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Hybridization in heliothine moths: impacts on reproduction, pheromone communication, and pest management

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Heliothine moths (Family Noctuidae : Subfamily Heliothinae) are ubiquitous crop pests with three documented species combinations known to hybridize: *Helicoverpa zea* x *Helicoverpa armigera*, *H. armigera* x *Helicoverpa assulta*, and *Heliothis virescens* x *Heliothis subflexa*. Hybrids can have advantageous traits, such as increased host range, resistance to insecticides, and increased mating success, posing serious threats to agriculture. However, deleterious traits of hybrids, such as developmental abnormalities or sterility, can be exploited for pest management. In this review, the characteristics of F1 hybrids and backcrosses are examined through a historical lens. Topics reviewed include developmental characteristics, sex pheromone synthesis and perception, mating and calling behavior, sperm morphology, gene expression, electrophysiological responses, structures of the central and peripheral nervous systems, hybrid sterility, and applications in pest control. Recommendations for future studies based on existing gaps of knowledge are given, as are proposed pest management strategies.

KEYWORDS

heliothine moths, Heliothinae, species hybridization, hybrid sterility, pheromone communication, prezygotic barriers, hybrid vigor, backcross hybrids

Introduction

Moths of the subfamily Heliothinae, often referred to as heliothine moths or heliothines, are highly cosmopolitan, with nearly 400 species identified across Africa, Asia, Europe, Oceania, and the Americas to date (Cho et al., 2008). Multiple species exist in overlapping geographic ranges, and where sympatry occurs, both prezygotic and postzygotic mating barriers may be present, resulting in reproductive isolation (Hardwick, 1965; Proshold and Lachance, 1974). While prezygotic mechanisms prevent interspecific mating, post-zygotic mechanisms such as hybrid inviability or sterility are only effective after mating has occurred. The species *Helicoverpa zea* and *Heliothis virescens*, which coexist in North America, exhibit irreversible locking of the genitalia upon mating

resulting in death of the mating pair – an example of prezygotic mechanical isolation (Hardwick, 1965). Although *H. virescens* can successfully mate with its congener *Heliothis subflexa*, which also resides in North America, their male offspring are sterile – an example of postzygotic isolation (Proshold and Lachance, 1974). In some cases, the fitness costs associated with interspecific mating have reinforced the evolution of prezygotic mechanisms to distinguish between different species before mating occurs (Hardwick, 1965). In heliothines (and many other insects), the emission and detection of specific volatile chemicals, such as pheromones and synomones, can perform this function. For example, the pheromone blend of *H. virescens* females contains the compound Z-9-tetradecenal (Z9-14:Ald), which repels male *H. zea* (Table 1; Figure 1; Vickers et al., 1991). When Z9-14:Ald is at a proportion of 15% relative to the principal component, Z-11-hexadecenal (Z11-16:Ald), upwind flight of males is significantly reduced, an effect referred to as "behavioral antagonism" (Table 1; Figure 1; Vickers et al., 1991; Baker, 2008). Therefore, Z9-14:Ald is said to be a behavioral antagonist of male *H. zea* (Baker, 2008). Similar heterospecific antagonism has been demonstrated between other sympatric species, such as antagonism of *H. virescens* males to Z-11-hexadecanyl acetate (Z11-16:OAc) produced by *H. subflexa*, or antagonism of *H. armigera* males when exposed to pheromone blends with abnormally high titres of Z9-14:Ald (Hillier and Baker, 2016). Pheromone-detecting olfactory sensory neurons (OSNs) are organized by "sensillar types", with *Heliothis* spp. typically exhibiting three predominant types (A, B, C) and *Helicoverpa* spp. exhibiting two (A and C) (Figure 1; Hillier and Baker, 2016). OSNs in type-A sensilla detect primary pheromone components (most often Z11-16:Ald), type-B OSNs detect secondary compounds, and type-C often house OSNs which are involved in heterospecific antagonism (Figure 1; Hillier and Baker, 2016).

Given the high fitness costs of some interspecific mating, prezygotic mating isolation *via* sex pheromone communication is under high selective pressure. Stabilizing selection would theoretically be favored in heliothine sex pheromone communication systems, with species becoming finely-tuned to the blends of conspecifics and blends remaining within tight parameters (Cardé & Baker, 1984; Baker, 1989). However, with random genetic mutations and epigenetic changes occurring in individuals within a population, and asymmetric sexual selection taking place, modifications in blend production and/or detection

TABLE 1 Female pheromone blends of major heliothine species (*Helicoverpa zea, Helicoverpa armigera, Helicoverpa assulta, Heliothis virescens*, and *Heliothis subflexa*) and the hybrid of *H. armigera* x *H. assulta* with percentage of compounds produced by females relative to the primary component (Z11-16:Ald for all except *H. assulta*, which has Z916:Ald as the primary component) and trace components.

	Helicoverpa zea	*Helicoverpa armigera*	*Helicoverpa assulta*	*Heliothis virescens*	*Heliothis subflexa*	*H. armigera x H. assulta*
Z11-16:Ald (Z-11-hexadecenal)	**100**	**100**	**6.5**	**100**	**100**	**100**
Z9-16:Ald (Z-9-hexadecenal)	**1.8**	**2.5**	**100**	Trace	**42.9**	4.0
Z7-16:Ald (Z-7-hexadecenal)	Trace	Trace		Trace		3.8
16:Ald (Hexadecenal)	Trace		Trace	25.6	Trace	
Z9-14:Ald (Z-9-tetradecenal)		**0.3**		**0.4–6**	Trace	
14:Ald (Tetradecenal)				Trace	Trace	
Z11-16:OH (Z-11-hexadecanol)			Trace		**5.8**	16.7
Z9-16:OH (Z-9-hexadecanol)			Trace			1.3
Z11-16:OAc (Z-11-hexadecanoate)			Trace		22.7	3.3
Z9-16:OAc (Z-9-hexadecanoate)			3051		9.9	0.3
Z7-16:OAc (Z-7-hexadecanoate)					3.6	

Adapted from Hillier and Baker (2016).
Bolded "100"s correspond to the primary component of the blend, while other bolded numbers correspond to the secondary components of the blend.

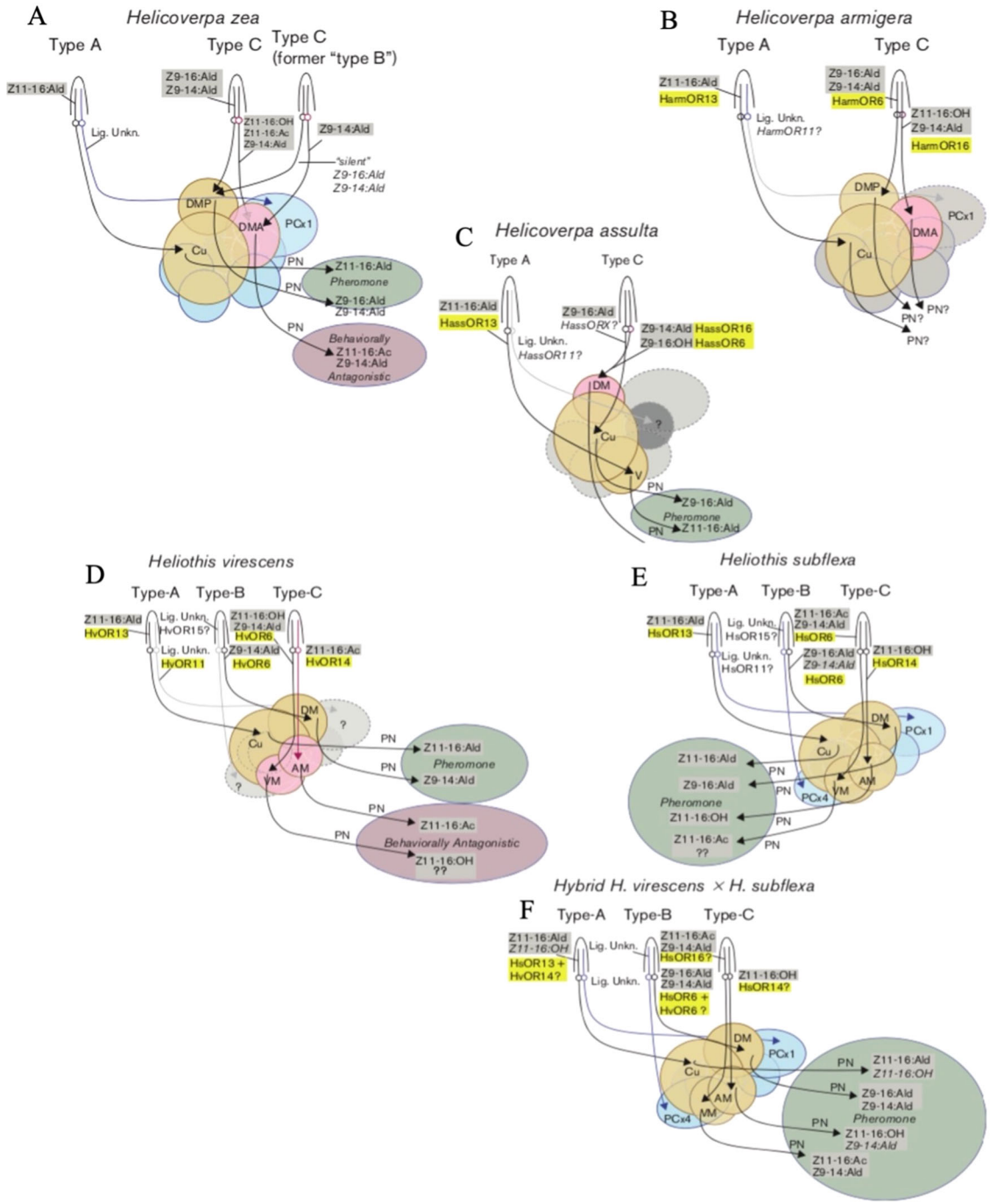

FIGURE 1
Diagrams of the olfactory pathways of male *H.zea* **(A)**, *H. armigera* **(B)**, *H. assulta* **(C)**, *H. virescens* **(D)**, *H. subflexa* **(E)**, and hybrid *H.virescens* x *H. subflexa* **(F)**. Sensilla are represented as conical structures labeled Type-A, Type-B, or Type-C. Olfactory sensory neurons (OSNs) are represented as lines inside the sensilla. The ligands known to produce responses within each OSN are highlighted in grey. The olfactory receptors (ORs) to which these ligands bind are highlighted in yellow. The projections of each OSN from neuronal cell bodies to areas of the brain are represented by arrows projecting from circles. Areas of the brain are represented by circular or ovoid shapes where Cu = cumulus, DM = dorsomedial glomeruli, AM = anteromedial glomeruli, VM = ventromedial glomeruli, DMA = anterior dorsomedial glomeruli, DMP = posterior dorsomedial glomeruli, PCx1 = posterior complex glomerulus 1, PCx4 = posterior complex glomerulus 4. The projections of projection interneurons (PN) are represented by black arrows. The behavioural responses elicited by particular pheromone components are represented by circular or ovoid shapes, where green = behavioral agonists (attraction) and red = behavioral antagonists (repulsion). Adapted from Hillier and Baker (2016).

can occur (Löfstedt et al., 1989; Phelan, 1992; Löfstedt, 1993; Jurenka et al., 1994; Haynes, 1997; Phelan, 1997a; Phelan, 1997b; Baker, 2002; Roelofs et al., 2002). Thus, despite the evolution of sex pheromones as prezygotic isolating mechanisms, hybridization of various heliothine species has been well-documented both in the wild (Anderson et al., 2016; Anderson et al., 2018) and under laboratory conditions (Hardwick, 1965; Laster, 1972; Wang and Dong, 2001). Heliothine hybrids were first extensively documented by Hardwick in 1965 in "The Corn Earworm Complex", primarily by attempting crosses between *H. zea*, *Helicoverpa armigera*, and *Helicoverpa punctigera* for the purpose of investigating the potential for hybrid species to be used for pest control strategies (Hardwick, 1965). For each pairing, a female of one species was placed in a jar with two males of another species, and attempted and completed copulations were recorded (Hardwick, 1965). The only successful mating resulting in viable eggs and offspring occurred between a male *H. zea* and female *H. armigera*, although these species sometimes had locked genitalia leading to death without reproduction (Figure 2; Hardwick, 1965). In addition to *H. zea* and *H. armigera*, two other major heliothine hybrids would be identified in the following decades: those between crosses of *H. virescens* and *H. subflexa* (Laster, 1972) and between crosses of *Helicoverpa assulta* and *H. armigera* (Figures 3, 4; Wang and Dong, 2001). The implications of these hybridizations on pheromone production and detection are important for understanding the history of speciation events in heliothine moths and for developing new pest management techniques as these hybrids emerge in the wild.

Furthermore, studying hybridization can provide insight into how changes in pheromone detection systems may be driven by phenotypic plasticity (Anderson, 2003; Andersson et al., 2007; Bailey and Zuk, 2008; Groot et al., 2009a; Groot et al., 2010). Studies in various insects, including heliothines, have shown that individuals can change their pheromone blends in response to the social environment (Bashir et al., 2003; Petfield et al., 2005; Kent et al., 2008; Krupp et al., 2008; Groot et al., 2009a; Groot et al., 2010). In the presence of hybrid species in hybrid zones, plastic non-hybrid individuals could potentially change their blends to prevent interspecific mating, allowing them to colonize new habitats and forcing pheromone detection in conspecifics to shift in response to the new blends (Crispo, 2008; Groot et al., 2010). Numerous studies on the three known heliothine hybrids (*H. zea* x *H. armigera*, *H. assulta* x *H. armigera*, and *H. subflexa* x *H. virescens*) have explored their genetics, physiology, and behavior, and provide insight into the plasticity of pheromone communication systems in wild populations.

