

Seasonal genetic variation and genetic structure of *Spodoptera exigua* in Liaoning Province, Northeast China: Insights from 11 years of microsatellite data (#107333)

1

First revision

Guidance from your Editor

Please submit by **10 Mar 2025** for the benefit of the authors .



Structure and Criteria

Please read the 'Structure and Criteria' page for guidance.



Custom checks

Make sure you include the custom checks shown below, in your review.



Author notes

Have you read the author notes on the [guidance page](#)?



Raw data check

Review the raw data.



Image check

Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files

Download and review all files from the [materials page](#).

1 Tracked changes manuscript(s)

1 Rebuttal letter(s)

5 Figure file(s)

2 Table file(s)

2 Other file(s)

! Custom checks

Field study



Have you checked the authors [field study permits](#)?



Are the field study permits appropriate?



Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. BASIC REPORTING
2. EXPERIMENTAL DESIGN
3. VALIDITY OF THE FINDINGS
4. General comments
5. Confidential notes to the editor

 You can also annotate this PDF and upload it as part of your review

When ready [submit online](#).

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your [guidance page](#).

BASIC REPORTING

-  Clear, unambiguous, professional English language used throughout.
-  Intro & background to show context. Literature well referenced & relevant.
-  Structure conforms to [Peerj standards](#), discipline norm, or improved for clarity.
-  Figures are relevant, high quality, well labelled & described.
-  Raw data supplied (see [Peerj policy](#)).

EXPERIMENTAL DESIGN

-  Original primary research within [Scope of the journal](#).
-  Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
-  Rigorous investigation performed to a high technical & ethical standard.
-  Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

-  **Impact and novelty is not assessed.** Meaningful replication encouraged where rationale & benefit to literature is clearly stated.
-  All underlying data have been provided; they are robust, statistically sound, & controlled.
-  Conclusions are well stated, linked to original research question & limited to supporting results.



The best reviewers use these techniques

Tip

Example

Support criticisms with evidence from the text or from other sources

Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.

Give specific suggestions on how to improve the manuscript

Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).

Comment on language and grammar issues

The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.

Organize by importance of the issues, and number your points

1. Your most important issue
2. The next most important item
3. ...
4. The least important points

Please provide constructive criticism, and avoid personal opinions

I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC

Comment on strengths (as well as weaknesses) of the manuscript

I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.

Seasonal genetic variation and genetic structure of *Spodoptera exigua* in Liaoning Province, Northeast China: Insights from 11 years of microsatellite data

Ming-Li Yu¹, Xian-Zhi Xiu¹, Jin-Yang Wang¹, Xin-Yi Cao¹, Fa-Liang Qin¹, Xing-Ya Wang^{Corresp., 1}, Li-Hong Zhou^{Corresp., 2}

¹ College of Plant Protection, Shenyang Agricultural University, Shenyang, Liaoning, China

² Institute of Flower, Liaoning Academy of Agricultural Sciences, Shenyang, Liaoning, China

Corresponding Authors: Xing-Ya Wang, Li-Hong Zhou
Email address: wangxingya20081@syau.edu.cn, vickyzh@163.com

Background: The beet armyworm (BAW), *Spodoptera exigua*, is a destructive migratory pest worldwide that has caused severe economic losses in China's major crop-producing regions. To control this pest effectively, it is crucial to investigate its seasonal genetic variation and population genetic structure in northern China. **Methods:** In this study, we used eight nuclear microsatellite loci to investigate the seasonal genetic diversity and genetic structure of BAW in Shenyang, Liaoning Province, Northeast China, from 2012-2022. **Results:** Microsatellite data revealed moderate levels of genetic variation among 50 seasonal populations of BAW sampled from 2012-2022, along with significant genetic differentiation among these populations. Neighbor-joining dendrograms, STRUCTURE analysis, and principal coordinate analysis (PCoA) revealed two genetically distinct groups: the SY2012-2018 group and the SY2019-2022 group. Our results revealed seasonal variation in the genetic subconstruction at this location, which may be related to the presence of different migratory individuals throughout the year. Accordingly, our unique insights into the population genetics of BAW will contribute to the development of effective management strategies for this migratory pest.

Seasonal genetic variation and genetic structure of *Spodoptera exigua* in Liaoning Province, Northeast China: Insights from 11 years of microsatellite data

Ming-Li Yu¹, Xian-Zhi Xiu¹, Jin-Yang Wang¹, Xin-Yi Cao¹, Fa-Liang Qin¹, Xing-Ya Wang^{1*} and Li-Hong Zhou^{2*}

¹ College of Plant Protection, Shenyang Agricultural University, Shenyang, Liaoning, 110866, P. R. China

² Institute of Flower, Liaoning Academy of Agricultural Sciences, Shenyang, Liaoning, 110161, P. R. China

*** Correspondence authors:**

Xing-Ya Wang
120 Dongling Road, Shenhe District, Shenyang, Liaoning, 110866, P. R. China.
Email: wangxingya20081@syau.edu.cn

Li-Hong Zhou
84 Dongling Road, Shenhe District, Shenyang, Liaoning, 110866, P. R. China.
Email: vickyzh@163.com

Abstract

Background: The beet armyworm (BAW), *Spodoptera exigua*, is a destructive migratory pest worldwide that has caused severe economic losses in China's major crop-producing regions. To control this pest effectively, it is crucial to investigate its seasonal genetic variation and population genetic structure in northern China.

Methods: In this study, we used eight nuclear microsatellite loci to investigate the seasonal genetic diversity and genetic structure of BAW in Shenyang, Liaoning Province, Northeast China, from 2012-2018.

Results: Microsatellite data revealed moderate levels of genetic variation among 50 seasonal populations of BAW sampled from 2012-2018, along with significant genetic differentiation among these populations. Neighbor-joining dendrograms, STRUCTURE analysis, and principal coordinate analysis (PCoA) revealed two genetically distinct groups: the SY2012-2018 group and the SY2019-2022 group. Our results revealed seasonal variation in the genetic subconstruction at this location, which may be related to the presence of different migratory individuals throughout the year. Accordingly, our unique insights into the population genetics of BAW will contribute to the development of effective management strategies for this migratory pest.

Key words: *Spodoptera exigua*; genetic structure; microsatellites; migration; seasonal variation; Welsh onion

Introduction

The beet armyworm (BAW), *Spodoptera exigua* (Lepidoptera: Noctuidae), is a major polyphagous pest affecting a wide range of crops, including vegetables, maize, cotton, soybeans, and ornamental plants (Adamczyk et al., 2009; Guo et al., 2010). In general, BAW larvae feed on the leaves of host plants, resulting in reduced crop yields and potential plant death. Originally from South Asia, this species is now widely distributed across the tropical and temperate regions of Europe, Africa, North America, and Asia (Wei et al., 2010). In China, BAW was first recorded in Beijing in the 1890s. It is widely distributed in the primary crop-producing regions of China and has caused significant economic losses in recent years. For example, the beet armyworm has spread to several provinces in North China and East China, infesting a total area exceeding 2.7 million hectares (Luo et al., 2000). This pest particularly affects Welsh onions in northern China, which infest over 8,000 hectares in Tianjin and result in a 30% reduction in annual Welsh onion production (Zheng et al., 2009; Zhu et al., 2010).

BAW is a polyphagous insect known for its high fecundity and long-distance flight capabilities (Feng et al., 2003; Adamczyk et al., 2009). Typically, the eggs of this species are laid on the undersides of leaves. Newly hatched larvae feed gregariously on the upper surfaces of the leaves, whereas third-instar larvae begin to feed solitarily. By the fourth instar, they start consuming a variety of plant parts, including leaves, petals, and pods. Pupae predominantly overwinter in the soil, with no overwintering occurring in South China. BAW can reproduce year-round, and no diapause behavior has been observed (Zheng et al., 2011). Previous studies have indicated that BAW migrates seasonally once a year in eastern China (Si et al., 2012). There were significant interannual and seasonal variations in the capture number of BAW in northern China from 2012 to 2022, and the biomass of BAW also increased significantly during these years, which will contribute to a deeper understanding of population dynamics in northern China and provide a theoretical basis for regional monitoring, early warning, and the development of effective management strategies for long-range migratory pests (Ma et al., 2024). In addition, chemical pesticides remain the primary method for pest control. However, the prolonged use of these

insecticides has led to the rapid development of resistance in BAW, notably to chlorinated hydrocarbons and carbamates (Chaufaux & Ferron, 1986). Therefore, effectively controlling this pest is difficult.

