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Population Genetics of the Western Bean Cutworm (Lepidoptera: Noctuidae) Across the United States

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ABSTRACT The western bean cutworm, *Striacosta albicosta* (Smith), is a secondary pest of maize (*Zea mays* L.) and dry beans (*Phaseolus vulgaris* L.) in the western United States. Recently, this insect has undergone a major territory expansion into the eastern United States and has become a pest throughout much of the Corn Belt. This study was instigated to examine the population genetics of this pest to facilitate control and resistance management, as well as to shed light on the current habitat expansion. *S. albicosta* individuals were collected from 24 different locations across the traditional and expanded range and amplified fragment length polymorphism analysis was conducted to assess genetic variability. In total, 90 markers were analyzed, encompassing >90% of genetic variation. G_{st} across all locations was moderately high ($G_{st} = 0.5032$). AMOVA analysis revealed that the majority of genetic variation was within locations (54%) and among locations within groups (45%) indicating genetic differentiation of subpopulations. The Mantel test revealed no correlation between geographic and genetic distance ($n = 548$; $r = 0.0015$; $P = 0.4350$). Locations sampled in the eastern United States did not exhibit any reduction in genetic variation in comparison to locations sampled in the western United States, so we conclude that no bottleneck event has occurred with this territory expansion.

KEY WORDS *Striacosta albicosta*, western bean cutworm, population genetics, AFLP

The western bean cutworm, *Striacosta albicosta* (Smith), is a pest of corn, *Zea mays* (L.), and dry beans, *Phaseolus vulgaris* (L.), throughout the west-central United States (Hoerner 1948, Blickenstaff and Jolley 1982). Damage caused by the western bean cutworm is variable, but up to 40% yield loss can occur in heavily infested cornfields (Appel et al. 1993) and up to 80% in heavily infested bean fields (Hoerner 1948). The western bean cutworm damages beans by feeding on the foliage and developing pods (Antonelli and O'Keeffe 1981). Corn is damaged through direct feeding on the ear, as well as indirectly through the entry of fungi at damage sites (Appel et al. 1993).

S. albicosta was first collected in Arizona and was then reported in Idaho, Utah, Colorado, New Mexico, Texas, Wyoming, Oklahoma, South Dakota, Kansas, Nebraska, Iowa, and parts of Mexico and Canada (Douglass et al. 1957, Appel et al. 1993). At the beginning of the 21st century the western bean cutworm began to expand its range into the eastern portion of the Corn Belt. Recently, the western bean cutworm has been reported in Minnesota (O'Rourke and Hutchison 2000) as well as Illinois, Missouri, Minnesota, Indiana, and Ohio (Rice 2000, Dorhout and Rice 2004, Rice and Dorhout 2006, Rice 2007, DiFonzo and Hammond 2008). In the summer of 2010 the moth was reported as far east as New York (Tooker and Fleischer 2010).

A recent genetic analysis of *S. albicosta* subpopulations from Wyoming, Nebraska, and Iowa revealed no evidence of a bottleneck effect, suggesting that the range expansion is more likely because of ecological factors than due to a founder population (Miller et al. 2009). Dorhout and Rice (2010) hypothesized that the *S. albicosta* has expanded eastward in response to a niche recently opened by the widespread planting of maize containing the Cry1Ab protein. The Cry1Ab protein controls for many types of Lepidopteran pests, but does not control *S. albicosta* (O'Rourke and Hutchison 2000). Therefore, it was hypothesized that insects such as the corn earworm, *Helicoverpa zea* (Boddie), outcompete *S. albicosta,* preventing it from becoming a pest species in the eastern Corn Belt before the introduction of *Bacillus thuringiensis* (Berliner) (Bt) maize. Other hypotheses for the recent expansion that are related to the adoption of Bt corn include the widespread use of conservation till practices and a reduction in the use of pesticides (Hutchison et al. 2011).

