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Population Distribution and Genetic Diversity of *Ostrinia furnacalis* (Guenée) in Northeast China †

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Abstract: The *Ostrinia furnacalis* (Guenée) as a major pest that affects the yield and quality of corn. In this study, the ISSR Molecular marker method was used to analyze the genetic strcture of corn borer population collected in different accumulated temperature areas of Heilongjiang Province. The results showed that the total genetic variation of the *Ostrinia furnacalis* mainly came from between populations and adapted to the low-temperature field ecological environment in order to follow the host maize. Therefore they had changes in genetic diversity and structure, which had expanded from the first accumulated temperate belt to the sixth accumulated temperate belt.

Keywords: Asian corn borer; genetic variation; ISSR; individual migration; accumulated temperature belt

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1. Introduction

Insects have a long evolutionary history as the first biological group to master the ability of flight [1,2]. They have strong adaptability to the environment and play an important role in the ecosystem due to their flight ability, diversified feeding habits, diverse reproductive methods and amazing fertility [3–5]. Lepidopteran insect corn borer (*Pyrausta nubilalis* (Hubern)), also called corn drill worm, belongs to the borer moth family, mainly including *Ostrinia furnacalis* and European corn borer [6,7]. The young corn borers destroy the stem tissue, affect nutrient transport, damage the plant, and the serious pest breaks the stem. They belong to the word pests, and mainly harm corn, sorghum, millet and other important food and cash crops [8]. China is one of the countries with the richest biodiversity in the world due to its vast territory and complex and diverse geomorphic landscape and climate types, but it is also a country suffering from serious agricultural pests [9]. Due to the influence of environmental factors, and the difference of host plant species, the Asian corn borer is a hazard in most areas of China. Although its adults mainly spread in close range, they have the potential of long-distance migration, and have different evolutionary characteristics in different geographical distribution areas. With the continuous increase of corn planting area in Heilongjiang Province, the ecological environment in the field has changed, and the Asian corn borer has presented a trend of distribution and diffusion.

In recent years, due to the wide application of molecular biology techniques and molecular markers, molecular phylogeography, which studies the formation mechanism of interspecific and intraspecific and intraspecific phylogeographic patterns at the molecular level, has been developed [10,11]. Genomic DNA extraction, primer screening, PCR amplification, sequencing, statistics and analysis were used to further clarify insect genetic diversity and population genetic structure from the perspective of molecular biology [12–14]. The main molecular markers include specific mitochondrial genes (*Co*I, *Co*II, *CyTB*, etc.), the random amplified polymorphic (RAPD), the amplified fragment length polymorphism (AFLP), the Simple Sequence Repeat (SSR), and the Inter-Simple Sequence repeat (ISSR), etc [15]. The ISSR is a method used to amplify the sequence between two adjacent SSR loci that are close to each other. The marker uses longer primers than RAPD, has higher stability and better repeatability, and has the characteristics of simple operation and high polymorphism [16,17]. In addition, the species can be distinguished without any known genomic information of the detected species, which has wider applicability. Saha used ISSR markers to analyze the genetic diversity of shellac resin secreting insects, and the results showed that the genetic diversity between shellac species was significant, which could be used for genetic improvement [18]. Hu used ISSR technology to study the genetic variation of Asian corn borer under biological control in the same territory, and the results showed that the genetic distance was significantly correlated with geographical distance [19].This indicates that ISSR technique can be applied to the analysis of insect genetic diversity and population genetic structure.

Analysis of genetic structure of different geographic populations of the Asian corn borer is of great significance for species evolution, dispersal and comprehensive control. The method of ISSR molecular markers was used in this study to analysis the genetic structrue of Asian corn borer population collected from different regions in Heilongjiang province. It not only to lays a good foundation for the analysis of the genetic structure of Asian corn borer population in Heilongjiang province, also provides a scientific theoretical basis for the rational use of chemical insecticides against Asian corn borer and the management of drug resistance of Asian corn borer.

2. Materials and Methods

2.1. The Selected for Insect

A total of 22 different geographic populations of Asian corn borer were collected in Heilongjiang Province (Table 1), of which 30 samples were collected from each region and stored in 80% alcohol. The basic information such as longitude, latitude, effective accumulated temperature and rainfall of the collection sites were recorded in detail.

Table 1. The population collection sites, labeling and test individuals of *Ostrinia furnacalis*.