Helicoverpa zea and *Helicoverpa armigera*

First laboratory studies of *H. zea* x *H. armigera* hybrids

The species *H. zea* and *H. armigera* are recently sympatric in regions of the Americas, particularly South America and the southern USA (Hillier and Baker, 2016). Following the discovery of the first *H. armigera* x *H. zea* crosses in the lab by Hardwick, Laster and colleagues would attempt a formal study on their intermating compatibility in 1985, inspired by previous research on *H. subflexa* x *H. virescens* (Laster, 1972; Laster et al., 1985). In their study, wild *H. armigera* females imported from Australia were

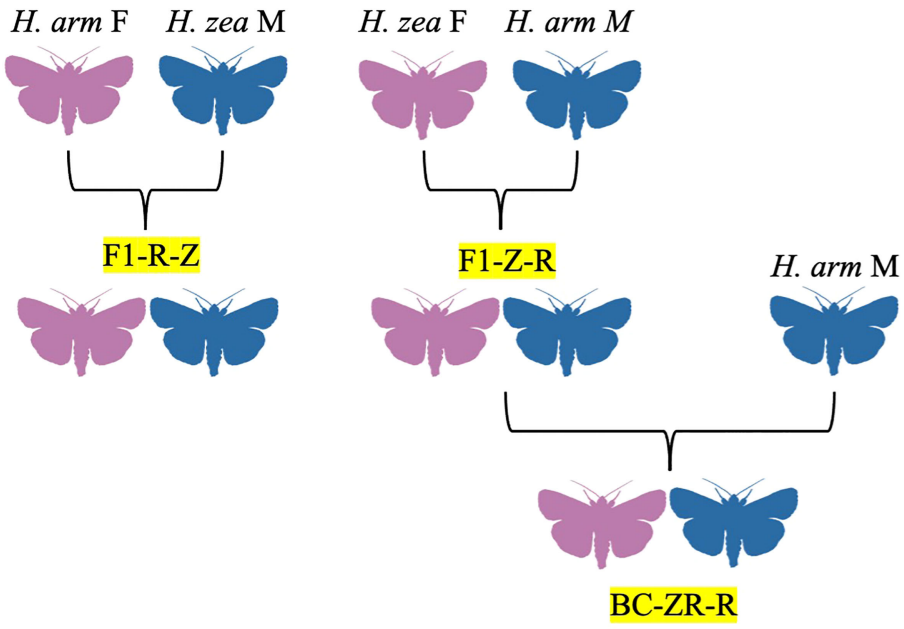

FIGURE 2
Hybrid family trees for *H. zea* x *H. armigera* crosses, identified by codes denoting generation, female parent, and male parent, in order. F1 = filial generation, BC = backcross generations, Z = *H. zea*, R = *H. armigera*.

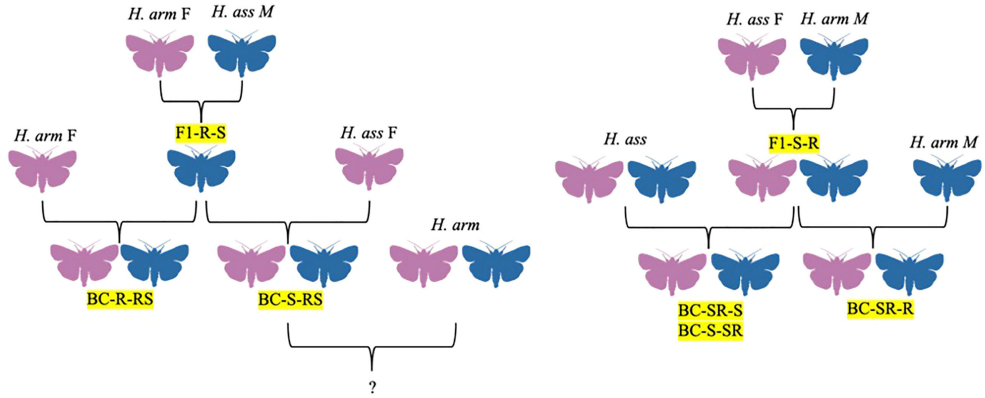

FIGURE 3
Hybrid family trees for *H. assulta* x *H. armigera* crosses, identified by codes denoting generation, female parent, and male parent, in order. F1 = filial generation, BC = backcross generations, S = *H. assulta*, R = *H. armigera*.

crossed with laboratory-reared male *H. zea* to see if sterile male offspring could be obtained, allowing for pest control *via* the sterile insect technique (Figure 2; Laster, 1972; Laster et al., 1985). Some evidence of male sterility was found in later filial generations, leading to further investigation in a 1995 study using wild *H. armigera* from Russia and laboratory-bred *H. zea* populations (Laster and Hardee, 1995). However, no backcross sterility was detected despite some differences in mating incidence, life-stage development, and pupal weights between certain crosses and some incidences of genital locking (Laster and Hardee, 1995). A similar study of wild *H. armigera* from China and lab-reared *H. zea* also found no evidence of sterility between any crosses (Laster and Sheng, 1995).

The viability of reciprocal crosses of *H. zea x H. armigera* was evaluated by Rios and colleagues in 2021, with the authors detecting hybrid vigor in some F1 hybrids and hybrid sterility in intercrosses of offspring from *H. armigera* females and *H. zea* males not previously documented by Laster, Sheng, and Hardee (Figure 2; Laster and Hardee, 1995; Laster and Sheng, 1995; Rios et al., 2021). Although the fecundity and egg survival of parental species was higher, most hybrids showed greater survival rates in the larval, pupal, and pre-pupal stages (Rios et al., 2021). Some hybrid crosses

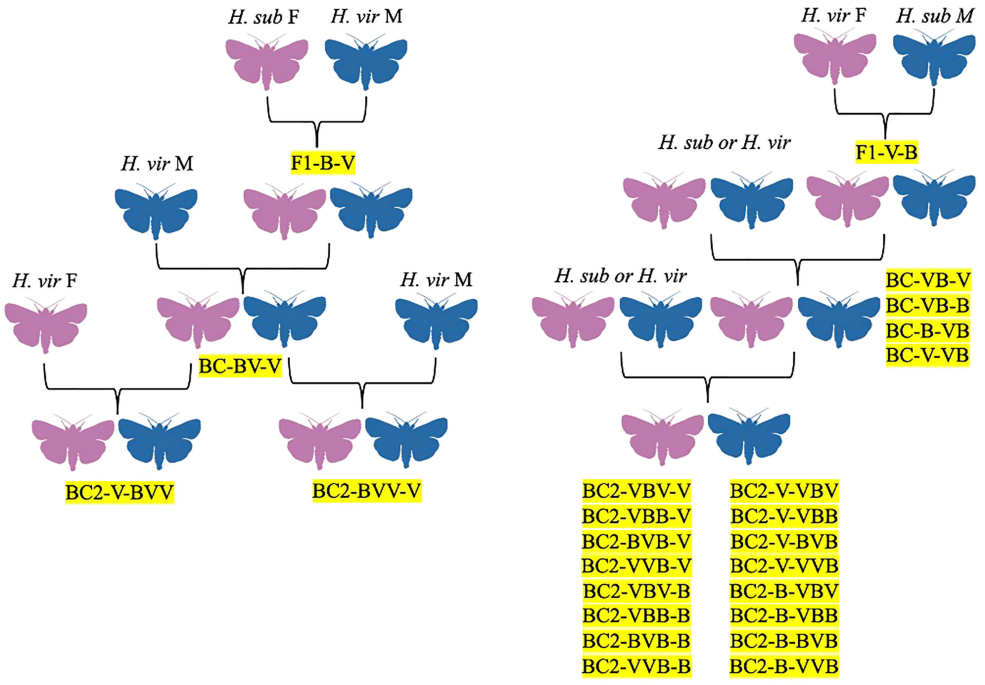

FIGURE 4
Hybrid family trees for *H. virescens* x *H. subflexa* crosses, identified by codes denoting generation, female parent, and male parent, in order. F1 = filial generation, BC = backcross generations, V = *H. virescens*, B = *H. subflexa*.

also showed unbalanced sex ratios; *H. armigera* male x *H. zea* female hybrids were 38.3% female, while backcrosses of these hybrid females and *H. armigera* males were 63.6% female (Rios et al., 2021). Female hybrids also tended to produce fewer females (Rios et al., 2021). The increased survival rates of hybrids in immature stages of development were not enough to overcome their lack of fecundity, a weakness which could counteract other benefits hybrids may have, such as shorter development time (Rios et al., 2021). The authors suggest that such limitations would prevent hybrids from flourishing in the wild, although a greater number of mating events over a longer time period could produce enough hybrids with advantageous traits (i.e., pesticide and disease resistance, polyphagy, thermal tolerance, high mobility) to cause issues (Rios et al., 2021). To prevent this, numbers of each parental species should ideally be maintained in equal numbers, and other IPM strategies employed, including: usage of predators, parasitoids, or microorganisms for population control; usage of genetically-modified crops; pheromone trapping and mating disruption with special attention to geographic variations in pheromone blend; use of insecticides against *H. zea* but not *H. armigera*, which exhibit some resistance; population monitoring particularly at the egg and larval stages; monitoring of resistance to pesticides and xenobiotics; and strategic crop rotation involving staggering of crops (Rios et al., 2021). For example, corn is the preferred host of *H. zea*, but soybeans, tomatoes, and cotton for *H. armigera*, and thus sowing of these crops could be alternated depending on geography and seasonality (Rios et al., 2021).

Discovery of wild *H. zea* x *H. armigera* hybrids

Although previous laboratory studies showed that *H. armigera* and *H. zea* could produce viable offspring, it was not until 2013 that a potential *H. armigera* and *H. zea* hybrid was found in the wild in Brazil by Anderson et al. (2016). Heliothines were collected from sixteen different countries between 2004–2014 and identified at the species level using mitochondrial gene sequencing. Some *H. zea* were collected from Brazil before 2013 (the year of the first *H. armigera* invasion to the Americas), and some after the invasion (Anderson et al., 2016). Genotyping-by-sequencing analysis identified one *H. zea* individual from Brazil as falling between the main cluster of *H. zea* and two clusters of *H. armigera*, making it a possible hybrid (Anderson et al., 2016). Additionally, *H. armigera* from Brazil were found to be more similar to populations of *H. zea* from Brazil than to other populations of *H. armigera* collected from Australia, China, India, or Uganda, denoting either potential hybridization or similar populations of origin between the two species (Anderson et al., 2016). In contrast, *H. zea* sampled both before and after the *H. armigera* invasion in 2013 did not differ from one another (Anderson et al., 2016). The only significant admixture between populations was found between Brazilian *H. zea* and USA *H. zea*, evidence of the founder effect caused by the initial foundation of the *H. zea* population when *H. armigera* first came to the Americas 1.5–2 million years ago (Behere et al., 2007). There was no evidence of greater gene flow from Brazilian *H. armigera* into Brazilian *H. zea* (Anderson et al., 2016). The researchers hypothesized that the genetic distinctions between *H. armigera* and *H. zea* could become more difficult to parse if *H. armigera* were to spread and hybridize in the Americas (Anderson et al., 2016). They also suggest that *H. zea* may have facilitated the arrival of *H. armigera* into the Americas by exchanging genetic material that would lessen the effects of a bottleneck or provide a source for advantageous traits (Anderson et al., 2016). One such advantageous trait could be resistance to the pyrethroid insecticide fenvalerate conferred by the *CYP337B3* gene, which was found to be under selection in the study (Joußen et al., 2012; Anderson et al., 2016). The CYP337B3 gene codes for the P450 enzyme capable of converting pyrethroid insecticides into nontoxic compounds, and its presence has been shown to give a 42-fold increase in fenvalerate resistance in *H. armigera* (Joußen et al., 2012). If a population of *H. armigera* had recently undergone a bottleneck, for example, pest control methods targeting insecticide resistance could be more effective than on a population undergoing purifying selection to get rid of deleterious alleles. Alternatively, it could increase the prevalence of favorable genes in a new habitat.

Population genetics of wild *H. zea* x *H. armigera* hybrids

In 2017, expanding on the study by Anderson and colleagues, Leite and colleagues used microsatellite markers to determine population structure and gene flow between Brazilian *H. armigera*, Brazilian *H. zea*, and US *H. zea* (Leite et al., 2017). Putative hybrids were highest during the years 2012–2013, with *H. armigera* populations showing the highest proportions of hybrids (up to 40%) compared to *H. zea* (up to 18%) (Leite et al., 2017). In both Brazil and the US, both species seemed to have random breeding patterns, but also showed greater genetic variation in Brazil. Although the genetic identities of *H. armigera* and *H. zea* are generally preserved in South America, most likely due to prezygotic isolation based on differences in genital morphology (Pogue, 2004), hybridization still occurs at low rates and hybrids could be better adapted to certain climates, pathogens, or pest control methods. Given that both species are widely distributed, have a high number of shared traits (including high fecundity, polyphagy, and high migration and dispersion), and with evidence of *H. armigera* being detected in Central America and Florida, the authors suggest further monitoring of species ranges and implementation of robust integrated pest management strategies (Leite et al., 2017).

Potential resistance to certain insecticides and transgenic crops is also concerning considering the polyphagous nature of both species (Leite et al., 2017). Pearce and colleagues published an investigation of the evolutionary trajectory of various gene families in the *H. zea*/*H. armigera* lineage (Pearce et al., 2017). They found that prior to the divergence of this lineage from the common ancestor around 1.5 million years ago, hundreds of gene families involved in detoxification of compounds (particularly pesticides or host plants), digestion, and gustatory receptors for CO_2 detection

were accumulated compared to lepidopterans with more narrow host ranges. However, differences have emerged, with *H. armigera* showing a higher number of pesticide and Bt crop resistance genes and wider host range, while *H. zea* has lost some genes for GRs, detoxification, and pesticide resistance (Pearce et al., 2017). In the last decade, *H. armigera* has invaded South America, is showing signs of invading Central and North America, and is largely displacing *H. zea* in Brazil (Pearce et al., 2017). The authors suggest that evidence of hybridization and introgression of *H. armigera* genes into *H. zea* could be occurring in South America, and the high level of similarity between species suggests that hybrids would not break down over time (Pearce et al., 2017). The additional GRs, genes for tolerance to plant defenses, and resistance to pyrethroids and Bt could be passed from *H. armigera* to *H. zea* and cause further crop damage (Pearce et al., 2017).

In 2018, Anderson and colleagues identified nine hybrid individuals from Brazil, eight being largely *H. armigera* (91–98%) with some introgression of *H. zea* genes, and the ninth individual showing greater similarity to *H. zea* (Anderson et al., 2018). In the ninth individual, the *H. armigera*-like regions contained genes for a gustatory receptor and esterase genes responsible for host range, and a cytochrome p450 enzyme gene (*CYP337B3*) involved with resistance to pyrethroids (Anderson et al., 2018). There is a potential that a combination of the traits that allowed *H. zea* to successfully adapt to the New World will mix with the traits that give *H. armigera* its greater ability to resist pesticides and transgenic crops and its wider host range to create a much more problematic pest (Anderson et al., 2018). Although this study suggests a low level of backcrossing of *H. armigera* with *H. zea* and thus a lower likelihood of *H. zea* inheriting these traits, high selection pressures still exist (Valencia-Montoya et al., 2020).

