

## ORIGINAL ARTICLE

# Genetic analysis of cabbage loopers, *Trichoplusia ni* (Lepidoptera: Noctuidae), a seasonal migrant in western North America

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amplified fragment length polymorphism, gene flow, genetic structure, long-range migration, mitochondrial DNA, population structure.

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**Abstract**

Long-range migrations of many wind-borne noctuid moths will have been influenced by the expansion of agriculture that provides greater availability of food plants along the migratory route. The migratory, agricultural pest, *Trichoplusia ni* (cabbage looper) over-winters in southern California and each summer migrates as far north as British Columbia. We explored the degree of genetic connectivity of populations over this migratory range. Preliminary investigation of seven mitochondrial gene regions found little to no variation among 13 populations, while partial regions of the NADH dehydrogenase subunits 1 and 4 in 42 individuals revealed eight and six haplotypes, respectively. The pattern of haplotype distribution indicated genetic homogeneity of persistent populations in California but weak differentiation among populations further north. Four highly variable amplified fragment length polymorphism primer combinations generated 167 polymorphic bands, with heterozygosity levels ranging from 0.250 to 0.302. Pairwise  $F_{ST}$  values and clustering analyses also showed similarity among populations in California with some differentiation among populations initiated from the annual migration. Overall, some differentiation occurs among temporary, annual migratory populations but no pattern occurs with distance from the source population. Population subdivision in British Columbia associated with greenhouses has the greatest impact on genetic differentiation.

**Introduction**

Migration has been defined as 'a relocation of the animal that is on a much greater scale, and involves movement of much longer duration, than those arising in its normal daily activities' (Dingle and Drake 2007). It has evolved independently as an adaptive strategy across a wide range of taxa and thus what we learn from studying migration in one taxon can improve our understanding in a vast number of other species (Dingle 2006).

Many insects migrate in the summer from low latitude, over-wintering areas, to northern environments (Dingle 2006). New techniques such as vertical looking entomological radars, now show that seasonally migrating moths use wind-borne migration to rapidly cover distances of up to 700 km in an 8-h period and both northern migra-

tion in the spring and southern migration in the autumn have been recorded (Chapman et al. 2010). In addition, genetic markers can be used to track populations from different source areas (Pashley et al. 1985; Pashley and Johnson 1986; Nagoshi et al. 2008; Sosa-Gómez 2004) and these contribute to an understanding of the patterns of long-distance migrations.

In western North America, cabbage looper moths, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) are an example of a species that is a major agricultural pest along its migratory route. Cabbage loopers are native to subtropical areas of North America, and are unable to survive over-winter when temperatures are 10°C or below (Toba et al. 1973; Mitchell and Chalfant 1984; Caron and Myers 2008). Thus, although resident populations are limited to areas south of 35° to 40° latitude in southern

California and Mexico, moths migrate annually to establish summer breeding populations as far north as Canada.

*Trichoplusia ni* is a polyphagous insect that feeds on many plants of economic importance, including crucifer crops. With the development of agriculture in northwestern North America in the last several hundred years, large areas of suitable food plants have become widely available during the summer for northern migrants west of the Cascade Mountains. This is an example of an insect species that has been influenced by anthropogenic plant range expansions and potentially increased gene flow among populations following agricultural development. The new opportunity for populations to mix fits the prediction of genetic homogeneity over large distances (Oliver 2006).

Although the nature of the migration is not known, observations from pheromone trapping programs along the cabbage looper migration pathway; Central Valley of California (Cahn et al. 2001), Linn County Oregon (McGrath 2008) and Langley British Columbia (Cervantes 2005), all show the first appearance of male moths in early to mid-May. This pattern suggests that a single, large migration event from over-wintering populations in southern California, Arizona and Mexico could be initiating the northern, seasonal populations at approximately the same time. If so, reasonably high genetic similarity among populations is predicted.

In Oregon and British Columbia, cabbage loopers appear to have two generations a year; one initiated by adult moths flying in late April to mid-May and another resulting from moths flying in late July and August. Few moths were captured after mid-August in Oregon (McGrath 2008) or after September in British Columbia while in some locations in Merced, California, moths were captured in October and even November, although for most locations the last peak of moth flight was in August (Cahn et al. 2001). Thus the population pattern is for immigrating moths to establish populations in large areas of newly available crops and increase in numbers over the summer. Conditions for further reproduction decline as crops are harvested and temperatures cool in the autumn.

While northern migration is easy to recognize by the occurrence of populations in areas that are too cold to support over-wintering populations, the existence of return southern migration is not easy to identify. Observations of return southern movements of cabbage loopers in the late summer and fall have been suggested from trapping data (Lingren et al. 1979, 1993; Debolt et al. 1984) but not proven. If southward movement of moths does not occur, northern movement is difficult to interpret as an evolutionary process. If it does occur, gene flow will be enhanced over the species range. Because

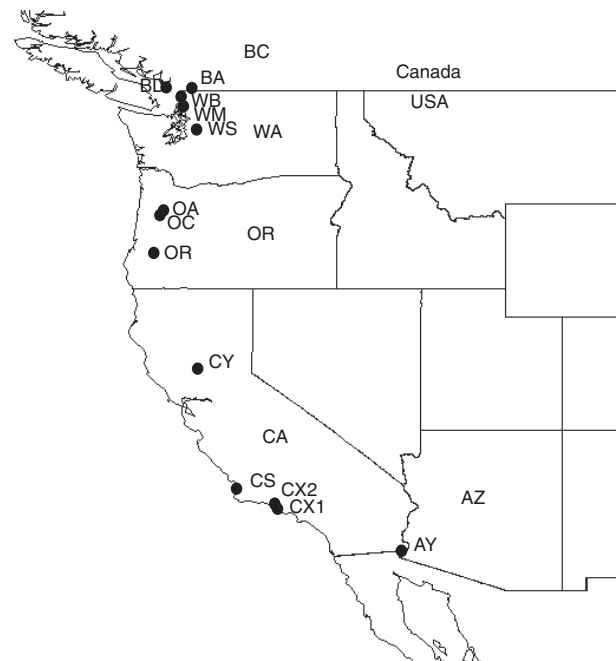
northern populations are temporary, the opportunities for directional selection are limited to one or two generations and thus genetic differentiation of southern migrants is unlikely to be strong.

To test the prediction of genetic homogeneity among temporary northern and permanent southern cabbage looper populations, we used mtDNA sequence variation in conjunction with nuclear loci, generated from the amplified fragment length polymorphism (AFLP) method to examine *T. ni* populations from California to British Columbia. We hypothesized that the annual migration of moths from California would result in genetic similarity of populations along the 1700 km migration route although random founder effects could create modest heterogeneity among different populations.

