1
Zimbabwe	Harm01	C	NA	NA	NA	NA	NA	NA	NA	NA	NA

Shaded cells indicate significant correspondence between the two independent methods: PCoA and nonspatial Bayesian analysis. From these analyses, two well-supported genetic clusters are resolved, indicated in the cells as green and not green. NA (not applicable).

Results

Helicoverpa armigera Haplotype Diversity and Population Structure

Fifty-four of the haplotypes samples in this study are identical to those haplotypes designated by Tay et al. (2017), although our data includes an additional 20 countries that had not been previously analyzed. Four of the COI haplotypes are new and described here for the first time (Table 2). The new haplotypes were found in the New World, Europe, and Africa. Specifically, haplotype 'Harm55' was found in Brazil, Columbia, Dominican Republic, Peru, and Puerto Rico; 'Harm56' in Spain; 'Harm57' in Italy, Portugal, and Peru; and 'Harm58' in Kenya, the Netherlands, and Peru (Fig. 1A). Given the ability of *H. armigera* to hybridize with *H. zea* (Laster and Sheng 1995, Anderson et al. 2018) all the new haplotypes were aligned to the complete mitochondrial sequences for *H. armigera* and *H. zea* to ensure that the new haplotypes did not originate from a mating of an *H. armigera* male to a female *H. zea*. All new haplotypes matched with numerous single-nucleotide polymorphisms (SNPs) to *H. zea* and few SNPs to *H. armigera* demonstrating that these results are not due to introgressed mitochondria from *H. zea*.

Similar to the results from Tay et al. (2017), the most common haplotype was Harm01, which was found in nearly every country sampled including all three individuals found in Florida (Figs. 1B and 2). As such, it is unclear what the geographic origin of Harm01 might have been. In an effort to resolve the origins of Harm01, and ultimately, how it might have migrated to Florida and throughout much of the world, several analyses were run leaving only the Florida Harm01 haplotype in the final matrix. To the degree that haplotype relatedness and geographic origin can be correlated, the origin of Harm01 and other haplotypes can be inferred (Avise et al. 1987). The assignments from Geneland, when applying the nonspatial model, place Harm01 among a group, out of four total haplotype clusters, with a generally southern and equatorial distribution found in Australia, Burkina Faso, Brazil, China, Cameroon, Spain, France, Ghana, India, Kenya, Madagascar, New Zealand, Pakistan, Peru, Puerto Rico, Palestine, Senegal, Chad, and Uganda (Fig. 1B; Table 3). However, the support for assignment to this cluster is low at 0.39 posterior probability (PP), with a nearly equivalent value to cluster 1 at 0.35 PP.

When applying a genetic distance-based approach in principle coordinates analysis (PCoA), the genetic clustering was similar between methods. The haplotype Harm01 was again part of a large worldwide group with low support of assignment as measured by the separation of individuals across coordinate two, which only explains 6.2% of the variance compared with coordinate one, which explains 69.7% of the variance. More interestingly, however, is the separation of haplotypes Harm03, Harm04, Harm57, and Harm58 from all other haplotypes. This separation among haplotype groups was also supported in the Bayesian analysis at 0.95 PP (Table 3). Divergence among these groups was further tested using the PhiPT method and found to be significant ($P < 0.001$). Within this genetic cluster, haplotypes were clearly separated along coordinate one into 1) Harm04, 2) Harm03 and Harm57, and 3) Harm58 (Fig. 3). As such, some inferences regarding geographic origin can be drawn

