



# Adaptive Introgression across Semipermeable Species Boundaries between Local *Helicoverpa zea* and Invasive *Helicoverpa armigera* Moths

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## Abstract

Hybridization between invasive and native species has raised global concern, given the dramatic increase in species range shifts and pest outbreaks due to anthropogenic dispersal. Nevertheless, secondary contact between sister lineages of local and invasive species provides a natural laboratory to understand the factors that determine introgression and the maintenance or loss of species barriers. Here, we characterize the early evolutionary outcomes following secondary contact between invasive *Helicoverpa armigera* and native *H. zea* in Brazil. We carried out whole-genome resequencing of *Helicoverpa* moths from Brazil in two temporal samples: during the outbreak of *H. armigera* in 2013 and 2017. There is evidence for a burst of hybridization and widespread introgression from local *H. zea* into invasive *H. armigera* coinciding with *H. armigera* expansion in 2013. However, in *H. armigera*, the admixture proportion and the length of introgressed blocks were significantly reduced between 2013 and 2017, suggesting selection against admixture. In contrast to the genome-wide pattern, there was striking evidence for adaptive introgression of a single region from the invasive *H. armigera* into local *H. zea*, including an insecticide resistance allele that increased in frequency over time. In summary, despite extensive gene flow after secondary contact, the species boundaries are largely maintained except for the single introgressed region containing the insecticide-resistant locus. We document the worst-case scenario for an invasive species, in which there are now two pest species instead of one, and the native species has acquired resistance to pyrethroid insecticides through introgression.

**Key words:** hybridization, secondary contact, insecticide resistance evolution, gene flow, invasive species.

## Introduction

Hybridization between local and introduced species is an underestimated threat to global agriculture (Mooney and Cleland 2001; Blair and Hufbauer 2010; Paine et al. 2016). Although hybridization is often maladaptive, introgressive hybridization can occasionally introduce adaptive variants and potentially contribute to the establishment and invasiveness of the newcomer species (Currat et al. 2008; Saarman and Pogson 2015; Hall 2016; Mesgaran et al. 2016). This makes invasive hybridization a major global concern impacting natural and agricultural systems, since species introductions are predicted to continue at an accelerating rate following trade, human movement, and climate change (Saarman and Pogson 2015; Paine et al. 2016). Simultaneously, invasive

species offer a unique opportunity to study evolutionary outcomes following secondary contact. Although secondary contact zones have played a major role in the study of reproductive isolation and speciation, it remains a challenge to disentangle the contribution of ancient and recent gene flow to the contemporary patterns of introgression (Barton and Hewitt 1985; Abbott et al. 2013). This is primarily because ancestral range reconstruction is challenging, and so it is hard to confidently estimate the extent of historical contact between species (Barton and Hewitt 1985; Abbott et al. 2013; Saarman and Pogson 2015). However, in the case of invasive species, the geographical reunion of divergent species can be observed in “real time.” This allows us to systematically test hypotheses regarding the possible outcomes of secondary

contact including fusion, reinforcement, hybrid speciation, and adaptive introgression.

The recent invasion by the Old World cotton bollworm *Helicoverpa armigera* of South America (Czepak et al. 2013), where it hybridizes with its sister species *H. zea* (Tay et al. 2013; Anderson et al. 2018), represents an excellent scenario in which to study the evolutionary trajectories following secondary contact. *Helicoverpa armigera* comprises two subspecies: *H. armigera conferta* in Oceania (Australia, New Zealand) and *H. armigera armigera* in its Old World native range, subsequently introduced to South America (Tay et al. 2013; Anderson et al. 2018) (hereafter *H. armigera* refers to *H. armigera armigera* unless otherwise specified). *Helicoverpa zea* is present throughout the temperate and tropical regions of North and South America. *Helicoverpa armigera* and *H. zea* are estimated to have diverged around 1.5 Ma (Behere et al. 2007; Pearce et al. 2017) as a result of a transoceanic dispersion of *H. armigera* into the New World, and thus represent a classic case of divergence due to allopatric distributions (Pearce et al. 2017; Anderson et al. 2018). There is no evidence of introgression between the species during this period of divergence until the recent incursions of *H. armigera* into Brazil (Pearce et al. 2017; Anderson et al. 2018). The invasion is thought to have resulted from international trade (Pearce et al. 2017; Anderson et al. 2018; Mallet 2018; Tay and Gordon 2019) and there is evidence for initial independent incursions, centered on numerous geographic locations (Tay et al. 2017; Arnemann et al. 2019). Multiple and likely ongoing introductions of *H. armigera* from the Old World mean that the genetic diversity of the New World populations is very high and possibly comparable to that in the species' ancestral range (Tay et al. 2017; Arnemann et al. 2019; Gonçalves et al. 2019).

Although the two species are very similar morphologically and their genomes share high levels of orthology, *H. armigera* has twice the genome-wide genetic diversity and a much greater (~10×) effective population size compared with *H. zea* (Mallet et al. 1993; Pearce et al. 2017). In addition, *H. zea* shows a relatively narrow host-plant range and is mainly known as a pest of corn and cotton, although it is also found on over 100 plants belonging to 29 families (Pearce et al. 2017; Anderson et al. 2018; Mallet 2018). *Helicoverpa armigera* is more devastating to agriculture and feeds on over 300 species belonging to 68 families (Pearce et al. 2017; Anderson et al. 2018; Mallet 2018). These differences in host range are associated with fewer gustatory receptor and detoxification genes in the *H. zea* genome, which showed no evidence for gain of any genes after the species diverged (Pearce et al. 2017).

Another substantial phenotypic difference between *H. armigera* and *H. zea* lies in their susceptibility to insecticides widely used for pest control (Anderson et al. 2018; Walsh, Jousse, et al. 2018). *Helicoverpa armigera* shows by far the highest number of reported cases of insecticide resistance worldwide, having evolved resistance against pyrethroids,

organophosphates, carbamates, organochlorines, and recently to macrocyclic lactone spinosad, and several *Bacillus thuringiensis* toxins (McCaffery 1998; Xu et al. 2005; Mahon et al. 2007; Joußen et al. 2012; Tay et al. 2015; Durigan et al. 2017; Pearce et al. 2017; Walsh, Jousse, et al. 2018). Importantly, the resistance toward pyrethroids in *H. armigera* is due to a unique P450 enzyme, CYP337B3, that is capable of metabolizing these pesticides into nontoxic hydroxyrethroids (Joußen et al. 2012). *Helicoverpa zea*, in contrast, remains susceptible to many major insecticides and tends to cause less damage than its Old World relative, although there are recent reports of resistance to *Bt* toxins (Moar et al. 2010; Brévault et al. 2015; Reisig and Kurtz 2018). Therefore, one of the major concerns following the invasion of *H. armigera* is the formation of novel hybrid ecotypes or the introduction of genes associated with insecticide resistance and host-range expansion.

Anderson et al. (2018) unambiguously identified nine genetically intermediate individuals using whole-genome sequences between the two species from 2013, at least 6–8 years after its possible initial introduction into Brazil, when significant crop damages were reported (Tay et al. 2013, 2017; Sosa-Gómez et al. 2016). Of the limited individuals analyzed, eight were genetically closer to *H. armigera* (between 91% and 98% *H. armigera*-like) with some introgressed haplotypes from *H. zea* scattered throughout the genome. The ninth individual resembled an F2 hybrid with stretches of homozygosity for each parental species, and all *H. armigera* surveyed individuals contained the CYP337B3 gene involved in pesticide resistance. The apparent rarity of backcrossing to *H. zea* in these individuals (Anderson et al. 2018) initially suggested that hybridization would not introduce novel insecticide resistance to *H. zea* as had been feared (Mallet 2018). However, intensive pesticide-based control programs in Brazil aimed at controlling the invasive *H. armigera* provide a scenario in which there is strong selection for adaptive introgression of insecticide resistance genes into *H. zea* (Leite et al. 2014; Durigan et al. 2017; Pinto et al. 2017). These efforts to control *H. armigera* may also have had the converse effect of facilitating the establishment of *H. armigera* due to differential survival enabled by its resistance alleles (Norris et al. 2015; Dermauw et al. 2018).

Here, we use whole-genome resequencing of individuals in two temporal samples, soon after the outbreak of *H. armigera* in Brazil (2012–2013) and more recent populations (2016–2017) from a region with large areas cultivating cotton, soybean, and corn to examine the evolutionary outcomes following secondary contact between the *Helicoverpa* sister species. We first identify genome-wide patterns of species distinctiveness and then explore signatures of introgression. We find that the species boundaries are strongly maintained in recent Brazilian populations. Then, we show that introgression is heterogeneous throughout the genome and that admixture has decreased over time in the Brazilian *H. armigera* population. Conversely, for *H. zea*, there is a single strong signature of introgression around a region containing a novel, chimeric gene implicated in insecticide resistance (CYP337B3 gene) which was previously only documented in *H. armigera*

and identified in the invasive population in Brazil (Anderson et al. 2018; Walsh, Jousen, et al. 2018).