## Materials and methods

### Specimen collections

*Trichoplusia ni* larvae were collected from 13 sites along the west coast of North America, including: Arizona (AZ), California (CA), Oregon (OR), Washington (WA), and British Columbia (BC) (Fig. 1, Table 1). Sites were selected through consultation with Agriculture Extension advisors that helped to identify regions where suitable crops were grown and the optimal times for collection of



**Figure 1** Geographic distribution of sites where *Trichoplusia ni* were collected along their migration route. Sampling localities were located in British Columbia (BC), Washington (WA), Oregon (OR), California (CA), and Arizona (AZ). Locality codes are defined in Table 1.

**Table 1.** Summary of collection dates, latitude and longitude coordinates, and the crops that *T. ni* larvae were collected from.

Sample locality code	City, state/province	Collection date	Latitude (N)	Longitude (W)	Crop
<b>AY</b>	<b>Yuma, AZ</b>	10 Nov 2006*	32°42.750'	114°42.300'	Cabbage
<b>CX1</b>	<b>Oxnard field 1, CA</b>	29–30 Jun 2006	34°12.561'	119°03.403'	Mixed†
<b>CX2</b>	<b>Oxnard field 2, CA</b>	29–30 Jun 2006	34°19.803'	119°08.339'	Cabbage
<b>CS</b>	<b>Santa Maria, CA</b>	27–28 Jun 2006	34°53.550'	120°30.853'	Broccoli
CY	Yuba, CA	22 Jun 2006	39°08.617'	121°53.098'	Tomato
OR	Roseburg, OR	24 Jul 2006	43°15.494'	123°26.415'	Broccoli
OC	Corvallis, OR	27 Jul 2006	44°34.384'	123°14.209'	Broccoli
OA	Albany, OR	27 Jul 2006	44°43.865'	123°07.455'	Broccoli
WS	Seattle, WA	29 Aug 2006	47°36.923'	121°55.206'	Mixed†
WM	Mount Vernon, WA	30 Aug 2006	48°24.270'	122°26.306'	Broccoli
WB	Bellingham, WA	30 Aug 2006	48°43.495'	122°28.622'	Mixed†
BD	Delta, BC	9 Aug 2006	49°06.723'	123°02.266'	Broccoli
BA	Abbotsford, BC	14 Sep 2006	49°05.046'	122°05.805'	Rutabaga

Populations in bold are resident populations. MtDNA analysis was done for all populations and AFLP analysis for all populations except AZ, WB, WM.

\**T. ni* larvae were reared for one generation in the laboratory before shipping to UBC.

†Mixed crucifer crops.

*T. ni*. Larvae were collected between May and September 2006, with the exception of those collected from AZ. Cooperators from the University of Arizona made the collections from AZ during November 2006. *Trichoplusia ni* were collected as larvae by sampling over a wide area and those used for the AFLP analysis were reared on artificial diet for a minimum of 1 day to ensure that no plant material was present in the gut. Larvae from AZ were reared in the laboratory for one generation at the University of Arizona, Yuma Agricultural Center prior to their arrival at the University of British Columbia (UBC) (Vancouver, BC, Canada). The gut and its contents were removed from larvae according to procedures outlined in Franklin et al. (2009) to prevent DNA degradation by enzymes found in the gut and larvae were frozen at their 4th or 5th instar of development. During collections, larvae were transported in a cryogenic vapour shipping tank (MVE Vapor Shipper, Minnesota Valley Engineering Inc., New Prague, MN, USA) held constant at  $-150^{\circ}\text{C}$  until arrival at UBC where the samples are stored long-term at  $-80^{\circ}\text{C}$ .

#### DNA isolation

DNA was isolated from a total of 161 larvae collected from these localities following procedures outlined in Sambrook et al. (1989) for analysis of two regions of the mitochondrial genome and nuclear AFLP markers (Franklin et al. 2009). DNA was extracted using phenol/chloroform extraction procedures outlined in Sambrook et al. (1989) in protocol 1: isolation of DNA for mammalian cells. The concentration and quality of DNA was verified with a spectrophotometer (Ultrospec 3000, Pharmacia Biotech,

Piscataway, NJ, USA) and by viewing DNA on a 0.8% agarose gel electrophoresis in  $1\times$  Tris-borate-EDTA.

#### Mitochondrial sequencing and analysis

To identify mitochondrial regions with a suitable level of variation for our current study, preliminary analyses were performed to examine the level of variation in seven mitochondrial gene regions (Table 2). Based on preliminary analyses, we chose to focus on a 487 bp region and a 402 bp region of NADH dehydrogenase subunits 1 and 4 (NAD1, NAD4), respectively. Three individuals were sequenced from each collection locality, with the exception of six individuals from the site in Arizona, for a total of 42 individuals (Table 1). Polymerase chain reactions (PCR) for all mitochondrial gene regions were performed with 50 or 100 ng of total genomic DNA, 0.2 mM dNTP (New England Biolabs, Ipswich, MA, USA), ( $1\times$ ) PCR buffer (Agilent Technologies Canada Inc., Toronto, ON, Canada), 1 or 2 units of Paq5000 (Agilent Technologies Canada Inc.), 20 pmol each primer (Eurofins MWG Operon, Huntsville, AL, USA). The PCR conditions used for amplification were as follows:  $95^{\circ}\text{C}$  for 5 min, cycled 35 times at  $94^{\circ}\text{C}$  for 1 min,  $55^{\circ}\text{C}$  for 1 min,  $72^{\circ}\text{C}$  for 1 min,  $72^{\circ}\text{C}$  for 10 min. PCR products were purified using Wizard<sup>®</sup> PCR Preps DNA Purification System (Promega, Madison, WI, USA) and sequenced with Sequitherm Excel II DNA Sequencing Kit (Interscience, Marham, ON, Canada) on a PTC 100 Thermal Cycler (MJ Research Inc. Waltham, MA, USA) or MultiGene Gradient Thermal Cycler (LabNet, Edison, NJ, USA) with the following conditions:  $95^{\circ}\text{C}$  for 3 min, cycled 24 times at  $95^{\circ}\text{C}$  for 30 s,  $58^{\circ}\text{C}$  for 15 s,  $70^{\circ}\text{C}$  for 30 s. Products

**Table 2.** MtDNA region, primer name and sequence, size of region (bp), the number of individuals sequenced (*n*), their sampling localities, and corresponding GenBank accession numbers.