Although existing evidence indicates no genetic bottleneck for *S. albicosta* (Miller et al. 2009), the recent expansion of *S. albicosta* populations and the

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Table 1. Collection information and genetic diversity by location

Numerical code	Location	Date collected	Collected by	No. individuals	Percent polymorphic loci	Nei's gene diversity (h)
34	Holyoke West, CO	8/23/07	L. Appel	27	55%	0.1908
37	Holyoke W., CO	8/23/07	L. Appel	24	26%	0.1036
15	Holyoke East, CO	8/23/07	L. Appel	20	77%	0.2995
4	Banner Co., NE	8/16/07	E. Lindroth	26	71%	0.2116
25	Lake Minatare, NE	9/5/08	L. Appel	22	65%	0.2613
20	Chase Co., NE	8/22/07	L. Appel	10	72%	0.3169
29	N 40 48.206′ W 101 45.460′ Perkins Co. NE	8/31/09	D. Boxler	16	35%	0.1491
16	SW ¼ 11-T11N-R37W Perkins Co. NE	8/27/07	L. Appel	10	44%	0.1741
28	NE ¼ 19 T10N-R41W Perkins Co. NE	9/2/09	C. Rusentrater	38	53%	0.1811
18	SE ¼ 8-T9N-R41W Perkins Co. NE	9/24/07	L. Appel	34	46%	0.1606
7	Broadwater, NE	9/3/08	L. Appel	21	65%	0.2195
17	Imperial, NE	8/22/07	L. Appel	22	68%	0.2749
8	Brandon, NE	8/22/08	L. Appel	12	75%	0.2855
39	Grainton, NE	8/23/07	L. Appel	13	71%	0.2658
30	N 41 26.209′ W 100 42.430′ Logan Co. NE	8/31/09	D. Boxler	28	86%	0.3131
26	Clay Center, NE	8/3/06	Unknown	31	13%	0.0427
5	Concord, NE	7/16/07	E. Lindroth	16	83%	0.3200
14	Boone Co., IA	2006	M. Rice	28	93%	0.3380
35	Hamilton Co., IA	2006	M. Rice	25	40%	0.1495
1	Rosemount, MN	7/24/06	B. Hutchison	30	87%	0.3570
38	Columbia Co., WI	8/19/08	E. Cullen	23	43%	0.1901
12	Door Co., WI	10/8/08	E. Cullen	22	94%	0.3380
24	La Porte Co., IN	9/2/08	M. Coomer	30	74%	0.3060
27	Ohio, various	8/21/08	A. Michaels	32	84%	0.2507

The location, date collected, collector, and no. of samples were recorded. Host plant information was collected for larvae and eggs, adults were captured in light or pheromone traps. Location data were recorded as GPS, soil map coordinates, or by town and/or county. All analyses performed in Popgene. A locus is defined as polymorphic if the frequency of the most common allele is <99%.

advent of companies producing genetically modified corn with traits effective against this pest necessitates better understanding of the genetics and gene flow of *S. albicosta* populations. Knowledge of the amount of variation and gene flow among populations is important in managing resistance to control methods. Better understanding of the population dynamics of this pest insect could potentially provide baseline data to aid in the control of resistance if it appears. Furthermore, this is a unique opportunity to examine the genetics of a native insect pest as it expands into new territory. The objective of this study was to further our understanding of the genetic variation of *S. albicosta* populations throughout its range and to provide an hypothesis for this expansion.

Materials and Methods

S. albicosta samples were collected from 24 different locations (Table 1; Figs. 1 and 2) throughout its range, including the areas in which it is newly established. The insects were primarily collected in the larval stage, although adults were also used in this study. Thirty insects per location is the recommended sample size (Bonin et al. 2007); the actual number of insects collected in this study varied between 10 (in locations into which the western bean cutworm has recently immigrated) to >30 in certain locations in the western United States. When larvae were collected, insects were taken from different plants to avoid collecting siblings. Adults were collected in light traps. Larvae were either kept alive on ice until they arrived at the laboratory for processing, or they were stored in 95–100% ethanol. Upon reaching the laboratory samples were stored at −80°C.

