UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Variation in sexual communication and its role in divergence of two host strains of the noctuid moth Spodoptera frugiperda

Unbehend, M.

Publication date
2013

Link to publication

Citation for published version (APA):
Unbehend, M. (2013). *Variation in sexual communication and its role in divergence of two host strains of the noctuid moth Spodoptera frugiperda*. [Thesis, externally prepared, Universiteit van Amsterdam].

Chapter 6

Evolution of reproductive isolation of *Spodoptera frugiperda*

Astrid T. Groot[1,2], Melanie Unbehend[1], Sabine Hänniger[1], Maria Laura Juárez[3], Silvia Kost[1] and David G. Heckel[1]

[1]Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany
[2]Sección Zoología Agrícola, Estación Experimental Agroindustrial Obispo Colombres, Las Talitas (T4104AUD), Tucumán, Argentina
[3]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, the Netherlands

Introduction

Spodoptera frugiperda, the fall armyworm, is a noctuid moth occurring in North and South America with two host strains (a corn- and a rice-strain) identified in the 1980s (Pashley et al. 1985; Pashley 1986). These two strains were originally characterized by a polymorphism in an esterase allozyme marker and three other strain-biased protein variants in larvae collected from corn fields and rice paddies in Puerto Rico (Pashley et al. 1985; Pashley 1986). Since then, several additional strain-biased or strain-diagnostic molecular markers have been identified: the two strains differ in mitochondrial DNA sequences in the cytochrome oxidase I (COI) and NADH dehydrogenase 1 (ND1) genes (Pashley 1989; Pashley and Ke 1992; Lu and Adang 1996; Levy et al. 2002; Meagher and Gallo-Meagher 2003; Prowell et al. 2004; Nagoshi et al. 2006a; Machado et al. 2008). There are also strain-biased and strain-specific amplified fragment length polymorphisms (AFLP) (McMichael and Prowell 1999; Busato et al. 2004; Prowell et al. 2004; Clark et al. 2007; Martinelli et al. 2007; Juárez et al. 2012), restriction length fragment polymorphisms (RFLP) (Lu et al. 1992), a so-called Frugiperda Rice (FR) repetitive nuclear DNA sequence, present in high copy number in the rice-strain and mostly lower copy number in the corn-strain (Lu et al. 1994; Nagoshi and Meagher 2003b; Nagoshi et al. 2008) and nucleotide polymorphisms within the triose phosphate isomerase gene (Tpi, Nagoshi 2010).

Recently, sex pheromone differences have been found among populations of the two strains (Groot et al. 2008; Lima and McNeil 2009; Unbehend et al. 2012). However, these differences were not consistent among studies, suggesting that geographic variation may be confounded with strain-specific variation, or that pheromones may vary within strains as well. The relative importance of the pheromone differences between the two strains still needs to be established, i.e. are all pheromone compounds in the pheromone glands behaviorally important and/or

are males of the two strains differentially attracted to the different pheromone blends? Since other physiological, developmental, and behavioral differences have been found among the strains (Pashley and Martin 1987; Pashley 1988b; Pashley et al. 1992; Pashley et al. 1995; Veenstra et al. 1995; Meagher et al. 2004; Schöfl et al. 2009; Groot et al. 2010; Meagher et al. 2011; Schöfl et al. 2011; Meagher and Nagoshi 2012), this overview integrates strain-specific variation in sexual communication (variation in the pheromone gland composition as well as variation in male response) with other possible pre- and postmating barriers that likely contribute to isolation of the two strains. First, we will show that the naming of the two strains is somewhat misleading, as the host specificity of the two strains is not as clear-cut as the names suggest. Then we will focus on the two types of prezygotic isolating mechanisms that have been demonstrated to differ between the two strains: a) the diel pattern of reproductive activity, and b) variation in sexual communication (both signal and response). In addition to the premating barriers, we also consider postmating barriers that may isolate the two strains. Finally, based on recent findings, we discuss a possible evolutionary scenario for the evolution of the two strains of *S. frugiperda*.

I. Are the two strains really host strains?

Allozyme differences at five loci, including one apparently strain-specific esterase allele, provided the first evidence of partial genetic differentiation of populations collected from adjacent corn and rice fields in Puerto Rico and Louisiana (Pashley et al. 1985; Pashley 1986). Differences in mitochondrial DNA RFLP patterns were also found among these populations (Pashley 1988a). Subsequently, the same genetic differences were found in populations collected from other host plants and localities, and used to assign them to either the corn- or rice-strain. The so-called corn-strain was found to infest mainly corn (i.e. maize, *Zea mays*), sorghum,

(*Sorghum bicolor* subsp. *bicolor*), and cotton (*Gossypium hirsutum*), whereas the so-called rice-strain was found mostly in rice (*Oryza sativa*), sugar cane (*Saccharum officinarum*), and grasses such as Johnson grass (*Sorghum halepense*), and Bermuda grass, (*Cynodon dactylon*). Genetic differentiation between these two strains has been confirmed in several regions in North and South America, using different molecular markers. The host associations of the two strains are summarized below.

1a. Host associations based on mitochondrial COI polymorphism

Among all molecular markers available to distinguish the two strains, the most widely used target mitochondrial DNA. For example, the two strains show differences in their cytochrome oxidase I (COI) gene and can be identified by a polymorphism in the restriction sites for SacI and AciI (both present in rice-strain and absent in the corn-strain), and for HinfI, BsmI and Msp1 (all present in corn-strain and absent in the rice-strain). The polymorphisms in SacI and MspI are used in most studies (Lu and Adang 1996; Levy et al. 2002; Meagher and Gallo-Meagher 2003; Nagoshi et al. 2006a). Based on the restriction site polymorphisms mentioned above, especially in the double digestion with SacI and MspI, the identity of the strains has been evaluated for different habitats and has been shown that the association is not always absolute.

Approximately 80% of individuals collected from corn habitats were identified as corn-strain and the remaining 20% as rice-strain (Pashley 1989; Lu and Adang 1996; Levy et al. 2002; Nagoshi et al. 2006a; Nagoshi et al. 2007b). However, exceptions from this percentage of distribution have been found as well: Prowell et al. (2004) identified samples collected from corn predominantly (i.e. 50% or more of the individuals) as rice-strain in French Guiana as well as in Louisiana. Nagoshi et al. (2006a) also found mostly rice-strain individuals in a sorghum field in Texas,

which is considered a corn-strain habitat. In the case of larvae collected from rice fields, up to 95% of individuals have been identified as rice-strain (Nagoshi and Meagher 2003a, 2004; Machado et al. 2008; Velez-Arango et al. 2008). Recently, Juárez et al. (2012) did not find a consistent pattern between the two strains and their respective host plants either (especially, in rice habitats), when using COI markers in South American populations.

