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Article type : Original Article

Submission: Molecular Ecology

Section: Ecological genomics

Influence of host plant, geography and pheromone strain on genomic differentiation in sympatric populations of *Ostrinia nubilalis*

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/mec.15234

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Abstract

Patterns of mating for the European corn borer (*Ostrinia nubilalis*) moth depend in part on variation in sex-pheromone blend. The ratio of (*E*)-11- and (*Z*)-11-tetradecenyl acetate (E11- and Z11-14:OAc) in the pheromone blend that females produce and males respond to differs between strains of *O. nubilalis*. Populations also vary in female oviposition preference for and larval performance on maize (C4) and non-maize (C3) host plants. The relative contributions of sexual and ecological trait variation to the genetic structure of *O. nubilalis* remains unknown. Host-plant use ($^{13}C/^{14}C$ ratios) and genetic differentiation were estimated among sympatric E and Z pheromone strain *O. nubilalis* males collected in sex-pheromone baited traps at 12 locations in Pennsylvania and New York between 2007 and 2010. Among genotypes at 65 single nucleotide polymorphism marker loci, variance at a position in the pheromone gland fatty acyl-reductase (*pgfar*) gene at the locus responsible for determining female pheromone ratio

(*Pher*) explained 64% of the total genetic differentiation between males attracted to different pheromones (male response, *Resp*), providing evidence of sexual inter-selection at these unlinked loci. Principal coordinate, Bayesian clustering, and distance-based redundancy analysis (dbRDA) demonstrate that host plant history or geography does not significantly contribute to population variation or differentiation among males. In contrast, these analyses indicate that pheromone response and *pgfar*-defined strain contribute significantly to population genetic differentiation. This study suggests that behavioral divergence likely plays a larger role in driving genetic variation compared to host plant-defined ecological adaptation.

Keywords: population genetics, sexual selection, reproductive isolation

1 | Introduction

Recent models of speciation predict that populations diverge primarily due to the accumulation of genetic changes coincident with local ecological adaptation (Diekmann & Doebeli 2000; Schluter 2001; Schluter & Conte 2009; Nosil 2012). For example, host plant shifts among herbivorous insects can increase genetic variance among sympatric subpopulations by disrupting random mating (Pashley 1989; Nason *et al*. 2002) or selecting for optimal responses to host plant defenses (Feder *et al*. 1998; Berlocher & Feder 2002; Drès & Mallet 2002). However, sexual selection can also contribute to population fragmentation and ultimately speciation by reducing the frequency of mating between individuals with variant sexual traits (Panhuis *et al*. 2001), thus arguably functioning as a force independent of natural selection (Safran *et al*. 2013). Sexual selection is capable of generating linkage disequilibrium (LD) between independently assorting loci due to assortative mating between mates with compatible attraction and response phenotypes (Kirkpatrick & Ravigne 2002; Kirkpatrick 2017). Divergent mating preferences for sexual traits are often one of the most important reproductive isolating

barriers in sympatric populations (Svensson *et al*. 2006; Dopman *et al*. 2010; Grace & Shaw 2011; Lackey & Boughman 2017), although whether divergent preferences arise through sexual selection alone is still a matter of debate (Servedio & Boughman). Furthermore, sexual and natural selection might interact in various ways during speciation, and opportunities to study both in unison may provide unique insights into the mechanisms by which reproductive isolation is established and maintained (Maan & Seehausen 2011; Butlin *et al*. 2012).

Populations of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), vary in ecological and sexual traits, and this species has emerged as a model for studying mechanisms of ecological diversification and the formation of incipient species (Dopman 2011; Lassance 2016; Kozak *et al*. 2017; Wang *et al*. 2017; Coates *et al*. 2018). *Ostrinia nubilalis* is a polyphagous lepidopteran native to Europe and western Asia. Multiple inadvertent introductions into North America on imported broomcorn from Europe occurred near Boston, Massachusetts, Amsterdam, New York, and St. Thomas, Ontario between 1909 and 1914 (Vinal 1917; Smith 1920; McLaine 1922; Caffrey & Worthley 1927). Populations subsequently expanded south and west to the Rocky Mountains (Chiang 1972; Showers 1993). Current *O. nubilalis* populations in the northeastern United States vary in the number of reproductive generations per year (voltinism) as well as sex pheromone communication systems (Roelofs *et al*. 1985; Glover *et al*. 1991; Sorenson *et al*. 1992). Specifically, E- and Z-strain females produce blends of 99:1 or 3:97 (*E*)-11- and (*Z*)-11-tetradecenyl acetate (11-14:OAc), respectively, (Klun & Brindley 1970; Kochansky *et al*. 1975; Roelofs *et al*. 1972), to which corresponding males of the two strains preferentially respond (Glover *et al*. 1987). Although pheromone blend specificity contributes to partial reproductive isolation (Liebherr & Roelofs 1975; Pélozuelo *et al*. 2007; Dopman *et al*. 2010), hybrid females producing a 65:35 blend of *E*11- to *Z*11-14:OAc are found in nature (Klun & Maini 1979; Roelofs *et al*. 1985; DuRant *et al*. 1995).

Genetic loci contributing to *O. nubilalis* sexual isolation are inherited independently (Löfstdt *et al*. 1989), with female pheromone blend and male response respectively controlled

by autosomal *Pher* and sex-linked *Resp* loci (Glover *et al*. 1990; Dopman *et al*. 2004). *Pher* corresponds to the pheromone gland fatty acyl reductase gene (*pgfar*; Lassance *et al*. 2010), and fixed amino acid changes between *pgfar-e* and *pgfar-z* alleles cause differences in the biosynthesis of isomeric components of female pheromone blends (Lassance *et al*. 2013). Pheromone strains and hybrids can be distinguished by a single nucleotide polymorphism (SNP) fixed between *pgfar-e* and *pgfar-z* alleles. Estimates of hybridization and positive assortative mating based on this autosomal marker vary among natural populations (Coates *et al*. 2013a). *Resp* is located within a region of the Z-chromosome that encodes a suite of genes putatively involved in neuronal development and function (Koutroumpa *et al*. 2016). In addition to *Resp*, a major QTL for voltinism differences between strains is located on the Z-chromosome (Dopman *et al*. 2004). Several loci on the sex chromosome (*Tpi*, *OR1* and *OR3*) show strong differentiation between E- and Z-strain individuals while other sex chromosomal and autosomal loci do not (Dopman *et al*. 2005; Dopman 2011; Lassance *et al*. 2011). Thus, the contribution of divergence in sexual traits (sexual selection), to genome-wide substructure among sympatric *O. nubilalis* populations remains relatively unknown.