To determine the potential effects of secondary contact between these species, Valencia-Montoya and colleagues performed whole-genome sequencing on populations of *H. armigera* and *H. zea* from shortly after the *H. armigera* invasion (2012–2013) and more recently (2016–2017). The researchers found that species boundaries were generally well-maintained, introgression is not specific to any regions of the *H. armigera* genome, and that admixture has decreased in Brazilian *H. armigera* (Valencia-Montoya et al., 2020). For *H. zea*, there was strong introgression of a region containing the insecticide resistance gene *CYP337B3*, which had previously only been found in invasive Brazilian *H. armigera* (Valencia-Montoya et al., 2020). In a principal component analysis (PCA), two individuals fell between the clusters of *H. zea* and *H. armigera*, and thus these individuals were suspected to be early generation hybrids. Out of 132 moths analyzed, 66 individuals had a closer relationship to *H. armigera* with 0–9.2% of their alleles derived from *H. zea*, while 27 individuals genetically closer to *H. zea* had 0–4.1% *H. armigera* ancestry, suggesting that hybridization was rare in more recent Brazilian populations (Valencia-Montoya et al., 2020). Using fixation index (F_{ST}), the researchers found only one peak of differentiation between *H. zea* from the US and Brazil corresponding to a region on chromosome 15 containing a locus for insecticide resistance, suggesting localized introgression (Valencia-Montoya et al., 2020). Phylogenetic comparisons between the genomes of Brazilian *H. zea* and *H. armigera* showed large-scale introgression in recent populations and discordant topology around the *CYP337B3* locus on chromosome 15 (Valencia-Montoya et al., 2020). With introgression only occurring around this region, the researchers concluded that introgression was not widespread in *H. zea*. However, there was also strong evidence of gene flow between Brazilian *H. zea* and *H. armigera* between 2013–2017, with introgression decreasing over time in *H. armigera* but increasing in *H. zea*, particularly of the *CYP337B3* gene, with the gene being present in 14/18 (77.8%) of *H. zea* in 2017 (Valencia-Montoya et al., 2020). In *H. armigera*, nearly entire chromosomes showed introgression in the earlier samples, particularly on chromosome 18, but shorter introgressed regions and lower effective migration rates in more recent samples, with indications of purifying selection against introgression of *H. zea* genes in *H. armigera* (Valencia-Montoya et al., 2020). There is evidence for strong positive selection of the *CYP337B3* locus in both *H. zea* and *H. armigera* (Valencia-Montoya et al., 2020). Overall, the evidence gathered by Valencia-Montoya and colleagues demonstrated that *H. zea* and *H. armigera* remain two distinct species and hybridization levels remain low, likely due to the incompatibility of genitalia and differences in sex pheromone composition. While both blends contain different proportions of Z11-16:Ald, Z-9-hexadecenal (Z9-16:Ald), and Z-7-hexadecenal (Z7-16:Ald), the *H. armigera* blend contains Z9-14:Ald, which is antagonistic to male *H. zea* (Table 2; Vickers et al., 1991). While the pheromone blends of the parental species are known, no studies have been published on pheromone blend composition produced by these hybrids. Despite differences in pheromone blends, there is still strong introgression of the *CYP337B3* locus from *H. armigera* into *H. zea*. This increase in introgression may have been driven by increased use of pesticides in 2012–2013 following the *H. armigera* invasion, selecting for early hybrids (Valencia-Montoya et al., 2020). The researchers warn that with global trade, invasive pests are becoming more common, and excessive pesticide use may drive selection for introgression and hybridization (Valencia-Montoya et al., 2020).

In 2020, Cordeiro and colleagues also used single nucleotide polymorphisms (SNPs) to detect *H. zea* x *H. armigera* hybrids in various crop fields in Brazil. The researchers found hybrids at 5 different sites, with 26 insects (15%) showing mixed ancestry and an average of 10% introgression (Cordeiro et al., 2020). The main gene flow between species was found to be from *H. zea* to *H. armigera* (Cordeiro et al., 2020). Variations in climate and landscape affected the rate of introgression into *H. armigera*, with the presence of maize, tree plantations, and soybeans being associated with higher introgression, *H. zea* being associated with maize, and *H. armigera* with soybean (Cordeiro et al., 2020). Although few hybridization events occur, large sections of the genome can be introgressed, and multiple hybridizations may create an adaptive bridge between the species and increase the fitness of hybrids (Cordeiro et al., 2020). The researchers point to the *CYP337B3v2* resistance gene, found in both *H. zea* and *H. armigera*, as a salient example, along with genes for detoxification and gustatory

TABLE 2 Summary of behavioral and electrophysiological responses of *H. assulta*, *H. armigera*, their hybrids, and backcross males from insect lines tested by Zhao et al. (2006).

	Behavioral Responses (Relative Attraction)		Electrophysiological Responses (EAG Amplitude)	
Male Offspring from Cross (Female x Male)	*H. assulta* blend	*H. armigera* blend	Z9-16Ald	Z11-16Ald
H. assulta x *H. assulta*	+++	–	+++	+++
H. armigera x *H. armigera*	+++	+++	+	++++
F1SR (*H. assulta* x *H. armigera*)	++++	+++	+	+++
F1RS (*H. armigera* x *H. assulta*)	+++	+++	+	+++
H. assulta x F1RS	+++	+	++	+++
H. armigera x F1RS	+++	+++	+	+++
F1SR x *H. assulta*	++	+	+++	+++
F1SR x *H. armigera*	+	++	+	++++

Adapted from Zhao et al. (2006).
The responses were classified as "–" indicating no response, or "+" indicating varying degrees of responses.

receptors that could be passed from *H. armigera* to *H. zea* (Pearce et al., 2017). They also suggest that rotation of crops may increase hybridization, and since *H. armigera* is resistant to pyrethroids and *H. zea* to the Cry1Ac Bt-protein in some soybean crops, resistance to each of these due to introgressions is likely to occur in areas where maize and soybeans are cultivated together (Cordeiro et al., 2020).

Helicoverpa armigera and *Helicoverpa assulta*

First laboratory studies of *H. armigera* x *H. assulta* hybrids

In addition to *H. zea*, *H. armigera* is also known to interbreed with *Helicoverpa assulta*, a major polyphagous pest of Africa, Asia, Australia, and Oceania (Figure 3). Although this hybrid has only been documented under laboratory conditions, the two do have overlapping habitats on these continents (Hillier and Baker, 2016). The first attempt to study *H. armigera* x *H. assulta* hybrids occurred in 1984, with the authors concluding at the time that the two species could not interbreed (Wang and Li, 1984; Wang and Dong, 2001). However, the original study had a small sample size, and thus Wang and Dong decided to re-attempt the experiment in 2001 with more robust methods to evaluate the potential for producing sterile hybrids that could be used for pest management (Figure 3; Wang and Dong, 2001). The researchers found that the hybrids of *H. armigera* females and *H. assulta* males were all males, although there was no evidence of hybrid sterility in either the offspring or the backcross of the hybrid offspring with *H. armigera* (Figure 3; Wang and Dong, 2001). However, the backcross offspring did show a distorted sex ratio of 4 males to 1 female, and the original *H. armigera* female x *H. assulta* male hybrid males exhibited greater signs of fitness than their parents, showing a possibility of using these crosses as a control mechanism (Wang and Dong, 2001). The researchers suggested further studies showing how the hybrids could compete against wild *H. armigera*.

Pheromone biosynthesis pathways in *H. armigera* x *H. assulta* hybrids

In 2005, Wang and colleagues further investigated the biosynthesis and composition of sex pheromones in *H. armigera* x *H. assulta* crosses. Both *H. armigera* and *H. assulta* use Z11-16:Ald and Z9-16:Ald in their blends, however, while Z9-16:Ald is the major component in *H. assulta* and Z11-16:Ald the minor component, Z11-16:Ald is the major component in *H. armigera*, with the minor components being Z9-16:Ald and Z9-14:Ald (Table 1; Figure 1; Hillier and Baker, 2016). In this study, *H. armigera* was found to have a 2.1:100 ratio of Z9-16:Ald to Z11-16:Ald, and *H. assulta* a 1739:100 ratio (Table 1; Wang et al., 2005). These ratios were close to the ratios of the precursor compounds for Z9-16:Ald and Z11-16:Ald, Z-9-hexadecanol (Z9-16:OH) and Z-11-hexadecanol (Z11-16:OH), respectively (Wang et al., 2005). The hybrids had a ratio of 4:100, which was closer to the *H. armigera* ratio but still significantly different (Table 1; Wang et al., 2005). Quantities of Z9-16:Ald, Z11-16:Ald, Z9-16:OH, Z11-16:OH, Z11-16:OAc, and Z-9-hexadecanoate (Z9-16:OAc) were also found in the hybrids (Table 1; Wang et al., 2005). Additionally, the relative amounts of Z9-16:OH and Z11-16:OH to Z11-16:Ald in the hybrid were much higher than in *H. armigera*, but their ratio to each other followed a similar pattern (Table 1; Wang et al., 2005). Small quantities of Z9-16:OAc and Z11-16:OAc were found in the hybrid gland extracts, with their ratios being similar to that of their corresponding alcohols in *H. armigera* but different from their

corresponding acetates in *H. assulta* (Table 1; Wang et al., 2005). Based on their analysis of the chemical composition of the pheromone blends and their precursors, the researchers deduced the biosynthetic pathways of Z11-16:Ald and Z9-16:Ald in both species and their hybrid, with the hybrids having a combination of the two pathways (Wang et al., 2005). The ratio of Z9-16:Ald to Z11-16:Ald in the hybrid is regulated by both the Δ11 and Δ9 desaturases, which act on both palmitic acid and stearic acid, respectively, leading to a greater ratio of Z9-16:Acid to Z11-16:Acid (Table 1; Wang et al., 2005). The researchers concluded that the sex pheromone biosynthetic pathways are under polygenic control, with desaturase genes being most primitive in *H. assulta*, followed by *H. zea*, and becoming most complex in *H. armigera* (Wang et al., 2005). The pheromone biosynthesis pathway of hybrids was generally found to be similar to the pathway of *H. zea*, with the hybrid being able to produce Z9-16:Ald as a secondary component much like in parental *H. armigera* (Table 1; Choi et al., 2002; Wang et al., 2005). While this study revealed the composition of the hybrid pheromone blend, no further research has been done to compare the responses of hybrids or parental species to the hybrid blends.

Morphology and sex ratios of *H. armigera* x *H. assulta* crosses

In 2005, Zhao and colleagues documented the development and morphological characteristics of *H. armigera* x *H. assulta* crosses. Out of 492 hybrid larvae resulting from the *H. armigera* female x *H. assulta* male crosses, 374 (76%) completed larval development and pupated, 171 of these (~46%) showing abnormal development (Figure 3; Zhao et al., 2005). While the normal hybrids were all males capable of backcrossing, the abnormal hybrids were sterile with undeveloped or malformed reproductive structures (Figure 3; Zhao et al., 2005). Normal male hybrids showed greater flight activity as adults compared to the parental species (Zhao et al., 2005). Larvae and pupae that grew into abnormal adults took longer to develop (Zhao et al., 2005). Crosses between *H. assulta* females and *H. armigera* males yielded normal offspring with a 1:1 sex ratio which could then be self-mated to form mostly normal offspring with a 1:1 sex ratio (Figure 3; Zhao et al., 2005). When female hybrid offspring from the female *H. assulta* x male *H. armigera* cross were mated with male *H. armigera*, the sex ratio of the resulting offspring was 0.58:1, with males outnumbering females (Figure 3; Zhao et al., 2005). The backcross of female *H. armigera* and the male offspring of female *H. armigera* x male *H. assulta* crosses resulted in a male-biased sex ratio and 43% sterility (Figure 3; Zhao et al., 2005). The sterility of F1 hybrids from crosses between *H. armigera* females and *H. assulta* males, as well as the sterility of two backcross hybrids (*H. armigera* females x F1 males from both reciprocal crosses) may have been due to interactions between the Z chromosome from *H. assulta* and autosomes from *H. armigera* (Zhao et al., 2005). This interaction may also explain the sex biases observed in the backcrosses stated (Zhao et al., 2005). The ability to create a higher ratio of sterile hybrid males through *H. armigera* x *H. assulta* crosses could be beneficial for pest control, but the authors note several limitations (Zhao et al., 2005). First, the presence of normal hybrid males with increased flight capacity could result in introduction of pests to new areas. Second, although the species could mate under laboratory conditions, hybrids have never been found in the wild and various prezygotic isolation barriers exist, including differences in host plant range, pheromone composition, and calling periods (Zhao et al., 2005).

Electrophysiological responses and brain morphology of *H. armigera* x *H. assulta* hybrids

In 2006, Zhao, Yan, and Wang further studied the behavior and electrophysiological responses of *H. assulta* x *H. armigera* crosses to sex pheromone blends. In the field, a 95:5 blend of Z9-16:Ald to Z11-16:Ald was found to be most attractive to *H. assulta* males, while a 97:3 blend was found to be most attractive to *H. armigera* males, with some variation of these ratios occurring in different regions (Table 2; Zhao et al., 2006). In response to the *H. armigera* blend (97:3 Z11-16:Ald to Z9-16:Ald), *H. assulta* males remained inactive, while males of both *H. armigera* x *H. assulta* crosses showed attraction to the blend similar to *H. armigera* (Table 2; Zhao et al., 2006). Very few of the crosses between *H. assulta* and the hybrid offspring of *H. assulta* females x *H. armigera* males responded to the *H. armigera* blend and they behaved similarly to male *H. assulta* (Table 2; Zhao et al., 2006). However, male offspring of crosses between *H. armigera* females x F1RS males (the offspring of *H. armigera* females x *H. assulta* males) and *H. armigera* males x F1SR females (the offspring of *H. assulta* female x *H. armigera* males) showed attraction to the sex pheromones, with the number of moths responding in the former group being very close to *H. armigera* (Table 2; Zhao et al., 2006). When electroantennograms (EAGs) were recorded in response to the *H. assulta* and *H. armigera* blends, the only significant differences were found to be between F1RS males, who showed lower responses than *H. assulta* and *H. armigera* at 10^4 and 10^5 ng/μl concentrations (Table 2; Zhao et al., 2006). EAGs were also recorded in response to individual components of pheromone blends (Table 2; Zhao et al., 2006). In *H. assulta* males, both Z9-16:Ald and Z11-16:Ald evoked dose-dependent responses but were especially high in the 10^3–10^5 ng/μl range (Table 2; Zhao et al., 2006). In *H. armigera* males, only Z11-16:Ald evoked dose-dependent responses, while Z9-16:Ald evoked weak responses (Table 2; Zhao et al., 2006). The normal F1 hybrids with both sex combinations showed similar dose-dependent responses to both Z11-16:Ald and Z9-16:Ald, with Z11-16:Ald evoking higher responses in each (Table 2; Zhao et al., 2006). Abnormal offspring from the female *H. armigera* x male *H. assulta* cross showed no significant responses to either compound (Table 2; Zhao et al., 2006). Comparisons between groups at the 10^5 ng/μl dose showed that *H. assulta* males showed significantly higher responses to Z9-16:Ald than *H. armigera*, while the response of male hybrids was similar to *H. armigera* males (Table 2; Zhao et al.,

2006). Male offspring from the backcross of *H. assulta* females x F1RS males and *H. assulta* males x F1SR females responded more strongly than *H. armigera* males, with the former cross showing a lower response than *H. assulta* but the latter exhibiting a similar response to *H. assulta* (Table 2; Zhao et al., 2006). Males of the F1SR female x *H. armigera* male and F1RS male x *H. armigera* female cross showed responses to Z9-16:Ald similar to male *H. armigera* (Table 2; Zhao et al., 2006). Males of the F1 hybrids with both sex/species combinations exhibited an intermediate response to Z11-16:Ald between that of the parent species (Table 2; Zhao et al., 2006). Males from the backcrosses of female *H. assulta* x F1RS males and male *H. assulta* x F1SR females had similar Z11-16:Ald responses to male *H. assulta*, while the response of males from the back crosses *H. armigera* males x F1SR females and *H. armigera* females x F1RS males were similar to those of male *H. armigera* (Table 2; Zhao et al., 2006). Flight experiments indicated that *H. armigera* genes tended to be dominant in the inheritance of sex pheromone responses, particularly to Z9-16:Ald (Table 2; Zhao et al., 2006).