Some of these shifts in strain distributions may be due to seasonal and temporal variation in the distributions of the two strains and in the distribution of available plant hosts or by different migration patterns of the two strains (Nagoshi et al. 2007a). For example, Nagoshi et al. (2007c) showed that the corn-strain predominated in collection from sorghum in the fall (March-June) in Brazil and reduced in frequency in spring (September-November), while in Florida rice-strain larvae predominated in collections made in the fall (September-November), and corn-strain larvae were mostly present in the spring season (February-April). In Louisiana, Pashley et al. (1992) found that corn strain populations were detected in the corn fields in the spring, while rice strain populations remained at low density on various grasses until late summer when they increased in number. Together, these findings suggest that the migration pattern of the two strains may not be the same (Nagoshi and Meagher 2004).

In Figure 1 we provide an overview of collections of the fall armyworm over a period of 27 years (from 1983 until 2010) from a number of different habitats. Most individuals from 17 out of 20 populations, sampled from predominantly rice habitats (rice and pasture/Bermuda grass), were identified as rice-strain, whereas most individuals from 29 out of 44 populations from predominantly corn habitats (corn, cotton and sorghum) were identified as corn-strain.

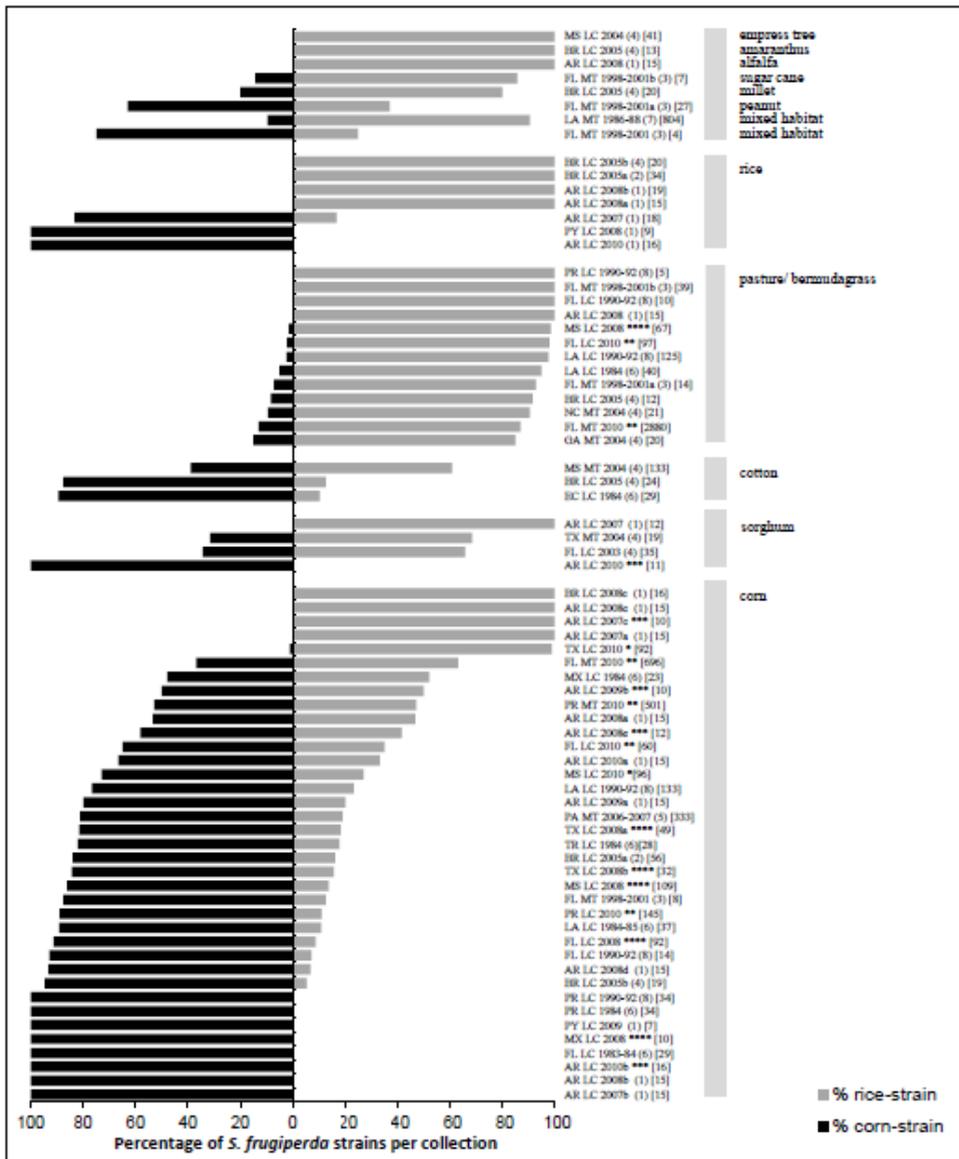**Figure 1.** Distribution of *Spodoptera frugiperda* host strains in different habitats and geographic regions. Each bar shows the percentage of the identified strains per collection, based on mitochondrial markers. Habitats are indicated on the right. Numbers in [ ] indicate total n of collection. MS-Mississippi, BR-Brazil, AR-Argentina, LA-Louisiana, FL-Florida, PY-Paraguay, PR-Puerto Rico, NC-North

Carolina, GA-Georgia, EC-Ecuador, TX-Texas, MX-Mexico, PA-Pennsylvania, TR-Trinidad; LC-larval collection, MT-male trapping experiments; Data from: * C. Blanco, ** S. Hänniger & M. Unbehend, *** M. L. Juárez, **** G. Schöfl, (1) Juárez et al. 2012b, (2) Machado et al. 2008, (3) Meagher & Gallo-Meagher 2003, (4) Nagoshi et al. 2006a, (5) Nagoshi et al. 2009, (6) Pashley 1989, (7) Pashley et al. 1992, (8) Prowell et al. 2004.

Although mitochondrial markers generally show a strong correlation between strain type and host plant, in many of collections this association is lacking, especially in predominantly corn habitats (see Figure 1).

1b. Host associations based on genome-wide AFLP markers

Although some studies have found a close association between the two strains and their host plants using AFLP markers (e.g., McMichael and Prowell 1999; Busato et al. 2004 in the USA and Brazil, respectively), others have not (e.g., Martinelli et al. (2007) in Mexico, Brazil, Argentina and the USA). Recently, we found that individuals from populations collected from corn plants tended to cluster together and showed a high degree of homogeneity (Juárez et al. 2013). This finding thus contrasts the trend found in the COI marker, where 15 out of 44 populations collected from corn (see Figure 1) showed a significant portion of rice-strain individuals. Individuals from the populations collected from rice formed three distinct groups and showed a much higher level of heterogeneity in their AFLP markers (Juárez et al. 2013). Overall, individuals collected from corn-strain habitats were clustered separately from individuals collected from rice-strain habitats, although there were some marked exceptions (Juárez et al. 2013).