Host-plant interactions have long been recognized as factors that impact the diversity within insect populations (Ehrlich & Raven 1964). Although *O. nubilalis* larvae can feed on over 200 monocot and dicot host plants (Hodgson 1928; Lewis *et al*. 1975), evidence supports some degree of local host plant specialization on cultivated maize and other plant species (O'Rourke *et al*. 2010). Laboratory choice tests suggest that neonates may prefer alternate weedy hosts over maize (Tate *et al*. 2006), but survivorship is highest on maize (Calcagno *et al*. 2007; Fisher *et al*. 2017). The relative proportion of carbon stable isotope ($\delta^{13}C$) integrated into developing insects is directly correlated with food source (DeNiro & Epstein 1978; Boutton *et al*. 1999), and indicates history of C3- or C4 photosynthetic-pathways for host plants ingested by *O. nubilalis* as larvae (Ponsard *et al*. 2004; Malausa *et al*. 2005). Analysis of $\delta^{13}C$ demonstrated that both *O. nubilalis* strains fed mostly on C4 plants (predominantly maize), but a higher proportion of males from traps baited with the E-strain pheromone lure fed on non-maize hosts as larvae in

North America (O'Rourke *et al*. 2010). Nevertheless, host plant attraction for *O. nubilalis* oviposition can be influenced by plant phenology (Savinelli *et al*. 1988; Spanger & Calvin 2000) and volatiles (Udayagiri & Mason 1995), and may contribute to variation in host plant associations among *Ostrinia* species within Europe (Bontemps *et al*. 2004; Bethenod *et al*. 2005; Malusa *et al*. 2008). In France, *Ostrinia* populations using the alternative pheromone blends of E11- and Z11-14:OAc were initially assumed to be E- and Z-strain *O. nubilalis*. Strong associations were demonstrated among moths using the Z-pheromone communication system for maize (C4) and the E-system moths for C3 host plants (Bontemps *et al*. 2004; Bourguet *et al*. 2014, which were accompanied by significant levels of genetic differentiation (Bethenod *et al*. 2005; Malausa *et al*. 2007). However, subsequent studies concluded that *Ostrinia* using the E-pheromone system in France were *O. scapulalis*, and a different species compared to sympatric Z-strain *O. nubilalis* (Frolov *et al*. 2007; Malausa *et al*. 2008; Calcagno *et al*. 2010). Thus, results of these European studies are not directly transferrable to E- and Z-strain *O. nubilalis* in North America which show significant, but less pronounced and spatially-dependent associations with C3 and C4 host plants, respectively (O'Rourke *et al*. 2010). The degree to which host plant preference is associated with any genetic differentiation within or between *O. nubilalis* pheromone strains in North America remains unknown.

Genetic drift may also contribute to differentiation among *O. nubilalis* populations. Based on anonymous microsatellite marker data, *O. nubilalis* from two locations in New York and Pennsylvania were genetically differentiated from sites in Ohio westward to Colorado (Kim *et al*. 2011). Although the authors suggested landscape features within northeastern states, including the Appalachian Mountains and fragmented crop land might serve as a partial barrier to gene flow, the possible influence of pheromone strain differences among geographic regions (Liebherr & Roelofs 1975; Glover *et al*. 1991) could not be ruled out. A separate study predicted a significant isolation-by-distance relationship when comparing only Z-strain populations along a north-south transect in the Midwest United States, although this pattern was driven by a small percentage of the sampled markers (15 of 194: Levy *et al*. 2015).

Of the handful of studies that address the relative roles of ecological adaptation, sexual selection, and genetic drift as drivers of genomic divergence, many find that sexual selection is potent only when coupled with ecological factors (van Doorn *et al*. 2009; Mann & Seehausen 2011; Wagner *et al*. 2012; Servedio & Boughman 2017), but in most systems limited details are available regarding ecologically and sexually relevant traits (Safran *et al*. 2013). By dissecting the short-term contributions of ecology, genetic drift, and sexual traits on patterns of genomic variation and gene flow among distinct subpopulations, subspecies, and incipient species, a broader understanding of their contributions to speciation across time scales may be gained. Using anonymous genomic SNP markers, this study estimates the influence of pheromone strain, host plant differences, and geographic distance on population genetic structure of *O. nubilalis* within the northeastern United States.

2 | Materials and Methods

2.1 | Collection and phenotypic differentiation

Pheromone lures with a blend of 97:3 or 1:99 synthetic E11- and Z11-14:OAc (Trécé Incorporated, Adair, OK) were used to separately bait one of a pair of Harstack wire cone traps at Pennsylvania and cloth Scentry traps at New York collection sites between 2007 and 2012 (12 sites total; Table 1; Fig. 1; Table S1). Males were captured during the second flight of the bivoltine strain (i.e. adult offspring of the overwintering generation) and frozen at -20°C. Samples were shipped on dry ice to the USDA-ARS, Corn Insects & Crop Genetics Research Unit, Ames, Iowa, where thorax tissue and all 4 wings were dissected from each moth. Wings were placed in individual 0.2 ml wells of a 96-well PCR plate lined with aluminum foil and stored at -20°C until used for carbon isotope analysis. Total genomic DNA was purified from each thorax using the Qiagen DNeasy Blood and Tissue Extraction kit according to manufacturer instructions (Qiagen, Hilden, Germany). DNA concentration in resulting extracts was quantified

on a NanoDrop2000 spectrophotometer (Thermo Scientific, Wilmington, DE), diluted to 10 ng μl^{-1} with deionized nuclease free water, and stored at -20°C.

Host plant history, C3 (non-maize) or C4 (probable maize), was assigned to each male collected in pheromone traps based on carbon stable isotope analysis. For this, the four wings were dissected from each trap collected male, and the ratio of 13C to 14C ($\delta^{13}C$) was estimated by continuous flow-isotopic ratio mass spectrometry (CF-IRMS) conducted at the University of California Berkley, Center for Stable Isotope Biogeochemistry (Berkley, CA). The results were converted to δ units relative to Pee Dee Belemnite carbonate ($\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$; R = the ratio of $^{13}C/^{12}C$ atomic percentage).

In addition, genetic assignment of pheromone strain was determined for each male based on SNP genotype of a marker at the *pgfar* locus. This assay detects alleles that are >98.7% correlated with E- or Z-pheromone strains (Coates *et al*. 2013a). Specifically, the *Taq*I PCR-RFLP assay was used to assign homozygous *pgfar-e*/*pgfar-e* and *pgfar-z*/*pgfar-z* genotypes to respective strains, and *pgfar-e*/*pgfar-z* heterozygotes to a hybrid class. Additionally, capture of males in pheromone traps baited with the E- or Z-strain lure was used as a proxy for pheromone response phenotype, given that males of the two strains strongly differ in their flight towards E and Z pheromone blends (Roelofs *et al*. 1987).

