

Adaptive introgression across semipermeable species boundaries between local and invasive *Helicoverpa* mega-pest moths

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Abstract

Cross-breeding between invasive and native species, has raised global concern given the dramatic increase in species range shifts and pest outbreaks due to climate change, development of suitable agroecosystems, and anthropogenic dispersal. Nevertheless, secondary contact between diverged 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 *H. zea* mega-pest moths in Brazil. We carried out whole-genome resequencing of *Helicoverpa* moths from Brazil in two temporal samples: soon after *H. armigera* invasion in 2013, and more recent populations from 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* admixture proportions were reduced between 2013 and 2017, suggesting a decline in hybridization rates. Recent populations also showed shorter introgressed tracks suggesting selection against admixture. In contrast to the genome-wide pattern, there was striking evidence for introgression of a single region including an insecticide-resistance allele from the invasive *H. armigera* into local *H. zea*, which increased in frequency over time but was localized within the genome. In summary, despite extensive gene-flow after secondary contact, the species boundaries are 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.

Key words: hybridisation, gene flow, secondary contact, pest

Introduction

Hybridization between local and introduced species is an underestimated threat to global agriculture (1–3). Although hybridization is often maladaptive, introgressive hybridization can occasionally introduce adaptive variants, and potentially contribute to establishment and invasiveness of the newcomer species (4–7). This makes invasive hybridization a worldwide cause of concern, since

species introductions are predicted to continue at an accelerating rate due to increases in human movement and trade, and changes in climate (1,4). Simultaneously, invasive species offer a unique opportunity to study evolutionary outcomes following secondary contact. While 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 (8,9). This is primarily because ancestral range reconstruction is challenging, and so it is hard to confidently estimate the extent of historical contact between species (4,8,9). 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, and adaptive introgression.

The recent invasion of the cotton bollworm moth *Helicoverpa armigera* from the Old World into South America, where it hybridizes with its sister species *H. zea*, represents an excellent scenario in which to study the evolutionary trajectories following secondary contact. *H. armigera* and *H. zea* are estimated to have diverged around 1.5 Mya as a result of a transoceanic dispersion of *H. armigera* into the New World, and thus, a classic case of allopatric speciation (10,11). There is no evidence of introgression between the species during this period of divergence until the recent incursion of *H. armigera* into Brazil (10,11), thought to have resulted from the fresh fruit and vegetable trade (10–12). 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 effective population size compared to *H. zea* (10). In addition, *H. zea* shows a narrower host-plant range and is mainly known as a pest of corn and cotton although is also found on over 100 plants belonging to 29 families (10–12). *H. armigera* is more devastating to agriculture and feeds on over 300 species belonging to 68 families (10–12). These differences in host range are associated with fewer gustatory receptor and detoxification genes in the *H. zea* genome (10).

Another significant phenotypic difference between *H. armigera* and *H. zea* lies in their susceptibility to insecticides widely used for pest control (11,13). *H. 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 (10,13–15). Importantly, the resistance toward pyrethroids in *H. armigera* is due to a unique P450 enzyme, *CYP337B3*, that is capable of metabolizing these pesticides into non-toxic hydro-pyrethroids (14). *H. zea*, in contrast, remains susceptible to many major insecticides and tends to cause less damage than its Old World relative (10,11). 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. (11) unambiguously identified nine hybrid individuals using whole-genome sequences between the two species from 2013 when *H. armigera* expanded in Brazil. Eight were genetically closer to *H. armigera* with some introgressed haplotypes from *H. zea* scattered throughout the genome. The ninth individual resembled an F1 hybrid with stretches of homozygosity for each parental species and containing the *CYP337B3* gene involved in pesticide resistance. The apparent rarity of backcrossing to *H. zea* in these individuals (11) initially suggested that hybridization would not introduce novel insecticide resistance to *H. zea* as had been feared (12). However, intensive pesticide-based control programs in Brazil directed to control the invasive *H. armigera* provide a scenario in which there is strong selection for adaptive introgression of insecticide resistance genes into *H. zea* (15–17). These

efforts to control *H. armigera* may also have had the perverse effect of facilitating the establishment of *H. armigera* due to differential survival in the presence of the *H. armigera* resistance allele.

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

Results

Sympatric *H. zea* and *H. armigera* post-incursion persist as two well-distinct species

We analysed whole-genome sequence data from 137 *Helicoverpa* moths of the complex *H. armigera*-*H. zea* in Brazil: 65 *H. armigera armigera* (hereafter *H. armigera*) and 27 *H. zea* distributed in two temporal samples: soon after *H. armigera* expansion in 2012-2013 (47 individuals hereafter 2013) and more recent populations from 2016-2017 (18 individuals hereafter 2017), along with nine individuals from an outgroup species *H. punctigera* endemic to Australia (Fig. 1A). Our sampling included three regions of sympatry in Brazil resulting from the invasion of *H. armigera* into local *H. zea* territory, four individuals of Brazilian *H. zea* pre-invasion (2006); as well as allopatric populations: *H. zea* from USA (8 individuals) and *H. armigera* from the Old World (9 individuals) which are all considered to be geographically or temporally isolated from the current Brazilian invasion (Fig. 1A, Tab. S1). *H. armigera* is present as two distinct sub-species/populations, *H. armigera conferta* (Australia) and *H. armigera armigera* (rest of world) (18) and we sampled 20 individuals from the Australian sub-species.

