Seasonal genetic variation and genetic structure of *Spodoptera exigua* in northern China on the basis of 11 years of microsatellite data (#107333)

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Seasonal genetic variation and genetic structure of *Spodoptera exigua* in northern China on the basis of 11 years of microsatellite data

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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 ten 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.

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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 ten 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: beet armyworm; genetic differentiation; genetic structure; microsatellites; migration; seasonal ~~genetic~~ variation

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 are significant interannual and seasonal variations in the capture number of BAW based on 11 years of monitoring data in northern China, 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 (Meinke & Ware, 1978; 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 (Aggarwa et a 007; Wang et al., 2007; Zhu et al., 2020). Previous studies have focused on investigating the genetic variability and structure of BAW across different spatial scales (Wang et al., 2014; Wang and Zhou, 2016; Niu et al., 2006; Zhou et al., 2017). For example, previous studies have indicated that both mtDNA and microsatellite data indicate low levels of genetic diversity among all populations. Moderate genetic differentiation among some BAW populations and two genetically distinct groups in western China has been detected (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 individuals of BAW in the 50 seasonal populations were collected via three sex pheromone traps (Pherobio Technology Co. Ltd., Beijing, China) in a Welsh onion field (123.57°N, 41.82°E) over a period of 11 years, from June to October 2012 to 2022 in Shenyang, Liaoning Province. The number of trapped moths was recorded weekly, and the trap cores were replaced every two weeks. 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.

MSI amplification and genotyping. In this study, individuals were genotyped vi[comment]ght loci (Spe06, Spe07, Spe08, Spe09, Spe10, Spe11, Spe12, and Spe15) from a set of eight 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 μL of DNA template, 1× EasyTaq® buffer (containing 2 mM $MgCl_2$; TransGen Biotech Co., Ltd., Beijing, China), and 0.4 μM 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 were 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). 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 (*Na*), effective number of alleles (*Ne*), Shannon's information index (*I*), observed heterozygosity (H_O), expected heterozygosity (*He*), 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, 2002), 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., 2010). 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).

148 The Markov chain Monte Carlo simulation was run 10 times for each value of *K*, with a total of 5
149 × 10^5 iterations following a burn-in period of 5 × 10^4. The most likely number of clusters was
150 determined via the *ΔK* approach (Evanno et al., 2005), as implemented in Structure Harvester
151 0.56.3 (Earl and vonholdt, 2012). The optimal alignment of the ten replicate analyses for the "best"
152 *K* was achieved via CLUMPP 1.1 (Jakobsson, 2007) and visualized with DISTRUCT 1.1 (Guillot
153 et al., 2012). Third, we conducted principal coordinate analysis (PCoA) on the basis of the
154 covariance of the genetic distance matrix via GenAl 6.41 (Piry et al., 1999). Fourth, we assessed
155 the hierarchical partitioning of genetic structure among groups through analysis of molecular
156 variance (AMOVA), which was performed via Arlequin 3.0 (Excoffier et al., 2005). As mentioned
157 previously, populations were grouped into (1) genetic structure, (2) year groups, and (3) month
158 groups.
159
160 **Results**
161 **Seasonal and interannual genetic variation**
162 In this study, we used 8 microsatellite loci to genotype 1095 individuals from 50-month
163 populations during eleven years from 2012-2022 in Shenyang, Liaoning Province, Northeast
164 China. Low null allele frequencies per locus were observed, with an average of 0.066 (Table S3).
165 Furthermore, the average F_{ST} values calculated with and without applying the ENA correction
166 were 0.159 and 0.145, respectively, and these values did not differ significantly. Therefore, the
167 presence of null alleles did not affect the F_{ST} estimations (Table S4). The average number of alleles
168 (*Na*) varied from 2.680 in spe06 to 10.300 in spe09, with an overall average of 6.270. The
169 maximum polymorphic information content (*PIC*) was 0.860 for spe09, whereas the minimum was
170 0.392 for spe06, resulting in an average *PIC* of 0.694. Eight microsatellite loci presented a low
171 inbreeding coefficient (F_{IS}) in most BAW populations. The mean observed heterozygosity was
172 0.569, whereas the expected heterozygosity was 0.597 (Table S5).
173 Overall, the eight microsatellite loci selected in this study were modestly polymorphic. Seasonal
174 genetic variation analysis indicated that the average number of alleles (*Na*) ranged from 2.625 in