1c. Host association based on mitochondrial and nuclear markers

Combining mitochondrial and nuclear markers with their different modes of inheritance, the rate and directionality of hybridization between the strains in the field can be identified. Prowell et al. (2004) analyzed populations from Louisiana, Florida, Puerto Rico, Guadeloupe, and French Guiana with different molecular markers (mitochondrial haplotype, esterase genotypes, AFLPs) and reported that 16% of the samples were potential hybrids due to discordance for at least one marker. The authors found evidence of crosses between the strains in both directions: when using mtDNA and esterase markers, 66% of the hybrids were inferred to be derived from rice-strain females mated with corn-strain males, i.e. RC hybrids, while in multilocus comparison using the three markers, 54% of the hybrids were RC hybrids and 46% were from the reciprocal cross, i.e. CR hybrids. In addition, Prowell et al. (2004) found that these hybrids occurred mostly in the corn habitats. Similar results were found by Saldamando and Vélez-Arango (2010) with Colombian populations. In contrast, Nagoshi and Meagher (2003b) and Nagoshi et al. (2006b), using mitochondrial haplotypes and the nuclear FR tandem-repeat sequence, found mainly RC-hybrids (56%), while CR-hybrids were rare or absent, and hybrids occurred in both corn and rice habitats.

Recently, Nagoshi (2010) identified 10 polymorphic diagnostic sites in the Z-linked (sex-linked) triose phosphate isomerase (*Tpi*) gene that can be associated with the corn- or rice-strain of the fall armyworm (as in Lepidoptera females are the heterogametic sex, ZW). With this marker, Nagoshi (2012) analyzed 12 populations (9 collected from corn and 3 from rice) with the COI marker, and then reanalyzed the same samples with the Tpi marker, and found that 60% and 7% of the COI-R typed individuals were Tpi-C in the corn and rice habitats, respectively (i.e. RC hybrids). The reverse constellation, COI-C and Tpi-R (i.e. CR hybrids), occurred in 8% and 22% of the CO1-C typed individuals from corn and rice

habitats, respectively. Like Nagoshi (2012), when we combined the COI marker with the Z-linked Tpi marker, we also found a high percentage of hybrids (43%) (Juárez et al. 2013). These hybrids consisted of four different combinations: RC (33 % of all hybrids), CR (5 % of all hybrids), CI (25 % of all hybrids), and RI (37 % of all hybrids). The I stands for a Tpi-intermediate haplotype, i.e. individuals in which corn and rice SNPs were present in similar proportions or heterozygous individuals in which SNPs showed the two alternative nucleotides. The latter individuals must be hybrid males, as in Lepidoptera the females carry only one copy of the Z-linked Tpi gene. Nagoshi (2010) and Nagoshi et al. (2012) also found this intermediate configuration in a very low frequency and proposed that they may represent hybrid individuals as well.

In summary, both types of hybrids seem to occur in nature, although recent studies suggest that the RC-hybrids are more common. These hybrids are mostly found in corn habitats, while other hybrids (CR, CI, RI) are mostly found in rice habitats. Overall, the two strains seem to be predominantly found in the habitats from where they were originally described, but significant exceptions have been found with all markers used. Therefore, our preliminary conclusion is that divergence between the strains is not likely due to host plant specialization, or at least not alone. We hypothesize that an interaction between ecological and behavioral mechanisms has contributed to reproductive isolation between the two strains (Groot et al. 2010).

II. Behavioral isolation mechanism 1: Timing of reproductive activity at night

Differences in the diel pattern of mating activity between strains would create a powerful barrier to hybridization. Strain-specific differences in the timing of reproductive activity of the two strains have been consistently found, independent

of the geographic origin of the strains (Pashley et al. 1992; Schöfl et al. 2009; Schöfl et al. 2011): the corn-strain is active early in the scotophase, while the rice-strain is active late in the scotophase. Schöfl et al. (2009) showed that different reproductive behaviors (calling, copulation and oviposition) are differentially inherited and thus under complex genetic control, and suggested the involvement of the circadian clock.

When testing whether allochronic separation causes assortative mating in the laboratory, Schöfl et al. (2011) found an interaction between strain-specific timing of mating and time-independent intrinsic preferences that influenced the mating choice of both strains. Furthermore, mate choice changed over time in consecutive nights and was influnced by the timing of introduction of the mating partners, i.e. at the onset of the scotophase or 6 hours after the onset of scotophase (Schöfl et al. 2011). In general, females were more restricted in their mate preference than males and approximately 30% of the isolation between both strains was generated by female mate preference, suggesting the involvement of a male-specific sex pheromone that mediate close-range courtship behavior (Schöfl et al. 2011). Also, this mate-choice experiment indicates that the level of assortative mating caused by allochronic separation alone is not strong enough to cause reproductive isolation between strains. Although the importance of differential timing of reproduction is probably not as strong as suggested by Pashley et al. (1992), the consistent timing differences between the strains, independent of the geographic origin, suggests that this behavioral difference could have a stronger influence as prezygotic isolation barrier than host plant choice. Therefore, we are tempted to argue that both strains are "timing strains" rather than "host strains."

III. Behavioral isolation mechanism 2: Variation in sexual communication

In the early 1990s, Pashley et al. (1992) found that males of both strains showed a slight preference for females of the same strain, 60-65% of corn- and rice-strain males being attracted to corn- and rice-strain females, respectively. These findings indicate that pheromone differences might be important for mate choice and cause assortative mating in the two strains, although Pashley et al. (1992) suggested that "pheromone chemistry may play a small role (if any) in strain separation". The sex pheromone composition of *S. frugiperda* females has been studied in different geographic regions (Mitchell et al. 1985; Tumlinson et al. 1986; Descoins et al. 1988; Batista-Pereira et al. 2006; Groot et al. 2008; Lima and McNeil 2009; Unbehend et al. 2013). While earlier studies mainly focused on the general composition of the female sex pheromone without distinguishing the two strains, later studies investigated strain-specific differences in the female pheromone composition (Groot et al. 2008; Lima and McNeil 2009; Unbehend et al. 2013).

In general, the fall armyworm sex pheromone consists of the primary sex pheromone component Z9-14Ac and the critical secondary sex pheromone Z7-12Ac (Tumlinson et al. 1986; Batista-Pereira et al. 2006; Groot et al. 2008; Lima and McNeil 2009). The behavioral effect of other secondary compounds in the female gland, is not clear so far (Tumlinson et al. 1986; Andrade et al. 2000; Fleischer et al. 2005; Groot et al. 2008; Unbehend et al. 2013). However, twice as many males were caught when Z11-16Ac or Z9-12Ac were added to the binary blend (Fleischer et al. 2005), suggesting at least a synergistic effect of these compounds. It has been shown that corn- and rice-strain females exhibit strain-specific differences in their relative amount of Z7-12Ac (relative to the amounts of other gland compounds), as well as in the relative amount of Z9-14Ac, Z11-16Ac

and Z9-12Ac, although the type of variation found seems to vary in different geographic regions (Groot et al. 2008; Lima and McNeil 2009; Unbehend et al. 2013).