## Results

### Sympatric *H. zea* and *H. armigera* Postinvasion Persist as Two Well-Distinct Species

We analyzed whole-genome sequence data from 137 moths from across the world: 90 *H. armigera armigera* (hereafter *H. armigera*), 39 *H. zea*, 2 hybrid individuals (hybrids considered as F1–F3), and 6 *H. punctigera*. The Brazilian individuals were collected in two temporal samples: soon after the *H. armigera* expansion in 2012–2013 (Sosa-Gómez et al. 2016) (59 individuals hereafter 2013) and more recent populations from 2016 to 2017 (37 individuals hereafter 2017). The Brazilian sampling included three regions of sympatry in Brazil that resulted from the invasion of *H. armigera* into local *H. zea* territory. We also included, as a baseline comparison, four preinvasion Brazilian *H. zea* individuals sampled in 2006 (Behere et al. 2007), as well as eight individuals of *H. zea* from allopatric (USA) populations. Additionally, nine individuals of *H. armigera* from the Old World which are all considered to be geographically or temporally isolated from the Brazilian invasion (fig. 1A and supplementary tables S1–S3, Supplementary Material online), as well as 14 *H. a. conferta* individuals from Australia, and six individuals from an outgroup Australian endemic species *H. punctigera* (fig. 1A).

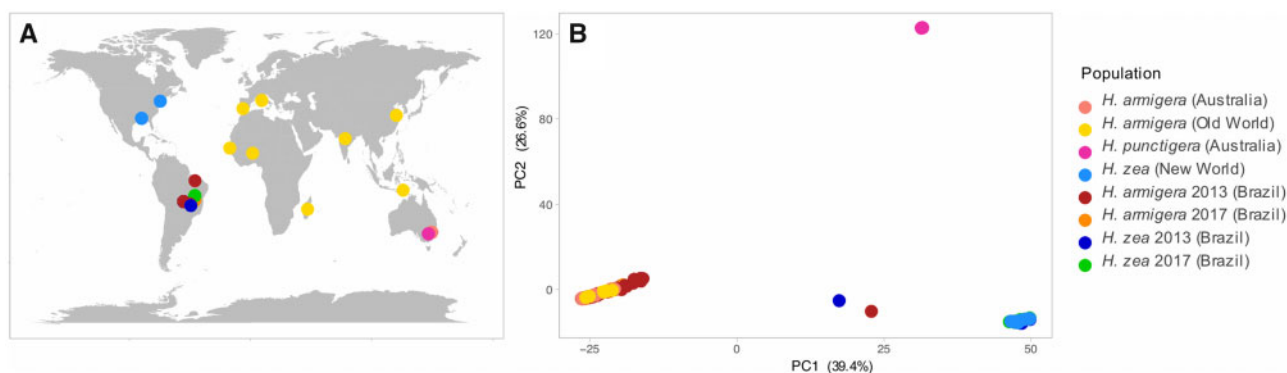
Principal component analysis (PCA) based on ~50,000 whole-genome single-nucleotide polymorphism (SNP) data separated *H. zea* from *H. armigera* on principal component 1, which explained 39.41% of the variation in the data (fig. 1B and supplementary fig. S1A, Supplementary Material online). Across the wide geographic range covered by our sampling, there was no evidence of population substructure. The reported separation between *H. armigera conferta* from Australia and *H. armigera armigera* from the rest of the Old World (Anderson et al. 2016, 2018) was evident when resuming at least the first 25 principal component axes (supplementary fig. S1B, Supplementary Material online). Two individuals fell between *H. armigera* and *H. zea*, likely

representing early generation hybrids. The remaining 97 individuals from Brazil clearly have a closer genetic affinity to one or other of the parental species. Consistent with PC1, ADMIXTURE (Alexander and Lange 2011) analyses showed strong differentiation between the two species, which is supported by the lowest cross-validation error of any assessed when the number of populations is  $K = 2$  (supplementary fig. S2, Supplementary Material online).

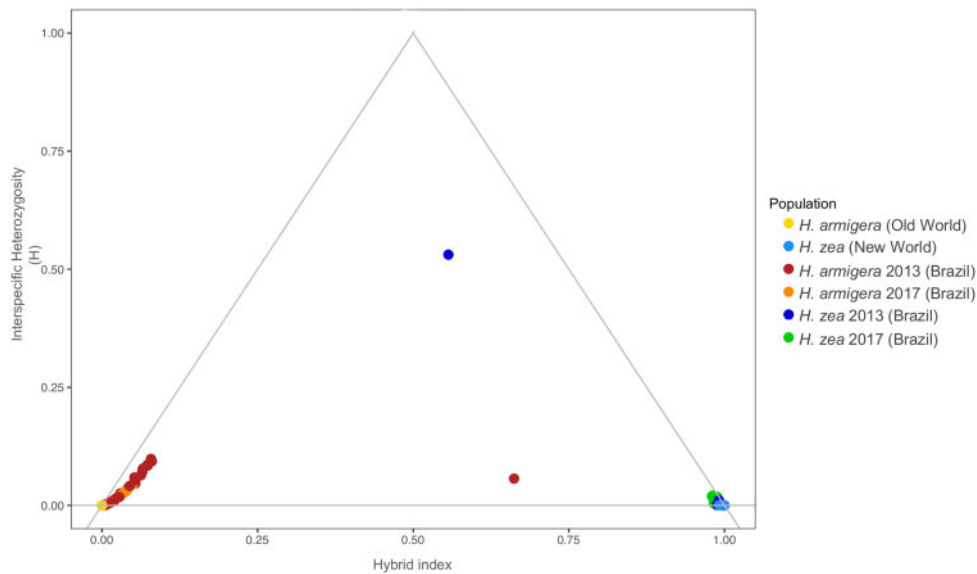
To classify parental and hybrid genotypes, we calculated the proportion of ancestry and heterozygosity based on SNPs that strongly segregated between allopatric populations of the species. In accordance with the PCA and ADMIXTURE results, the classification of Brazilian individuals identified only two early generation hybrids (fig. 2). One individual resembled an F2 hybrid, whereas the other individual resembled an F3 hybrid, both from 2013. All remaining individuals grouped with one of the parental species, although with various fractions of their genome coming from the other species. (Proportions of ancestry are listed in supplementary table S4, Supplementary Material online). A total of 66 individuals were closer to *H. armigera*, with ~0–9.2% of alleles derived from *H. zea*. Meanwhile, the 27 individuals that grouped with *H. zea* samples had ~0–4.1% of *H. armigera* ancestry (supplementary table S4, Supplementary Material online). The tight within-species clustering and low prevalence of intermediate individuals indicated that almost none of the recently sampled individuals resulted from contemporary hybridization, suggesting that hybridization is rare on a per-individual basis in recent populations.

### Reduced Interspecific Differentiation in Sympatry around a Single Genomic Region

We characterized patterns of divergence between sympatric and allopatric pairs of *H. zea* and *H. armigera* populations using the fixation index  $F_{ST}$ . We found similar high genome-wide differentiation between *H. zea* and *H. armigera* for allopatric and recent sympatric populations with a mean autosome-wide  $F_{ST}$  of  $0.474 \pm 0.001$  (mean  $\pm$  SE) and  $0.496 \pm 0.001$ , respectively (supplementary fig. S3 and table S1,



**FIG. 1.** Sampling locations and PCA. (A) Sampling locations of *Helicoverpa* individuals. (B). Genetic PC1 and PC2 of ~50,000 genome-wide SNPs. Color codes indicate different samples and species based on mitochondrial sequences and the groups used for the analyses. *Helicoverpa armigera* (Australia) refers to the subspecies *H. armigera conferta* and *H. armigera* (Old World) to the subspecies *H. armigera armigera* in the rest of the Old World. *Helicoverpa zea* (New World) comprises Brazilian preinvasion and United States, and finally, *H. armigera* 2013/2017 and *H. zea* 2013/2017 refer to the postinvasion sympatric populations in Brazil.



**Fig. 2.** Classification of hybrid and parental genotypes. Classification is based on the hybrid index and interspecific heterozygosity for *Helicoverpa zea* and *H. armigera* individuals. The hybrid index and the interspecific heterozygosity were calculated based on  $\sim 1,000,000$  SNPs that are fixed differences between allopatric populations of the parental species.