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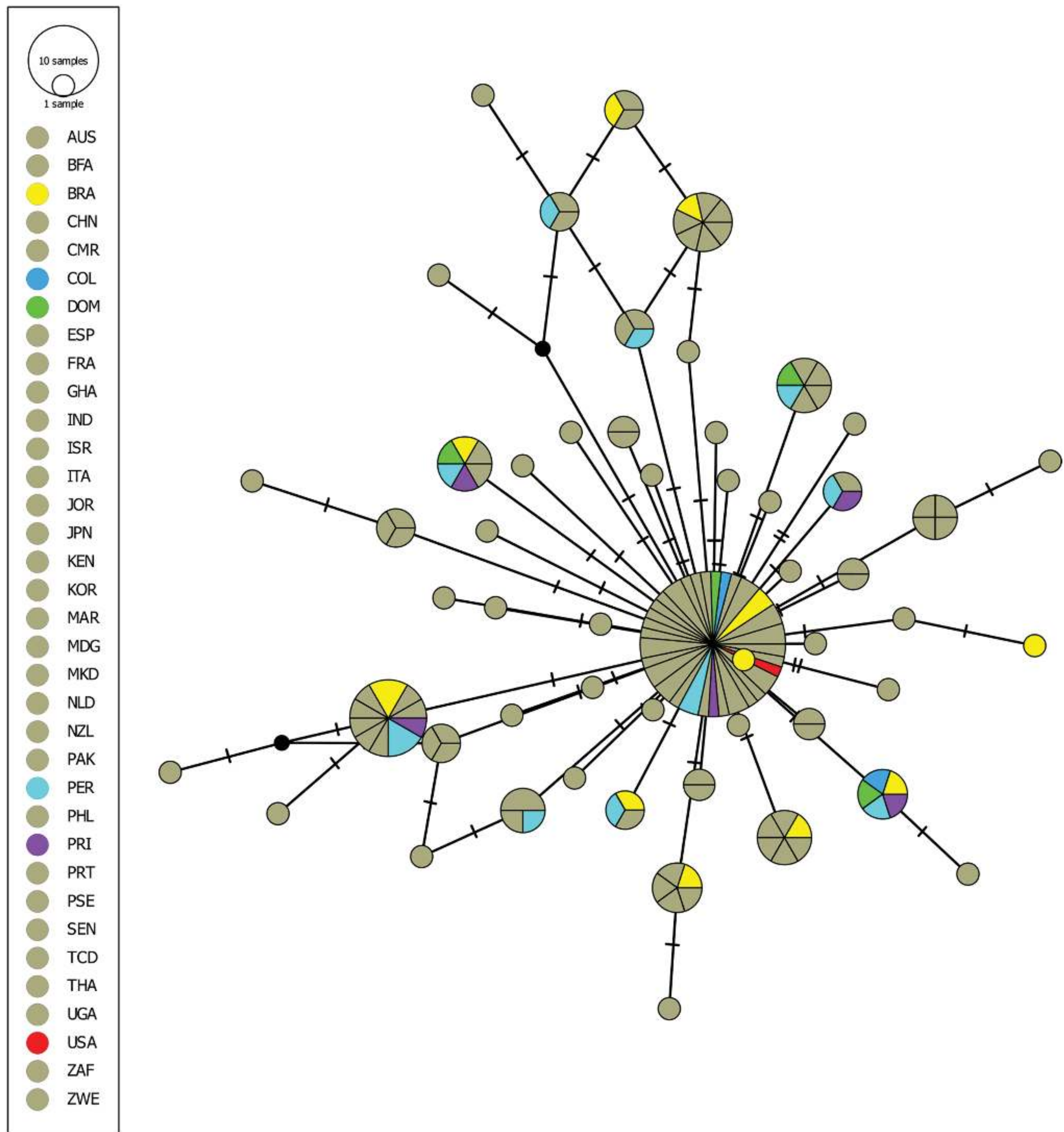

Fig. 2. Network of CO1 sequences with country of origin. Nodes are scaled by number of individuals sharing the same haplotype. Country codes are found in Supp Table S1 [online only].

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between these groups and within PCoA cluster A and Bayesian nonspatial cluster 2 (Fig. 1A; Table 3).