SY1408 to 7.625 in SY2009 and SY2209 (average = 6.270). The effective number of alleles (*Ne*) ranged from 1.766–4.295 (average = 3.510). The observed heterozygosity (*Ho*) ranged from 0.382–0.813, whereas the expected heterozygosity (*He*) 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. Furthermore, the interannual genetic variation analysis revealed that the mean observed heterozygosity (*Ho* = 0.564) was comparable to the mean expected heterozygosity (*He* = 0.627) across all the BAW populations. The estimates of microsatellite genetic variation varied among the populations. For example, the unbiased expected heterozygosity (*uHe*) ranged from 0.546 in 2014 to 0.680 in 2020. The average number of effective alleles (*Ne*) across the different BAW populations was 3.884. Additionally, the average observed number of alleles (*Na*) across microsatellite loci ranged from 6.500 in SY2012 to 10.750 in SY2019, with an overall mean value of 8.864 (Table 1).

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 did not indicate genetic differentiation, as evidenced 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 neighbor-joining tree and Bayesian clustering analyses, which were based on data from 1095 individuals, indicated the presence of two distinct groups, corroborating the effectiveness of the PCoA method.

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 F_{CT} for K = 2 was 0.217. The variation rates of the two groups were similar and statistically significant. Therefore, the group with K = 2 was considered the optimal grouping for these 50 BAW populations (Table 1). Consistent with the results from the NJ tree, PCoA, and STRUCTURE analyses, the AMOVAs also supported the existence of two distinct genetic groups.

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. Originally from South Asia, it is now widely distributed across many major crop-producing areas in China. Understanding seasonal genetic variation and genetic structure can offer valuable insights into the evolutionary and ecological processes of this species. In this study, the microsatellite markers used presented a high average number of alleles (*Na*), ranging from 2.625 to 7.625, 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). The microsatellite markers used in this study exhibited a high number of alleles, indicating that they are potentially informative tools

for population genetics analysis of this species (Kalinowski 2004; Rueda et al. 2011). The microsatellite data indicated moderate levels of genetic variation among all 50 BAW seasonal populations. Seasonal genetic variation analysis indicated that the average number of alleles (*Na*) ranged from 2.625 in SY1408 to 7.625 in SY2009 and SY2209 (average = 6.270). The effective number of alleles (*Ne*) ranged from 1.766–4.295 (average = 3.510). This result is consistent with those of previous studies (Niu et al., 2006; Wang et al., 2014; Wang et al., 2016). This high level of diversity is likely due to BAW not experiencing significant founder effects, genetic bottlenecks, or strong selection pressures (e.g., from insecticides) in this region. 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 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). 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). 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. In this study, neighbor-joining dendrograms, STRUCTURE

283 analysis, and principal coordinate analysis (PCoA) were used to identify two genetically distinct
284 groups: the SY2012-2018 group and the SY2019-2022 group. We also investigated the seasonal
285 genetic diversity and genetic structure of BAW in Shenyang, Liaoning Province, Northeast China,
286 via six temporal samples collected over 11 years. POPTREE, PCoA, STRUCTURE, and
287 AMOVAs, which are based on microsatellite data, divided all individuals into two clusters: the
288 SY2012-2018 group and the SY2019-2022 group (Fig 2-Figure 5). One clade corresponded to 28
289 populations collected from 2012-2018, and the second clade was composed of the remaining 22
290 populations collected from 2019-2022. This may be attributed to either relatively recent population
291 expansion or the capacity of migratory populations to sustain genetic diversity over time. Such
292 turnover can impact the control of BAW, particularly in terms of pesticide resistance, as it may
293 lead to the rapid spread of resistance alleles (Che et al., 2015)
294 Resistance to insecticides in insects exemplifies evolutionary adaptation to environmental changes
295 (Ffrench–Constant et al., 2004). For several decades, cultural and chemical control methods have
296 been employed to prevent the spread and damage caused by BAW. This pest has developed
297 resistance to certain insecticides. For example, it has a long history of exposure to carbamate
298 pesticides and has exhibited a high level of resistance to methomyl in California, USA, since 1989
299 (Meinke and Ware, 1978). Therefore, quantifying the level of resistance to insecticides and
300 investigating the distribution of resistance genes in relation to the genetic structure and gene flow
301 among Chinese BAW populations are essential. Understanding the dispersal ability, genetic
302 structure, and population demography of this pest is crucial for both elucidating the theoretical
303 aspects of its evolution and effectively implementing pest forecasting systems. In the future, we
304 will investigate the population genetic differentiation and structure of BAW at the genomic level
305 to reveal its evolutionary relationships and reconstruct its population history. This research
306 enhances our understanding of how BAW adapts to climate and ecological factors at the genomic
307 level. Additionally, by elucidating the influence of monsoon patterns on the migration dynamics
308 of BAW, we can improve predictions regarding the magnitude, timing, and geographic distribution
309 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.

