

Genetic structure and gene flow among European corn borer populations from the Great Plains to the Appalachians of North America

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- Abstract**
- 1 Earlier population genetic spatial analysis of European corn borer *Ostrinia nubilalis* (Hübner) indicated no genetic differentiation even between locations separated by 720 km. This result suggests either high dispersal resulting in high gene flow or that populations are not in migration–drift equilibrium subsequent to their invasion of the central U.S.A. in the 1940s.
 - 2 To discriminate among these two possibilities, samples were collected at 12 locations in eight states from New York to Colorado, a geographic scale that is three-fold greater than previously tested. Eight microsatellite markers were employed to estimate genetic differentiation and gene flow among these populations, and to test for isolation-by-distance.
 - 3 Although pairwise F_{ST} estimates were very low, there was a significant isolation-by-distance relationship.
 - 4 Wright's neighbourhood area (i.e. the surface area covered by a panmictic group of individuals within a larger continuous distribution) was calculated as 433 km², and the radius indicates that approximately 13% of *O. nubilalis* adults disperse a net distance >12 km per generation from their natal source.
 - 5 Analyses indicated significant differentiation between the north-eastern region (New York and Pennsylvania) and the region combining sample locations from Ohio to Colorado, suggesting the potential for isolation of populations by topographic barriers in the Northeast.
 - 6 Taken together, the results suggest that *O. nubilalis* exhibits substantial gene flow over long distances and that the lack of genetic differentiation between populations across hundreds of kilometres is not simply a result of migration–drift disequilibrium arising from the recent range expansion.

Keywords Dispersal, European corn borer, gene flow, genetic structure, invasive, landscape, migration, neighbourhood, *Ostrinia nubilalis* (Lepidoptera: Crambidae), population genetics.

Introduction

The European corn borer *Ostrinia nubilalis* (Hübner) was introduced at least thrice to North America from Europe in the early 20th Century (Caffrey & Worthley, 1927), and then proceeded

to invade nearly all corn producing regions of the U.S.A. and Canada east of the Rocky Mountains (Showers, 1979; Mason *et al.*, 1996). It is a chronic pest of corn that causes substantial losses on an annual basis in many areas, but it is quite difficult to control with chemical insecticides because of its ability to bore into the stalk where it is sheltered from contact sprays (Mason *et al.*, 1996; Rice & Pilcher, 1998). The introduction of transgenic *Bacillus thuringiensis* (Bt) corn in the U.S.A. in 1997 has revolutionized *O. nubilalis* management,

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providing essentially 100% protection from this pest over the entire season (Graeber *et al.*, 1999; Ma & Subedi, 2005).

The very high adoption rate of this technology in the U.S.A. (James, 2010) and the resulting high selection pressure increases the risk that resistance will evolve in an *O. nubilalis* population, followed by geographic spread of the resistance trait and loss of the yield protection provided by Bt transgenic technology (Bravo & Soberón, 2008; Tabashnik *et al.*, 2008a, b, 2009; MacIntosh, 2009; Lopez *et al.*, 2010). To slow resistance development, the U.S. Environmental Protection Agency requires growers to plant a refuge of nonBt corn serving as a nursery for susceptible moths that would mate with any resistant moths surviving and emerging from Bt plants (EPA, 2001; Ives & Andow, 2002; Bravo & Soberón, 2008; Tabashnik *et al.*, 2008a, 2009). The rate at which resistance, or any other local adaptation, may develop and spread depends critically on gene flow (Slatkin, 1987; Caprio & Tabashnik, 1992; Andow, 2002; Manel *et al.*, 2003; Sisterson *et al.*, 2004). Therefore, to improve the modelling of resistance development and to forecast the spread of Bt resistant moths so that rational control strategies may be implemented if resistance does arise, it would be useful to understand *O. nubilalis* dispersal behaviour and dispersal distances relative to the location and timing of mating and oviposition (Dalecky *et al.*, 2006a; Reardon *et al.*, 2006a; Bailey *et al.*, 2007; Tyutyunov *et al.*, 2008). Population genetics analyses are useful for examining patterns of dispersal over even great geographic scales (Kim *et al.*, 2006; Jiang *et al.*, 2007, 2010; Nagoshi *et al.*, 2009).

Ostrinia nubilalis is distributed through much of the U.S. Corn Belt as an essentially continuous population without obvious environmental barriers to adult movement. Genetic differentiation can take place within a continuous distribution based on distance alone, depending on effective dispersal distances of individuals within the population, resulting in a genetic pattern of isolation-by-distance (Wright, 1943; Slatkin, 1987). To conceptualize functional populations within a continuous distribution, Wright (1946) introduced the idea of the neighbourhood. Neighbourhood size is defined as the number of local individuals within the larger population that interact as if they are effectively in a panmictic (i.e. random mating) population. The neighbourhood area is the two-dimensional space occupied by those individuals (Wright, 1946; Slatkin & Barton, 1989; Rousset, 1997; Allendorf & Luikart, 2007). Knowing the neighbourhood area of *O. nubilalis* within the larger continuous distribution is important for determining the most efficient sampling strategy to monitor development of resistance to Bt corn (Ives & Andow, 2002; Kim *et al.*, 2009) because, conceptually, it is a measure of the typical distance that genes move per generation.

