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Genetic homogeneity between populations of cotton bollworm from Xinjiang, China

Dongmei Wang[a,*,1], Xianming Yang[b,1], Haiqiang Li[a], Akedan Wuwaishi[a], Ruifeng Ding[a], Haobin Li[a], Hongsheng Pan[a], Jian Liu[a], Yao Xu[a], Yanhui Lu[b]

[a] Institute of Plant Protection, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, China
[b] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

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ABSTRACT

We studied the population structure of cotton bollworm (CBW), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), in Xinjiang, the largest cotton-growing region in China, using a fragment of cytochrome c oxidase subunit I (COI) gene. Alignments of all 192 COI sequences revealed 28 haplotypes including 23 in southern Xinjiang, 5 in eastern Xinjiang and 13 in northern Xinjiang. Negative and significant values of neutrality tests for the Tajima's D and Fu's FS parameters, combined with the high values of haplotype diversity (H_d), low values of nucleotide diversity (π) and a high number of low frequency haplotypes indicated a recent demographic expansion of Xinjiang CBW populations. Analysis of molecular variation (AMOVA) indicated low and non-significant genetic structure, regardless of geographical scale or crop, with most of genetic variation occurring within local CBW populations. Pairwise F_{ST} analyses also indicated low genetic differentiation. This demographic event and high gene flow could be responsible for the low genetic structure currently found. CBW populations in Xinjiang need to be considered as one panmictic unit in its management, especially for the design of refuges to delay the development of resistance by this migratory pest to transgenic Bt (*Bacillus thuringiensis*) cotton.

Introduction

The cotton bollworm (CBW), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is an important insect pest in cotton, corn, wheat, soybeans, peanuts, vegetables, and many other crops in China, where it causes important crop losses when at high population levels (Wu and Guo, 2005). Since the late 1990s, the regional population of CBW has been greatly suppressed by the introduction of Bt (*Bacillus thuringiensis*) cotton expressing the Cry1Ac toxin (Wu et al., 2008). However, if this pest is subject to intense selection for resistance to these Bt toxins, it will develop resistance, and Bt cotton will lose its control efficiency on CBW (Jin et al., 2015; Li et al., 2010). Hence, preventative management of CBW Bt resistance is crucial for the long-term sustainable use of Bt cotton.

There are three agroecologically different cotton-growing areas in China: the Changjiang River Region (southern China), the Yellow River Region (northern China), and the Xinjiang Region (northwestern China) (Wu and Guo, 2005). In the Yellow River Region, Bt cotton accounts for < 10% of all host crops of CBW, and other non-Bt crops such as corn, peanut, wheat and vegetables provide a refuge for susceptible genotypes of CBW, effectively delaying its development of Bt resistance (Jin et al., 2015). Because the CBW moth has the capacity for long-distance migration (Feng et al., 2004, 2005, 2009), the function of non-cotton refuges is usually assessed at a regional scale. Xinjiang, the largest cotton-growing region in China, accounts for one sixth of the area of the country. Currently, it's planting area and total yield of cotton equal > 60% of all the cotton in China (National Bureau of Statistics of China, 2016). Previous studies have found only limited gene flow between CBW in Xinjiang and CBW from the Changjiang River or the Yellow River regions (Chen, 2010). These findings suggest that CBW migration does not commonly occur between Xinjiang and those other two regions. Hence, a specific strategy of CBW resistance management in Xinjiang is needed.

Xinjiang is divided into southern, eastern and northern areas by the Tianshan Mountain. Ratios of Bt and non-Bt cotton, and different other host crops of CBW differ among these three parts of Xinjiang. To date, the genetic structure of CBW has only been studied in few locations in Xinjiang. For example, low genetic differentiation and high gene flow among hosts and locations were observed in Kashgar, Turpan, Shanshan (Turpam), and Shihezi CBW populations (Deng, 2012). However, in

* Corresponding author.
E-mail address: wdm872@sina.com (D. Wang).
[1] These authors contributed equally to this work.

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Table 1
The samples of cotton bollworm *Helicoverpa armigera* collected in Xinjiang, China from 2013 to 2015. Sample location, host, numbers and Genbank number are shown in this table.

Region	Location	Code	Host plant	No. individuals	Latitude	Longitude	Sample date	Genbank number
Southern Xinjiang	Aksu	1	Corn, *Zea mays*	8	41.07	80.35	2014.08.26	KY411166-KY411173
	Aksu	2	Corn	8	41.05	80.36	2015.08.17	KY411174-KY411181
	Bohu	3	Tomato, *Solanum lycopersicum*	7	41.02	86.61	2015.07.21	KY411272, KY411323 KY411185-KY411188
	Bugur	4	Wheat, *Triticum aestivum*	8	41.91	84.61	2013.07.17	KY411189-KY411196
	Bugur	5	Wheat	6	41.93	84.62	2015.08.24	KY411197-KY411202
	Hoton	6	Corn	9	36.86	81.60	2013.08.02	KY411203-KY411211
	Korla	7	Corn	9	41.46	85.52	2014.08.20	KY411212-KY411220
	Korla	8	Corn	5	41.43	85.60	2015.08.01	KY411221-KY411225
	Kuqa	9	Corn	7	41.01	80.20	2015.08.24	KY411226-KY411232
	Shule	10	Corn	7	38.33	77.21	2013.07.28	KY411233-KY411239
	Shule	11	Corn	5	38.31	77.26	2015.07.26	KY411240-KY411244
	Yuli	12	Corn	5	41.32	86.22	2015.07.23	KY411245-KY411245
Eastern Xinjiang	Hami	13	Corn	9	42.66	93.95	2013.08.12	KY411250-KY411258
	Turpan	14	Velvetleaf, *Abutilon theophrasti*	14	42.88	89.02	2013.07.28	KY411259-KY411259
Northern Xinjiang	Bole	15	Corn	8	44.89	82.18	2013.07.20	KY411273-KY411280
	Bole	16	Tomato	6	44.93	82.21	2015.07.27	KY411281-KY411286
	Changji	17	Cotton, *Gossypium hirsutum*	12	44.11	87.28	2013.08.25	KY411287-KY411298
	Fukang	18	Watermelon, *Citrullus lanatus*	7	44.18	88.32	2015.07.17	KY411299-KY411305
	Fukang	19	Tomato	12	44.35	86.40	2015.08.14	KY411306-KY411317
	Jinghe	20	Corn	6	44.57	82.41	2015.07.28	KY411318-KY411323
	Karamay	21	Sunflower, *Helianthus annuus*	6	45.53	84.99	2015.08.14	KY411324-KY411329
	Kuytun	22	Tomato	8	44.76	84.80	2015.07.23	KY411330-KY411337
	Manas Xian	23	Corn	10	44.24	86.40	2013.08.25	KY411338-KY411347
	Shihezi	24	Cotton	10	44.43	85.35	2013.08.27	KY411348-KY411357

