Insect science

Insect Science (2020) 00, 1–10, DOI 10.1111/1744-7917.12798

ORIGINAL ARTICLE

Landscape genetic analyses reveal host association of mitochondrial haplotypes in the Asian corn borer, *Ostrinia furnacalis*

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Abstract Crop expansion often leads to high pest pressure. These pests may have fitness trade-offs related to host use, and some host-associated genotypes may benefit and increase in frequency. However, evidence concerning the effect of host availability on spatial distribution and frequency of mitochondrial haplotypes is scarce. We studied genetic variation of the Asian corn borer, *Ostrinia furnacalis* (Guenée), across a large area during 2 years (2016 and 2017). Mitochondrial sequence data were obtained from 530 individuals collected from 79 locations in Shandong Province, China. In total, 155 haplotypes were found based on the combined cytochrome oxidase subunit 1 (COI) and COII genes. Three haplotypes (H2, H12, and H23) were dominant, whereas most of the other haplotypes occurred in low frequency. A haplotype network showed that the 155 haplotypes can be grouped into three clusters. Haplotype clusters seemed to be randomly distributed. The frequency of H12 (in Cluster 1) was positively correlated with maize crop proportion, but negatively correlated with other crops (primarily vegetables, oilseed crops, and cotton) at all spatial scales (1-, 3-, and 5-km radius). Cluster 2 had haplotype H23, and this cluster was negatively correlated with semi-natural habitats. Cluster 3 had no dominant haplotype and was not affected by landscape factors. We conclude that H12 may be a maize-associated haplotype. Further study is needed to verify the possibility that the carriers of this haplotype may possess some fitness trade-offs. Our study highlights the importance of host availability in *O. furnacalis* haplotype distribution and frequency.

Key words agricultural landscape; corn pest; haplotype network; host-associated haplotype; mitochondrial sequence

Introduction

Landscape genetic studies mainly evaluate the role of habitat features in shaping the population genetic structure of individual species (e.g., Keyghobadi *et al*., 2005). Reports on landscape genetics of herbivorous insects have mainly focused on the effects of habitat fragmentation or habitat loss (Segelbacher *et al*., 2010). However, little attention has been paid to the effects of agriculture simplification on pest genetics.

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Agricultural intensification has converted natural habitats and semi-natural habitats (SNH) within agricultural landscapes into arable fields, resulting in a loss of habitat heterogeneity (Rusch *et al*., 2016). Simplification of the agricultural landscape is widely expected to exacerbate yield losses to pest species (Tscharntke *et al*., 2005; Grab *et al*., 2018). Exploring the relationship between landscape features and the genetics of insect pests is important to help design management strategies for pest control.

Insect herbivores show dynamic interactions with their food plants. These herbivores can develop distinct host races (Guldemond *et al*., 1994; Calcagno *et al*., 2007; Agarwala & Choudhuri, 2014), and many herbivores are known to have some degree of host specialization (Dorchin *et al*., 2009; Medina, 2012). Insect diet breadth is related to host plant availability. Host-specific adaptation and associated trade-offs in performance are favored if the suitable host is easy to find (Jaenike, 1990). On the molecular level, host specialization is usually reflected by the occurrence of a particular genotype; for example, different mitochondrial haplotypes are often associated with different hosts (Shufran *et al*., 2000; Anstead *et al*., 2002; Joyce *et al*., 2016).

Mitochondrial DNA (mtDNA) has been widely used as a marker in molecular ecology and evolution studies. Effective neutrality is one of the major assumptions underlying analyses of mtDNA population data to infer demographic patterns (Galtier *et al*., 2009). However, increasing evidence indicates that mtDNA does not always neutrally evolve (Bazin *et al*., 2006; Dowling *et al*., 2008; Galtier *et al*., 2009). Selection may be operating directly on mtDNA variability or may be mediated through cytonuclear interactions (Grant *et al*., 2006).

Food stress is one of the most obvious environmental stressors (Ballard & Pichaud, 2014). There is experimental evidence that diet can influence mitochondrial functions in *Drosophila* flies harboring specific mtDNA haplotypes (Pichaud *et al*., 2013). Moreover, flies with distinct haplotypes had different life-history traits (James & Ballard, 2003). In natural populations, food availability can be a major selective force (Forbes *et al*., 2009; Ballard & Pichaud, 2014). Additionally, haplotype frequency can be used as an indicator of genetic variation between populations from different geographical locations (Grant *et al*., 2006), and spatial variation in host distribution often leads to divergence in host use between populations. Therefore, host availability in landscapes may affect haplotype frequency, which is not yet fully understood.

The Asian corn borer (ACB, *Ostrinia furnacalis* [Guenée], Crambidae) is an important pest of maize in Asia (Nafus & Schreiner, 1991; Kojima *et al*., 2010). It annually causes significant reductions in maize yield, and also damages sorghum, millet, and cotton. With the widespread cultivation of maize in Asia, the ACB has become highly adapted to this host plant (Kojima *et al*., 2010). In China, the ACB is widely distributed from north to south. It overwinters as diapausing larvae and exhibits freeze tolerance in cold areas (Xie *et al*., 2015). In regions with extensive cultivation of maize, the ACB has 1–7 generations per year (Wang *et al*., 2005).