Principal component analysis (PCA) based on 70,000 whole-genome single-nucleotide polymorphism data separated *H. zea* from *H. armigera* on principal component 1 which explained 17.2% of the variation in the data (Fig. 1B). Across the wide geographic range covered by our sampling there was no evidence of population substructure. The well-known separation between *H. armigera conferta* from Australia and *H. armigera armigera* from the rest of the Old World was evident resuming the first 100 principal component axes (Fig. S1). Three individuals fell between *H. armigera* and *H. zea*, likely representing early generation hybrids. The remaining 96 individuals from Brazil clearly have a closer genetic affinity to one or other of the parental species. Consistent with PC1, ADMIXTURE (19) analyses showed strong differentiation between the two species, which is supported by the lowest cross-validation error (CV) of any assessed when the number of populations is K=2 (Fig. S2).

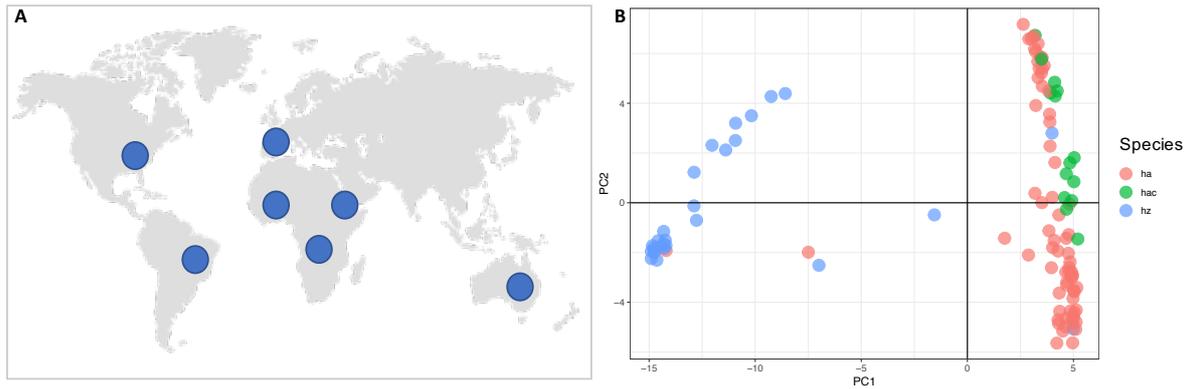


Figure 1. A. Sampling locations of *Helicoverpa* individuals. **B.** Genetic PC1 and PC2 of 70,099 genome-wide SNPs. Color codes indicate the species classification as *H. armigera armigera* (ha), *H. armigera conferta* (hac), and *H. zea* (hz) based on mitochondrial sequences.

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 three early generation hybrids (Fig. 2). One individual resembled an F2 hybrid from 2013, while the other two individuals seemed closer to F3 hybrids from 2013 and 2017, respectively. 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 listed in Tab. S2-3). A total of 65 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 (Table S3). The tight within-species clustering and low prevalence of intermediate individuals indicated that almost none of the recently sampled individuals resulted from contemporary hybridisation, suggesting that hybridisation is rare on a per-individual basis.