Since *H. assulta* females from different geographic areas have different sex pheromone blend ratios and males different responses, differences in blend specificity between regions may cause diversification of species. Alternatively, lower specificity in *H. armigera* could lead to widespread random mating, thereby reducing diversification. Males of *H. assulta* and *H. armigera* showed no significant differences in EAG responses, but *H. assulta* males still showed strong dose-dependent responses to Z9-16:Ald, and *H. armigera* a much weaker response (Table 2; Zhao et al., 2006). OSNs of *H. assulta* were tuned to Z9-16:Ald and co-occurred in the sensilla with neurons tuned to Z9-14:Ald, the secondary pheromone component in the blend of *H. virescens* females (Figure 1; Zhao et al., 2006). Few or none of these Z9-16:Ald-tuned neurons existed in *H. armigera* (Figure 1; Zhao et al., 2006). In the case of Z11-16:Ald, males of both species displayed strong dose-dependent EAG responses, while males of both F1 hybrids showed intermediate responses compared to the parental generation (Table 2; Zhao et al., 2006). Overall, *H. armigera* males are mostly sensitive to Z11-16:Ald, while male *H. assulta* are sensitive to both Z11-16:Ald and Z9-16:Ald (Table 2; Figure 1; Zhao et al., 2006). The researchers concluded that genes on autosomal chromosomes, not sex chromosomes or cytoplasmic factors, are likely responsible for sex pheromone responses in hybrid offspring, but that further research was needed to conclusively determine the pattern of inheritance (Zhao et al., 2006).

In 2017, Xu and colleagues did further work on inheritance of the pheromone sensory system in *H. armigera* and *H. assulta*. The researchers compared olfactory responses to pheromone components in the periphery and antennal lobes of males and found that pheromone responses to two sex pheromones were controlled by a major gene, with the allele from *H. armigera* being dominant as suggested by Zhao, Yan, and Wang in 2006 (Zhao et al., 2006; Xu et al., 2017). The male specific sensilla found in both species were also found in the male hybrids and backcrosses, in addition to two new subtypes of sensilla with broader responses (Xu et al., 2017). They hypothesized that when female *H. armigera* and male *H. assulta* hybridize, male hybrids can successfully backcross with female *H. armigera*, leading to introgression from *H. assulta* into *H. armigera* (Figure 3; Xu et al., 2017). All *H. armigera* individuals had more A type sensilla than C type, while the opposite was found in *H. assulta* (Table 3; Xu et al., 2017). The F1 (female *H. armigera* x male *H. assulta*) and BC2 (F1 x female *H. assulta*) exhibited the same sensilla patterns as *H. armigera*, but the BC1 (F1 x female *H. armigera*) backcrosses were comprised of approximately half *H. armigera*-like individuals and half *H. assulta*-like individuals (Table 3; Xu et al., 2017). While the Type A sensilla in *H. armigera* and *H. assulta* only respond to Z11-16:Ald, 8% of the

TABLE 3 Sensillar types and the compounds to which they are responsive in *H. armigera*, *H. assulta*, and their F1 hybrids and BC1 (F1 x female *H. armigera*) or BC2 (F1 x female *H. assulta*) backcrosses as studied by Xu et al. (2017).

	Type A	Expanded A	Type B1	Type B2	Type C1	Type C2	Type C3	Expanded C
H. armigera	***Z11-16:Ald***			*Z9-14:Ald* Z11-16:OH	Z9-16:Ald *Z9-14:Ald* Z11-16:OH Z11-16:OAc	Z9-16:Ald *Z9-14:Ald* Z11-16:OH		
H. assulta	*Z11-16:Ald*		*Z9-14:Ald*	*Z9-14:Ald* Z9-16:OH	***Z9-16:Ald*** ***Z9-14:Ald*** ***Z9-16:OH*** ***Z11-16:OH***	**Z9-16:Ald** ***Z9-14:Ald*** **Z9-16:OH**	**Z9-16:Ald** ***Z9-14:Ald***	
F1	***Z11-16:Ald***	*Z11-16:Ald* Z9-14:Ald Z11-16:OH		Harm: *Z9-14:Ald* Z11-16:OH	Hass: Z9-16:Ald *Z9-14:Ald* Z9-16:OH Z11-16:OH Harm: Z9-16:Ald *Z9-14:Ald* Z11-16:OH Z11-16:OAc	Harm: Z9-16:Ald *Z9-14:Ald* Z11-16:OH		Z9-16:Ald *Z9-14:Ald* Z9-16:OH Z11-16:OH Z11-16:OAc

(Continued)

TABLE 3 Continued

	Type A	Expanded A	Type B1	Type B2	Type C1	Type C2	Type C3	Expanded C
BC1	***Z11-16:Ald***		*Z9-14:Ald*	Harm: *Z9-14:Ald* Z11-16:OH Hass: *Z9-14:Ald* Z9-16:OH	**Hass: Z9-16:Ald *Z9-14:Ald* Z9-16:OH Z11-16:OH**	**Hass: Z9-16:Ald *Z9-14:Ald* Z9-16:OH Harm: Z9-16:Ald *Z9-14:Ald* Z11-16:OH**		
BC2	***Z11-16:Ald***			Harm: *Z9-14:Ald* Z11-16:OH Hass: *Z9-14:Ald* Z9-16:OH	Harm: Z9-16:Ald *Z9-14:Ald* Z11-16:OH Z11-16:OAc	Harm: Z9-16:Ald *Z9-14:Ald* Z11-16:OH		Z9-16:Ald *Z9-14:Ald* Z9-16:OH Z11-16:OH Z11-16:OAc

The bolded compounds represent the sensillar type with the most dominant response profile (either Type A or Type C) while the italicized compounds represent the chemical to which the sensillar type had the greatest EAG response.

Type A sensilla in F1 (named expanded A), responded to Z11-16:OH and Z9-14:Ald (Table 3; Xu et al., 2017). The Type C sensilla were divided into two subgroups in *H. armigera* (Harm C1, Harm C2) and three in *H. assulta* (Hass C1, Hass C2, Hass C3), and all were found in F1 and all backcrosses (Table 3; Xu et al., 2017). An "expanded C" subtype of sensilla was discovered F1 and BC2, which were responsive to a wider array of compounds than Type C sensilla (Table 3; Xu et al., 2017). In F1, 35.6% of Type C sensilla had *H. armigera*-like responses, 46.7% *H. assulta*-like responses, while 17.8% were the extended C type (Table 3; Xu et al., 2017). In BC1 and BC2, the majority of Type C sensilla responded as *H. assulta* and *H. armigera*, respectively (Table 3; Xu et al., 2017). Calcium-imaging studies of male brains showed that the α glomerulus (responsive to Z11-16:Ald) was the largest in *H. armigera*, while the β glomerulus (responsive to Z9-16:Ald) was largest in *H. assulta* (Xu et al., 2017). In F1 and BC2, male brains had similar topography and α:β glomerulus ratio to *H. armigera* (Xu et al., 2017). In BC1 males, half were more similar to *H. assulta* and the other half to *H. armigera* regarding the same criteria (Xu et al., 2017). The macroglomerular complexes (MGC) of parents and hybrids were classified into α, β, and γ glomeruli and 3D imaging showed that F1 had a similar topography to *H. armigera*, although the β glomerulus was larger in F1 (Xu et al., 2017). Overall, the F1 and BC2 sex pheromone responses were similar to *H. armigera*, albeit with some broader responses (Xu et al., 2017). The researchers suggest that the expanded Type C sensilla, which responded to five compounds in comparison to three or four in the parental species, could be a product of genetic recombination (Xu et al., 2017). Two different pheromone receptors co-expressed or co-localized in the Type C olfactory sensory neurons (OSNs) could change the compounds to which they are tuned (Xu et al., 2017).

The work of Xu et al. (2017) provides a link between changes in sensillar profiles and antennal lobe morphology, proving that hybridization can alter olfactory pathways at both levels of the nervous system (peripheral and central). The emergence of a new subtype of sensilla could provide hybrids with an adaptational advantage; since the pheromone blends of females are under less selective pressure and therefore more susceptible to variation, the ability of males to detect these new variations could improve their fitness (Xu et al., 2017). Female *H. armigera* x male *H. assulta* hybrids are easier to produce than the opposite hybrid, and these hybrids are more likely to be attracted to *H. armigera* females based on their responses to their blend (Xu et al., 2017). These factors, in combination with the high success of the BC2 backcross, mean BC2 would likely be the dominant cross in the wild (Xu et al., 2017). Since BC2 individuals have an *H. armigera*-like male olfactory system and female sex pheromone synthesis, they could potentially backcross with *H. armigera*, allowing *H. assulta* genes to introgress, new types of sensilla with broader tuning to emerge, and *H. armigera* fitness to increase (Xu et al., 2017). The success of *H. armigera* as an Old World pest may be attributed to similar mechanisms, and their increased fitness under these circumstances could lead to evasion of existing pest management strategies reliant on pheromones (Xu et al., 2017). Furthermore, the potential introgression of olfactory receptor genes and the presence of more broadly-tuned sensillae could lead to altered sensitivity to novel pheromone blends, resulting in changes in male preference and eventual speciation within hybrid zones (Xu et al., 2017).

Genetics of *H. armigera* x *H. assulta* crosses

To date, the only genetic study done on *H. armigera* x *H. assulta* crosses was by Guo and colleagues in 2022, analyzing the connection between gene expression and the divergence of pheromone sensing in the two species (Guo et al., 2022). Allele-specific expression of cis-regulatory genes (segments of non-coding DNA which regulate transcription of neighboring genes, such as enhancers, promoters, or silencers) and trans-regulatory genes (segments of coding DNA which regulate transcription of distant genes) was monitored in F1 hybrids (Wang et al., 2019; Guo et al., 2022). The crosses of *H. armigera* females and *H. assulta* males produced some fertile males, some abnormal males, and all

abnormal females, while the reciprocal cross produced fertile males and females (Figure 3; Guo et al., 2022). Parental males and normal hybrid males from both crosses had strong electrophysiological responses to Z11-16:Ald, but abnormal males had a very low response (Guo et al., 2022). For Z9-16:Ald and Z9-14:Ald, *H. assulta* males had the strongest responses, abnormal male hybrids almost no response, and all other hybrid males intermediate responses (Guo et al., 2022). Transcriptome reads identified greater expression of *H. assulta* genes in all F1 hybrids, suggesting that more cis-regulatory gene changes are fixed in this species (Guo et al., 2022). Principal component analysis revealed large variances between sterile and normal hybrids, and *H. armigera* genes were more variable in their expression in sterile F1 hybrids and *H. armigera* female x *H.assulta* male hybrid males (Guo et al., 2022). The most variable genes in the abnormal hybrids had functions relating to sex pheromone detection, reproduction, and development, which are likely the cause of differences in pheromone perception, sterility, and abnormal morphology in these hybrids (Guo et al., 2022). Abnormal hybrids had more mis-expressed genes in their antennae, particularly genes with pheromone reception, explaining their low electrophysiological responses (Guo et al., 2022). Even normal hybrids of the *H. assulta* female x *H. armigera* male crosses had abnormal gene expression in antennae (Guo et al., 2022). Incompatibility of genes on the chromosomes of F1 hybrids was the likely cause of mis-expressed genes, leading to developmental defects and divergences in regulatory patterns in different hybrids (Guo et al., 2022). Antennal gene expression was sexually dimorphic, with many all cis-regulatory genes being more expressed in males and more trans-regulatory genes being expressed in females, potentially contributing to sexual dimorphism in pheromone detection (Guo et al., 2022). Trans-regulatory genes are more pleiotropic, usually affecting multiple phenotypic traits and leading to increased variation (Guo et al., 2022). Female antennae must be adapted to a wide range of host plant volatiles, making trans-regulatory genes more appropriate for this function, while the more tightly-regulated cis-regulatory genes in male antennae are more conducive to being finely-tuned to species-specific female sex pheromone blends (Guo et al., 2022). Cis-regulatory elements generally have larger and faster effects on gene expression, which could be advantageous for males if changes in female sex pheromone blends require rapid adaptation *via* changing the expression of ORs (Guo et al., 2022). Hybrid males were the most accurate predictors of regulatory patterns, particularly the *H. armigera* female x *H. assulta* male hybrids, so these hybrids were used to study regulatory patterns of olfactory receptor genes (i.e., ORs, odorant binding proteins, sensory neuron membrane proteins) (Guo et al., 2022). The results suggested that cis-regulation of genes is an important factor in differences in olfactory sensation between *H. armigera* and *H. assulta* males, particularly *HarmOR13* (responsible for detecting Z11-16:Ald the major component of the *H. armigera* blend) and *HassOR14* (responsible for detecting Z9-16:Ald, the major component of the *H. assulta* blend), which were both cis-regulated and more highly expressed in the hybrid antennae than their counterparts in the other parental species (Table 1; Guo et al., 2022). However, it is important to consider that other non-OR genes or transcription factors may be important in male adaptation and could take effect even before changes in female pheromone blends occur (Unbehend et al., 2021). As such, many yet-to-be identified genes and gene functions may influence olfactory sensitivity and pheromone preference. For example in *Ostrinia nubilalis*, the cis-regulatory element bric à brac, encoded by the *bab1* gene expressed early in antennal development, is the key locus for variation in male preference (Unbehend et al., 2021).

Heliothis virescens and Heliothis subflexa

First laboratory studies of *H. virescens* x *H. subflexa* hybrids

Arguably the most-studied heliothine hybrid is the *Heliothis virescens* x *Heliothis subflexa* cross. The hybrid was first investigated by Laster (1972) as a potential method for controlling crop damages *via* genetic suppression, with the two species being chosen due to their close relation and overlapping ranges in Mississippi, USA, although they do overlap in other parts of the Americas (Laster, 1972; Hillier and Baker, 2016). While *H. virescens* females and *H. subflexa* males mated but did not produce offspring in Laster's experiments, *H. subflexa* females and *H. virescens* males readily mated and produced offspring (F1) (Figure 4; Laster, 1972). Male offspring were sterile, and backcrossing hybrid females (F1) with *H. virescens* males passed this sterility onto their male offspring (BC1) (Figure 4; Laster, 1972). Using *H. virescens* males in the crosses always produced offspring, while all other combinations of *H. virescens*, *H. subflexa*, F1, BC1, and BC2 individuals did not result in offspring (Figure 1; Laster, 1972). This data provided evidence that control of *H. virescens* could be achieved using sterile F1 males from the *H. subflexa* female x *H. virescens* male cross (Figure 4; Laster, 1972). However, since F1 females could mate with *H. virescens* males, the authors suggested that characteristics of their offspring (such as pesticide resistance and feeding habits) needed to be monitored in case F1 females were accidentally released during sterile-male programs (Figure 4; Laster, 1972).