DNA Extraction. Before the DNA extraction process was initiated, specimens were first washed in 70% ethanol and then in nanopure water. The head and gut were removed from larval specimens to avoid contamination with any food sources. In adult specimens, DNA was extracted from the head and thorax.

DNA was isolated from western bean cutworm larvae using a cetyl trimethylammonium bromide (CTAB; Sigma-Aldrich, St. Louis, MO) protocol (Alamalakala et al. 2009) modified from Doyle and Doyle (1987). Extracted DNA was suspended in 50 μl 1× TE buffer (10 mM Tris-HCl; 0.1 mM EDTA) and stored at −20°C. DNA concentration and purity was determined using the Nanodrop 2000 (Thermo Scientific, Wilmington, DE). Extracted DNA was then diluted with nanopure water to a concentration of 20–100 ng/μl.

Amplified Fragment Length Polymorphism. Genetic variation was analyzed using a modified amplified fragment length polymorphism (AFLP) procedure (Clark et al. 2007, Krumm et al. 2008) based on Vos et al. (1995), which consists of four basic steps, restriction digestion, adapter ligation, preamplification, and selective amplification.

Restriction Digestion and Adapter Ligation. Seven microliters of diluted DNA template was mixed with 0.0625 μl *Eco*RI, 0.125 μl *Mse*I (New England Biolabs, Ipswich, MA), 1.25 μl 1× NEBuffer four (New England Biolabs; 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol; original buffer concentrations of 10× were

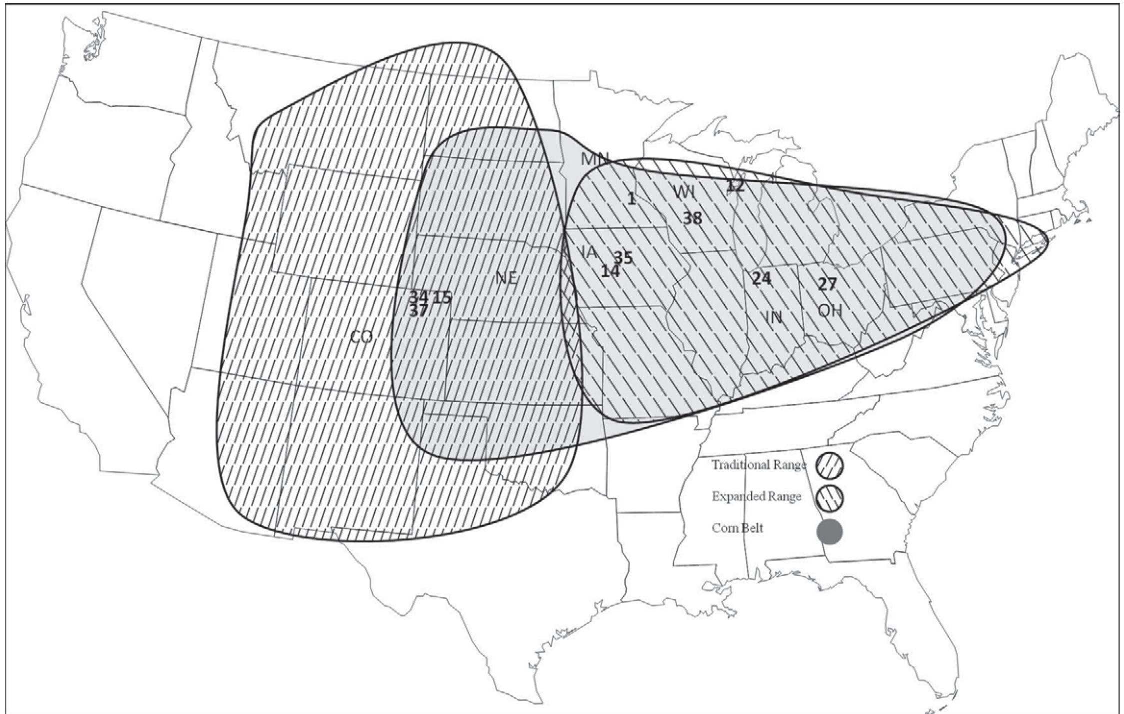

Fig. 1. Map of collection locations across the United States. Highlighted areas depicting the traditional range of the *S. albicosta*, the expanded range, and the Corn Belt. Collection locations for the state of Nebraska are show in detail in Fig. 2.