Disentangling geographic from strain-specific variation

Extractions of the pheromone glands of females from a colony, that was initiated with larvae collected in Florida, revealed that rice-strain females produce significantly higher relative amounts of Z7-12Ac and Z9-12Ac, and lower relative amounts of Z11-16Ac, than corn-strain females (Groot et al. 2008). However, laboratory rice-strain females originating from Louisiana contained lower relative amounts of the major component Z9-14Ac, as well as larger relative amounts of Z7-12Ac and Z11-16Ac, compared to laboratory corn-strain females from Louisiana (Lima and McNeil 2009). Taken together, only Z7-12Ac showed consistent strain-specific variation in females from Florida and Louisiana (Groot et al. 2008; Lima and McNeil 2009; Unbehend et al. 2013). This is not too surprising, as Z7-12:Ac is a critical component for male attraction and thus likely to be under stabilizing selection. The importance of Z11-16Ac and Z9-12Ac in the attraction of fall armyworm males is not completely understood yet, but their variation suggests that these components are not under strong stabilizing selection.

Geographic variation in the strain-specific pheromone composition of females from Florida and Louisiana may be related to different haplotype profiles in Floridian and Louisianan corn-strain populations. There seem to be two main migration routes of the fall armyworm, based on haplotype patterns in the corn-strain (Nagoshi et al. 2008; Nagoshi et al. 2010). These patterns suggest an eastern migration route, i.e. populations originating from Puerto Rico and Florida move northwards to Georgia, and a western migration route, i.e. populations from Texas move northeastwards to Louisiana, Mississippi, Alabama and Pennsylvania

(Nagoshi et al. 2008; Nagoshi et al. 2009). If no other geographic effects influence the female pheromone, then pheromone profiles of females from Texas, Louisiana, Mississippi, Alabama and Pennsylvania may be more similar to each other than to pheromone profiles of females from Florida, Puerto Rico and Georgia.

In fall armyworm females from Brazil, another minor sex pheromone component, E7-12Ac, was identified to be attractive to Brazilian males in the field (Batista-Pereira et al. 2006). Addition of E7-12Ac to binary blends, containing Z9-14Ac and Z7-12Ac, significantly increased the number of males captured in Brazil, i.e. from an average of 70 males per trap to an average of 100 males per trap (Batista-Pereira et al. 2006). The fact that E7-12Ac has not been found in females from Florida, Louisiana or French Guyana (Descoins et al. 1988; Groot et al. 2008; Lima and McNeil 2009) suggests the existence of geographic variation in female pheromone production. In conclusion, the two *S. frugiperda* strains do differ in their female sex pheromone composition (Groot et al. 2008; Lima and McNeil 2009; Unbehend et al. 2013), but geographic variation seems to influence the strain-specific pheromone production. To disentangle geographic from strain-specific variation, additional strain-specific pheromone extractions of different populations from North and South America will be necessary.

Variation in pheromone composition within the strains

In addition to strain-specific and geographic variation in the pheromone composition, pheromone differences within females of both strains have been observed between artificially-reared and field-collected females (Unbehend et al. 2013). Females of both laboratory strains produced significantly lower relative amounts of the major pheromone component Z9-14Ac and usually higher relative amounts of Z7-12Ac, Z11-16Ac and Z9-12Ac, compared to the field-collected females, although strain-specific pheromone variation was maintained (Unbehend

et al. 2013). To estimate how much within-strain variation occurs in nature, we analyzed the pheromone composition of females from seven different corn-strain families, originating from single pair matings of individuals that were collected one generation earlier from a corn field in Florida (Marr 2009). The females of these families exhibited significant differences in their pheromone composition compared to our laboratory populations (Marr 2009). The variation of Z9-14Ac, Z7-12Ac, Z11-16Ac and Z9-12Ac was strongly heritable and a broad-sense heritability analysis showed that the variation in gland compounds within the different families is determined mainly by genetic rather than environmental effects (Marr 2009). However, the within-strain variation found in laboratory- and field-females, in addition to the geographic variation, indicates that laboratory rearing and environmental factors influence the pheromone composition of females. The challenge is to determine which factors may cause variation in the pheromone composition and why. Understanding the cause of variation in the pheromone composition and its genetic control will be important to understand how variation in sexual communication influences reproductive isolation and how sexual communication systems may evolve (Baker and Cardé 1979; Löfstedt 1993; Butlin and Trickett 1997; Ritchie 2007; Bergen et al. 2012).

Male response to strain-specific pheromone

The existence of strain-specific sex pheromone blends can only contribute to differentiation between the strains if this leads to differential attraction of fall armyworm males in the field. Although several trapping experiments of *S. frugiperda* males have been conducted in the field (Mitchell et al. 1985; Tumlinson et al. 1986; Meagher and Mitchell 1998; Andrade et al. 2000; Batista-Pereira et al. 2006), only one investigated strain-specific differences in the male attraction toward different pheromones (Pashley et al. 1992). In Louisiana fields containing both host plants, 60% of all rice-strain males trapped in pheromone traps were

attracted to a virgin rice-strain female, while 65% of all trapped corn-strain males were caught in traps baited with virgin corn-strain females (Pashley et al. 1992). Thus, males of both strains exhibited only a slight bias toward females of their own strain in mixed habitats, suggesting that strain-specific sexual communication is a weak prezygotic isolation barrier (Pashley et al. 1992). Similarly, Lima and McNeil (2009) argued that is quite unlikely that strain-specific sex pheromone differences alone "would be sufficient to ensure reproductive isolation of the two strains."