Region	Primer name	Primer (5'-3')	Size (bp)	<i>n</i>	No. variable sites	Sampling localities	GenBank Accession No.
CYTB	REVCB2H*	TGAGGACAAATATCATTTTGAGGW	500	6	1	OR, OA, WS, WB, CS	GQ183958–GQ183963
	REVCBJ*	ACTGGTCGAGCTCCAATTCATGT					
COI	COI&IIF†	GGATTCATTGTTTGAGCTC	549	2	0	OA, WS	GQ183955–GQ183956
	COIR	CATTATATGAATGTTTCAGCWGG					
TRNL2, COII	COIF	CCWGTGAACATTCATATAATG	546	3	0	CS, OA, WS	GQ184054–GQ184056
	COI&IIR†	CGCAAATTTCTGAACATTGTCC					
NAD5	NAD5F‡	CTGGAATTGCCGCTAATTATG	440	5	1	CS, OR, OA, WS, BD	GQ184048–GQ184051
	NAD5R‡	ATCTCCCTCTAATTACTC					
NAD1	NAD1F	AGGGAGTTCGATTAGTTTCAGC	487	42	7	All	GQ183964–GQ184005
	N1N12595§	GTAGCATTTTTAACTTTATTAGAACG					
NAD4	NAD4F	TTAAATATTCTCGAGAAACTCC	402	42	6	All	GQ184006–GQ184047
	N4N8727§	AAATCTTTRATTGCTTATTCWTC					

For each gene region sequenced, forward primers are listed first and reverse primers are listed second.

\*Primers from Simmons and Weller (2001).

†Primers designed from previously sequenced *T. ni* from Japan (GenBank Accession No. AB158623).

‡Primers designed from previously sequenced *T. ni* from Japan (GenBank Accession No. AB158627).

§Primers from Salvato et al. (2008).

were loaded on a 5.5% polyacrylamide gel (SequaGel XR, National Diagnostics, Distributor Diamed Lab Supplies Ltd, Mississauga, ON, Canada) and electrophoresis was performed for 10 h on a LI-COR 4200 automated sequencer (LI-COR Inc., Lincoln, NE, USA).

All mitochondrial sequences were submitted to GenBank (see Table 2). Mitochondrial sequences were scored using BaseImagIR Image Analysis Version 04.1 (LI-COR Inc.) and aligned using BioEdit Sequence Alignment Editor (Hall 1999). Individuals with haplotypes that showed unique variation were sequenced a second time to confirm the sequence variation.

Haplotype diversity (*h*) (Nei 1987) and nucleotide diversity ( $\pi$ ) (Nei 1987) were calculated for gene regions NAD1 and NAD4 and for the concatenated sequence of these two regions using DnaSP (Rozas et al. 2003). TCS version 1.21 was used to construct a haplotype network based on combined analysis of gene regions NAD1 and NAD4 (Clement et al. 2000). Analysis of molecular variance (AMOVA) was performed to test for genetic structuring among populations from AZ to BC using the  $\phi_{ST}$  statistic (Excoffier et al. 1992). Significance was assessed under the null hypothesis of no genetic structure using 999 random permutations of the data (GenALEX6; Peakall and Smouse 2006).

### AFLP genotyping and analysis

Amplified fragment length polymorphism analysis was performed on 15 individuals from each of nine localities and 14 individuals from a locality near Seattle Washington due to the low number of larvae found at this field

locality (Table 1). Other field localities were not included in AFLP analysis because larval densities were too low at these locations. Procedures followed those outlined in Franklin et al. (2009), with preamplification primers of EcoR1 + C and Mse + A. Four primer combinations listed in Table 3 were used for the final amplification.

To ensure the repeatability of our results, we took several precautions as outlined in Bonin et al. (2004). Tissue from late instar larvae (4th and 5th instar) was used because changes in DNA methylation with tissue type and age can lead to differences in AFLP banding patterns (Donini et al. 1997). Larvae were flash frozen and stored at  $-80^{\circ}\text{C}$  to ensure that DNA molecules remained intact. The quality of isolated DNA was determined by viewing it on a 0.8% agarose gel electrophoresis and spectrophotometer (Ultraspec 3000) and low quality samples were excluded from AFLP analysis.

A pilot study outlined in Franklin et al. (2009) was undertaken to select primer combinations with a suitable level of variation and to ensure the repeatability of the results. In the pilot study, negative controls were run on

**Table 3.** AFLP primer combinations and the number of scored fragments for *T. ni* populations surveyed from the west coast of North America in 2006.

Primer combination	EcoR1	Mse1	No. of scored fragments
1	Eco + CGG	Mse + ATGG	64
2	Eco + CGG	Mse + AGCT	37
3	Eco + CCG	Mse + ACCG	55
4	Eco + CGA	Mse + ACCG	65

all gels to check for contamination and replicate amplification was performed on 11 individuals from independent extractions for six of the screened primer combinations. Replicate amplifications were run side-by-side with the original amplification and visualized on a LI-COR automated sequencer (LI-COR Inc.). The repeatability of our pilot study was high (>99%) and there was no contamination of samples.

In the present study, we discarded low quality samples from scoring, ran negative controls, and performed a repeatability test on nine previously typed individuals. We used 50–700 bp IRDye 700 and 800 commercial ladder (LI-COR Inc.) to size our markers for all gels visualized on the LI-COR automated sequencer (LI-COR Inc.). Scoring procedures followed those recommended in Bonin et al. (2004), with automated scoring using SAGA 2.0 (LI-COR Inc.) in conjunction with the data checked by hand. The genotype error rate estimated by running previously typed samples side-by-side with newly amplified samples from previously extracted DNA was 2.4%.

The percentage of polymorphic loci (%P) and expected heterozygosity ( $H_e$ ) were calculated using AFLP-SURV 1.0 (Vekemans et al. 2002). Expected heterozygosity was estimated for all loci using a Bayesian approach with nonuniform prior distribution of allele frequencies and assuming Hardy-Weinberg genotypic proportions (Zhivotovskiy 1999). This approach is favoured for dominant markers because it provides reasonable estimates of the null allele frequency at each locus (Bonin et al. 2007). Pairwise  $F_{ST}$  values were calculated for all population comparisons using loci that were polymorphic at the 5% level in AFLP-SURV 1.0. Significance was tested by comparing the observed  $F_{ST}$  value to a distribution of  $F_{ST}$  values based on 1000 random permutations of individuals among existing populations (Vekemans et al. 2002). The significance level was adjusted using the Bonferroni correction to account for multiple comparisons.

A Mantel test of matrix correspondence (Mantel 1967) in GenAEx version 6.1 (Peakall and Smouse 2006) was used to test for isolation-by-distance following methods of Smouse et al. (1986), by examining the correlation between matrices of Nei's genetic distance calculated according to methods outlined in Lynch and Milligan (1994) (AFLP-SURV 1.0) and geographic distance. Geographic distances were calculated from latitude and longitude coordinates based on a modified version of the Haversine Formula implemented in GenAEx version 6.1. To test for significance 999 random permutations of the data were performed.