Control practices usually focus on summer maize because of its economic importance and large planting area. Before damaging summer maize, the ACB can feed on spring maize or other wild plants. The ACB mtDNA shows high haplotype diversity, with 2–4 lineages of haplotypes based on mtDNA sequences (Hoshizaki *et al*., 2008, Li *et al*., 2014). Genetic variation in the ACB may be related to use of different host plants. However, a field study that sampled different host plants did not find evidence of host specialization (Wang *et al*., 2018). It is unclear whether host availability affects the haplotype frequency of ACB populations. Here, we sampled ACB across a large area in Shandong Province, China. We combined mtDNA data (cytochrome oxidase subunit 1 [COI] and COII genes) analyses with geographical information systems (GIS) techniques to test the hypothesis that the proportion of host habitats in landscapes may affect haplotype frequency of the ACB.

Materials and methods

Sampling

We sampled maize fields across 79 counties in Shandong Province, China (Fig. 1 and Table S1). The area is located near the East China Sea in the Lower Yellow River (34°22.9′–38°24.0′N, 114°47.5′–122°42.3′E) and has a continental monsoon climate. Precipitation is concentrated in July and August. This province is a typical agricultural production area. This region mainly produces annual crops (wheat, maize, oil rape) and greenhouse crops. The locations of the sampling sites were recorded using a Magellan handheld GPS receiver, a latitude−longitude projection, and WGS84 datum.

The sampling protocol consisted of collecting ACB larvae from maize plants in fields at least 21 d prior to preharvest. Sampling began on September 20 in 2016 and September 22 in 2017, and lasted for 1 week per year. The sampling period corresponded to the time when ACB larvae are preparing to overwinter in maize stems. We arbitrarily selected one maize field (area > 1000 m^2) in each site. At each of the sampling fields, we arbitrarily

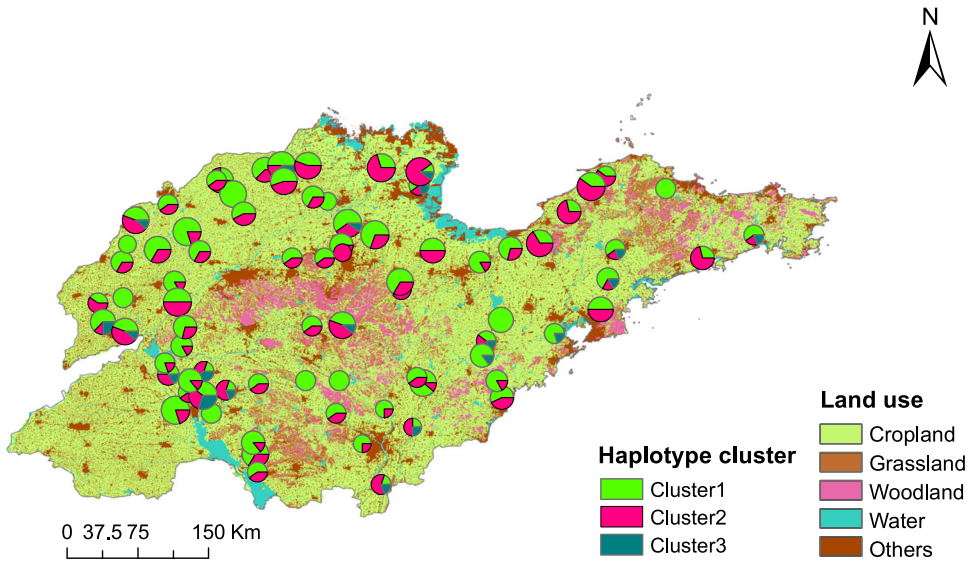

Fig. 1 Map of the sampling area and sampling locations in Shandong Province, China. The mitochondrial DNA (mtDNA) haplotype clusters are also indicated on the map as pie charts with the center of the pie charts corresponding to the position where fields were sampled. Pie slices correspond to haplotype cluster 1 (green), cluster 2 (magenta), or cluster 3 (cyan).

sampled 50 plants, cut them open, and examined the ACB larvae. Samples were stored in 95% ethanol until they were processed in the laboratory. In total, we prepared 530 individuals for genetic analyses; there were 140 individuals from 2016 and 390 from 2017. Differentiation between yearly populations estimated by F_{ST} was not significant ($F_{ST} = -0.0021$, $P > 0.05$). Therefore, the samples from both years were pooled together, and 4–10 individuals (mean $\pm$ SD $= 6.7 \pm 2.0$) per site were sequenced.

DNA extraction, amplification, and sequencing

We extracted genomic DNA using the DNeasy Tissue Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. We sequenced two partial regions of the mitochondrial genes COI and COII. The COI fragments were amplified using the primer pair COI_F (5′-CAAGAAGAATCGTTGAAAATGGAGC-3′) and COI_R (5′-TGGAAGTTCGTTATATGAATGTTCTGC-3′), and the COII fragments were amplified using the primer pair COII_F (5′-CCACCGGCAGAACATT CATAT-3′) and COII_R (5′-GACCATTACTTGCTT TCAGTCATC-3′) (Li *et al*., 2014). Amplification reactions were performed with TransTaq® HiFi DNA Polymerase (TRANS, Beijing, China) in 25-μL aliquots containing 1 μL genomic DNA, forward and reverse primers (1 μL each), 5 μL 10 $\times$ TransTaq® HiFi Buffer I, 4 μL deoxynucleotide triphosphate, 0.5 μL HiFi DNA polymerase, and 12.5 μL ddH_2O. Polymerase chain reactions (PCRs) were conducted using an S1000 Thermal Cycler (BIO-RAD, Hercules, CA, USA) under the following conditions: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1.5 min; and 72 °C for 10 min. PCR products were purified using a PCR purification kit (Qiagen Inc.) and were sequenced by Sangon Biotechnological Co., Ltd. (Shanghai, China).