Kim *et al.* (2009) examined genetic variation at eight microsatellite loci to estimate gene flow along 720-km transects in the central Corn Belt of the U.S.A., although they found that, even at these long distances, populations were undifferentiated, and there was no significant isolation-by-distance pattern. Typically, a lack of genetic differentiation is caused by high gene flow over the spatial dimensions tested. In the case of *O. nubilalis*, however, it might also be caused by a lack of genetic drift–migration equilibrium among populations

resulting from its westward range expansion across the U.S.A. several decades ago (Slatkin, 1987; Hutchison & Templeton, 1999). Temporal analyses (Wang & Whitlock, 2003) in four locations in Iowa suggested that high gene flow may best explain the observed lack of spatial differentiation (Kim *et al.*, 2009). It is important to conclude which of these two alternatives is valid because, if gene flow is as high as suggested by the previous data, it has important implications for predicting the rate of geographic spread of resistance. High gene flow also limits options and time-frames for effective mitigation measures once resistance is detected, and an awareness of these limits is essential for realistic planning. Conversely, if the observed spatial homogeneity of genetic variation is a remnant signature of the North American invasion event, then reliable spatial estimates of gene flow will be difficult to obtain at this stage.

In the present study, we sampled and genotyped *O. nubilalis* at a geographic scale (2179 km, maximum), which is approximately three-fold greater than in the previous study. Our premise is that if genetic differentiation and isolation-by-distance patterns are not observed at such a large scale, it is probably because there has been insufficient time subsequent to the invasion of the continent for distant populations to have accumulated genetic differences via drift (Slatkin, 1987, 1993; Wang & Whitlock, 2003). On the other hand, genetic differentiation and a pattern of isolation-by-distance at this larger spatial scale would support the idea that high gene flow is responsible for the observed lack of genetic structure at smaller scales rather than insufficient time for genetic drift to generate differences (Hutchison & Templeton, 1999; Wang & Whitlock, 2003). Finding an appropriate geographic scale over which isolation-by-distance can be detected (Hellberg, 2009) is important for estimating neighbourhood size, and hence the neighbourhood area and dispersal distance. In the present study, we calculated the neighbourhood area of *O. nubilalis* based on the observed isolation-by-distance relationship reported across much of its range in the U.S.A. from Colorado to New York.

Materials and methods

Geographic samples of *O. nubilalis*

Adult male European corn borers were sampled at 12 sites from Colorado to New York (Fig. 1). One of the locations, near Arcadia, Iowa (AIA), was sampled during the second flight in 2006 as part of a previous study (Kim *et al.*, 2009). The collection from Oaks Corners, New York (OCNY), was made in 2008. All other samples were collected during the second flight in 2007. Temporal analysis in single locations indicated very little genetic change over two generations (Kim *et al.*, 2009), and thus the two populations sampled in different years can be used for the spatial analysis in the present study. Samples from Illinois, Indiana and Ohio were collected from grassy adult aggregation sites (Showers *et al.*, 1976; Sappington & Showers, 1983) with a sweep net. All other locations were sampled with cone-style traps (Reardon *et al.*, 2006b; Kim *et al.*, 2009), each baited with a Z-race pheromone lure (Trécé Inc., Adair, Oklahoma).



Figure 1 Geographic locations of *Ostrinia nubilalis* adult samples. SCO, Sterling, Colorado; YCO, Yuma, Colorado; NPNE, North Platte, Nebraska; AIA, Arcadia, Iowa; MIL, Monmouth, Illinois; WIL, Waterloo, Illinois; WIN, Wanatah, Indiana; FIN, Frankfort, Indiana; CCIN, Columbia City, Indiana; BOH, Bowersville, Ohio; RPA, Rockspring, Pennsylvania; OCNY, Oaks Corners, New York.

Genotyping

Genomic DNA from individual moths was extracted using the Puregene Core Kit (Qiagen, Valencia, California) in accordance with the manufacturer's instructions. Eight European corn borer microsatellite loci, On-T2, On-T3 and On-T4 from Kim *et al.* (2008), and D63, D65, D145, D243 and T81 from Dalecky *et al.* (2006b), were selected to infer the population genetic structure based on low frequencies of null alleles and deviation from Hardy–Weinberg equilibrium (HWE), adequate polymorphism, and ease of scoring and multiplexing, as previously described by Kim *et al.* (2009). The microsatellites were amplified by polymerase chain reaction (PCR) in two separate multiplex reactions for each insect: multiplex 1: D63, D65, D145, D243 and T81; multiplex 2: On-T2, On-T3 and On-T4. The loci were amplified from 57 to 60 individuals per population (except OCNY, where $n = 33$) using the Qiagen Multiplex Kit in accordance with the protocol previously described by Dalecky *et al.* (2006a). The PCR fragments were analyzed by capillary gel electrophoresis and genotypes were determined as previously described by Kim *et al.* (2009). Ambiguous genotypes were determined by visual inspection. When necessary (approximately 5% of moths), individual loci were re-amplified with single primer pairs and re-analyzed to clarify ambiguous genotypes.

