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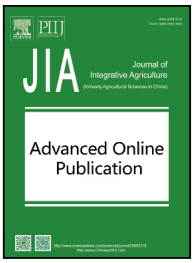

RESEARCH ARTICLE

Genetic structure of the invasive Colorado potato beetle *Leptinotarsa decemlineata* populations in China

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Abstract:
The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say, is an infamous invasive species worldwide. It was first found in Xinjiang Uygur Autonomous Region of China in 1993 and spread to Northeast China in 2013. To better understand the genetic structure and the diffusion path of their populations in China, we used nine polymorphic microsatellite loci to elucidate the genetic diversity, genetic structure and gene flow among nine CPB populations across Xinjiang and Northeast China. The results show that: (1) Two genetically separated clusters were identified by phylogenetic tree, principal coordinate analysis (PCoA) and Bayesian cluster method. Cluster one contained populations from Xinjiang, China. Cluster two contained populations from Northeast China. A genetic differentiation existed between the two clusters. (2) Three populations in Northeast China hold an obvious genetic differentiation according to the phylogenetic tree and PCoA, indicating that multiple introductions may occur in Northeast China. (3) The ALT population in Xinjiang showed a closer genetic relationship with the populations in Northeast China which may be due to the fact that they collectively originated in neighboring Russia. (4) Among all populations, ML and WS had obvious gene migrations with TC, indicating that the inland populations are most likely to originate from Tacheng, Xinjiang.

Keywords: Colorado potato beetle, genetic variation, alien invasive species, microsatellite marker

1. Introduction

Invasive biological problems have become increasingly prominent with globalization process. Invasive alien species can destroy the local ecological balance and cause serious damage to agroforestry (Carlton 1996), even directly harm human health, e.g., red imported fire ant (Zhang *et al.* 2005). The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), is an infamous quarantine invasive pest, which seriously harms the production of potatoes (*Solanum tuberosum* L.) and

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other Solanaceae crops (Li 2013), usually causing 30–50% yield losses in potato cultivation. CPB is highly resistant to traditional insecticides and has a spectacular ability to adapt to a variety of solanaceaeous plants under variable climates (Schoville *et al.* 2018), making them difficult to control (Alyokhin *et al.* 2008).

CPB originated from the eastern slopes of the Rocky Mountains in the United States in the 1850s, feeding on its original host: *Solanum rostratum* Dunal (Weber 2003; Izzo *et al.* 2018). In 1877, it crossed the Atlantic and spread rapidly over Europe. By 1920, it settled throughout Europe except the United Kingdom (De Wilde and Hsiao 1981) and continued to spread eastward at a rate of about 100 km per year (Zhang 2010). CPB entered Central Asia in the 1970s and in 1993, they invaded Xinjiang, China (Zhang 1996). At present, CPB is distributed in more than 40 countries in Europe, Africa, Asia, and North America. The world's major distribution range is 15–55°N of North America and 33–60°N of Eurasia (Guo *et al.* 2014).

In 1993, CPB invaded the Ili Valley and Tacheng areas of Xinjiang Uygur Autonomous Region, China. It had spread eastwards to Mulei County in Xinjiang through a total of 38 counties and cities by 2006 (Liu 2012). In 2000, an outbreak occurred in the Primorsky Krai region of Russia, which is the border region of Northeast China (Zhang *et al.* 2010), and then Heilongjiang Province and Jilin Province were seriously threatened. In July 2013, China's quarantine officers discovered CPB in Chunhua Town, Hunchun City, Jilin Province (Guo 2014), which is less than 1 km away from the Sino-Russian border. CPB was discovered in Baoqing County and Mudanjiang City in Heilongjiang Province in 2016 (Zhao and Qing 2016). In this study, we sampled part of these occurred areas.