A spatial prior was applied to the Bayesian analyses of haplotype cluster assignments to test the significance of geographic origin to the genetic structure. The assignments were compared between spatial and nonspatial models and found to differ in cluster membership and support values (Table 3). The spatial model did not contain any assignment values above 0.61 PP. The supported clusters found for the genetic-only approaches were not retained when a spatial prior was applied. This result suggests that any genetic structure that may have been shaped by geographic isolation in the past has been largely broken down through natural and/or human-influenced migration. This result is also probably influenced by the limited power of the single mitochondrial marker used in this study.

Haplotype–Host Associations in the New World

For 54 New World samples, reliable records were available regarding the plant species that the specimen was collected from or intercepted on (Table 1; Supp Table S1 [online only]). This plant species association information was then integrated with the genetic

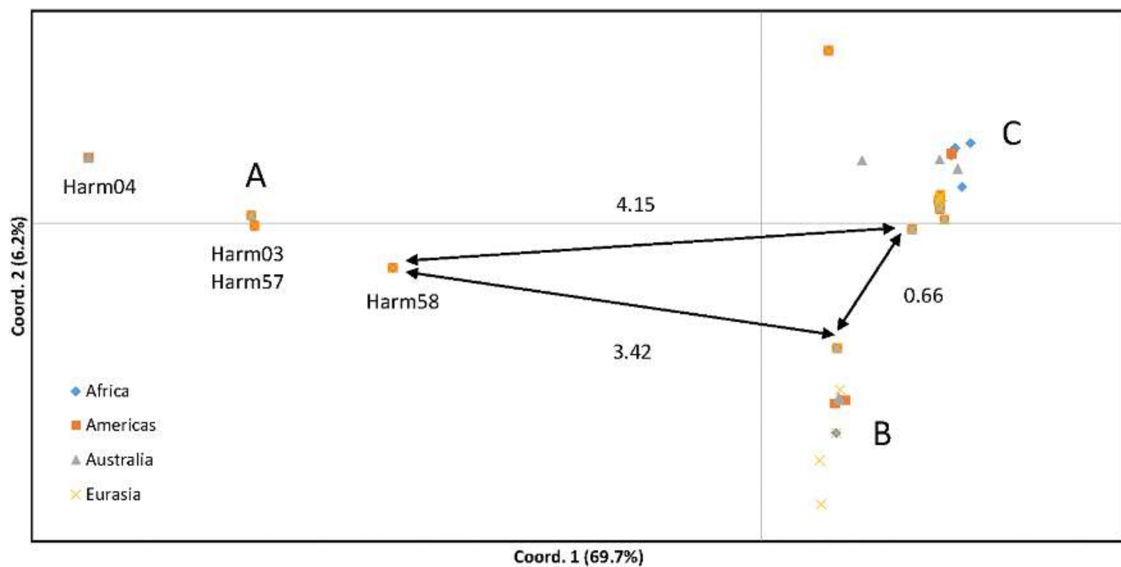

Fig. 3. PCoA plot with all haplotypes, linear PhiPT calculated between clusters, all linear PhiPT values are significantly different than random at the $P < 0.001$ level.

distance-based and Bayesian analyses to see whether any association between haplotype and host could be found (Table 4).

In the Bayesian analysis, geographic location, allele frequency, and host plant species were treated as priors to determine which parameters were correlated with population genetic structuring. No single parameter provided a well-supported partitioning of the data when analyzed individually. However, when combined in a single analysis, a geographic partitioning of the data into a Caribbean cluster, Colombian cluster, and Peruvian cluster was well supported (Table 4). This result suggests that haplotypes are both broadly distributed and do not have strong host preferences but that regional differences in crops contribute to a geographic partitioning of the data. The best-supported data partition did not cluster by host plant or family of host plants suggesting that New World *H. armigera* has not evolved host specialization since its introduction.

The distance-based approach indicated a similar result with all haplotypes having been found associated with pea (Fig. 4). Interestingly, Harm55 was not found outside the New World but clusters with PCoA group C and Bayesian genetic cluster 1 (Table 3). In either clustering arrangement, haplotype Harm55 resolves with other haplotypes in a worldwide distribution similar to Harm01 in the PCoA analyses (Table 3).