Populations of short-lived organisms can adapt to seasonal changes through various mechanisms, including genetic polymorphisms and phenotypic plasticity. These populations typically harbor significant adaptive genetic variation, enabling them to respond rapidly to environmental shifts (Brennan et al., 2019). The genetic variation and population genetic structure of a species can be influenced by numerous factors, including climate change, ecological conditions, natural barriers, migration patterns, and human activities (Fairley et al., 2000; Prugh et al., 2008; Pauls et al., 2013; Nater et al., 2013). Currently, a range of molecular markers are utilized to illuminate the biogeography and evolutionary history of this species (Susanta, 2006). Owing to its moderate evolutionary rate and distinct evolutionary pattern, the *cytochrome c oxidase subunit I* (COI) gene is well suited for reconstructing species phylogenies (Hebert et al., 2003; Wang et al., 2014). Owing to their high codominance and significant polymorphism, microsatellite markers have been widely utilized in population genetics studies (Aggarwal et al., 2007; Wang et al., 2007; Zhu et al., 2020). Previous studies using amplified fragment length polymorphism (AFLP) and mitochondrial genes (e.g., mtDNA *COI* and *Cytb*) have indicated low levels of genetic variation and differentiation among BAW populations, with no clear phylogeographic structure (Niu et al., 2006; Wang et al., 2014; Wang and Zhou, 2016; Zhou et al., 2017). In contrast, two genetically distinct groups have been identified in Iran and western China (Golikhajeh et al., 2018; Wang et al., 2020). These studies provide valuable information for understanding the dispersal patterns and causes of outbreaks of pest species. However, accurate assessments of the genetic diversity and population genetic structure of this pest across large temporal scales in northern China have not been performed.

In the present study, we investigated the seasonal genetic variation and structure of BAW in northern China. We utilized microsatellite loci to assess genetic variation, genetic differentiation, and population structure across 50 seasonal populations of BAW collected from October 2012 to

2022 in Shenyang, Liaoning Province. Additionally, we discuss potential management strategies for this species. This research aims to deepen our understanding of the population genetics of this moth and provide a robust theoretical foundation for developing effective pest management strategies.

Materials & Methods

Sample collection and DNA extraction. A total of 1095 male adult BAW individuals of 50 seasonal populations were collected via three sex pheromone traps (Pherobio Technology Co. Ltd., Beijing, China) in a Welsh onion field, covering an area of approximately one hectare (123.57°N, 41.82°E) over a period of 11 years, from June to October 2012 to 2022 in Shenyang, Liaoning Province. The distance between the three traps set in the field is 30 meters. The traps used were cylindrical plastic devices, approximately 30 cm in height and 18 cm in diameter, with 16 one-way entrances on the top designed to capture pests. The pheromones used in this study were contained in small PVC lures (Pherobio Technology Co., Ltd., Beijing, China). The *cis*-9, *trans*-12-tetradecadienyl acetate and *cis*-9-tetradecadienol are the key components of pheromones (Ma et al., 2024). The number of trapped male adult BAW individuals was recorded weekly, and the trap lures were replaced every two weeks. Samples were collected on private land with permission of the land owners (Guo-Qing Xu). All the BAW samples were preserved in 95% ethanol at −20 °C and stored at the Plant Protection College, Shenyang Agricultural University, Shenyang, China. Details regarding the locations of the populations and the number of samples are provided in Table S1. The samples were collected from private land with the permission of the landowners, and none of the field surveys in this study involved endangered or protected species. Genomic DNA was extracted from individual samples via Qiagen's DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's protocols.

Microsatellite amplification and genotyping. In this study, individuals were genotyped via eight loci (Spe06, Spe07, Spe08, Spe09, Spe10, Spe11, Spe12, and Spe15) from a set of polymorphic microsatellite loci provided by Kim et al. (2012). Each microsatellite locus was assigned a unique

fluorophore for fluorescent tagging of the DNA. For these isolated microsatellites, each PCR mixture consisted of 1.0 units of EasyTaq DNA polymerase, 2.5 mM dNTP mixture, 0.5 μ L of DNA template, 1 $\times$ EasyTaq® buffer (containing 2 mM $MgCl_2$; TransGen Biotech Co., Ltd., Beijing, China), and 0.4 μ M of each primer, which was labelled with the fluorochromes HEX or FAM (Sangon Biotech, Shanghai, China). The PCR amplification conditions were as follows: initial denaturation at 94 °C for 4 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 30 seconds. A final extension was performed at 72 °C for 5 minutes. After amplification, the products were visualized at Sangon Biotech Co., Ltd. (Shanghai, China) via an ABI 3730XL automated sequencer (Applied Biosystems, Foster City, CA, USA). The microsatellite alleles were analysed with GeneMapper 4.0 software (Applied Biosystems). The raw reads of the amplified fragment length from 1095 individuals of BAW are shown in Table S2.

Microsatellite data analyses

Genetic variation and genetic differentiation. Micro-Checker 2.2.3 was utilized to detect errors and null alleles in BAW microsatellite genotypes, excluding individuals with missing data (Van Oosterhout et al., 2004). In the basic data table, missing genetic data are replaced with “0”. When Structure software was used for group analysis, the missing data were replaced with “-9”. Genotypic linkage disequilibrium (LD) was assessed for all pairs of loci across populations via Genepop 3.4 with exact probability tests (Raymond & Rousset, 1995). An exact test for Hardy–Weinberg equilibrium (HWE) was conducted for each locus as well as for all loci within each population.

Genetic diversity indices, including the mean number of alleles (N_a), effective number of alleles (N_e), Shannon's information index (I), observed heterozygosity (H_o), expected heterozygosity (H_e), and unbiased expected heterozygosity (uH_e), were assessed via GenAlEx 6.5 (Peakall and Smouse, 2006). The allelic richness (A_R), fixation index (F_{ST}), and inbreeding coefficient (F_{IS}) among populations were calculated via FSTAT 2.9.3.2 (Goudet, 1995), whereas the polymorphism information content (PIC) was computed via Cervus 2 (Hearne et al., 1992). To assess the degree

of genetic differentiation between pairs of BAW populations, we calculated pairwise F_{ST} values via Arlequin 3.0 (Excoffier et al., 2005) and created associated heatmaps of these values via R statistical software 3.0.2 (Dean and Nielsen, 2007).

Temporal genetic structure. To investigate the temporal population structure of BAW, we followed a stepwise process. First, we used POPTREE 2 to construct an unrooted tree via the neighbor-joining method (Takezaki et al., 2009). Next, we employed a Bayesian clustering model to assess the degree of genetic structure and admixture among populations. The software STRUCTURE 2.3 (Evanno et al., 2005) was utilized to identify clusters of genetically similar populations. We specified an initial range of potential genotype clusters (K) from 1-10 under the admixture model, assuming correlated allele frequencies among populations (Falush et al., 2003). The Markov chain Monte Carlo simulation was run 10 times for each value of K , with a total of 5×10^5 iterations following a burn-in period of 5×10^4 . The most likely number of clusters was determined via the ΔK approach values and the mean posterior probability ($\ln p(D)$) values (Evanno et al., 2005), as implemented in Structure Harvester 0.56.3 (Earl and vonholdt, 2012). The final results were visualized as bar plots by finding the optimal alignment of the ten replicate analyses of the ‘best’ K in CLUMPP 1.1 (Jakobsson & Rosenberg, 2007) and visualized with DISTRUCT 1.1 (Guillot et al., 2012). Third, we conducted principal coordinate analysis (PCoA) on the basis of the covariance of the genetic distance matrix via GenAl 6.41 (Piry et al., 1999). Fourth, we assessed the hierarchical partitioning of genetic structure among groups through analysis of molecular variance (AMOVA), which was performed via Arlequin 3.0 (Excoffier et al., 2005). As mentioned previously, populations were grouped into (1) genetic structure, (2) year groups, and (3) month groups.

Results

Seasonal and interannual genetic variation

In this study, we used 8 microsatellite loci to genotype 1095 individuals from 50-month populations from 2012-2022 in Shenyang, Liaoning Province, Northeast China. Low null allele

frequencies per locus were observed, with an average of 0.066 (Table S3). Furthermore, the average F_{ST} values calculated with and without applying the EN correction were 0.159 and 0.145, respectively, and these values did not differ significantly. Therefore, the presence of null alleles did not affect the F_{ST} estimations (Table S4). The average number of alleles (N_a) varied from 2.680 in spe06 to 10.300 in spe09, with an overall average of 6.270. The maximum polymorphic information content (PIC) was 0.860 for spe09, whereas the minimum was 0.392 for spe06, resulting in an average PIC of 0.694. Eight microsatellite loci presented a low inbreeding coefficient (F_{IS}) in most BAW populations. The mean observed heterozygosity was 0.569, whereas the expected heterozygosity was 0.597 (Table S5).