Based on molecular markers, it has played an important role during the research of the spread of agricultural pests (Cao *et al.* 2016, 2017; Wu *et al*. 2019). In order to effectively monitor and control the spread of CPB, it is very necessary to analyze and predict its diffusion routes and the source of populations (Boiteau *et al.* 2003). Isozyme variation analysis revealed a large genetic difference between Mexican and American populations, indicating that Mexico is the source of invasive populations (Jacobson 1983), but the latest research shows that the pest populations are most likely to originate from the southern plains of the United States (Izzo *et al.* 2018). Based on mtDNA marker, most of the CPB populations in the United States were mainly derived from the same mitochondrial family (Zehnder 1992); AFLP markers revealed that there were 20 haplotypes of five populations in the United States but only one haplotype of eight populations in Europe. Genetic diversity of CPBs after reaching the Eurasian continent was reduced significantly (Grapputo 2005); SSR markers for CPB studies in Xinjiang, China revealed that after CPB invaded China in 1993, the genetic diversity had declined significantly and was divided into three genetic groups (Liu 2010; Yang 2010; Zhang *et al.* 2013).

With the further spread of CPB to Northeast China, genetic information of its population remains unknown. Therefore, we need to use molecular markers to analyze the genetic structure of CPB populations in Northeast China and its relationship with populations in Xinjiang, which will provide a theoretical basis for its proliferation, prevention and control.

In this study, we used microsatellite markers with nine microsatellite loci (Grapputo 2006) to investigate the genetic diversity, genetic relationships and gene flow of populations from nine populations to infer the origins and diffusion of CPB populations in China.

2. Materials and methods

2.1. Sampling methods

The experimental samples were CPB adults and larvae from 6 populations (Altay City (ALT), Tacheng City (TC), Wusu City (WS), Houcheng County (HC), Xinyuan County (XY)and Mulei County (ML)) in Xinjiang, and 3 populations (Baoqing County (BQ), Mudanjiang City (MDJ) and Chunhua Town (CH)) in Northeast China with 13–30 individuals per population, totaling 232 (Table 1). All samples were soaked in 100% ethanol and stored in a freezer at –40°C.

2.2. Experimental methods

DNA was extracted using the 6 legs of each adult or the head of each larvae using DNeasy Blood & Tissue Kit (Qiagen, Germany) following the procedure recommended by manufacturer. Final concentration of DNA was between 50 and 200 ng μL^{-1} (measured by Nano Drop 2000 (Thermo Scientific, USA). PCR amplifications used the nine pairs of polymorphic microsatellite primer (Grapputo 2006). They were LdA11b, LdB8b, LdE10e, LdE11c, LdAC5-2, LdAC5-22, LdGA4-5, LdGA4-18, LdGA5-30 (Appendix A), synthesized by Sangon, Shanghai, China. Reaction products were detected by ABI3730XL DNA Sequencer (Tianyi Huiyuan, Beijing). The original data were analyzed using GeneMapper v.3.2 to determine the length of the allele fragments and the genotypes of each locus. The data were summarized in the Excel table for further analysis.

2.3. Statistical and genetic analyses

Data evaluation The null alleles caused the population to deviate from the Hardy-Weinberg (H-W) equilibrium.

Table 1 The sample information from nine geographic populations in China[1)]

Population	Location	Lat.	Long.	*n*	C_T	F_{DY}	C_Y
ALT	Altay City, Xinjiang Autonomous Region	47°50′	88°12′	28	2009	2003	6
TC	Tacheng City, Xinjiang Autonomous Region	46°46′	82°59′	30	2018	1993	16
WS	Wusu City, Xinjiang Autonomous Region	44°28′	84°41′	24	2009	1997	12
HC	Huocheng County, Xinjiang Autonomous Region	43°99′	80°88′	30	2016	1993	23
XY	Xinyuan County, Xinjiang Autonomous Region	43°32′	84°00′	30	2017	1994	23
ML	Mulei County,Xinjiang Autonomous Region	43°48′	90°21′	30	2018	2003	6
BQ	Baoqing County, Heilongjiang Province	46°32′	132°19′	17	2016	2016	0
MDJ	Mudanjiang City, Heilongjiang Province	44°55′	129°63′	30	2016	2016	0
CH	Chunhua Town, Jilin Province	43°26′	131°19′	13	2013	2013	0

[1)] Lat., sampling latitude; Long., sampling longitude; *n*, sample size; C_T, collecting time; F_{DY}, first year detected; C_Y, years of colonization.

Therefore, in this study of microsatellite markers, we used FreeNA (Chapuis and Estoup 2007) with 10 000 bootstraps to check for null alleles. The H-W equilibrium and linkage disequilibrium tests were subsequently performed using GenePop v.4.7.0 (Rousset 2008).