Statistical analysis

Within-population genetic diversity was gauged by allelic diversity per locus per population, observed heterozygosity

(H_o) and unbiased estimates of expected heterozygosity (H_E) (Nei, 1987) under Hardy–Weinberg assumptions using the Microsatellite Toolkit (Park, 2001) and FSTAT, version 2.9.3 (Goudet, 1995). F -statistics (Weir & Cockerham, 1984) and R_{ST} (Goodman, 1997) for each locus, as well as pairwise F_{ST} estimates, were calculated by FSTAT, version 2.9.3 (Goudet, 1995); significance values were calculated using a permutation approach implemented in the same software. A significant difference between observed and expected heterozygosity results in a significant F_{IS} value, and may indicate the presence of null alleles, the Wahlund effect or some other anomaly. F_{ST} estimates are potentially in the range 0–1, and are a measure of how genetically different two populations are at selectively neutral loci, with an F_{ST} of 0 indicating that no genetic differentiation has occurred, and a value of 1 indicating that the two populations share no genotypes in common.

Linkage disequilibrium between locus pairs, and deviation from HWE for each locus and population were checked by the exact probability test approach (Guo & Thompson, 1992) using GENEPOP, version 4.0.6 (Raymond & Rousset, 1995). The sequential Bonferroni correction was applied when determining significance in multiple comparisons (Rice, 1989). Excessive deviation from HWE would indicate violation of the assumptions of population genetics analyses such as nonrandom mating or a lack of selective neutrality.

The possible presence of null alleles was assessed using MICRO-CHECKER (Van Oosterhout *et al.*, 2004). Null alleles are caused by mutations in the flanking region of a microsatellite locus, resulting in lack of binding by one of the PCR primers and a consequent lack of amplification of the locus. They are

a problem because non-amplification of one allele in a heterozygote results in only one allele being detected and a false inference that the individual is a homozygote for the allele that did amplify. Null alleles are especially frequent in Lepidoptera (Megléc *et al.*, 2004; Zhang, 2004). Because most of the loci and populations appeared to possess a low frequency of null alleles (see Results), all pairwise F_{ST} were corrected by the ENA (excluding null alleles) procedure of Chapuis and Estoup (2007) using the FREENA software package (<http://www1.montpellier.inra.fr/URLB/>). All values of F_{ST} reported in the present study are ENA-corrected values, except where noted.

The software GENALEX, version 6.1 (Peakall & Smouse, 2006) was used to carry out a principal coordinate (PCo) analysis using the ENA-corrected pairwise F_{ST} estimates among *O. nubilalis* samples and a covariance matrix. A scatter diagram was plotted based on factor scores along the two PCo axes accounting for the most variation. Such a diagram facilitates visualization of geometric relationships among *O. nubilalis* samples.

Isolation-by-distance (Wright, 1943) was inferred from the regression of $F_{ST}/(1 - F_{ST})$ on geographic distance between all pairs of sample locations. Because the sampling scheme was closer to one-dimensional (i.e. linear) than two-dimensional (Fig. 1), untransformed distance (km) was used for regression rather than the logarithm of distance, as recommended by Rousset (1997). The relationship between matrices for two variables, and the probability that there is no relationship based on 9999 permutations of samples, were calculated using the Matrix Comparison option in NTSYSPC, version 1.70 (Rohlf, 1992).

On the basis of the results of the PCo analysis, the 12 locations we sampled were divided into two regions: (i) north-eastern, consisting of Rocksring, Pennsylvania (RPA) and Oaks Corners, New York (OCNY), and (ii) central-western, consisting of the remaining ten sites from Ohio to Colorado. Analysis of molecular variance (AMOVA) for the hierarchical partitioning of genetic variation among populations and regions, and calculation of regional F -statistics (F_{RT} , F_{SR} , F_{ST} , F_{IS} and F_{IT}) and their significance using the permutation approach, were carried out using the programme GENALEX (Peakall & Smouse, 2006). An AMOVA provides an estimate of the proportion of genetic diversity within and between populations, or among groups of populations that are categorized based on criteria such as region.

Neighbourhood size is given by $4\pi D\sigma^2$, where D is population density and σ^2 is the one-way variance in parent–offspring dispersal distance (Wright, 1946; Slatkin & Barton, 1989; Rousset, 1997; Sumner *et al.*, 2001; Manel *et al.*, 2003). This value ($4\pi D\sigma^2$) can be estimated from an isolation-by-distance relationship as the inverse of the slope of pairwise genetic differences [expressed as $F_{ST}/(1 - F_{ST})$] regressed on pairwise geographic distances (Slatkin & Barton, 1989; Rousset, 1997, 2000; Cabe *et al.*, 2007). Neighbourhood area is given by $4\pi\sigma^2$ and represents the area of a circle anywhere within which the parents of a zygote could have been drawn at random from a panmictic population (Endler, 1979; Allendorf & Luikart, 2007; Ishida, 2009). Thus, neighbourhood area reflects variation in net dispersal distances of individuals from their natal source (King & Murtaugh, 1997). Wright's neighbourhood area circle

contains 86.5% of individuals dispersing from its centre per generation, and its radius is 2σ (Wright, 1946; Boshier *et al.*, 1995; King & Murtaugh, 1997; Kitamura *et al.*, 2008). Therefore, the radius, which represents a measure of gene flow, can be calculated from an estimate of $4\pi D\sigma^2$ if an independent demographic estimate of D can be obtained (Rousset, 1997; Sumner *et al.*, 2001).