Overall, the eight microsatellite loci selected in this study were modestly polymorphic. The average number of alleles (N_a) ranged from 2.625 in SY1408 to 7.625 in SY2009 and SY2209 (average = 6.270). The effective number of alleles (N_e) ranged from 1.766–4.295 (average = 3.510). The observed heterozygosity (H_o) ranged from 0.382–0.813, whereas the expected heterozygosity (H_e) ranged from 0.359–0.673. The unbiased expected heterozygosity (uHe) ranged from 0.367 in SY1408 to 0.750 in both SY2106 and SY2206 (Table S5). Furthermore, the interannual genetic variation analysis revealed that the mean observed heterozygosity ($H_o = 0.569$) was comparable to the mean expected heterozygosity ($H_e = 0.597$) across all the BAW populations (Table 1). The estimates of microsatellite genetic variation varied among the populations. example, the unbiased expected heterozygosity (uHe) ranged from 0.546 in 2014 to 0.680 in 2020. The average number of effective alleles (N_e) across the different BAW populations was 3.510. The average observed number of alleles (N_a) across microsatellite loci ranged from 6.500 in SY2012 to 10.750 in SY2019, with an overall mean value of 6.270 (Table 1). Additionally, the highest number of private alleles was detected in SY1709 and SY2008 ($A_p = 0.164$).

Table 1

Population genetic differentiation

On the basis of the microsatellite data, pairwise F_{ST} values for genetic differentiation ranged from 0 to 0.473, with 1,038 out of 1,225 comparisons showing significant differences. Overall, a high

level of genetic differentiation among the populations was observed, with an average F_{ST} of 0.1452. Only a few pairwise F_{ST} comparisons revealed no genetic differentiation, as indicated by the low pairwise F_{ST} values (Figure 1).

Figure 1

Temporal genetic structure

POPTREE analysis based on microsatellite data

A comparison of samples taken at different times of the year revealed that the BAW genetic signature population in Shenyang significantly increased throughout the year. On the basis of microsatellite data, the unrooted neighbor-joining tree, which included the 50 BAW seasonal populations, revealed two major clades: the SY2012-2018 group and the SY2019-2022 group (Figure 2). One clade corresponded to 28 populations collected from 2012–2018, and the second clade was composed of the remaining 22 populations collected from 2019–2022.

Figure 2

Bayesian clustering

Using microsatellite data, we applied a clustering algorithm in STRUCTURE 2.3.3 to analyse the relationships among the 50 BAW populations in Shenyang (Figure 3). The mean $LnP(D)$ values gradually increased from $K = 2$, suggesting that this is likely the optimal number of primary clusters. The highest value of ΔK was reached at $K = 2$. This result was consistent with the hypothesis that these populations could be divided into two groups: the SY2012-2018 group and the SY2019-2022 group (Figure 3, Figure 4). One clade corresponded to 28 populations collected from 2012–2018, and the second clade was composed of the remaining 22 populations collected from 2019–2022. This finding aligned with the results from the NJ phylogenetic tree analyses.

Figure 3

Figure 4

Principal coordinate analysis (PCoA)

Population-based PCoA was performed on the basis of Nei's genetic distance matrix derived from the allele frequencies of the eight microsatellite markers in the 50 BAW populations (Figure 5).

The first and second axes explained 37.19% and 62.1% of the total variance, respectively. The PCoA results indicated there were two genetic groups, which were consistent with those shown above for the neighbor-joining tree (Figure 2) and Bayesian clustering was performed on the same data (Figure 3), which were based on data from 50 BAW seasonal populations.

Figure 5

Analysis of molecular variance (AMOVA)

The global AMOVA of the microsatellite genotype data from the 50 BAW populations indicated that most genetic variation was partitioned between populations and individuals within those populations. Approximately 82.12% of the total genetic variation was attributed to individuals within populations, whereas 17.88% was attributed to variation among populations. Interannual AMOVA indicated that 15.36% of the total genetic variation could be attributed to differences between major groups, whereas the majority of the variation (81.16%) was due to variation within populations. When $K = 2$, AMOVA revealed that 21.74% of the total genetic variation was explained by differences between major groupings, with the most variation (73.13%) occurring within populations. Additionally, the largest genetic difference among groups ($F_{CT} = 0.217$) for $K = 2$ was observed among the three different groups. Therefore, the group with $K = 2$ was considered the optimal grouping for these 50 BAW populations (Table 2). These results suggest that the BAW populations in Liaoning are composed of two distinct genetic groups, which is consistent with the findings from the NJ tree, PCoA, and STRUCTURE analyses.

Table 2

Discussion

A comprehensive understanding of the genetic makeup of migratory pest populations is crucial for developing forecasting tools, biosecurity protocols, and sustainable management practices (Simon and Peccoud, 2018). Despite this importance, there is still a lack of critical insights into long-range dispersal events influenced by allelic drift and migration (Rosetti and Remis, 2012). BAW is a polyphagous species that feeds on more than 300 plant species, indicating its significant adaptive potential (Adamczyk et al., 2009; Guo et al., 2010). Originally from South Asia, it is now widely

distributed across many major crop-producing areas in China (Wei et al., 2013; Luo et al., 2000). Understanding seasonal genetic variation and genetic structure can offer valuable insights into the evolutionary and ecological processes of this species.

N_e and N_a are critical genetic diversity parameters in pest control. For example, high values of N_a typically indicate that pest populations possess strong adaptive capacity and significant potential for resistance evolution in response to control measures, thus necessitating more refined management approaches. Understanding N_e in pest populations is crucial for evaluating the potential for resistance development and gauging the effectiveness of control strategies. In addition, a low value of N_e may increase the susceptibility of a population to resistance evolution, potentially accelerating the accumulation and spread of resistance genes. Therefore, both the genetic diversity index directly influences the genetic fitness of populations and the rate of resistance development, making the monitoring of these indicators essential for predicting control effectiveness and devising more effective management strategies. In this study, the microsatellite markers used presented a high average number of alleles (N_a), indicating that they are potentially informative tools for population genetics analysis of this species (Kalinowski 2004; Rueda et al. 2011). Populations frequently exhibit substantial adaptive genetic variation, enabling rapid responses to environmental changes (Bitter et al., 2019; Brennan et al., 2019). Fluctuating selection can result in stable oscillations in the relative abundance or frequency of various alleles within a population, especially when these alleles correspond to phenotypes adapted to the differing environments encountered throughout the year (such as winter and summer morphs; Bergland et al., 2014). Seasonal genetic variation analysis revealed that the average number of alleles (N_a) ranged from 2.625 in SY1408 to 7.625 in SY2009 and SY2209 (average = 6.270), and the effective number of alleles (N_e) ranged from 1.766–4.295 (average = 3.510). In contrast, several studies have indicated low levels of genetic diversity in other migratory Lepidoptera, such as monarch butterflies (*Danaus plexippus*) (Lyons et al., 2012). In addition, private alleles are crucial in understanding genetic differentiation between populations, species origin, and evolutionary history. Notably, the highest number of private alleles in SY1709 and SY2008, which indicated

that the two populations are genetically distinct, likely evolved in isolation, and have different genetic traits, potentially due to separate evolutionary histories and different environmental pressures. Both populations may have adapted to different environments or ecological niches. The private alleles in each population may reflect unique adaptations to local conditions, suggesting that each population has evolved in response to different selective pressures.

Rapid climate change is likely to produce a range of new selection pressures on populations (Hoffmann and Carla, 2011). Evolutionary adaptation could be an important way for natural populations to counter rapid climate change (Kanarek and Webb, 2010). Widespread insect species often exhibit considerable genetic variation in various traits that are likely involved in climatic adaptation (e.g., seasonal timing) (Bradshaw & Holzapfe) and resistance to stressful climatic conditions (Hoffmann et al., 2003). In general, species capable of dispersal exhibit minimal genetic differentiation between populations. While BAW is a significant agricultural pest, there is limited information available regarding its dispersal ability (Fu et al., 2017). Migration typically homogenizes genetic differentiation among populations (Wei et al., 2013). Our previous work revealed asymmetric migration between the eastern and western BAW populations in China, with the eastern population exhibiting a greater proportion of potential migrants. Additionally, the East Asian monsoon in the eastern range facilitates BAW migration and promotes gene flow (Wang et al., 2023). However, the seasonal population genetic differentiation of this pest has rarely been studied. In this study, a high level of genetic differentiation among the seasonal populations was detected (average $F_{ST} = 0.1452$) (Figure 1). Spatiotemporal separation can lead to random genetic drift and adaptive mutations, ultimately resulting in reproductive isolation and speciation (Wang et al., 2023). Genetic divergence is likely to be particularly pronounced in genes associated with migration and those subjected to strong selection pressures.