Following the discovery of this hybrid by Laster (1972); Proshold and Lachance (1974) investigated the fertility of hybrids, as well as the genetic and physiological mechanisms underlying their sterility. Both parental crosses from the Laster (1972) experiment were studied, and the hybrids resulting from these crosses were backcrossed to males or females of the parental species, although only offspring resulting from the hybrids crossed with *H. virescens* were used for further backcrosses (Figure 4; Proshold and Lachance, 1974). The progeny of hybrids x *H. virescens* were BC1 while the progeny of BC1 x *H. virescens* were BC2 (Figure 4; Proshold and Lachance, 1974). From replications of the parental crosses, all produced offspring, but the

H. subflexa females x *H. virescens* males were the most successful hybrid cross, with 75.4% mating and 56% of females producing progeny (Figure 4; Proshold and Lachance, 1974). Both types of hybrid larvae had similar survival rates as *H. virescens* larvae, with 87% pupating (Proshold and Lachance, 1974). Male F1 hybrids from both parental crosses mated readily with females of either species (89.6–97.2% of the time), but hybrid males backcrossed with *H. virescens* females tended to mate more frequently (~3 times) than those backcrossed with *H. subflexa* females (~2 times) (Figure 4; Proshold and Lachance, 1974). Although hybrid males from the *H. virescens* male x *H. subflexa* female cross were more fertile (2–6.2% egg hatch) than the *H. virescens* female x *H. subflexa* male cross (<1%), none of these males could induce a normal egg-laying response in the females they mated with (Proshold and Lachance, 1974). Most hybrid males from both crosses were sterile since they could not transfer eupyrene (nucleated) sperm to females to fertilize their eggs (Proshold and Lachance, 1974). Regarding female F1 hybrids, those from the *H. subflexa* female x *H. virescens* male cross had low mating success and egg-laying, but high percentages of eggs hatching (Figure 4; Proshold and Lachance, 1974). Female F1 hybrids from the *H. subflexa* male x *H. virescens* female cross were more successful at mating and egg-laying but had only moderate egg-hatching rates (Figure 4; Proshold and Lachance, 1974). The BC1 male offspring from crosses of hybrid females (*H. virescens* males x *H. subflexa* females) and male *H. virescens* were sterile or semi-sterile, delivering no or irregular sperm in most instances, but BC1 females were fertile (Figure 4; Proshold and Lachance, 1974). Crosses between BC1 females x *H. virescens* males produced sterile males and fertile females (BC2), while crosses between BC1 males x *H. virescens* females produced fertile males and females (Figure 4; Proshold and Lachance, 1974). The cross between *H. virescens* females x *H. subflexa* males became more fertile with each generation, so BC2 individuals were partially or fully fertile (Figure 4; Proshold and Lachance, 1974). When hybrid females and their female offspring were crossed with *H. virescens* males, male offspring showed increased fertility (Figure 4; Proshold and Lachance, 1974). Both *H. virescens* and *H. subflexa* males had 2n = 62 chromosomes, and thus each gamete should contain 31 chromosomes (Proshold and Lachance, 1974). However, only 20–28 chromosomes were found in all types of hybrid males, while the other chromosomes were unpaired (Proshold and Lachance, 1974). The lack of pairing was attributed to desynapsis, a failure in the fusion of chromosome pairs due to homologous chromosomes separating after meiosis (Proshold and Lachance, 1974). Overall, the researchers found that reproductive capacity in both male and female hybrids was reduced, with males being sterile or semi-sterile and females having reduced fecundity or fertility (Proshold and Lachance, 1974). Male sterility was attributed to the absence of eupyrene sperm in females who mated with hybrid males, possibly due to lower production or abnormal activity of the sperm (Proshold and Lachance, 1974). These deficiencies were attributed to desynapsis, as continued backcrossing of fertile BC1 hybrid females reintroduced chromosomal homology, allowing some BC2 males to be fertile (Figure 4; Proshold and Lachance, 1974). Desynapsis was also the explanation for reduced egg hatches in hybrid females (Proshold and Lachance, 1974).

Mating experiments further indicated that the female mating urge was not adequately served by hybrid males, particularly in the case of female *H. subflexa* which tended to only mate with one normal male but had to mate several times with hybrid males (Proshold and Lachance, 1974). The authors suggested that hybrid females could be used to suppress *H. virescens* males in the field since they produced sterile male offspring when crossed (Proshold and Lachance, 1974). However, key differences in the mating behaviors and fertility of each hybrid pose challenges. While the female *H. subflexa* x male *H. virescens* hybrid females tended to enter diapause more often than regular females and mated poorly with normal males, they did produce sterile BC1 males and fertile BC1 females, who could then be crossed with male *H. virescens* to produce more sterile BC2 male hybrids (Figure 4; Proshold and Lachance, 1974). Alternatively, while the female *H. virescens* x male *H. subflexa* hybrids did not diapause, mated readily, and had reduced fertility, BC1 and BC2 offspring could have fertility restored, making them less suitable for population control (Figure 4; Proshold and Lachance, 1974).

In a 1975 follow-up study, Proshold and LaChance studied sperm production and transfer by the *H. subflexa* x *H. virescens* hybrid males compared to their parent species (Proshold et al., 1975). While both parent and hybrid males produced the same number of spermatophores, when the hybrids mated with females, only apyrene (non-nucleated) sperm were delivered to the female spermatheca (Proshold et al., 1975). Although it would seem that hybrid males incapable of delivering viable sperm to females would have no influence on female oviposition and calling, and thus no benefit in population suppression, hybrid males could make up for this insufficiency with hybrid vigor, outcompeting irradiated males traditionally used for population control (Proshold et al., 1975). Further research the same year concluded that abnormal morphology of hybrid male sperm (such as the presence of two-tailed sperm) and lower counts of eupyrene sperm were likely the cause of their sterility (Richard et al., 1975). This would later be corroborated by a 1977 study by Karpenko and Proshold, which showed that females crossed with BC1 males (the offspring of crosses between hybrid females and *H. subflexa* males) did not often receive eupyrene sperm, although females mated with BC2 males were twice as likely to receive eupyrene sperm than from BC1 males (Figure 4; Karpenko and Proshold, 1977). Laster and colleagues were able to maintain sterile hybrid males and fertile hybrid females over 40 generations by mating backcross hybrid females with *H. virescens* males (Figure 4; Laster et al., 1976). Their research led to the development of a potential population suppression strategy: fertile hybrid females released in sufficient numbers could outcompete native *H. virescens* females, mating with normal *H. virescens* males to create more sterile male hybrids (Figure 5; Laster et al., 1976; Parvin et al., 1976). The principle was that over many generations, more normal *H. virescens* females would die unmated and sterile males would dominate, leading to death of the native *H. virescens* population (Figure 5; Laster et al.,

1976; Parvin et al., 1976). Their model was further supported by the discovery that offspring of hybrid crosses had mating behaviors similar to those of normal *H. virescens* and that pheromone blends of both hybrid females and *H. virescens* females were equally attractive to *H. virescens* males in trapping experiments (Laster et al., 1977; Pair et al., 1977; Laster et al., 1978). Furthermore, female progeny of backcrosses between *H. virescens* males and hybrid females (*H. subflexa* female x *H. virescens* male) were found to attract *H. virescens* males equally as well as normal *H. virescens* females (Figures 4, 5; Tingle et al., 1978). Male progeny of these backcrosses were attracted to both *H. virescens* females and their pheromone extracts, while F1 hybrid males did not respond to the pheromone (Tingle et al., 1978). However, in 1979, Makela and Huettel made amendments to the Laster-Parvin model, as Laster and colleagues had incorrectly assumed that *H. virescens* populations would be driven to extinction regardless of the ratio of hybrid to native moths upon release (Figures 5, 6; Laster et al., 1976; Parvin et al., 1976; Makela and Huettel, 1979). Makela and Huettel proposed that in the Laster-Parvin model, the ratio of hybrid females to native *H. virescens* would remain constant, assuming no immigration occurs (Figures 5, 6; Makela and Huettel, 1979). With this assumption in mind, the success of the model would be dependent on the insemination rate of *H. virescens* males and the growth rate of the population (Figures 5, 6; Makela and Huettel, 1979). In application, migration would likely decrease the ratio of hybrid females and backcross offspring to native *H. virescens*, leading to diminishing hybrid populations and thus a diluted effect (Figures 5, 6; Makela and Huettel, 1979). Furthermore, the effects of selection against hybrid females and assortative mating in the wild would need to be considered (Figures 5, 6; Makela and Huettel, 1979). To maintain the population suppression effects of the hybrids, several releases of fertile hybrid females would likely need to be done with careful monitoring of the ratio of the hybrid and native populations throughout the program (Figures 5, 6; Makela and Huettel, 1979).

The viability of the Laster-Parvin model (and the further corrections by Makela and Huettel) were investigated by Cibrian-Tovar and Mitchell, specifically by comparing the courtship behaviors of parental *H. subflexa* and *H. virescens* to their hybrids and backcrosses (Figures 5, 6; Cibrian-Tovar and Mitchell, 1991). Mating behaviors of male and female *H. subflexa*, *H. virescens*, and backcross hybrids (*H. virescens*

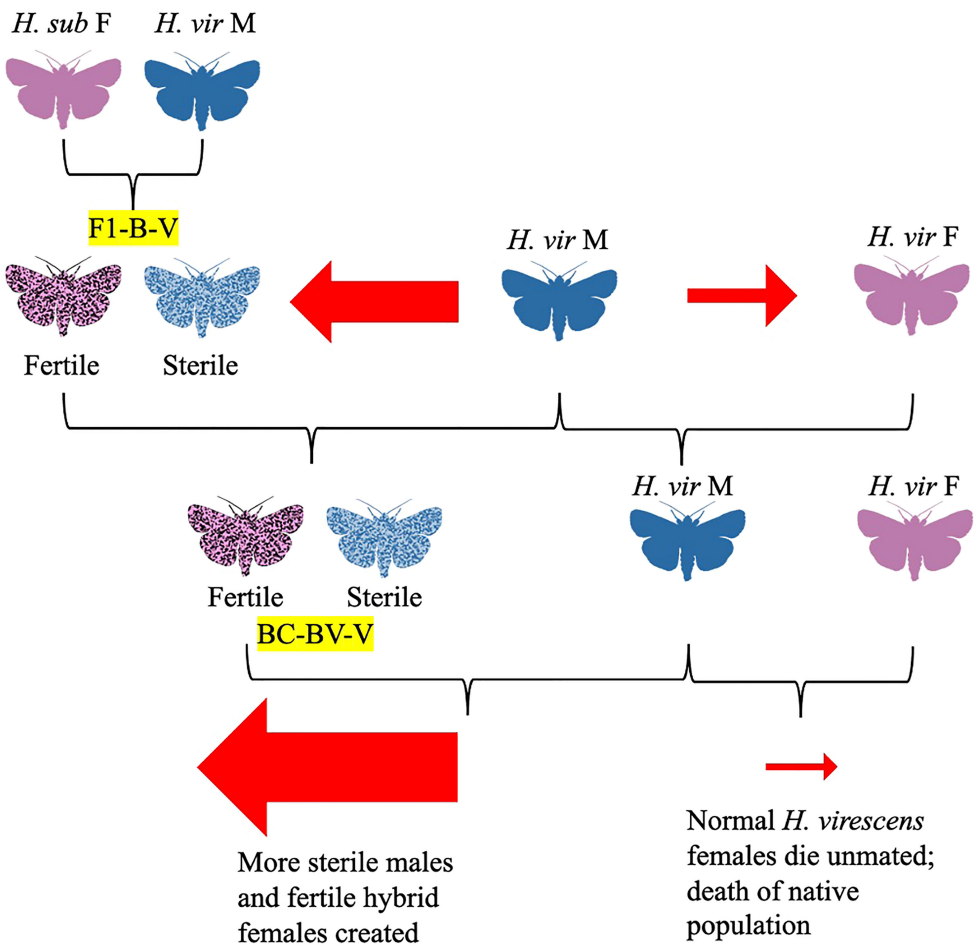

FIGURE 5
Diagram depicting the Laster-Parvin Model (Laster et al., 1976; Parvin et al., 1976). According to this model, the hybrid female to native female ratio would increase over generations.

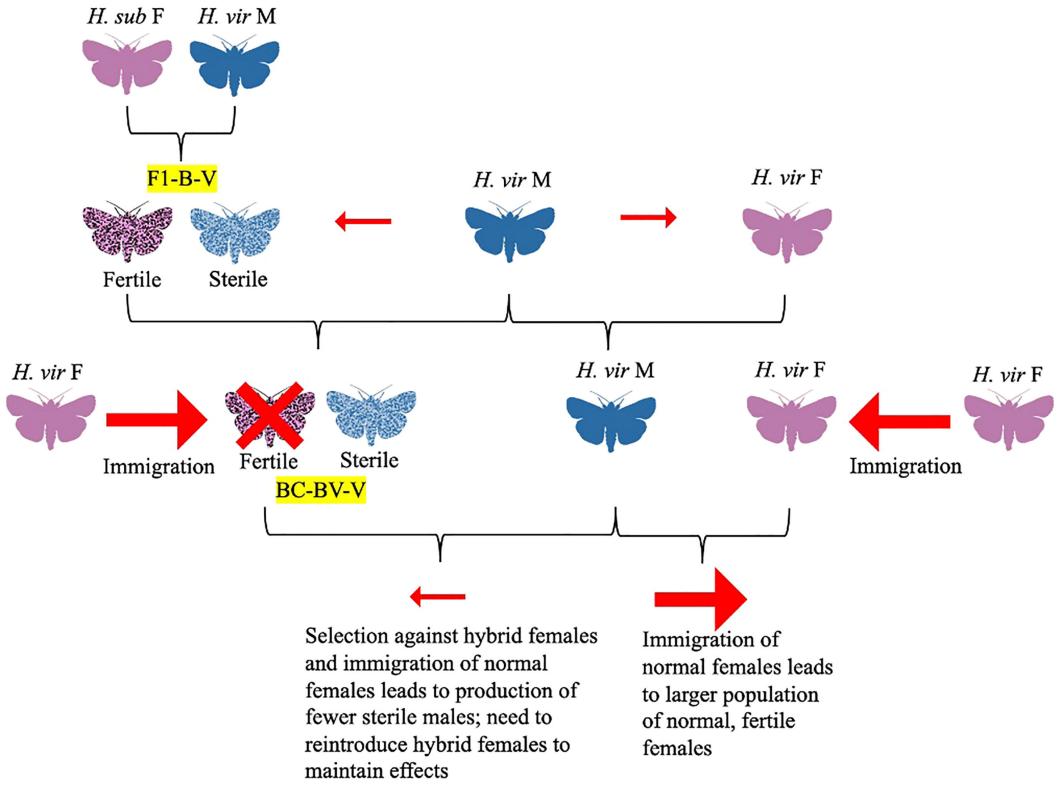

FIGURE 6
Diagram depicting the Makela-Huettel Model (Makela and Huettel, 1979). In the Laster-Parvin model, the hybrid female to native female would be constant over generations, assuming no immigration of females (Laster et al., 1976; Parvin et al., 1976). However, the Makela-Huettel model proposes that the success of the hybrid sterility program would depend on the insemination rate of *H. virescens* males and growth rate of the population (must be hybrid>native). Immigration of native females would likely decrease the hybrid female to native female ratio. Selection against hybrid females and assortative mating also need to be assessed. To maintain the effects of the hybrid sterility program, several releases of hybrid females (ideally from BC7 or greater) should be accompanied by monitoring of hybrid to native female ratio.