most areas of Xinjiang, the genetic diversity and differentiation of CBW populations are unknown, and this knowledge is crucial for the management of Bt resistance. In this study, using a mitochondrial DNA marker, we investigated the population structure of 20 CBW populations distributed throughout southern, eastern and northern Xinjiang, and our results revealed a genetic homogeneity among these populations.

Materials and methods

Sample collection

From 2013–2015, we collected a total of 192 CBW larvae from southern (84), eastern (23), or northern (85) Xinjiang, China. CBW larvae were morphologically identified and confirmed by the sequence results of a fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI) amplified using primers described below (LepF1/LepR1). The coordinates for each collection site and the host plant species from which the CBW were collected are summarized in Table 1. CBW populations occurred at low densities in cotton fields due to the wide-scale adoption of Bt cotton. Therefore, most CBW larvae were collected from other host plants, including *Triticum aestivum* L. (wheat), *Zea mays* L. (corn), *Solanum lycopersicum* L. (tomato), *Helianthus annuus* L. (sunflower) *Citrullus lanatus* (Thunb.) Matsum. & Nakai (watermelon) and *Abutilon theophrasti* Medik. (velvet leaf). Collected larvae were individually stored at −20 °C.

DNA extraction and mitochondrial DNA sequencing

Genomic DNA was extracted using Insect DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.A.) according to the manufacturer's instructions. All DNA samples were amplified and sequenced with the primers LepF1 (5′-ATTCAACCAATCATAAAGATATTGG-3′) and LepR1 (5′-TAAACTTC TGGATGTCCAAAAAATCA-3′) to yield a 658-bp fragment of the mitochondrial COI gene. PCRs were performed on a Veriti machine (Applied Biosystems, Foster City, CA, USA) in a 25 µl reaction volume containing 0.75 units of Takara Ex Taq polymerase (Takara Bio Inc., Japan), 1 × Ex Taq Buffer (including 2 mM of $MgCl_2$; Takara), 0.2 mM of dNTPs (Takara), 1 µl of DNA (concentration not estimated) and 0.5 mM each of the oligonucleotide primers. The thermal profile used included an initial denaturation step at 94 °C for 3 min followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 49 °C for 30 s, and extension at 72 °C for 1 min. A 10 min final extension at 72 °C was added at the end of the cycle to increase copy number. Negative controls were included in both DNA isolation and PCR reactions. After verification via gel electrophoresis, the PCR templates were purified (OMEGA) and then sequenced in both directions at the Sangon Shanghai Co., Ltd. (Shanghai, China).

We also tested the presence of *Wolbachia* in all CBW samples using the primers 81f (5′-TGGTCCAATAAGTGATGAAGAAAC-3′) and 691r (5′-AAAAATTAAACGCTACTCCA-3′) which amplify *Wolbachia* surface protein wsp (Zhou et al., 1998). PCR reaction was performed in 25 µl reaction volume containing 0.75 units of Takara Ex Taq polymerase (Takara Bio Inc., Japan), 1 × Ex Taq Buffer (including 2 mM of $MgCl_2$; Takara), 0.2 mM of dNTPs (Takara), 1 µl of DNA (concentration not estimated) and 0.5 mM each of the oligonucleotide primers. Cycling conditions were 94 °C for 3 min followed by 40 cycles of 94 °C for 30 s, 52 °C for 45 s and 72 °C for 1 min and finally 72 °C for 7 min. Approximately 7 µl of the obtained PCR product was verified via gel electrophoresis. A negative and positive control was included in the PCR.

Analysis

Sequences were assembled with CodonCode Aligner 3.6.1 (CodonCode, Dedham, MA, USA) and manually edited before creating consensus sequences. The resulting consensus sequences of all individuals were aligned using Clustal X 2.0.11 (Larkin et al., 2007). All population genetic parameters such as number of haplotypes (N_h), nucleotide diversity (π), and haplotype diversity (H_d) were calculated using the program DNASP v5 (Librado and Rozas, 2009). An analysis of molecular variation (AMOVA) implemented in ARLEQUIN v3.01 (Excoffier et al., 2005) was used to test the hierarchic genetic structure of the populations. TCS v1.21 was used to generate a haplotype network using statistical parsimony (Clement et al., 2000). Correlation between pairwise F_{ST} and geographic distance was analyzed by the web version of Genepop (Raymond and Rousset, 1995; Rousset, 2008).