Microsatellite locus polymorphism information content (P_{IC}) refers to the value of a marker used to detect polymorphism in a population which depends on the number of alleles detected and their frequency distribution. According to the method of Botstein *et al.* (1980), a site is highly polymorphic when P_{IC}>0.5; moderately polymorphic when 0.25<P_{IC}<0.5, and has low polymorphism when P_{IC}<0.25. Among the nine microsatellite loci in this study, there were three highly polymorphic sites and six moderate polymorphic sites with an average of 0.47, which show suitability for assessing the genetic diversity of CPB.

Genetic diversity Fstat 2.9.3 (Goudet 2001) and Microsatellite Toolkit (Trinity College Dublin, Ireland) were used to analyze the genetic diversity of each geographical populations (Goudet 2001), including the number of alleles (N_A), allele richness (A_R), observation heterogeneity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}) and P_{IC}. Statistical significance test was completed by SPSS 17.0. We tested evidence of a recent decrease of effective population size by bottleneck effect using Program BOTTLENECK 1.2.02 (Piry *et al.* 1999) with a two-phase model of mutation with 30% multistep mutations and 1 000 iterations.

Population relationship Fstat 2.9.3 (Goudet 2001) was used to calculate the *F*-statistics (F_{ST}). which is pairwise based on the frequency difference of genotype distribution to measure the genetic differences among populations. The size of this value depends on the degree of differentiation between populations. The Nei's genetic distance (*D′*) was used to measure the genetic distance between populations (Nei 1972), calculated by Popgene32 (Yeh 1999). The neighbour-joining (NJ) method was used to construct the phylogenetic tree between populations based on *D'* using MEGA7 (Kumar 2016).

To assess genetic relationships among all coral samples, principal coordinate analyse (PCoA) was conducted in GENALEX version 6.5 (Peakall and Smouse 2006) based on codominant genotypic distance among pairs of all individuals and populations. We delimited the major genetic clusters of all populations with the Bayesian Software STRUCTURE 2.3 (Pritchard *et al.* 2000) with a burn-in of 200 000 followed by 2 000 000 MCMC iterations of the admixture model. The optimum number of genetic clusters was estimated using the Delta *K* method (Evanno *et al.* 2005) using the online tool STRUCTURE Harvester ver. 0.6.93 (Earl and Vonholdt 2012). The membership coefficient matrices (*Q* matrices) of replicated runs for each *K* were combined using CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) with the Greedy algorithm, and then visualized using the Program DISTRUCT 1.1 (Rosenberg 2004). The analysis of molecular variance (AMOVA) and fixation indices were performed by Arlequin 3.51 (Excoffier *et al.* 2005). AMOVA was performed to assess the genetic variance partitioned into 3 levels (among groups, among populations within groups and within populations) based on the 2 clusters defined by STRUCTURE, with 1 000 permutations to test for significance.

Isolation by distance was tested to examine the correlation between geographical distance and genetic distance (estimated as F_{ST}/(1–F_{ST}) and *D′*) over all samples, as well as among each cluster defined by STRUCTURE, and actual districts using Mantel procedure, as implemented in IBD Software (Bohonak 2002), with 10 000 randomizations.

Gene flow BAYESASS 3.0 (Wilson and Rannala 2003) was used to estimate contemporary migration rates among populations. A Markov Chain Monte Carlo (MCMC) resampling method was run with 10 000 000 iterations, discarding the first 1 000 000 iterations and sampling every 1 000 iterations from the remaining 9 000 000 iterations, producing a sample of 9 000 observations from the chain that will be used to estimate parameters. The result was visualized using the R package circlize 0.4.8 (Gu 2014).