Density of *O. nubilalis* adults per km² was calculated from flush sample data for the first flight of 2004 in Marshall County, Iowa, as reported in table 3 of Sappington (2005). The data were collected from grassy roadside ditches, where moths preferentially aggregate during the day (Showers *et al.*, 1976; Sappington & Showers, 1983), and were segregated by the presence of corn on one, both, or neither side of the road. To obtain an estimate of the mean density of moths, the data for these categories were adjusted for the proportion of farmland acres planted to corn (0.427) in Marshall County (http://www.city-data.com/county/Marshall_County-IA.html) and summed, yielding an estimate of 37 moths per 100 m of roadside ditch (assuming typical 3-m wide ditches). In much of rural Iowa, farmland is laid out in 1-mile² (1.609-km²) grid-like sections, with roads (and accompanying ditches) on each of the four sides. Under the simplifying assumption that the density of adult *O. nubilalis* is represented adequately by those found in the grass around these sections, we calculated a mean of 919 adults per km². Some moths aggregate in grass in fencerows and waterways within the sections, and a small proportion may remain in the cornfield itself as well, so that any calculations made under this assumption will tend to underestimate the true number of total moths per km². On the other hand, not all parts of all ditches are equally suitable for adult habitation. Although sample locations were chosen without previous information being available on moth abundance, stretches of ditch with a habitat clearly unsuitable for adults were avoided (Sappington, 2005). Thus, the number of moths per linear km of ditch observed in the flush samples probably overestimates the true density along the entire length of the ditches. Given these caveats, the estimate of density in the present study is imprecise, although probably not unreasonable.

Results

Allele frequency and within-population diversity

A total of 86 alleles across eight microsatellite loci were observed for 682 *O. nubilalis* individuals from the 12 locations sampled (Table 1). The number of alleles per locus ranged from 7 in On-T2 to 18 in T81 (mean 10.8). Fourteen of 86 alleles were unique to a single location. Most of the unique alleles were observed in north-eastern sites: seven in Rocksring, Pennsylvania (RPA) and four in Oaks Corners, New York (OCNY). However, all unique alleles occurred at very low frequencies (<0.025), except one On-T2 allele found only in Oaks Corners, New York (OCNY) that was sampled at a frequency of 0.061. There was no evidence of linkage disequilibrium between any loci within or across populations (data not shown).

Mean frequencies of null alleles for each locus ranged from 0.000 for D243 to 0.109 for On-T4 (Table 1). Reduction in observed heterozygosity from the expected heterozygosity

Table 1 Characteristics of each microsatellite locus for 12 geographic *O. nubilalis* samples from the U.S.A., including total number of alleles, mean estimated frequency of null alleles and estimates of F -statistics (F_{IS} , F_{IT} and F_{ST}) and R_{ST}

Locus	Number of total alleles	Mean $\pm$ SD estimated frequency of null alleles ^a	F_{IS}^b	F_{IT}^b	F_{ST}^c	R_{ST}
On-T2	7	0.084 $\pm$ 0.080	0.218***	0.222***	0.004*	-0.001
On-T3	10	0.063 $\pm$ 0.026	0.157***	0.157***	0.000 ^{NS}	-0.003
On-T4	10	0.109 $\pm$ 0.049	0.273***	0.277***	0.005***	-0.003
D63	11	0.023 $\pm$ 0.022	0.013**	0.062**	0.007*	0.000
D145	9	0.026 $\pm$ 0.020	0.067*	0.067**	0.000 ^{NS}	-0.005
D65	13	0.042 $\pm$ 0.032	0.112***	0.112***	0.000 ^{NS}	0.007
D243	8	0.000 $\pm$ 0.000	-0.051 ^{NS}	-0.052 ^{NS}	-0.000 ^{NS}	-0.000
T81	18	0.068 $\pm$ 0.038	0.170***	0.173***	0.004*	0.002
All loci	86	—	0.141***	0.143***	0.003***	0.001

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

^aNull allele frequency for each locus was estimated for each of the 12 samples using the EM algorithm (Dempster *et al.*, 1977) and then averaged.

^bAlleles randomized within samples, and testing for Hardy–Weinberg equilibrium within samples. Based on 999 randomizations.