diluted to 1×), 0.125 μl bovine serum albumin (New England Biolabs), and nanopure water for total volume of 12.5 μl and incubated at 37°C for 2.5 h in a polymerase chain reaction (PCR) thermal cycler (Applied Biosystems, Carlsbad, CA). The resulting fragments were then incubated at 25°C for 8 h with a ligation mixture of 0.15 μl T4 DNA ligase (New England Biolabs), 1× T4 DNA ligase buffer (New England Biolabs), 0.5 μl *Eco*RI prepared adapter, 0.5 μl *Mse*I prepared adapter, and 3.35 μl nanopure water. Before ligation, adapters were prepared by separately incubating 1.0 μg/μl *Eco*RI and 0.5 μg/μl *Mse*I oligonucleotides (Table 2) (Integrated DNA Technologies, www.idtdna.com) with 1× NEBuffer four in a thermal cycler for one cycle of 65°C for 10 min, 37°C for 10 min, and 25°C for 10 min. The final ligation product was diluted 1:10 using 1× TE buffer.

Preamplification. Then, 1.5 μl of the ligation product was incubated with 10 μl Preamplification Primer Mix II (containing preamplification primers; LI-COR Biosciences, Lincoln, NE), 0.25 μl Bullseye *Taq*DNA polymerase (MidSci, St. Louis, MO), 1.25 μl 1× PCR buffer (100 mM Tris-HCl, 500 mM KCl), and 0.75 μl $MgCl_2$. The PCR program consisted of 20 cycles (30 s at 94°C, 1 min at 56°C, 1 min at 72°C). The final preamplification template mixture was diluted 20-fold with nanopure water.

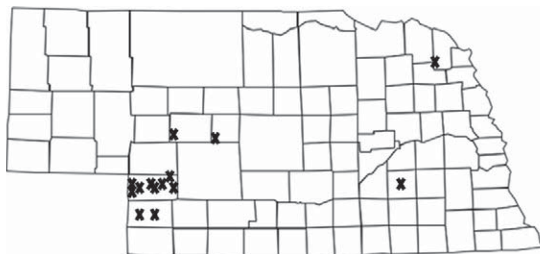

Fig. 2. Map of collection locations within Nebraska.

Selective Amplification. Reaction volumes containing 4.1 μl nanopure water, 1.2 μl 1× PCR buffer, 0.72 μl $MgCl_2$, 0.08 μl Bullseye *Taq*DNA polymerase (MidSci, St. Louis, MO), 2.0 μl *Mse*I primer (Integrated DNA Technologies), 0.4 μl *Eco*RI IRD-700 labeled primer (Integrated DNA Technologies), 0.4 μl of dNTPs (Applied Biosystems), and 2.0 μl of the preamplification template were amplified via PCR. The PCR program consisted of one cycle (30 s at 94°C, 30 s at 65°C, 1 min at 72°C), 12 cycles (30 s at 94°C, 1 min at 72°C), and 23 cycles (30 s at 94°C, 30 s at 56°C, 1 min at 72°C). A list of primers used and their sequences is shown in Table 2. Finally, 2.5 μl stop solution (LI-COR Biosciences) was added to the PCR product. The product was then denatured for 2 min at 94°C and immediately stored at −20°C.