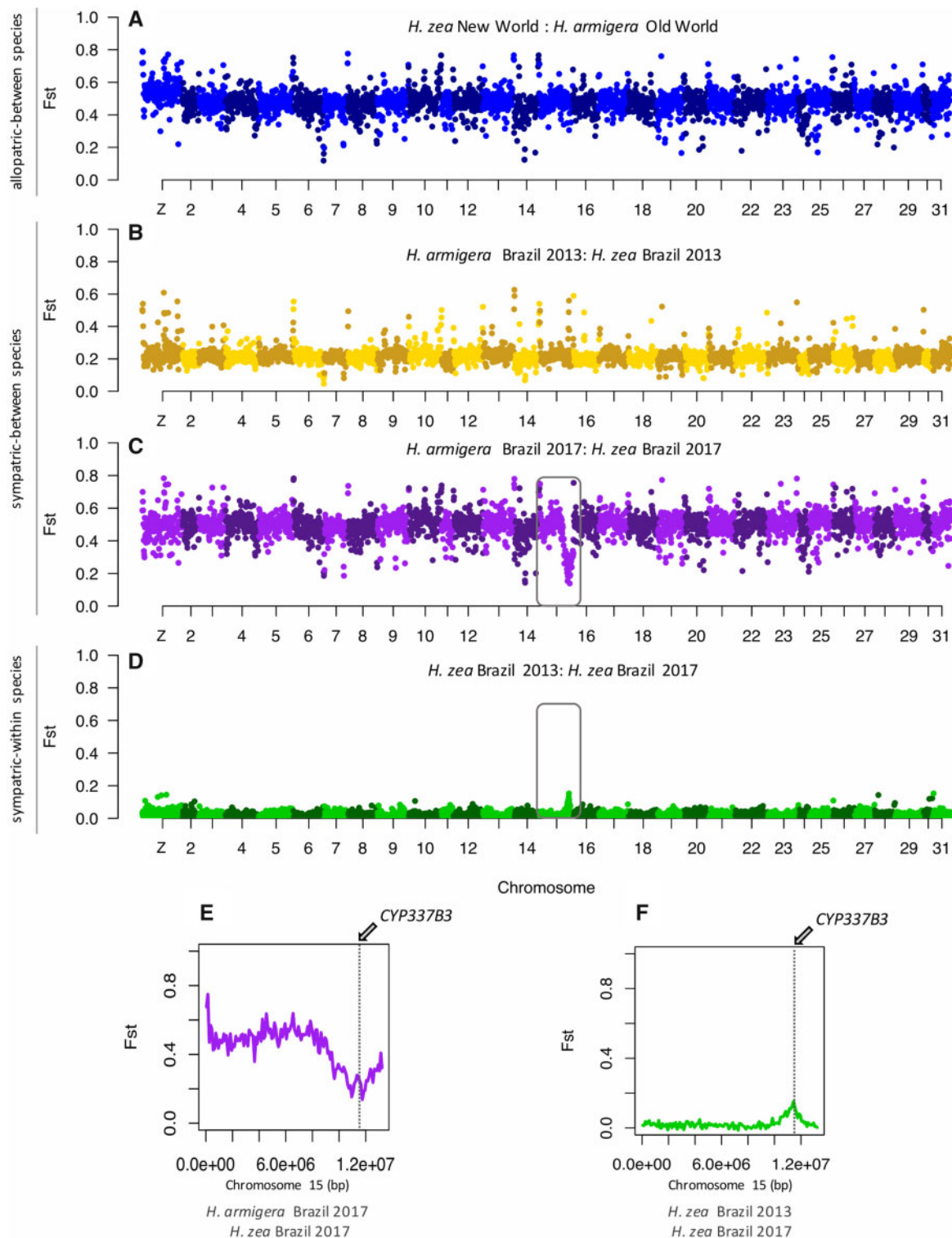
Supplementary Material online). By contrast,  $F_{ST}$  values for intraspecific comparisons were low, consistent with a clear genetic identity within species (supplementary fig. S3, Supplementary Material online). Indeed, the  $F_{ST}$  between the invasive Brazilian and Old World *H. armigera* was  $0.0062 \pm 0.0001$  (supplementary fig. S3A, Supplementary Material online), and the pairwise  $F_{ST}$  between Brazilian *H. zea* and North American *H. zea* averaged  $0.0118 \pm 0.0002$  (supplementary fig. S3A, Supplementary Material online).

To compare differentiation across the genome, we calculated  $F_{ST}$  values in 100-kb nonoverlapping windows. Interspecific  $F_{ST}$  values for allopatric ( $0.474 \pm 0.0012$ ) and recent sympatric ( $0.489 \pm 0.0068$ ) populations were consistently high throughout the genome except for a single region on chromosome 15 (fig. 3C–F). This region included the insecticide resistance gene *CYP337B3*, whereby  $F_{ST}$  significantly dropped up to 0.14 ( $-\log_{10}[P \text{ value}] = 13.96$ ) for the recent sympatric Brazilian populations of *H. armigera* and *H. zea* (fig. 3C and E). Intraspecific  $F_{ST}$  values across the genome between Brazilian and Old World *H. armigera* populations were also consistently low (2013:  $0.0107 \pm 0.001$ ; 2017:  $0.0162 \pm 0.0016$ ), although there was a slight peak at the *CYP337B3* locus (2013:  $F_{ST} = 0.11$ ,  $-\log_{10}(P \text{ value}) \gg 30$ ; 2017:  $F_{ST} = 0.19$ ,  $-\log_{10}(P \text{ value}) \gg 30$ ) (supplementary fig. S3B, Supplementary Material online). Between *H. zea* from United States and Brazil, there was also only one peak of differentiation ( $-\log_{10}[P \text{ value}] \gg 30$ ), corresponding to the same region on chromosome 15 which contains the insecticide-resistant locus and that showed low differentiation for the interspecific comparison between Brazilian *H. zea* and *H. armigera* (fig. 3D). Although both  $F_{ST}$  estimates at a genome-wide scale and across windows showed that differentiation between species is high between recent sympatric ( $0.489 \pm 0.0068$ ) and allopatric ( $0.474 \pm 0.0012$ ) populations,

these patterns of reduced between-species and elevated within-species differentiation around the same region hint at recent localized introgression.

### Widespread Phylogenetic Discordance Is Consistent with Recent Gene Flow

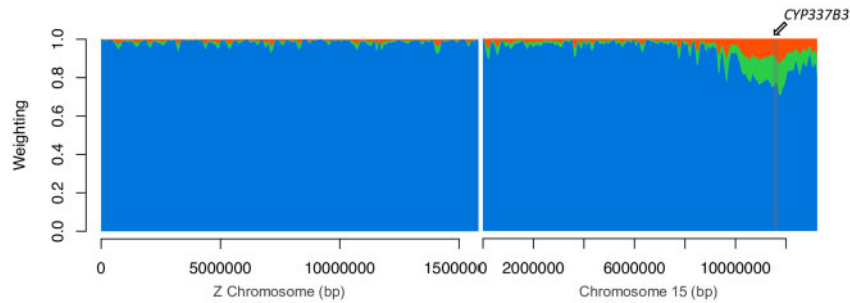
We explored species phylogenies across the genome using Twisst (Martin and Van Belleghem 2017), which quantifies the frequency of alternative topological relationships among all individuals in sliding windows, in this case, of 50 SNPs. These phylogenetic comparisons across the genome indicated large-scale introgression among recent samples of Brazilian *H. armigera* and *H. zea* (fig. 4). The three possible unrooted topologies that describe the relationships between allopatric and sympatric populations were represented across the genome (supplementary fig. S4, Supplementary Material online). Around 60% of the windows had completely sorted genealogies (i.e., weighting of 1), most of which matched the expected species branching order (blue in fig. 4 and supplementary fig. S4, Supplementary Material online). Around 90% of the topologies are concordant with the species tree (supplementary fig. S4, Supplementary Material online). The two discordant topologies are likely explained by incomplete lineage sorting of the nonstructured populations of *H. zea* and *H. armigera* (green and orange in supplementary fig. S4, Supplementary Material online). However, the topology that groups recent *H. zea* and *H. armigera* together from Brazil is more common and is consistent with a recent burst of gene flow between sympatric species and introgression (green in fig. 4 and supplementary fig. S4, Supplementary Material online). In addition, there is a correlation between the weights of the topology grouping the Brazilian populations (green in fig. 4 and supplementary fig. S4, Supplementary Material online) and the tree



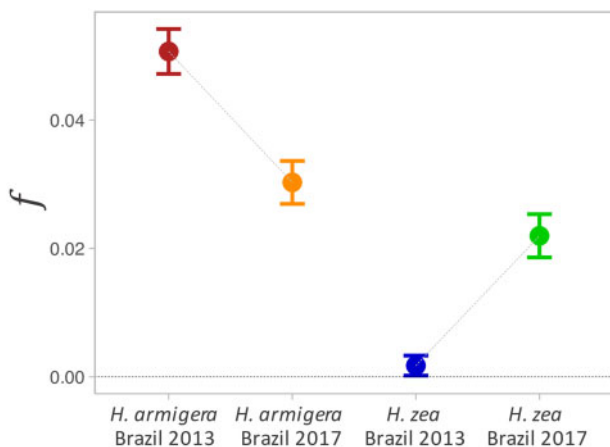
**Fig. 3.**  $F_{ST}$  scans across the genome.  $F_{ST}$  across 100-kb genomic windows between sympatric and allopatric populations of *Helicoverpa armigera* and *H. zea*. Inter-specific comparisons between allopatric populations (A) and sympatric populations during the outbreak in 2013 (B) and more recent populations in 2017 (C). Intra-specific comparison between *H. zea* 2013 and 2017 populations (D). Squares on (C) and (D) highlight chromosome 15 and (E) and (F) zoom in on  $F_{ST}$  values on chromosome 15, showing the location of the *CYP337B3* gene.

grouping *H. zea* 2017 and *H. armigera* from the Old World, which is expected given that there is not population substructure within *H. armigera*. Notably, from these regions of phylogenetic incongruence, the longest continuous interval with a discordant topology was on chromosome 15,

around the *CYP337B3* locus (fig. 4). Therefore, given that introgression seems to be restricted to a single region, the similar frequencies of the nonspecies phylogenies suggest that introgression in recent populations of *H. zea* is not widespread.



**Fig. 4.** Topology weightings across the Z-chromosome and chromosome 15. Twist analysis on 50 SNPs windows for a species topology with the relations: ((Old World *Helicoverpa armigera*, Brazilian *H. armigera* 2017) (New World *H. zea*, Brazilian *H. zea* 2017)). Blue represents the weight of the species topology ((Old World *H. armigera*, Brazilian *H. armigera* 2017) (New World *H. zea*, Brazilian *H. zea* 2017)). Green ((Brazilian *H. zea* 2017, Brazilian *H. armigera* 2017) (New World *H. zea*, Old World *H. armigera*)) and orange ((Brazilian *H. zea* 2017, Old World *H. armigera*) (New World *H. zea*, Brazilian *H. armigera* 2017)) are alternative topologies to the species tree. See [supplementary figure S4, Supplementary Material](#) online, for explanations of the topologies.