^cTesting for population differentiation under assumption of random mating within samples. The statistic used is the exact G -test. (Goudet *et al.*, 1996).

under HWE, indicated by the F_{IS} estimate, was significant for all but one locus, and ranged from -0.051 in D243 to 0.273 in On-T4. Null alleles were likely present at more than one locus in all *O. nubilalis* samples except Oaks Corners, New York

(OCNY), and all samples deviating from HWE were estimated to have had at least one locus containing a null allele (Table 2). The On-T4 locus appeared to have null alleles present in ten of 12 populations, whereas there was no evidence for null

Table 2 Characteristics of geographic populations of *Ostrinia nubilalis*: sample size (N), allelic diversity (mean number of alleles per locus), observed (H_O) and expected (H_E) heterozygosity, and F_{IS} per sample over all loci

Location	Geographical positioning system coordinates	N	Allelic diversity	H_O	H_E	F_{IS}^a	Number (identity) of loci deviating from HWE	P^b	Number (identity) of loci with null allele ^c
Sterling, Colorado	N40 40.222 W103 09.518	59	6.4	0.485	0.587	0.175*	1 (On-T4)	0.0000	3 (On-T4, D65, T81)
Yuma, Colorado	N40 13.177 W102 42.294	60	6.4	0.529	0.585	0.096 ^{NS}	2 (On-T4, T81)	0.0000	2 (On-T4, T81)
North Platte, Nebraska	N41 05.378 W100 43.490	57	6.1	0.479	0.574	0.166*	2 (On-T3, On-T4)	0.0000	3 (On-T3, On-T4, T81)
Arcadia, Iowa	N42 02.860 W95 04.366	60	6.1	0.526	0.609	0.136*	2 (On-T4, T81)	0.0000	3 (On-T3, On-T4, T81)
Monmouth, Illinois	N40 52.430 W90 37.944	60	6.0	0.540	0.584	0.077 ^{NS}	1 (T81)	0.0075	1 (T81)
Waterloo, Illinois	N38 14.696 W90 02.171	60	6.5	0.535	0.614	0.129*	3 (On-T2, On-T4, T81)	0.0000	2 (On-T2, On-T4)
Wanatah, Indiana	N41 23.159 W86 54.079	59	6.9	0.494	0.584	0.156*	3 (On-T2, On-T4, D65)	0.0000	5 (On-T2, On-T3, On-T4, D65, T81)
Frankfort, Indiana	N40 14.814 W86 32.607	58	6.2	0.502	0.591	0.151*	1 (On-T3)	0.0019	4 (On-T2, On-T3, On-T4, D65)
Columbia City, Indiana	N41 04.990 W85 23.637	58	6.2	0.484	0.596	0.189*	3 (On-T2, On-T4, T81)	0.0000	5 (On-T2, On-T3, On-T4, D65, T81)
Bowersville, Ohio	N39 38.735 W83 49.946	59	6.2	0.530	0.610	0.133*	3 (On-T2, D63, D65)	0.0000	3 (On-T2, On-T4, D63)
Rockspring, Pennsylvania	N40 42.196 W77 59.036	59	7.5	0.536	0.648	0.174*	3 (On-T2, On-T4, D65)	0.0000	3 (On-T4, D65, T81)
Oaks Corners, New York	N42 0.934 W77 0.011	33	7.0	0.578	0.628	0.082 ^{NS}	0	0.4335	0

^aSignificance level for F_{IS} within samples based on 1920 randomizations. Indicative adjusted nominal level (5%, marked with an asterisk if significant at this level) for multiple testing is 0.00052.

^bProbability that sample-wide deviation from Hardy–Weinberg equilibrium (HWE) is by chance alone, based on Fisher's method.

^cBased on MICRO-CHECKER analysis (Van Oosterhout *et al.*, 2004).

alleles at the D243 locus in any of the samples. Exact tests for deviations from HWE across all loci revealed that ten of 12 samples were significantly out of equilibrium after correction for multiple testing. All deviations from HWE were in the direction of deficiency of heterozygotes (Table 2), as expected if the deviations are caused by one or more null alleles. Such consistent heterozygote deficiency is striking, although a high frequency of null alleles plague microsatellites in Lepidoptera (Megl  cz *et al.*, 2004; Zhang, 2004; Van't Hof *et al.*, 2007) and has been previously observed in *O. nubilalis* as well (Dalecky *et al.*, 2006b; Kim *et al.*, 2008), making development of suitable markers for population genetics studies difficult (Coates *et al.*, 2005). Analyses of families from laboratory crosses of moths originating in central Iowa have confirmed the presence of null alleles for at least On-T2, On-T3, On-T4, D63 and D65 (K. S. Kim and T. W. Sappington, unpublished data).

Allelic diversity, H_E and H_O all indicate high levels of genetic diversity across all populations (Table 2). Allelic diversity ranged from 6.0 in Monmouth, Illinois (MIL) to 7.5 in Rocksring, Pennsylvania (RPA), averaging 6.5. H_E ranged from 0.574 in North Platte, Nebraska (NPNE) to 0.648 in Rocksring, Pennsylvania (RPA), averaging 0.601. There were no significant differences in genetic diversity across locations (KW statistic = 3.589, P = 0.980 for allelic diversity; KW statistic = 3.001, P = 0.9981 for H_E).