Data Scoring and Analysis. One microliter samples were electrophoresed through a KB^{Plus} 6.5% polyacrylamide gel (LI-COR Biosciences) and the bands were detected via infrared florescence using a laser scanning machine (LI-COR model 4200S-2, LI-COR Biosciences, Lincoln, NE). An IRD-700 labeled 50–700 bp size standard was used to estimate fragment

Table 2. AFLP adapters and primers used

Oligonucleotide	Purpose	Sequence (5′-3′)
*Eco*RI-forward adapter	Adapter ligation	CTCGTAGACTGCGTACC
*Eco*RI-reverse adapter	Adapter ligation	AATTGGTACGCAGTCTAC
*Mse*I-forward adapter	Adapter ligation	GACGATGAGTCCTGAG
*Mse*I-reverse adapter	Adapter ligation	TACTCAGGACTCAT
*Eco*RI primer	Preamplification	GACTGCGTACCAATTC
*Mse*I primer	Preamplification	GATGAGTCCTGAGTAA
E-ACG	Selective amplification	GACTGCGTACCAATTC + ACG
M-CAG	Selective amplification	GATGAGTCCTGAGTAA + CAG
M-CTG	Selective amplification	GATGAGTCCTGAGTAA + CTG
Primer pair	Number of loci	Fragment sizes
M-CAG + E-ACG	49	42–305 bp
M-CTG + E-ACG	41	28–257 bp

Adapter and primer sequences taken from Vos et al. (1995). Primer combinations for selective amplification. Number of markers scored per primer pair and fragment sizes of markers scored.

size. Gels were scored using the program SAGA MX 3.2 (LI-COR Biosciences). The data were converted to matrix form for further analysis, with a one indicating band presence and a 0 indicating absence.

Before performing statistical analyses, the error rate was calculated for AFLP markers following Pompanon et al. (2002). In total, 30 samples were selected randomly and replicated three times. Each locus was then examined for mismatches among the three replicates. The error rate was calculated by dividing the number of mismatches at a particular locus by the number of individuals replicated (Pompanon et al. 2005, Bonin et al. 2007). Loci with an error rate >0.1 were rejected.

The data were first assessed to determine whether the number of loci used was sufficient to explain the genetic variation among *S. albicosta* subpopulations using DBOOT v. 1.1 (Coelho 2001). For all genetic analyses, sampled locations were divided into groups (Table 3). The first group consisted of locations sampled inside the historical range of the *S. albicosta*, while the second group contained locations sampled into which *S. albicosta* has recently expanded.

The population genetics software Popgene v. 1.32 (Yeh and Boyle 1997) was used to assess genetic diversity at the group level, the intralocation level, and the whole population level. Hardy–Weinberg equilibrium was assumed. For each individual location, the percentage of polymorphic loci and Nei's Gene Diversity were calculated. At the group and whole population levels Nei's G_{st} was calculated. Genetic structure in subdivided populations is characterized by values such as F_{st} and G_{st}, which range from 0 to 1. A value of 0 would indicate no subdivision of the whole population, while a value of one would indicate nearly complete genetic isolation (structure) of subpopulations (locations) (Nei 1987). Popgene was also used to construct a matrix of genetic distances between locations using Nei's unbiased measures of genetic identity and genetic distance (Nei 1978).

Table 3. Nei's G_{st}

	States included	G_{st}
Western midwest United States (traditional range)	Nebraska, Colorado	0.5121
Eastern midwest United States (expanded range)	Iowa, Wisconsin, Minnesota, Indiana, Ohio	0.4194
All locations	Nebraska, Colorado, Iowa, Wisconsin, Minnesota, Indiana, Ohio	0.5032

Analyses for groups of locations performed in Popgene. Locations were grouped according to traditional and expanded habitat.

The software package Arlequin v. 3.1 (Excoffier et al. 2005) was used to conduct the analysis of molecular variance (AMOVA) as well as the calculation of Wright's *F*-statistics. The AMOVA tests for genetic structure at the among groups, within groups, and within location levels; 1,000 permutations of the data were run to test the significance of the variance components. Arlequin v. 3.1 was also used to perform the Mantel test, which was used to determine any correlation between genetic and geographic distance. The geographic distance matrix was constructed by measuring distances between locations in Google Earth v. 5.2.1. The genetic distance matrix from Popgene v. 1.32, using Nei's unbiased measures of genetic distance, was imported into Arlequin.