3. Results

3.1. Genetic diversity of populations

Among nine microsatellite loci, five out of the 324 locus-locus pairs showed linkage disequilibrium, while six out of 81 loci-population pairs deviated H-W equilibrium after Bonferroni correction. The genetic diversity parameters of all populations were shown in Table 2. The statistical results revealed the N_A, A_R, H_E of all populations in China were 3.62, 3.29 and 0.54, respectively. N_A, A_R and H_E average values of six populations in Xinjiang (ALT, TC, WS, ML, HC and XY) were 3.41, 3.10 and 0.52. The highest values were from the ALT (4.00, 3.55 and 0.60), and the lowest were from XY (2.89, 2.71 and 0.47). The average values of three populations in Northeast China (BQ, MDJ and CH) were 4.04, 3.65 and 0.60, respectively. The highest values were from the MDJ (5.22, 4.33 and 0.61), and the lowest were from CH (3.11, 3.09 and 0.58). The H_E of populations in Northeast China was significantly higher than populations in Xinjiang (ANOVA, P=0.023), but there was no significant difference with A_R (ANOVA, P=0.112).

F_{IS} refers to the probability that an individual's allele is from the same ancestor, and we used it to reflect the degree of inbreeding that occurs within populations. It was shown that populations with negative F_{IS} were HC, XY, MDJ and CH. A significant bottleneck effect resulted in a reduction in effective population size (Appendix B), with a corresponding decrease in the number of alleles and heterozygosity at the polymorphic locus and a faster decrease in allele numbers than heterozygosity (Nei and Chakraborty 1975). So, H_O of the population is greater than H_E, and population exhibits heterozygous excess (Piry *et al.* 1999). F_{IS} of the other populations were positive and only WS population had the inbreeding coefficients greater than 0.1.

3.2. Genetic relationship among populations

The mean value of pairwise F_{ST} for population genetic disparity was 0.127 (minimum 0.009, maximum 0.237), and mean of D' was 0.217 (minimum 0.022, maximum 0.516). Both F_{ST} and D' indicated that populations in Xinjiang showed relatively high genetic differentiation from the populations in Northeast China except for the relationship of ALT with BQ. The differentiation within populations in Northeast China was greater than the differentiation of populations in Xinjiang. The WS and ML populations showed the lowest differentiation (F_{ST}=0.009, D'=0.022), and they had the lowest genetic differentiation with TC among all the three introductory locations; The genetic relationship between HC and XY was also relatively closer (F_{ST}=0.025, D'=0.031) (Table 3).

The unrooted NJ phylogenetic tree showed that all populations were clearly split into two branches (Fig. 1). It can be observed that China's populations were roughly divided into two genetic clusters. The first branch included all populations in Xinjiang (ALT, TC, WS, ML, HC and XY), where WS and ML populations were relatively closer, as two sister populations derived from TC. The second branch contained all the populations from Northeast China with relatively longer branch length.

PCoA for populations and individuals were shown in Fig. 2. For populations, percentages of variation explained by principal coordinate 1 (Coord.1) and principal coordinate 2 (Coord.2) were 52.60 and 20.75%, respectively, in Fig. 2-A, explained 72.04% of the total variation between populations, showing clearly cluster and separation of populations. The MDJ, CH and BQ populations were clearly separated from other populations while ALT is equidistant to most populations (Fig. 2-A). For individuals, the cumulative percentages of the first two eigen values was 29.11%, showing a similar, but more gradual relationship compered with PCoA of populations (Fig. 2-B).

The Bayesian cluster analysis detected similar genetic structure. We plotted the distribution probability of grouping nine populations when Delta K values were 2, 3 and 4 (Fig. 3). The most suitable grouping value was obtained at K=2 (Appendix C), which suggested there were 2 clusters of

Table 2 Summary data of genetic diversity of nine geographic populations in China[1)]

Population	n	N_A	A_R	H_O	H_E	F_{IS}	P_{IC}
ALT	28	4.00±1.73	3.55±1.32	0.59±0.03	0.60±0.05	0.02	0.52
TC	30	3.00±1.12	2.87±1.03	0.49±0.03	0.49±0.05	0.01	0.41
WS	24	3.11±1.27	2.94±1.05	0.40±0.03	0.53±0.05	0.24	0.44
HC	30	3.78±1.32	3.35±0.89	0.58±0.03	0.51±0.06	–0.13	0.45
XY	30	2.89±1.27	2.71±0.99	0.48±0.03	0.47±0.06	–0.01	0.39
ML	30	3.67±0.87	3.19±0.80	0.47±0.03	0.49±0.06	0.05	0.43
BQ	17	3.78±1.79	3.54±1.42	0.58±0.04	0.60±0.04	0.03	0.51
MDJ	30	5.22±1.79	4.33±1.17	0.70±0.03	0.61±0.05	–0.16	0.54
CH	13	3.11±0.93	3.09±0.90	0.67±0.04	0.58±0.05	–0.17	0.49

[1)] n, sample size; N_A, number of alleles; A_R, allelic richness; H_O, observed observed heterogeneity, H_E, expected heterogeneity; F_{IS}, inbreeding coefficient; P_{IC}, polymorphism information content.
Data are mean±SD.

