# Genetic variability and structure of populations of *Homoeosoma nebulella* (Denis et Schiffermüller) (Lepidoptera: Pyralidae) in northern China

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In recent years, there have been significant outbreaks of the European sunflower moth (ESM) *Homoeosoma nebulella* in northern China, causing enormous agricultural losses to local farmers. Although some control measures have been investigated, there is limited knowledge about genetic structure among ESM populations which may be related to pest outbreak. In this study, we analyzed the genetic variation and genetic structure of ESM. Our results showed that genetic differentiation was low among populations (2.73%), while it was high within individuals (60.73%), and among individuals within populations (32.59%). Dendograms based on $F_{st}$ and genetic distance revealed that populations from Inner Mongolia and Xinjiang Uyghur Autonomous Region were first clustered and subsequently grouped with populations from Heilongjiang province. The outbreaks of ESM in Inner Mongolia may be caused by dispersal of ESM from Xinjiang Uyghur Autonomous Region.

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## 1. Introduction

European sunflower moth (ESM) is a Palaearctic species that is widely distributed from northern China to Western Europe (Zagatti *et al.* 1991). ESM is the most common insect pest of sunflower (*Helianthus annuus* L.) in northern China and the region has experienced large agricultural losses as a result of this pest in recent years. ESM as a pest insect was investigated during 2007–2009 (Cao *et al.* 2010a). The first generation of ESM begins to emerge from early July and the second-generation moths begin to appear in August. The life cycle of the ESM is as follows: eggs hatch within 3 to 5 days, larval stage (four instars) lasts 22 days, larvae pupate in soil for 6 to 7 days, and the flight period of the adults lasts for 7 to 10 days. ESM females oviposit in cultivated sun-

Table 1. Sampling list of the 11 locations where *Homoeosoma nebulella* was collected on sunflowers in August and September 2008 and 2009. Abbreviations: Alt = altitude (m); $N_t$ = no. of traps.

| Provinces | Locations | Code | *N* | E | Alt | $N_t$ |
|---|---|---|---|---|---|---|
| Xinjiang | Aletai | Alt | 47°52' | 88°80' | 1,325 | 25 |
| | Wulumuqi | Wlmq | 43°77' | 87°72' | 800 | 60 |
| | Shihezi | Shz | 44°27' | 85°94' | 700 | 60 |
| Neimenggu | Hangjin | Hj | 41°03' | 107°14' | 1,040 | 28 |
| | Linhe | Lh | 40°46' | 107°24' | 1,042 | 48 |
| | Wuyuan | Wy | 41°12' | 108°28' | 1,200 | 46 |
| Heilongjiang | Mudanjiang | Mdj | 44°38' | 129°34' | 120 | 35 |
| | Daqing | Dq | 47°55' | 122° 29' | 196 | 35 |
| | Ganna | Gn | 47°55' | 123°29' | 196 | 32 |

flowers as soon as blooming begins. The eggs hatch and the young larvae tunnel into the developing heads and feed on the developing seeds and floral structures. Larval feeding also contributes to secondary infection by *Rhizopus* head rot (Royer & Walgenbach 1987). When mature, the larvae spin down to the ground and enter the soil, where they form a silken cocoon.

Genetic studies could provide useful information regarding the potential for large-scale insect pest control, particularly in terms of the use of the sterile insect technique (Dale *et al.* 1995, Vreysen *et al.* 1998, Cheng & Aksoy 1999). Similar studies have been performed for the diamondback moth, *Plutella xylostella* (Zhou *et al.* 2010), the mosquito *Anopheles gambiae* (Pinto *et al*. 2002), the boll weevil, *Anthonomus grandis* (Black & Sappington 2004), a weed plant, *Chenopodium album* (Aper *et al.* 2010) and the tsetse fly, *Glossina palpalis gambiensis* (Solano *et al.* 2000). For the ESM, we had previously isolated nine microsatellite polymorphic loci from genomic DNA (Cao *et al.* 2010b). However, its genetic structure and dispersal behavior had not been previously reported; therefore, we report here on a study of the genetic differentiation of ESM populations in northern China.