The program PAUP v. 4.01b (Swofford 2001) was used to construct an unweighted pair-group method with arithmetic average distance tree to depict genetic relationships among locations. The BOODP program (Coelho 2001) was used to conduct bootstrap analysis of 1,000 pseudoreplicates to assess the dependability of the unweighted pair-group method with arithmetic average cluster analysis.

Potential population genetic structure was analyzed using the software package Structure (Pritchard et al. 2000). Structure uses the Markov chain Monte Carlo algorithm to assign individuals to clusters. A burn-in and MCMC rate of 10,000 each was performed for each value of K. The analysis was replicated five times for each value of K and the ln likelihood was averaged. Data were analyzed with no a priori assumption of structure. The range of possible K-values tested was between 1 and 24. The ln likelihood of various values of K were calculated to determine the true number of clusters. Additionally, the amount of admixture (α) was estimated.

Table 4. Nei's gene diversity statistics for subdivided populations

H_t	H_c	H_s	G_{st}	G_{cs}
0.4682	0.2372	0.4599	0.0178	0.4843

Hierarchical pop analysis performed in Popgene.

H_t, heterozygosity of the total; H_c, heterozygosity of groups; H_s, heterozygosity of subpopulations; G_{cs}, genetic diversity of subpopulations relative to groups; G_{st}, genetic diversity of subpopulations relative to the total.

Table 5. AMOVA

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among groups	1	201.780	−0.05415 Va	−0.32
Among locations within groups	22	4112.111	7.85125 Vb	45.77
Within locations	525	4912.755	9.35763 Vc	54.55
Total	548	9226.647	17.15473	

Analyses performed in Arlequin v. 3.1 for three levels of pop subdivision. Locations were grouped for this analysis as described in Table 3.

Results

In total, 548 individuals from 24 locations of *S. albicosta* across the United States were analyzed (Table 1). After rejecting 12 loci for a high error rate, the two primer combinations resulted in a total of 90 loci, ranging in size from 28 to 305 bp (Table 2). The coefficient of variation, calculated to determine the amount of genetic variability encompassed by the number of individuals and loci in our study (Coelho 2001), showed that our data set accounted for >90% of genetic variability; therefore, 90 was a sufficient number of markers for a robust statistical analysis. In general, >90% of genetic variability is considered suitable for analysis (Clark et al. 2007, Krumm et al. 2008).

Analysis for individual locations revealed a significant range of genetic diversity within locations. The number of polymorphic loci ranged from 13% (Clay Center, NE) to 94% (Door County, WI) within individual locations (Ξ = 64%; Table 1). A locus is considered polymorphic when the frequency of the most common allele is less than or equal to 0.99 (Nei 1987). Nei's gene diversity (Nei 1987) fell between 0.0427 and 0.3570 (Table 1).

Analysis of multiple locations in Popgene (Nei 1973, McDermott and McDonald 1993) revealed a moderately high level of differentiation (G_{st} = 0.5032) (Table 3). G_{st} values within groups were variable, although it was slightly lower between locations in newly established territory (G_{st} = 0.4194) than among locations within the historical range (G_{st} = 0.5121). When Popgene was used to calculate Gst using a hierarchical model with both locations and groups of locations, the total G_{st} became 0.0178 while the G_{cs} was 0.4843 (Table 4).

AMOVA analysis revealed the highest percentage of variation (54.55%) to have been within locations; 45.77% of variation was among locations within groups; only −0.32% of variation was accounted for by differences among the three groups (Table 5). The groups of locations for the AMOVA analysis may be found in Table 3. No haplotypes were shared between locations. The fixation indices, as calculated by Arlequin (Excoffier et al. 1992), also support a moderately high degree ($F_{st} = 0.4545$; $P = 0.000$) of differentiation among locations. *F*-statistics were tested for significance by conducting 1,000 permutations of locations, groups, and individuals (Excoffier et al. 1992). The fixation indices F_{st} (correlation among groups within the whole population) and F_{sc} (correlation of haplotypes within groups relative to the whole population; $F_{sc} = 0.4562$, $P = 0.000$) were statistically significant, while F_{ct} (correlation of haploypes within locations relative to the containing group; $F_{ct} = -0.0032$, $P = 0.000$) was not.