2.2 | Population genetic differentiation

A set of 108 anonymous biallelic SNP markers was used to genotype each male by single base extension assay products analyzed on a Sequenom MassARRAY® located at the Iowa State University, Center for Plant Genomics, Ames, IA. In brief, loci containing *a priori* defined SNPs were amplified in four PCR multiplexes (W1, W2, W3 and W4; Coates *et al*. 2011), and subsequently used as template for SBE assays that incorporated allele-specific mass-modified dideoxynucleotides using the iPLEX-Gold Mastermix (Sequenom; Tang *et al*. 1999). Genotypes

were determined from raw Sequenom MassARRAY® output as described by Coates *et al*. (2013b).

Processed Sequenom SNP and *pgfar* PCR-RFLP genotype data were assessed for Hardy–Weinberg equilibrium (HWE), with departures of observed heterozygosity (H_O) from expected heterozygosity (H_E) across collection sites tested for significance ($\alpha = 0.05$) using Markov chain exact tests implemented in the Arlequin software package (v. 3.5.2; Excoffier *et al*. 2005). HWE tests were performed for E-, Z- and hybrid *pgfar* PCR-RFLP-defined partitions within location. Loci were subsequently excluded from further analyses if monomorphic, genotypic data were missing for ≥ 25% of individuals, or genotypic proportions showed a significant departure from HWE expectation in ≥ 20% of populations. Exceptions were made in instances where no HWE departures were predicted across all E- or Z-strain populations. For loci that passed the preceding filters, Fisher's exact tests were conducted to predict linkage disequilibrium (LD) between all pairs of unphased loci, and significance determined by 10,000 Markov Chain permutation steps using Arlequin 3.2.2 (Excoffier *et al*. 2005). Any pair of SNP loci in LD were compared to known map positions on a consensus *O. nubilalis* genetic linkage map (Levy *et al*. 2015). Linkage groups (LGs) were assigned to physical location on *Bombyx mori* chromosomes by querying the *B. mori* genome assembly database with *O. nubilalis* contig sequences from which SNP markers were developed (Coates *et al*. 2011) using the BLASTx algorithm at KAIKOBASE (Shimomura *et al*. 2009). Additionally, the *B. mori* fatty acyl-reductase involved in bombykol production (Moto *et al*. 2003; GenBank accession BAC79425) is the nearest ortholog to *O. nubilalis pgfar* (Lassance *et al*. 2010), and was used predict the relative orthologous position in *O. nubilalis*. All BLAST database results were filtered for *E*-values ≤ 10^{-50}.

Locus-by-locus F_{ST}, F_{IS}, and F_{IT} estimates were made based on homozygous *pgfar* genotypes of males partitioned by having been collected in traps baited with E11- or Z11-14:OAc pheromone lure (male pheromone response) or by *pgfar* genotype. Global *F*-statistics and analysis of molecular variance (AMOVA) were estimated for genotypes partitioned by

pheromone response and *pgfar* genotype, as well as by host plant history (C3 or C4), across the 12 collection sites. Pairwise F_{ST} estimates were made between collection sites, and within collection site between pheromone response, *pgfar* genotype, and host plant history. Significance threshold (false discovery rate) for each statistic was adjusted for multiple testing using the BH procedure (Benjamini & Hochberg 1995). All estimates of subpopulation differentiation were assessed using Arlequin v. 3.5.2 (Excoffier *et al*. 2005). A filtered SNP dataset of 64 loci (omitting *pgfar*) was used in all instances, except for comparisons of pheromone response in which the corresponding SNP dataset of 65 loci (including *pgfar*) was also assessed.

Principal coordinate analysis (PCoA) was conducted using the covariance matrix of pairwise F_{ST}s using GENALEX v. 6.503 (Peakall & Smouse 2006). Pairwise F_{ST} estimates were made for 52 groups (n = 1,421 genotypes) using the 65 SNP loci that included the *pgfar* locus, and again with 64 loci after excluding the *pgfar* locus. The 52 groups represent the three *pgfar* genotypes in the 12 collection sites for each year; note that there were no homozygous *pgfar-e*/*pgfar-e* individuals in two of the collections (Table 1), so those combinations were not represented in the analyses. Pairwise F_{ST} estimates were also made for the same 52 *pgfar* delimited groups with collection sites further partitioned by the two host plant history categories of C3 and C4 (n = 1,411 individuals), creating 91 total groups; note that there were no individuals with C3 host plant history in 13 of the *pgfar* plus collection site groups, so those combinations were not represented in the analyses. Factor scores along the two principal coordinates that accounted for the most variation were used to construct a scatter diagram to visualize genetic relationships among populations.

2.3 | Bayesian clustering

Population structure was inferred using a model-based clustering method implemented by STRUCTURE 2.3.4 (Prichard *et al*. 2000; Falush *et al*. 2003; Hubisz *et al*. 2009). STRUCTURE

provides likelihood estimates of the number of distinct populations (*K*) from unlinked genetic marker data using a Markov Chain Monte Carlo (MCMC) method. Estimates were obtained by iteratively running STRUCTURE 2.3.4 with *K* values from 1 to 10. Each run consisted of 10 replications with 1×10^7 iterations preceded by a burn-in of 10^5 iterations. Runs used an admixture model of individual ancestry and a uniform prior for ALPHA. The LOCPRIOR option (Hubisz *et al*., 2009) was used on genotypes partitioned into 12 collection sites (1,421 genotypes, 12 partitions by location), pheromone response combined across the 12 sample sites (1,421 genotypes, 2 partitions by collection in traps baited with E11- or Z11-14:OAc pheromone lure), and C3 and C4 host plant history (δ^{13}C estimates for C4 host = -8 to -17 and C3 host = -22 to -32). All runs were conducted using the set of 64 SNP genotypes (*pgfar* omitted), then repeated using the set of 65 SNP genotypes (*pgfar* included). For all STRUCTURE results, the median value of the estimated log probability for each clustering analysis, conditional on *K*, ln Pr(X|*K*), was used to compute the posterior probability of *K*, Pr(*K*|X), assuming a uniform *prior* distribution for *K*. Optimized values for *K* were derived from the resulting population co-ancestries (*Q*-matrix) using the *ΔK* statistic (Evanno *et al.* 2005). Individual *Q*-matrix data were visualized using Distruct (Rosenberg 2004).