The model-based clustering method implemented in STRUCTURE 2.2 (Pritchard et al. 2000; Falush et al. 2007) was used to determine patterns of genetic structure. Simulations were run under the admixture ancestry

model with correlated allele frequencies. To estimate the number of clusters ( $K$ ), the following settings were used: burn-in 100 000 steps, run length of 200 000 steps, and 10 replicate simulations of each  $K$ -value ( $K = 1-10$ ). The ad hoc statistic  $\Delta K$  developed by Evanno et al. (2005) was used to identify the most probable number of clusters.

To incorporate spatial information, we used a Bayesian clustering method with a spatially explicit prior implemented in TESS 2.1 (Chen et al. 2007). AFLP loci were coded according to the method described in Evanno et al. (2005) for dominant markers. To detect the maximum number of clusters ( $K_{max}$ ) we performed an analysis under the admixture model for  $K$  from 1 to 10, using 50 replicate simulations for each  $K$ , with a burn-in of 10 000 sweeps, run-length of 50 000 sweeps, and an admixture parameter  $\alpha = 1$ . Two values of the spatial interaction parameter were examined ( $\psi = 0.7$  and 0.9), with higher values giving greater importance to spatial interactions. The deviance information criterion (DIC) was computed for each run and the mean DIC for the 10 runs with the lowest DIC was computed for each value of  $K$ , as an indicator of how well the model fit the data.  $K_{max}$  was identified from the inflexion point in a plot of the mean DIC against  $K$ -values from 1 to 7. Preliminary runs with  $K > 7$  provided a poor fit to the data and were not included in the final analysis. Results are reported based on the spatial prior  $\psi = 0.7$ , since results were similar for both values tested. CLUMPP version 1.1.1 (Jakobsson and Rosenberg 2007) was used to correct for label switching and to identify the mean cluster membership for individuals based on the 10 replicate simulations in STRUCTURE and TESS for the number of clusters identified by the  $\Delta K$  statistic and DIC values, respectively. DISTRUCT (Rosenberg 2004) was used to display the results.

## Results

### Specimen collections

Overall, there was a trend for *T. ni* to be collected later in the summer with increasing latitude, which is indicative of when larvae could be found at these collection sites (Table 1). Larval collections from AZ to BC were performed from May to November 2006. At the southernmost site (AY) larvae were not collected until November because *T. ni* are at very low densities during the summer in AZ due to the hot, dry conditions.

### Mitochondrial sequencing

Preliminary examination showed little to no variation in mitochondrial gene regions COI, COII, CYTB, and NAD5 and therefore they were not used to assess the population structure of *T. ni* (Table 2). Instead, we sequenced a total



of 889 bp of mtDNA from 42 field collected *T. ni*, of which 487 bp were from the NAD1 gene and 402 bp were from the NAD4 gene. We identified seven variable nucleotide sites in the NAD1 gene and six variable sites in the NAD4 gene, all of which resulted in synonymous changes. Nucleotide diversity ( $\pi$ ) was highest in populations from CY and WS (Table 4). Nucleotide composition was highly A/T skewed, with mean base pair frequencies for NAD1 and NAD4 of A: 0.46, C: 0.12, G: 0.09, T: 0.33 and A: 0.43, C: 0.15, G: 0.08, T: 0.33, respectively.

A total of eight haplotypes were found for the NAD1 region and six for the NAD4 region. Overall 12 haplotypes were identified from the combined analysis of NAD1 and NAD4 regions, of which eight were private haplotypes (E-L) (Table 4). All populations, with the exception of OA, WM, and WB, had more than one haplotype present. The most common haplotypes (A and C) were both present in 6 of the 13 populations surveyed from BC to AZ.

A haplotype network was constructed based on the 12 haplotypes from the combined analysis of NAD1 and NAD4 genes (Fig. 2). The haplotype network was comprised of two common haplotypes (A and C) that were found in 29 of the individuals surveyed. No distinct geographical separation of haplotypes appeared in the network. AMOVA results indicated marginal support for genetic subdivision of the populations, with 13% of the variation attributable to population subdivision ( $\phi_{ST} = 0.13$ ,  $P = 0.053$ ).

### AFLP genotyping

A total of 221 AFLP fragments ranging in size from 73 to 531 bp were scored from four primer combinations in 149 individuals. The number of fragments generated from each primer combination ranged from 37 to 65 (Table 3). Of the 221 fragments scored 15 loci were monomorphic and 39 had a band frequency <5% or >95%. The percentage of polymorphic bands  $\geq 5\%$  level ranged from 67.9% to 86.0% in the populations surveyed, with WS having the lowest number of polymorphic bands and CX1, the most southern population, having the highest (Table 4). The mean expected heterozygosity under Hardy-Weinberg proportions was 0.284, with the lowest observed in the WS population (0.250) and the highest in the OC population (0.302) (Table 4).

Pairwise  $F_{ST}$  values between populations ranged from 0.0008 to 0.1121 (Table 5).  $F_{ST}$  values indicated a lack of differentiation among the populations surveyed from CA where cabbage loopers over-winter, but significant differentiation existed among the majority of CA populations and those north of CA. Significant differentiation was also observed among most populations surveyed from OR, WA, and BC. Although the two populations from BC were significantly differentiated, one of the populations was not different from one of the CA populations. The highest level of differentiation was observed between populations at the localities BA and WS. Populations at these

**Table 4.** Summary of descriptive statistics for mitochondrial and nuclear markers for *T. ni* collected from 13 sampling localities on the west coast of North America.