Genetic structure within and among *O. nubilalis* samples

The global estimate of F_{ST} across all loci and all populations was very low but significant (Table 1) (uncorrected F_{ST} = 0.003; ENA-corrected F_{ST} for null alleles = 0.004). Corrected pairwise F_{ST} estimates across all loci among the 12 *O. nubilalis* samples ranged from -0.004 to 0.012 (Table 3). Permutation tests indicated that allelic differentiation was significant in 19 of the 66 pairwise comparisons (Table 3). Most of the significant pairwise differentiation involved populations at the extreme eastern [Oaks Corners, New York (OCNY) and Rocksring,

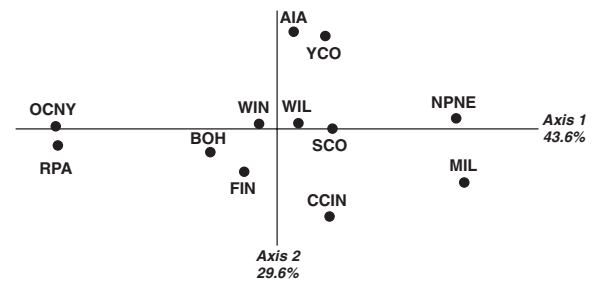


Figure 2 Scatter diagram of factor scores from a principal coordinate analysis of genotype data from eight microsatellite loci across samples of *Ostrinia nubilalis* from 12 locations (Fig. 1). The percentage of total variation attributed to each axis is indicated. SCO, Sterling, Colorado; YCO, Yuma, Colorado; NPNE, North Platte, Nebraska; AIA, Arcadia, Iowa; MIL, Monmouth, Illinois; WIL, Waterloo, Illinois; WIN, Wanatah, Indiana; FIN, Frankfort, Indiana; CCIN, Columbia City, Indiana; BOH, Bowersville, Ohio; RPA, Rocksring, Pennsylvania; OCNY, Oaks Corners, New York.

Pennsylvania (RPA)] and western [Yuma, Colorado (YCO)] sample locations. Surprisingly, a different Colorado location, Sterling (SCO), showed no differentiation from any other location, whereas the centrally-located Monmouth, Illinois (MIL) population was significantly differentiated from a number of populations including relatively nearby samples from Arcadia, Iowa (AIA) and Franklin, Indiana (FIN).

Relationships among *O. nubilalis* samples based on microsatellite genotype data were visualized by PCo analysis (Fig. 2). The first two axes together accounted for 73.2% of the variation. Factor scores along axis 1 clearly highlight the relative genetic distance of the north-eastern [Rocksring, Pennsylvania (RPA) and Oaks Corners, New York (OCNY)] populations from the central and western samples. The Monmouth, Illinois (MIL) and North Platte, Nebraska (NPNE) samples stood out at the other extreme of axis 1.

Table 3 Corrected F_{ST} estimates (below diagonal) and significance of permutation tests for allelic differentiation (above diagonal) across eight microsatellite loci in pairwise comparisons of the *Ostrinia nubilalis* samples from 12 locations of the U.S.A.

	SCO	YCO	NPNE	AIA	MIL	WIL	WIN	FIN	CCIN	BOH	RPA	OCNY
SCO	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
YCO	0.003	—	NS	NS	*	*	NS	**	*	NS	**	*
NPNE	-0.003	0.002	—	NS	NS	NS	NS	*	NS	NS	***	**
AIA	0.002	0.003	0.003	—	**	NS	NS	NS	**	NS	**	*
MIL	0.001	0.008	-0.002	0.010	—	NS	NS	*	NS	NS	***	**
WIL	0.002	0.007	0.002	0.004	0.006	—	NS	NS	NS	NS	*	NS
WIN	-0.001	0.005	0.000	0.004	0.004	-0.001	—	NS	NS	NS	*	NS
FIN	0.000	0.011	0.005	0.004	0.006	0.002	0.001	—	NS	NS	NS	NS
CCIN	0.001	0.009	0.001	0.011	0.001	0.004	0.004	0.001	—	NS	NS	*
BOH	0.000	0.003	0.002	0.006	0.004	0.002	0.000	0.001	-0.001	—	NS	NS
RPA	0.003	0.009	0.011	0.007	0.012	0.007	0.005	0.004	0.005	-0.001	—	NS
OCNY	0.004	0.007	0.010	0.008	0.012	0.005	0.000	0.004	0.007	-0.001	-0.004	—

* p < 0.05, ** p < 0.01, *** p < 0.001. Probability values are based on 999 permutations.

NS, not significant.

SCO, Sterling, Colorado; YCO, Yuma, Colorado; NPNE, North Platte, Nebraska; AIA, Arcadia, Iowa; MIL, Monmouth, Illinois; WIL, Waterloo, Illinois; WIN, Wanatah, Indiana; FIN, Frankfort, Indiana; CCIN, Columbia City, Indiana; BOH, Bowersville, Ohio; RPA, Rocksring, Pennsylvania; OCNY, Oaks Corners, New York.

Although axis 2 accounted for almost 30% of the total variation in the data, the relative positions of the *O. nubilalis* samples along that axis did not show any obvious clustering or directionality related to geography.

There is a significant genetic isolation by geographic distance relationship over all samples ($y = 0.00159 + 0.000002515x$; $P = 0.002$; $r^2 = 0.136$). The isolation-by-distance relationship disappears ($r^2 = 0.0282$, $P = 0.110$) when both Pennsylvania and New York are excluded based on their clustering in the PCo analysis (Fig. 2), suggesting that differences between the eastern *O. nubilalis* samples and others may drive the isolation-by-distance relationship. However, when the Sterling, Colorado (SCO) population near the western extreme of the geographic range sampled also is excluded, based on its surprising lack of any significant pairwise F_{ST} estimates [compared with nearby Yuma, Colorado (YCO)] (Table 3), the isolation-by-distance relationship was significant with a slope similar to that when all locations are included ($y = 0.00204 + 0.000002510x$; $P = 0.044$; $r^2 = 0.108$).