2.4 | Ecological and behavioral relationships

Possible isolation-by-distance (IBD) (Slatkin 1993) was assessed by regressing pairwise genetic distance (based on estimates of F_{ST} among 12 sample locations) on geographic (km) distance. IBD analyses were performed using GENALEX (Peakall & Smouse, 2006) with the associated probabilities determined using Mantel's Test, and significance determined based on 999 permutations. The significance of any differences in the distribution of δ^{13}C estimates between *pgfar*-defined E-, Z- and hybrid (3 partitions) or pheromone response (2 partitions) grouped by sample site were made via single-factor ANOVA using SAS 9.2 (SAS Institute, Cary, NC, USA). Additionally, a distance-based redundancy analysis (dbRDA; Legendre & Anderson

1999; Oksanen *et al*. 2017) was used to test the relative roles of host plant use (C3:C4), sexual traits (*pgfar* or pheromone response), and geographic distance on population genetic structure. Redundancy analysis allows for multiple predictor variables to be analyzed simultaneously and each parsed into distinct factors unlike the partial Mantel Test (Legendre & Legendre 1999; Legendre & Fortin 2010). At each sampling site, males were classified as E- or Z-strain in two different ways: 1) by pheromone response (collected in traps baited with E11- or Z11-14:OAc pheromone lure), and 2) by male *pgfar* genotype (*pgfar-e/-e* as E-strain individuals and *pgfar-z/-z* as Z-strain individuals; all *pgfar-e/-z* were excluded). Since very few E-strain individuals were collected at Northampton, PA (Nor), and due to proximity (~5 km) of this site to Lehigh, PA (Leh), Nor was removed from analysis, resulting in 22 total populations (11 E- and 11 Z-strain). For each population, the mean $\delta^{13}C$ value was calculated, and the matrix of geographic distances (km) was transformed into component eigenvectors for each location using the PCNM function in the VEGAN R package (http://cc.oulu.fi/~jarioksa/softhelp/vegan/). Subsequent dbRDA was performed using the CAPSCALE function in the VEGAN R package (http://cc.oulu.fi/~jarioksa/softhelp/vegan/html/capscale.html). The constrained matrix was composed of multilocus F_{ST} estimates [from the dataset of 64 SNPS (*pgfar* excluded)], and the predictor variables were set to pheromone (a binary trait, 0 = same pheromone, 1 = different pheromone), mean $\delta^{13}C$, and the first three geographic distance eigenvectors. Significance of each predictor (while accounting for other factors) was determined by permutation (N = 1000). The dbRDA was replicated using latitude and longitude as factors instead of the eigenvectors to verify that the results were not influenced by the geographic distance transformation. Partial Mantel Tests were also performed in VEGAN using distance matrices and conditioning pairs of factors (geographic distance, pheromone difference, and mean $\delta^{13}C$ difference) sequentially on each other. *P*-values were corrected for multiple testing using the Bonferroni method. In addition, an individual-based analysis was performed in which genetic distance was calculated as the number of allelic differences between two individuals (allelic dissimilarity) in the PPOPR package (Kamvar *et al*. 2014). The predictor variables in the dbRDA were pheromone (*pgfar* or

pheromone response), $\delta^{13}C$ value, and the first four distance eigenvectors (due to the larger number of eigenvectors in the individual dataset).

3 | Results

3.1 | Collection and phenotypic differentiation

A total of 1,421 adult male *O. nubilalis* were collected at 12 trap locations in New York and Pennsylvania from 2007 to 2010 (Fig. 1), with 704 and 717 collected from E- and Z-pheromone baited traps, respectively (Table 1). Results of the *pgfar*-specific PCR-RFLP genotyping assay yielded three distinct genotypes which were assigned to E-strain (*pgfar-e*/*pgfar-e*; $n = 355$), Z-strain (*pgfar-z*/*pgfar-z*; $n = 711$), or a heterozygote class (*pgfar-e*/*pgfar-z*; $n = 355$; Table 1; doi:10.5061/dryad.kj34mq9). Assessment of temporal (year-to-year) change in heterozygosity at *pgfar* across locations in 2009 vs 2010 indicated a significant difference ($P = 0.043$; Fig. S1A). The $\delta^{13}C$ values calculated for 1,415 males from which isotope readings were collected showed a bimodal distribution, with mode peaks at -10 and -28 (Fig. S1B**).** The estimates of $\delta^{13}C$ between -8 to -17 and -22 to -32 were respectively assigned to the C4 ($n = 1,160$; 82.0% of total) and C3 ($n = 255$; 18.0%) groups. The host plant history of five individuals with $\delta^{13}C$ estimates ranging between -18 to -21 were considered ambiguous and were excluded from further analyses involving host plant. For this dataset, the proportion of males derived from C3 host plants did not differ significantly between 2009 and 2010 ($P = 0.109$; Fig. S1C). These male pheromone response, *pgfar*-defined pheromone strain, and host plant history partitions of the data were used in subsequent analyses.

3.2 | Population genetic differentiation

Of the 108 SNP markers, 65 (including *pgfar*) passed stringency criteria and were retained within the final dataset (Dryad database accession doi:10.5061/dryad.kj34mq9**)**. Of those markers that were excluded, 31 were not in HWE. Subsequent locus-by-locus F_{ST} estimates indicated five SNP loci, *pgfar*, contig00078.322, contig05852.999, contig06634.250, and contig06864.843, differed significantly between groups of males defined by response to E- or Z-strain pheromone lure (Table S2A**)**. Similarly, markers contig00078.322, contig01257.497, contig01453.160, contig05858.945, contig06634.250, and contig06864.843 showed locus-by-locus differentiation between pheromone strains defined by *pgfar* (Table S2B; significance of *pgfar* ignored). The *pgfar* locus accounted for 64% of the total genetic variation between samples trapped with different pheromone lures, and the SNP marker locus contig01257.497 accounted for ≥ 10% of the total genetic variance in both locus-by-locus analyses. Tests of independence among the 65 filtered loci detected LD only between *pgfar* and contig01257.497 ($P < 0.0001$; results of other locus pairs not shown). Secondary confirmation of linkage came from the mapping of ortholog positions. Specifically, the predicted genome position of the nearest *B. mori* ortholog of *O. nubilalis pgfar*, BAC78425 (Lassance *et al*. 2010), is synonymous with the *B. mori* gene model BGIGMGA010458 (*E*-values ≤ 8×10^{-39}; 84.5 to 100% identity across exons) located on *B. mori* chromosome 12 (positions 2,426,074 to 2,437,943). The nearest ortholog to *O. nubilalis* SNP marker locus contig01257.497 is a ubiquitin conjugating enzyme E2 (*E*-values ≤ 10^{-56}; 80.0 to 100% identity across exons) on *B. mori* chromosome 12 positions 5,150,695 to 5,153,581 or ~ 2.7 Mbp away from the *pgfar* ortholog. The SNP marker contig01257.497 was previously positioned on LG11 of an *O. nubilalis* genetic map, and also assigned to an orthologous position on *B. mori* chromosome 12 (Levy *et al*. 2015). Contig06721.1113 was the only SNP marker on the Z-chromosome and F_{ST} estimates did not differ significantly between strains defined either by pheromone response (Table S2A) or *pgfar* (Table S2B).