Population genetic analysis

A region of 1082 bases in the mtDNA COI gene (deposited in GenBank under accession nos. MK374375–MK374764, MK919708–MK919892) and a region of 738 bases of the COII gene (accession nos. MK374796–MK375185, MK919893–MK920077) were sequenced. To check that the sequences obtained from the COI–II fragments were genuine mtDNA sequences and not nuclear mtDNA sequences, the sequences were translated into amino acids using the invertebrate mitochondrial code, and were screened for stop codons and numerous amino acid changes. DNA polymorphism was examined using DnaSP ver. 5.10 (Librado & Rozas, 2009). Tajima's D (Tajima, 1989) was calculated from the total number of segregating sites and was used to assess the evidence for population expansion. The statistical parsimony network (also known as the TCS network) of haplotypes was analyzed using Popart ver. 1.7 (Clement *et al*., 2002; Leigh

& Bryant, 2015). Isolation by distance (IBD) analysis was performed using Mantel tests (1 000 permutations) in GenALEx ver. 6.5 (Peakall & Smouse, 2006; Peakall & Smouse, 2012) to determine if there was a correlation between genetic and geographic distances.

Landscape data

Land-use maps of the sampling sites were obtained from a digital map provided by Landsat-8 with 30-m resolution in 2015. Habitats around the focal field were classified into five general categories: cropland, woodland, grassland, water, and residential areas. Then, cropland was further classified into two parts: maize and other crops (mainly vegetables, oilseed crops, and cotton). Land-use classifications were ground-truthed at every site. Landscape variables were estimated at a radius of 1, 3, or 5 km using ArcGIS ver. 10 (ESRI, Redlands, CA, USA).

It is often important for landscape ecology studies to incorporate a broad range of spatial scales as multiple-scale studies can gain better understanding of ecological phenomena. These spatial scales were chosen by biologically based criteria such as colonization and dispersal of ACB adults. This pest species was demonstrated to have high dispersal ability (45.5 km) but individuals often move locally (<4 km) (Wang *et al*., 1994). For each spatial scale, we measured the percent cover of maize, other crops (i.e., vegetables, oilseed crops, and cotton), and SNH. SNH are usually defined as any habitat containing a community of non-crop plant species (Pfister *et al*., 2017). In our analysis, SNH included woodland and grassland habitats. The proportion of maize was inversely related to the cover of other crops, and negatively correlated with SNH at most spatial scales. Therefore, in the modeling analyses, we modeled each landscape variable separately.

Relationships between haplotype frequency and landscape factors

We performed all analyses using the statistical program R ver. 3.4.3 (R Core Team, 2018). First, we grouped the haplotypes into three clusters (Clusters 1, 2, and 3) and calculated the frequency of each cluster per site. In addition, we calculated the frequencies of three dominant haplotypes (H2, H12, and H23) per site. Then, we used generalized linear mixed effects models in GLMM package "lme4" (Bates *et al*., 2015) with binominal distributions for proportion data (Zuur *et al*., 2009). Four landscape variables were treated separately as fixed effects: proportion of maize, other crops (i.e., vegetables, oilseed crops, and cotton), cropland (i.e., combined maize and other crops), and SNH. Sampling number was used as a random effect. The model analyses were separately conducted at each spatial scale. We constructed candidate models to evaluate the importance of each landscape variable for haplotype frequency, and compared them with the null model (intercept only). We performed model selection based on Akaike's information criterion for small sample sizes (AICc), and ranked alternative models using model probabilities known as Akaike weights (wi) (package "MuMIn"44) (Bartoń, 2018). If one of the models was strongly supported (i.e., wi >0.90), we based inferences on that model. However, in cases where no single model was superior, coefficients for each predictor variable were derived from the competing models using a model averaging procedure (Burnham & Anderson, 2002).

Results

Haplotype diversity

A 1820-bp fragment of the mtDNA COI–COII subunit was obtained from all of the sequenced larvae. Among all of the sequences, there were 131 segregating sites. A total of 155 haplotypes were found (Figs. 1,2; Supplementary Material). Relatively high haplotype diversity (Hd = 0.943), nucleotide diversity (Pi = 0.00535), and average number of nucleotide differences (k = 9.739) were found. Tajima's D statistic was used to test the deviation from neutrality and yielded a D value of −1.44, which was not statistically significant (at the 0.05 level) for a sample size of 530 individuals.