Understanding population genetic structure provides valuable insights into the evolutionary and ecological processes of species. Microsatellite data reveal a temporal population structure, where genetic turnover leads to a distinct temporal genetic pattern (Che et al., 2015). Rapid genetic turnover in populations of *Bemisia tabaci* was also investigated in an agricultural landscape in the

lockyer Valley, Australia. There was significant temporal variation in local genetic composition from the beginning to the end of the season, and the temporal genetic structure greatly exceeded the spatial structure. Additionally, two genetic clusters were obtained (Dinsdale et al., 2012). Aphids also displayed rapid temporal genetic changes and low variation in population structure between sample sites (Sunnucks et al., 1997), as well as temporal genetic changes in *Helicoverpa virescens* (Han and Caprio, 2004). These studies suggest that agricultural pest insects can be strongly influenced by cropping practices, as well as spatial and temporal factors. Similar findings have been reported in the diamondback moth *Plutella xylostella*, where climatic variables contribute to genetic differentiation between temperate and subtropical regions (Wei et al., 2013). In this study, we investigated the seasonal genetic diversity and distinctly genetic structure of BAW in Shenyang, Liaoning Province, Northeast China (Fig 2-Figure 5). One clade corresponded to 28 populations collected from 2012-2018, and the second clade was composed of the remaining 22 populations collected from 2019-2022. Genetic turnover in populations of BAW were occurred. The factors causing genetic turnover may be multifaceted. One reason is related to the local rapidly changing climatic conditions (temperature, humidity, precipitation, etc.), host nutrition, natural enemies and other factors (Ma et al., 2024). For example, the average annual temperature was higher than the long-term average in 2019, ranking as the fifth warmest year since 1951. The national average temperature was 10.34 °C, which was 0.79 °C higher than the long-term average. The temperature in Northeast China also significantly increased, with all four seasons being warmer, especially spring and autumn (Li et al., 2020). Additionally, precipitation patterns may affect the migration and settlement of BAW. Areas with abundant precipitation may provide more suitable habitats, promoting the spread of the population and gene exchange (Han, 2004). In 2019, the national average precipitation was 645.5 millimeters, which was 2.5% greater than the long-term average. The Northeast Region receives more precipitation, characterized by an early start and late end of the rainy season and increased rainfall (Li et al., 2020). Humidity also affects the survival and reproduction of BAW. Higher humidity may be beneficial for the survival and development of its larvae, thereby affecting the genetic diversity of the population (Han, 2004).

The planting ratio and distribution of different crops may affect the population dynamics of insects. Large-scale planting of a single crop may provide abundant food resources, promoting the reproduction and spread of insects, thereby affecting its genetic structure (Avisé et al., 1987). In addition, genetic drift as the season progresses may have contributed to changes in genotype frequencies. Such explanations may be related to temperature and/or farming practices (i.e. insecticides). In 2019, the first capture date was earlier than in previous years, the trapped amounts of BAW and migration duration is obviously higher than previous years trapped by sex pheromone traps in Shenyang. The damage caused by BAW larvae is significantly more severe compared to previous years. Therefore, multiple applications of chemical pesticides (e.g., emamectin) have been carried out. Resistance selection pressure causes a change in the frequency of specific genes in the population, resulting in genetic turnover. This change is usually due to the frequent spread of resistance genes, resulting in a gradual increase in the proportion of resistant individuals in the population, thus altering the genetic makeup of the population. This genetic turnover can impact the effective control of BAW. For example, monitoring genotype aggregation allows for early detection of changes in the genetic composition of pest populations, such as the emergence of resistance (Che et al., 2015). This enables pest managers to quickly adjust their strategies, helping prevent the escalation of infestations or the development of resistance.

Resistance to insecticides in insects exemplifies evolutionary adaptation to environmental changes (Ffrench-Constant et al., 2004). For several decades, cultural and chemical control methods have been employed to prevent the spread and damage caused by BAW. Currently, there are more than 586 instances of pesticide resistance among BAW populations worldwide (Akhtar et al., 2011), with resistance to new chemistries emerging in several Asian countries (Che et al., 2015; Ahmad et al., 2018). Therefore, quantifying the level of resistance to insecticides and investigating the distribution of resistance genes in relation to the genetic structure and gene flow among BAW populations in China are essential. In addition, understanding the dispersal ability, genetic structure, and population demography of this pest is crucial for both elucidating the theoretical aspects of its evolution and effectively implementing pest forecasting systems. In the future, we

will investigate the population genetic differentiation and structure of BAW at the genomic level to reveal its evolutionary relationships and reconstruct its population history in the future. This research enhances our understanding of how BAW adapts to climate and ecological factors at the genomic level. Additionally, by elucidating the influence of monsoon patterns on the migration dynamics of BAW, we can improve predictions regarding the magnitude, timing, and geographic distribution of immigrant pest populations in China. Thus, while characterizing the fine-scale seasonal genetic structure of BAW in northern China, our work will also clarify its large-scale temporal migration dynamics and provide vital information for refining monitoring, forecasting, and integrated pest management (IPM) strategies.

Conclusions

This study provides further data on the seasonal genetic variation and genetic structure of BAW in northern China. The results support moderate levels of genetic variation and two genetically distinct groups among 50 BAW seasonal populations from 2012-2018. These unique insights into BAW population genetics will aid in the development of strategies for managing this highly migratory pest.

Acknowledgements

We are grateful to associate researcher Xianming Yang at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, for providing valuable comments and suggestions. We are also grateful to many experts for providing important information about BAW and for their enthusiastic help during our field surveys.

Additional Information and Declarations

Funding

This work was supported by the Science & Technology Fundamental Resources Investigation Program (Grant No. 2023FY100500), the Natural Science Foundation of Liaoning Province of

China (No. 2023-MS-209), and the National Key R & D Program of China (2021YFD1400200).

Grant Disclosures

The following grant information was disclosed by the authors:
the Science & Technology Fundamental Resources Investigation Program (Grant No. 2023FY100500), the Natural Science Foundation of Liaoning Province of China (No. 2023-MS-209), and the National Key R & D Program of China (2021YFD1400200).

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

Ming-Li Yu and Xian-Zhi Xiu conceived and designed the experiments, analysed the data, wrote the paper, and contributed reagents/materials/analysis tools.

Ming-Li Yu¹ and Jin-Yang Wang performed the experiments, analysed the data, wrote the paper, and prepared the figures and/or tables.

Ming-Li Yu, Xian-Zhi Xiu, Xin-Yi Cao and Fa-Liang Qin performed the experiments and analysed the data.

Xing-Ya Wang and Li-Hong Zhou contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, and approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers): Field experiments were approved by the Chinese Academy of Agricultural Sciences (project number: 201003025).

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in Table S2.

Supplemental Information

The following information was supplied regarding the data availability:

The data are available in the Supplemental Information.

References

Adamczyk JrJJ, Williams MR, Reed JT, Hubbard DW, Hardee DD. 2009. Spatial and temporal occurrence of beet armyworm (Lepidoptera: Noctuidae) moths in Mississippi. *Florida Entomologist* **86**: 229–232. DOI 10.1653/0015-4040(2003)086[0229:satoob]2.0.co;2.

Aggarwal RK, Hendre PS, Varshney RK, Bhat PR, Krishnakumar V, Singh L. 2007. Identification, characterization and utilization of EST–derived genic microsatellite markers for genome analyses of coffee and related species. *Theoretical and Applied Genetics* **114**: 359–372. DOI 10.1007/s00122-006-0440-x.

Ahmad M, Farid A and Saeed M. 2018. Resistance to new insecticides and their synergism in *Spodoptera exigua* (Lepidoptera: Noctuidae) from Pakistan. *Crop Protection* **107**: 79–86. DOI 10.1016/j.cropro.2017.12.028.

Akhtar M, Mahadevan S, Paquet A, Arthropod pesticide resistance database. Available: <http://www.pesticideresistance.org/> [19 June 2011].

Avice J C, Arnold J, Ball R M, Bermingham E, Lamb T, Neigel J E, Reeb C A, Saunders N C. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology Evolution and Systematics* **18**: 489-522. DOI 10.1146/annurev.es.18.110187.002421.

Bergland AO, Behrman EL, O'Brien KR, Schmidt PS, Petrov DA. 2014. Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in *Drosophila*. *PLoS genetics* **10** :11. DOI 10.1371/journal.pgen.1004775.

- Bitte MC, Kapsenberg L, Gattuso JP, Pfister CA. 2019. Standing genetic variation fuels rapid adaptation to ocean acidification. *Nature Communications* **10**: 1–10. DOI 10.1038/s41467-019-13767-1.
- Bradshaw WE, Holzapfel CM. 2008. Genetic response to rapid climate change: it's seasonal timing that matters. *Molecular Ecology* **17**, 157–166. DOI 10.1111/j.1365-294X.2007.03509.x.
- Brennan RS, Garrett AD, Huber KE, Hargarten H, Pespeni MH. 2019. Rare genetic variation and balanced polymorphisms are important for survival in global change conditions. *Proceedings of the Royal Society B* **286**: 20190943. DOI 10.1098/rspb.2019.0943.
- Chaufaux J, Ferron P. 1986. Sensibilite differente de deux populations de *Spodoptera exigua* Hub. (Lepid., Noctuidae) aux baculovirus et aux pyrethrinoides de synthese. *Agronomie* **6**: 99–104. DOI 10.1051/AGRO:19860109.
- Che W, Huang J, Guan F, Wu Y and Yang Y. 2015. Cross-resistance and inheritance of resistance to emamectin benzoate in *Spodoptera exigua* (lepidoptera: noctuidae). *Journal of Economic Entomology*. **108**: 1–6. DOI 10.1093/jee/tov168.
- Che W, Huang J, Guan F, Wu Y, Yang Y. 2015. Cross-resistance and inheritance of resistance to emamectin benzoate in *Spodoptera exigua* (lepidoptera: noctuidae). *Journal of Economic Entomology* **108**:1–6. DOI 10.1093/jee/tov168.
- Dean CB, Nielsen JD. 2007. Generalized linear mixed models: A review and some extensions. *Lifetime Data Analysis* **13**: 497–512. DOI 10.1007/s1098 5-007-9065-x
- Dinsdale A, Schellhorn NA, Barro PD, Buckley YM and Riginos C. 2012. Rapid genetic turnover in populations of the insect pest *Bemisia tabaci* Middle East: Asia Minor 1 in an agricultural landscape. *Bulletin of Entomological Research* **102**: 539–549. DOI 10.1017/S0007485312000077.
- Earl DA, vonHoldt BM. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resource* **4**: 359–361. DOI 10.1007/s1268 6-011-9548-7.