Number	Code	Longitude and Latitude	Accumulated Temperature Belts *	Rainfall
1	SQA	46.87 N, 127.50° E	2400—2700	577.00
2	MDN	44.07 N, 131.12° E	>2700	530.00
3	HBA	48.23 N, 126.52° E	2300—2500	500.00
4	MML	44.90 N, 130.50° E	2500—2600	530.00
5	HSB	47.29 N, 131.85° E	2300—2500	642.60
6	SAD	46.42 N, 125.30° E	2500—2700	419.70
7	HSC	45.37 N, 126.32° E	>2700	481.00
7	JJD	45.25 N, 131.13° E	2500—2700	427.90–542.50
9	HYL	46.33 N, 129.55° E	2500—2700	555.60
10	QTL	46.39 N, 123.41° E	>2700	392.60
11	HFZ	45.84 N, 128.83° E	2500—2700	579.70
12	HBX	45.75 N, 127.48° E	>2700	570.00

13	MNA	44.25 N, 129.47° E	>2700	400.00–600.00
14	HWC	44.92 N, 127.15° E	>2700	625.00
15	QNH	48.48 N, 124.87° E	2300—2500	450.80
16	SWK	46.83 N, 126.50° E	2300—2500	500.00
17	JFJ	47.26 N, 132.03° E	2700—2750	339.50
18	YTL	46.98 N, 128.02° E	2100—2300	630.00
19	JHL	45.75 N, 133.97° E	2300—2500	566.20
20	HLJ	47.33 N, 123.18° E	2500—2700	469.80
21	SQG	46.68 N, 126.10° E	2300—2500	477.00
22	HAH	50.25 N, 127.48° E	1800—2000	450.00—550.00

* Note: The accumulated temperature belt is the sum of the daily average temperature during the continuous period of the daily average temperature ≥ 10 °C in one year, that is the sum of the active temperature, referred to as the accumulated temperature. It is an indicator to study the relationship between temperature and the rate of development of biological organisms.

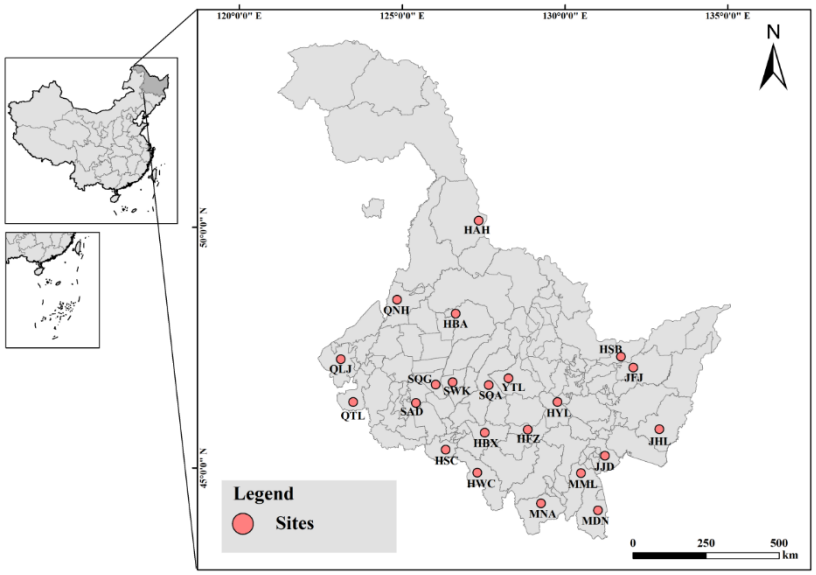

Figure 1. The distribution map of geographical populations of *Ostrinia furnacalis.*

2.2. Extraction of Total DNA and Detection of Concentration and Purity

The total DNA of *Ostrinia furnacalis* was extracted by animal DNA extraction kit (Takara Biomedical Technology, Co., Ltd., Dalian, China), and refer to the kit instructions for the extraction method. The extracted original DNA solution was placed in double-distilled water, and use the UV spectrophotometer to detect the radio of OD260/OD280 and its DNA mass concentration. The OD value was between 1.7 and 1.9, and then extracted DNA was stored at −20 °C for storage.

2.3. Establishment of ISSR-PCR Reaction System

The optimized ISSR-PCR reaction system (25 μL) was used in this experiment. Different renaturation temperatures were set according to different annealing temperatures of primers, and a total of 40 cycles were set (Table 2).

Table 2. The PCR reaction system and program.

PCR	25 μL	Program(°C)	Time
Template	1	94	5 min
Taq DNA Polymerase	0.125	94	45 s
Buffer	2.5	40–61	1.5 min
dNTP	2	72	1.5 min
P2	1	72	10 min
DdH_2O	18.375	4	∞

2.4. Screening of Primers for ISSR-PCR Amplification of Ostrinia furnacalis

This research institute in 100 ISSR primers all primers see University of British Columbia provide standard primer sequences, the sifting primer sequences for use in Asian corn borer.

