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Mitochondrial Genetic Diversity in the Bird Cherry-Oat Aphid *Rhopalosiphum padi* (L.) in China

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ABSTRACT

Mitochondrial DNA (mtDNA) is an important genetic marker in population and evolutionary biology. To test the feasibility of two mitochondrial gene markers (COI and Cytb) for *Rhopalosiphum padi*, we collected 275 individuals of the insect species from 15 locations, which cover most of the species' distribution range in China, and analyzed the diversity of the two genes. Seven COI haplotypes and 15 Cytb haplotypes were identified by 13 and 36 polymorphic sites, respectively. Across the entire samples, the average haplotype diversities (H_d) of COI and Cytb were 0.491 and 0.607, and the nucleotide diversities (π) of COI and Cytb were 0.147% and 0.160%, respectively. Relatively low levels of genetic diversity and genetic differentiation were observed among all *R. padi* populations based on the two genes. Moreover, parsimony networks of the COI and Cytb haplotypes of *R. padi* all supported a single clade. Although the nucleotide variation of mitochondrial genes has been used in other insect species, reviewing the recent literatures on mitochondrial diversity in aphid species, we found that the population and evolutionary biology of aphids including *R. padi*, could not be elucidated by analyzing mtDNA alone, mostly because of the low genetic variation of mitochondrial genetic markers among populations. We suggest the combined use of mtDNA and other genetic markers, such as microsatellites, to overcome the low genetic information provided by mtDNA in evolutionary studies on aphid populations.

Keywords: COI, Cytb, Gene markers, mtDNA, Population biology.

INTRODUCTION

Population genetics has undergone considerable progress over the past decades. Population biology can effectively reveal the micro-evolution and ecological adaption strategies of insect pests in agroecosystems (Harrison, 1989; Gueguen *et al*., 2010; Zheng *et al*., 2013). Generally, a good population genetic study starts with an appropriate genetic marker, including mitochondrial, multilocus nuclear, and single-locus nuclear markers, which are commonly used in molecular population biology studies (Sunnucks, 2000). Mitochondrial DNA (mtDNA), representing a very small fraction of the organism's genome, is a popular marker of molecular diversity in animals. It has strict maternal transmission (Birky, 2001) with high mutation rates due to a limited repair system (Brown *et al*., 1979). A simple, conserved structure (Mandal *et al*., 2014), lack of genetic recombination, and relatively infrequent rearrangements are also characteristics of mtDNA (Mandal *et al*., 2014). In natural environments, insect population genetics can be affected by various factors, including geographical

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distance, migration, the host plant, reproductive mode, among others (Llewellyn *et al*., 2003; Miller *et al*., 2003; Duan *et al*., 2017). Native insects usually have high genetic diversity, while invasive and/or exotic species often show reduced genetic diversity (Puillandre *et al*., 2008; Li *et al*., 2015).

The bird cherry-oat aphid, *Rhopalosiphum padi* (L.), is an important wheat pest (Van Emden and Harrington, 2007) affecting yield and quality, as it can transmit the Barley Yellow Dwarf Virus (BYDV). This virus can become epidemic over a large area, leading to serious economic losses due to damage to wheat production (Leather *et al*., 1989). *R. padi* has been distributed among most wheat-producing regions worldwide (Wang *et al*., 2016) and has been reported in almost all wheat-growing regions in China (Duan *et al*., 2017). Martinez-Torres *et al*. (1996; 1997) examined the genetic variation of *R. padi* among European populations and different breeding systems via restriction enzyme analysis of mtDNA. Our previous study using microsatellite marker assays showed significant genetic differences between populations with obligate parthenogenesis and those with cyclical parthenogenesis, as well as an association between significant genetic structures and different reproductive modes (Duan *et al*., 2017). These differences are also significantly related to the synergistic effect of Isolation-By-Distance (IBD) (Duan *et al*., 2017). There are no previous studies on the mitochondrial genetics of *R. padi* in China.

In the present study, we aimed to evaluate the feasibility of using mtDNA markers for the study of *R. padi* population genetics, by selecting two mitochondrial genes [Cytochrome Oxidase subunit I (COI) and Cytochrome b (Cytb)], and analyzing mitochondrial genetic diversity of 15 *R. padi* geographical populations throughout the main wheat-producing regions of China revealed by these two genes. We also planned to discuss the possible factors involved in the limitation of mtDNA diversity in *R. padi* with regard to population and phylogeographic studies.