- Evanno GS, Regnaut SJ, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611–2620. DOI 10.1111/j.1365-294X.2005.02553.x.
- Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetic data analysis. *Evolutionary Bioinformatics online* **1**: 47–50. DOI 10.1143/JJAP.34.L418.
- Fairley TL, Renaud TM, Conn JE. 2000. Effects of local geographic barriers and latitude on population structure in *Anopheles punctipennis* (Diptera: Culicidae). *Journal of Medical Entomology* **5**: 754–60. DOI 10.1603/0022-2585-37.5.754.
- Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**: 1567–1587. DOI 10.1093/genetics/164.4.1567.
- Feng, HQ, Wu KM, Cheng DF, Guo YY. 2003. Radar observation of the autumn migration of the beet armyworm, *Spodoptera exigua*, and other moths in northern China. *Bulletin of Entomological Research* **93**: 115–24. DOI 10.1079/BER2002221.
- Ffrench–Constant RH, Daborn PJ, Goff GL. 2004. The genetics and genomics of insecticide. *Trends in Genetics* **20**: 163–170. DOI 10.1016/j.tig.2004.01.003.
- Fu X, Feng H, Liu Z, Wu K. 2017. Trans-regional migration of the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), in North-East Asia. *PLoS One* **12**: e0183582. DOI 10.1371/journal.pone.0183582.
- Fuentes-Contrera E, Espinoza JL, Lavandero B, Ramírez CC. 2008. Population genetic structure of codling moth (Lepidoptera: Tortricidae) from apple orchards in central Chile. *Journal of Economic Entomology* **101**: 190–198. DOI 10.1603/0022-0493(2008)101[190:PGSOCM]2.0.CO;2.
- Golikhajeh N, Naseri B, Razmjou J, Hosseini R and Aghbolaghi MA. 2018. Genetic variation of beet armyworm (lepidoptera: noctuidae) populations detected using microsatellite markers in Iran. *Journal of Economic Entomology* **111**: 1404–1410. DOI 10.1093/jee/toy050.

- Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* **86**: 485–486. DOI 10.1093/oxfordjournals.jhered.a111627.
- Guillot, G., Renaud, S., Ledevin, R., Michaux, J. & Claude, J. 2012. A unifying model for the analysis of phenotypic, genetic and geographic data. *Systematic Biology* **61**: 897–911. DOI 10.1093/sysbio/sys038.
- Guo JY, Wu G, Wan FH. 2010. Activities of digestive and detoxification enzymes in multiple generations of beet armyworm, *Spodoptera exigua* (Hübner), in response to transgenic Bt cotton. *Journal of Pest Science* **83**: 453–460. DOI 10.1007/s10340-010-0315-4.
- Han Q, Caprio MA. 2004. Evidence from genetic markers suggests seasonal variation in dispersal in *Heliothis virescens* (Lepidoptera: Noctuidae). *Environmental Entomology* **33**: 1223–1231. DOI 10.1603/0046-225X-33.5.1223.
- Hearne CM, Ghosh, S, Todd, JA. 1992. Microsatellites for linkage analysis of genetic traits. *Trends in Genetics* **8**: 288–301. DOI 10.1016/j.sbspro.2012.01.068.
- Hebert PDN, Cywinska A, Ball SL, Dewaard R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* **270**: 313–321. DOI 10.1098/rspb.2002.2218.
- Hoffmann AA, Carla MS. 2011. Climate change and evolutionary adaptation. *Nature* **470**, 480–485. DOI 10.1038/nature09670.
- Jakobsson M, Rosenberg NA. 2007. CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801–1806. DOI 10.1093/bioinformatics/btm233.
- Kalinowski S. 2004. Do polymorphic loci require large sample sizes to estimate genetic distances? *Heredity* **94**:33–36. DOI 10.1038/sj.hdy.6800548.
- Kanarek A, Webb C. 2010. Allee effects, adaptive evolution, and invasion success. *Evolutionary Applications* **3**: 122–135. DOI 10.1111/j.1752-4571.2009.00112.x.
- Kim M, Kim H, Kwon DH, Lee S. 2012. Isolation and characterization of microsatellite loci from *Spodoptera exigua* (Lepidoptera: Noctuidae). *Applied Entomology and Zoology* **47**: 149–152.

DOI 10.1007/s13355-012-0094-x.

Lavandero B, Miranda M, Ramirez CC, Fuentes-Contreras E. 2009. Landscape composition modulates population genetic structure of *Eriosoma lanigerum* (Hausmann) on *Malus domestica* Borkh in central Chile. *Bulletin of Entomological Research* **99**: 97–105. DOI 10.1017/S0007485308006196.

Li Y, Zeng HL, Wang GF. 2020. Climatic Characteristics and Major Meteorological Events over China in 2019. *Meteorological Monthly* **46**: 547–555. DOI 10.7519/j.issn.1000-0526.2020.04.009.

Luo LZ, Cao YZ, Jiang XF. 2000. The occurrence and damage characteristics analysis of the beet armyworm. *Plant Protection*. **26**: 37–39. DOI 10.3969/j.issn.0529-1542.2000.03.017.

Lyons JJ, Pierce AA, Barribeau SM, Sternberg ED, Mongue AJ, De Roode, JC. 2012. Lack of genetic differentiation between monarch butterflies with divergent migration destinations. *Molecular Ecology* **21**: 3433–3444. DOI 10.1111/j.1365-294X.2012.05613.x.

Ma HT, Zhou LH, Tan H, Xiu XZ, Wang JY, Wang XY. 2024. Population dynamics and seasonal migration patterns of *Spodoptera exigua* in northern China based on 11 years of monitoring data. *PeerJ*. **12**: e17223. DOI 10.7717/peerj.17223.

Nater A, Arora N, Greminger MP, van Schaik CP, Singleton I. 2013. Marked population structure and recent migration in the critically endangered *Sumatran orangutan* (*Pongo abelii*). *Journal of Heredity*. **1**: 2–13. DOI 10.1093/jhered/ess065.

Niu CW, Zhang QW, Ye ZH, Luo LZ. 2006. Analysis of genetic diversity in different geographic populations of the beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) with AFLP technique. *Acta Entomologica Sinica* **49**: 867–873. DOI 10.1016/S1872-2067(06)60034-X.

Pauls SU, Nowak C, Bálint M, Pfenninger M. 2013. The impact of global climate change on genetic diversity within populations and species. *Molecular Ecology* **22**: 925–946. DOI 10.1111/mec.12152.

Peakall ROD, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288–295. DOI:

10.1111/j.1471-8286.2005.01155.x.

Piry S, Luikart G, Cornuet, JM. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*. **90**: 502–503. DOI 10.1093/jhered/90.4.502.

Prugh LR, Hodges KE, Sinclair ARE, Brashares JS. 2008. Effect of habitat area and isolation on fragmented animal populations. *Proceedings of the National Academy of Sciences of the United States of America*. **52**: 20770–20775. DOI 10.1073/pnas.0806080105.

Raymond M, Rousset F. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*. **86**: 248–249. DOI 10.1093/oxfordjournals.jhered.a111573.

Rosetti N, Remis MI, 2012. Spatial genetic structure and mitochondrial DNA phylogeography of argentinean populations of the grasshopper *Dichroplus elongatus*. *PLoS One* **7**: e40807. DOI 10.1371/journal.pone.0040807

Rueda EC, Sommer J, Scarabotti P, Markariani R, Ortí G. 2011. Isolation and characterization of polymorphic microsatellite loci in the migratory freshwater fish *Prochilodus lineatus* (Characiformes: Prochilodontidae). *Conservation Genetics Resources*. **3**: 681–684. DOI 10.1007/s12686-011-9432-5.

Si SY, Zhou LL, Wang SL, Jiang XF, Xu ZF, Mu W, Wang DS, Wang XP, Chen HT, Yang YH, Ji XC. 2012. Progress in research on prevention and control of beet armyworm, *Spodoptera exigua* in China. *Chinese Journal of Applied Entomology* **49**: 1432–1438.