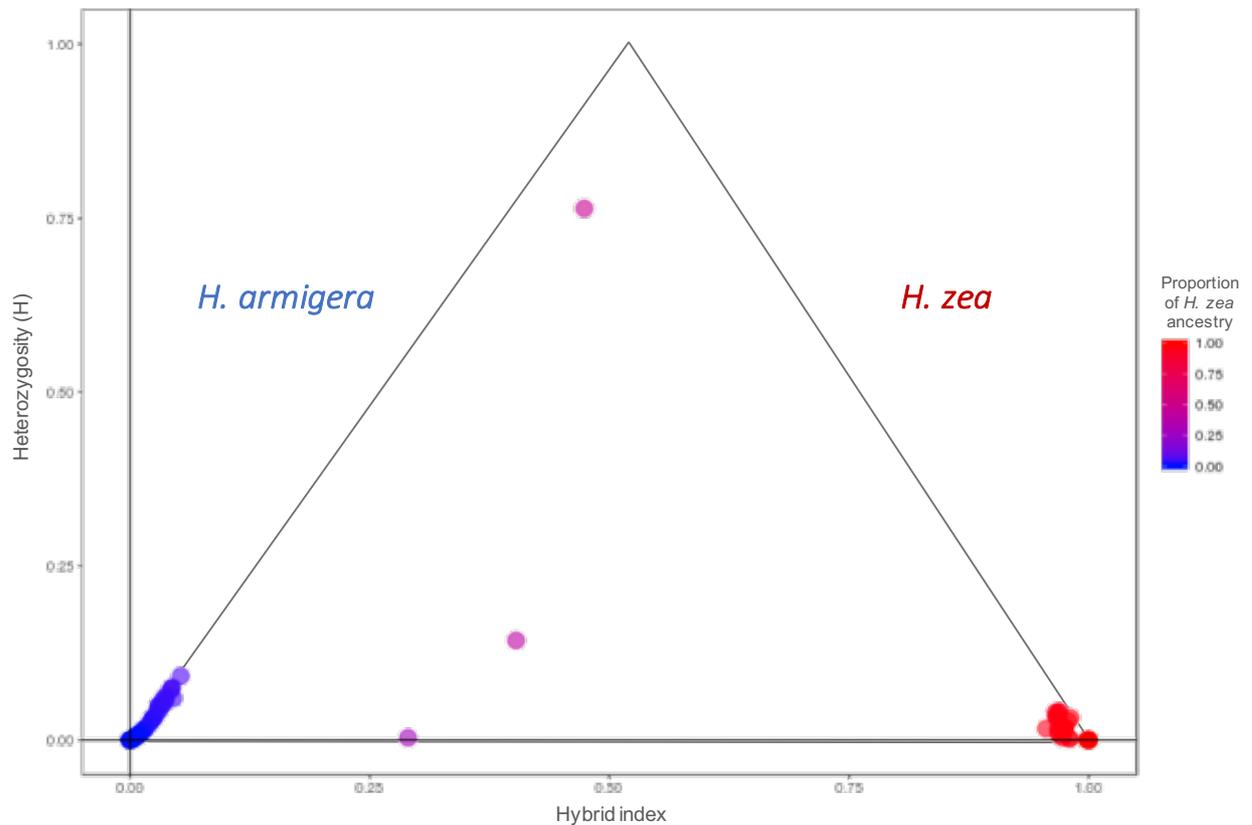


Figure 2. Classification of hybrid and parental genotypes based on the hybrid index and interspecific heterozygosities for *H. zea* and *H. armigera* individuals. The hybrid index and the interspecific heterozygosity were calculated based on SNPs that are fixed differences between allopatric populations of the species.

Reduced interspecific divergence 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 *Fst*. We found similar high genome-wide differentiation between *H. zea* and *H. armigera* for allopatric and recent sympatric populations with a mean autosome-wide *Fst* of 0.474 ± 0.001 and 0.496 ± 0.001 , respectively (Fig. S3, Tab. S1). By contrast, *Fst* values for intraspecific comparisons were low, consistent with a clear genetic identity within species (Fig. S3). Indeed, the *Fst* between the invasive Brazilian and Old World *H. armigera* was 0.0062 ± 0.0001 , and the pairwise *Fst* between Brazilian *H. zea* and North American *H. zea* averaged 0.0118 ± 0.0002 .

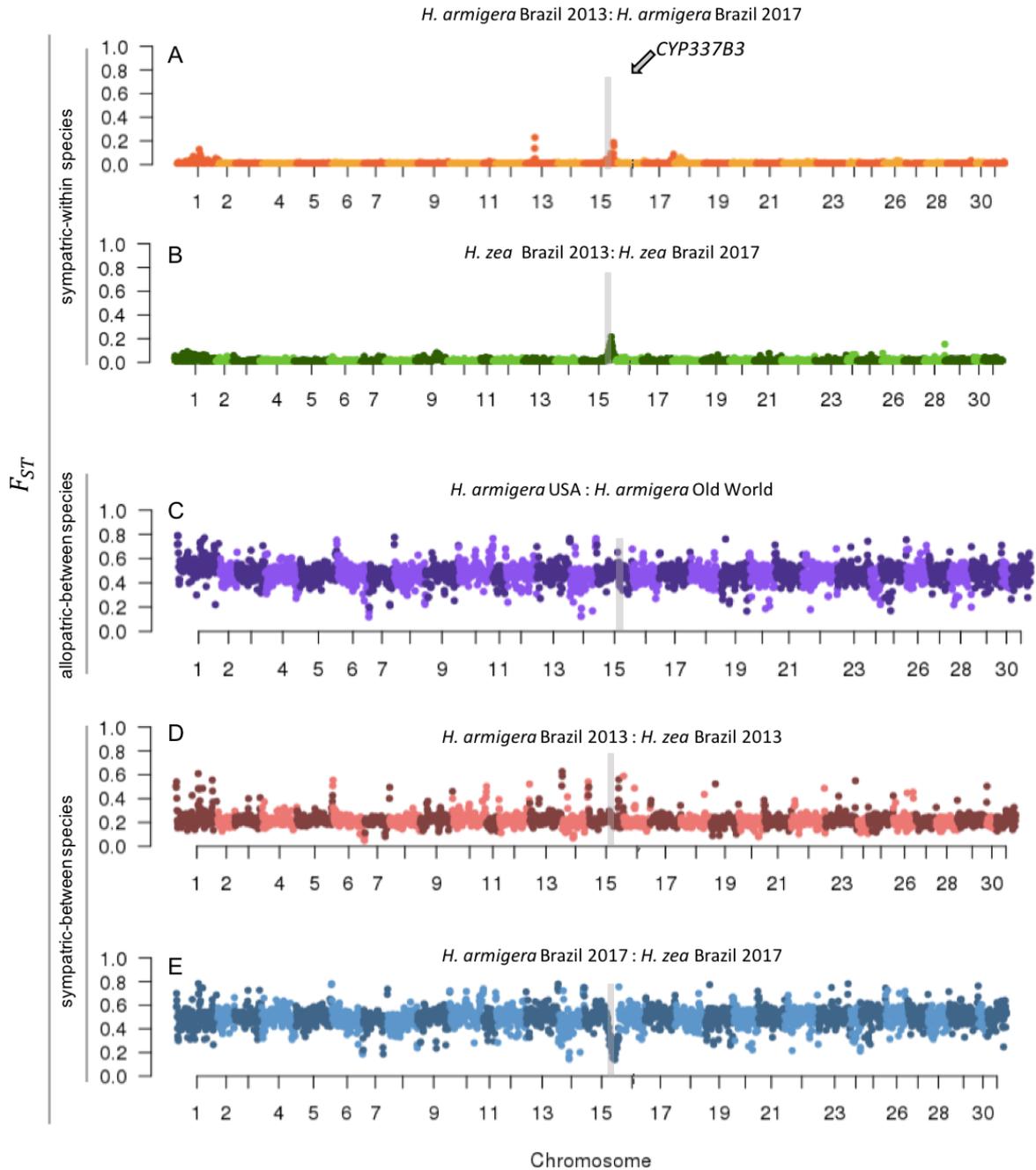


Figure 3. F_{ST} across 100kb genomic windows between sympatric and allopatric populations of *H. armigera* and *H. zea*. Graded grey regions show the location of *CYP337B3* gene.