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Additional Information and Declarations

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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 Yu[1] 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.

364

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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. *indicates significant differences following Bonferroni correction. See Table S1 for population codes.

Figure 2 Unrooted neighbor–joining phylogenetic tree based on microsatellite data from 14 populations of *Spodoptera exigua* in northern China. The numbers next to the nodes represent bootstrap values.

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 lnP(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. 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.

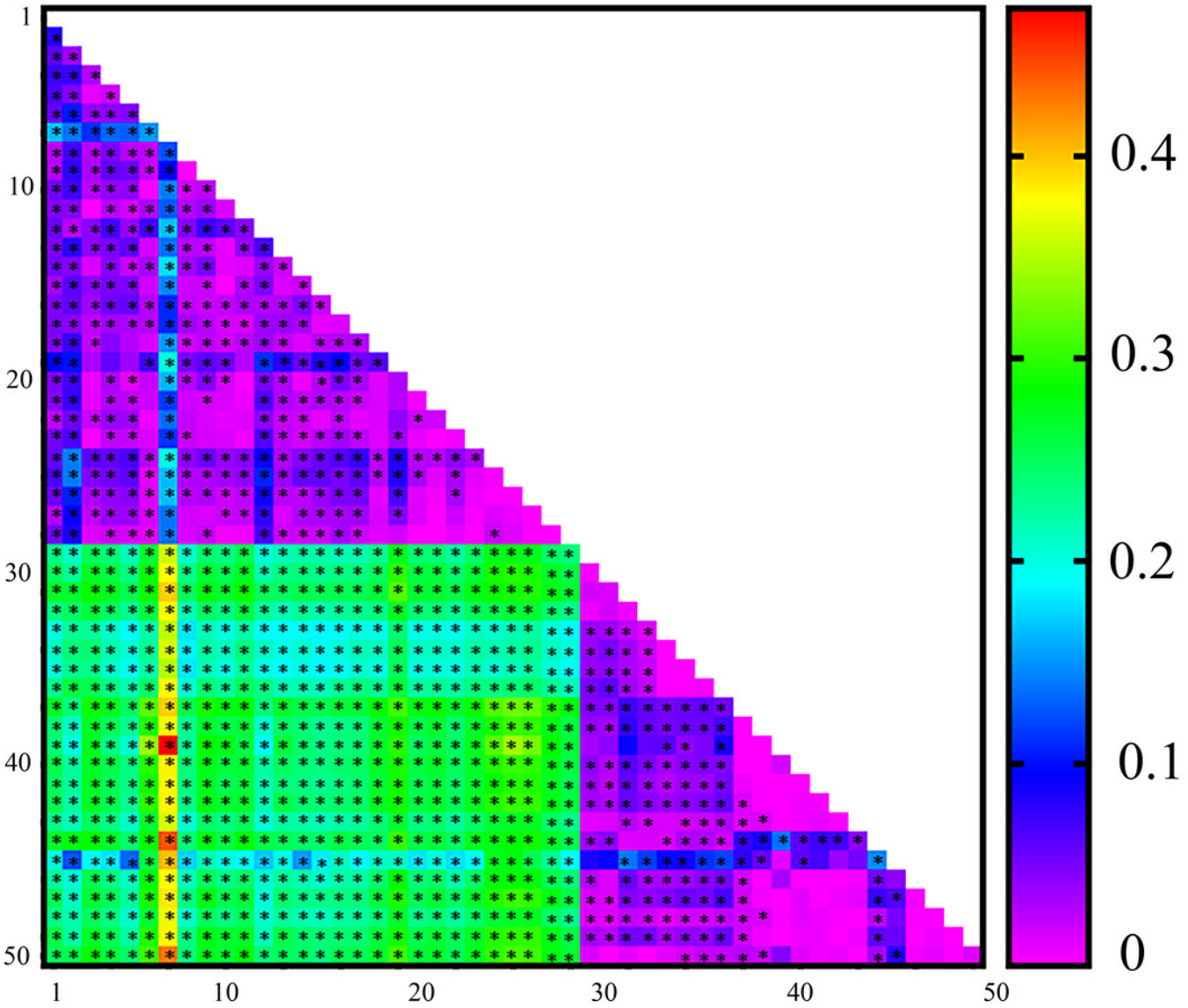
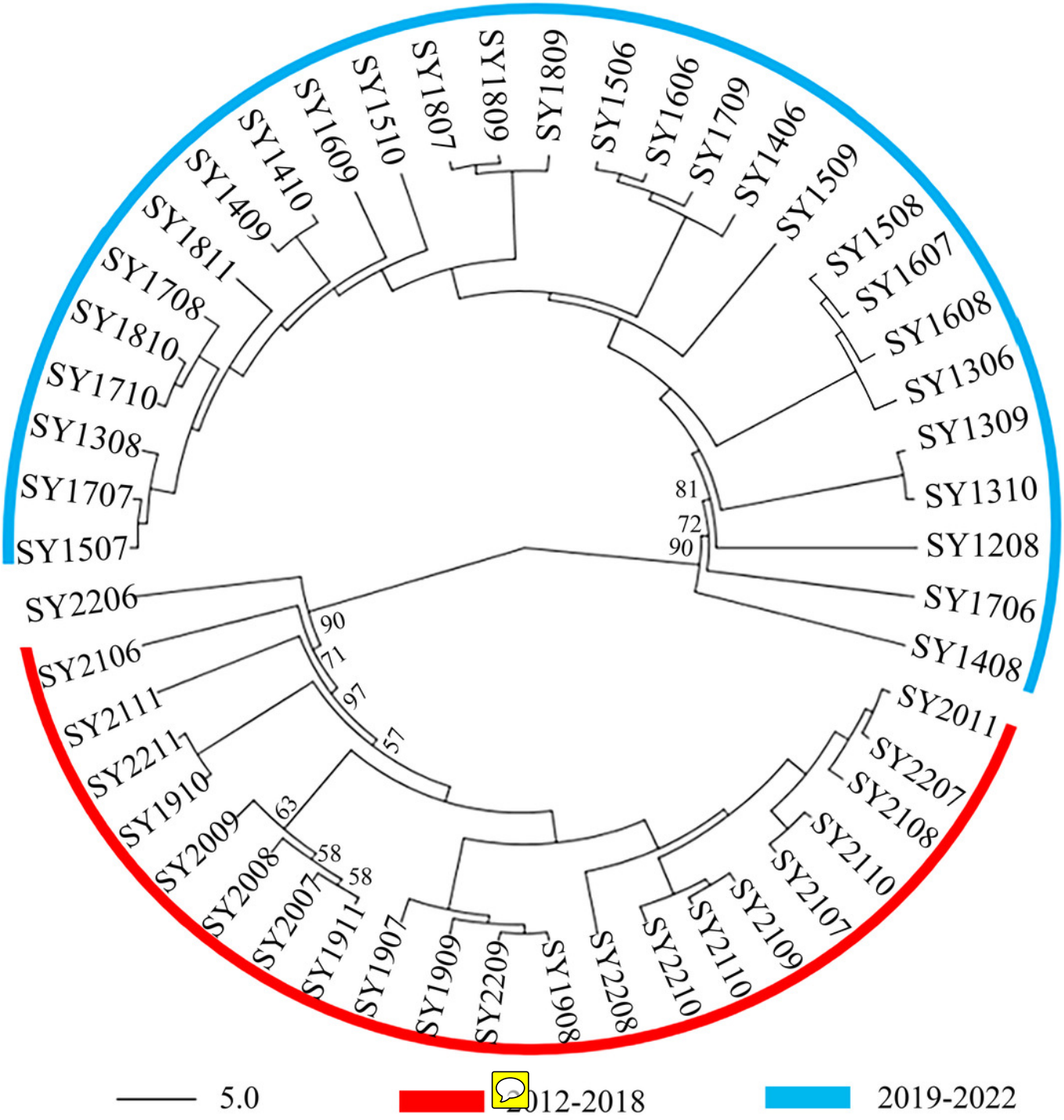