Discussion

Genetic Structure and the Origin and Translocation of *H. armigera* to the New World

As *H. armigera* spread across Brazil from the initial introduction, it has been displacing the native *H. zea*, presumably due to superior pesticide resistance mechanisms (e.g., Pinto et al. 2015, Pearce et al. 2017). A similar situation among *H. armigera* haplotypes appears to be taking place throughout the New World and elsewhere around the world as the haplotype Harm01 was found from nearly every location (Fig. 1B; Table 2). This suggests that a selective sweep may account for the ubiquity of Harm01 throughout the range of *H. armigera*, including newly invaded areas. This is consistent with the findings from other studies (Tay et al. 2013, 2017), including one that also analyzed markers associated with fenvalerate resistance (Anderson et al. 2016).

Given its widespread geographic distribution, the geographic origins of Harm01 cannot currently be determined. In contrast, because the haplotypes in PCoA cluster A (Fig. 3) are more geographically isolated and more clearly separated from other haplotype groups, some inferences can be drawn about their geographic origin and translocation to the New World. Haplotype Harm03 is found in Australia, Burkina Faso, Brazil, China, France, India, and Uganda. Thus, this haplotype was probably translocated or migrated from Africa, Australia, and/or Eurasia into South America, specifically Brazil. The closely related haplotype Harm04 is found in Australia, Brazil, and Uganda. Unlike Harm01 in the New World, these haplotypes have not spread beyond Brazil. Other authors have suggested that if the introduction into Brazil was a result of natural dispersal, then Africa, given its close proximity, may have been the origin of these haplotypes (Tay et al. 2013). For haplotype Harm57, the origin appears to be Mediterranean with collections from Italy, Portugal, and Peru. Similarly, Harm58 seems to have a Euro-African origin with populations from Kenya, the Netherlands, and Peru. Kenya supplies 35% of Europe's cut flowers, of which the majority are transported through Amsterdam (Veselinovic 2015); however, as Kenya grows in importance in the floriculture industry, it has begun to trade directly with other countries (Escritt 2016), so neither scenario can be ruled out as a potential source.

The Harm57 and Harm58 haplotypes were found to be present in Peru but not elsewhere in the New World, including Brazil. This could indicate that a separate introduction into Peru was part of the establishment of *H. armigera* in the New World (Fig. 1A). Since 2014, *H. armigera* has been of the most commonly intercepted pests on pea from Peru (Gilligan et al. 2019), suggesting that *H. armigera* was well established in that country sometime prior to 2014. As such, this secondary introduction has become an important, yet poorly described, potential source for the spread of *H. armigera* in the New World. Secondary introductions increase the genetic diversity of invading populations and thus may worsen future control efforts (e.g., Walsh et al. 2018).

Patterns of Haplotype–Crop Associations

Similar to geography, host preference does not appear to be driving genetic structure among the rapidly expanding range of *H. armigera*

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Table 4. Results from Bayesian analyses of host–haplotype associations