Haplotype grouping

The haplotype network of combined genes displayed three clear haplotype groupings (Clusters 1, 2, and 3) (Fig. 2). Cluster 1 contained the most haplotypes (n = 98), and included 59.6% of the total samples. The second largest was Cluster 2, which had 45 haplotypes and included 35.5% of the samples. The smallest was Cluster 3, with only 12 haplotypes and 4.9% of the samples. These haplotype clusters seemed to be randomly distributed and showed no obvious geographic pattern (Fig. 1). There were three common haplotypes found in 39.6% of the individuals (n = 210): H2, H12 (both belonging to Cluster 1), and H23 (Cluster 2). Most of the haplotypes were found in a maximum of three individuals, with 102 of the haplotypes only found in a single individual

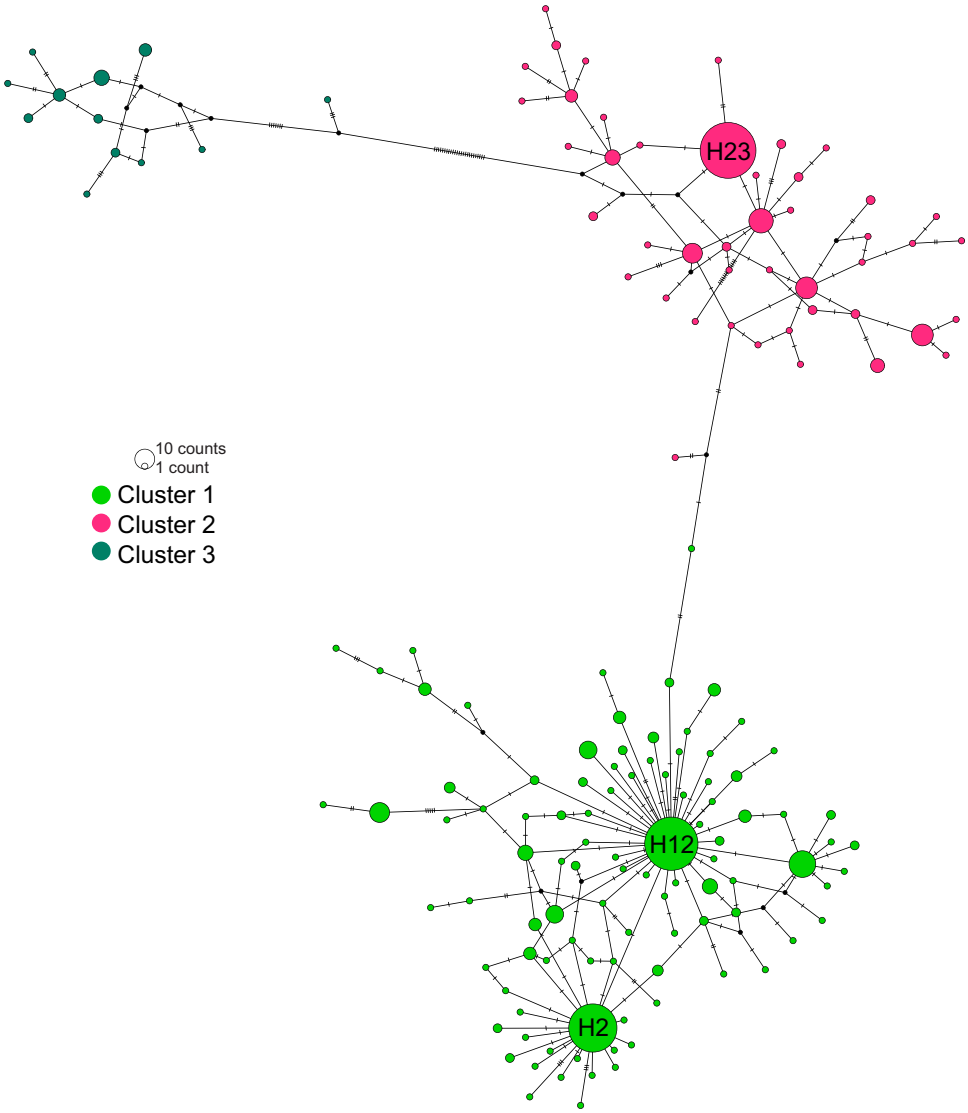

Fig. 2 Network showing the most parsimonious evolutionary relationships among haplotypes. Three clusters of haplotypes are indicated. Circles representing haplotypes are proportional to the number of individuals per haplotype. Each hatch mark along a connecting line represents a change of one base pair.

and therefore at a single location. A significant positive relationship was found between genetic and geographic distances for the 530 individuals ($r = 0.027$, $P = 0.017$), which indicated a very weak role of geographic isolation in the genetic structure of the ACB.

Relationships between landscape factors and haplotype frequency

Landscape variables were included in most of the competing models (Table 1). However, effects of landscape variables on Cluster 3, H2, and H23 were weak because null models (intercept only) were also competing models ($\triangle$AICc < 2, Table 1). SNH at a 1-km scale were significantly associated with frequencies of Clusters 1 and 2; however, there was a positive relationship between SNH and Cluster 1, but a negative relationship between SNH and Cluster 2 (Table 2). For the dominant haplotypes, H12 was best predicted by landscape variables. The results of model averaging showed that H12 was positively correlated with the proportions of maize cover, but negatively correlated with proportions of other crops (i.e., vegetables, oilseed crops, and cotton). Such results were

Table 1 Summary of the competing models for estimating the effects of maize proportion, other crops proportion (vegetables, oilseed crops and cotton), cropland proportion (maize + other crops) and semi-natural habitats (SNH) proportion on the frequency of haplotypes across three spatial scales (1, 3 and 5 km).