The results of the Mantel test (Mantel 1967) confirmed that there is no significant correlation between geographic and genetic distance in the *S. albicosta* locations sampled ($n = 548$; $r = 0.0015$; $P = 0.4350$). Testing for significance was accomplished by permuting the rows and columns of the matrices 1,000 times.

The unweighted pair-group method with arithmetic average (Sneath and Sokal 1973) dendrogram (data not shown) revealed almost no relationship between genetic similarity and geographic location. Geographically distant locations, such as Holyoke, CO (location 34) and Hamilton Co. IA (location 35) are indicated as genetically similar while other genetically similar locations are also close geographically, such as Banner Co. NE (location 4) and Broadwater, NE (location 7).

Structure analysis revealed the real number of clusters (K) to be 22. The ln likelihood of K = 22 was ln(−15,703.5). Admixture was low with $\alpha = 0.0306$.

Discussion

S. albicosta from different locations exhibit genetic differentiation. Nei's G_{st} revealed more genetic isolation among western locations (G_{st} = 0.5121) than among eastern locations (G_{st} = 0.4194). This indicates that there is more gene flow among eastern locations than among western locations. Hierarchical analysis of *S. albicosta* populations revealed that most of the differentiation among locations was because of differentiation within groups (G_{cs} = 0.4843) rather than between groups (G_{st} = 0.0178). Therefore, while there is genetic differentiation among *S. albicosta* subpopulations, it is not driven by differences between eastern and western locations. This suggests that expansion into the eastern United States has thus far had little impact on genetic differentiation. Alternatively, the influx of individuals into the eastern Corn Belt could be ongoing. If yearly weather patterns cause individuals from the western United States to move east, this could provide enough gene flow to prevent significant genetic differentiation.

While the "ideal" sample size per subpopulation is 30 according to Bonin et al. (2007), it is not always possible to obtain this number with natural populations. *S. albicosta* is a secondary pest and not always

present in large numbers. While every effort has and should be made to obtain as many individuals as possible per location, genetic information obtained from subpopulations with fewer individuals may still be informative (Singh et al. 2006).

AMOVA results provide support for the idea that the majority of genetic variation is within locations. However, nearly as much variation among locations within groups indicates there is genetic differentiation of *S. albicosta* within groups. The fixation index F_{ct} was not significant, indicating that genetic diversity does not lie between groups and suggesting that genetic differentiation of subpopulations does not follow an east–west pattern.

The Mantel test and the unweighted pair-group method with arithmetic average dendrogram indicated that geographic and genetic distances were uncorrelated; suggesting that genetic relatedness of *S. albicosta* is not affected by geographic distance. However, the moderately high values of both G_{st} and F_{st} can be interpreted to mean that the amount of gene flow among locations within and between groups is low. Results from the program Structure suggest that collection locations roughly correspond to actual subpopulations. Bayesian analysis also indicated that admixture among locations is low. When α is close to zero, individuals are essentially from one population or another. When $\alpha > 1$ there is significant admixture (Pritchard et al. 2000).

Given that *S. albicosta* has undergone rapid range expansion within the past decade, it is likely that any gene flow seen between the western and eastern United States represents colonization events rather than interbreeding between locations. As there were no state records of *S. albicosta* in many eastern states before the year 2000 (Estes 2004, DiFonzo and Hammond 2008), it is extremely unlikely that there were previously established populations east of Nebraska that would result in interbreeding.