To evaluate whether fall armyworm males exhibit strain-specific attraction towards females of their own strain, we conducted wind tunnel choice assays and male trapping experiments in Florida (Unbehend et al. 2013). Wind tunnel experiments without plant volatiles revealed that *S. frugiperda* males from laboratory populations show no strain-specific attraction to virgin females of their own strain. Interestingly, males of both strains were mainly influenced by the timing of female calling, and did not discriminate among calling females (Unbehend et al. 2013). However, when testing pheromone lures mimicking the four-component pheromone blend of Floridian corn-strain females (i.e. 100% Z9-14Ac, 13% Z11-16:Ac, 2% Z7-12Ac, 1% Z9-12Ac), 74% of all trapped corn-strain males in a corn field were attracted to this corn-strain lure, and only 26% to the rice-strain lure, i.e. 100% Z9-14:Ac, 8% Z11-16:Ac, 4% Z7-12:Ac, 2% Z9-12:Ac (Figure 2). In rice fields, such a strong strain-specific attraction was not found, and only 59% of all trapped corn-strain males were attracted to the synthetic corn-strain lure, while 41% were attracted to the rice-strain lure (Figure 2). This result suggests that strain-specific attraction to different lures depends on the respective (volatile) environment, and hints to a synergistic effect of sex pheromones and host plant volatiles. However, similar to corn-strain males, rice-strain males were also mostly attracted to the synthetic corn-strain lure in the corn field and 76% of all trapped rice-strain males were caught in traps baited with the corn-strain lure (Figure 2).
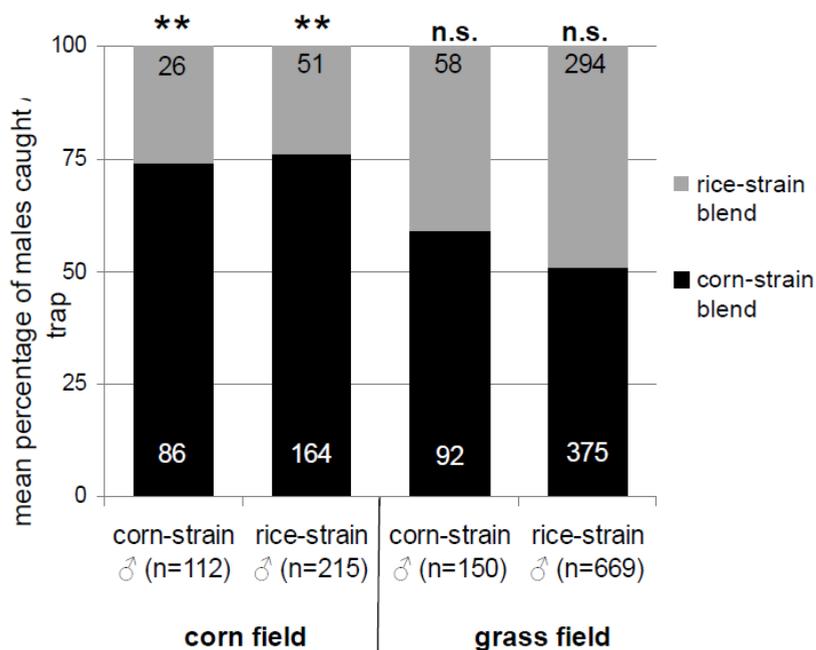
Figure 2. Mean percent of corn-strain and rice-strain males caught in sex pheromone traps baited with synthetic pheromone lures in a corn field and a grass field in Florida. The corn-strain blend consisted of 300 µg Z9-14:OAc, which was considered 100%, 6 µg (2%) Z7-12:OAc, 39 µg (13%) Z11-16:OAc and 3 µg (1%) Z9-12:OAc. The rice-strain blend was constructed in a similar way, only with 12 µg (4%) Z7-12:OAc , 24 µg (8%) Z11-16:OAc and 6 µg (2%) Z9-12:OAc. Numbers in the bars indicate total number of males caught. See Unbehend et al. (2013) for more details. ** $P < 0.01$; * $P < 0.05$; NS: not significant.

The pheromone traps that were baited with the so-called rice-strain lure (100% Z9-14:Ac, 8% Z11-16:Ac, 4% Z7-12:Ac, 2% Z9-12:Ac) did not specifically attract rice-strain males in a grass field and only 49% of all trapped rice-strain males were attracted to the rice-strain lure (Unbehend et al. 2013). Together, these results

indicate that corn-strain lures are most attractive for both strains in a corn habitat, while there is no preference for a corn- or rice-strain lure in a rice habitat.

Importance of different pheromone components for male attraction in the field

To assess strain-specific male response towards the different pheromone components, we also evaluated the importance of single pheromone components in the attraction of corn- and rice-strain males in a corn and a grass field in Florida (Unbehend et al. 2013). As mentioned earlier, fall armyworm males can vary in their attraction towards E7-12Ac, but show stable geographic-independent attraction towards binary blends containing Z9-14Ac and Z7-12Ac (Tumlinson et al. 1986; Andrade et al. 2000; Fleischer et al. 2005; Batista-Pereira et al. 2006; Unbehend et al. 2013). We tested different doses of the critical secondary component Z7-12Ac and found that corn-strain males were most responsive to lures containing 2% Z7-12Ac, whereas rice-strain males showed a wider response range, as they were attracted to lures containing 2%, 4% or 10% Z7-12:Ac (Unbehend et al. 2013; Figure 3). This strain-specific male response is consistent with the strain-specific female pheromone production in Florida, at least in the corn-strain, because corn- and rice-strain females produce around 2% and 4% Z7-12Ac, respectively (Groot et al. 2008; Unbehend et al. 2013). These results suggest that fall armyworm corn-strain males in Florida are adapted to the strain-specific female pheromone differences in the amount of Z7-12Ac, i.e. 2% versus 4%. To our knowledge, this is the first time that such a small change in a pheromone component has such a pronounced effect on male attraction.
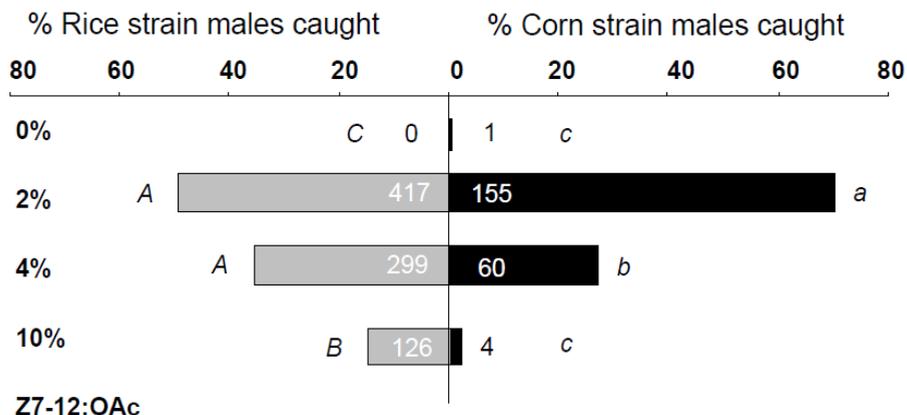
Figure 3. Strain-specific response of *Spodoptera frugiperda* males towards different doses of Z7-12:OAc added to 300 µg Z9-14:OAc in a corn and grass field in Florida. Different letters next to the bars indicate significant differences. Numbers in the bars indicate total number of males caught. See Unbehend et al. (2013) for more details.

The relative importance of Z11-16Ac is still unclear. In Costa Rica, the ternary blend of Z11-16Ac, Z9-14Ac and Z7-12Ac captured marginally more males than the binary blend of Z9-14:Ac and Z7-12:Ac in one test and marginally fewer in another, although neither effect was statistically significant (Andrade et al. 2000). Similarly, addition of Z11-16Ac to binary blends did not significantly increase trap catches in Brazil (Batista-Pereira et al. 2006) or Florida (Tumlinson et al. 1986; Unbehend et al. 2012). However, trapping experiments in Pennsylvania indicate that the addition of Z11-16Ac, together with Z9-12Ac, enhances male attraction to Z9-14Ac and Z7-12Ac (Fleischer et al. 2005), and field experiments in Florida showed that addition of Z11-16Ac, together with Z9-12Ac, to Z9-14Ac and Z7-12Ac changed the response of corn- and rice-strain males in different fields (Unbehend et al. 2013).