2.5. Statistical Analysis

The PCR products were detected by 2.0% agarose gel electrophoresis and photographed by gel imaging system. The electrophoretic map obtained was analyzed by Quantity One software, and the bands at the migration location of each sample were counted. The bands at the same location were marked as '1' and those without bands were marked as '0' to establish a binary data matrix. The binary data were analyzed by POPGEN32 to obtain the similarity coefficient matrix of each Asian corn borer population. Software A was used to calculate and analyze the genetic similarity coefficient between different populations, according to which the UPGMA system cluster diagram was obtained. The UPGMA method was used for cluster analysis of 22 Asian corn borer populations and the cluster map was made.

3. Results

3.1. Amplification Results and Primer Screening

From University of British Columbia provide standard of 100 ISSR primers in the final were selected 14 primer sequences for Asian corn borer, and finally confirmed by gradient experiments the optimum annealing temperature (Table 3).

Table 3. Primers used for the ISSR amplification in this study.

Number	Primers Sequences	Abbreviation	Tm(°C)
807	AGA GAG AGA GAG AGA GT	(AG)8T	54.0
810	GAG AGA GAG AGA GAG AT	(GA)8T	51.8
818	CAC ACA CAC ACA CAC AG	(CA)8G	53.4
825	ACA CAC ACA CAC ACA CT	(AC)8T	53.0
826	ACA CAC ACA CAC ACA CC	(AC)8C	50.1
835	AGA GAG AGA GAG AGA GYC	(AG)8YC	59.0
836	AGA GAG AGA GAG AGA GYA	(AG)8YA	54.2
847	CAC ACA CAC ACA CAC ARC	(CA)8RC	54.2
848	CAC ACA CAC ACA CAC ARG	(CA)8RG	54.2
849	GTG TGT GTG TGT GTG TYA	(GT)8YA	56.9
855	ACA CAC ACA CAC ACA CYT	(AC)8YT	52.1
878	GGA TGG ATG GAT GGA T	(GGAT)4	50.4
880	GGA GAG GAG AGG AGA	(GGAGA)3	50.4
890	VHV GTG TGT GTG TGT GT	VHV(GT)7	52.8

Note: B = T, C or G; D = A, T or G; W = A or T; Y = C or T.

3.2. Genetic Diversity Analysis

The DNA fingerprints of 660 Asian corn borer DNA samples from 22 populations in Heilongjiang province were obtained by PCR amplication with 14 ISSR primers (Figure 2). A total of 42 clear bands were amplified by 14 primers, with an average of 3 bands per primer, including 41 polymorphic bands, and the ratio of polymorphic bands was 97.62%.

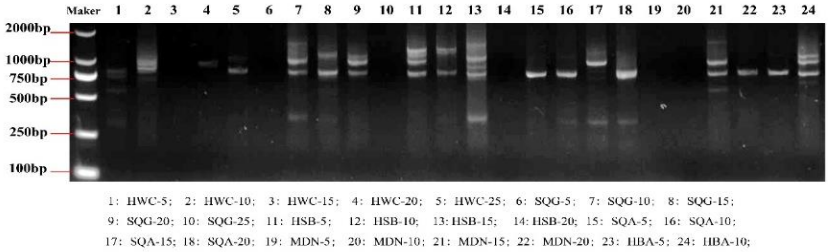

Figure 2. The result of electrophoretic for ISSR-PCR.

3.3. Genetic Differentiation between Populations

The total gene diversity (*Ht*) was 0.3236, and the genetic diversity (*Hs*) within the population was 0.2094, indicating that the total genetic variation mainly came from the inter-population. The level of differentiation (*Gst*) between different populations was calculated based on the total genetic diversity and genetic diversity within populations. The *Gst* of the 22 analyzed populations was 0.3531, indicating that 35.31% of the total genetic variation existed between populations, the genetic variation within populations was 64.69%, and the gene flow (*Nm*) of each generation between populations was 0.9161.

3.4. Genetic Distance

The UPGMA method was used to analyze the genetic distance of 22 populations and make cluster graphs (Figure 3). The regions that clustered into one branch first had the closest genetic distance and the highest genetic background similarity. As can be seen from the figure, the Asian corn borer in SQG, SAD and QNH had the longest genetic distance from other regions.

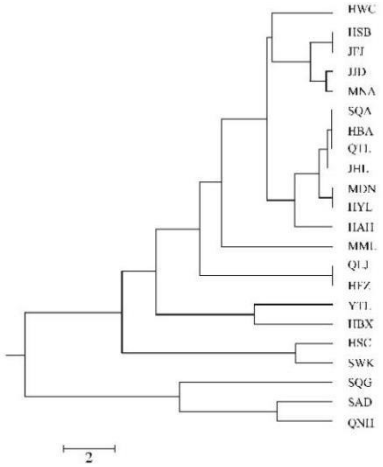

Figure 3. Cluster analysis tree diagram of *Ostrinia furnacalis* population based on UPGMA.