The lack of correlation between genetic and geographic distance could also simply be an indication that not enough time has elapsed since initial colonization for genetic drift to exert any influence on these subpopulations. At this point in time, population expansion is still on-going. In the summer of 2010 moths were captured as far east as Long Island (Waldron 2010), and they may yet move farther up the East Coast. *S. albicosta* is univoltine, indicating that if expansion began in the 1990s the moth has been in new territory for $<$20 generations. Given the incremental expansion eastward, it is possible that future genetic analyses will reveal a pattern of gene flow that more closely resembles the stepping-stone model of migration (Hedrick 2000).

A common feature of many population expansions is the founder effect (Hedrick 2000). A few individuals from the parent population move to a new location and found a separate, breeding subpopulation. This new subpopulation is genetically isolated from the parent population, leading to a significant difference in allele composition and genetic diversity (Hedrick 2000). Typically, the founder effect leads to lower genetic diversity in the subpopulation in the new location than is seen in the parent population. If *S. albicosta* locations in the east were subject to the founder effect, we could expect to see a significant reduction in heterozygosity as well as a significant genetic isolation from western locations. However, these data show that there is no reduction in heterozygosity among eastern locations. Eastern locations also do not exhibit more genetic isolation from western locations than western locations do from one another. Miller et al. (2009) came to a similar conclusion by analyzing differences in the NADH dehydrogenase gene in the mitochondrial DNA of four subpopulations of *S. albicosta* from Wyoming, Nebraska, and Iowa. Their study revealed no significant heterogeneity between subpopulations, and they rejected the hypothesis that *S. albicosta* subpopulations in the east are subject to a bottleneck effect. It is possible that a genetically diverse group, perhaps from multiple locations, colonized the new territory. Like Miller et al. (2009) we conclude that as there is no evidence of a bottleneck effect; therefore, it is unlikely that *S. albicosta* evolved the ability to overcome some barrier, but rather that there was a previous barrier to population expansion that is no longer present.

Several hypotheses have been suggested to explain the expansion. There may not be a single factor responsible for the rapid increase in *S. albicosta* numbers; several recent changes in farming practices could be acting in concert. The most striking fact is that *S. albicosta* territory expansion coincided with the widespread adoption of maize expressing proteins from the bacteria Bt as well as a gene conferring glyphosate tolerance. Bt corn and glyphosate-tolerant soybeans have altered agricultural practices in the Midwest in many ways, including tillage practices (Fawcett and Towry 2003, Uri 1999, Stinner and House 1990), pesticide application (Pilcher et al. 2002), and pest composition (Dorhout and Rice 2010).

The expansion that *S. albicosta* populations have undergone in the past decade is remarkable. One of the goals of this study was to attempt to shed light on why *S. albicosta* underwent this rapid expansion. Whether this insect has evolved the ability to overcome some ecological barrier, or whether some barrier to territory expansion been recently removed is one of the more interesting questions in crop pest entomology at the moment. These data lend support to the hypothesis that the western bean cutworm is expanding because some obstacle is no longer present.

Western bean cutworm should continue to be monitored as it colonizes new territory. It will be important not only because this is an opportunity to study an insect as it undergoes habitat expansion, but also because it will be important to monitor locations for resistance to the Cry1 F protein. The potential for selection for resistance to Cry1 F exists, as Catangui and Berg (2006) showed that *S. albicosta* is not entirely controlled by this protein. *S. albicosta* in the eastern United States locations should also be monitored over time to determine whether additional genetic isolation from the western locations is occurring. For this

study, *S. albicosta* samples were collected only from corn. Future studies should examine western bean cutworm from geographic areas representing the full geographic extent of its range as well as from corn and dry beans for potential host plant strains.

It is likely that a combination of factors has played a part in the recent increase in *S. albicosta* numbers and damage levels. A reduction in the use of pesticides, recent conversion to low-till farming, and a change in the species composition in corn ears have likely all coincided in a way that benefits the survival of *S. albicosta.* When combined with genetic evidence showing no founder effect, we conclude that *S. albicosta* is responding to changes in agricultural practices rather than evolving to overcome an obstacle.

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