The compound Z9-12:Ac has been reported to occur in glands of females from North and South America (Descoins et al. 1988; Batista-Pereira et al. 2006; Groot et al. 2008). In Costa Rica and Florida, fall armyworm males were attracted to traps containing only Z9-12Ac (Jones and Sparks 1979; Andrade et al. 2000). When conducting experiments where we added different relative amounts of Z9-12Ac to the binary blend of Z9-14:Ac and Z7-12:Ac, we found that all tertiary blends containing Z9-12Ac were similarly attractive as the binary blends without Z9-12Ac, both in corn-and rice-strain habitats in Florida (Unbehend et al. 2013). However, since the addition of Z9-12Ac and Z11-16Ac to binary blends also changed the attraction of corn- and rice-strain males in Florida (Unbehend et al. 2013), a synergistic effect of Z9-12Ac in combination with other components cannot be excluded.

In summary, corn- and rice-strain males in Florida were mostly attracted to a corn-strain pheromone blend, at least in corn fields. Thus, there may be synergistic effects of host plant volatiles and sex pheromone components in corn fields. In grass fields, we did not find a preference for a corn- or a rice-strain pheromone blend in either strain. Strain-specific responses were found towards different doses of Z7-12Ac added to the major pheromone component Z9-14Ac, where corn-strain males were mostly attracted to 2% Z7-12Ac and rice-strain males were attracted to a wider range (2-10%). Together, these data suggest that strain-specific differences in the sexual communication of both strains do not cause assortative mating in Florida and thus are a weak prezygotic isolation barrier between the corn- and the rice-strain.

IV. Level and direction of hybridization between the two strains

The fact that hybridization between the two strains can be observed in the field raises the question: are these strains in the process of divergence or convergence?

RC-hybrid females have been found to be less likely to mate with any kind of male (C, R, RC or CR) and to produce a lower number of egg masses when they do mate (Pashley and Martin 1987; Whitford et al. 1988; Groot et al. 2010). Interestingly, RC-hybrid males did not show this deficiency and mated readily with all types of females (C, R and CR) (Groot et al. 2010). The fact that RC hybrid females are found to be mostly sterile in laboratory experiments seems to conflict with the field observation where mainly RC hybrids are found (see Section I). However, this contradiction makes the "reproductive problem" of RC hybrid females a perfect postzygotic isolation barrier: if the most abundant individuals are at the same time the least fertile ones, gene flow is maximally prevented at this stage. This thus indicates that these strains are in the process of divergence rather than convergence. Since reproductive isolation barriers are believed to act sequentially in the life cycle of hybridizing taxa (Schemske 2000; Ramsey et al. 2003), postzygotic phenomena like the evolution of RC hybrid female sterility do not appear to be the first step in speciation. However, given the existence of RC hybrid females in the field, while in the laboratory these hybrid females are hardly able to reproduce, this hybrid incompatibility represents an essential contribution to the process of speciation between the two strains.

V. Possible evolutionary scenarios on reproductive isolation in the two strains

Since the host association of the two strains does not seem to be as strict as early studies indicated, ecological specialization does not seem the most likely cause of differentiation between the two strains in *S. frugiperda*. Other factors may have influenced a host association between the strains. One of these factors may be the presence of competitors or natural enemies on the ancestral host as has been suggested for other phytophagous insects (Berlocher and Feder 2002). Pashley et

al. (1995) reported that over a two year period, fall armyworm larval mortality caused by parasites, predators and pathogens was higher in pastures than in corn fields. For this reason, the corn habitat may constitute a more protected environment than the rice habitats.

On the basis of the distribution of the two strains, particularly the distribution of the respective hybrids, and the behavioral differences between the two strains, we hypothesize that the rice strain is the ancestral strain and corn the derived strain (Juárez et al. 2013). Higher levels of genetic and behavioral homogeneity observed in the corn- than the rice-strain suggests that the corn-strain went through a bottleneck, i.e. that the corn-strain arose from a few individuals. Additionally, in corn fields a significant portion of rice-strain individuals as well as hybrids are found, specifically RC hybrids, while in rice fields the percent of corn-strain individuals or hybrids is generally much lower (Prowell et al. 2004; Saldamando B and Vélez-Arango 2010). The observation that males of both strains are mostly attracted to a corn-strain sex pheromone blend in corn fields, while this preference is not found in rice fields, is consistent with these results. Hybrid incompatibility is between R mothers and C fathers and not vice versa, i.e. RC hybrids are incompatible with any kind of male, whereas CR hybrids produce fertile and viable offspring. Together, these findings suggest that the rice-strain is the ancestral strain and the corn-strain is the derived strain.

Conclusion

In reviewing many studies on the host plant association of the two strains, host associations do not seem to be consistent when the mitochondrial COI marker is considered. In corn fields, more rice strain individuals seem to be found than vice versa, and RC hybrids are also mostly found in corn habitats. Thus, habitat isolation alone does not seem to be strong prezygotic isolation barrier between the

corn- and the rice-strain. Similarly, strain-specific differences in the sexual communication system of both strains alone do not appear strong enough to cause assortative mating between strains. However, differences in diel patterns of reproductive behaviors seem to be much more consistent than host-plant associations or differential sexual communication between the strains. Since a shift in timing can immediately inhibit gene flow, the strains may be "timing strains" rather than "host strains" or "pheromone strains". Furthermore, the postmating barrier of RC hybrid female sterility seems to be most likely a key element in the divergence of these two strains.

Acknowledgements

References

Andrade, R., C. Rodriguez, and A. C. Oehlschlager. 2000. Optimization of a pheromone lure for *Spodoptera frugiperda* (Smith) in Central America. *Journal of the Brazilian Chemical Society* 11:609-613.

Baker, T. C., and R. T. Cardé. 1979. Endogenous and exogenous factors affecting periodicities of female calling and male sex pheromone response in *Grapholitha molesta* (Busck). *J. Insect Physiol.* 25:943-950.

Batista-Pereira, L. G., K. Stein, A. F. de Paula, J. A. Moreira, I. Cruz, M. D. Figueiredo, J. Perri, and A. G. Correa. 2006. Isolation, identification, synthesis, and field evaluation of the sex pheromone of the Brazilian population of *Spodoptera frugiperda*. *J. Chem. Ecol.* 32:1085-1099.

Bergen, E. L., J. T. Rowell, F. Gould, and M. R. Servedio. 2012. Stochasticity in sexual selection enables divergence: implications for moth pheromone evolution. *Evol Biol (N Y)* 39:271-281.

Berlocher, S. H., and J. L. Feder. 2002. Sympatric speciation in phytophagous insects: Moving beyond controversy? *Annu. Rev. Entomol.* 47:773-815.

Busato, G. R., A. D. Grutzmacher, A. C. de Oliveira, E. A. Vieira, P. D. Zimmer, M. M. Kopp, J. D. Bandeira, and T. R. Magalhaes. 2004. Analysis of the molecular

structure and diversity of *Spodoptera frugiperda* (JE smith) (Lepidoptera: Noctuidae) populations associated to the corn and rice crops in Rio Grande do Sul State, Brazil. *Neotrop. Entomol.* 33:709-716.

Butlin, R., and A. J. Trickett. 1997. Can population genetic simulations help to interpret pheromone evolution? Pp. 548–562 *in* R. T. Carde, and A. K. Minks, eds. *Insect pheromone research: New directions*. Chapman and Hall, New York.