## 2. Materials and methods

### 2.1. Sample collection

We sampled 417 ESM individuals from 11 locations across three provinces located at longitude 85º94'–129º34', latitude 40º27'–47º55', ranging in height above sea level from 120 m to 1,325 m (Table 1). The numbers of traps per population are shown in Table 1.

All samples of pheromone-trapped males were collected between August and September during 2008 and 2009, and were preserved in 95% non-denatured ethanol. Plastic basins baited with red rubber septum dispensers were used. Each yellow basin was 24 cm in diameter and contained a water-detergent mixture to capture the moths. The rubber septum dispenser was fixed 2 cm above the water surface in the basin, and all the basins were placed 150 cm above the ground at the intervals of 100 m.

### 2.2. DNA extraction and amplification

All ESM individuals were separately prepared to isolate total DNA for PCR and analyzed subsequently. The individuals were pestled to a powder in liquid nitrogen and submerged in CTAB extraction buffer {CTAB 2% (m/v), Tris-Hcl 100 mmol/L (ph 8.0), EDTA 20 mmol/L (ph 8.0), Nacl 1.4 mol/L}. The mixture was extracted using phenol/chloroform and the DNA precipitated with ethanol and then dissolved inTE buffer (ph 8.0) (Tris-Hcl 10 mmol, EDTA 1 mmol). The DNA concentration was detected using an ultraviolet spectrophotometer (UV-2802H).

### 2.3. PCR amplification

PCR was used to amplify four microsatellite loci (ESM116, ESM264, ESM172 and ESM66) (Cao

Table 2. Summary of genetic variation for four loci. Abbreviations: $N$ = sample number; $n_a$ = observed no. of alleles; $n_e$ = effective no. of alleles; $H_o$ = observed heterozygosity; $H_e$ = expected heterozygosity; Nei = Nei's unbiased heterozygosity; $Het_{aver}$ = average heterozygosity; $I$ = Shannon's information index.

| Locus | $N$ | $n_a$ | $Sn_e$ | $H_o$ | $H_e$ | Nei | $Het_{aver}$ | $I$ |
|---|---|---|---|---|---|---|---|---|
| ESM264 | 368 | 27 | 6.10 | 0.46 | 0.84 | 0.84 | 0.78 | 2.36 |
| ESM116 | 368 | 52 | 22.57 | 0.48 | 0.96 | 0.96 | 0.88 | 3.44 |
| ESM66 | 308 | 28 | 13.74 | 0.50 | 0.93 | 0.93 | 0.81 | 2.85 |
| ESM172 | 354 | 17 | 7.53 | 0.69 | 0.87 | 0.87 | 0.73 | 2.23 |
| Mean | 350 | 31 | 12.48 | 0.53 | 0.90 | 0.90 | 0.80 | 2.72 |

*et al.* 2010b). PCR was carried out in 15 µL reaction volumes and the PCR amplification conditions contained: 10 ng genomic DNA, 1.5 mM MgCl (for ESM264 and ESM172), 2.5 mM MgCl2 (for ESM66 and ESM116), 200 µM dNTP, 0.4 µM of each primer and 0.5 U Taq polymerase (Genstar), in a total volume of 15 µL. Cycling conditions were as follows: denaturation at 94ºC for 5 min; 34 cycles of 30 s at 94ºC; locus-specific Ta for 30 s; 72ºC for 30 s; and a final extension at 72ºC for 5 min (Cao *et al.* 2010b). Amplified fragments were separated on an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA), and allele sizes were estimated using the ABI GeneMarker and Genotyper software.

