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Short communication

Effects of habitat fragmentation on genetic diversity and population differentiation of *Liposcelis bostrychophila* badonnel (Psocoptera: Liposcelididae) as revealed by ISSR markers

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ABSTRACT

Liposcelis bostrychophila is a globally important stored product pest that is adapted to many habitats and is difficult to control due to insecticide resistance. We evaluated effects of habitat fragmentation on the genetic diversity and differentiation of *L. bostrychophila* populations. These genetic data were collected in Wuhu city, China using inter-simple sequence repeat (ISSR). A total of 108 discernible DNA fragments were detected, 107 were polymorphic and the percentage of polymorphic bands (PPB) was 99.1%. Shannon's information index (*I*) and Nei's gene diversity (*He*) from four populations were ordered (high to low) as herbstore > flourmill > bedroom > library. Population differentiation ($Gst = 0.41$) was average in these populations. Analysis of molecular variation (AMOVA) showed that variation existed mainly within populations (68.1%). At the same time, gene flow ($Nm = 0.72$) was interpreted to be moderate. Cluster analysis showed a small degree of genetic distance among different isolates and permitted grouping the isolates of *L. bostrychophila* into three distinct clusters. The study demonstrated clear genetic effects of habitat fragmentation on *L. bostrychophila* populations. Genetic diversity decreased and genetic differentiation increased between remnant populations when habitats were fragmented. Gene flow is not impeded by habitat fragmentation due to the impact of human activities. These findings are of great use for the prevention and control of this pest.

1. Introduction

The psocid *Liposcelis bostrychophila* Badonnel is a serious pest worldwide that causes economic losses to stored grains and other commodities (Lienhard, 1990; Turner, 1994, 1998). It is parthenogenic, small (~1.0 mm long), wingless, and produces proteins that are allergens for humans (Lin et al., 2004; Perlman et al., 2015; Fukutomi et al., 2012). Repeated use of chemical pesticides for control has led to rapid development of pesticide resistance. This makes *L. bostrychophila* difficult to control using conventional chemical treatments (Ding et al., 2002; Nayak and Collins, 2008; Nayak et al., 2002).

The physiological mechanisms used by *L. bostrychophila* to develop insecticide resistance and successfully adapt to complex, stressful environments could be associated with aspects of genetic diversity and population differentiation. Therefore, understanding the genetic diversity and genetic differentiation may provide insight into improved pest management strategies. Dan-Dan Wei et al. (2012) investigated the population genetics of Chinese populations of *L. bostrychophila* and found that genetic differentiation was not significantly correlated with geographic distance (Wei et al., 2012). Nevertheless, genetic diversity and differentiation of *L. bostrychophila* still merits additional genetic study because of its extensive distribution.

Habitat fragmentation is increasing in the genetic structure of many populations (Culley et al., 2007), and can negatively impact populations by reducing genetic diversity and decreasing gene flow. This causes a decline in the population's ability to adapt in response to changing environments (Cordingley et al., 2015; Kruess and Tscharntke, 1994; Mona et al., 2014; Rhodes and Chesser, 1994). Reduction in effective population size and individual fitness could ultimately lead to population extinction (Raijmann et al., 1994). However, recent reports indicate that *L. bostrychophila* can be found in large numbers on flour products in houses (Turner, 1986, 1987, 1994). *L. bostrychophila* frequents human habitats that are

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constantly being altered (Chin et al., 2010). It is unclear if *L. bostrychophila* genetic diversity and differentiation are affected by the frequency or magnitude of habitat fragmentation.

Previous studies of *L. bostrychophila* population genetics were conducted using allozymes, randomly amplified polymorphic DNA (RAPDs) and mitochondrial DNA (*cytb*) (Ali and Turner, 2001; Mikac and Clarke, 2006; Wei et al., 2012). Microsatellite markers have been characterized for *Liposcelis. decolor* (Mikac, 2006), while microsatellites are found in many other Arthropod genomes (Fagerberg et al., 2001). Inter simple sequence repeat (ISSR) makers are based on microsatellite markers and inherited in a Mendelian fashion. ISSR could be able to produce reliable and reproducible bands because of long primer sequences and high annealing temperature (Zietkiewicz et al., 1994). Compared with microsatellite markers, inter simple sequence repeat marker performance for more polymorphic loci. ISSR markers have been applied to assess genetic diversity and population differentiation in insects, such as *Lepidoglyphus destructor*, *Sogatella furcifera* and *Acyrthosiphon. pisum* and *Pemphigus. obesinymphae* (Abbot, 2001; Ge et al., 2014; Liu et al., 2014; Zietkiewicz et al., 1994). Thus, ISSR technology was employed here to study population genetics and differentiation of *L. bostrychophila*.