AMOVA analysis among the 12 *O. nubilalis* samples revealed that most of the genetic variation was partitioned to within- and among individuals (Table 4). Genetic differences were examined between the north-eastern region [Rockspring, Pennsylvania (RPA) and Oaks Corners, New York (OCNY)] and the region combining all locations from Ohio westward. The difference was low but significant ($F_{RT} = 0.006$) and was six-fold higher than that among populations within region ($F_{SR} = 0.001$), supporting the relative isolation of *O. nubilalis* populations between the north-eastern and the central-western regions, which are separated by the Appalachian Mountains.

Neighbourhood size for *O. nubilalis* ($4\pi D\sigma^2$) was calculated as the inverse of the isolation-by-distance regression slope: $1/0.000002515 \approx 400\,000$. Given the density (D) estimate of 919 adults/km², variance in dispersal distance (σ^2) was calculated as approximately 34, and neighbourhood area ($4\pi\sigma^2$) as approximately 430 km². The radius of the neighbourhood area (2σ) is thus approximately 12 km. This means that after dispersal, approximately 87% of adults are located within 12 km of the egg mass from which they originated. Conversely, it means that approximately 13% of *O. nubilalis* adults disperse a net distance >12 km from their natal site per generation.

Discussion

In the present study, we found low, but significant, genetic differentiation between *O. nubilalis* populations from a number of locations across the northern U.S.A. We also found a significant isolation-by-distance relationship across the locations when examined at the level of population means. This relationship is weak but, together with the results of temporal analyses (Kim *et al.*, 2009), we tentatively conclude that the lack of genetic differentiation between locations along the full length of two 720-km transects in the central Corn Belt in our earlier study is largely a result of high gene flow in the geographic region studied rather than to invasion history.

Other studies reporting low genetic differentiation among *O. nubilalis* populations in the Great Plains of the U.S.A. (Krumm *et al.*, 2008) and in France (Bourguet *et al.*, 2000a; Martel

et al., 2003; Leniaud *et al.*, 2006; Malausa *et al.*, 2007) are consistent with our conclusion of high gene flow over long distances. Furthermore, the significant isolation-by-distance relationship that we observed allowed us to calculate neighbourhood size and neighbourhood area for *O. nubilalis*. The estimates are of course approximate but provide an indication of the scale at which dispersal and gene flow are occurring in this species. The estimate of neighbourhood area is large, being almost one-third the mean size of an Iowa county. The neighbourhood radius of 12 km represents the net displacement of only approximately 87% of adults, so that 13% disperse >12 km per generation. The ability to disperse such distances is consistent with observations from mark-recapture experiments where dispersal to 49 km was demonstrated (Showers *et al.*, 2001), as well as with species and trait range expansion data (Caffrey & Worthley, 1927; Chiang, 1972; Showers *et al.*, 1995), water crossings (Caffrey & Worthley, 1927; Bretherton & Chalmers-Hunt, 1989; Langmaid & Young, 2006), and flight mill studies (Dorhout *et al.*, 2008).

Estimates of *O. nubilalis* effective population size from temporal analyses of genetic variation were large (Kim *et al.*, 2009). Similarly, our estimate of the number of individuals comprising a panmictic breeding group (approximately 400 000), or neighbourhood size, is quite large, such that the rate of differentiation through genetic drift is expected to be slow. Our finding of significant isolation-by-distance despite the genetic homogenization that may have followed in the wake of the range expansion across the northern U.S.A., which ended 30–40 years ago, suggests that drift has had time to create genetic differences and that gene flow is restricted over the large geographic scale tested. Nevertheless, the low slope of the isolation-by-distance regression line, in combination with several other lines of evidence noted above, suggests that gene flow in *O. nubilalis* commonly occurs over great distances. However, the sampled landscape in the Midwest Corn Belt is relatively uniform and without major topographical features. It is possible that the significant isolation-by-distance pattern we observed is the result of greater genetic isolation of the two eastern populations where the landscape is not so uniform and where there may be more opportunity for genetic drift to affect neutral variation. The Appalachian Mountains are a significant landscape barrier to gene flow and dispersal for many faunal groups, including insects (Schultheis *et al.*, 2002; Heilveil & Berlocher, 2006).

There are some anomalies in the results obtained in the present study emphasizing the complexity of gene flow and dispersal in *O. nubilalis*. For example, closer examination of the Monmouth, Illinois (MIL) population revealed significant differentiation from a number of other populations relatively nearby (354 and 396 km), whereas it was not differentiated from other populations much further away geographically (848 and 1056 km). Not surprisingly, the Yuma, Colorado (YCO) population near the western extreme of the range sampled was genetically differentiated from several other populations. Oddly, however, the nearby (64 km) Sterling, Colorado (SCO) population was not significantly differentiated from any other population, including the most distant locations in the North-east (>2100 km). The reasons for such anomalies are unclear.