Figure 2

Figure 2

Unrooted neighbor–joining phylogenetic tree based on microsatellite data from 14 populations of *Spodoptera exigua* in northern China. The numbers next to the nodes represent bootstrap values.


SY1809
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SY1706
SY1408
SY2011
SY2207
SY2108
SY2110
SY2107
SY2109
SY2110
SY2210
SY2208
SY1908
SY2209
SY1909
SY1907
SY1911
SY2007
SY2008
SY2009
SY1910
SY2211
SY2111
SY2106
SY2206
SY1507
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SY1308
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SY1810
SY1708
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SY1409
SY1410
SY1609
SY1510
SY1807
SY1809
81
72
90
90
71
97
57
63
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5.0
2012-2018
2019-2022

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 lnP(*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.

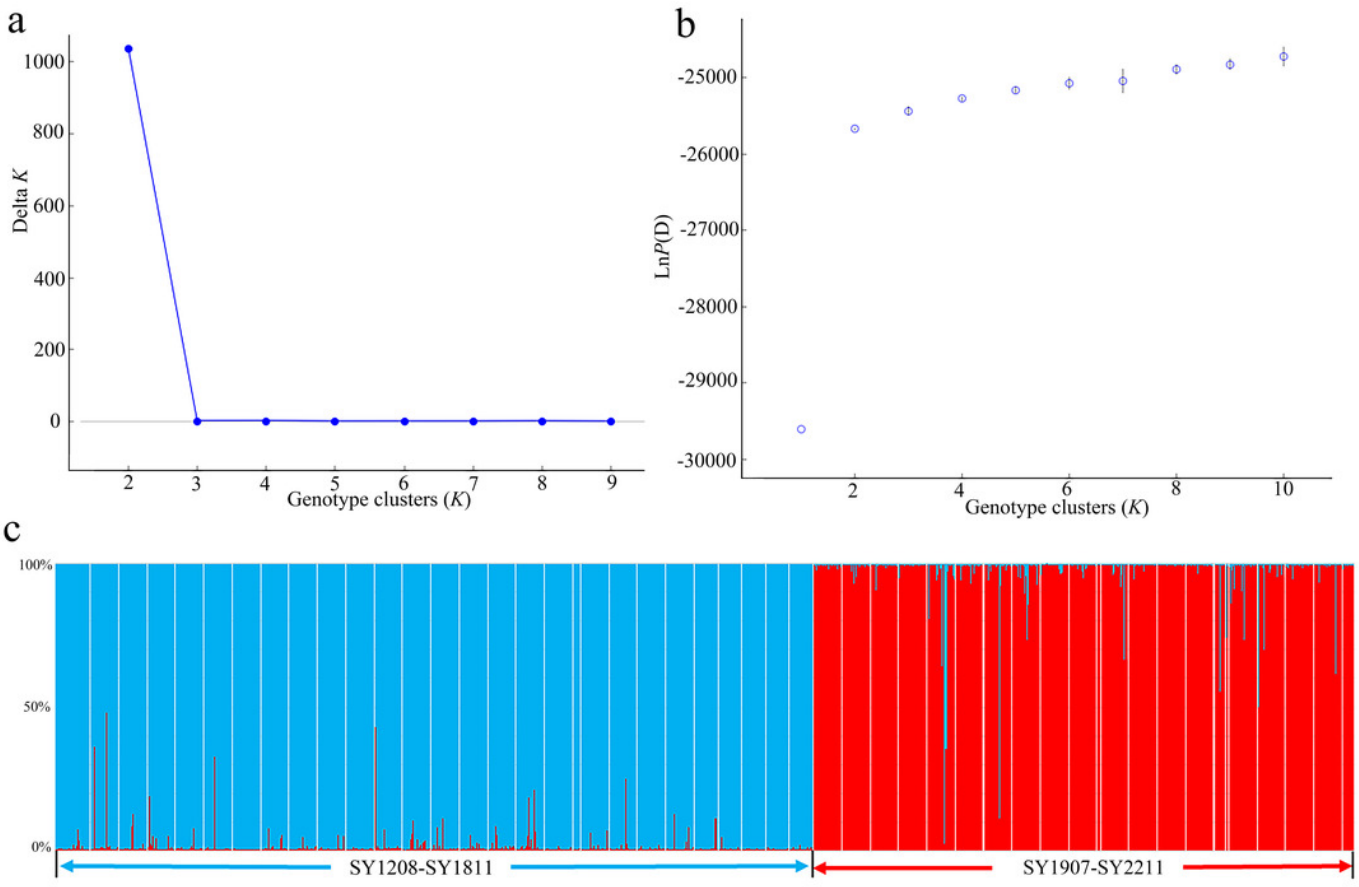

Figure 4

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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).

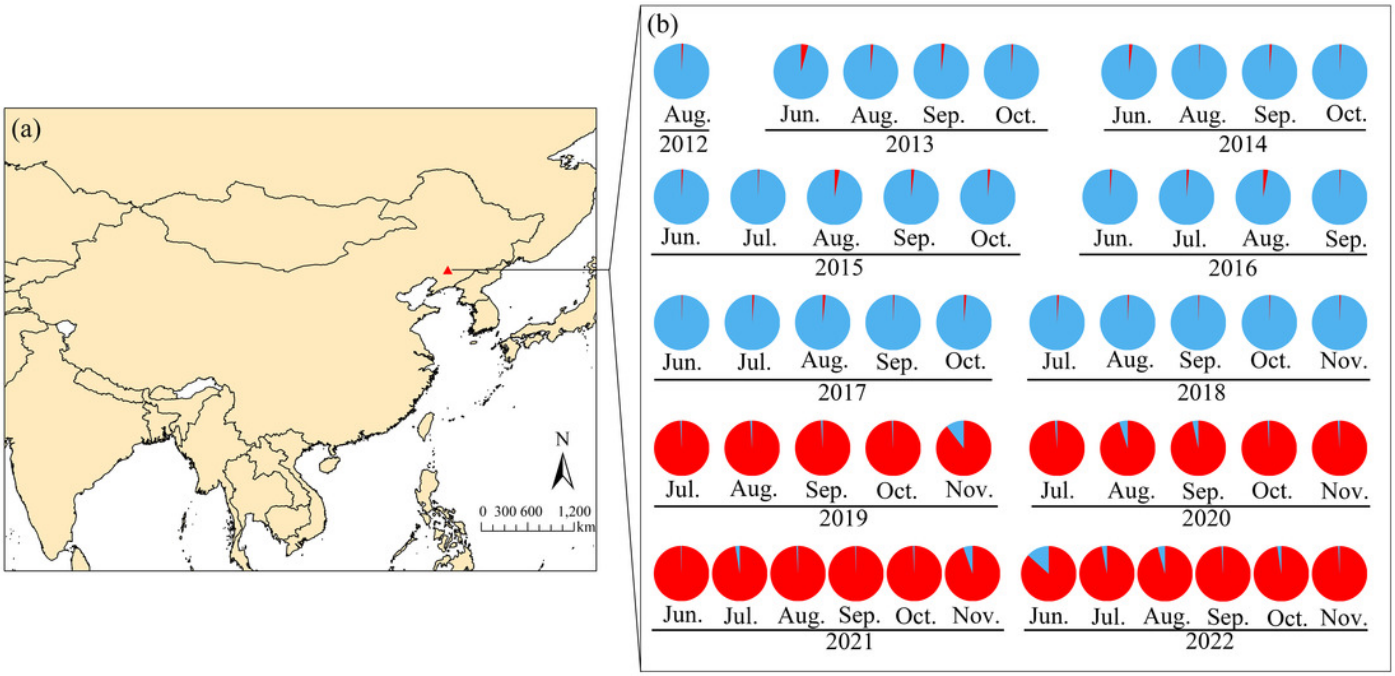

Figure 5

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.