Simon JC, Peccoud J. 2018. Rapid evolution of aphid pests in agricultural environments. *Current Opinion In Insect Science* **26**: 17–24. DOI 10.1016/j.cois.2017.12.009.

Sunnucks P, DeBarro PJ, Lushai, G, Maclean N, Hales D. 1997. Genetic structure of an aphid studied using microsatellites: cyclic parthenogenesis, differentiated lineages and host specialization. *Molecular Ecology* **6**: 1059–1073. DOI 10.1046/j.1365-294X.1997.00280.x.

Susanta KB. 2006. Molecular marker systems in insects: current trends and future avenues. *Molecular Ecology*. **15**: 3087–3113. DOI 10.1111/j.1365-294X.2006.03014. x.

- Takezaki N, Nei, M, Tamura, K. 2009. POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. *Molecular Biology and Evolution* **27**: 747–752. DOI 10.1093/molbev/msp312.
- Van Oosterhout C, Hutchinson WF, Will DP, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**: 535–538. DOI 10.1111/j.1471-8286.2004.00684.x.
- Wang XY, Wang MM, Chen C, Wang XQ. 2020. Genetic variation and phylogeographic structure of *Spodoptera exigua* in western China based on mitochondrial DNA and microsatellite markers. *PLoS One*. **15**: e0233133. DOI 10.1371/journal.pone.0233133.
- Wang XY, Zhou LH, Zhong T, Xu GQ. 2014. Genetic variation and phylogeographic structure of *Spodoptera exigua* in the welsh onion producing areas of North China. *Journal of Applied Entomology* **138**: 612–622. DOI 10.1111/jen.12102.
- Wang XY, Zhou LH. 2016. Genetic diversity and population history among geographic populations of *Spodoptera exigua* in North China based on mtDNA *Cytb* gene sequences. *Acta Ecologica Sinica* **36**: 2337–2347. DOI 10.5846/stxb201410041952.
- Wang XY, Yang XM, Zhou LH, Wyckhuys KAG, Jiang S, Nguyen Van Liem, Le Xuan Vi, Abid Ali, Kongming Wu. 2022. Population genetics unveils large-scale migration dynamics and population turnover of *Spodoptera exigua*. *Pest Management Science* **78**: 612–625. DOI 10.1002/ps.6670
- Wang YM, Shen ZR, Gao LW. 2007. Microsatellite markers and their application in aphid population biology. *Acta Entomologica Sinica* **50**: 621–627. DOI 10.3321/j.issn:0454-6296.2007.06.012.
- Wei J, Chen HT, Cui JH, Zhou SH. 2010. Bibliometric analysis on the study of *Spodoptera exigua* from 1989 to 2010 in China. *Journal of Changjiang Vegetables* 124–127. DOI 0.3865/j.issn.1001-3547.2010.18.040.
- Wei SJ, Shi BC, Gong Y J, Jin GH, Chen XX, Meng XF. 2013. Genetic structure and demographic history reveal migration of the diamondback moth *Plutella xylostella* (Lepidoptera:

609 Plutellidae) from the southern to northern regions of China. *PLoS One* **8**:e59654. DOI
 610 10.1371/journal.pone.0059654.

611 Zheng XL, Cong XP, Wang XP, Lei CL. 2011. A review of geographic distribution, overwintering
 612 and migration in *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). *Journal of the*
 613 *Entomological Research Society* **13**: 39–48.

614 Zheng XL, Wang P, Wang XP, Lei C.L. 2009. Main biological habits, occurrence reason analyses
 615 and control of *Spodoptera exigua* in welsh onion. *Journal of Changjiang Vegetables*. 4–7.
 616 DOI 10.3865/j.issn.1001-3547.2009.18.002.

617 Zhou LH, Wang XY, Xu GQ, Lei JJ. 2017. Mitochondrial DNA phylogeography of *Spodoptera*
 618 *exigua* across a broad geographic area in China. *Journal of Applied Entomology* **141**: 527–
 619 539. DOI 0.1111/jen.12371.

620 Zhu G, Gu X, Wang S, Zhang Y, Hu X, Xu W. 2010. Occurrence and integrated pest management
 621 of beet armyworm, *Spodoptera exigua* in green Chinese onion in Tianjin. *Journal of*
 622 *Changjiang Vegetables*. 96–100. DOI 10.3865/j.issn.1001-3547.2010.18.031.

623 Zhu KX, Jiang S, Han L, Wang MM, Wang XY. 2020. Fine-scale genetic structure of the
 624 overwintering *Chilo suppressalis* in the typical bivoltine areas of northern China. *PLoS One*
 625 **15**: e0233133. DOI 10.1371/journal.pone.0243999.

Figure legends

Figure 1 Heatmap of pairwise F_{ST} values estimated from microsatellite data for 50 *Spodoptera exigua* populations collected in Shenyang, Liaoning Province in northern China. The colours indicate F_{ST} values ranging from purple for lower values to red for higher values. *indicates significant differences following Bonferroni correction. See Table S1 for population codes.

Figure 2 Unrooted neighbor-joining phylogenetic tree based on microsatellite data from 50 populations of *Spodoptera exigua* in northern China. The numbers beside the nodes indicate bootstrap support values above 50%.

Figure 3 Population structure analysis of 1095 individuals collected from 50 seasonal populations of *Spodoptera exigua* in northern China on the basis of eight microsatellite loci. The likelihood of the data is plotted against the number of genetic clusters (K) for (a) the mean posterior probability values (mean $\ln P(D)$ values) and (b) ΔK values. (c) Individual Bayesian assignment probabilities for $K = 2$ are shown, with each individual represented by a single vertical line. The sampling location codes can be found in Table S1.

Figure 4 Seasonal sampling locations of *Spodoptera exigua* and distribution of microsatellite lineages in northern China. The red triangle indicates the sampling site in Shenyang, Liaoning Province (a). Lineage 1 is represented in blue, whereas Lineage 2 is shown in red (b). Groups identified by STRUCTURE analysis of the microsatellite data are indicated. The population codes can be found in Table S1. The monitoring and sampling position map was created via ArcGIS Pro (<https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview>) on the basis of geographic coordinates. The base map utilized in the analysis originates from the World Bank (<https://datacatalog.worldbank.org/search/dataset/0038272>).

Figure 5 Principal coordinate analysis (PCoA) illustrating the relationships among 50 *Spodoptera exigua* seasonal populations in northern China, based on the genetic distance matrix of F_{ST} values derived from microsatellite data. The population codes are provided in Table S1.

Tables

Table 1 Seasonal genetic variation in *Spodoptera exigua* based on eight microsatellite loci in Shenyang, Liaoning Province, Northeast China from 2012-2022

Table 2 Results of the molecular variance analysis (AMOVA) for microsatellite markers

Figure 1

Figure 1

Heatmap of pairwise F_{ST} values estimated from microsatellite data for 50 *Spodoptera exigua* populations collected in Shenyang, Liaoning Province innorthern China. * indicates significant differences following Bonferroni correction. See Table S1 for population codes.