**Fig. 5.** Genome-wide proportion of admixture. Proportion of admixture ( $f$ ) estimated over time for *Helicoverpa armigera* and *H. zea* in Brazil estimated as an  $F_4$  ratio for the genealogy ((Australian *H. armigera conferta*, Old World *H. armigera*; X; New World *H. zea*)).

### Introgression Is Biased toward Specific Genomic Regions and Has Decreased over Time in *H. armigera* and Increased in *H. zea*

To test for genetic admixture, we calculated Patterson's  $D$ -statistics, which test for an imbalance of discordant SNPs of derived alleles that is indicative of introgression (Green et al. 2010; Patterson et al. 2012). We found strong signals of gene flow between Brazilian *H. zea* and *H. armigera* for 2013 and 2017 samples (table 1). We then examined  $f$ , the proportion of the genome that has been shared between species (Green et al. 2010), and observed a decreased  $f$  over time in *H. armigera* (fig. 5 and supplementary table S2, Supplementary Material online). The fraction of ancestry derived from their sibling species was higher in *H. armigera* populations than for *H. zea* in 2013, suggesting asymmetric introgression early after the invasion (fig. 5).

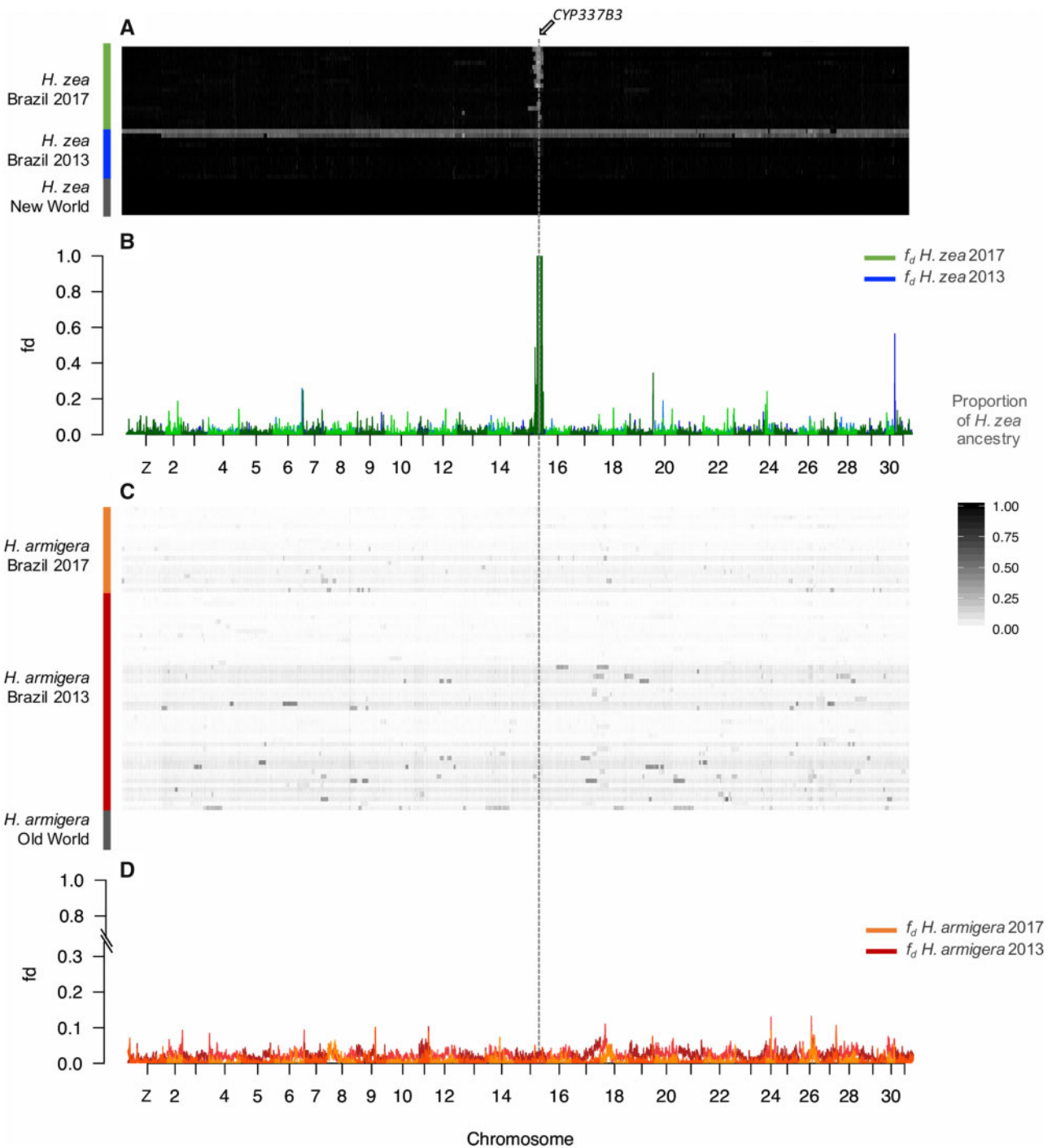
To locate introgressed loci, we used the summary statistic  $f_d$  since it is better suited than  $f$  to quantify admixture in small genomic regions and which is roughly proportional to the effective migration rate (Martin et al. 2015). As expected from

**Table 1.** Results of  $D$ -Statistic.

Target Population	$D$ -Statistic	Z-Score	P Value
<i>H. armigera</i> 2013 <sup>a</sup>	0.1203 ± 0.005015	23.986	<0.0001
<i>H. armigera</i> 2017 <sup>a</sup>	0.0259 ± 0.004774	5.427	<0.0001
<i>H. zea</i> 2013 <sup>b</sup>	0.0068 ± 0.004373	1.551	0.1209
<i>H. zea</i> 2017 <sup>b</sup>	0.0554 ± 0.010163	5.455	<0.0001

NOTE.—Admixture test for Brazilian populations of *Helicoverpa* in 2013 and 2017. Model used for *H. armigera* populations (((A, B)C)D): (((x, *H. armigera* Old World) *H. zea* New World) *H. punctigera*). Model used for *H. zea* populations (((A, B)C)D): (((x, *H. zea* New World) *H. armigera* Old World) *H. punctigera*). A significantly positive Z-score implies gene flow between A and C. A significantly negative Z-score implies gene flow between B and C. P values <0.05 indicates  $D$ -statistic significantly different from 0.

the genome-wide  $f$ ,  $f_d$  values also decreased over time in *H. armigera* populations (fig. 6). However, the proportion of admixture estimated in sliding windows showed considerable heterogeneity in the extent of introgression across the genome (fig. 6). In both temporal samples and species, admixture was minimal on the Z-chromosome ( $P$  value <  $1 \times 10^{-4}$ ), indicating a strong barrier to introgression (fig. 6B and D). For Brazilian *H. armigera*, we found high heterogeneity in admixture proportion in the autosomes, with peaks scattered throughout different chromosomes (fig. 6D). Some regions, including the entire sequence of chromosome 15, exhibited  $f_d$  values below the genome-wide average ( $P$  value <  $1 \times 10^{-4}$ ), implying localized barriers to gene flow. Conversely, in local *H. zea* populations,  $f_d$  values were lower throughout the genome compared with the invasive *H. armigera* in 2013 ( $P$  value <  $2.2 \times 10^{-6}$ ). Yet, for recent *H. zea* populations, we found a peak showing a higher proportion of admixture (fig. 6B), than any peak in *H. armigera* and reflecting almost complete replacement. This peak was located across the region on chromosome 15 containing the CYP337B3 gene responsible for pyrethroid resistance, and that initially was only present in the invasive species (fig. 6). Additionally, gene scans of *H. zea* revealed that CYP337B3 was present in 14 out of 18 *H. zea* individuals from 2017 (frequency  $\sim 0.70$ ) (supplementary table S4, Supplementary Material online). Therefore, the insecticide resistance locus has not swapped to fixation yet, although  $f_d$  suggested a

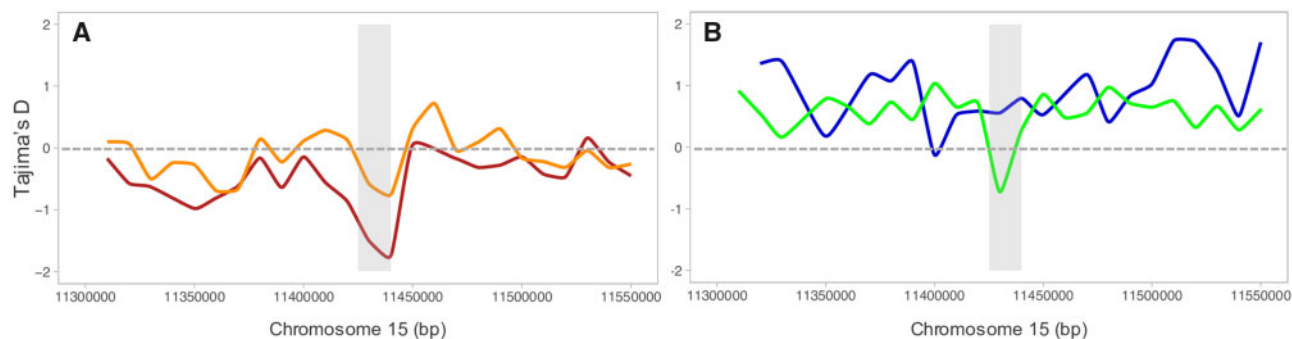


**FIG. 6.** Proportion of admixture across the genome. ( $f_d$ ) and introgressed tracks per individual. (A) Admixture tracks displaying *Helicoverpa zea* ancestry in 100-kb windows for individuals of *H. zea* 2013, *H. zea* 2017, and *H. zea* (New World). (B) Proportion of admixture  $f_d$  across 100-kb windows, for populations of *H. zea* 2013 (blue) and *H. zea* 2017 (green). (C) Admixture tracks displaying *H. zea* ancestry in 100-kb windows for individuals of *H. armigera* 2013, *H. armigera* 2017, and *H. armigera* (Old World). (D) Proportion of admixture  $f_d$  across 100-kb windows, for populations of *H. armigera* 2013 (dark red) and *H. armigera* 2017 (orange).