To compare differentiation across the genome, we calculated F_{ST} -values in 100-kb non-overlapping windows. Interspecific F_{ST} -values for allopatric and sympatric populations were consistently high throughout the genome except for a single region on chromosome 15 (Fig. 3C-E). This region included the insecticide-resistance gene *CYP337B3*, whereby F_{ST} dropped to 0.14 for the sympatric Brazilian

populations of *H. armigera* and *H. zea*. Intraspecific *Fst*-values across the genome between Brazilian and Old World *H. armigera* populations were also consistently low, although there was a slight peak at the *CYP337B3* locus (Fig. 3A). Between *H. zea* from USA and Brazil, there was also only one peak of differentiation, 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. 3B). Although both *Fst* estimates at a genome-wide scale and across windows show that differentiation between species is virtually the same for sympatric and allopatric populations, these patterns of reduced between-species and elevated within-species differentiation around the same region, hint at recent localized introgression.

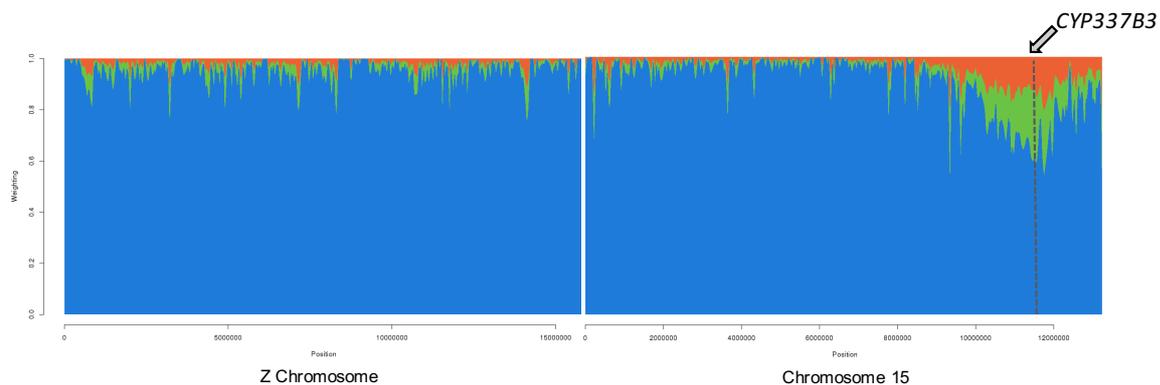


Figure 4. Topology weightings across the Z chromosome and chromosome 15 for a species topology with the relations: ((Old World *H. armigera*, Brazilian *H. armigera* 2017) (North American *H. zea*, Brazilian *H. zea* 2017)). Blue represents the weight of the species topology ((Old World *H. armigera*, Brazilian *H. armigera* 2017) (North American *H. zea*, Brazilian *H. zea* 2017)). Green ((Brazilian *H. zea* 2017, Brazilian *H. armigera* 2017) (North American *H. zea*, Old World *H. armigera*)) and orange ((Brazilian *H. zea* 2017, Old World *H. armigera*) (North American *H. zea*, Brazilian *H. armigera* 2017)), are alternative topologies to the species tree. See Fig. S4 for explanations of the topologies.

Widespread phylogenetic discordance is consistent with recent gene flow

We explored species phylogenies across the genome using Twisst (20), which quantifies the frequency of alternative topological relationships among all individuals in sliding windows 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 (Fig. S4). 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, Fig. S4). The other two topologies are likely explained by incomplete lineage sorting of the non-structured populations of *H. zea* and *H. armigera* (Green and orange in Fig. S4). However, the second most common topology clustered together Brazilian *H. zea* and invasive *H. armigera*, which is consistent with a recent burst of gene-flow between sympatric species (Green in Fig. 4, Fig. S4). 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).

Introgression is biased to specific genomic regions and has decreased over time

To test for genetic admixture, we calculated Patterson's D statistics which considers a significant imbalance of discordant gene trees of derived alleles as indicative of introgression (21,22). We found strong signals of gene-flow between Brazilian *H. zea* and *H. armigera* for 2013 and 2017 samples (Tab. 1). We then examined f , the proportion of the genome that has been shared between species (21), and observed a strong trend to a decreased f over time in *H. armigera* (Fig. 5). The fraction of ancestry derived from their sibling species was higher in *H. armigera* populations than for *H. zea* in 2013, suggesting asymmetrical introgression early after the invasion (Fig. 5).

Table 1. Results of D-statistic, a formal four-populations test of admixture.

Target population	D-statistic	Z-score	P-value
<i>H. armigera</i> 2013 ^a	0.1203 ± 0.005015	23.986	< 0.0001
<i>H. armigera</i> 2017 ^a	0.0259 ± 0.004774	5.427	< 0.0001
<i>H. zea</i> 2013 ^b	0.0068 ± 0.004373	1.551	0.1209
<i>H. zea</i> 2017 ^b	0.0554 ± 0.010163	5.455	< 0.0001

^aModel used for *H. armigera* populations ((A,B)C)D): (((x, *H. zea* New World) *H. armigera* Old World) *H. punctigera*).

^bModel used for *H. zea* populations ((A,B)C)D): (((x, *H. armigera* Old World) *H. zea* New World) *H. punctigera*). A significantly positive Z-score implies gene flow between A and C or B and D. A significantly negative Z-score implies gene-flow between C and D or B and C. P-values <0.05 indicates D-statistic significantly different from 0.