4. Discussion

In recent years, in order to better understand the geographic pattern and evolution process of gene lineages of different insect groups, researchers have gradually deepened their research on the genetic diversity and genetic differentiation of insect populations in different regions. Duan et al. [20]used 10 microsatellite loci to study the genetic diversity

and differentiation of 13 geographic populations of *Acanthoscelides obtectus*, which provided valuable information for the understand of occurrence and spread. Wei et al. used grid the unit analyzed the diversity pattern of scale insects in China, and found [21] that temperature and precipitation were the main factors affecting the distribution in China through principal component analysis. Peng et al. [22] assessed the differences in life history and table parameters of *Rhopalosiphum padi* populations in 6 geographic regions of China, revealing the complexity of local genetic adaptation of aphids. In this study, a total of 660 genomes were successfully extracted from Asian corn borer samples form 22 different regions. By comparing with the standard ISSR primers, we successfully screened 14 amplification primers. The amplification results showed that the proportion of polymorphic bands was 97.62%. The gene diversity (*Ht*) of the total population of Asian corn borer was 0.3236, and the genetic diversity within the population was 0.2094, it indicated their total genetic variation may mainly come from among the populations. The results of differentiation level G_{ST} among different populations showed that there was 35.31% variation among populations, while the genetic variation within populations was 64.69%. It indicated that with the change of geographical location and climate, there was genetic variation among Asian corn borer populations in different accumulated temperate zones. There was a large genetic variation among different individuals of the same population, which may be due to the strong adaptability of Asian corn borer to the changes of environment. Previous studies have proved that gene flow between populations is a measure of population genetic differentiation, and $Nm < 1$ indicates that gene flow is the main influencing factor [23]. In this study, the *Nm* of Asian corn borer was 0.9161. Therefore, there is genetic differentiation between populations caused by individual migration among corn borers. That provides a research basis for exploring the spatial distribution characteristics and group characteristics of Asian corn borer in northeastern China.

With the combination of population genetics and systems biology, the genetic evolution of insects has made more extensive research on the relationship between onlookers and macrocosm. Zhang et al. [24] found the ecological characteristics of the invasive species *Amphiareus obscuriceps* P. and the fluctuation of sea level during the Pleistocene through phylogeny, genetic diversity and genealogical geography analysis, and determined its genetic structure and population history. Combined with phylogenetic tree, ancestral state reconstruction, and ancestral distribution area reconstruction, Ye et al. [25] found that Cenozoic temperature changes played an important role in regulating the evolution of Holarctic water striders. Li et al. [26] found that dispersal events played an important role in the distribution of extant species, geological and climatic changes by sequencing the genes of 10 genera and 45 species of Chinese Aeromachini Tutt, constructing a phylogenetic tree, estimating divergence time, and reconstructing ancestral regions. It was an important factor driving the current species distribution pattern. In this study, the genetic distance analysis of 22 populations by UPGMA method showed that the genetic similarity coefficient was higher in other regions except SQG, SAD and QNH. The results showed that Asian corn borer had migrated to a great extent in most areas of Heilongjiang Province in Northeast China, and frequent gene exchange occurred with the individual migration between populations. Therefore, with the change of accumulated temperate zone and different climate, the promotion of maize varieties and the migration of corn borer may be important factors affecting the genetic structure of Asian corn borer.

5. Conclusions

In this study, we analyzed the genetic diversity of Asian corn borer in different accumulated temperature zones in Heilongjiang Province, Northeastern China by using ISSR Molecular marker technology. The result of the total genetic diversity of the population and the genetic diversity index within the population showed that the total genetic varian of Asian corn borer in 22 regions mainly came from among the populations, and the results of differnetiation levels among different populations showed that there were variations among Asian corn borer populations in different accumulated temperate zones. The

analysis of the gene flow and population genetic distance of each generation among Asian corn borer populations, it was shown that the genetic similarity between the populations decreased with the decrease of regional daily accumulated temperature, and had a high genetic similarity coefficient in the adjacent accumulated temperature zone. Consequently, our research showed that the Asian corn borer adapts to the field ecological environment with lower temperature in order to follow the host cron, resulting in changes in genetic diversity and genetic structure, and expand from the initial one or two accumulated temperate zone to the five or six accumulated temperate zone. Therefore, our findings provide a useful basis for further research on revealing the genetic evolution of insects based on spatial geographic distribution.

Author Contributions: F.Y. designed this study; C.L., S.Y. and Y.W. collected materials; D.M., H.C. and Y.W. performed experiments; P.W. and D.M. analyzed the data and made figures; P.W. and F.Y. drafted manuscript; F.Y. provided reagents and materials and supervised the study; F.Y., H.F. and Y.M. reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement:

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