Based on the entire dataset of genotypes from 65 SNP loci (inclusive of *pgfar*), F_{ST} estimates between the 12 collection sites were significant for 13 of the 66 (19.7%) pairwise comparisons (Table S3A). This same set of genotypes was partitioned across sample sites based on response to either E- or Z-strain pheromone lures (n = 24 groups). Resulting F_{ST} estimates ranged from -0.09583 to 0.05852 and 144 of 276 comparisons (52.2%) were significant, of which 125 (86.8%) were significant between groups responded to different lures (Table S3B). Eleven of the 12 (92%) pairwise F_{ST} comparisons were significant between sympatric (within location) groups collected in E- or Z-pheromone-baited traps. The global estimate of differentiation between E- and Z-strain males across locations was also significant (F_{ST} = 0.0322, P < 0.0001), and AMOVA implicated pheromone response as accounting for 3.22% of the total variation between subpopulations (Table S4A). Conversely, omission of the *pgfar* locus from comparisons of pheromone response resulted in a lower number of significant pairwise F_{ST} estimates (15.2%; Table S3C), and a reduced global estimate of subpopulation differentiation (F_{ST} = 0.0047, P < 0.0001; Table S4B). Interestingly, significant genotypic differences between sympatric E- and Z-strains were only detected at northern collection sites (Oak, Int, Ran, Plan and Roc; Fig. 1) where pairwise F_{ST} estimates were ≤ 0.0087; Table S3C). Five of 12 (42%) pairwise F_{ST} comparisons were significant between sympatric (within location) E- and Z-pheromone response groups, and were located at a significantly higher latitude (mean 41.79° N) than those that were non-significant (mean latitude 40.54° N; $T_{4.78}$=2.81, P = 0.039).

Pairwise F_{ST} estimates from 64 SNP loci (excluding *pgfar*) between E- and Z-strains identified by homozygous genotypes at the *pgfar* marker locus across 21 trap locations where the strains co-occurred (Table 1) were significant in 210 of 276 comparisons (76.1%; Table S3D). Among these, F_{ST} estimates between E- and Z-strains within and across sites were significant in 113 of 132 instances (85.6%; 10 of 11 sympatric E-Z comparisons). Global differentiation across locations between E- and Z-strain males based on *pgfar* genotype was low but significant (F_{ST} = 0.0085, P < 0.0001), and AMOVA implicated *pgfar*-defined pheromone strain as accounting for 6.78% of the total variation (Table S4C).

Fifty-five of 276 (19.9%) pairwise F_{ST} estimates were significant between partitions of the SNP genotype data defined by both collection site and host plant history (C3 or C4) (Table S3E). Twenty-six of these 55 (47.3%) significant comparisons were between genotypes assigned to different host plant history groups, including within three sites [Interlaken (Int), Ransom (Ran), and Rockspring (Roc)]. Overall global estimates of F_{ST} between C3 and C4 host plant history groups across all collection sites was low and non-significant (F_{ST} = 0.0004; P = 0.1779; Table S4D).

There was no significant correlation of pairwise F_{ST} estimates with geographic distance as tested by the IBD model (P = 0.4280; Fig. 2). The results of principal coordinate analyses (PCoA) based on genotype data from all 65 SNP loci (including *pgfar*) indicated that factor scores along Coordinate 1, accounted for 25.1% of the total variation, and grouped E-, Z- and heterozygote individuals into unique clusters along the Coordinate 1 axis (Fig. 3A). This contrasted with results of PCoA based on SNP data from the 64 loci excluding the *pgfar* locus, where factor scores for E-, Z- and heterozygotes groups were not distinct (Fig. 3B). PCoA revealed no discernable pattern in clustering of groups that accounted for *pgfar* genotype (pheromone strain) and location when assigned to C3 or C4 host plant history groups (Fig. 3C).

3.3 | Bayesian clustering

An iterative Bayesian simulation approach estimated the optimal number of clusters (K), or subpopulations, represented by multilocus *O. nubilalis* SNP genotypes. STRUCTURE 2.3.4 predicted an optimal number of K = 3 both when *pgfar* was either included (65 loci; ΔK = 47.7) and excluded (64 loci; ΔK = 36.3) from the genotypic dataset partitioned by pheromone response as the *prior*. Co-ancestries (clusters) were relatively homogenous between groups based on E- and Z-strain response using the dataset with 64 loci (Fig. S2A). In contrast, inclusion of *pgfar* (65 loci) resulted in a mean proportion of Cluster 1 among E-pheromone response males of 0.7786 compared to 0.0451 among Z-pheromone response males (Fig. S2A).

Assessment of these differences in assignment of co-ancestries revealed that the proportion of homozygous *pgfar-z/-z* males that responded to the Z-strain lure (mean = 0.8972 ± 0.0512; Table S5) was significantly greater than the proportion of homozygous *pgfar-e/-e* genotypes that responded to the E-strain lure (0.4305±0.2249; one-way ANOVA: $F = 49.12$, $P < 0.0001$, d.f. = 1, 22).

Partitioning data using the 12 collection sites as *a priori* information under the LOCPRIOR option yielded similar results. Specifically, for 65 loci (including the *pgfar* locus), STRUCTURE 2.3.4 estimated $K = 3$ ($\Delta K = 28.5$) with clusters 1 and 2 corresponding to E- (E-pheromone response-*pgfar-e/-e*) and Z-strain (Z-pheromone response- *pgfar-z/-z*), respectively (Fig. S3). Use of 64 loci (*pgfar* omitted) resulted in $K = 2$ ($\Delta K = 29.4$), and no structure among pheromone response groups. Approximately equal mean proportions individual co-ancestries (population *Q*-score) of 0.850 ± 0.240 and 0.830 ± 0.241 were assigned to Cluster 1 for *pgfar-e/e* and *pgfar-z/-z* genotypes, respectively (remaining data not shown).