males mated with hybrid females derived from *H. virescens* males x *H. subflexa* females) were observed in a wind tunnel (Figure 4; Cibrian-Tovar & Mitchell, 1991). When observing pre-copulatory calling behavior, backcross females exhibited similar behaviors to normal *H. virescens* females, and their calling period overlapped with that of *H. subflexa* females, making the calling periods of the backcrosses and parental species indistinguishable (Cibrian-Tovar and Mitchell, 1991). Backcross males were much less active than *H. virescens* males, and in mating experiments, 20–25% were not responsive to calling virgin *H. subflexa* or backcross females (Cibrian-Tovar and Mitchell, 1991). Similar to *H. virescens* males but unlike *H. subflexa* males, backcross males did not touch the ovipositors of calling females with their antenna, and likely only attracted females with displays from hair-pencil glands, structures which secrete sex pheromones (Cibrian-Tovar and Mitchell, 1991). Only one-third of backcross males mated successfully, their poor performance attributed to their relative flight inactivity, inability to move parallel to the calling females, and improper copulation (Cibrian-Tovar and Mitchell, 1991). Many unsuccessful males spent more time copulating or sitting on the walls of the wind tunnel than successful males (Cibrian-Tovar and Mitchell, 1991). When comparing between *H. virescens*, *H. subflexa*, and backcross insects, their flight orientation, landing, and copulatory behaviors were very similar, but they differed mostly in closer-range behaviors (Cibrian-Tovar and Mitchell, 1991). Because backcross males from earlier generations (1st to 6th) were found to be much more inactive than their parental species, while later generations (7th and beyond) were comparable to normal *H. virescens* males, the authors suggested the use of 7th generation or greater backcross hybrids for field suppression of *H. virescens*, as well as further research into post-mating behaviors (Figure 4; Cibrian-Tovar and Mitchell, 1991).

Abnormalities in sperm and consequences for sterility of *H. virescens* x *H. subflexa* hybrids

Throughout the 1980s, further evidence was gathered indicating abnormal sperm as a contributor to hybrid sterility (Goodpasture et al., 1980; Lachance and Karpenko, 1983; Miller et al., 1986; Lachance and Olstad, 1988; Degrugillier, 1989;

Degrugillier and Newman, 1993). In 1980, Goodpasture and colleagues found that chromosome pairing remained normal in later generations of *H. virescens* x *H. subflexa* backcrosses and the presence of multiple-tailed sperm diminished (Goodpasture et al., 1980). However, sperm production remained abnormal to the point where parental hybrid males (*H. subflexa* females x *H. virescens* males) could be distinguished from backcross males (F1 hybrid females x *H. virescens* males) by sperm morphology (Figure 4; Goodpasture et al., 1980). The authors suggested that these abnormalities could be used to screen for normal and hybrid males in field experiments and hybrid sterility programs (Goodpasture et al., 1980). In 1983, LaChance and Karpenko subjected backcross males to antiviral treatments and extreme heat and still detected hybrid sterility, suggesting that a microorganism was not responsible for hybrid sterility (Lachance and Karpenko, 1983). In 1986, Miller and colleagues presented evidence that abnormalities in mitochondrial function, particularly RNA metabolism, played a factor in hybrid male sterility (Miller et al., 1986). This was corroborated by a 1988 study by LaChance and Olstad which found abnormal mitochondrial derivatives in many backcross males (Lachance and Olstad, 1988). Studies by Degrugillier (1989) and Degrugillier and Newman (1993) pointed to the presence of virus-like particles (VLPs) in the spermatocyst and follicle cells of *H. virescens*, *H. subflexa*, F1 (*H. virescens* males x *H. subflexa* females and the reciprocal cross), and backcross males (F1 females x *H. virescens* males) (Figure 4; Degrugillier, 1989; Degrugillier and Newman, 1993). In the parental species, infection was dependent on age, ranging from 4–8% of young males to 90% in older males, while prevalence in backcross males was 100% (Degrugillier, 1989). There was a high correlation between the presence of VLPs in certain males and abnormal sperm, and many F1 and BC1 males presented with multiple-tailed eupyrene sperm (Figure 4; Degrugillier and Newman, 1993). The VLPs were hypothesized to be fragments of a hereditary virus that has become integrated into the genomes of various *Heliothis* and *Helicoverpa* species *via* retrotransposons (Degrugillier and Newman, 1993). Despite this, the authors also suggest that VLPs could appear in backcross spermatocysts secondarily due to stressors, such as the presence of organisms resembling rickettsia bacteria in the testes and sperm, although this would contradict the findings of Lachance and Karpenko (1983).

Genetics, electrophysiology, and behavior in relation to sex pheromones and hair-pencil compounds in *H. virescens* x *H. subflexa* hybrids

In addition to reproductive isolating mechanisms such as gametic isolation, hybrid sterility, and hybrid viability, the effects of behavioral isolation (specifically sex pheromone blend composition) have been investigated in relation to the hybridization of *H. subflexa* and *H. virescens*. In 1993, Teal and Oostendorp studied the inheritance of hair-pencil glands in *H. subflexa* x *H. virescens* hybrids (Teal and Oostendorp, 1993). Hair-pencil glands are structures found on some male lepidopterans which are responsible for secreting sex pheromones that attract conspecific females and repel conspecific males (Hillier and Vickers, 2004; Hillier and Baker, 2016). Male *H. virescens* have hair-pencil glands, while male *H. subflexa* do not (Brazzel et al., 1953). Their hybrids and some backcrosses were found to exhibit hair-pencil glands similar to those of *H. virescens*, suggesting that the presence of hair-pencils is a dominant sex-linked trait of males controlled by the Z chromosome (Teal and Oostendorp, 1993). Despite this, a 1995 follow-up study by the pair found that production of hair-pencil pheromones was under autosomal control and that pheromone blends of backcross males were actually more similar to *H. subflexa* in some cases (Teal and Oostendorp, 1995). The hair-pencil glands of parental *H. virescens* males had 16:OAc and 16:OH in a 4:1 ratio as their primary components, while *H. subflexa* males showed minute amounts of 16:OH and traces of other compounds in their abdomens, although none were characteristic of the species (Teal and Oostendorp, 1995). The average ratio of 16:OAc and 16:OH in *H. subflexa* female x *H. virescens* male hybrids, their reciprocal cross, and backcross males was approximately 1:4, showing greater similarity to *H. subflexa* males in all cases (Figure 4; Teal and Oostendorp, 1995). Therefore, the production of these compounds was deduced to be under the control of dominant autosomal genes from the *H. subflexa* genome. This work in male hybrids was compared to previous work by Klun and colleagues (1982) in hybrid females, in which females produced from crosses between *H. subflexa* females x *H. virescens* males and backcrosses of these hybrid females to *H. virescens* males were shown to produce sex pheromone blends similar to *H. virescens* females (Figure 4; Klun et al., 1982; Teal and Oostendorp, 1995). Thus, the authors concluded that unlike males, the regulation of sex pheromone production in hybrid females is under the control of dominant autosomal genes from the *H. virescens* genome (Klun et al., 1982; Teal and Oostendorp, 1995). Later research would confirm that such dominant genes include those which control the ratios of 14-carbon aldehydes in the female pheromone blend and the activity of several biosynthetic enzymes in the female pheromone glands (acetyl transferase, desaturases, and a fatty acyl reductase or alcohol oxidase) (Teal and Tumlinson, 1997; Groot et al., 2009b). Further research into pheromone production by Teal and Oostendorp found that while F1 females from the *H. subflexa* females x *H. virescens* males cross had similar pheromone blends to *H. virescens* females, they have no periodicity in their calling period, which could explain the poor mating efficacy of this hybrid as previously documented, although not all hybrid females are affected (Figure 4; Proshold and Lachance, 1974; Karpenko and Proshold, 1977; Teal and Oostendorp, 1995). However, in the reciprocal cross (*H. subflexa* male x *H. virescens* female), periodicity of pheromone calling

matched the parental species, which the authors hypothesized was the likely cause of their mating success with both (Figure 4; Proshold and Lachance, 1974; Teal and Oostendorp, 1995). Only when the hybrid females were injected with pheromonotropic substances could they adequately produce blends in amounts similar to or greater than the parental species, suggesting the presence of hybrid vigor (Teal and Oostendorp, 1995). The inadequate production and mature eggs observed in these hybrids lead the authors to conclude that hybridization could lead to reproductive arrest, perhaps due to a lack of juvenile hormone (Proshold and Lachance, 1974; Teal and Oostendorp, 1995).

Although previous work had proven that the offspring of hybrid females backcrossed with *H. virescens* males could produce a pheromone blend identical to normal *H. virescens* females, and thus compete with these normal females in hybrid sterility-based control programs (Klun et al., 1982; Cibrian-Tovar and Mitchell, 1991; Teal and Tumlinson, 1997), and that hybrid males could produce hair-pencil compounds (male courtship pheromones) similar to those of *H. subflexa*, comprehensive behavioral studies were lacking. In 2006, Vickers investigated the effects of female blend composition on the responses of hybrid males. In the wild, reproductive isolation between *H. virescens* and *H. subflexa* is maintained by *H. subflexa* females producing specific levels of acetates (including Z11-16:OAc) to attract *H. subflexa* males and repel male *H. virescens*, and *H. virescens* females failing to produce adequate levels of Z9-16:Ald and Z11-16:OH, which are needed to attract *H. subflexa* males (Table 1; Figure 1; Groot et al., 2006; Vickers, 2006a). Hybrids (both *H. virescens* male x *H. subflexa* female and *H. virescens* female x *H. subflexa* male, but particularly the former) were found to be highly attracted to blends of Z11-16:Ald, Z9-16:Ald, and Z11-16:OH in a 1:0.5:0.1 ratio with or without the addition of 0.1 Z11-16:OAc, which was also found to be attractive to *H. subflexa* males (Figure 1; Figure 4; Vickers, 2002; Vickers, 2006a). Replacing Z9-16:Ald with 0.1 Z9-14:Ald (an important compound in the *H. virescens* blend) made the blend significantly less attractive to *H. virescens* male x *H. subflexa* female hybrids, but not the reciprocal cross, although there was some variability between individuals (Figure 1; Figure 4; Vickers, 2006a). These results indicated that the inheritance of sex pheromone olfactory reception genes was co-dominant or exhibited incomplete dominance (Vickers, 2006a). This characteristic was also evident in electrophysiological data, which indicated that olfactory pathways in hybrids were equally tuned to Z9-16:Ald and Z9-14:Ald (Figure 1; Vickers, 2006a). The hybrids were also much more attracted to blends containing Z11-16:OH, suggesting a dominant effect of *H. subflexa* genes on sex pheromone perception (Figure 1; Vickers, 2006a). However, not all hybrid males needed Z11-16:OH to show a response, indicating variability in male behavior and OSN composition on the antennae (Vickers, 2006a). The B-type and C-type sensillae of hybrid males showed a range of similarities to each parental species, and males with sensillae more similar to those of *H. virescens* could be able to respond to blends lacking Z11-16:OH (Figure 1; Vickers, 2006a). The responses of hybrids to Z11-16:OAc also indicated variability in C-type sensillae, as it repelled certain hybrid males but had no effect on most others (Figure 1; Vickers, 2006a). Z11-16:OAc may function behaviorally as an attractant in blends (i.e. produced by female *H. subflexa* to attract conspecific males) or more frequently to antagonize heterospecific males (i.e. it is inhibitory to male *H. zea* and male *H. virescens* when presented in otherwise attractive blends) (Vickers, 2002; Groot et al., 2006; Hillier and Baker, 2016). Electrophysiological recordings from OSNs in trichoid sensillae showed that hybrid males produced from crosses between female *H. subflexa* and male *H. virescens* had spike amplitudes and OSN co-compartmentalization more similar to *H. subflexa*, while dose-response profiles were mostly intermediate between the two parental species (Figure 1; Figure 4; Baker et al., 2006). In A-type hybrid sensillae, there was a response to Z11-16:OH not present in either parental species (Figure 1; Baker et al., 2006). Hybrid males showed higher sensitivity to Z9-14:Ald (a compound in the *H. virescens* blend) in B-type sensillae OSNs normally responsive to Z9-16:Ald and seemed to be able to substitute between Z9-14:Ald and Z9-16:Ald sensitivity unlike either of their parental species (Table 1; Figure 1; Baker et al., 2006). This shift between the two compounds was hypothesized to be a result of two different types of pheromone receptors being co-expressed on the same OSNs, with variation in the hybrid population being attributed to different co-expression of parental alleles or receptors being coded for by different genes (Baker et al., 2006). The C-type sensillae of hybrid males also contained OSNs which showed decreased cross-sensitivity to Z11-16:OAc and Z9-14:Ald compared to parental *H. subflexa* males, although they still retained more similarity to this species than to *H. virescens* (Figure 1; Baker et al., 2006). To further the understanding of *H. subflexa* x *H. virescens* hybrid olfactory processing, the projections of pheromone-receptive neurons to specific areas of the brain was investigated (Vickers, 2006b). The hybrid A-type sensillae OSNs detecting Z11-16:Ald/Z11-16:OH projected to the cumulus, just like the A-type sensillae of the parental species, although the parental species only detect Z11-16:Ald (Figure 1; Vickers, 2006b). The hybrid B-type sensillae OSNs detecting Z9-16:Ald/Z9-14:Ald projected to the dorsomedial glomeruli (DM), just like the parental species, although the detection of Z9-16:Ald is absent in parental *H. virescens* and detection to Z9-14:Ald weak in parental *H. subflexa* (Figure 1; Vickers, 2006b). The hybrid C-type sensillae detecting Z11-16:OAc/Z9-14:Ald projected to the ventromedial glomeruli (VM), while the hybrid C-type sensillae detecting Z11-16:OH projected to the anteromedial glomeruli (AM) (Figure 1; Vickers, 2006b). Once again, these projections were the same as the parental species, but parental *H. virescens* detect Z11-16:OH/Z9-14:Ald and Z11-16:OAc, while parental *H. subflexa* detect Z11-16:OAc/Z9-14:Ald and Z11-16:OH, making the hybrid C-

type sensillae identical to *H. subflexa* (Figure 1; Vickers, 2006b). Based on the evidence gathered cross the three studies, the researchers concluded that *H. subflexa* genetic factors were dominant in determining pheromone blend preference in males (Baker et al., 2006; Vickers, 2006a; Vickers, 2006b).