maximal admixture proportion, which may be explained by a large excess of ABBA patterns due to the recent gene flow. The introgressed sequence was similar to that found in European *H. armigera* (window 11,435,001–11,440,000,  $d_{XY} = 0.0064$ ,  $-\log_{10}(P \text{ value}) = 2.14$  genome-wide  $d_{XY} = 0.040 \pm 0.29$ ) compared with African ( $d_{XY} = 0.025$ ,  $-\log_{10}(P \text{ value}) = 2.15$ , genome-wide  $d_{XY} = 0.040 \pm 0.029$ ,

$Z = 86.918$ ,  $P \text{ value} < 2.2 \times 10^{-6}$ ) and Asian *H. armigera* ( $d_{XY} = 0.021$ ,  $-\log_{10}(P \text{ value}) = 1.85$ , genome-wide  $d_{XY} = 0.053 \pm 0.031$ ,  $Z = 49.094$ ,  $P \text{ value} < 2.2 \times 10^{-6}$ ).

Increased  $F_{ST}$  values in both within-species comparisons around the CYP337B3 locus might also be a result of within-species population dynamics. However, the joint estimation of  $F_{ST}$  and  $f_d$  allowed us to disentangle linked selection in



**FIG. 7.** Tajima's  $D$  in 10-kb windows along the region in chromosome 15 containing *CYP337B3* gene for populations of *Helicoverpa armigera* and *H. zea* in Brazil. (A) Tajima's  $D$  for *H. armigera* 2013 (dark red) and for *H. armigera* 2017 (orange). (B) Tajima's  $D$  for *H. zea* 2013 (blue) and for *H. zea* 2017 (green) containing *CYP337B3* gene. Gray shading indicates the location of *CYP337B3*.

*H. armigera* from differentiation due to admixture in *H. zea*.  $F_{ST}$  is expected to be highly heterogeneous even in the absence of gene flow, with elevated values in regions of the genome that experience strong linked selection (Jakobsson et al. 2013; Martin et al. 2015). Therefore, the weak  $F_{ST}$  peak within *H. armigera* is likely an artifact of the recent selective sweep in this region in chromosome 15 (Walsh, Jousen, et al. 2018) (supplementary fig. S3, Supplementary Material online). In contrast, the  $F_{ST}$  peak on chromosome 15 in the within-species comparison of *H. zea* populations pre- and post-*H. armigera* invasion coincided with the  $f_d$  peak in recent *H. zea* populations, implying that differentiation was caused by introgression.

We then calculated the proportion of introgressed ancestry across the genome of all individuals using a data set of diagnostic SNPs that differentiate the two species and estimated a hybrid index in 100-kb sliding windows (fig. 6A). To disentangle the role of neutral recombination and selection shaping the size of the admixed blocks over time, we characterized the extent of linkage disequilibrium and estimated a genome-wide recombination rate. Then, we used these parameters to simulate the expected distribution of block sizes under neutral recombination following (Racimo et al. 2015). Across individuals, there was evidence for long regions acquired through introgression (fig. 6A). We found that temporal samples showing a higher proportion of admixture (fig. 5) also exhibited stronger linkage disequilibrium ( $P$  value  $< 2.2 \times 10^{-6}$ ) (supplementary fig. S6, Supplementary Material online). In the recent *H. zea* population, the mean introgressed block size was higher in 2017 (509 kb) compared with 2013 ( $Z = 5.23$ ,  $P$  value  $< 1 \times 10^{-4}$ ) (supplementary fig. S7, Supplementary Material online) and the admixed haplotypes are restricted to chromosome 15 (fig. 6A and supplementary fig. S6, Supplementary Material online). Therefore, consistent with the  $f_d$  analyses, recent individuals of Brazilian *H. zea* exhibited a single clearly introgressed block around the *CYP337B3* locus.

By contrast, in early *H. armigera*, long introgressed regions were pervasive, indicating introgression of almost whole chromosomes. Particularly long admixture tracks derived from *H. zea* on chromosome 18 seemed to be persistent across most *H. armigera* individuals in 2013

and 2017. Nevertheless, we observed shorter introgressed blocks ( $Z = 7.79$ ,  $P$  value  $< 1 \times 10^{-4}$ ) and a lower effective migration rate ( $Z = 289.18$ ,  $P$  value  $< 0.001$ ) in recent populations of *H. armigera*. Similarly, the extent of Linkage Disequilibrium (LD) was greater in the 2013 population ( $P$  value = 0.002) (supplementary fig. S6, Supplementary Material online). Moreover, the mean introgressed block size in the recent *H. armigera* population was around 227 kb, significantly shorter ( $Z = 7.88$ ,  $P$  value  $< 1 \times 10^{-4}$ ) than the theoretically predicted mean length of 470 kb after 36 generations (4 years between 2013 and 2017) of neutral recombination (supplementary fig. S7, Supplementary Material online). Thus, suggesting that the admixed regions have been removed faster than expected just from recombination over time, thus suggesting strong purifying selection against introgression in *H. armigera*.

To characterize recent selection around the *CYP337B3* locus, we calculated Tajima's  $D$  on 10-kb windows across the region on chromosome 15 containing this gene. There was a trough in Tajima's  $D$  consistent with a selective sweep at the location of *CYP337B3* from invasive *H. armigera* in 2013 (fig. 7A and supplementary table S3, Supplementary Material online) that is absent in the nonintrogressed *H. zea* from the same period (fig. 7B and supplementary fig. S5, Supplementary Material online), suggesting strong selection on this locus. In recent populations of Brazilian *H. armigera*, Tajima's  $D$  is negative in the vicinity of the insecticide resistance allele (Tajima's  $D = -0.60$ ) although not lower compared with the genome-wide average (Tajima's  $D \pm$  SD:  $-0.98 \pm 0.36$ ) (fig. 7A). In contrast, recent populations of *H. zea* harboring the *CYP337B3* haplotype exhibited significantly lower Tajima's  $D$  along this region (Tajima's  $D = -0.72$ ; genome-wide Tajima's  $D \pm$  SD:  $0.57 \pm 0.59$ ) suggesting recent positive selection on this introgressed locus (fig. 7B and supplementary table S3, Supplementary Material online).

## Discussion

We have used whole-genome sequencing of 137 individuals to document the interaction between native and invasive crop pest species. This has demonstrated that these species



are maintaining their differentiation in the face of hybridization, showing evidence for a reduction in admixture over time in the invasive species and localized admixture in the native. Indeed, a single locus associated with insecticide resistance shows dramatic evidence for an introgressed selective sweep from *H. armigera* into *H. zea*. Whole-genome sequencing, therefore, offers an opportunity to track the fate of invasive species in the face of hybridization. In this particular case, we are witnessing the emergence of a new paradigm where insecticide resistance or other adaptive traits can be passed rapidly between species in the native range, potentially leading to the formation of a “megapest” species. This has serious implications for pest control and agricultural crop production both in South America and globally, as the movement of pests is not unidirectional.

### Sympatric *H. zea* and *H. armigera* Persist as Two Well-Differentiated Species

In secondary contact after strict allopatric speciation, there are a number of possible outcomes, with barriers to gene flow potentially being either strengthened or broken down and resulting in the fusion of the divergent species. Despite pervasive introgression following invasion and secondary contact between *H. armigera* and *H. zea*, we found that the species boundaries are strongly maintained. Most individuals showed some proportion of introgression, but early hybrids and backcrosses were scarce, suggesting low levels of ongoing hybridization. Differentiation between species is roughly the same between allopatric and sympatric populations except for a single “pocket” of introgression driven by strong selection. Therefore, from the myriad of possible outcomes after secondary contact, our results do not lend support to *H. armigera* and *H. zea* fusing back into one species, nor formation of a new hybrid species, at least not in the time frame examined here. On the contrary, the predominance of parental forms, the rarity of hybrids, particularly in the recent samples, and the distribution of introgressed block sizes suggest selection against most of the introgressed variation.