Use of host plant history (C3 or C4 designation) based on $\delta^{13}C$ estimates as *a priori* information for Bayesian clustering of *O. nubilalis* genotypes across 64 SNP loci (excluding *pgfar*) resulted in an optimum K of 3 ($\Delta K = 42.0$). There was no difference in the mean proportion of co-ancestries assigned to Cluster 1 between C3 and C4 plants for comparisons either with E- (C3: 0.892 ± 0.148; C4: 0.873 ± 0.147) or Z-strain (C3: 0.836 ± 0.218; C4: 0.837 ± 0.226) defined by *pgfar* genotype (Fig. S2B). Bayesian clustering of genotypes from 65 SNP loci (including *pgfar*) resulted in an optimum $K = 3$ ($\Delta K = 569.6$) with shared co-ancestries differentiated into clusters corresponding to *pgfar-e*/*-e*, *pgfar-e*/*-z* and *pgfar-z*/*-z* despite $\delta^{13}C$ estimates (C3 or C4 host plant history) having been set as the *prior* (Fig. S2B).

3.4 | Ecological and behavioral relationships

The overall proportions of individuals with $\delta^{13}C$ values in the range of -24 to -32 (C3 host plant history group) among *pgfar-e*/*pgfar-e* (E-strain), *pgfar-z*/*pgfar-z* (Z-strain), and *pgfar-e*/*pgfar-z* (E-/Z-strain hybrids) were 0.161, 0.203, and 0.151, respectively. Single factor ANOVA showed no significant difference in the proportion of homozygous male *pgfar* genotypes assigned to the $\delta^{13}C$ bin (-8 to -32) across populations ($F = 3.42 \times 10^{-5}$, $P = 1.0000$, d.f. = 2, 22), between groups defined as *pgfar* homozygotes across locations ($F = 1.75 \times 10^{-5}$, $P = 0.9967$, d.f. = 1, 23), or strain defined by pheromone response across locations ($F = 2.59 \times 10^{-5}$, $P = 0.9981$, d.f. = 2, 22).

Analyses of dissimilarity matrices using dbRDA showed that F_{ST} estimates (genetic differentiation) among populations differed significantly only by pheromone strain. E- and Z-strain pairwise comparisons had significantly higher F_{ST} estimates when classified by pheromone response or by *pgfar* genotype (Fig. 4A, B; Table 2), but not by host plant (Fig. 4C). These dbRDA analyses also indicated F_{ST} estimates were not significant for host plant history (C3:C4; $P \geq 0.268$) or geographic distance ($P \geq 0.369$). Similarly, latitude and longitude (used as distance factors) or using partial Mantel tests indicated that pheromone strain (response and *pgfar* genotype) significantly contributed to genetic variance, but host plant history did not (Table S6A, 6B; Table S7). Lastly, males responding to different pheromone lures, from different locations, or with different C3:C4 ratios showed significant levels of mean allelic differentiation; however, the effect of host plant history (C3:C4; $\eta^2 = 0.0009$) was weaker than those of distance ($\eta^2 = 0.0017$) or pheromone response ($\eta^2 = 0.0034$; Table S8).

4 | Discussion

In many diverging populations of Lepidoptera, ecological factors such as host plant use co-vary with differences in sexual signals (Groot *et al*. 2008; Joyce *et al*. 2016), but in few cases has the

relative contribution of these factors to genetic structure been tested (Prowell *et al*. 2004; Gouin *et al*. 2017). *Ostrinia nubilalis* is an emerging model for speciation dynamics due to sympatric ecotypes or strains that vary in voltinism and sex-pheromone communication (Dopman *et al*. 2010; Olsson *et al*. 2010; Lassance 2016; Kozak *et al*. 2017; Coates *et al*. 2018). Ecological differences in host plant preference occur between closely related *O. nubilalis* strains in North America (O'Rourke *et al*. 2010) and among *Ostrinia* species in Europe (Bourguet *et al*. 2000a, 2014; Thomas *et al*. 2003; Pélozuelo *et al*. 2004; Bethenod *et al*. 2005; Pelozuelo *et al*. 2007; Frolov *et al*. 2012). Although pheromone system and host plant use co-vary in *Ostrinia* (Alexandre *et al*. 2013), elucidating the relative contribution of various selective forces to differentiation in the presence of high rates of gene flow remains a challenge.

Our current study suggests that gene flow among northeastern populations of *O. nubilalis* remains relatively high at neutral genetic loci, as inferred by mostly non-significant pairwise F_{ST} estimates between collection sites (Table S3A) and by IBD modeling (Fig. 2). Similarly, our dbRDA analyses suggest that geographic distance did not contribute significantly to variation among collection sites (Table 2; Table S6, S7). This conclusion is supported by earlier population genetics studies where low pairwise F_{ST} estimates and shallow IBD slopes suggested high gene flow over long distances (Bourguet *et al.* 2000b; Kim *et al*. 2009, 2011; Frolov *et al*. 2012). Additionally, in the current study we detected no significant impact of host plant history on *O. nubilalis* population genetic structure (Table S4D) or in PCoA (Fig. 3C**)**. Likewise, dbRDA-based eigenvectors for variance in C3:C4 ratios among collection sites (Table 2; Table S6) and partial Mantel Tests (Table S7) were not significant. Finally, the proportion of individual co-ancestries assigned to subpopulations in Bayesian clustering analysis were strongly influenced by variation at the *pgfar* locus, not host plant despite the latter beings used as the *prior* (Fig. S2B). Together, these results indicate that host plant history is not a strong ecological factor impacting *O. nubilalis* population structure in North America. The scenario in Europe appears to be markedly different between the sympatric sibling species, *O. nubilalis* and *O. scapulalis*. Specifically, *Ostrinia* from France that use the E-pheromone communication

system are *O. scapulalis* which predominantly feed and develop on mugwort (Malausa *et al*. 2007; Frolov *et al*. 2007), whereas *O. nubilalis* are Z-strain and mostly feed on maize. Thus, conclusions from the current study regarding the basis of ecological diversification between E- and Z-strain *O. nubilalis* may not be transferrable to the sibling species in Europe.

Our study suggests that pheromone strain has a greater effect on *O. nubilalis* genetic differentiation than either geography or host plant use. Specifically, pheromone strain of captured males (*pgfar* homozygous genotype) or pheromone response phenotype (*Resp*) each represent significant factors accounting for *O. nubilalis* population genetic variance. This conclusion is most clearly drawn from dbRDA, wherein pheromone response phenotype and *pgfar* genotype are the most significant eigenvectors determining degree of genetic variance when compared to host plant history- and geography-based eigenvectors (Table 2; Fig. 4; Table S6, S8). Even though genetic loci determining female pheromone signal and corresponding male response are not physically linked in all species of Lepidoptera studied (Groot *et al*. 2015), including *O. nubilalis* (Roelofs *et al*. 1987; Löfstedt *et al*. 1989; Dopman *et al*. 2004), these loci remain in LD (Kirkpatrick & Ravigne 2002; Kirkparick 2017). Several models have been proposed to explain the co-evolution of genetic loci contributing to mate attraction and selection (Groot *et al*. 2016), but lack of information on the genomic architecture of these traits hinders current research (Coates *et al*. 2018). Although we used capture of male *O. nubilalis* in traps baited with strain-specific pheromone lures as a proxy for pheromone attraction phenotype (*Resp*), a direct measure of allelic variation at the *Resp* locus is lacking. The Z-chromosome-linked *Resp* locus (Dopman *et al*. 2004) is located within a suite of genes putatively involved in neurogenesis (Koutroumpa *et al*. 2016). The specific gene or mutation(s) involved, or genetic markers, most strongly correlated with male response are yet to be determined. Thus, variation at the causal locus, and therefore potential impacts of selection could not be tested directly and remain the subject of future work.