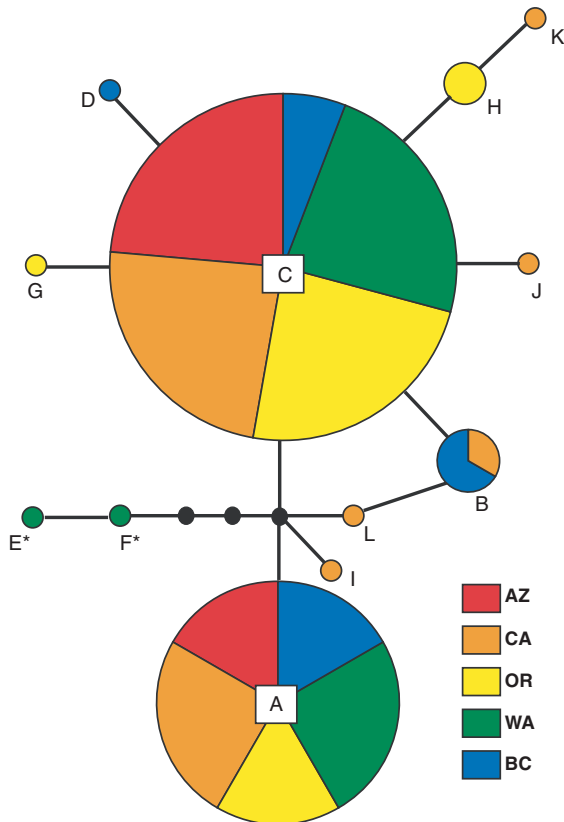
Locality	Mitochondrial DNA									Nuclear DNA			
	NAD1			NAD4			NAD1 and NAD4			AFLP			
	<i>n</i>	Haplotype*	$\pi$ †	$h$ ‡	Haplotype*	$\pi$ †	$h$ ‡	Haplotype*	$\pi$ †	$h$ ‡	<i>n</i>	%P	He
AY	6	M	0.0000	0.00	U(2), V(4)	0.0027	0.53	A(2), C(4)	0.0012	0.53	–	–	–
CX1	3	M(2), N	0.0014	0.67	U, V(2)	0.0033	0.67	A, B, C	0.0023	1.00	15	86.0	0.298
CX2	3	M(2), N	0.0014	0.67	U, V, Z	0.0033	1.00	A, C, L	0.0023	1.00	15	77.8	0.280
CS	3	M	0.0000	0.00	U, V(2)	0.0033	0.67	A, C(2)	0.0015	0.67	15	81.9	0.278
CY	3	M, S, T	0.0027	1.00	V, X, Y	0.0050	1.00	I, J, K	0.0038	1.00	15	82.4	0.286
OR	3	M, S(2)	0.0014	0.67	U, V(2)	0.0033	0.67	A, H(2)	0.0023	0.67	15	76.9	0.265
OC	3	M(2), R	0.0014	0.67	U, V(2)	0.0033	0.67	A, C, G	0.0023	1.00	15	83.3	0.302
OA	3	M	0.0000	0.00	V	0.0000	0.00	C	0.0000	0.00	15	83.7	0.301
WS	3	M, P, Q	0.0027	1.00	V, W(2)	0.0050	0.67	C, E, F	0.0038	1.00	14	67.9	0.250
WM	3	M	0.0000	0.00	U	0.0000	0.00	A	0.0000	0.00	–	–	–
WB	3	M	0.0000	0.00	V	0.0000	0.00	C	0.0000	0.00	–	–	–
BD	3	M(2), N	0.0014	0.67	U, V(2)	0.0033	0.67	A, B, C	0.0023	1.00	15	84.2	0.300
BA	3	M, N, O	0.0027	1.00	U, V(2)	0.0033	0.67	A, B, D	0.0030	1.00	15	79.6	0.275

The number of individuals (*n*), haplotypes, nucleotide diversity ( $\pi$ ), and haplotype diversity ( $h$ ) are reported for mitochondrial DNA from a 487 bp region of NAD1 and 402 bp region of NAD4 and 889 bp for combined analysis of these two regions. The % polymorphic loci at the 5% level (%P) and expected heterozygosity under Hardy-Weinberg proportions (He) are reported for AFLP data.

\*Numbers in brackets denote the number of individuals represented by a particular haplotype when ambiguous.

†Nucleotide diversity (Nei 1987).

‡Haplotype diversity (Nei 1987).



**Figure 2** Haplotype network of 12 mtDNA haplotypes based on gene regions NAD1 and NAD4. Forty-two specimens were collected from locations in Arizona (AZ), California (CA), Oregon (OR), Washington (WA), and British Columbia (BC). The size of each circle is proportional to the number of individuals. Letters within the circles correspond to the haplotype shared by those individuals. Lines connecting circles represent a single nucleotide change between haplotypes and black dots between circles represent a single unobserved nucleotide change inferred from parsimony analysis. Asterisks (\*) denote haplotypes that are unique to the sample from the Seattle Washington population.

localities also showed the highest levels of differentiation compared to all other populations surveyed.

Amplified fragment length polymorphism loci that had low polymorphism (i.e., present <5 or >95% of individuals) were excluded from all spatial analysis due to their potential to bias parameter estimates (Lynch and Milligan 1994). Results from STRUCTURE identified three clusters ( $K = 3$ ) to be the most probable number (Fig. 3). The bar plot revealed that the WS population was most distinct from the other populations with high representation in a cluster type that had low representation in all other populations (Fig. 4). Populations surveyed from CA showed mixed membership into two groups, with an average membership of 0.446 and 0.444 in the two clusters. Individuals from populations BA, OR, and OC had moderately high assignment to one of these two clusters

and all other populations showed a mixed membership into the two groups. The most probable number of clusters identified by TESS Bayesian cluster analysis was four ( $K = 4$ ). When compared to the results of STRUCTURE, a similar pattern of spatial structure was observed, except a portion of individuals from the BA population had membership into a fourth cluster. Consistent with gene flow, a mantel test showed no relationship between geographic and genetic distance for *T. ni* populations from CA to BC ( $r = -0.077$ ,  $P = 0.35$ , Fig. 5).

## Discussion

The modest levels of geographic structure in *T. ni* populations from Arizona to British Columbia, suggest high migratory connectivity for this moth species. Patterns arising are consistent with significant movement of *T. ni* from southern, over-wintering populations, with some genetic differentiation occurring among temporary populations in northern regions initiated by annual immigration of moths.

Our AFLP results from the clustering analyses and pairwise  $F_{ST}$  values indicate a lack of geographic structure among California populations. Warm temperatures in southern California allow *T. ni* populations to persist year-round (Mitchell and Chalfant 1984) and our findings suggest that gene flow connects these populations. Moths are capable of dispersing at least 500–700 km in a single night on prevailing wind currents or high level jet streams (Drake and Farrow 1988; Gatehouse 1997) and therefore it is not surprising that *T. ni* populations in California separated by as much as 600 km remain connected.