Response variable	Scale (km)	Model	K	LL	AICc	△AICc	Weight
Cluster 1	1	SNH	3	−134.05	274.41	0.00	1
	3	(Intercept only)	2	−139.57	283.29	0.00	0.42
		SNH	3	−139.11	284.55	1.26	0.22
		Cropland	3	−139.23	284.79	1.50	0.20
		Maize	3	−139.48	285.28	1.99	0.16
	5	(Intercept only)	2	−139.57	283.29	0.00	0.53
		Cropland	3	−139.30	284.92	1.63	0.23
		SNH	3	−139.51	284.93	1.64	0.23
Cluster 2	1	SNH	3	−130.00	266.32	0.00	1
	3	(Intercept only)	2	−134.26	272.68	0.00	1
	5	(Intercept only)	2	−134.26	272.68	0.00	1
Cluster 3	1	(Intercept only)	2	−57.02	118.19	0.00	0.39
		Cropland	3	−56.16	118.65	0.45	0.31
		Other crops	3	−56.86	120.05	1.86	0.15
		Maize	3	−56.94	120.20	2.00	0.14
	3	SNH	3	−55.28	116.88	0.00	0.43
		Cropland	3	−55.46	117.25	0.37	0.35
		(Intercept only)	2	−57.02	118.19	1.31	0.22
	5	Cropland	3	−55.74	117.81	0.00	0.31
		SNH	3	−55.75	117.82	0.00	0.31
		(Intercept only)	2	−57.02	118.19	0.38	0.26
		Other crops	3	−56.68	120.27	1.87	0.12
H2	1	(Intercept only)	2	−89.09	182.33	0.00	0.48
		Other crops	3	−88.63	183.59	1.26	0.26
		Cropland	3	−88.64	183.60	1.27	0.26
	3	(Intercept only)	2	−89.09	182.33	0.00	0.34
		Maize	3	−89.15	182.62	0.29	0.30
		Other crops	3	−88.40	183.13	0.80	0.23
		SNH	3	−88.99	184.30	1.97	0.13
	5	(Intercept only)	2	−89.09	182.33	0.00	0.42
		Maize	3	−88.64	183.59	1.26	0.22
		Other crops	3	−88.82	183.96	1.62	0.19
		SNH	3	−88.89	184.10	1.77	0.17
H12	1	Other crops	3	−95.11	196.55	0.00	0.58
		Maize	3	−95.43	197.18	0.63	0.42
	3	Other crops	3	−95.31	196.95	0.00	0.53
		Maize	3	−95.42	197.16	0.21	0.47
	5	Other crops	3	−94.39	195.11	0.00	0.69
		Maize	3	−95.18	196.69	1.59	0.31
H23	1	(Intercept only)	2	−104.50	213.17	0.00	1
	3	(Intercept only)	2	−104.50	213.17	0.00	0.45
		Other crops	3	−104.30	214.93	1.76	0.19
		Cropland	3	−104.31	214.94	1.77	0.19
		SNH	3	−104.34	215.01	1.85	0.18
	5	(Intercept only)	2	−104.50	213.17	0.00	1

K, number of parameters; LL, log-likelihood of the model; AICc, Akaike's information criterion second order; △AICc, the difference between AICc values of the best ranked model and model tested; Weight, Akaike weight of the model.

Table 2 Generalized linear mixed effects models relating haplotype clusters and dominant haplotypes to landscape variables as predictors. Landscape variables include maize proportion, other crops proportion (vegetables, oilseed crops and cotton), cropland proportion (maize + other crops) and semi-natural habitats (SNH).

	Estimate ± SE	*P*
Cluster 1		
(Intercept)	0.38 ± 0.11	< 0.001
SNH at 1 km	7.81 ± 2.91	0.007
Cluster 2		
(Intercept)	−0.61 ± 0.11	< 0.001
SNH at 1 km	−6.88 ± 2.86	0.016
Cluster 3		
(Intercept)	−4.35 ± 1.86	0.020
SNH at 3 km	−12.82 ± 9.62	n.s.
Cropland at 3 km	−3.71 ± 2.40	n.s.
H2		
(Intercept)	−2.17 ± 0.39	< 0.001
Maize at 3 km	1.06 ± 0.77	n.s.
Other crops at 3 km	−0.91 ± 0.78	n.s.
SNH at 3 km	−1.05 ± 2.44	n.s.
H12		
(Intercept)	−1.37 ± 0.64	0.031
Other crops at 5 km	−2.05 ± 0.01	< 0.001
Maize at 5 km	1.59 ± 0.01	< 0.001
H23		
(Intercept)	−2.08 ± 0.56	< 0.001
Other crops at 3 km	−0.64 ± 1.02	n.s.
Cropland at 3 km	−0.78 ± 1.24	n.s.
SNH at 3 km	1.57 ± 2.73	n.s.