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 (23). As expected from the genome-wide f , f_d -values also decreased over time in populations of sympatric *H. armigera* and *H. zea* (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 in the Z chromosome, particularly in *H. armigera*, indicating a strong barrier to introgression (Fig. 6). For Brazilian *H. armigera* we found high heterogeneity in admixture proportion in the autosomes, with peaks scattered throughout different chromosomes (Fig. 6). Some regions, including the entire chromosome 15, exhibited f_d -values below the genome-wide average, implying strong localised barriers to gene-flow. Conversely, in local *H. zea* populations, f_d values were lower throughout the genome compared to the invasive *H. armigera* in 2013. Yet, for recent *H. zea* populations, we found a peak showing a higher proportion of admixture (Fig. 6), 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 ~0.70) (Tab S3).

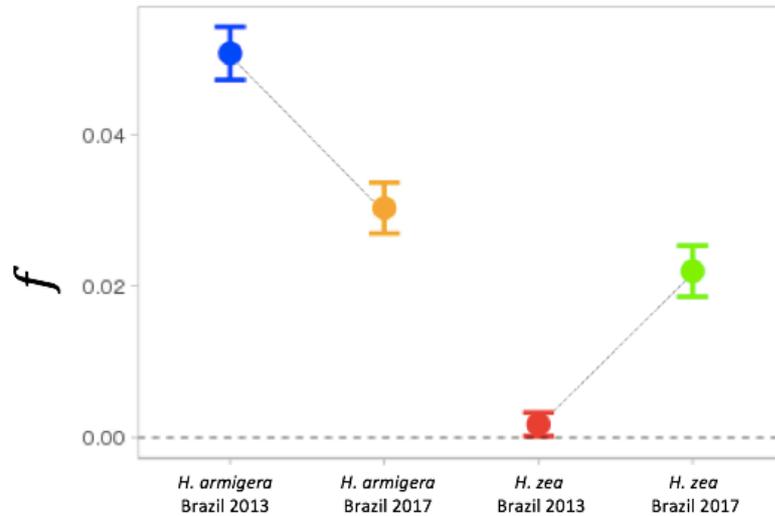


Figure 5. Proportion of admixture (f) estimated over time for *H. armigera* and *H. zea* in Brazil estimated as an F4 ratio for the genealogy ((Australian *H. armigera conferta*, Old World *H. armigera*; X; North-American *H. zea*)).

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 *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 (23,24). Therefore, the F_{st} peak within *H. armigera* is likely an artefact of the recent selective sweep in this region (13). 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 *H. zea* 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. 6). Across individuals, there was evidence for long regions acquired through introgression (Fig. 6). Consistent with the f_d analyses, recent individuals of Brazilian *H. zea* exhibited a single clearly introgressed block on chromosome 15, 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 a general pattern of shorter introgressed blocks in recent populations of *H. armigera* implying that admixed regions have been substantially removed and broken up by recombination over time.