Our results suggest that sexual selection is acting concurrently on unlinked loci contributing to female pheromone signaling and corresponding male response phenotypes in *O. nubilalis*. These characters appear to have a more significant impact on inter-strain genetic divergence and strain maintenance than the ecological factor of host plant use. These results are consistent with the observed high rates of assortative mating caused by male response to female pheromone and selection against hybrid pheromone blends (Linn *et al*. 1997; Dopman *et al*. 2010). However, observed levels of hybridization (as evidenced by heterozygosity at *pgfar*; Fig. S1A) suggests assortative mating is incomplete and gene flow is occurring between E- and Z-strains. Hybridization likely explains why genome-wide structuring was not detected among strains when the *pgfar* locus genotype was removed from analyses. Future studies using a greater number of anonymous markers could clarify whether population differentiation in *O. nubilalis* is limited to *Pher* and *Resp*, and genome regions physically linked to these loci. It is also possible that other islands of differentiation in the genome are associated with loci promoting assortative mating (Royer & McNeil 1992; Lassance & Löfstedt 2009) including differences in circadian timing (Liebherr & Roelofs 1975; Levy *et al*. 2018).

Although we found no evidence that host-plant use contributes to genetic structure, sexual selection alone is not necessarily the singular evolutionary force maintaining *O. nubilalis* pheromone strains. Ecological variation in seasonal timing (voltinism) in conjunction with pheromone stain may also contribute to high genetic differentiation on the *O. nubilalis* Z-chromosome compared to on the autosomes (Levy *et al*. 2015; Kozak *et al*. 2017). This phenomenon may be due in part to a rearrangement (probably an inversion; Wadsworth *et al*. 2015) of a segregating region of the Z-chromosome which differs in frequency most among individuals that differ in pheromone strain and voltinism ecotype (Kozak *et al*. 2017). Voltinism is strongly correlated with latitude in Z-strain *O. nubilalis* (Showers 1981). Based on SNP markers (excluding the *pgfar* locus), we detected significant F_{ST}s between E- and Z-pheromone response groups collected in the same location mostly at northern sites (Int, Oak, Pla, and Ran) where the strains are also known to differ in voltinism (bivoltine E- and univoltine Z-strain;

Glover *et al*. 1991; Calvin & Song 1994). These results suggest that interactions between certain ecological and sexual traits may reinforce one another to limit gene flow in *O. nubilalis*, and the subsequent development of reproductive isolation may be a multivariate process dependent on both natural and sexual selection (Maan & Seehausen 2011; Butlin *et al*. 2012).

In this study, we found that female pheromone blend and male response contributes to a greater proportion of the genetic differentiation among populations of *O. nubilalis* in the northeastern United States compared to geographic distance or host plant. These conclusions were drawn from congruent results from Bayesian clustering, PCoA, IBD modeling, and dbRDA. Data from this and earlier studies suggest that sexual selection may function as a component maintaining the observed level of genetic differentiation between sympatric pheromone strains in North America, but whether it is sufficient by itself to maintain the pheromone strains over evolutionary time remains unclear. The relative contributions of male pheromone response and voltinism on genomic variance between *O. nubilalis* pheromone strains may be more precisely quantifiable upon the future development of genetic markers that detect or are tightly linked to causal mutations. Regardless, these data are important for understanding the relative contributions of ecological factors (host plant use), distance (geographic isolation), and sexual selection (maternal female pheromone blend and male response) in forming barriers to gene flow between *O. nubilalis* subpopulations, which may be propelling them along different evolutionary trajectories.

Acknowledgments

This research was a joint contribution from the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) (CRIS Project 5030-22000-018-00D), and the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Project 3543), This research was funded by the National Science Foundation (DEB-1257251; E.B.D.),

and cooperative agreement 58-5030-7-066 between USDA-ARS and Tufts University. We thank Natalie Wertish and Miranda Dietz for carrying out portions of the genotyping assays used in this study. This article reports the results of research only and any mention of products or services does not constitute an endorsement by USDA-ARS. USDA-ARS is an equal opportunity employer and provider. Graham Stone, Astrid Groot, and two anonymous reviewers provided helpful comments on the manuscript.

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Author Contributions

BSC designed experiments, analyzed data, and wrote the manuscript with contributions from other authors; GMK, KSK, and YW each analyzed portions of the data; JS conducted laboratory experiments and collected data; SJF designed strategy for location of traps and collection of samples; EBD and TWS provided advice and contributed to writing portions of the manuscript.

Data accessibility

High throughput single nucleotide polymorphism genotype data for all samples are available at doi:10.5061/dryad.kj34mq9.

Table 1. Sample locations within and across years for 12 sites in the northeast United States where *Ostrinia nubilalis* E- and Z-pheromone strains are sympatric. Males were captured in pheromone traps at each location baited with either an E-lure (99:1 E-:Z11-14:OAc) or Z-lure (3:97 E-:Z11-14:OAc). Pheromone strain of the same males was determined independently based on *pgfar* genotype (homozygous *pgfar-e*/*-e* and *pgfar-z*/*-z*; heterozygous *pgfar-e*/*-z*). The count of males from with larval host plant as determined from wing stable carbon isotope content ratio of $^{13}C/^{14}C$ (i.e., $\delta^{13}C$) are indicated within brackets [C3 ($\delta^{13}C$ range of -22 to -32); C4 ($\delta^{13}C$ range of -8 to -17)]. □ = Harstack wire cone trap; ● = cloth Scentry trap.

Table 2. Distance-based redundancy analysis (dbRDA) of eigenvectors (EV) for the relative contributions to *Ostrinia nubilalis* population genetic structure of pheromone strain [assigned based on **A)** male response (attraction) to either E-strain lure (99:1 E-:Z11-14:OAc) or Z-strain lure (3:97 E-:Z11-14:OAc), or **B)** *pgfar* genotype], host plant history (C3:C4), and geographic distance (km) (*pgfar* genotype excluded). The sum of squares (Sum of Sq.), *F*-statistic based on 1 and 16 degrees of freedom ($F_{1,16}$), proportion of the total variance attributed to each component (η^2), and associated *P*-values are shown.