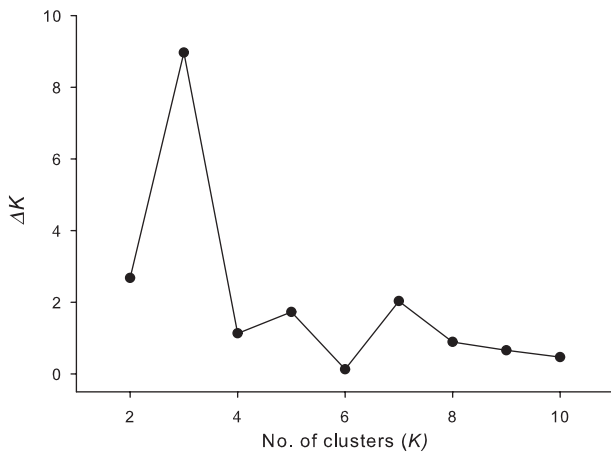
In areas north of California in which field populations are not expected to over-winter, pairwise  $F_{ST}$  comparisons based on AFLP markers indicate that the majority of populations are weakly, genetically differentiated. Consistent with these findings, AMOVA results based on mtDNA variation show marginal support for genetic subdivision of populations. Nonsignificant pairwise  $F_{ST}$  comparisons between distant populations however indicate a lack of genetic differentiation in some cases. For example the California population, CX1 is not differentiated from the British Columbia population, BD more than 1700 km away. In addition, no significant relationship between isolation and distance along the proposed migration path occurred. The lack of isolation by distance but statistically significant differentiation among temporary northern populations and California source populations could be due to founder events and random genetic drift during the migration process. On a local scale, the two populations from BC were genetically differentiated and this might be the result of cabbage loopers that over-winter in some greenhouses there (Franklin and Myers 2008; Franklin et al. 2010).

**Table 5.** Pairwise  $F_{ST}$  values (below the diagonal) and geographic distances (km) (above the diagonal) between *T. ni* populations collected from localities along the west coast of North America.

Population	CX1	CX2	CS	CY	OR	OC	OA	WS	BD	BA
CX1	–	15	153	604	1070	1210	1220	1510	1690	1670
CX2	0.0025	–	140	588	1060	1190	1200	1490	1670	1660
CS	0.0008	0.0138	–	488	964	1100	1120	1420	1590	1580
CY	0.0149	0.0092	0.0139	–	476	614	630	942	1110	1100
OR	0.0282*	0.0381*	0.0398*	0.0337*	–	147	166	499	652	656
OC	0.0089	0.0112	0.0208*	0.0156	0.0264*	–	20	353	505	509
OA	0.0207*	0.0218*	0.0265*	0.0276*	0.0552*	0.0205*	–	334	487	490
WS	0.0687*	0.0696*	0.0830*	0.0665*	0.0696*	0.0671*	0.0964*	–	186	164
BD	0.0040	0.0152*	0.0176*	0.0325*	0.0273*	0.0103	0.0236*	0.0560*	–	69
BA	0.0316*	0.0332*	0.0492*	0.0514*	0.0605*	0.0365*	0.0453*	0.1121*	0.0282*	–

1000 random permutations were used to test for significant genetic differentiation between populations.

\*Populations were genetically differentiated at a significance level of  $P < 0.001$ .

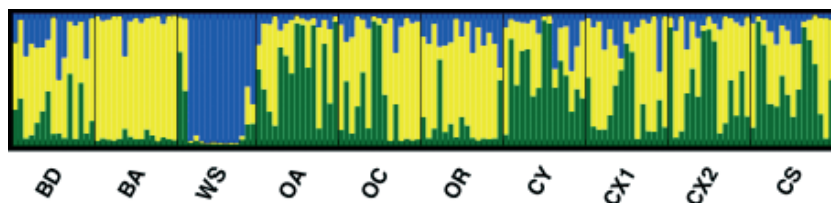


**Figure 3**  $\Delta K$ -values calculated according to the method outlined in Evanno et al. (2005) from the clustering results obtained from STRUCTURE for each cluster size ( $K$ ) from 2 to 10. The peak  $\Delta K$ , which indicates the most probable number of clusters was obtained at  $K = 3$ .

Our results are consistent with those from other migratory insects that have observed genetic homogeneity among some populations separated by large geographic distances (Daly and Gregg 1985; Johnson 1987; Peterson and Denno 1998; Mun et al. 1999; Zhou et al. 2000;

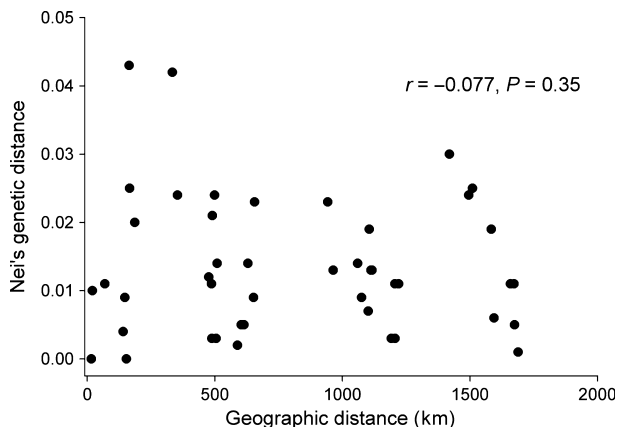
Llewellyn et al. 2003; Vandewoestijne and Baguette 2004; Scott et al. 2005). Also in agreement Peterson and Denno (1998) found with allozyme data a generally weak relationship between genetic and geographic distance for 16 highly mobile insect species. This is in contrast to insects such as the Green Oak leafroller, *Tortrix viridana* L. (Lepidoptera: Tortricidae) and the Pine processionary moth, *Thaumetopoea pityocampa* Denis & Schiffmüller (Lepidoptera: Thaumetopoeidae) that are widely distributed, but disperse short distances and exhibit strong geographic structure (Salvato et al. 2002; Schroeder and Degen 2008). Low population densities of *T. ni* were observed along the proposed migration route in 2006 (personal observation) and this could have led to smaller founding populations and genetic differentiation among many of the surveyed populations. This year contrasts to 2008 in which populations at least in Oregon were exceptionally high (McGrath 2008). A future study over multiple years would be required to determine if genetic variation was associated with population density variation.

Although our data show that gene flow occurs across the survey range, the Seattle, Washington population (WS) was genetically distinct from the other populations. Two of the three mitochondrial haplotypes were only found in this sample, heterozygosity was the lowest in



**Figure 4** Results of STRUCTURE clustering analysis ( $K = 3$ ) for 149 *Trichoplusia ni* collected from British Columbia (BD, BA), Washington (WS), Oregon (OA, OC, OR), and California (CY, CX1, CX2, CS). Labels below represent locality codes for collection sites that are defined in Table 1. Individuals are represented by vertical lines, divided into coloured segments that represent their inferred membership into each of the  $K$  clusters.





**Figure 5** Relationship between Nei's genetic distance and geographic distance among populations of *T. ni* from California to British Columbia.

this population, and pairwise  $F_{ST}$  values and clustering results of AFLP data indicated that this population was genetically distinct. It is possible that this particular population was established by a human associated introduction from another source population (Loxdale and Lushai 1999) such as larvae and pupae being transported with cut cabbage or cruciferous transplants infested with eggs and larvae (Lingren et al. 1979) or entry through a marine port.