Our goal was to quantify the genetic structure of *L. bostrychophila* populations and provide answers for the following questions: Has habitat fragmentation affected *L. bostrychophila* genetic diversity and genetic differentiation? Is *L. bostrychophil*a gene flow reduced by isolation in fragmented habitats? These questions might explain why *L. bostrychophila*, with a broad tolerance to environmental stress, can successfully adapt to complex environments. The information may be useful in regard to *L. bostrychophila* chemical resistance and helpful for improving control strategies.

2. Materials and methods

2.1. Sample collecting

Collecting locations were selected in Wuhu, China, including a herbstore, a flourmill, a home bedroom and a library. The psocids sampled from storage premises containing flour, storage premises containing traditional Chinese medicinal materials, mattress dust and deep litter of library corresponding to collecting locations, respectively. Eventually, 20 populations were obtained and all population samples are shown in Table 1. Slide-mounted psocids were identified morphologically as *L. bostrychophila* (Li, 2002) by using an optical microscope (BX51, Olympus, Tokyo, Japan). To make sure only *L. bostrychophila* is involved, DNA was initially extracted as template from each population, and identification was reconfirmed by the cytochrome b (*cytb*) sequence (Simons et al., 1994). Reference sequences for each *L. bostrychophila* population were deposited in the Gen Bank (KT873481-KT873486).

2.2. DNA extraction

DNA was extracted from 20 individuals per population which were cloned from a single female *L. bostrychophila* using the protocols described by Jia et al. (2009) (Jia et al., 2009) and all DNA samples were stored at −80 °C for later use.

Table 1
Sampling location, data and other information of *L. bostrychophila* sampled.

Environment	Location	Populations	Collect data
Storage environment	Herbstore	6	Apr 2015
	Flourmill	5	Apr 2015
Living environment	Bedroom	5	May 2015
Working environment	Library	4	May 2015

2.3. ISSR amplification

A total of 40 ISSR primers were selected according to the public ISSR primers by the University of British Columbia in Canada. Different combinations of $MgCl_2$ concentration and annealing temperatures were analyzed to achieve the optimal amplification conditions. Eventually, 9 ISSR primers (Table 2) that produced clear and reproducible bands were selected for the amplification of all samples. ISSR amplification reactions mixtures were implemented in 25 μL volume comprising 0.1 U Taq Polymerase/μL; 500 μM dNTP; 20 mM Tris-HCl; 100 mM KCl; 3 mM $MgCl_2$, 0.5 μM primer, 1 μL template DNA and complemented by deionized water. The PCR amplification was performed under the following conditions: initial denaturation at 94 °C for 5min, 35 cycles of denaturation at 94 °C for 45s, annealing at the specific annealing temperature for 45s and extension at 72 °C for 1.5 min. Additional cycle of 10 min at 72 °C was used for extension of primers. PCR products were separated on 1.2% agarose gel at 70 V for 1 h. The gel image was recorded by Zel Boc Xr (Bio-Rad). All amplifications were repeated 3 times to verify reproducibility of the results.

2.4. Data analysis

Amplified fragments were automatically recorded using Gene Tools from SynGene software. Each sample was scored as 1 for presence or 0 for absence. Statistical analysis was performed on fragments from intensely stained unambiguous, clear bands were used for statistical analysis, as well as fragments from reproducible weak bands. Genetic parameters of *L. bostrychophila*, including the observed number of alleles (*Na*), effective number of alleles (*Ne*), number of bands, percentage of polymorphic bands (PPB), Nei's gene diversity (*He*), Shannon's information index (*I*), genetic differentiation (*Gst*) (Nei, 1978) and gene flow (*Nm*), Nei's (1978) genetic identity and Nei's genetic distance (Nei, 1972), were calculated by POPGENE version 1.32 assuming Hardy-Weinberg equilibrium. To assess the overall distribution of diversity within *L. bostrychophila* populations, analysis of molecular variance (AMOVA) was used (Excoffier et al., 1992) to quantify variation inter and intra populations. Input data files for AMOVA version 1.55 programs were generated using DCFA 1.1 (Zhang and Ge, 2002), and the number of permutations was determined at 1000 for significance analysis. Clustering analysis of all samples was performed using the unweighted pair-group method with arithmetic average (UPGMA) via the NTSYS-pc version2.1.

3. Results and discussion

3.1. Genetic diversity

Of the 40 ISSR primers tested, 9 primers were highly polymorphic and produced unambiguous and reproducible amplification profiles. There were 108 loci generated from all primers, resulting in an average of 12 bands per primer (Fig. 1). The percentage of polymorphic loci was 99.1% indicating high species level genetic diversity. Due to human activities, there was incomplete isolation within populations. Among four populations, PPB ranged from 82.4% to 30.6% (Table 3). The values of Shannon's (at the population level) varied from a high of 0.42 ± 0.24 to a low of 0.18 ± 0.28, with similar results for other indexes (Table 3). The levels of genetic diversity in four populations of *L. bostrychophila* are ordered (high to low) as herbstore > flourmill > bedroom > library. In the present study,