			Host + haplotype + geography				Haplotype			Haplotype + host							Haplotype + geography		
Haplotype	Host species	Country of origin	Pop1	Pop2	Pop3	Assign	Pop1	Pop2	Assign	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Assign	Pop1	Pop2	Assign
Harm01	Pepper	Dominican Republic	0	1	0	2	0.37	0.63	2	0.16	0.26	0.10	0.14	0.12	0.22	2	0.29	0.71	2
Harm01	Sage	Peru	0	0	1	3	0.37	0.63	2	0.18	0.15	0.14	0.17	0.15	0.22	6	0.41	0.59	2
Harm01	Waxflower	Peru	0	0	1	3	0.32	0.68	2	0.16	0.12	0.14	0.15	0.22	0.21	5	0.41	0.59	2
Harm01	Cucumber	Puerto Rico	0	1	0	2	0.32	0.68	2	0.31	0.15	0.15	0.17	0.09	0.15	1	0.28	0.72	2
Harm01	Pigeon pea	Puerto Rico	0	1	0	2	0.32	0.68	2	0.15	0.16	0.12	0.19	0.20	0.18	5	0.28	0.72	2
Harm01 (30)	Pea	Peru	0	0	1	3	0.31	0.69	2	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2
Harm02	Bean	Peru	0	0	1	3	0.46	0.54	2	0.18	0.14	0.15	0.19	0.12	0.22	6	0.41	0.59	2
Harm02 (2)	Pea	Peru	0	0	1	3	0.50	0.50	1_2	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2
Harm05	Pea	Peru	0	0	1	3	0.46	0.54	2	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2
Harm10	Pea	Peru	0	0	1	3	0.62	0.38	1	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2
Harm14	Pepper	Dominican Republic	0	1	0	2	0.40	0.60	2	0.16	0.26	0.10	0.14	0.12	0.22	2	0.29	0.71	2
Harm14	Pea	Peru	0	0	1	3	0.41	0.59	2	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2
Harm35	Pea	Peru	0	0	1	3	0.48	0.52	2	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2
Harm39	Pea	Peru	0	0	1	3	0.46	0.54	2	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2
Harm44	Oregano	Peru	0	0	1	3	0.72	0.28	1	0.15	0.38	0.09	0.14	0.11	0.13	2	0.41	0.59	2
Harm44 (2)	Pea	Peru	0	0	1	3	0.70	0.30	1	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2
Harm54	Pea	Peru	0	0	1	3	0.63	0.37	1	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2
Harm55	Basil	Colombia	1	0	0	1	0.28	0.72	2	0.15	0.12	0.12	0.26	0.16	0.19	4	0.30	0.70	2
Harm55	Oregano	Peru	0	0	1	3	0.27	0.73	2	0.15	0.38	0.09	0.14	0.11	0.13	2	0.41	0.59	2
Harm55	Sorghum	Puerto Rico	0	1	0	2	0.30	0.70	2	0.13	0.14	0.08	0.12	0.38	0.15	5	0.28	0.72	2
Harm55 (3)	Pea	Peru	0	0	1	3	0.30	0.70	2	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2
Harm58	Pea	Peru	0	0	1	3	0.83	0.17	1	0.11	0.10	0.41	0.16	0.13	0.09	3	0.41	0.59	2

Columns after country of origin are the results from the best-supported data partition for each parameter combination indicating the posterior probability of individual membership to each cluster. Numbers in parentheses in the haplotype column indicate the number of individuals with a given haplotype found on that host with only a single haplotype–host combination used in the analyses. Reduction in PP for the haplotype only column was the result of having to trim the number of SNPs from the complete data set (due monomorphic loci) used in Table 3.

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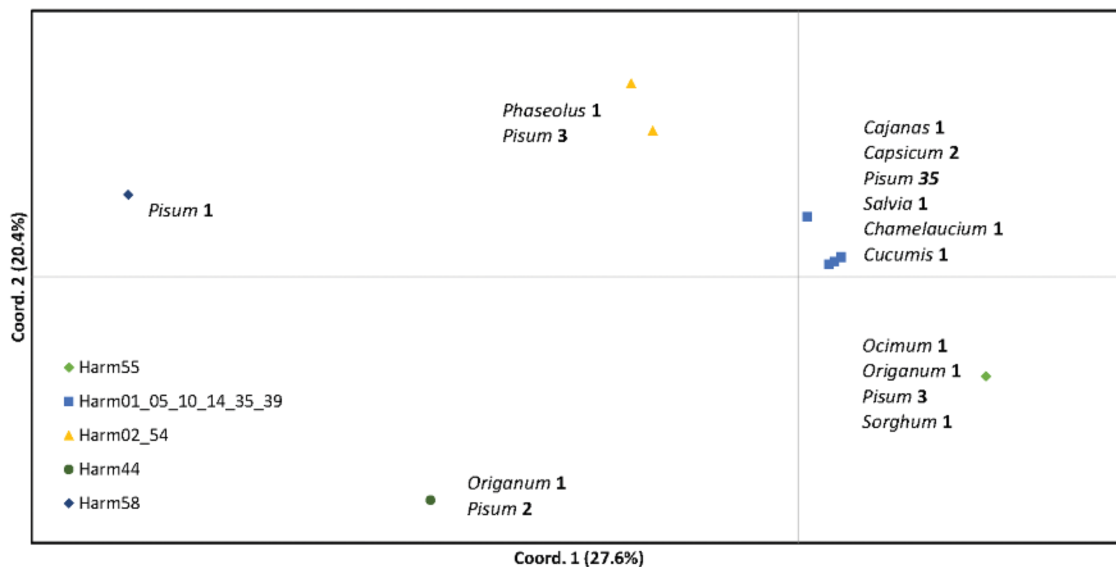