Estimate, fixed-effect parameters; *P*, significant levels (n.s. means not significant). The presented predictors are those retained in the models after model selection. They are shown here together with the radius of impact.

relatively consistent at a 1-, 3-, or 5-km radii (Tables 1 and 2).

Discussion

This study focused on the effect of host availability on haplotype frequency, a topic that has received limited attention. The most important finding is that the frequency of H12 was positively affected by the proportion of maize. This indicates that H12 is probably a maize-associated haplotype. In North China, maize proportion in the landscape represents the intensification of agriculture, because the wheat–maize rotation is the main planting pattern. Maize is the most widely planted crop in Shandong, and approximately 3.67 million hectares were planted in 2016 (Shandong Municipal Bureau of Statistics, 2017). The relationship between H12 and maize planting may reflect adaptation of the ACB to its host. Host plant is one of various potential drivers of adaptive evolution of mtDNA. For example, *Eurosta solidaginis* populations using different goldenrod species as hosts had different mtDNA haplotypes (Brown *et al*., 1996). The fall armyworm, *Spodoptera frugiperda*, is a major agricultural pest with a wide host range. Its populations can be subdivided into corn and rice strains, which can be reliably distinguished by mitochondrial haplotyping (Nagoshi *et al*., 2007).

The haplotype distribution in the sampling area indicated no apparent geographic pattern of haplotypes. Only a weak effect of IBD at an individual level was found. Limited by the small sample size at each site, we could not precisely evaluate IBD at a population level. The potential for geographic isolation may not exist when more individuals mix in one population. The distribution of haplotypes can result from many factors, including dispersal propensity. It was demonstrated that the ACB is highly mobile (Wang *et al*., 1994; Shirai, 2009). Despite its flight capability, short-range dispersal is most prevalent in the ACB (Wang *et al*., 1994). Local habitats might have a major influence on the dispersal pattern, and thus could affect haplotype distribution.

In nature, ACB populations usually feed on sorghum or wild plants before colonizing maize. A previous study showed that gene flow of the ACB was frequent among different host populations; thus, no host specialization was found (Wang *et al*., 2018). However, laboratory experiments showed that ACB larvae still maintain their feeding preference on native hosts (Yuan *et al*., 2013). Whether the ACB populations in fields differ in host use is unclear. In this study, association between a common haplotype and areas with maize indicates that maize is the most suitable host of the ACB. Our recent study showed that the ACB tended to aggregate in maize-rich landscapes (Dong *et al*., 2020). Therefore, host availability may play a key role in the spatial distribution of haplotypes. Although our data show a significant correlation between H12 frequency and areas with maize, it does not necessarily mean that selection directly operates on the mtDNA gene. The cytonuclear interactions could potentially result in hitchhiking of the mtDNA haplotype (Drovetski *et al*., 2012).

We found a large number of haplotypes, and most haplotypes only occurred in a single sampling location. Similar situations were also found in other studies of ACB populations (Hoshizaki *et al*., 2008; Li *et al*., 2014). The

high dispersal ability may contribute to the large diversity of haplotypes, because gene mutations often spread before giving rise to new haplotypes (Freeland *et al*., 2011); that is, genetic variations become widespread through dispersal. Another possible explanation for the high haplotype diversity is that some wild crop ancestors may serve as a refuge for archaic ACB genotypes, as shown in corn leafhoppers (Bernal *et al*., 2019). Clusters 1 and 2 were only associated with SNH at a 1-km scale; however, at larger spatial scales (i.e., 3 and 5 km), we did not find any significant influence of SNH. The relationship between haplotype frequency and SNH was unclear. Future studies should focus on targeted geographic areas and test the role of SNH.

In this study, we collected samples from a large number of locations ($n = 79$) but a small number of individuals (4–10) per site. This was a compromise between sampling a broad geographical range and genotyping a realistic number of samples (Freeland *et al*., 2011). It is possible that not all haplotypes were sampled from any particular population. However, genetic–environment association methods may be more robust with smaller sample sizes per location if many locations that vary in the environmental features are sampled (De Mita *et al*., 2013). Our haplotype data were obtained across a large area and in different years. Therefore, we believe that these data yield valuable information and could be useful for future long-term studies.

Host factors are often assumed to affect the mtDNA variation in ACB populations from different geographic locations. However, no previous empirical study has supported this assumption. Our findings provide evidence that crop area can affect the mtDNA haplotype frequency in ACB populations. The association between haplotype frequency and host availability suggest evolutionary responses of this species that promoted host adaptation.

Acknowledgments

This study was supported by the National Key R&D Program of China (2017YFD0200400), the National Natural Science Foundation of China (31800349) and the Key R&D Program of Shandong Province (2018GNC111019), Agricultural Science and Technology Innovation Project of Shandong Academy of Agricultural Sciences (CXGC2016A09).

Disclosure

All authors have seen and agree with the contents of the manuscript and there is no conflict of interest, including specific financial interest and relationships and affiliations relevant to the subject of manuscript.

References

Agarwala, B.K. and Choudhuri, P.R. (2014) New host races of *Aphis gossypii* (Insecta: Hemiptera: Aphididae) from northeast India. *Current Science*, 107, 1172–1177.