### 2.4. Analysis of microsatellite data

Microsatellite data from the populations were analyzed using GenePop 3.2 (Raymond & Rousset 1995). Basic descriptive population genetic statistics included observed allelic number and effective allelic number, observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), $F$-statistic and Shannon's Information Index. Tests of deviation from Hardy–Weinberg proportions at each locus and of linkage disequilibrium between pairs of loci were also determined by exact tests ($\chi^2$ and $G^2$) using GenePop and Microchecker software with Bonferroni correction. Allele frequencies were calculated by using Microsat. The Microchecker program (Oosterhout *et al*. 2004) was used to check for errors owing to large allele dropout and stuttering. Pairwise $F_{ST}$ (a measure of population differentiation that takes into account microsatellite allele size) was calculated using Arlequin (Excoffier *et al.* 2006). An analysis of molecular variance (AMOVA) test was used to partition the genetic variation in an across-population and a within-population component. Significance tests in Arlequin were performed with 1023 permutations. A genetic distance matrix of pairwise $F_{ST}$ values was also used to perform a hierarchical AMOVA with Arlequin.

Based on the value of $F_{ST}$ obtained by the polymorphic markers, one dendrogram was constructed using Mega 3.0 (Kumar *et al.* 2004) and the other one was obtained from GenePop, based on Nei's regular and unbiased genetic distance measures.

## 3. Results and discussion

A total of 417 ESM individuals collected from 11 locations (Table 1) were identified and genotyped in this study. Of these individuals 308, 368, 354 and 368 were successfully genotyped at four loci (ESM66, ESM116, ESM172 and ESM264) respectively (Table 2).

There were a total of 124 alleles in four loci, ESM66, ESM116, ESM172 and ESM264. These had 28, 52, 17 and 27 alleles, respectively, with a mean of 31 alleles. The effective alleles ranged from 6.10 to 22.57, with a mean of 12.48; $H_o$ ranged from 0.46 to 0.69, with a mean of 0.53; $H_e$ ranged from 0.84 to 0.96, with a mean of 0.90; and Shannon's Information Index ranged from 2.23 to 2.85, with a mean of 2.72. The genetic diversities are detailed in Table 2.

The four loci are highly polymorphic across the 11 geographic populations. The measures of $H_o$ were lower than the measures of both $H_e$ and Nei's genetic distance. For the ESM samples,

Table 3. Summary of *F* statistics. Abbreviatons: *N* = number of samples; $F_{is}$ = individuals within subgroups); $F_{it}$ = individuals within total; $F_{st}$ = subgroups within total.

| Locus | *N* | $F_{is}$ | $F_{it}$ | $F_{st}$ |
|---|---|---|---|---|
| ESM264 | 368 | 0.44 | 0.48 | 0.07 |
| ESM116 | 368 | 0.47 | 0.51 | 0.07 |
| ESM66 | 308 | 0.46 | 0.53 | 0.13 |
| ESM172 | 354 | 0.04 | 0.19 | 0.15 |
| Mean | 350 | 0.36 | 0.43 | 0.11 |

analysis by Micro-checker indicated that there was no evidence for scoring errors owing to large allele dropout or stutter at any locus, although it might be that there are null alleles in some samples. The estimated frequency of null alleles for the four loci (ESM264, ESM66, ESM172 and ESM116) was 17.2%, 33.7%, 11.5% and 26%, respectively. No significant linkage disequilibrium was detected between loci from any of the populations.

Seven cases disaccorded with HWE in total of 44 cases (i.e. 4 loci in 11 geographical populations), whereas there were two cases disaccording with HWE according to Micro-checker. A test of overall genetic variation across populations indicated the other three loci showed significant deviation from HWE, except the ESM116 locus. Values for $F_{IS}$, $F_{IT}$ and $F_{ST}$ are shown in Table 3. Wright (1978) suggested that if $F_{ST} = 0$, then the two populations showed no differentiation, when $F_{ST}$ = 0.05–0.15, the populations were moderately differentiated, and when $F_{ST}$ = 0.15–0.25, the populations showed high differentiation. In our study, different populations showed moderate genetic differentiation according to the mean