Figure 1 Topographic map of geographic locations for 12 collection sites (location abbreviations in Table 1 and coordinates in Table S1). □ = Harstack wire cone trap; ● = cloth Scentry trap.

Figure 2 Influence of geographic distance on genetic differentiation between *Ostrinia nubilalis* subpopulations (collection sites; Table 1). Tests for an isolation-by-distance model between pairwise genetic distance (F_{ST}) and geographic distance. Associated R^2 (goodness of fit) and *P*-values estimated from 1,000 iterations performed using Mantel Tests.

Figure 3 Scatter plot of factor scores along the major Principal Coordinates (PCs) accounting for the greatest variation among *Ostrinia nubilalis* population-derived single nucleotide polymorphism (SNP) genotype data partitioned into **A)** pheromone strain using 65 SNP markers including the *pgfar* locus, **B)** pheromone strain using 64 SNP markers excluding the *pgfar* locus, and **C)** host plant history using 64 SNP markers excluding the *pgfar* locus. □ = Harstack wire cone trap; ● = cloth Scentry trap.

Figure 4 Influence of ecological factors on the genetic differentiation between *Ostrinia nubilalis* subpopulations (collection sites; Table 1) based on dbRDA analyses (64 loci, excluding *pgfar* genotypes). Range of F_{ST} estimates between E- and Z-strain when partitioned by pheromone lure (trap), homozygous genotypes from the *pgfar* genetic marker, or C3:C4 ratio (host plant). Significance as determined by dbRDA (Table 2) are shown for each comparison.

Table 1. Sample locations within and across years for 12 sites in the northeast United States where *Ostrinia nubilalis* E- and Z-pheromone strains are sympatric. Males were captured in pheromone traps at each location baited with either an E-lure (99:1 *E*-:Z11-14:OAc) or Z-lure (3:97 *E*-:Z11-14:OAc). Pheromone strain of the same males was determined independently based on *pgfar* genotype (homozygous *pgfar-e*/*-e* and *pgfar-z*/*-z*; heterozygous *pgfar-e*/*-z*). The count of males from with larval host plant as determined from wing stable carbon isotope content ratio of $^{13}C/^{14}C$ (i.e., $\delta^{13}C$) are indicated within brackets [C3 ($\delta^{13}C$ range of -22 to -32); C4 ($\delta^{13}C$ range of -8 to -17)]. □ = Harstack wire cone trap; ● = cloth Scentry trap.

#	ID	Location	Year	Trap counts		Pheromone strain [host plant C3/C4]		
				E-lure	Z-lure	*pgfar-e*/*-e*	*pgfar-z*/*-z*	*pgfar-e*/*-z*
1	Bed09	□Bedminster, PA	2009	21	48	4 [0; 4]	47 [11;36]	18 [5;13]
2	Bro10	●Brooklawn, PA	2010	48	48	26 [0;;26]	43 [0;43]	27 [3;24]
3	Dru09	□Drums, PA	2009	48	48	10 [1;; 9]	65 [20;45]	21 [3;18]
4	Int07	●Interlaken, NY	2007	46	48	42 [15;;27]	46 [6;40]	6 [2; 4]
5	Lan08	□Landisville, PA	2008	32	45	18 [0;18]	46 [6;40]	13 [0;13]
	Lan09	□Landisville, PA	2009	48	45	15 [1;14]	49 [21;25]	29 [6;23]
	Lan10	□Landisville, PA	2010	48	48	29 [3;26]	46 [16;33]	21 [4;17]
6	Leh10	□Lehigh, PA	2010	20	48	9 [0; 9]	46 [19;27]	13 [2;11]
7	Nor10	□Northampton, PA	2010	3	48	0 [0; 0]	44 [8;36]	7 [2; 5]
8	Oak07	●Oaks Corners, NY	2007	48	48	22 [0;22]	49 [1;49]	25 [1;24]
9	Pla09	□Plains, PA	2009	48	18	20 [5;15]	16 [6;10]	30 [4;26]
	Pla10	□Plains, PA	2010	28	11	19 [1;18]	8 [3; 5]	12 [0;12]
10	Por09	□Port Matilda, PA	2009	48	18	16 [1;15]	19 [4;15]	31 [0;31]
11	Ran09	□Ransom, PA	2009	47	48	0 [0; 0]	48 [13;35]	47 [4;43]
	Ran10	□Ransom, PA	2010	44	22	36 [1;35]	20 [3;16]	10 [0;10]
12	Roc08	□Rockspring, PA	2008	32	32	24 [0;24]	33 [0;33]	7 [0; 7]
	Roc09	□Rockspring, PA	2009	47	46	30 [28; 2]	42 [1;41]	21 [8;13]
	Roc10	□Rockspring, PA	2010	48	48	35 [1;34]	44 [2;42]	17 [2;15]
				704	717	355 [57;298]	711 [140;571]	355 [46;309]

Table 2. Distance-based redundancy analysis (dbRDA) of eigenvectors (EV) for the relative contributions to *Ostrinia nubilalis* population genetic structure of pheromone strain [assigned based on **A)** male response (attraction) to either E-strain lure (99:1 *E*-:*Z*11-14:OAc) or Z-strain lure (3:97 *E*-:*Z*11-14:OAc), or **B)** *pgfar* genotype], host plant history (C3:C4), and geographic distance (km) (*pgfar* genotype excluded). The sum of squares (Sum of Sq.), *F*-statistic based on 1 and 16 degrees of freedom ($F_{1,16}$), proportion of the total variance attributed to each component (η^2), and associated *P*-values are shown.

A) Strain assigned by pheromone response (males in lure-baited traps)

Component eigenvector	Sum of Sq.	$F_{1,16}$	*P*	η^2
Pheromone strain (response)	0.00021459	2.5390	0.03497	0.115
Mean C3:C4	0.00010939	1.2943	0.26873	0.059
Geographic distance EV1	0.00005160	0.6105	0.73826	0.028
Geographic distance EV2	0.00004956	0.5864	0.71528	0.027
Geographic distance EV3	0.00008757	1.0361	0.36963	0.047
Residual	0.00135226			

B) Population by *pgfar* genotype

Component eigenvector	Sum of Sq.	$F_{1,16}$	*P*	η^2
Pheromone strain (*pgfar*)	0.0010539	4.4362	0.001998	0.189
Mean C3:C4	0.0001699	0.7151	0.596404	0.030
Geographic distance EV1	0.0001569	0.6603	0.697303	0.028
Geographic distance EV2	0.0002069	0.8711	0.489510	0.037
Geographic distance EV3	0.0001993	0.8389	0.495504	0.035
Residual	0.0038010