Clark, P. L., J. Molina-Ochoa, S. Martinelli, S. R. Skoda, D. J. Isenhour, D. J. Lee, J. T. Krumm, and J. E. Foster. 2007. Population variation of the fall armyworm, *Spodoptera frugiperda*, in the Western Hemisphere. *Journal of Insect Science* 7.

Descoins, C., J. F. Silvain, B. Lalannecassou, and H. Cheron. 1988. Monitoring of crop pests by sexual trapping of males in the French West-Indies and Guyana. *Agric Ecosyst Environ* 21:53-65.

Fleischer, S. J., C. L. Harding, P. E. Blom, J. White, and J. Grehan. 2005. *Spodoptera frugiperda* pheromone lures to avoid nontarget captures of *Leucania phragmatidicola*. *J. Econ. Entomol.* 98:66-71.

Groot, A. T., M. Marr, D. G. Heckel, and G. Schofl. 2010. The roles and interactions of reproductive isolation mechanisms in fall armyworm (Lepidoptera: Noctuidae) host strains. *Ecol. Entomol.* 35:105-118.

Groot, A. T., M. Marr, G. Schofl, S. Lorenz, A. Svatos, and D. G. Heckel. 2008. Host strain specific sex pheromone variation in *Spodoptera frugiperda*. *Front Zool* 5.

Jones, R. L., and A. N. Sparks. 1979. (Z)-9-Tetradecen-1-ol acetate - secondary sex pheromone of the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae). *J. Chem. Ecol.* 5:721-725.

Juárez, M. L., M. G. Murua, M. G. Garcia, M. Ontivero, M. T. Vera, J. C. Vilardi, A. T. Groot, A. P. Castagnaro, G. Gastaminza, and E. Willink. 2012. Host association of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn and rice strains in Argentina, Brazil, and Paraguay. *J. Econ. Entomol.* 105:573-582.

Juárez, M. L., G. Schöfl, M. T. Vera, J. C. Vilardi, M. G. Murua, E. Willink, S. Hänniger, D. G. Heckel, and A. T. Groot. 2013. Population genetic analysis of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn and rice strains from Argentina, Brazil and Paraguay. *Submitted*.

Levy, H. C., A. Garcia-Maruniak, and J. E. Maruniak. 2002. Strain identification of *Spodoptera frugiperda* (Lepidoptera : Noctuidae) insects and cell line: PCR-RFLP of cytochrome oxidase C subunit I gene. *Fla. Entomol.* 85:186-190.

Lima, E. R., and J. N. McNeil. 2009. Female sex pheromones in the host races and hybrids of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Chemoecology* 19:29-36.

Löfstedt, C. 1993. Moth pheromone genetics and evolution. *Phil Trans Royal Soc London B* 340:167-177.

Lu, Y. J., and M. J. Adang. 1996. Distinguishing fall armyworm (Lepidoptera: Noctuidae) strains using a diagnostic mitochondrial DNA marker. *Fla. Entomol.* 79:48-55.

Lu, Y. J., M. J. Adang, D. J. Isenhour, and G. D. Kochert. 1992. RFLP analysis of genetic variation in North American populations of the fall armyworm moth *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Mol. Ecol.* 1:199-207.

Lu, Y. J., G. D. Kochert, D. J. Isenhour, and M. J. Adang. 1994. Molecular characterization of a strain-specific repeated DNA sequence in the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Insect Mol. Biol.* 3:123-130.

Machado, V., M. Wunder, V. D. Baldissera, J. V. Oliveira, L. M. Fiuza, and R. N. Nagoshi. 2008. Molecular characterization of host strains of *Spodoptera frugiperda* (Lepidoptera : Noctuidae) in Southern Brazil. *Ann. Entomol. Soc. Am.* 101:619-626.

Marr, M. 2009. Differences in pheromone composition between the two strains of the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Pp. 64.*http://www.clib-jena.mpg.de/ theses/ice/ICE09003.pdf*. Friedrich Schiller University, Jena.

Martinelli, S., P. L. Clark, M. I. Zucchi, M. C. Silva-Filho, J. E. Foster, and C. Omoto. 2007. Genetic structure and molecular variability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected in maize and cotton fields in Brazil. *Bull. Entomol. Res.* 97:225-231.

McMichael, M., and D. P. Prowell. 1999. Differences in amplified fragment-length polymorphisms in fall armyworm (Lepidoptera : Noctuidae) host strains. *Ann. Entomol. Soc. Am.* 92:175-181.

Meagher, R. L., and M. Gallo-Meagher. 2003. Identifying host strains of fall armyworm (Lepidoptera: Noctuidae) in Florida using mitochondrial markers. *Fla. Entomol.* 86:450-455.

Meagher, R. L., and E. R. Mitchell. 1998. Phenylacetaldehyde enhances upwind flight of male fall armyworm (Lepidoptera : Noctuidae) to its sex pheromone. *Fla. Entomol.* 81:556-559.

Meagher, R. L., and R. N. Nagoshi. 2012. Differential feeding of fall armyworm (Lepidoptera: Noctuidae) host strains on meridic and natural diets. *Ann. Entomol. Soc. Am.* 105:462-470.

Meagher, R. L., R. N. Nagoshi, C. Stuhl, and E. R. Mitchell. 2004. Larval development of fall armyworm (Lepidoptera : Noctuidae) on different cover crop plants. *Fla. Entomol.* 87:454-460.

Meagher, R. L., R. N. Nagoshi, and C. J. Stuhl. 2011. Oviposition choice of two fall armyworm (Lepidoptera: Noctuidae) host strains. *J. Insect Behav.* 24:337-347.

Mitchell, E. R., J. H. Tumlinson, and J. N. McNeil. 1985. Field evaluation of commercial pheromone formulations and traps using a more effective sex pheromone blend for the fall armyworm (Lepidoptera, Noctuidae). *J. Econ. Entomol.* 78:1364-1369.

Nagoshi, R. N. 2010. The fall armyworm Triose Phosphate Isomerase (Tpi) gene as a marker of strain identity and interstrain mating. *Ann. Entomol. Soc. Am.* 103:283-292.

Nagoshi, R. N. 2012. Improvements in the identification of strains facilitate population studies of fall armyworm subgroups. *Ann. Entomol. Soc. Am.* 105:351-358.

Nagoshi, R. N., J. J. Adamczyk, R. L. Meagher, J. Gore, and R. Jackson. 2007a. Using stable isotope analysis to examine fall armyworm (Lepidoptera : Noctuidae) host strains in a cotton habitat. *J. Econ. Entomol.* 100:1569-1576.

Nagoshi, R. N., S. J. Fleischer, and R. L. Meagher. 2009. Texas is the overwintering source of fall armyworm in central Pennsylvania: implications for migration into the northeastern United States. *Environ. Entomol.* 38:1546-1554.

Nagoshi, R. N., and R. L. Meagher. 2003a. Fall armyworm FR sequences map to sex chromosomes and their distribution in the wild indicate limitations in interstrain mating. *Insect Mol. Biol.* 12:453-458.