Further research on the genetic mechanisms underlying sex pheromone production in the *H. virescens* x *H. subflexa* hybrid was conducted in 2009 by Groot and colleagues. In this study, *H. virescens* females were crossed with *H. subflexa* males, and the resulting F1 hybrid females were crossed with either *H. subflexa* males (S-backcrosses) or *H. virescens* males (V-backcrosses) (Figure 4; Groot et al., 2009b). Normally, male *H. virescens* respond to Z11-16:Ald and Z9-14:Ald, the primary and secondary components of the conspecific female blend, respectively (Table 1; Figure 1). Male *H. subflexa* primarily respond to Z9-16:Ald and Z11-16:OH and show an enhanced response to Z11-16:OAc, a compound which repels *H. virescens* (Table 1; Figure 1). In the V-backcrosses, Hs chromosome 24 was associated with higher production of Z9-16:Ald (from the *H. subflexa* blend), while Hs chromosome 7 was associated with lower production of Z9-14:Ald (from the *H. virescens* blend) (Groot et al., 2009b). In the S-backcrosses, the presence of Hv chromosomes 14, 15, 19, and 22 was associated with higher production of Z9-14:Ald (from the *H. virescens* blend) and its precursor tetradecenal (14:Ald), while Hv chromosomes 19 and 24 were associated with lower production of Z9-16:Ald (from the *H. subflexa* blend) (Table 1; Groot et al., 2009b). Decreased production of acetates from the *H. subflexa* blend was associated with Hv chromosomes 4 and 22 (Groot et al., 2009b). Based on this analysis, it was confirmed that female sex pheromone production is under the control of several quantitative trait loci (QTL), regions of DNA consisting of several genes that can be expressed in different combinations to produce a phenotypic trait with varying degrees (Members of the Complex Trait Consortium, 2003; Groot et al., 2009b). Many of these QTL were responsible for the activity of several biosynthetic enzymes, such as acetyl transferase, desaturases, and a fatty acyl reductase or alcohol oxidase, and corroborated earlier evidence that dominant genes from the *H. virescens* genome influence female sex pheromone production (Teal & Oostendorp, 1995; Teal and Tumlinson, 1997; Groot et al., 2009b).

In 2010, another analysis of QTL was performed by Gould and colleagues, this time focusing on sex pheromone perception rather than production (Groot et al., 2009b; Gould et al., 2010). As in the study by Groot and colleagues in 2009, *H. virescens* females were crossed with *H. subflexa* male hybrids, and females resulting from this cross were mated with either *H. virescens* males (creating Hv-BC offspring) or *H. subflexa* males (creating Hs-BC offspring) (Figure 4; Groot et al., 2009b; Gould et al., 2010). This study found that the responses of males to key components from female pheromone blends were controlled by a single QTL (Gould et al., 2010). Hv-BC and Hs-BC males flew towards a source of Z9-16:Ald at rates of 54% and 40%, respectively (Gould et al., 2010).The researchers found that responses to these compounds were under the control of at least four OR genes on chromosome 27, based on an analysis of backcrosses (Gould et al., 2010). Females with Hs-c27 were repeatedly backcrossed to *H. virescens* males, and 5th and 15th generation males with Hs-c27 had significantly higher attraction to blends containing Z9-16:Ald (Gould et al., 2010). Backcross individuals heterozygous for Hs-c27/Hv-c27 tended to respond less to blends with Z11-16:OAc, while 67% responded to blends without Z11-16:OH (Gould et al., 2010). In backcross individuals homozygous for Hv-c27, 25% responded less to Z11-16:OAc, while 77% of backcross individuals homozygous for Hs-c27 responded to blends without Z11-16:OH, much like heterozygous individuals (Gould et al., 2010). Pure *H. virescens* B-type OSNs were activated by Z9-14:Ald, not Z9-16:Ald, pure *H. subflexa* B-type OSNs were more sensitive to Z9-16:Ald, but backcross individuals with all *H. virescens* genes but having Hs-c27 had their B-type OSNs respond like pure *H. subflexa* individuals (Figure 1; Gould et al., 2010). Pure *H. virescens* C-type OSNs responded to Z11-16:OH and Z11-16:OAc, and pure *H. subflexa* C-type OSNs only responded strongly to Z11-16:OH, while backcrosses with all *H. virescens* genes but having Hs-c27 had C-type OSNs respond similarly to pure *H. subflexa* individuals (Figure 1; Gould et al., 2010). The gene HR14 which binds Z11-16:OAc was shown to have a significant effect on differences in response profiles between species, while the genes HR16 (which binds Z11-16:OH), HR15 and HR6 were also suspected to be responsible for differences in sensitivity to Z9-16:Ald and Z9-14:Ald (Gould et al., 2010). Different combinations of these genes in hybrids could result in variability of response profiles in males, thereby affecting speciation and subsequent isolation, as could the introduction of novel mutations (Gould et al., 2010). In 2021, Cao and colleagues showed that a single point mutation in the OR6 gene, which is primarily responsible for detecting Z9-16:Ald in *H. subflexa* and Z9-14:Ald in *H. virescens*, could alter the response profile of *H. virescens* to become more like that of *H. subflexa*, but not the other way around (Table 1; Figure 1; Cao et al., 2021). Thus, the *H. subflexa* OR6 must have evolved from the *H. virescens* OR6 (Cao et al., 2021). Evidence from Cao, Gould, and colleagues provides a genetic mechanism for the emergence of new sex pheromone tuning profiles and responses in males (Gould et al., 2010; Cao et al., 2021). This plasticity would allow males to adapt and optimize detection of novel female blends, providing a selective advantage not only to these males, but to females producing these blends.

Conclusion

The study of hybridization in heliothines is significant not only for the development of pest management programs, but in discerning evolutionary relationships between species and how they may have emerged due to the plasticity of pheromone blend production and detection (Yang and Wang, 2020). The ability to create sterile hybrids, such as with *H. virescens* and *H. subflexa*,

presents a mechanism to suppress *H. virescens* populations. Normal *H. virescens* females will "waste" their reproductive capacity on mating with normal *H. subflexa* males, producing sterile males and reproductive females who can pass their sterility onto further generations by mating with normal *H. virescens* males (Figure 4; Laster, 1972). Autosomal genes from the *H. subflexa* genome for the production of hair-pencil compounds were dominant over those of *H. virescens* in their hybrids (Teal and Oostendorp, 1995a). In contrast, sex pheromone production in females of this cross is under the control of dominant autosomal genes from the *H. virescens* genome, meaning they could compete with normal *H. virescens* females in the wild (Klun et al., 1982; Teal & Oostendorp, 1995b). Hybrid males could outcompete normal males due to such hybrid vigor, particularly 7th generation or greater backcross hybrids which were found to be on par with normal *H. virescens* males (Figures 4–6; Proshold et al., 1975; Cibrian-Tovar & Mitchell, 1991). However, several measures need to be taken in such sterile hybrid programs. The amendments of Makela and Huettel to the Laster-Parvin model must be observed by coordinating several releases of hybrid females (ideally 7th generation or greater) while monitoring growth rates of the native and hybrid population, immigration of native females, potential selection against hybrid females, and potential assortative mating (Figures 5, 6; Laster et al., 1976; Parvin et al., 1976; Makela and Huettel, 1979). Additionally, reproductive F1 hybrid females can mate with normal *H. virescens* males to create sterile offspring, so these offspring must be monitored for pesticide resistance and feeding habits, lest they become a more destructive pest than the parental species (Figures 5, 6; Laster, 1972). Finally, not all hybrids are equally suited to sterile male programs, with reciprocal crosses having different advantages and disadvantages (Proshold and Lachance, 1974). The mechanism of sterility in these hybrids remains uncertain, with the question of sperm abnormalities being caused by live organisms (such as bacteria or viruses) or genomic integration of VLPs left open. If studied further, the cause could be understood, although the use of sperm abnormalities to screen for hybrids in the wild could currently be adopted even without this knowledge (Goodpasture et al., 1980; Lachance and Karpenko, 1983).

While hybrids may be beneficial for pest management in the case of *H. virescens x H. subflexa*, the presence of *H. zea* x *H. armigera* hybrids in the wild poses an issue, as the latter may be able to pass beneficial genes on to its hybrid offspring (Leite et al., 2017; Pearce et al., 2017; Cordeiro et al., 2020; Valencia-Montoya et al., 2020). Studies have shown that *H. armigera* has accumulated genes for Bt crop resistance, insecticide resistance (i.e., *CYP337B3*), and wider host range, while *H. zea* has lost genes for GRs, and detoxification of insecticides and host plant compounds (Pearce et al., 2017). Given that both species have high reproductive and dispersive abilities and are both polyphagous, a combination of their most advantageous traits in a hybrid could create a problematic pest (Leite et al., 2017). Additionally, with *H. zea* potentially preventing bottlenecking in *H. armigera* populations, insecticidal control could be less effective, leading to excessive pesticide use which could further drive introgression and hybridization (Anderson et al., 2016; Valencia-Montoya et al., 2020). This effect could be strengthened in areas where mixed cropping and frequent rotation of maize and soybean occurs (Cordeiro et al., 2020). Interestingly, not much work has been done on production and detection of sex pheromones or hair-pencil compounds in *H. armigera* x *H. zea* hybrids, leaving a potential area for future research with additional urgency added by the emergence of hybrids in the wild. In addition to existing genetic studies, electrophysiological and behavioral data would be beneficial.

Much like *H. virescens* x *H. subflexa*, hybrids of *H. armigera* and *H. assulta* have not been found in the wild although the biased sex ratios of F1 hybrids (which produce all males) and backcrosses (which have a 4:1 ratio of males to females) could aid in controlling the parental species in a release program (Figure 3; Wang & Dong, 2001). However, prezygotic isolation barriers could make mating difficult in the wild after initial release (Zhao et al., 2005). Even if release was successful, with pheromone responses in *H. armigera* x *H. assulta* crosses being controlled by a dominant gene from the *H. armigera* genome, the hybrids would likely be more attracted to their blend, potentially introducing some *H. assulta* genes into *H. armigera* populations leading to increased fitness in wild *H. armigera* and potential evasion of pest management (Zhao et al., 2006; Xu et al., 2017). Hybrid males could also reach new geographic areas due to their increased flight capacity, destroying more crops (Zhao et al., 2005). Since *H. assulta* females from different areas can have different blend variations, these hybrid males with compatible variations in blend detection could mate with them, leading to diversification (Zhao et al., 2006). Alternatively, greater perception diversity in hybrid males could lead to random mating, thereby lowering diversification (Zhao et al., 2006). Much like *H. zea x H. armigera* hybrids, data is lacking on the behavioral responses to sex pheromone or hairpencil gland compounds in *H. assulta x H. armigera* hybrids, and genetic data is scarce (Guo et al., 2022). Genetic studies will be important, as introgression of *H. assulta* genes could change blend production and preference, as evidenced by the emergence of new subtypes of trichoid sensilla in *H. armigera* x *H. assulta* hybrids, and males able to detect new blends could have increased fitness (Zhao et al., 2006; Xu et al., 2017). In addition to potentially aiding with sterility-based control programs, these traits of hybrids could lead to changes in blend preference and production (Groot et al., 2010).

Hybridization studies have provided important insights into mechanisms of speciation in heliothines (as summarized in Table 4), particularly changes in sex pheromone responses driven by genetic inheritance. However, a limitation of these studies is that they do not explore changes that can occur within the lifetime of an organism, i.e., phenotypic plasticity. Significant gaps in our knowledge of how epigenetic changes or post-translational modifications could affect sex pheromone detection remain. Performing studies focusing on such plasticity could help determine why heliothine sex pheromone communication

TABLE 4 Summary of literature review organized by codes indicating generation and crosses.

Cross	Gen-F-M	
H. zea x *H. armigera*	F1-R-Z	· Viable offspring of P1, some genitalia locking of parents (Hardwick, 1965). · Some male sterility in later filial generations (Laster et al, 1985). · No backcross sterility, some differences in mating, development, pupal weights, some genital locking (Laster & Hardee, 1995). · No sterility (Laster & Sheng, 1995). · Potential hybrid found in Brazil in 2013 (Anderson et al., 2016). · Hybridization and introgression occurring, potentially of pesticide resistance genes (*CYP337B3*), gustatory receptor, detoxification, and esterase genes influencing host range (Anderson et al., 2016; Leite et al., 2017; Pearce et al., 2017; Anderson et al., 2018; Cordeiro et al., 2020; Valencia-Montoya et al., 2020). · Hybrids had greater survival in some life stages, but lack of fecundity and egg survival (Rios et al., 2021).
	F1-Z-R	· No offspring produced from P1 (Hardwick, 1965). · Offspring produced from P1 with unbalanced sex ratios (~40% F) (Rios et al., 2021). · had greater survival in some life stages, but lack of fecundity and egg survival (Rios et al., 2021).
	BC-ZR-R	· Offspring produced with unbalanced sex ratios (~60% F) (Rios et al., 2021).
H. assulta x *H. armigera*	F1-R-S	· Viable offspring of P1 (Wang & Dong, 2001; Wang and Dong, 2001). 100% M, no sterility, greater fitness than parents (Wang and Dong, 2001). · Normal males had greater flight capacity than parents, but ~46% of male hybrids showed abnormal development, sterile with undeveloped/malformed reproductive structures (Zhao et al., 2005). · Attraction to *H. arm* blend (see Table 2) (Zhao et al., 2006). Same sensilla as *H. arm*, had expanded A type responsive to Z11-16:OH and Z9-14:Ald, all C-type sensilla found as well as expanded C-type, similar topography, and a:b ratio to *H. arm*, sex pheromone responses closer to *H. arm* (Xu et al., 2017). · P1 cross produced some fertile males, some abnormal males, all abnormal females (Guo et al., 2022). Normal males had high response to Z11-16:Ald and moderate to Z9-16:Ald and Z9-14:Ald, abnormal males low response to all (Guo et al., 2022). · Greater expression of *H. ass* genes, more cis-regulatory genes fixed in species, males more accurate predictors of gene regulation (Guo et al., 2022).
	BC1-R-RS	· 4 M:1 F (Wang and Dong, 2001). · 43% sterility (Zhao et al., 2005). · Female blend closer to *H. arm* (4 Z9-16:Ald: 1 Z11-16:Ald), similar biosynthetic pathway to *H. zea* that represents a mix of both parental species' pathways (Wang et al., 2005). · Responded similarly to *H. arm* to *H. arm* blend (see Table 2) (Zhao et al., 2006). · Sensilla: half are *H. arm*-like, half *H. ass*-like, all C-type sensilla found, half had similar topography and a:b ratio to *H. arm*, and half to *H. ass* (Xu et al., 2017).
	BC1-S-RS	· (See Table 2) (Zhao et al., 2006). Same sensilla as *H. arm*, all C-type sensilla found as well as expanded · C-type, similar topography, and a:b ratio to *H. arm*, sex pheromone responses closer to *H. arm*, could be dominant cross in wild? (Xu et al., 2017). · Could mate with *H. arm*, have introgression of *H. ass* genes, increased fitness, broader tuning, evasion of pest mgmt., speciation? (Xu et al., 2017).
	F1-S-R	· Normal offspring of P1, 1:1 sex ratio, could be self-mated to form normal offspring with 1:1 sex ratio (Zhao et al., 2005). Attraction to *H. arm* blend (see Table 2) (Zhao et al., 2006). · P1 cross produced some fertile males and females (Guo et al., 2022). · Normal males had high response to Z11-16:Ald and moderate to Z9-· · 16:Ald and Z9-14:Ald (Guo et al., 2022). · Greater expression of *H. ass* genes, more cis-regulatory genes fixed in species, males more accurate predictors of gene regulation (Guo et al., 2022).
	BC-SR-S	· Behaved similarly to *H. ass* males in response to *H. arm* blend (see Table 2) (Zhao et al., 2006).
	BC-S-SR	· Behaved similarly to *H. ass* males in response to *H. arm* blend (see Table 2) (Zhao et al., 2006).
	BC-SR-R	· 0.58 F: 1 M (Zhao et al., 2005). Responded similarly to *H. arm* to *H. arm* blend (Zhao et al., 2005). · (See Table 2) (Zhao et al., 2006).