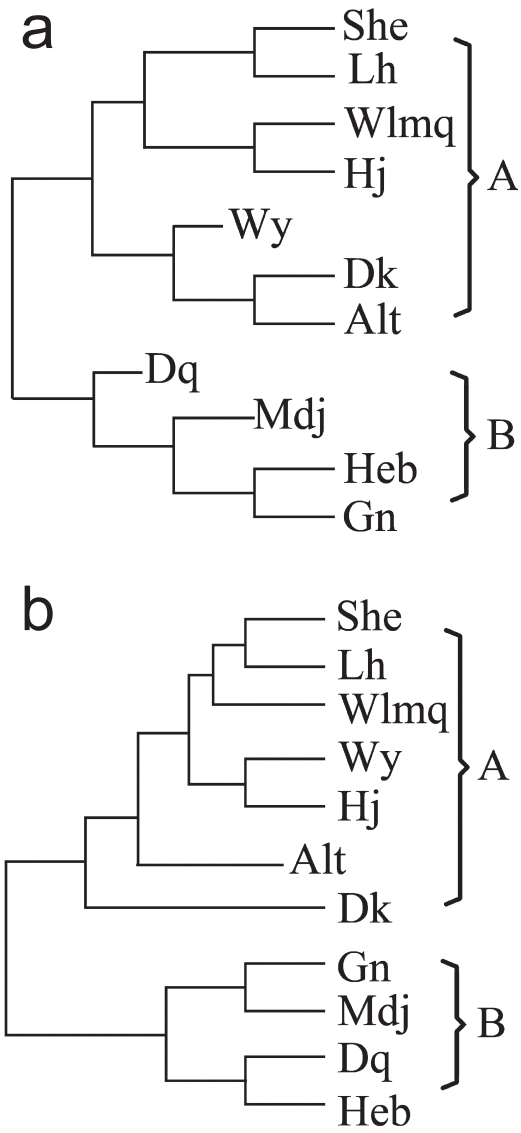


Fig. 1. Dendrograms showing relationships among 11 populations of *Homoeosoma nebulella*. – a. UPMGA dendrogram of Mega cluster. – b. GenePop cluster.

Table 4. AMOVA design and results for genetic variation at different levels. Abbreviations: SS = sum of squares; VC = variance components; %V = percentage of variation; *F* = multilocus *F*-statistics, the values were significant (permutation tests, *p* 0.05).

| Source of variation | *df* | SS | VC | %V | *F* |
|---|---|---|---|---|---|
| Among groups | 2 | 15.97 | 0.04 | 2.73 | 0.03 |
| Among populations within groups | 8 | 25.04 | 0.05 | 3.59 | 0.04 |
| Among individuals within population | 173 | 289.18 | 0.44 | 32.95 | 0.35 |
| Within individuals | 184 | 147.50 | 0.80 | 60.73 | 0.39 |
| Total | 367 | 477.69 | 1.32 | | |

Table 5. Nei's original measures of genetic identity (above diagonal) and genetic distance (below diagonal) of populations.