The clustering analysis implemented in STRUCTURE and TESS and pairwise  $F_{ST}$  values also indicated that the Abbotsford, British Columbia population (BA) was somewhat distinct. In the last 30 years, the development of vegetable greenhouses that potentially provide refugia for over-wintering cabbage looper moths could have contributed to this. Although a winter cleanup in which greenhouse temperatures are reduced, crops are removed, and houses are fumigated, eliminates most cabbage looper eggs, larvae, and moths; pupae can sometimes survive 2 weeks at 10°C and moths still mate after emergence if the greenhouse is again heated (Caron and Myers 2008). In this situation, populations occur in the greenhouses before they can exist in the fields (Cervantes 2005). Greenhouse populations are frequently under strong selection from the use of the microbial insecticides based on the toxin produced by *Bacillus thuringiensis* (*Bt*) with resulting increased selection for resistance in some cases (Janmaat and Myers 2003; Franklin and Myers 2008). Movement of moths from greenhouse populations could influence the genetic structure of surrounding field populations and contribute to greater distinctiveness of the BA population. Additional work on the local genetic structure of greenhouse and field populations in British Columbia suggests that this is likely to be the case (Franklin et al. 2010).

*Trichoplusia ni* larvae were not present in Yuma, Arizona (AY) during the summer when all other populations were collected due to high temperatures and the lack of suitable crops. Our collaborators collected larvae from Arizona during November when temperatures were cooler and crucifer field crops existed in the area. We predicted that this population might be genetically distinct from the other populations surveyed due to its location further east and the unsuitable summer temperatures. However, the two most common mitochondrial haplotypes (A and C) observed in other populations were also present in the Arizona population. This supports the notion of genetic connectivity between this population and other populations surveyed.

As predicted by the 'moving-deme' hypothesis (Loxdale and Lushai 1999, 2001) geographic differentiation can remain low among widely separated populations because maternal haplotypes remain grouped in migratory species as individuals move together to and from seasonal migration grounds. In support of this hypothesis, the migratory monarch butterfly, *Danaus plexippus* L. (Lepidoptera: Nymphalidae) populations in North America show low levels of mitochondrial variation (Brower and Boyce 1991) similar to what we have observed here.

In future, it would be useful to extend our study over multiple years to test for temporal changes in migratory patterns of *T. ni* in years of high and low population densities. Furthermore, in search of an explanation for the distinct geographic structure of the Washington population, it would be important in the future to examine the migration patterns of *T. ni* from regions further east. Understanding migratory connectivity in *T. ni* will allow predictions as to the outcomes of natural versus human induced changes that occur in different habitats at different times of the year. From an applied perspective, knowledge of the strong migratory connectivity in *T. ni* may inform predictions of when populations will be problematic in crops and aid in the management of *Bt* resistance. By sharing information among agencies that monitor cabbage loopers along the migratory route, growers could anticipate annual variation in the density of the species. In addition, knowledge of changes in the exposure of southern populations of cabbage loopers to *Bt*, for example through the wider use of *Bt* cotton, could alert northern growers to the potential for increased resistance of immigrants.

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### Conflict of interest

The authors declare no conflict of interest.

### Literature cited

- Bonin, A., E. Bellemain, P. Bronken Eidesen, F. Pompanon, C. Brochmann and P. Taberlet. 2004. How to track and assess genotyping errors in population genetic studies. *Molecular Ecology* **13**:3261–3273.
- Bonin, A., D. Ehrich, and S. Manel. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology* **16**:3737–3758.
- Brower, A. V. Z., and T. M. Boyce. 1991. Mitochondrial-DNA variation in monarch butterflies. *Evolution* **45**:1281–1286.
- Cahn, M., K. Brittan, and C. Pickel. 2001. Pest management grants final report. <http://www.cdpr.ca.gov/docs/pestmgmt/grants/00-01/.../00-0192S.pdf>
- Caron, V., and J. H. Myers. 2008. Positive association between resistance to *Bacillus thuringiensis* and overwintering survival of cabbage loopers, *Trichoplusia ni* (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* **98**:317–322.
- Cervantes, V. (2005) Population Ecology of *Trichoplusia ni* in Greenhouses and the Potential of *Autographa californica* Nucleopolyovirus for their Control. M.Sc. Thesis. Department of Plant Science. University of British Columbia, Vancouver, BC.
- Chapman, J. W., R. L. Nesbit, L. E. Burgin, D. R. Reynolds, A. D. Smith, D. R. Middleton, and J. K. Hill. 2010. Flight orientation behaviors promote optimal migration trajectories in high-flying insects. *Science* **327**:682–685.
- Chen, C., E. Durand, F. Forbes, and O. Francois. 2007. Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes* **7**:747–756.
- Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**:1657–1659.
- Daly, J. C., and P. Gregg. 1985. Genetic variation in *Heliothis* in Australia: species identification and gene flow in two pest species *H. armigera* (Hubner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* **75**:169–184.
- Debolt, J. W., T. J. Henneberry, W. W. Wolf, and P. D. Lingren (1984) Release, recovery, and dispersal of adults. In P. D. Lingren, and G. L. Green, eds. *Suppression and Management of Cabbage Looper Populations*, pp. 19–25. U.S. Department of Agriculture, Technical Bulletin No 1684.
- Dingle, H. 2006. Animal migration: is there a common migratory syndrome? *Journal of Ornithology* **147**:212–220.
- Dingle, H., and V. A. Drake. 2007. What is migration? *BioScience* **57**:113–121.
- Donini, P., M. L. Elias, S. M. Bougourd, and R. M. D. Koeberner. 1997. AFLP fingerprinting reveals pattern differences between template DNA extracted from different plant organs. *Genome* **40**:521–526.
- Drake, V. A., and R. A. Farrow. 1988. The Influence of atmospheric structure and motions on insect migration. *Annual Review of Entomology* **33**:183–210.
- Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**:2611–2620.
- Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction sites. *Genetics* **131**:479–491.
- Falush, D., M. Stephens, and J. K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* **7**:574–578.
- Franklin, M. T., and J. H. Myers. 2008. Refuges in reverse: the spread of *Bacillus thuringiensis* resistance to unselected greenhouse populations of cabbage loopers *Trichoplusia ni*. *Agricultural and Forest Entomology* **10**:119–127.
- Franklin, M. T., J. H. Myers, and C. E. Ritland. 2009. Distinguishing between laboratory-reared and greenhouse- and field-collected *Trichoplusia ni* (Lepidoptera: Noctuidae) using the amplified fragment length polymorphism method. *Annals of the Entomological Society of America* **102**:151–157.
- Franklin, M. T., C. E. Ritland, and J. H. Myers. 2010. Spatial and temporal changes in genetic structure of greenhouse and field populations of cabbage looper, *Trichoplusia ni*. *Molecular Ecology* **19**:1122–1133.
- Gatehouse, A. G. 1997. Behavior and ecological genetics of wind-borne migration by insects. *Annual Review of Entomology* **42**:475–502.
- Hall, T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**:95–98.
- Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**:1801–1806.
- Janmaat, A. F., and J. Myers. 2003. Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni*. *Proceedings of the Royal Society of London Series B: Biological Sciences* **270**:2263–2270.
- Johnson, S. J. 1987. Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda* in the western hemisphere. *Insect Science and its Application* **8**:543–549.
- Lingren, P. D., T. J. Henneberry, and A. N. Sparks (1979) Current knowledge and research on movement of the cabbage looper and related looper species. In R. L. Rabb, and G. G. Kennedy, eds. *Movement of Highly Mobile Insects: Concepts and Methodology in Research*, pp. 394–405. University Graphics, North Carolina State University, Raleigh.