Table 3 Genetic differentiation F_{ST} (upper right) and genetic distance D' (lower left) among 9 populations in China[1)]

	ALT	TC	WS	HC	XY	ML	BQ	MDJ	CH
ALT	****	0.1323	0.1615	0.1305	0.1125	0.1222	0.1283	0.3156	0.2722
TC	0.0928	****	0.0704	0.0584	0.1075	0.0778	0.1941	0.4373	0.3268
WS	0.0962	0.0527	****	0.1082	0.1653	0.0222	0.2491	0.5164	0.2642
HC	0.0871	0.0478	0.0790	****	0.0311	0.1016	0.1860	0.3600	0.3341
XY	0.0846	0.0936	0.1264	0.0250	****	0.1526	0.2042	0.3075	0.3412
ML	0.0847	0.0647	0.0095	0.0816	0.1262	****	0.2664	0.4814	0.3041
BQ	0.0651	0.1287	0.1378	0.1187	0.1406	0.1622	****	0.2063	0.2312
MDJ	0.1479	0.2272	0.2306	0.1928	0.1866	0.2374	0.1028	****	0.3460
CH	0.1351	0.1983	0.1495	0.1935	0.2126	0.1851	0.1164	0.1620	****

[1)] These two kinds of values were shaded from high to low by deep red to deep blue respectively.

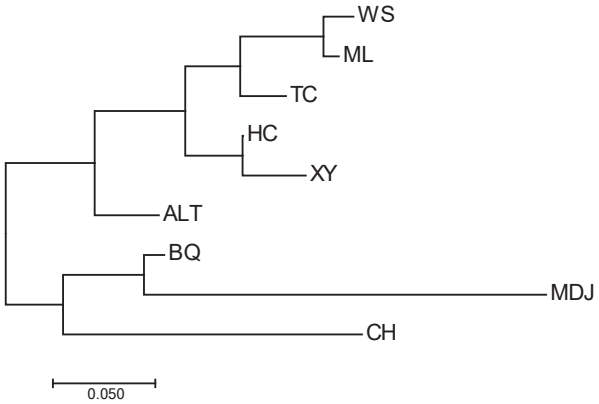

Fig. 1 The unrooted Neighbour-Joining phylogenetic tree based on Nei's genetic distance (D') for 9 Colorado potato beetle populations in China. Legend refers to D'.

all populations, clearly distinguished the populations in Northeast China from Xinjiang. The ALT population showed a low proportion of admixed individuals. Populations in Xinjiang appeared to be separated at K=3. The MDJ population appeared to be separated from Northeast China at K=4.

Based on the two clusters by STRUCTURE, the AMOVA indicated that most of the variation observed resided within populations with 82.73% of the total variation, and inter-population variation accounted for 7.88% within groups, and 9.39% could be explained by differences among groups (Table 4). The variation among populations within groups was lower than the variation within each group, which supported the rationality of clusters by STRUCTURE.

The Mantel tests between geographical distance and two kinds of genetic distances (F_{ST}/(1–F_{ST}) and D') conducted over all nine populations exhibited significant correlation among all populations (r=0.521 and 0.576, P=0.019 and 0.006, respectively), but not significant within populations neither in Xinjiang (r=–0.220 and –0.150, P=0.245 and 0.279, respectively) nor in Northeast China (r=–0.969 and –0.958, P=0.320 and 0.330, respectively).

3.3. Gene flow

The probability of migration within populations was plotted with a Chord diagram in Fig. 4 (see Appendix D for details). The results revealed a high level of contemporary gene flow within TC, WS and ML, as well as HC and XY. However, for all populations, the highest value was the self-assignment probability (0.6752–0.9029). This means that most populations were occupied by resident individuals. A low but watchable level of contemporary gene flow appeared at ALT (from BQ), CH (from BQ) and XY (from TC) while the other pairwise migration rates were not obvious (≤0.0531) besides the self-assignment rates.