The *Helicoverpa* sibling species exhibit both prezygotic and postzygotic barriers that can account for the low rates of natural hybridization (Hardwick 1965; Laster and Sheng 1995; Berg et al. 2014; Mallet 2018). Despite experiments having demonstrated no instances of sterility in *H. armigera* × *H. zea* hybrids in reciprocal crosses inbred for two generations or in lines backcrossed for four, they exhibit strong assortative mating (Hardwick 1965; Laster and Sheng 1995). Mating is particularly difficult to obtain between an F1 hybrid and *H. zea* (AZ × Z), which can account for the lower admixture proportion we observed in *H. zea* populations in 2013. Traits linked to prezygotic isolation include morphological differences in the male and female genitalia as well as divergent pheromonal bouquets (Hardwick 1965; Laster and Sheng 1995; Berg et al. 2014). A possible outcome of mating between *H. zea* and *H. armigera* is to “lock up,” leading to death without reproduction of the individuals involved (Hardwick 1965). This is apparently due to differences in the length of an elaborate inflating appendix in the genitalia, which makes the male unable to successfully withdraw his aedeagus extension

from the female (Hardwick 1965; Laster and Sheng 1995; Mallet 2018). Although this can also occur in intraspecific mates, this clogging is more common in interspecific matings (Hardwick 1965; Mallet 2018). Furthermore, behavioral tests in *Helicoverpa* moths showed that female pheromones and their ratios are used as critical signals to avoid interspecific matings (Fadamiro and Baker 1997; Quero et al. 2001; Berg et al. 2014). Even though *H. zea* and *H. armigera* both use Z-11-hexadecenal and Z-9-hexadecenal as the primary sex pheromones emitted by females and detected by males, the ratio of the latter to the former is about 3-fold higher in *H. armigera* (Fadamiro and Baker 1997; Quero and Baker 1999; Quero et al. 2001; Berg et al. 2014). Therefore, both mechanical and chemical reproductive isolations likely play a role in assortative mating and the reduction of successful interspecific mating.

In addition to prezygotic isolation, selection against hybridization could be the result of intrinsic postzygotic barriers, including Bateson–Dobzhansky–Müller incompatibilities (BDMIs) or alternatively “pathway” incompatibilities. BDMIs cause reduced fitness in hybrids but can be broken down by recombination in backcross progeny (Bomblies et al. 2007; Lee et al. 2008; Schumer et al. 2018). Conversely, “pathway” incompatibilities reduce fitness in recombinant hybrids in which coadapted alleles become separated (Lindtke and Buerkle 2015). Simulations and empirical data show that BDMIs usually have an even, genome-wide effect, whereas “pathway” incompatibilities produce distinct localized barriers to introgression (Lindtke and Buerkle 2015; Martin et al. 2019). Our results show a genome-wide reduction of admixture over time in *H. armigera* and strongly localized adaptive introgression in *H. zea*. Furthermore, the mean length of introgressed blocks was shorter than expected due to neutral recombination across generations, suggesting pervasive selection against hybridization likely as a result of widespread BDMIs throughout the genome. Moreover, patterns of differentiation captured by  $F_{ST}$  also suggested highly polygenic barriers that maintain *H. armigera* and *H. zea* as distinctive species despite the recent burst in gene flow. BDMIs are more likely to act as polygenic barriers and are a central mechanism underlying reproductive isolation of species formed through allopatric divergence, as in the case of *H. armigera* and *H. zea* (Bomblies et al. 2007; Lee et al. 2008). Therefore, we hypothesize that barrier loci like BDMIs accumulated rapidly during the 1.5 My of allopatric divergence, contributing to selection against admixture across the genome upon secondary contact. In summary, our findings highlight that species can be unambiguously different but coupled through the interchange of adaptive genes.

### Rapid Adaptive Introgression of an Insecticide Resistance Gene

Several lines of evidence suggested rapid adaptive introgression between the *Helicoverpa* sibling species after the *H. armigera* invasion in South America. In order to provide evidence for adaptive introgression, the introgressed haplotype needs to be shown to be responsible for a phenotype with a beneficial impact on fitness and to have increased in

frequency under the action of selection. The candidate region containing CYP337B3 appears to satisfy both requirements. First, pesticide resistance toward pyrethroids is due to the unique P450 enzyme encoded by this gene (Joußen et al. 2012; Walsh, Joussen, et al. 2018). This chimeric enzyme arose independently, at least eight times, from unequal crossover events between two parental P450 genes (Walsh, Joussen, et al. 2018), and is capable of metabolizing pyrethroids to nontoxic compounds in larvae and adults (Joußen et al. 2012). Indeed, the exclusive presence of CYP337B3 has been shown to confer a 42-fold resistance to fenvalerate in *H. armigera* (Joußen et al. 2012). Furthermore, Durigan et al. (2017) showed that CYP337B3 is associated with the very low mortality of larvae when sprayed with the pyrethroids deltamethrin and fenvalerate in invasive *H. armigera* in Brazil.

Second, the CYP337B3 introgression into *H. zea* and subsequent increase in frequency coincided with an intensification in pesticide use in Brazil directed to control outbreaks generated by the invasive *H. armigera* (Leite et al. 2014; Durigan et al. 2017; Pinto et al. 2017). In the 2012/2013 growing season, cotton, soybean, and maize crops were impacted by *Helicoverpa* species at unusually high infestations resulting in billion dollar losses to Brazilian agriculture (Durigan et al. 2017). Initially, the species was presumed to be *H. zea*, but during this season, growers reported a reduced efficacy of insecticides despite the increase in sprays and the known susceptibility of *H. zea* to pesticides (Durigan et al. 2017). The resistant individuals were subsequently confirmed as *H. armigera*, confirming its successful spread and establishment after being introduced into Brazil from ~2007 onward (Tay et al. 2013, 2015; Lopes-da-Silva et al. 2014; Sosa-Gómez et al. 2016). Furthermore, the survival and establishment of *H. armigera* in the studied region during the 2012/2013 season may have been facilitated by a decline in the use of Bt cotton cultivars during this season due to problems with the quality of fibers produced. Since *H. armigera* was detected in 2013, the frequency and dosages of insecticide sprayings, including pyrethroids, dramatically increased (Durigan et al. 2017). Thus, the survival of early hybrids and backcrosses enabling introgression is likely related to the rapid scale-up of insecticide-based control measures in Brazil after the outbreak generated by the expansion of *H. armigera*. The observation of early generation hybrids mostly during the outbreak in 2013 and not in recent samples, as well as the selective sweep in early *H. armigera* around CYP337B3, further supports this hypothesis. Furthermore, our population genomic analyses demonstrate a signature of recent selection at the introgressed allele in *H. zea* that is not present in the background of the ancestral allele. Meanwhile, *H. zea* populations, which were susceptible to pyrethroids, seem to have responded to insecticide selection pressure by co-opting resistance from *H. armigera* through adaptive introgression.

The introgression of a gene involved in insecticide resistance from *H. armigera* into *H. zea* joins a series of animal examples of rapid adaptive introgression triggered by human-mediated alteration of fitness landscapes. These examples include the European house mouse, *Simulium* black flies,

and *Anopheles* mosquitoes whereby the increase in pesticide exposure acted as a selective force sufficient to drive introgression across species boundaries (Djogbénu et al. 2008; Adler et al. 2010; Song et al. 2011; Clarkson et al. 2014; Norris et al. 2015). Remarkably, in all these cases, adaptive introgression occurred from the species with a higher effective population size into the species with lower  $N_e$  and lower genetic diversity. This pattern has also been found in natural populations in which the selective agent is not human mediated. For instance, adaptive introgression of color pattern genes from *Heliconius melpomene* into *H. timareta* because of natural selection for mimicry (Pardo-Díaz et al. 2012; Edelman et al. 2019), tidal marsh adaptations from *Ammospiza caudacauda* into *A. nelson* sparrow (Walsh, Kovach, et al. 2018), and female-competitive traits from *Jacana spinose* into *J. jacana* (Lipshutz et al. 2019). This pattern is expected given that species with higher  $N_e$  harbor more standing variation—the raw material for adaptation—so introgression is less likely to add beneficial variants (Barton 2010; Jensen and Bachtrog 2011). Moreover, small populations have a higher mutational load because they are more prone to accumulate deleterious mutations, whereas large populations are less subjected to drift (Kimura and Ohta 1969; Barton 2010). However, the most famous example of adaptive introgression from Neanderthals and Denisovans into out-of-Africa human populations seems to be in the reverse direction, undermining the generality of this pattern and highlighting the bidirectional nature of introgression (Green et al. 2010; Patterson et al. 2012; Gazave et al. 2014; Huerta-Sánchez et al. 2014; Mafessoni and Prüfer 2017). Of note, Neanderthals were established in Eurasia when humans were expanding into Europe, they suffered from inbreeding depression (Harris and Nielsen 2016), and although less is known about introgression from humans to Neanderthals and Denisovans, there is evidence of gene flow in this direction (Green et al. 2010; Patterson et al. 2012; Huerta-Sánchez et al. 2014; Prüfer et al. 2014).

The most comprehensive genomic study of rapid adaptive introgression resulting from pesticide pressure is between *Anopheles gambiae* and *An. coluzzi*, the major malaria vectors in Africa (Clarkson et al. 2014; Fontaine et al. 2015; Norris et al. 2015). Several studies have shown introgression of mutations conferring resistance to pyrethroids from *An. gambiae* into *An. coluzzi* (Djogbénu et al. 2008; Clarkson et al. 2014; Norris et al. 2015). The CYP337B3 trickle from *H. armigera* into *H. zea* coincided with an increase in pesticide spraying, similar to introgression of insecticide resistance alleles in *Anopheles* overlapping with the start of a significant insecticide-treated bed net distribution campaign in Mali (Norris et al. 2015). After introgression of the insecticide-resistant genes, these alleles almost reached fixation in 4 years in *An. coluzzi* populations (Norris et al. 2015), which is a temporal scale that strikingly resembles introgression and near fixation of the CYP337B3 loci in *H. zea* in Brazil.