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TABLE 4 Continued

Cross	Gen-F-M	
H. virescens x *H. subflexa*	F1-B-V	· P1 readily mated and produced offspring (Laster, 1972). · P1 had 75.4% mating (64.2% with eupyrene sperm), 56% females produced progeny (Proshold and Lachance, 1974). · Males sterile or semi-sterile due to inability to transfer eupyrene sperm, could be used for pest control (Laster, 1972; Proshold and Lachance, 1974). · 87.4% pupation, male F1 hybrids mated readily with females of either species, 2–6.2% egg hatch but female oviposition was abnormal, females had low mating success and egg-laying but higher egg-hatching, all hybrid males had missing or unpaired chromosomes (desynapsis)? and didn't satiate female mating urge as well (Proshold and Lachance, 1974) · Female hybrids mated poorly and laid few eggs, although their hatch rate was high, entered diapause more often (Proshold and Lachance, 1974) · Only delivered apyrene sperm, outcompete irradiated males? (Proshold et al., 1975). · Abnormal morphology of sperm and lower counts of normal sperm responsible for sterility (Richard et al., 1975; Karpenko and Proshold, 1977). · Males did not respond to *H. vir* female pheromone blend (Tingle et al., 1978). · Males had different sperm than backcross males (F1 x *H. vir*), 75.9–100% defective, abnormalities decrease with more generations (Goodpasture et al., 1980). · VLPs found in spermatocyst and follicle cells, many multiple-tailed eupyrene sperm – due to retrotransposons or stressors? (Degrugillier, 1989; Degrugillier & Newman, 1993). · All males had hair-pencil glands, blends were more similar to *H. sub* (1:4 16:OAc to 16:OH) but still unique (Teal and Oostendorp, 1993; Teal and Oostendorp, 1995). · Female hybrid blend similar to *H. vir* female, pheromone production (particularly 14-carbon aldehydes) under control of dominant autosomal genes from *H. vir* (Klun et al., 1982; Teal and Oostendorp, 1995). · V-line has no periodicity in calling period, could explain poor mating with parents. Could produce more pheromone than parents when injected with pheromonotropic substances (hybrid vigor)? Possibly in reproductive arrest due to low JH (Teal and Oostendorp, 1995). · Male hybrids highly attracted to blends of Z11-16:Ald, Z916:Ald, and Z11-16:OH in a 1:0.5:0.1 ratio with or without the addition of 0.1 Z11-16:OAc, which was also found to be attractive to *H. sub* males (Vickers, 2002; Vickers, 2006a). Replacing Z9-16:Ald with 0.1 Z9-14:Ald (an important compound in the *H. vir* blend) made the blend significantly less attractive. Much more attracted to blends containing Z11-16:OH, suggesting a dominant effect of *H. sub* genes on sex pheromone perception (Vickers, 2006a). · Spike amplitudes and OSN co-compartmentalization more similar to *H. sub*, while dose-response profiles were mostly intermediate between the two parental species (Vickers, 2006a). · A-type hybrid sensillae = response to Z11-16:Ald (both parents) and Z11-16:OH (neither parent) (Figure 1; Baker et al., 2006). Projected to cumulus, just like parents (Vickers, 2006b). · B-type sensillae = response to Z9-16:Ald (like *H. sub*) and Z9-14:Ald (both parents), higher sensitivity to Z9-14:Ald (a compound in the *H. vir* blend) in B-type OSNs normally responsive to Z9-16:Ald, seemed to be able to substitute between Z9-14:Ald and Z9-16:Ald sensitivity unlike either of their parental species – coexpression of ORs? Projected to dorsomedial glomeruli, just like parents (Vickers, 2006b). · The C-type sensillae = response to Z11-16:OAc/Z9-14:Ald (like *H. sub*) and Z11-16:OH (like *H. sub*), contained OSNs which showed decreased cross-sensitivity to Z11-16:OAc and Z9-14:Ald compared to parental *H. sub* males, although they still retained more similarity to this species than to *H. vir* (Figure 1; Baker et al., 2006). Projected to ventromedial glomeruli (Z11-16:OAc/Z9-14:Ald) and anteromedial glomeruli (Z11-16:OH), just like parents, more similar to *H. sub* (Vickers, 2006b).
	BC-BV-V	· Males sterile or semi-sterile delivering no or irregular sperm with only 2.5% average egg hatch, females fertile so need monitoring of female offspring in case of accidental female F1 release needed, all hybrid males had missing or unpaired chromosomes (desynapsis)? and didn't satiate female mating urge as well (Laster, 1972; Proshold and Lachance, 1974). · Mating behaviors similar to normal *H. vir*, females attracted *H. vir* males equally as well as *H. vir* females, males attracted to both *H. vir* females and their extracts (Laster et al., 1977; Pair et al., 1977; Laster et al., 1978; Tingle et al., 1978). · BC females behaved similarly to normal *H. vir* females, calling period overlapped with *H. sub* females; BC males were less active than *H. vir* males, 20–25% not responsive to calling virgin BC females, only 1/3 mated successfully, and differed in close-range mating behaviors. BC7 and greater were comparable to normal *H. vir* males – ideal for control (Cibrian-Tovar & Mitchell, 1991). · Males had different sperm than F1 hybrid males, 25.4–100% defective, abnormalities decrease with more generations (Goodpasture et al., 1980). · Microorganism likely not responsible for BC male sterility – thought to be abnormalities in mitochondrial function, particularly RNA metabolism (Miller et al., 1986; Lachance and Olstad, 1988). · VLPs found in spermatocyst and follicle cells, prevalence 100% in BC males, many multiple-tailed eupyrene sperm – due to retrotransposons or stressors? (Degrugillier, 1989; Degrugillier & Newman, 1993). · All males had hair-pencil glands, blends were intermediate (Teal and Oostendorp, 1993; Teal and Oostendorp, 1995). · Female BC1 blend similar to *H. vir* female, pheromone production (particularly 14-carbon aldehydes) under control of dominant autosomal genes from *H. vir* (Klun et al., 1982; Teal and Oostendorp, 1995).
	BC-V-BV	· Hybrid males backcrossed with *H. vir* females tended to mate more frequently (~3 times) than those backcrossed with *H. sub* females (~2 times) (Proshold and Lachance, 1974).
	BC2-V-BVV	· Males and females fertile when crossed with *H. vir*, all hybrid males had missing or unpaired chromosomes (desynapsis)? and didn't satiate female mating urge as well (Proshold and Lachance, 1974). Males twice as likely as BC1 males to deliver sperm (Karpenko and Proshold, 1977).

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TABLE 4 Continued

Cross	Gen-F-M	
	BC2-BVV-V	· Males sterile, females fertile when crossed with *H. vir*, all hybrid males had missing or unpaired chromosomes (desynapsis)? and didn't satiate female mating urge as well (Proshold and Lachance, 1974). All males had hair-pencil glands (Teal and Oostendorp, 1993; Teal and Oostendorp, 1995).
	F1-V-B	· Mating occurred in P1, but no viable offspring (Laster, 1972). 50.3% mating (only 25.1% with eupyrene sperm), 20% of females produced progeny (Proshold and Lachance, 1974). · Most males sterile or semi-sterile due to inability to transfer eupyrene sperm, 87.5% pupation, <1% egg hatch but female oviposition was abnormal, females were more successful at mating and egg-laying but moderate egg-hatching (Proshold and Lachance, 1974). · Male F1 hybrids mated readily with females of either species but preferred *H. vir*, female hybrids mated readily, high egg-laying and moderate egg hatching rates (Proshold and Lachance, 1974). · Crosses became more fertile over generations until BC2 were partially or fully fertile, all hybrid males had some unpaired chromosomes (desynapsis)? and didn't satiate female mating urge as well (Proshold and Lachance, 1974). · Only delivered apyrene sperm, outcompete irradiated males? (Proshold et al., 1975). Abnormal morphology of sperm and lower counts of normal sperm responsible for sterility (Richard et al., 1975; Karpenko and Proshold, 1977). · VLPs found in spermatocyst and follicle cells, many multiple-tailed eupyrene sperm – due to retrotransposons or stressors? (Degrugillier, 1989; Degrugillier & Newman, 1993). · All males had hair-pencil glands, blends were more similar to *H. sub* (1:4 16:OAc to 16:OH) but still unique (Teal and Oostendorp, 1993; Teal and Oostendorp, 1995). · Female hybrid blend similar to *H. vir* female, pheromone production (particularly 14-carbon aldehydes) under control of dominant autosomal genes from *H. vir* (Klun et al., 1982; Teal and Oostendorp, 1995). · S-line has periodicity in calling period, could explain good mating with parents. Could produce more pheromone than parents when injected with pheromonotropic substances (hybrid vigor)? Possibly in reproductive arrest due to low JH. (Teal and Oostendorp, 1995). · Male hybrids highly attracted to blends of Z11-16:Ald, Z916:Ald, and Z11-16:OH in a 1:0.5:0.1 ratio with or without the addition of 0.1 Z11-16:OAc, which was also found to be attractive to *H. sub* males (Vickers, 2002; Vickers, 2006a). Replacing Z9-16:Ald with 0.1 Z9-14:Ald (an important compound in the *H. vir* blend) did not make the blend significantly less attractive to *H. vir* female x *H. sub* male hybrid. The hybrids were also much more attracted to blends containing Z11-16:OH, suggesting a dominant effect of *H. sub* genes on sex pheromone perception (Vickers, 2006a).
	BC BC-VB-V BC-VB-B BC-B-VB BC-V-VB	· Hybrid males backcrossed with *H. vir* females tended to mate more frequently (~3 times) than those backcrossed with *H. sub* females (~2 times) (Proshold and Lachance, 1974). · VLPs found in spermatocyst and follicle cells in males from F1 F x *H. vir* M, many multiple-tailed eupyrene sperm – due to retrotransposons or stressors? (Degrugillier, 1989; Degrugillier & Newman, 1993). · V-backcrosses (VB-V): Hs chromosome 24 = higher Z9-16:Ald production, Hs chromosome 7 = lower Z9-14:Ald (Groot et al., 2009b) · S-backcrosses (VB-S): Hv chromosomes 14, 15, 19, 22 = higher Z9-14:Ald & 14:Ald production, Hv chromosomes 19, 24 = lower Z9-16:Ald, Hv chromosomes 4, 22 = lower acetates (ex: Z11-16:OAc) (Groot et al., 2009b)
	BC2 BC2-VBV-V BC2-VBB-V BC2-BVB-V BC2-VVB-V BC2-VBV-B BC2-VBB-B BC2-BVB-B BC2-VVB-B BC2-V-VBV BC2-V-VBB BC2-V-BVB BC2-V-VVB BC2-B-VBV BC2-B-VBB BC2-B-BVB BC2-B-VVB	· All hybrid males had some unpaired chromosomes (desynapsis)? and didn't satiate female mating urge as well (Proshold and Lachance, 1974). · Crosses between BC1 females x *H. vir* males produced sterile males and fertile females, while crosses between BC1 males x *H. vir* females produced fertile males and females (Proshold and Lachance, 1974). · The cross between *H. vir* females x *H. sub* males became more fertile with each generation, so BC2 individuals were partially or fully fertile (Proshold and Lachance, 1974). · Males twice as likely as BC1 males to deliver sperm (Karpenko and Proshold, 1977). · For hybrid F x *H. sub* M = 0% hair-pencil, S type (Teal and Oostendorp, 1993; Teal and Oostendorp, 1995). · For hybrid F x *H. vir* M = 100% hair-pencil, I type (Teal and Oostendorp, 1993; Teal and Oostendorp, 1995). · Hv-BC males: 54% flew to blend containing Z9-16:Ald, likely inherited gene from *H. sub* on c27 (Gould et al., 2010). · Hs-BC males: 40% flew to blend containing Z9-14:Ald, likely inherited gene from *H. vir* on c27 (Gould et al., 2010). · Females with Hs-c27 were repeatedly backcrossed to *H. vir* males, 5th gen males with Hs-c27 had significantly higher attraction to Z9-16:Ald blend, 15th gen too (Gould et al., 2010). · BC heterozygous: 80% responded less to blend with Z11-16:Oac, 67% responded to blend without Z11-16:OH (Gould et al., 2010). BC Hv-c27 homo: 25% responded less to Z11-16:OAc (Gould et al., 2010). · BC Hs-c27 homo: 77% responded to blend without Z11-16:OH (no diff to hetero) (Gould et al., 2010). · Pure Hv B-type activated by Z9-14:Ald, not Z9-16:Ald; Pure Hs B-type more sensitive to Z9-16:Ald; Hv except Hs-c27 B-type responded like pure Hs (Gould et al., 2010). · Pure Hv C-type responded to Z11-16:OH and Z11-16:Oac, Pure Hs only strongly to Z11-16:OH, Hv with Hs-c27 similarly to Hs (Gould et al., 2010).

The first letter of the code indicates the generation, the second letter the female parent, and the third letter the male parent. F1, filial generation; BC, backcross generations.

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systems are not under stabilizing selection as expected (Löfstedt et al., 1989; Phelan, 1992; Löfstedt, 1993; Jurenka et al., 1994; Haynes, 1997; Phelan, 1997a; Phelan, 1997b; Baker, 2002; Roelofs et al., 2002).

Author contributions

Concept and draft of manuscript written by VI. VI collected and analyzed all relevant literature, and developed graphics and tables for data presentation. NH assisted in discussions for concept development and in editing of the manuscript. Both authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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