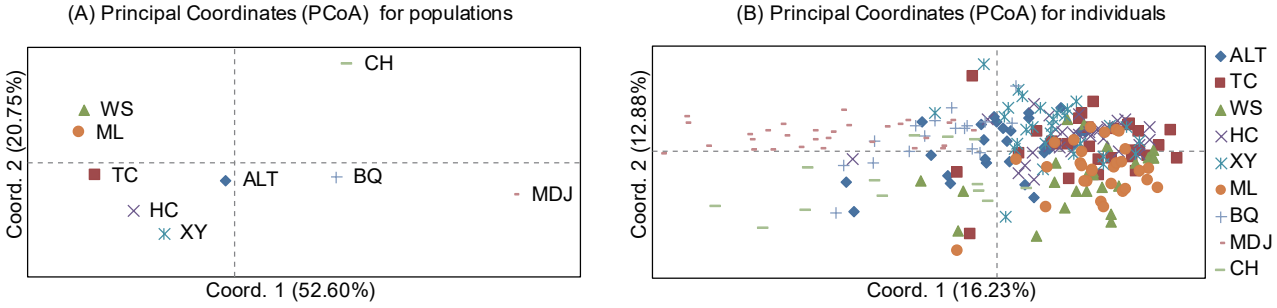

Fig. 2 Genetic structure of Colorado potato beetle populations by principal coordinate analysis (PCoA) for populations (A) and individuals (B).

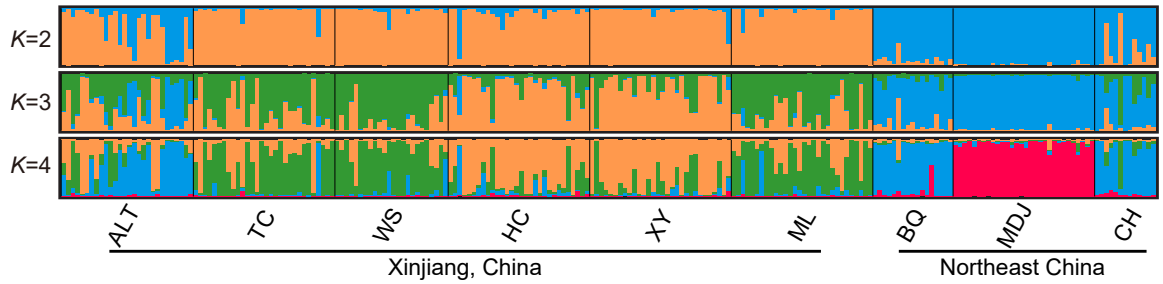

Fig. 3 Bayesian clustering of the multilocus genotypes of Colorado potato beetle individuals of nine geographical populations for *K*=2, 3 and 4. Each individual is denoted by a narrow vertical bar and its proportional membership in each of *K* cluster is represented by a different color. The most appropriate clustering was obtained at *K*=2.

Table 4 Analysis of molecular variance (AMOVA) of different populations and groups by STRUCTURE

Source of variation	*df*	Fixation index	Percentage of variance (%)	*P*-value
Among groups	1	0.17	9.38	0
Among populations within groups	7	0.09	7.88	0
Within populations	455	0.09	82.73	0.015

4. Discussion

4.1. Genetic diversity

Genetic diversity is one of the most important factors affecting the invasion of alien species. China is the eastern frontier region of the invasion of CPB in Eurasia and currently has the lowest level of genetic diversity in the world (A_R=3.25, H_E=0.54). The A_R and H_E averages for the United States, Russia and Estonia are 6.4 and 0.68, respectively, by Grapputo (2006) with the same nine microsatellite loci. Genetic diversity decreased by 51.5 and 23.5% of populations in Xinjiang and 43.0 and 11.8% of the populations in Northeast China as measured by A_R and H_E. Genetic diversity has also declined in studies of American and sub-European populations. In this study, XY population has the lowest genetic diversity in China.