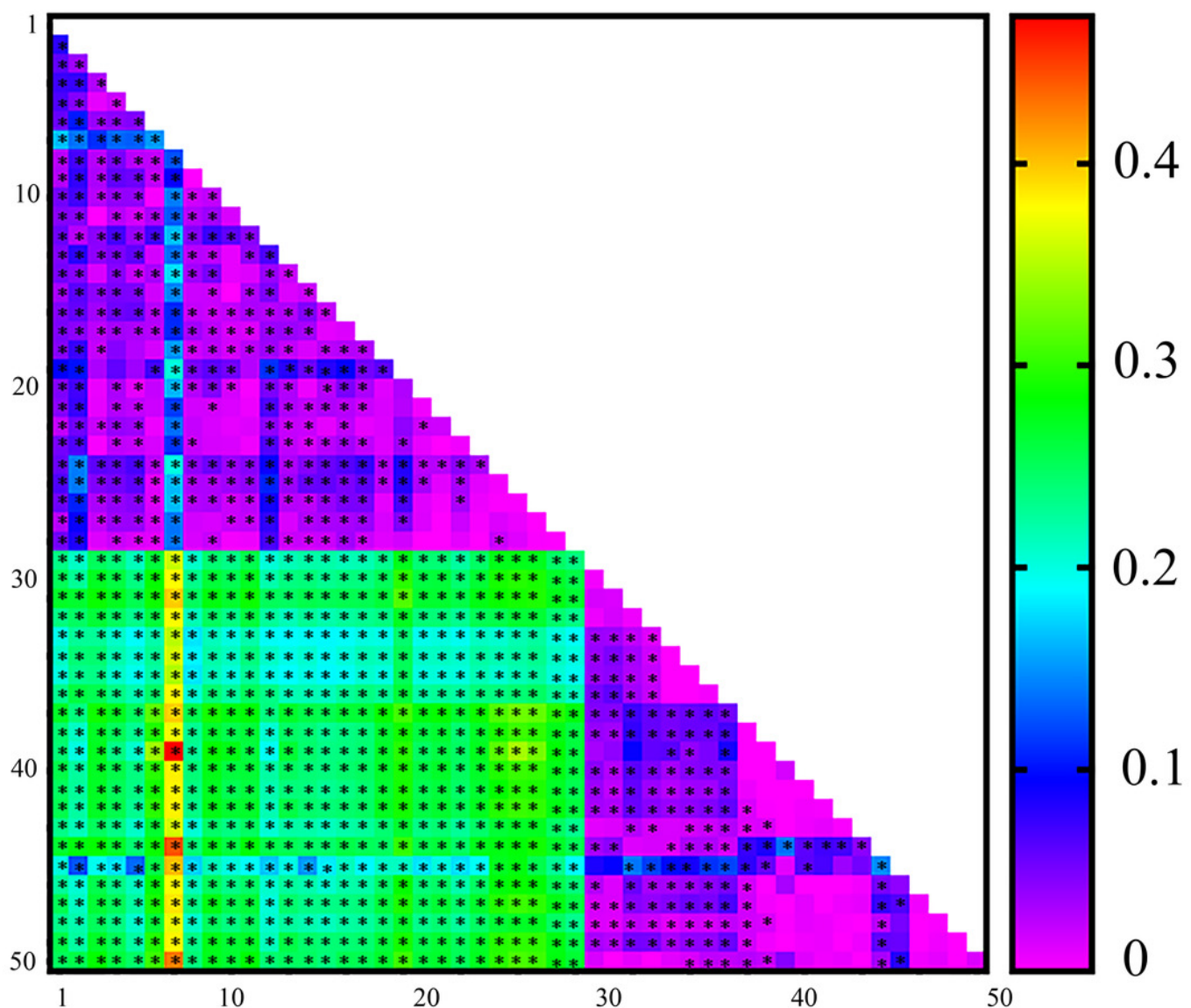


Figure 2

Figure 2

Unrooted neighbor-joining phylogenetic tree based on microsatellite data from 1  populations of *Spodoptera exigua* in northern China. The numbers next to the nodes represent bootstrap values.

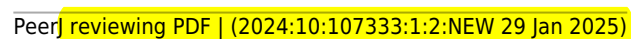


Figure 3

Figure 3

Population structure analysis of 1095 individuals collected from 50 seasonal populations of *Spodoptera exigua* in northern China on the basis of eight microsatellite loci. The likelihood of the data is plotted against the number of genetic clusters (K) for (a) the mean posterior probability values (mean $\ln P(D)$ values) and (b) ΔK values. (c) Individual Bayesian assignment probabilities for $K = 2$ are shown, with each individual represented by a single vertical line. The sampling location codes can be found in Table S1.

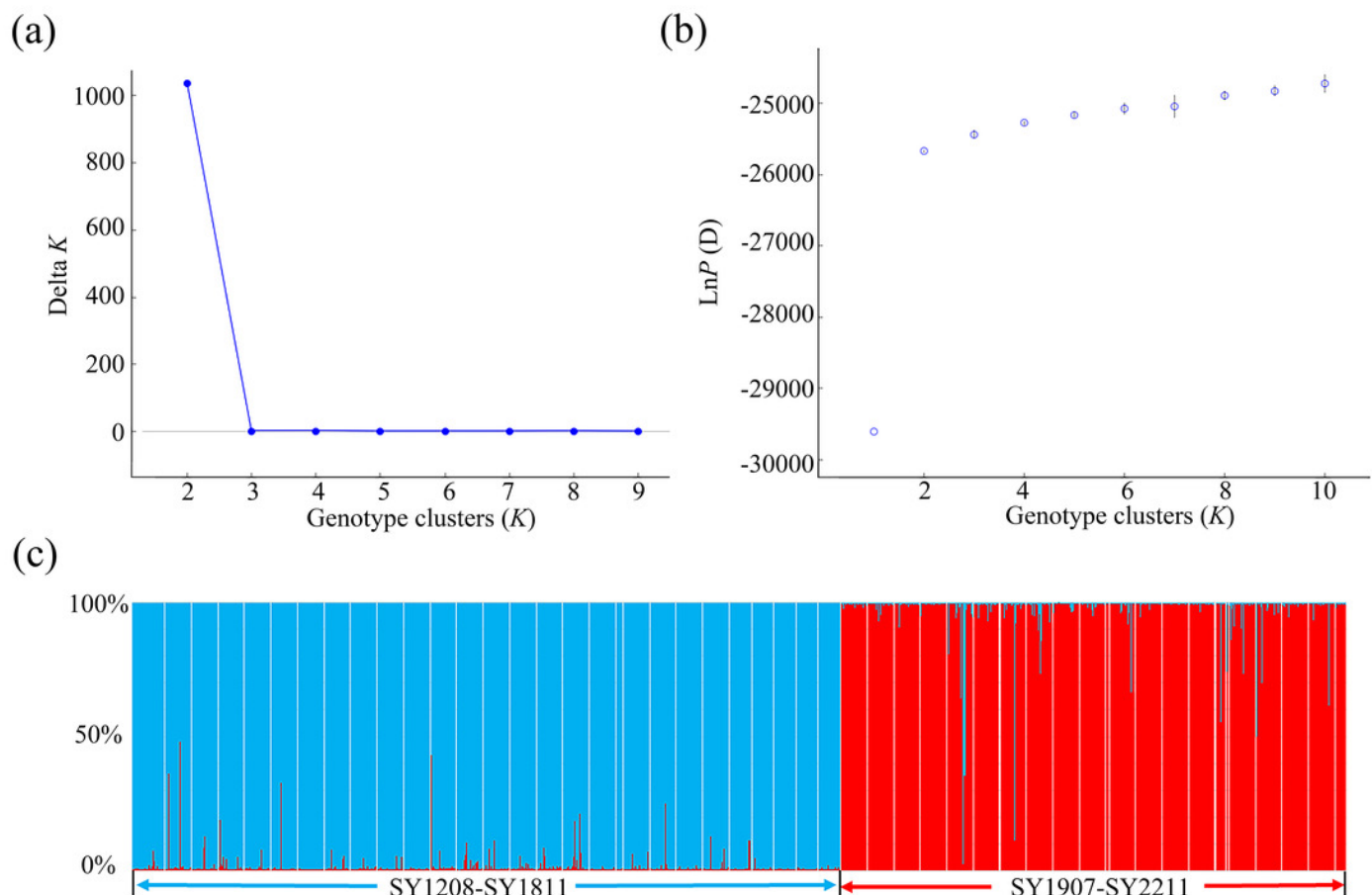


Figure 4

Figure 4

Figure 4 Seasonal sampling locations of *Spodoptera exigua* and distribution of microsatellite lineages in northern China. The red triangle indicates the sampling site in Shenyang, Liaoning Province (a). Lineage 1 is represented in blue, whereas Lineage 2 is shown in red (b). Groups identified by STRUCTURE analysis of the microsatellite data are indicated. The population codes can be found in Table S1. The monitoring and sampling position map was created via ArcGIS Pro (<https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview>) on the basis of geographic coordinates. The base map utilized in the analysis originates from the World Bank (<https://datacatalog.worldbank.org/search/dataset/0038272>).