Despite the similitudes between *Anopheles* and *Helicoverpa* examples of rapid adaptive introgression, a critical difference relies on the speciation mechanism underlying the divergence between the sibling species. *Anopheles gambiae*

and *An. coluzzi* diversified in sympatry only about 400,000 years ago (Clarkson et al. 2014; Fontaine et al. 2015), whereas *H. armigera* and *H. zea* differentiated in strict allopatry for 1.5 Ma and came into contact only in the recent 2013 invasion (Pearce et al. 2017; Anderson et al. 2018). Accordingly, the genomes of *An. gambiae* and *An. coluzzi* exhibit very low differentiation except for two large regions that show exceptional divergence known as “genomic islands” of diversification (Clarkson et al. 2014). The insecticide-resistant alleles are located within one of these major diverged regions, and its introgression resulted in the homogenization of the entire island, though no apparent impact on reproductive isolation (Clarkson et al. 2014). Conversely, in *H. armigera* and *H. zea*, the differentiation is not restricted to “islands” but rather, we observed consistently high  $F_{ST}$  values throughout the genome. Furthermore, incompatibilities are more likely to accumulate in allopatry, and therefore are expected to be more pervasive in *Helicoverpa* than in *Anopheles* sibling species (Bank et al. 2012). This suggests that in the face of intense selective pressure, adaptive variation can rapidly move across species boundaries even when differentiation and incompatibilities are pervasive across the genome, and not only between slightly differentiated species as in *Anopheles* mosquitos.

#### Asymmetric Introgression at the Front of an Invasion

We have primarily focused on the adaptive introgression of CYP337B3 from the invasive *H. armigera* into local *H. zea* even though we observed a higher effective migration of alleles from *H. zea* into *H. armigera*. This pattern is in agreement with theoretical models of secondary contact after invasions that account for demographic processes, which predict greater introgression of neutral genes from the local into the foreign species (Currat et al. 2008; Mesgaran et al. 2016; Quilodrán et al. 2019). This asymmetry is partly explained by a demographic imbalance between the two species at the wavefront, where the invading species is at lower densities (Currat et al. 2008; Hall 2016; Mesgaran et al. 2016). Therefore, it is likely that early invading *H. armigera* individuals had few available conspecific mates and thus were involved in more heterospecific crosses.

Most of the admixed alleles in the invasive *H. armigera* are likely to be neutral (Currat et al. 2008). However, introgression can also provide variants that have already been tested by natural selection and in precisely the geographic area in which they are already adapted (Martin and Jiggins 2017). Both models and empirical data show that genes from the resident species associated with local adaptation are easily introgressed into the invading species (Currat et al. 2008; Saarman and Pogson 2015). Contrastingly, genes from the invading species introgress into the local species only if they are under very strong positive selection in the local environment (Currat et al. 2008), as appears to be the case for the CYP337B3 gene. For genes with a large effect on fitness and a clear signal of admixture localized into a single region, it is reasonable to suggest that the increased frequency of the introgressed haplotypes was driven by strong positive selection. However, if introgression is pervasive, it is challenging to

distinguish introgressed alleles that are beneficial from neutral genes that have also cross species boundaries. Although *H. zea* has developed resistance to two-toxin *Bt* maize and cotton (Cry1A and Cry2a) in North America (Reisig and Kurtz 2018), no single specific genes or mutations have yet been identified. Therefore, there are currently no plausible candidates for adaptive introgression from *H. zea* into *H. armigera*, nor has any *Bt* resistance been observed in *H. zea* in South America. Nonetheless, studies on gene flow between *H. zea* populations across North, Central, and South America would be valuable to understand the possible movement of resistance genes such as the CYP337B3 gene introgressed from *H. armigera*. Further temporal monitoring of the invasive *H. armigera* populations in Brazil will permit the detection of fine-scale selection on any introgressed alleles that may be identified.

#### Practical Implications of Rapid Evolution Triggered by Invasive Hybridization

The pervasive introgression between local *H. zea* and invasive *H. armigera* demonstrates the risks of human-mediated dispersion and selection. Invasive species are a major cause of crop loss and a global threat to food security (Blair and Hufbauer 2010; Paine et al. 2016). With increased geographic connectedness via trade, the threat of invasive species arriving in countries where they were previously absent is expected to increase (Saarman and Pogson 2015; Paine et al. 2016). In pest invasions, special attention has been given to newcomer species that can rapidly proliferate as they no longer face their natural enemies (Sakai et al. 2001; Clavero and García-Berthou 2005; Reisig and Kurtz 2018). However, given the evidence of bidirectional introgression following invasion, the importance of the local species should not be underestimated when developing effective biosecurity programs. Through introgression, local species can provide the invasive species with genes associated with local adaptation, thus contributing to its settlement. In addition, hybridization with the local species can reduce allee effects in the introduced species (Mesgaran et al. 2016). Indeed, there is compelling evidence that species that hybridize have a higher likelihood of successful establishment (Currat et al. 2008; Saarman and Pogson 2015; Mesgaran et al. 2016).

In addition to the human-mediated introduction of species, there is growing evidence that anthropogenically altered selection regimes drive exceptionally rapid adaptation (Song et al. 2011; Norris et al. 2015). Agricultural pests are subjected to massive insecticidal pressure, which drives selection for rapid development of resistance (Clarkson et al. 2014; Norris et al. 2015). Our observations suggest that increased insecticide spraying in Brazil to control *H. armigera* in the early stages of invasion acted as a selective force driving introgression of CYP337B3, by temporarily elevating the fitness of hybrids over that of the insecticide-susceptible *H. zea*. Furthermore, the selection regimes are rapidly changing in Brazil, and the widespread use of *Bt*-crops has considerably increased following the expansion of *H. armigera*. Although there is field-evolved resistance to some *Bt* toxins for *H. zea* in North America, this appears to result from a downregulation of midgut protease activity that reduces toxin activation

and is associated with fitness costs (Moar et al. 2010; Brévault et al. 2015; Reisig and Kurtz 2018), so that, as noted above, it appears unlikely there will be any adaptive introgression of specific *Bt*-resistance-related genes into *H. armigera*, whose far greater genetic diversity already includes many specific resistance alleles with little or no fitness costs. In any case, the fate of the genes that cross species barriers will also be strongly affected by the demographic dynamics of these species which have substantially declined since 2012 along with other species of Noctuid moths (dos Santos et al. 2017; Piovesan et al. 2018; Fonseca-Medrano et al. 2019). These population declines seem to be driven by a large-scale selection regime linked to “El Niño-Southern Oscillation Effect” and other components of global change (dos Santos et al. 2017; Piovesan et al. 2018; Fonseca-Medrano et al. 2019). On a finer scale, invasive species substantially change native communities and ecosystems, and their expansion is often correlated with serious declines of closely related species (Bradley et al. 2019; Brzeziński et al. 2019; Larson et al. 2019). Understanding the fine and large-scale factors affecting these demographic changes may contribute to explain the variance in hybridization rates and the idiosyncratic responses to selection observed in each species. Thus, neglecting the interplay between selection due to local environmental heterogeneity in pesticide use and selection imposed by different components of global change can obscure the prediction of the species’ evolutionary responses and the long-term dynamics of hybridization in the sympatric populations of these *Helicoverpa* moths.

Hybridization and introgression represent serious risks associated with invasive species and understanding the mechanisms that determine their impact is necessary for the effective management of biological invasions. Recent advances in whole-genome sequencing give us a chance to monitor the rapid evolution of invasive and local species with important practical implications to design agricultural management programs. As whole-genome data accumulate for biological invasions, the consequences of invasive species in adaptive evolution can be systematically characterized.

## Materials and Methods

### Sample Collection and DNA Extraction

*Helicoverpa armigera* and *H. zea* moths were collected between 2012 and 2017 from three different localities in Brazil. The samples from 2012 and 2013 are referred to as “2013,” and the samples collected between 2016 and 2017 were treated as “2017” or recent populations. The Brazilian samples included 67 *H. armigera*, 27 *H. zea*, and 2 early hybrids closer to *H. zea*, from Bahia, Planaltina, and Mato Grosso states. As complementary groups for the admixture analyses, we included 4 *H. zea* preinvasion from 2006 in Brazil, 8 *H. zea* from the United States, 9 *H. armigera armigera* from Asia, Europe, and Africa, and 14 *H. armigera conferta* and 6 *H. punctigera* from Australia which are all considered to be unrelated to the current *H. armigera* invasion in Brazil. Sample collection data are listed in [supplementary tables S1–S4](#), [Supplementary Material](#) online. All samples were

preserved in ethanol and RNAlater or stored at  $-20^{\circ}\text{C}$  following collection. DNA was extracted using DNeasy blood and tissue kits (Qiagen) before quantification with a Qubit 2.0 fluorometer (Thermo Fisher Scientific).