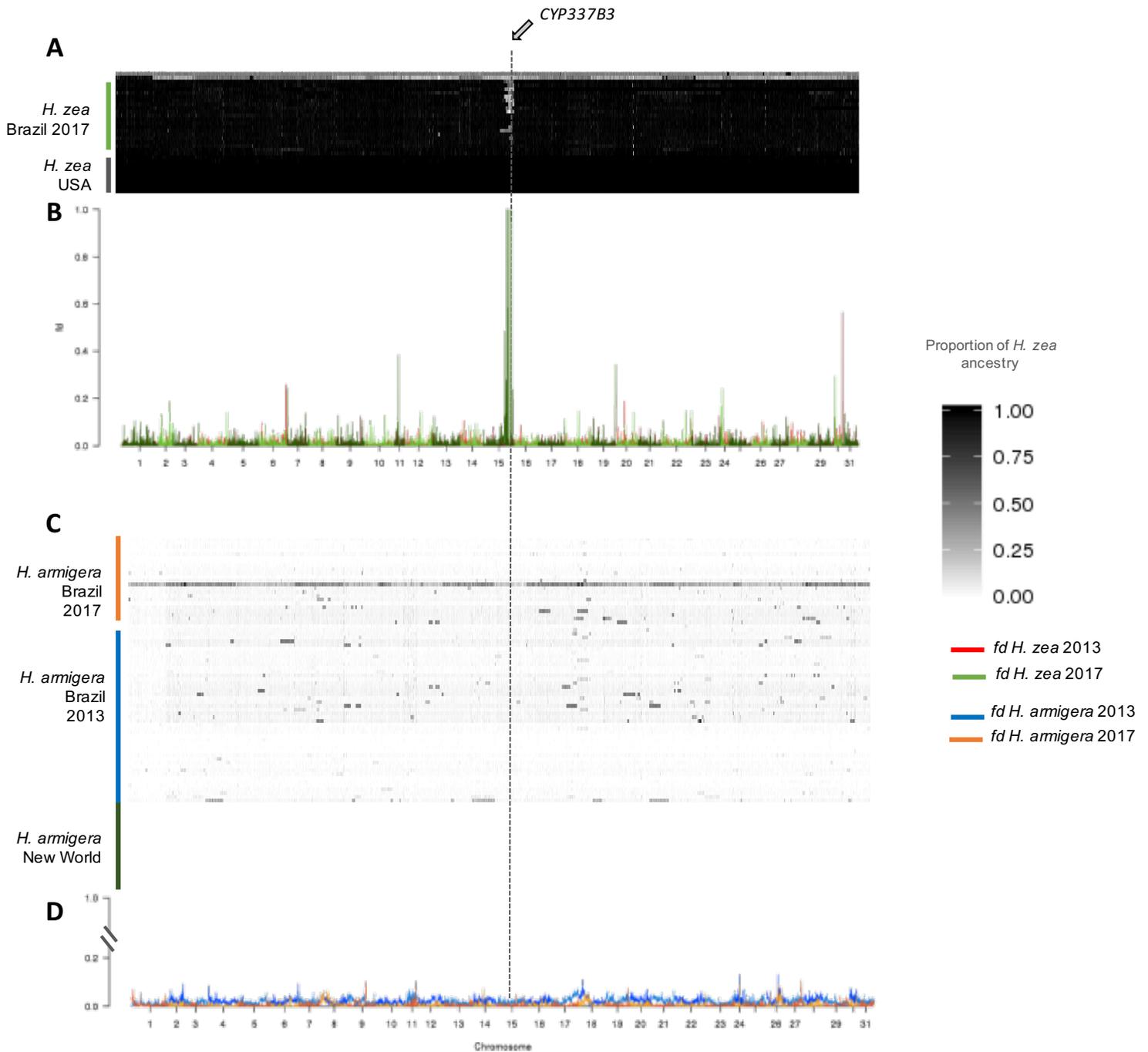


Figure 6. Proportion of admixture and introgressed tracks per individual. Arrow indicating position of *CYP337B3* **A.** Proportion of *H. zea* ancestry in 100kb windows for individuals of *H. zea* 2017 and *H. zea* from the USA. **B.** Proportion of admixture f_d across 100kb windows, for populations of *H. zea* 2013 (red) and *H. zea* 2017 (green). **C.** Proportion of *H. zea* ancestry in 100kb windows for individuals of *H. armigera* 2013 and *H. armigera* 2017. **D.** Proportion of admixture f_d across 100kb windows, for populations of *H. armigera* 2013 (blue) and *H. armigera* 2017 (orange).

Evidence for a recent selective sweep around *CYP337B3* in early *H. armigera* in Brazil

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 strong selective sweep at the location of *CYP337B3* from invasive *H. armigera* in 2013 that is absent in the non-introgressed *H. zea* from the same period (Fig. 7). In recent populations, the signature of selection in Brazilian *H. armigera* is not significant which may reflect a current population contraction (Fig. S5).

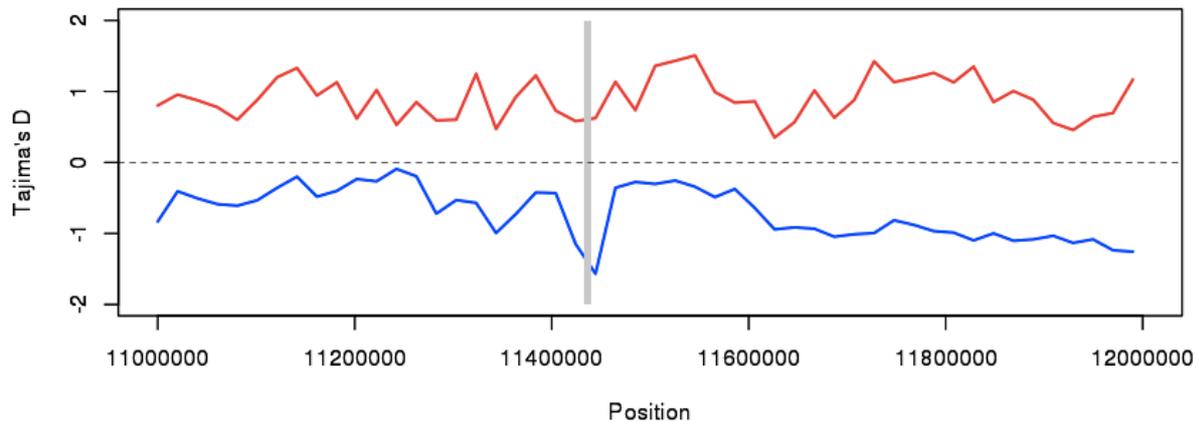


Figure 7. Tajima's D along the region in chromosome 15 containing *CYP337B* for populations of *H. armigera* and *H. zea* in 2013. Red corresponds to *H. zea* 2013 and blue to *H. armigera* 2017. Grey shading indicates the location of *CYP337B3* and the dashed line Tajima's D = 0.

Discussion

We have used whole genome sequencing of 137 individuals to document the interaction between a native and invasive species of crop pest. This has demonstrated that these species are maintaining their differentiation in the face of hybridisation, indeed there is evidence for a reduction in admixture over time. However, in contrast 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 hybridisation. In this particular case the invasion suggests a double blow for agriculture, with both a new pest species and a newly resistant native pest species both now present in South America.

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 hybridisation. Differentiation between species is roughly the same between allopatric and sympatric populations except for a single "pocket" of introgression apparently driven by strong selection. Therefore, from the myriad 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. On the contrary, the predominance of parental forms and the rarity of hybrids suggest strong selection against hybridization.