Nagoshi, R. N., and R. L. Meagher. 2003b. FR tandem-repeat sequence in fall army-worm (Lepidoptera : Noctuidae) host strains. *Ann. Entomol. Soc. Am.* 96:329-335.

Nagoshi, R. N., and R. L. Meagher. 2004. Behavior and distribution of the two fall armyworm host strains in Florida. *Fla. Entomol.* 87:440-449.

Nagoshi, R. N., R. L. Meagher, J. J. Adamczyk, S. K. Braman, R. L. Brandenburg, and G. Nuessly. 2006a. New restriction fragment length polymorphisms in the cytochrome oxidase I gene facilitate host strain identification of fall armyworm (Lepidoptera : Noctuidae) populations in the southeastern United States. *J. Econ. Entomol.* 99:671-677.

Nagoshi, R. N., R. L. Meagher, K. Flanders, J. Gore, R. Jackson, J. Lopez, J. S. Armstrong, G. D. Buntin, C. Sansone, and B. R. Leonard. 2008. Using haplotypes to monitor the migration of fall armyworm (Lepidoptera : noctuidae) corn-strain populations from Texas and Florida. *J. Econ. Entomol.* 101:742-749.

Nagoshi, R. N., R. L. Meagher, and D. A. Jenkins. 2010. Puerto Rico fall armyworm has only limited interactions with those from Brazil or Texas but could have substantial exchanges with Florida populations. *J. Econ. Entomol.* 103:360-367.

Nagoshi, R. N., R. L. Meagher, G. Nuessly, and D. G. Hall. 2006b. Effects of fall armyworm (Lepidoptera: Noctuidae) interstrain mating in wild populations. *Environ. Entomol.* 35:561-568.

Nagoshi, R. N., M. G. Murua, M. Hay-Roe, M. L. Juarez, E. Willink, and R. L. Meagher. 2012. Genetic characterization of fall armyworm (Lepidoptera: Noctuidae) host strains in Argentina. *J. Econ. Entomol.* 105:418-428.

Nagoshi, R. N., P. Silvie, and R. L. Meagher. 2007b. Comparison of haplotype frequencies differentiate fall armyworm (Lepidoptera : Noctuidae) corn-strain populations from Florida and Brazil. *J. Econ. Entomol.* 100:954-961.

Nagoshi, R. N., P. Silvie, R. L. Meagher, J. Lopez, and V. Machado. 2007c. Identification and comparison of fall armyworm (Lepidoptera : Noctuidae) host strains in Brazil, Texas, and Florida. *Ann. Entomol. Soc. Am.* 100:394-402.

Pashley, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera, Noctuidae) - a sibling species complex. *Ann. Entomol. Soc. Am.* 79:898-904.

Pashley, D. P. 1988a. Current status of fall armyworm host strains. *Fla. Entomol.* 73:227-234.

Pashley, D. P. 1988b. Quantitative genetics, development, and physiological adaptation in host strains of fall armyworm. *Evolution* 42:93-102.

Pashley, D. P. 1989. Host-associated differentiation in army worms (Lepidoptera: Noctuidae): an allozymic and mitochondrial DNA perspective. Pp. 103-114 *in* H. D. Loxdale, and J. Den Hollander, eds. *Electrophoretic studies on agricultural pests, Systematics Association*. Clarendon, Oxford.

Pashley, D. P., A. M. Hammond, and T. N. Hardy. 1992. Reproductive isolating mechanisms in fall armyworm host strains (Lepidoptera, Noctuidae). *Ann. Entomol. Soc. Am.* 85:400-405.

Pashley, D. P., T. N. Hardy, and A. M. Hammond. 1995. Host effects on developmental and reproductive traits in fall armyworm strains (Lepidoptera, Noctuidae). *Ann. Entomol. Soc. Am.* 88:748-755.

Pashley, D. P., S. J. Johnson, and A. N. Sparks. 1985. Genetic population structure of migratory moths - the fall armyworm (Lepidoptera, Noctuidae). *Ann. Entomol. Soc. Am.* 78:756-762.

Pashley, D. P., and L. D. Ke. 1992. Sequence evolution in mitochondrial ribosomal and ND-1 genes in Lepidoptera - Implications for phylogenetic analysis. *Mol. Biol. Evol.* 9:1061-1075.

Pashley, D. P., and J. A. Martin. 1987. Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera, Noctuidae). *Ann. Entomol. Soc. Am.* 80:731-733.

Prowell, D. P., M. McMichael, and J. F. Silvain. 2004. Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera : Noctuidae). *Ann. Entomol. Soc. Am.* 97:1034-1044.

Ramsey, J., H. D. Bradshaw, and M. Scheffer. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* 57:1520-1534.

Ritchie, M. G. 2007. Sexual selection and speciation. *Annu Rev Ecol Evol Syst* 38:79-102.

Saldamando B, C. I., and A. M. Vélez-Arango. 2010. Host plant association and genetic differentiation of corn and rice strains of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Colombia. *Neotrop. Entomol.* 39:921-929.

Schemske, D. W. 2000. Understanding the origin of species. *Evolution* 54:1069-1073.

Schöfl, G., A. Dill, D. G. Heckel, and A. T. Groot. 2011. Allochronic separation versus mate choice: nonrandom patterns of mating between fall armyworm host strains. *Am. Nat.* 177:470-485.

Schöfl, G., D. G. Heckel, and A. T. Groot. 2009. Time-shifted reproductive behaviours among fall armyworm (Noctuidae: *Spodoptera frugiperda*) host strains: evidence for differing modes of inheritance. *J. Evol. Biol.* 22:1447-1459.

Tumlinson, J. H., E. R. Mitchell, P. E. A. Teal, R. R. Heath, and L. J. Mengelkoch. 1986. Sex pheromone of fall armyworm, *Spodoptera frugiperda* Smith, J.E. - Identification of components critical to attraction in the field. *J. Chem. Ecol.* 12:1909-1926.

Unbehend, M., S. Hänniger, R. L. Meagher, D. G. Heckel, and A. T. Groot. 2012. Pheromonal divergence between two strains of *Spodoptera frugiperda*. *Jornal of Chemical Ecology* 39:364-376.

Veenstra, K. H., D. P. Pashley, and J. A. Ottea. 1995. Host-plant adaptation in fall armyworm host strains - comparison of food consumption, utilization, and detoxication enzyme activities. *Ann. Entomol. Soc. Am.* 88:80-91.

Velez-Arango, A. M., R. E. Arango I, D. Villanueva M, E. Aguilera G, and C. I. Saldamando B. 2008. Identification of *Spodoptera frugiperda* biotypes (Lepidoptera: Noctuidae) through using mitochondrial and nuclear markers. *Rev Colomb Entomol* 34:145-150.

Whitford, F., S. S. Quisenberry, T. J. Riley, and J. W. Lee. 1988. Oviposition preference, mating compatibility, and development of 2 fall armyworm strains. *Fla. Entomol.* 71:234-243.