### Whole-Genome Resequencing and SNP Genotyping

Illumina libraries were produced following the manufacturer’s instructions, and 100-bp paired-end reads were generated (Illumina HiSeq. 2000, and Novogene, Biological Resources Facility, Australian National University, Australia). For BR samples, libraries were prepared an adapted tn5 transposase protocol (Picelli et al. 2014), and 150-bp paired-end sequences were generated (Illumina HiSeq Xten, BGI, China) (Picelli et al. 2014). Raw sequence reads were aligned to the *H. armigera* genome using BWA v 0.7.12. Reads were trimmed when quality in at least two bases fell below Q30 using VCFTOOLS v. 0.1.15, and only uniquely aligning reads were included in the analysis. Resulting BAM files were sorted before duplicate reads were annotated using Picard v. 1.138 and indexed with SAMTOOLS v. 1.3.1 (Li et al. 2009). HaplotypeCaller in GATK v. 3.7 (McKenna et al. 2010) was used to call SNPs in individual samples, and we used GenotypeGVCFs in GATK (McKenna et al. 2010) to estimate genotypes across all individuals simultaneously, implementing a heterozygosity value of 0.01. We used VCFTOOLS v. 0.1.15 (Danecek et al. 2011) to calculate mean coverage statistics for each sample. We further filtered our SNP data set to include a minor allele count  $\geq 2$ , mean site depth  $\geq 5$  and  $\leq 200$ , missing data per site  $\leq 0.50$ , and Phred-scale mapping quality  $\geq 30$ , resulting in 17,257,450 genome-wide biallelic SNPs for the 150 individuals.

### Classification of Parental and Hybrids Individuals

We conducted a genetic PCA and discriminant analysis of principal components to visualize clustering of parental and hybrid genotypes using  $\sim 50,000$  SNP markers randomly sampled across all chromosomes with the packages “adegenet” (Jombart 2008), “Poppr” (Kamvar et al. 2014), and “vcfR” (Knaus and Grünwald 2017) in R v.3.3.2.

We further classified individuals as parental *H. zea* and *H. armigera*, hybrids or backcrosses based on the interspecific heterozygosity and the proportion of ancestry from parental species. We estimated the proportion of the genome that was ancestrally derived from *H. zea* using SNPs that strongly segregated between that species and *H. armigera armigera*. These diagnostic SNPs were determined via an association test in PLINK v1.90b (Purcell et al. 2007), taking only variants where  $P$  value  $< 3.83 \times 10^{-14}$ , thereby limiting the maximum number of alleles to fixed alleles from either *H. zea* or *H. armigera*. We used only preincursion allopatric populations of *H. zea* from Brazil and the United States and *H. armigera armigera* from the Old World for the association test resulting in  $\sim 1,000,000$  ancestry-informative SNPs. We calculated the proportion of ancestry and interspecific heterozygosity for the individuals using custom python scripts considering the sum of *H. zea* alleles divided by all alleles and the proportion of heterozygous sites (<https://github.com/wvalencia-montoya/helicoverpa-project>, last accessed April 29, 2020).

### Genomic Differentiation between and within Species

To estimate genome-wide differentiation, we calculated  $F_{ST}$  using the `popgenWindows.py` script from the repository ([https://github.com/simonhmartin/genomics\\_general](https://github.com/simonhmartin/genomics_general), last accessed April 29, 2020) in 100-kb nonoverlapping windows. We calculated  $F_{ST}$  between allopatric populations of *H. zea* and *H. armigera* and sympatric postinvasion populations for the two temporal samples 2013 and 2017. To assess outliers, we calculated Z-scores and  $-\log_{10} P$  values. In order to test for differences between populations, we used a permutation-based independence test implemented in the R package, `coin` (Hothorn et al. 2008).

### Phylogenetic Weighting

We used `Twisst` (Martin and Van Belleghem 2017) to explore evolutionary relationships across the genome of allopatric and sympatric recent populations of *H. zea* and *H. armigera*. We performed phasing and imputation of missing bases using `Beagle` (Browning and Browning 2007) with 10,000-bp step size and 100-kb overlapping sliding windows. Maximum likelihood trees for `Twisst` were generated with the `phymL_sliding_window.py` script (Martin and Van Belleghem 2017) with the GTR model in 50 SNPs sliding windows in `PhyML` (Guindon et al. 2010).

### Introgression across the Genome

To test for the genome-wide extent of admixture resulting from hybridization between *H. armigera* and *H. zea*, we performed the  $D$ -statistic test implemented in `AdmixTools` v. 5.1 with a block jackknife size of 61 bp (Patterson et al. 2012). We considered that  $D$ -statistic estimates were indicative of significant introgression when Z-scores  $\geq 2.5$ , and thus with a  $P$  value of  $\leq 0.05$ . To estimate the genome-wide proportion of admixture, we conducted an  $F_4$  ratio incorporating *H. armigera conferta* from Australia as the fifth population (description of the model in [supplementary table S2, Supplementary Material](#) online) and using `AdmixTools` v. 5.1 with a block jackknife size of 61 bp (Patterson et al. 2012).

For *H. armigera*-like individuals, we implemented a model phylogenetic model with the following branching: (((Old World *H. armigera*, Brazilian *H. armigera* Date 1 or Date 2) North American *H. zea*) *H. punctigera*) and (((Old World *H. armigera*, Australian *H. armigera conferta*) North American *H. zea*) *H. punctigera*) as a control ([supplementary fig. S1, Supplementary Material](#) online). For *H. zea* populations, we considered the model (((North American *H. zea*, Brazilian *H. zea* Date 1 or Date 2) Old World *H. armigera*), *H. punctigera*). To detect introgression in specific regions across the genome, we calculated  $f_d$  in 100-kb sliding windows with the `ABBA-BABA.py` scripts as in Martin et al. (2015) and implementing the same topology models used for the  $D$ -statistic.

To further characterize introgressed regions from either parental species across the genome of individuals, we calculated the proportion of ancestry in sliding windows, using custom python scripts. The calculations incorporated the set of SNPs ancestry-informative SNPs with hybrid index averages across 100-kb nonoverlapping windows. Following Anderson et al. (2018), we considered a window as

significantly introgressed with a  $P$  value threshold  $< 5 \times 10^{-7}$ . Differences in block length per date were evaluated using permutation tests. Sliding windows averages of the proportion of ancestry were plotted using custom R scripts, and using “`ggplot2`” (Wickham 2009), “`Hmisc`” (Harrell and Dupont 2019), and “`Rmisc`” (Hope 2013) packages.

To test if the change over time of the block length is faster than would be expected due to neutral recombination, we calculated the expected ancestry block size using a formula from (Racimo et al. 2015). Because of recent introgression, the lengths of admixed tracts may be highly correlated, violating the assumption of exponentially distributed block sizes (Liang and Nielsen 2014). However, because there is no certainty about the time of the invasion, we used this framework as an approximation to a null expectation under neutrality. We assumed nine generations per year (Walsh TK, personal communication), 4 years since admixture (from 2013 to 2017), and the admixture proportions calculated from the  $F_4$ -ratio tests ([fig. 5 and supplementary table S2, Supplementary Material](#) online). To estimate local recombination rate, we generated a consensus haplotype based on the phased data set of the nonadmixed *H. armigera* individuals, using `SAMTOOLS` v. 1.3.1 (Li et al. 2009) and `FastaAlternateReferenceMaker` within `GATK` v. 3.7 (McKenna et al. 2010). We inferred the population recombination parameter using `LDhlemet` (Chan et al. 2012). By incorporating the recombination rate, the initial admixture proportion, and the generation time, we performed 10,000 simulations of the distribution of the admixture tract length using the parameters obtained from (Racimo et al. 2015) equation and compared with the observed distribution using independence permutation tests (Hothorn et al. 2008). Furthermore, we run two additional sets of simulations varying generation time to account for realistic variation in this parameter in warmer (up to 12 generations) and cooler climates (3 generations including diapause). We did not infer a population recombination parameter for *H. zea* because of the lack of a high-quality, chromosome-assembly reference genome.

To characterize linkage disequilibrium, we included parsimony SNPs, thinning the phased data to about 25% of its original size using the software package `MapThin` (<http://www.staff.ncl.ac.uk/richard.howey/mapthin/>, last accessed April 29, 2020). We used `PLINK` v1.90b (Purcell et al. 2007) to calculate  $r^2$  within 1-kb windows for *H. armigera* and within 2-kb windows for *H. zea* because disequilibrium decayed at a slower rate (Seymour et al. 2016). To test for differences in the extent of LD between temporal samples, we applied a two-sided Wilcoxon signed-rank test using the mean differences across 10-bp windows.

To confirm introgression of *CYP337B3*, all individuals of *H. zea* 2017 were screened for the presence of this gene as in Joußen et al. (2012). Additionally, we mapped reads of recent individuals of *H. zea* to the complete coding sequence of *CYP337B3* and confirmed presence with the Integrative Genome Viewer `IGV_2.4.14` (Robinson et al. 2011).

### Tajima's $D$

Tajima's  $D$  was calculated in sliding windows of 10 kb in `VCFTOOLS` v. 0.1.15 (Danecek et al. 2011). We computed

mean and standard deviation of Tajima's *D* values for the Brazilian populations: *H. armigera* (2013), *H. armigera* (2017), *H. zea* (2013), and *H. zea* (2017) including individuals with the CYP337B3 haplotype and *H. zea* (2017), following Norris et al. (2015). We plotted Tajima's *D* using ggplot and ggalt along a region in chromosome 15, whereby CYP337B3 gene extends from ~11,436,000 to 11,440,000 bp.

## Supplementary Material

Supplementary data are available at *Molecular Biology and Evolution* online.

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