Fig. 4. PCoA plot of all New World haplotypes with plant association data. Host plants are grouped by genus and numbers indicate the number of individuals found with a given host. Points on the graph are colored coded by haplotype or closely related grouping of haplotypes.

in the New World (Fig. 4; Table 4). Nevertheless, some patterns are notable among the broadly distributed haplotypes. The most striking of these is the comparison between the group consisting of haplotypes Harm01, Harm05, Harm10, Harm14, Harm35, Harm39, and the group containing haplotype Harm55. The Harm01 group, which is made up of several closely related haplotypes, has been found primarily (90%) on pea, whereas the single haplotype Harm55 has been found with pea only 50% of the time. Thus, it would seem that Harm55 has a greater host breadth than the more genetically diverse cluster of haplotypes related to Harm01. Why Harm55 is only found in the New World is uncertain, but its increased frequency there might be related to host choice or environmental parameters such as pesticide resistance.

When examining host plant trends worldwide, *H. armigera* is found almost exclusively with food plants in New World interceptions, whereas interceptions from elsewhere in the world are found mostly with cut flowers (Table 1). These differences in host plant are probably a reflection of differences in trade commodity types from these countries and could provide some guidance on how to implement targeted inspections in the future. This trend is also found in the Bayesian analyses of the New World hosts, wherein a haplotype + host + country interaction was supported. These results should, however, be interpreted with caution as only a fraction of possible host data was included from the broad host range of *H. armigera*, and sample sizes were relatively small.

Our results indicate that *H. armigera* is polyphagous in the New World and will opportunistically host on different crop plant species regardless of haplotype as it expands its geographic range. Identical haplotypes recovered from different host plant species in this study provide some evidence that multiple *H. armigera* haplotypes could be occurring sympatrically on host plants; however, more detailed field studies are required to confirm this finding. As such further research on the role of hosts and environment in structuring populations of *H. armigera* in newly established habitats is justified, including the use of larger genomic data sets and multiyear in-field surveys to improve inferences regarding population structure, migration, and host selection. Being able to associate haplotype/genotype with a given host(s), geographic region, and/or environmental parameter would be a very useful tool for targeted management practices.

Conclusions

The exact mode of *H. armigera* translocation to the New World is not known. As such, the source populations of the Puerto Rico and Florida invasions cannot be unequivocally determined. Nonetheless, two well-supported genetic groups are currently represented in the New World, with the origin of PCoA group A possibly being Euro-African given the geographic distribution of Harm57 and Harm58, and PCoA group B + C with an uncertain origin given its worldwide distribution. Both haplotypes are now represented in South America and parts of the Caribbean, suggesting separate introductions into Brazil and Peru. Regardless, the spread of both haplotype groups throughout the New World has proceeded rapidly and with serious losses to crop production. Additional work is needed to understand the biological differences uniquely associated with the two divergent haplo groups, possibly providing targeted management strategies. Furthermore, given the introductions of multiple polyphagous haplotypes of *H. armigera* into South America, and its subsequent overlap with the range of *H. zea*, the New World could become a hotspot for the evolution and eventual export of new megapest lineages to other parts of the world.

Supplementary Data

Supplementary data are available at *Annals of the Entomological Society of America* online.

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