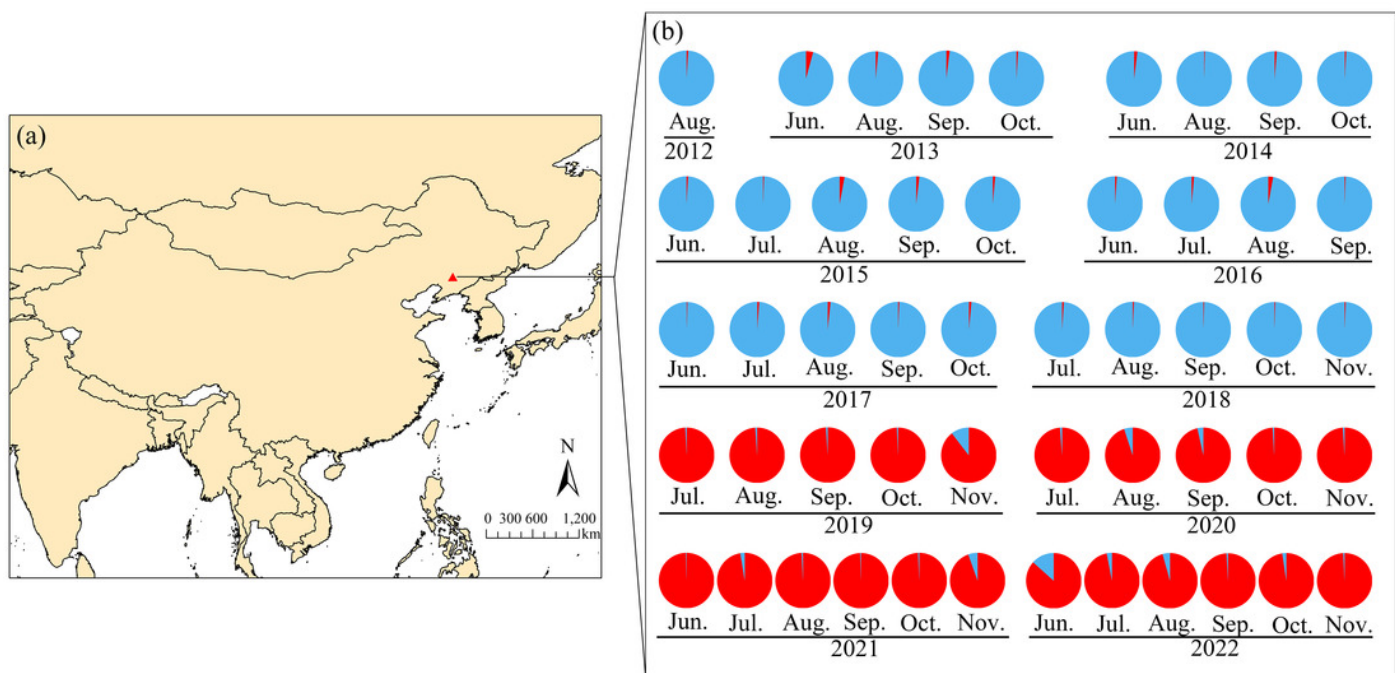


Figure 5

Figure 5

Principal coordinate analysis (PCoA) illustrating the relationships among 50 *Spodoptera exigua* seasonal populations in northern China, based on the genetic distance matrix of F_{ST} values derived from microsatellite data. Population codes are provided in Table S1.

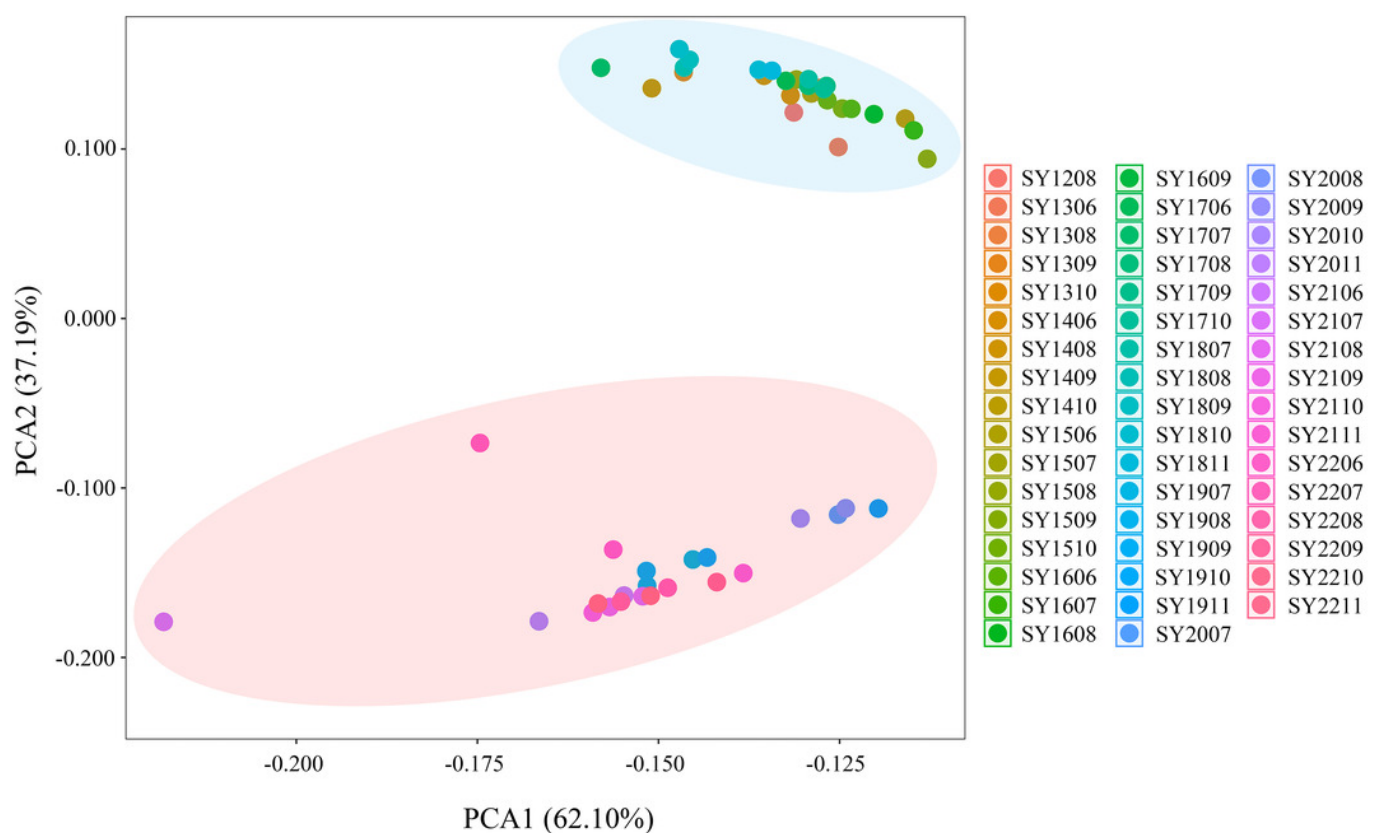


Table 1 (on next page)

Table 1

Seasonal genetic variation in *Spodoptera exigua* based on eight microsatellite loci in Shenyang, Liaoning Province, Northeast China from 2012-2022

Table 1 Seasonal genetic variation in *Spodoptera exigua* based on eight microsatellite loci in Shenyang, Liaoning Province, Northeast China from 2012-2022

Pop	N_a	N_e	I	H_o	H_e	uHe	A_p	H_s
SY2012	6.500	4.295	1.411	0.546	0.658	0.669	0.125	0.6100
SY2013	8.625	3.886	1.425	0.553	0.631	0.634	0.1250	0.6344
SY2014	8.000	2.943	1.212	0.452	0.543	0.546	0.2500	0.5470
SY2015	7.875	3.807	1.362	0.529	0.618	0.621	0.2500	0.6210
SY2016	9.125	3.777	1.403	0.526	0.612	0.615	0.3750	0.6159
SY2017	8.375	3.869	1.359	0.511	0.601	0.604	0.2500	0.6040
SY2018	7.625	3.675	1.279	0.521	0.561	0.564	0.1250	0.5643
SY2019	10.750	4.064	1.517	0.557	0.656	0.659	0.7500	0.6593
SY2020	10.625	4.209	1.527	0.688	0.677	0.680	0.6250	0.6801
SY2021	9.375	4.092	1.500	0.647	0.668	0.671	0.1250	0.6714
SY2022	10.625	4.105	1.527	0.670	0.668	0.671	0.2500	0.6710
Mean	6.270	3.510	1.272	0.569	0.597	0.619	0.052	0.6211

Abbreviations: N_a , observed number of alleles; N_e , effective number of alleles; I , Shannon’s information index; H_o , observed heterozygosity; H_e , expected heterozygosity; uHe , unbiased expected heterozygosity; A_p , number of private alleles; H_s , gene diversity.

Table 2(on next page)

Table 2

Results of the molecular variance analysis (AMOVA) for microsatellite markers

1 **Table 2** Results of the molecular variance analysis (AMOVA) for microsatellite markers

Source of variation	<i>d.f.</i>	Sum of squares	Variance components	Percentage variation (%)	<i>F</i> -statistics
Global analysis					
Among populations	49	1327.779	0.561 Va	17.88	
Within populations	2140	5510.564	2.575 Vb	82.12	$F_{ST}=0.179^{***}$
Total	2189	6838.343	3.136	100.00	
Hierarchical AMOVA ($K = 11$)					
Among groups	10	1042.527	0.487Va	15.36	$F_{ST} = 0.188^{***}$
Among populations within groups	39	285.207	0.111Vb	3.49	$F_{SC} = 0.041^{***}$
Within populations	2140	5510.564	2.575Vc	81.16	$F_{CT} = 0.153^{***}$
Total	2189	6838.343	3.173		
Hierarchical AMOVA ($K = 2$)					
Among groups	1	825.349	0.766 Va	21.74	$F_{ST} = 0.269^{***}$

Among populations within groups	48	502.430	0.181Vb	5.13	$F_{SC} = 0.066^{***}$
Within populations	2140	5510.564	2.575Vc	73.13	$F_{CT} = 0.217^{***}$
Total	2189	6838.343	3.521	100.00	

2 *d.f.*, degrees of freedom; $^{***}P < 0.001$: significance level. Abbreviations: F_{CT} genetic differences among groups, F_{SC} genetic differences within a group, F_{ST}
3 genetic differences among populations.

4
5
6