Alien species often experience the founder effect and bottleneck effect in the process of invasion and spread, resulting a decline in genetic diversity but this did not disrupt the successful invasion. This phenomenon is called “genetic paradox” (Allendorf and Lundquist 2003). This phenomenon is common in invasive species. Dlugosch and Parker (2008) counted more than 80 invasive species and found that the founder effect reduced the gene richness and heterozygosity of species in the invaded land by 15.5 and 18.7%, respectively. Grapputo *et al.* (2005) found that 13 European CPB invasive populations were accounted for only 1 of the 20 haplotypes of the North American origin populations based on the mtDNA, showing obvious bottleneck effects. However, a more detailed investigation is needed because the decline in genetic diversity found by us are not directly comparable. However, multiple introductions

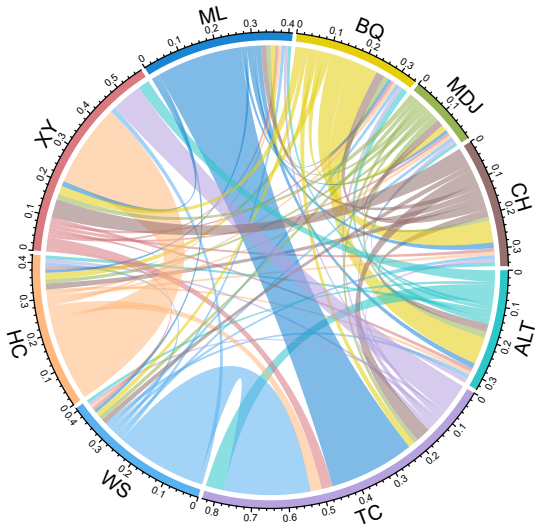

Fig. 4 The migration rates between populations of Colorado potato beetle plotted with a chord diagram. The self-assignment rates were removed when plotting. Sectors with different colors represent different populations and the thickness of links indicates the migration rates of the populations with the same lighter color.

as the key to successful species invasion will offset the reduction of genetic diversity to some extent (Sakai *et al.* 2001). For instance, the worldwide invasive lizard, *Anlis sagrei*, invaded Florida more than eight times, making its genetic variability exceed even that in its origin place (Kolbe 2004), which may be probably due to the mixture of haploids from multiple sources providing an opportunity for intra-population variation. Similarly, multiple introductions may have enabled CPB to resist the decline in genetic diversity, helping to some extent the spread of CPB in Xinjiang and Northeast China.

CPB was accidentally brought into Europe over a century. Genetic diversity has been greatly reduced, but it has not hindered its further spread. It is the fact that CPB is resistant to the cold weather in northern Europe (Popova and Semenov 2013; Hiiesaar *et al.* 2014). According to the current climatic conditions, it is predicted in the future that the central and northeastern China is a potential distribution area for CPB (Wang *et al.* 2017). In China, CPB adapts to wild plants including *Solanum rostratum* D. and *Hyoscyamus niger* L., an independent host for a whole life cycle. In addition, CPB feeds on eggplant and tomato (Li 2013). Therefore, from a biological and ecological point of view, the genetic diversity of CPB is sufficient for its further spread in the Eurasian continent.

In our study, according to H_E, the genetic diversity of CPB in TC, XY and ML populations were significantly lower than that of other populations (ANOVA, P=0.01). The Tacheng to Mulei area is located in the north of the Tianshan Mountains in Xinjiang. Of all the regions from which samples were taken, the environmental conditions in these regions are the worst for CPB. The climate is dry and the annual precipitation, an important factor limiting CPB distribution (Li 2016), is only about 150 mm. Moreover, the vast territory and sparse population are not conducive to the natural or anthropogenic migration of CPB. Therefore, these three regions have the lowest genetic diversity.