The *Helicoverpa* sibling species exhibit both prezygotic and postzygotic barriers that can account for the low rates of natural hybridisation (12,18,25,26). Traits linked to prezygotic isolation include morphological differences in the male and female genitalia as well as divergent pheromonal bouquets (18,25,26). A frequent outcome of mating between *H. zea* and *H. armigera* is to ‘lock up’, leading to death without reproduction of the individuals involved (18). This is due to differences in the length of an elaborate inflating appendix in the genitalia, which makes the male unable to successfully withdraw his penis extension from the female. Although this can also occur in intraspecific mates, this clogging is far more common in interspecific matings (12,18). Furthermore, behavioural tests in *Helicoverpa* moths showed that female pheromones and their ratios are used as critical signals to avoid interspecific matings (26–28). Even though both *H. zea* and *H. armigera* use Z-11-hexadecenal and (Z)-9-hexadecenal as the primary sex pheromones, the ratio of these compounds emitted from the female glands is about 3-fold higher in *H. armigera* (26–29). Therefore, both mechanical and chemical reproductive isolation likely play a role in assortative mating and the reduction of successful interspecific matings.

In addition to pre-zygotic isolation, selection against hybridization could be the result of intrinsic postzygotic barriers including Bateson-Dobzhansky-Müller (BDMIs) or “pathway” incompatibilities. BDMIs cause reduced fitness in hybrids but can be broken down by recombination in backcross progeny (30–32). Conversely, “pathway” incompatibilities reduce fitness in recombinant hybrids in which co-adapted alleles become separated (33). Simulations and empirical data show that BDMIs usually have an even, genome-wide effect whereas “pathway” incompatibilities produce distinct localised barriers to introgression (33,34). Our results showed a genome-wide reduction of introgression over time, thus suggesting pervasive selection against hybridization likely as a result of widespread BDMIs throughout the genome. Moreover, patterns of differentiation captured by *Fst* 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* (30,32). Therefore, we hypothesize that barrier loci like BDMIs accumulated rapidly in the 1.5 My of allopatric divergence and once in secondary contact these incompatibilities contribute to selection against admixture across the genome. In summary, our findings highlight that species can be unambiguously different but coupled by 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, there should be evidence that the introgressed haplotype is responsible for a phenotype with a beneficial impact on fitness, and that it has 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 (13,14). This chimeric enzyme arose independently at least eight times from unequal crossover events between two parental P450 genes (13), and is capable of metabolizing pyrethroids to non-toxic compounds in larvae

and adults (14). Indeed, the exclusive presence of *CYP337B3* has been shown to confer a 42-fold resistance to fenvalerate in *H. armigera* (14). Furthermore, Durigan et al. (15) showed that *CYP337B3* is associated with the very low mortality of larvae when sprayed with the pyrethroids deltamethrin and fenvalerate in invasive *H. armigera 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* (15–17). In the 2011/2012 and 2012/2013 growing seasons, cotton, soybean, and maize crops were attacked by *Helicoverpa* species at unusually high infestations resulting in billion dollar losses to Brazilian agriculture (15). Initially, the species was presumed to be *H. zea*, but during these seasons, growers reported a reduced efficacy of insecticides despite the increase in sprays and the known susceptibility of *H. zea* to pesticides (15). Research then confirmed that the resistant individuals were *H. armigera* which successfully invaded and established in the New World, resulting in the third major Brazilian pest outbreak in 30 years (15–17). Since *H. armigera* was detected in 2013, the frequency and dosages of insecticide sprayings including pyrethroids dramatically increased (15). 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. The hypothesis of recent selection on this locus is supported by our population genomic analysis demonstrating 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 mice, *Simulium* black flies, and *Anopheles* mosquitos whereby the increase in pesticide exposure acted as a selective force sufficient to drive introgression across species boundaries (35–39). Remarkably, in all these cases adaptive introgression occurred from the species with a higher effective population size into the species with lower *Ne* and lower genetic diversity. This pattern has also been found in natural populations whereby the selective agent is not human-mediated. For instance, adaptive introgression of colour pattern genes from *Heliconius melpomene* into *H. timareta* because of natural selection for mimicry (40), tidal marsh adaptations from *Ammospiza caudacauta* into *A. nelson* sparrow (41), and female-competitive traits from *Jacana spinose* into *J. jacana* (42). This pattern is expected given that species with higher *Ne* harbour more standing variation which is the raw material for adaptation and introgression is thus less likely to add beneficial variants (43,44). Moreover, small populations have a higher mutational load because they are more prone to accumulate deleterious mutations whereas large populations are less subject to drift, and therefore new beneficial mutations are less likely to be lost (44,45). However, the most famous example of adaptive introgression from Neanderthals and Denisovans into out-of-Africa human populations is in the reverse direction, undermining the generality of this pattern and highlighting the bidirectional nature of introgression (21,22,46). Of note, Neanderthals were established in Eurasia when humans were expanding into Europe, and less is known about introgression from humans to Neanderthals (21,22,46).

The most comprehensive genomic study of rapid adaptive introgression resulting from pesticide pressure is between *Anopheles gambiae* and *A. coluzzi*, the major malaria vectors in Africa (35,37,47).

Several studies have shown introgression of mutations conferring resistance to pyrethroids from *A. gambiae* into *A. coluzzi* (35–37). The *CYP337B3* trickle from *H. armigera* into *H. zea* coincided with increased in pesticide spraying, similar to introgression of insecticide resistance alleles in *Anopheles* coinciding with the start of a significant insecticide-treated bed net distribution campaign in Mali (37). After introgression of the insecticide-resistant genes, these alleles almost reached fixation in four years in *A. coluzzi* populations (37) 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. *A. gambiae* and *A. coluzzi* diversified in sympatry only 400,000 years ago (35,47), whereas *H. armigera* and *H. zea* differentiated in strict allopatry for 1.5 Mya and came into contact only in the recent 2013 invasion (10,11). Accordingly, the genomes of *A. gambiae* and *A. coluzzi* exhibit very low differentiation except for two large regions that show exceptional divergence known as "genomic islands" of diversification (35). 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 (35). Conversely, in *H. armigera* and *H. zea* the differentiation is not restricted to "islands" but rather we observed consistently high *Fst* 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 (48). This suggests that in the face of intense selective pressure, adaptive variation can rapidly cross 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 (5,7). This asymmetry is partly explained by a demographic imbalance between the two species at the wave front, where the invading species is at lower densities (5–7). It is likely that early invading *H. armigera* individuals had few available conspecific mates, and thus, were involved in more heterospecific crosses. A rapid increase in pyrethroid resistant *H. armigera* would have been favoured by the pesticide regime described above, consistent with the lower proportion of introgression in the larger recent population of *H. armigera* in Brazil.