| ID# | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *** | 0.74 | 0.75 | 0.45 | 0.48 | 0.43 | 0.45 | 0.42 | 0.46 | 0.43 | 0.48 |
| 2 | 0.30 | *** | 0.70 | 0.39 | 0.58 | 0.63 | 0.48 | 0.53 | 0.39 | 0.38 | 0.54 |
| 3 | 0.29 | 0.35 | *** | 0.38 | 0.55 | 0.42 | 0.66 | 0.48 | 0.39 | 0.37 | 0.68 |
| 4 | 0.79 | 0.93 | 0.95 | *** | 0.44 | 0.43 | 0.34 | 0.54 | 0.68 | 0.61 | 0.25 |
| 5 | 0.74 | 0.54 | 0.60 | 0.82 | *** | 0.65 | 0.48 | 0.51 | 0.56 | 0.37 | 0.65 |
| 6 | 0.84 | 0.47 | 0.87 | 0.85 | 0.43 | *** | 0.47 | 0.48 | 0.48 | 0.33 | 0.42 |
| 7 | 0.79 | 0.73 | 0.41 | 1.07 | 0.73 | 0.75 | *** | 0.51 | 0.31 | 0.40 | 0.61 |
| 8 | 0.87 | 0.63 | 0.74 | 0.61 | 0.67 | 0.73 | 0.68 | *** | 0.53 | 0.60 | 0.47 |
| 9 | 0.79 | 0.95 | 0.94 | 0.39 | 0.58 | 0.73 | 1.17 | 0.64 | *** | 0.61 | 0.25 |
| 10 | 0.84 | 0.97 | 0.99 | 0.50 | 1.00 | 1.11 | 0.91 | 0.52 | 0.50 | *** | 0.24 |
| 11 | 0.74 | 0.61 | 0.38 | 1.38 | 0.43 | 0.86 | 0.50 | 0.76 | 1.39 | 1.46 | *** |

# ID for the populations: 1=Shz, 2=Wlmq, 3=Lh, 4=Gn, 5=Wy, 6=Dk, 7=Alt, 8=Dq, 9=Mdj, 10=Heb, 11=Hj (see Table 1).

value of $F_{ST}$ (0.11, Table 3). According to the AMOVA results (Table 4), the percentage of variation among groups and among populations within groups was 2.73% and 3.59%, respectively, whereas the percentage of variation among individuals within populations and within individuals was 32.95% and 60.73%, respectively. Fixation indices were $F_{IS}$ = 0.3517, $F_{SC}$ = 0.03690, $F_{CT}$ = 0.02730 and $F_{IT}$ = 0.39271. Genetic differentiation existed mainly within individuals. $F_{ST}$ pairwise differences were relatively low and ranged from –0.00330 to 0.15195 among populations. The highest value of $F_{ST}$ (0.152) was observed between the Alt and Mdj populations, whereas the negative value was observed between the Hj and Lh populations. Estimates of Nm are often taken as face values of the approximate number of migrants moving among populations. In our experiment, due to Nm (2.1281) > 1, different populations showed moderate genetic differentiation (Wright 1931). However, these estimates are not necessarily indicative of gene flow (Bossart & Prowell 1998). Nei's genetic identity ranged from 0.2375 (Hj and Heb) to 0.7491 (Shz and Lh), genetic distance ranged from 0.2888 (Shz and Lh) to 1.4376 (Hj and Heb) (Table 5). Accordingly, genetic distance and genetic identity are identical in our experiment.

Based on the value of $F_{ST}$ obtained by the polymorphic markers, dendrograms were constructed using Mega 3.0 and GenePop to understand the relationships among the 11 populations. The dendrograms classified the populations in two main clusters, A and B (Fig. 1). Cluster A contains seven populations whereas cluster B contains four populations. Under each of these main clusters, some populations grouped further into subclusters. For instance, subclusters Shz and Lh, Wlmq and Hj, and Wy, Dk and Alt occur in cluster A. The two dendrograms are generally consistent, with little differentiation. In the two dendrograms, four populations within Heilongjiang Province constitute cluster B, whereas seven populations within Inner Mongolia and Xinjiang Uyghur Autonomous Region appear in cluster A, which could indicate that dispersal is frequent and gene flow is relative high between Inner Mongolia and Xinjiang Uyghur Autonomous Region. The same conclusion is reached with Nei's original measures of genetic identity and genetic distance (Table 5).

Therefore, these results indicate that genetic diversity was high among individuals, whereas it was relatively low among populations. Analysis of $F_{ST}$ genetic distances showed that the populations from Inner Mongolia and Xinjiang Uyghur Autonomous Region might be of the same phylogeographical race. Outbreaks of the ESM in Inner Mongolia might be due to dispersal of ESM from Xinjiang Uyghur Autonomous Region.

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