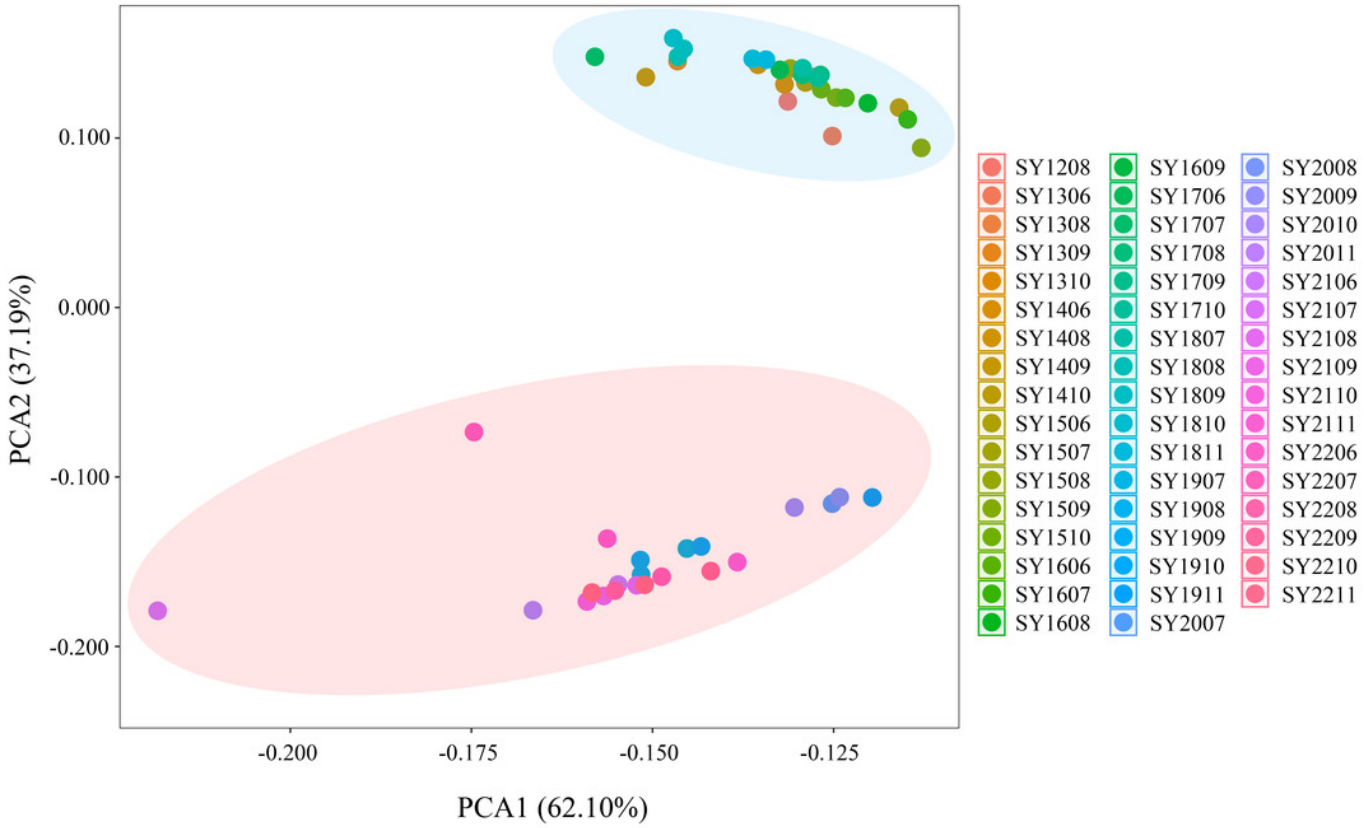

Table 1(on next page)

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	*Na*	*Ne*	*I*	H_0	*He*	*uHe*	*Ap*	*Hs*
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; *Ap*, number of private alleles; H_S, gene diversity.

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Table 2(on next page)

Table 2

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

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

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage variation (%)	*F*-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	

d.f., degrees of freedom; $^{***}P < 0.001$: significance level.