Table 4 Analysis of molecular variance among *Ostrinia nubilalis* samples from 12 locations in the U.S.A.

Source of variation	d.f.	Sums of squares	Mean sums of squares	Estimated variance	%
Among regions	1	7.854	7.854	0.015	0.62%
Among populations	10	31.424	3.142	0.003	0.14%
Among individuals	670	1843.434	2.751	0.345	14.22%
Within individuals	682	1406.000	2.062	2.062	85.02%
Total	1363	3288.711	—	2.425	100%
<i>F</i> -statistics	Value	<i>P</i> (random $\geq$ data)	—	—	—
<i>F</i> _{RT}	0.006	0.001	—	—	—
<i>F</i> _{SR}	0.001	0.073	—	—	—
<i>F</i> _{ST}	0.008	0.001	—	—	—
<i>F</i> _{IS}	0.143	0.001	—	—	—
<i>F</i> _{IT}	0.150	0.001	—	—	—

Probability estimation is based on 999 permutations across the full data set.

For comparison among regions, Rockspring, Pennsylvania, and Oaks Corners, New York, together were considered as a single region and the remaining ten locations were grouped as a single region.

Cornfields in Colorado and western Nebraska are usually irrigated and can be relatively isolated. Thus, facultative ranging in search of suitable oviposition and adult aggregation sites may result in differences in distance and patterns of dispersal compared with regions further east where rainfall and the percentage of corn in the landscape are greater. Theoretically, human-mediated transport can provide a means of dispersal, although, currently, there is no obvious mechanism for large-scale long-distance transport of *O. nubilalis* by normal human agricultural practices. Although transport in harvested seed corn ears may be possible (Prasifka *et al.*, 2006), survival in harvested commercial grain is effectively nil (Wilde, 1976). Regardless, the environments of the Yuma, Colorado (YCO) and Sterling, Colorado (SCO) locations do not differ in any noticeable way.

Population genetics studies of *O. nubilalis* in France, where post-invasion disequilibrium is not an issue, have revealed a strikingly similar pattern. For example, in the microsatellite-based population genetics study performed by Malausa *et al.* (2007), one sampled locale was significantly differentiated from populations only 12, 18 and 22 km away, and yet was not differentiated from populations 480 and 509 km away. As previously hypothesized (Kim, *et al.*, 2009), landscape barriers to *O. nubilalis* movement may differentially isolate some populations in a way that generates patterns unrelated to simple isolation-by-distance, a phenomenon referred to as 'chaotic genetic patchiness' (Johnson & Black 2006; Selkoe *et al.*, 2010). Although *O. nubilalis* adults, both mated and unmated, make local flights for many reasons throughout their life (Dorhout *et al.*, 2008), most long-range displacement of *O. nubilalis* probably occurs the first night after emergence, when at least a certain proportion of unmated females (but not males) engage in a true migratory flight (Reardon *et al.*, 2006a; Dorhout *et al.*, 2008). Direction and distance during *O. nubilalis* long-duration flight is almost certainly affected by wind (Caffrey & Worthley, 1927; Mikkola, 1986; Showers *et al.*, 1995, 2001), and local differences in wind patterns may affect patterns of gene flow and contribute to genetic patchiness.

In conclusion, the results obtained in the present study are consistent with a pattern of high gene flow in *O. nubilalis* in the absence of topographic barriers (e.g. much of the Corn Belt) and more moderate gene flow where topographic barriers exist

(north-eastern U.S.A.). These patterns have implications for resistance management for *O. nubilalis* in Bt corn. Restricted gene flow can promote evolution of resistance in isolated populations but results in slow expansion of the trait throughout the species range. High gene flow, on the other hand, can inhibit evolution of resistance by swamping out resistance alleles, although it allows rapid expansion of the resistance trait once established (Peck *et al.*, 1999; Ives & Andow, 2002; Morjan & Rieseberg, 2004). Although pooling samples from widely separated locations in the Corn Belt to increase the power of *F*₂ screens to detect resistance alleles (Bourguet *et al.*, 2003; Stodola *et al.*, 2006) appears justified given the high gene flow in this part of the country (Kim *et al.*, 2009), the potential for restricted gene flow in the north-eastern U.S.A. revealed in the present study suggests that more intensive monitoring may be needed. To our knowledge, no resistance monitoring at all is currently conducted in the north-east because of the relatively low percentage of hectareage planted to corn, although this may be the area at highest risk of resistance development. We have begun studies in the Northeast aiming to systematically examine the effects of its fragmented agricultural landscapes and topographic barriers on gene flow in *O. nubilalis*, which will permit modelling the risk of resistance evolution specific to this region.

Acknowledgements

We thank M. E. O'Rourke and S. J. Fleischer for providing samples from New York and Pennsylvania, and R. Ritland for organizing and conducting the collection of moths in Colorado, Nebraska and Iowa. We thank J. Gibson, L. Fraser and M. Minner for technical assistance in the laboratory, as well as N. Miller for insightful comments and suggestions during the course of the study. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). USDA is an equal opportunity provider and employer.

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Accepted 27 February 2011