4.2. Genetic relationship

CPB has invaded the Ili Valley and Tacheng areas on the border between China and Kazakhstan in 1993 (Zhang 1996), and rapidly invaded toward the east interior. Based on our STRUCTURE clustering results at K=2, WS and ML constituted one genetic cluster with TC. Their similar genetic backgrounds implied that all interior populations originate from the Tacheng areas, not Ili Valley combining with the historical record (Zhang 1996; Liu *et al.* 2012), and the result is similar with Zhang *et al.* (2013) but ALT, which may be due to different introduction. ALT population maintained a equidistant genetic differentiation with other populations which means ALT may be the closest population to the ancestor and has a low level of variation. This may also serve as an explanation for its highest genetic diversity in Xinjiang, China. Populations from TC to ML located on the northern slope of Tianshan Mountains, have common or relatively close ancestors, which means that TC may act as a bridgehead for inland spread, and they become independent families due to the isolation by Gurbantunggut Desert and the Tianshan Mountains. In addition, unlike ALT, TC and HC may have a common ancestor, similar to Zhang *et al.* (2013) by phylogenetic tree and Bayesian clusters at K=2, indicates that isolation by Tianshan Mountains further drove the genetic differentiation. It can be speculated that environmental heterogeneity (including altitude) contributes more to genetic distance than geographic distance, considering that the IBD test is not significant in Xinjiang.

The CPB outbreaked in Chunhua Town, Jilin Province in 2013 (Guo 2014) as well as Heilongjiang Province in 2016 (Zhao *et al.* 2016). The second cluster includes BQ, MDJ and CH (Fig. 3), which indicates that the populations in Northeast China had obvious genetic variation after spreading more than 3 000 km. However, they still keep a certain similarity with ALT which provides evidence for their European origin, not a re-invasion from the United States.

The three populations in Northeast China have a higher level of differentiation when compared within the Xinjiang populations (TC, WS and ML) according to the phylogenetic tree and F_{ST}. The invasion of CPB into Northeast China did not occur long compared with the colonial history in Xinjiang. Why did the three populations in Northeast China form a moderate level of differentiation whereas the TC, WS and ML populations in Xinjiang did not? Furthermore, no significant correlation within Northeast China populations was found by IBD test. Therefore, we speculate that each of Northeast China populations may have different population sources and different sources brought different genetic information. These sources include the border areas of Russia's Khabarovsk Krai and Primorsky Krai. At the same time, we ruled out the artificial spread of CPB from Xinjiang to Northeast China.

4.3. Gene flow

We found that there was obvious gene flow with TC, WS and ML in Xinjiang. This indicates that the TC invasion point is the main source of inland populations in Xinjiang. ML is the eastern frontier region after the colonization event. All the way from TC to ML is the flat north slope of Tianshan Mountains. CPB can rely on the west wind prevailing in Xinjiang and a continuum of potato and yellow thorns which permits genetic communication. This allows a very serious risk of CPB to spread to the east. The HC and XY populations are affected by the geographical isolation of the Tianshan Mountain, and it's unlikely to have gene flow with other populations.

There is no obvious geographical isolation of the three populations in Northeast China, however, no obvious gene flow was detected. CPB in Northeast China has been detected in border areas such as Hunchun City, Suifenhe City, Dongning City and Hulin City until 2013, which may be the initial stage of biological invasion and various populations have not settled stably, so that there is no further diffusion and gene communication and this is the most critical period for prevention and control. If the various populations are

fully mixed, their genetic diversity will be further improved, which will give CPB better adaptability.

The gene flow between CPB populations in Xinjiang and in Northeast China is minimal. However, gene flow from BQ in Northeast China to ALT in Xinjiang was significantly higher than the other two northeast populations. CPB is mainly transmitted through trade and can be carried by fruits, vegetables, logs, packaging materials, vehicles and CPB-related researchers from infested areas. For example, China's port quarantine has repeatedly intercepted adult insects imported from the United States (Zhang *et al.* 2010). However, the obvious gene flow of the BQ to ALT may also be caused by homologous ancestors because they are all close to Russia. Therefore, we temporarily believe that there was no direct genetic interaction of CPB between Northeast China and Xinjiang in the absence of more reference populations.

5. Conclusion

Populations in Xinjiang have the lowest genetic diversity in China but do not affect their further spread according to the perspective of environmental adaptability. Among all populations, ML has close genetic relationship with TC and WS indicating that TC population is the source of the interior populations (WS and ML). CPB in Northeast China may have multiple introductions from the Khabarovsk Krai and Primorsky Krai in Russia. ALT in Xinjiang maintains similarity with Northeast populations, revealing the fact that they share the same European source. There is weak genetic communication between the three populations in Northeast China indicating that this is a critical period implementing preventive and control measures.

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Appendices associated with this paper can be available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

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