Anstead, J., Burd, J. and Shufran, K. (2002) Mitochondrial DNA sequence divergence among *Schizaphis graminum* (Hemiptera: Aphididae) clones from cultivated and noncultivated hosts: haplotype and host associations. *Bulletin of Entomological Research*, 92, 17–24.

Ballard, J.W.O. and Pichaud, N. (2014) Mitochondrial DNA: more than an evolutionary bystander. *Functional Ecology*, 28, 218–231.

Bartoń, K. (2018) MuMIn: multi-model inference. R package version 1.40.4 ed.

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 79668.

Bazin, E., Glemin, S. and Galtier, N. (2006) Population size does not influence mitochondrial genetic diversity in animals. *Science*, 312, 570–572.

Bernal, J.S., Dávila-Flores, A.M., Medina, R.F., Chen, Y.H., Harrison, K.E. and Berrier, K.A. (2019) Did maize domestication and early spread mediate the population genetics of corn leafhopper? *Insect Science*, 26, 569–586.

Brown, J.M., Abrahamson, W.G. and Way, P.A. (1996) Mitochondrial DNA phylogeography of host races of the goldenrod ball gallmaker, *Eurosta solidaginis* (Diptera: Tephritidae). *Evolution*, 50, 777–786.

Burnham, K.P. and Anderson, D.R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer-Verlag, New York. 488 pp.

Calcagno, V., Thomas, Y. and Bourguet, D. (2007) Sympatric host races of the European corn borer: Adaptation to host plants and hybrid performance. *Journal of Evolutionary Biology*, 20, 1720–1729.

Clement, M., Snell, Q., Walker, P., Posada, D. and Crandall, K. (2002) TCS: Estimating gene genealogies. *Parallel and Distributed Processing Symposium, International Proceedings*, 184.

De Mita, S., Thuillet, A.C., Gay, L., Ahmadi, N., Manel, S., Ronfort, J. and Vigouroux, Y. (2013) Detecting selection along environmental gradients: analysis of eight methods and their effectiveness for outbreeding and selfing populations. *Molecular Ecology*, 22, 1383–1399.

Dong, Z., Zhang, Q., Li, L., Lu, Z., Li, C., Ouyang, F., Tscharntke, T., Yu, Y. and Men, X. (2020) Landscape agricultural simplification correlates positively with the spatial

distribution of a specialist yet negatively with a generalist pest. *Scientific Reports*, 10, 344.

Dorchin, N., Scott, E.R., Clarkin, C.E., Luongo, M.P., Jordan, S. and Abrahamson, W.G. (2009) Behavioural, ecological and genetic evidence confirm the occurrence of host-associated differentiation in goldenrod gall-midges. *Journal of Evolutionary Biology*, 22, 729–739.

Dowling, D.K., Friberg, U. and Lindell, J. (2008) Evolutionary implications of non-neutral mitochondrial genetic variation. *Trends in Ecology & Evolution*, 23, 546–554.

Drovetski, S.V., Kitaysky, A.S., Mode, N.A., Zink, R.M., Iqbal, U. and Barger, C. (2012) mtDNA haplotypes differ in their probability of being eliminated by a mass die-off in an abundant seabird. *Heredity*, 109, 29–33.

Forbes, A.A., Powell, T.H.Q., Stelinski, L.L., Smith, J.J. and Feder, J.L. (2009) Sequential sympatric speciation across trophic levels. *Science*, 323, 776–779.

Freeland, J., Petersen, S. and Kirk, H. (2011) *Molecular Ecology (Second Edition)*, John Wiley & Sons, Chichester, UK. 449 pp.

Galtier, N., Nabholz, B., GlÉmin, S. and Hurst, G.D.D. (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology*, 18, 4541–4550.

Grab, H., Danforth, B., Poveda, K. and Loeb, G. (2018) Landscape simplification reduces classical biological control and crop yield. *Ecological Applications*, 28, 348–355.

Grant, W.S., Spies, I.B. and Canino, M.F. (2006) Biogeographic evidence for selection on mitochondrial DNA in North Pacific walleye pollock *Theragra chalcogramma*. *Journal of Heredity*, 97, 571–580.

Guldemond, J.A., Tigges, W.T. and De Vrijer, P.W.F. (1994) Host races of *Aphis gossypii* (Homoptera: Aphididae) on cucumber and chrysanthemum. *Environmental Entomology*, 23, 1235–1240.

Hoshizaki, S., Washimori, R., Kubota, S., Ohno, S., Huang, Y., Tatsuki, S. and Ishikawa, Y. (2008) Two mitochondrial lineages occur in the Asian corn borer, *Ostrinia furnacalis* (Lepidoptera: Crambidae), in Japan. *Bulletin of Entomological Research*, 98, 519–526.

Jaenike, J. (1990) Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*, 21, 243–273.

James, A.C. and Ballard, J.W.O. (2003) Mitochondrial genotype affects fitness in *Drosophila simulans*. *Genetics*, 164, 187–194.

Joyce, A.L., Sermeno Chicas, M., Serrano Cervantes, L., Paniagua, M., Scheffer, S.J. and Solis, M.A. (2016) Host-plant associated genetic divergence of two *Diatraea* spp. (Lepidoptera: Crambidae) stemborers on novel crop plants. *Ecology and Evolution*, 6, 8632–8644.