- Lingren, P. D., V. M. Bryant Jr, and J. R. Raulston. 1993. Adult feeding host range and migratory activities of com earworm, cabbage looper, and celery looper (Lepidoptera: Noctuidae) moths as evidenced by attached pollen. *Journal of Economic Entomology* **86**:1429–1439.
- Llewellyn, K. S., H. D. Loxdale, R. Harrington, C. P. Brookes, S. J. Clark, and P. Sunnucks. 2003. Migration and genetic structure of the grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as revealed using microsatellites. *Molecular Ecology* **12**:21–34.
- Loxdale, H. D., and G. Lushai. 1999. Slaves of the environment: the movement of herbivorous insects in relation to their ecology and genotype. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* **354**:1479–1495.
- Loxdale, H. D., and G. Lushai (2001) Use of genetic diversity in movement studies of flying insects. In I. P. Woiwod, D. R. Reynolds, and C. D. Thomas, eds. *Insect Movement: Mechanisms and Consequences*, pp. 361–386. CABI Publishing, Wallingford, UK.
- Lynch, M., and B. G. Milligan. 1994. Analysis of population genetic structure with RAPD markers. *Molecular Ecology* **3**:91–99.
- Mantel, N. 1967. Detection of disease clustering and a generalized regression approach. *Cancer Research* **27**:209–220.
- McGrath, D. 2008. VegNet Newsletter 4 June 2008 <http://extension.oregonstate.edu/linn>
- Mitchell, E. R., and R. B. Chalfant. 1984. Biology, behaviour and dispersal of adults. In P. D. Lingren, and G. L. Green, eds. *Suppression and Management of Cabbage Looper Populations*, pp. 14–18. Technical bulletin/Agricultural Research Service, USDA.
- Mun, J. H., Y. H. Song, K. L. Heong, and G. K. Roderick. 1999. Genetic variation among Asian populations of rice planthoppers, *Nilaparvata lugens* and *Sogatella furcifera* (Hemiptera: Delphacidae): mitochondrial DNA sequences. *Bulletin of Entomological Research* **89**:245–253.
- Nagoshi, R. N., R. L. Meagher, and K. Flanders. 2008. Using haplotypes to monitor the migration of fall armyworm (Lepidoptera: Noctuidae) corn-strain populations from Texas and Florida. *Journal of Economic Entomology* **101**:742–749.
- Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Oliver, J. C. 2006. Population genetic effects of human-mediated plant range expansions on native phytophagous insects. *Oikos* **112**:456–463.
- Pashley, D. P., and S. J. Johnson. 1986. Genetic population structure of migratory moths: the Velvetbean Caterpillar (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **79**:26–30.
- Pashley, D. P., S. J. Johnson, and N. A. Sparks. 1985. Genetic population structure of migratory moths: the fall armyworm (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **78**:756–762.
- Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**:288–295.
- Peterson, M. A., and R. F. Denno. 1998. The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. *American Naturalist* **152**:428–446.
- Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**:945–959.
- Rosenberg, N. A. 2004. DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* **4**:137–138.
- Rozas, J., J. C. Sanchez-DelBarrio, X. Messeguer, and R. Rozas. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**:2496–2497.
- Salvato, P., A. Battisti, S. Concato, L. Masutti, T. Patarnello, and L. Zane. 2002. Genetic differentiation in the winter pine processionary moth (*Thaumetopoea pityocampa-wilkinsoni* complex), inferred by AFLP and mitochondrial DNA markers. *Molecular Ecology* **11**:2435–2444.
- Salvato, P., M. Simonato, A. Battisti, and E. Negrisola. 2008. The complete mitochondrial genome of the bag-shelter moth *Ochrogaster lunifer* (Lepidoptera, Notodontidae). *BMC Genomics* **9**.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning, a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Schroeder, H., and B. Degen. 2008. Spatial genetic structure in populations of the green oak leaf roller, *Tortrix viridana* L. (Lepidoptera, Tortricidae). *European Journal of Forest Research* **127**:447–453.
- Scott, K. D., N. Lawrence, and C. L. Lange. 2005. Assessing moth migration and population structuring in *Helicoverpa armigera* (Lepidoptera: Noctuidae) at the regional scale: example from the Darling Downs, Australia. *Journal of Economic Entomology* **98**:2210–2219.
- Simmons, R. B., and S. J. Weller. 2001. Utility and evolution of Cytochrome b in insects. *Molecular Phylogenetics and Evolution* **20**:196–210.
- Smouse, P. E., J. C. Long, and R. R. Sokal. 1986. Multiple regression and correlation extensions of the mantel test of matrix correspondence. *Systematic Zoology* **35**:627–632.
- Sosa-Gómez, D. R. 2004. Intraspecific variation and population structure of the Velvetbean Caterpillar, *Anticarsia gemmatialis* Hübner, 1818 (Insecta: Lepidoptera: Noctuidae). *Genetics and Molecular Biology* **27**:378–384.
- Toba, H. H., A. N. Kishaba, R. Pangaldan, and P. V. Vail. 1973. Temperature and the development of the cabbage looper. *Annals of the Entomological Society of America* **66**:965–973.
- Vandewoestijne, S., and M. Baguette. 2004. Demographic versus genetic dispersal measures. *Population Ecology* **46**:281–285.
- Vekemans, X., T. Beauwens, M. Lemaire, and I. Roldan-Ruiz. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology* **11**:139–151.
- Zhivotovskiy, L. A. 1999. Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology* **8**:907–913.
- Zhou, X., O. Faktor, S. W. Applebaum, and M. Coll. 2000. Population structure of the pestiferous moth *Helicoverpa armigera* in the eastern Mediterranean using RAPD analysis. *Heredity* **85**:251–256.