Most of the admixed alleles in the invasive *H. armigera* are likely to be neutral (5). 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 (49). Both models and empirical data show that genes from the resident species associated with local adaptation are easily introgressed into the invading species (4,5). Contrastingly, genes from the invading species introgress into the local species only if they are under very strong positive selection in the local environment (5), 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 introgressed. *H. zea* has developed resistance to two toxin Bt maize and cotton (Cry1A and Cry2a) (50)

and these may be plausible candidates for adaptive introgression from *H. zea* into *H. armigera*. Further temporal monitoring of the invasive *H. armigera* populations in Brazil will permit the detection of fine-scale selection on introgressed alleles.

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 (1,3). With increased geographic connectedness via trade, the threat of invasive species arriving to countries where they were previously absent is expected to increase (1,4). In pest invasions, special attention has been given to newcomer species that can rapidly proliferate as they no longer face their natural enemies (50–52). 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 (7). Indeed, there is compelling evidence that species which hybridize have a higher likelihood of successful establishment (4,5,7).

In addition to human-mediated introduction of species, there is growing evidence that anthropogenically altered selection regimes drive exceptionally rapid adaptation (37,39). Agricultural pests are subjected to massive insecticidal pressure, which drives selection for rapid development of resistance (35,37). 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*. Therefore, 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 the future of agricultural planning. 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 2016-2017 were treated as “2017” or recent populations. The Brazilian samples included 66 *H. armigera* and 34 *H. zea* from Bahia, Planaltina, and Mato Grosso states. As complementary groups for the admixture analyses, we included 8 *H. zea* from the United States, 9 *H. armigera armigera* from Asia, Europe, and Africa, and 20 *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 Table S1. All samples were preserved in ethanol/RNA later or stored at -20°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). Raw sequence reads were aligned to the *H. armigera* genome using BWA v 0.7.12 (54). Reads were trimmed when quality in at least two bases fell below Q30, 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 (55). HaplotypeCaller in GATK v. 3.7 (56) was used to call SNPs in individual samples, and we used GenotypeGVCFs (56) to estimate genotypes across all individuals simultaneously, implementing a heterozygosity value of 0.01. We used VCFTOOLS v. 0.1.15 (57) to calculate mean coverage statistics for each sample. We further filtered our SNP dataset to include a minor allele count ≥ 2 , mean site depth ≥ 5 and ≤ 200 , missing data per site ≤ 0.50 , and Phred-scale mapping quality ≥ 30 ; resulting in 17'257,450 genome-wide bi-allelic SNPs for the 150 individuals.

Classification of parental and hybrids individuals

We conducted a genetic principal component analysis (PCA) to visualize clustering of parental and hybrid genotypes using 70,099 SNP markers across all chromosomes with the packages "adegenet" (58), "Poppr" (59) and "vcfR" (60) 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 (61), 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 pre-incursion allopatric populations of *H. zea* from the USA and *H. armigera armigera* from the Old World for the association test resulting in 454,000 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>).

Genomic divergence between and within species

To estimate genome-wide differentiation we calculated Weir and Cockerham Weighted Fst in VCFTOOLS v. 0.1.15 (57) in 100kb non-overlapping windows. We calculated Fst between allopatric populations of *H. zea* and *H. armigera* and sympatric post-incursion populations for the two temporal samples 2013 and 2017.

Phylogenetic weighting

We used Twisst (20) to explore evolutionary relationships across the genome of allopatric and sympatric populations of *H. zea* and *H. armigera*. We perform phasing and imputation of missing bases using Beagle (62) 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 (20) with the GTR model in 50 SNPs sliding windows in Phyml (63).

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 (22). We considered that D-statistic estimates were indicative of significant introgression when z-scores ≥ 2.5 , and thus with a p-value of ≤ 0.05 . To estimate the genome-wide proportion of admixture we conducted an *F4*-ratio incorporating *H. armigera conferta* from Australia as the fifth population (description of the model in Tab. S2) and using Admixtools v. 5.1 with a block jackknife size of 61 bp (22).

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 (Fig. S1). 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 *fd* in 100kb sliding windows with the ABBA-BABA.py scripts as in Martin et al. (23) 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 non-overlapping windows. Sliding windows averages of the proportion of ancestry were plotted using custom R scripts, and using “ggplot2” (64), “Hmisc” (65), and “Rmisc” (66) packages.

To confirm introgression of CYP337B3, all individuals of *H. zea* 2017 were screened for the presence of this gene as in Joußen et al. (14). 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 (67).

Tajima’s D

Tajima’s D was calculated in sliding windows of 10 kb in VCFTOOLS v. 0.1.15 (57). Calculations were restricted to chromosome 15, from position 11,000,000 to 12,000,000 bp, whereby CYP337B3 gene extends from ~11,436,000 to 11,440,000 bp.

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