Keyghobadi, N., Roland, J. and Strobeck, C. (2005) Genetic differentiation and gene flow among populations of the alpine butterfly, *Parnassius smintheus*, vary with landscape connectivity. *Molecular Ecology*, 14, 1897–1909.

Kojima, W., Fujii, T., Suwa, M., Miyazawa, M. and Ishikawa, Y. (2010) Physiological adaptation of the Asian corn borer *Ostrinia furnacalis* to chemical defenses of its host plant, maize. *Journal of Insect Physiology*, 56, 1349–1355.

Leigh, J.W. and Bryant, D. (2015) Popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6, 1110–1116.

Li, J., Coates, B.S., Kim, K.S., Bourguet, D., Ponsard, S., He, K. and Wang, Z. (2014) The genetic structure of Asian corn borer, *Ostrinia furnacalis*, populations in China: Haplotype variance in northern populations and potential impact on management of resistance to transgenic maize. *Journal of Heredity*, 105, 642–655.

Librado, P. and Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451–1452.

Medina, R.F. (2012) Implications of host-associated differentiation in the control of pest species. *Insect Outbreaks Revisited* (eds. P. Barbosa, D.K. Letourneau & A.A. Agrawals), pp. 291–310.Wiley-Blackwell, Hoboken.

Nafus, D.M. and Schreiner, I.H. (1991) Review of the biology and control of the Asian corn borer, *Ostrinia furnacalis* (Lep: Pyralidae). *Tropical Pest Management*, 37, 41–56.

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

Peakall, R. and Smouse, P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288–295.

Peakall, R. and Smouse, P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, 28, 2537–2539.

Pfister, S.C., Sutter, L., Albrecht, M., Marini, S., Schirmel, J. and Entling, M.H. (2017) Positive effects of local and landscape features on predatory flies in European agricultural landscapes. *Agriculture Ecosystems & Environment*, 239, 283–292.

Pichaud, N., Messmer, M., Correa, C.C. and Ballard, J.W.O. (2013) Diet influences the intake target and mitochondrial functions of *Drosophila melanogaster* males. *Mitochondrion*, 13, 817–822.

R Core Team (2018) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rusch, A., Chaplin-Kramer, R., Gardiner, M.M., Hawro, V., Holland, J., Landis, D., Thies, C., Tscharntke, T., Weisser, W.W., Winqvist, C., Woltz, M. and Bommarco, R. (2016) Agricultural landscape simplification reduces natural pest

control: A quantitative synthesis. *Agriculture Ecosystems & Environment*, 221, 198–204.

Segelbacher, G., Cushman, S.A., Epperson, B.K., Fortin, M.J., Francois, O., Hardy, O.J., Holderegger, R., Taberlet, P., Waits, L.P. and Manel, S. (2010) Applications of landscape genetics in conservation biology: concepts and challenges. *Conservation Genetics*, 11, 375–385.

Shandong Municipal Bureau of Statistics (2017) *Shandong Statistical Yearbook*. China Statistic Press, Beijing.

Shirai, Y. (2009) Laboratory evaluation of flight ability of the Oriental corn borer, *Ostrinia furnacalis* (Lepidoptera: Pyralidae). *Bulletin of Entomological Research*, 88, 327–333.

Shufran, K., Burd, J., Anstead, J. and Lushai, G. (2000) Mitochondrial DNA sequence divergence among greenbug (Homoptera: Aphididae) biotypes: evidence for host-adapted races. *Insect Molecular Biology*, 9, 179–184.

Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123, 585–595.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I. and Thies, C. (2005) Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management. *Ecology Letters*, 8, 857–874.

Wang, K.Q., Liu, X.L. and Shao, T.Y. (2018) Analysis of the genetic diversity and host specialization of *Ostrinia furnacalis* (Lepidoptera: Crambidae) in Northeast China. *Acta Entomologica Sinica*, 61, 1054–1066.

Wang, Z., He, K. and Yan, S. (2005) Large-scale augmentative biological control of Asian corn borer using *Trichogramma* in China: A success story. *Proceedings of the Second International Symposium on Biological Control of Arthropods, Davos, Switzerland*, 487–494.

Wang, Z., Li, B., Wang, Y., Li, W. and Pan, Y. (1994) Studies on behaviour of dispersal and possibility of migration in adult overwintering generation Asian corn borer by using release-and-recapture technique. *Journal of Plant Protection*, 21, 25–31.

Xie, H.C., Li, D.S., Zhang, H.G., Mason, C.E., Wang, Z.Y., Lu, X., Cai, W.Z. and He, K.L. (2015) Seasonal and geographical variation in diapause and cold hardiness of the Asian corn borer, *Ostrinia furnacalis. Insect Science*, 22, 578–586.

Yuan, Z., Guo, J., Wang, Z., He, K. and Bai, S. (2013) Feeding preference of the Asia corn borer larvae for different host plants. *Acta Phytophylacica Sinica*, 40, 205–210.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. and Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer-Verlag New York, New York. 574 pp.

Manuscript received December 23, 2019
Final version received April 26, 2020
Accepted April 30, 2020

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Material Haplotypes of Asian corn borer bases on combined genes of mtDNA COI and COII.

Table S1. List of Asian corn borer, *Ostrinia furnacalis*, samples used for molecular work.