MATERIALS AND METHODS

Insect Sampling

R. padi samples were collected from wheat fields (*Triticum aestivum* L.) in the major wheat-producing areas of China from May to August 2013 (Table 1). We sampled 15 *R. padi* geographical populations. To ensure the representativeness of the samples, only

Table 1. Sample information for 15 *R. padi* geographical populations in China.

Province	Location	Population code	Sample size	Latitude	Longitude	Date
Chongqing	Beibei	CQB	30	29° 49′ N	106° 25′ E	2013.04.02
Henan	Nanyang	HNN	19	33° 14′ N	112° 36′ E	2013.04.18
Anhui	Chuzhou	AHC	22	32° 21′ N	118° 20′ E	2013.04.21
Hubei	Wuhan	HBW	24	30° 29′ N	114° 19′ E	2013.04.14
	Zaoyang	HBZ	20	32° 08′ N	112° 47′ E	2013.04.16
Shaanxi	Xianyang	SAX	27	34° 17′ N	108° 05′ E	2013.07.18
	Hanzhong	SAH	21	33° 11′ N	107° 27′ E	2013.04.08
Shandong	Heze	SDH	18	35° 10′ N	115° 29′ E	2013.05.04
	Zibo	SDZ	21	37° 06′ N	118° 02′ E	2013.05.10
Shanxi	Taigu	SXT	22	37° 25′ N	112° 34′ E	2013.05.27
	Hongtong	SXH	23	36° 13′ N	111° 41′ E	2013.05.28
Hebei	Baoding	HBB	20	38° 49′ N	115° 26′ E	2013.06.07
Jilin	Baicheng	JLB	18	45° 39′ N	122° 52′ E	2013.07.10
Qinghai	Xining	QHX	18	36° 38′ N	101° 37′ E	2013.08.14
Xizang	Lasa	XZL	24	29° 38′ N	91° 02′ E	2013.08.10

one apterous adult aphid was collected per site, and the distance between each site was at least 30 m. Each geographical population consisted of at least 15 collection sites to obtain a sufficient number of aphids for the analyses. All samples were preserved in absolute ethanol and stored at –20°C prior to the study.

DNA Extraction

Genomic DNA was extracted from single aphid individual using the EasyPure™ Genomic DNA Kit (TransGen Biotech Co., Ltd., Beijing, China). DNA extraction was performed according to the bench protocol for animal tissues. DNA was eluted in deionized water and stored at –20°C.

PCR Amplification and Mitochondrial Gene Sequencing

Four mitochondrial gene-based primers were used in the analysis. The mitochondrial *COI* gene was amplified using the primers LepF (5′-ATTCAACCAATCATAAAGATATTGG-3′) and LepR (5′-TAAACTTCTGGATGTCCAAAAAATCA-3′) (Foottit *et al*., 2008) and the mitochondrial *Cytb* gene was amplified using the primers CP1 (5′-GATGATGAAATTTTGGATC-3′) and CP2 (5′-CTAATGCAATAACTCCTCC-3′) (Harry *et al*., 1998).

All PCR amplifications were carried out in a total volume of 25 µL containing 12.5 µL 2X Taq Mastermix (CoWin Biotech., Beijing, China), 2.0 µL of each oligonucleotide primer (0.2 µM), 2 µL genomic DNA (10–30 ng μL^{-1}) and 6.5 µL ultra-pure water. The thermal profile consisted of an initial denaturation step at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 51 (COI) or 48°C (Cytb) for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The amplified fragments were electrophoresed on 1.0% agarose gels, checked under UV light and sequenced on the ABI 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Data Analysis

The sequencing data were aligned using ClustalX version 2.0 (Larkin *et al*., 2007). The aligned COI and Cytb sequences were 585 and 666 bp in length, respectively. Population genetic parameters, including the number of haplotypes, number of polymorphic sites, π, and H_d, were calculated using the DnaSP, version 5.0, software (Librado and Rozas, 2009). Median-Joining (MJ) networks of mtDNA haplotypes were constructed using the Network software based on statistical parsimony (Bandelt *et al*., 1999).

RESULTS

Sequence Variation

A statistical analysis using the *COI* gene (585 bp) and of the *Cytb* gene (666 bp) from 275 *R. padi* individuals revealed low levels of π among individuals and populations of *R. padi* in China. No insertions or deletions were found in either of the two mitochondrial genes. A total of 13 variable sites were inferred from the *COI* gene, three of which were parsimoniously informative sites, while the *Cytb* gene contained 36 variable sites, seven of which were parsimoniously informative sites.

Mitochondrial Gene Haplotypes

We obtained seven haplotypes for the *COI* gene (GenBank accession numbers KX827346–KX827352) and 15 haplotypes for the *Cytb* gene (GenBank accession numbers KX827353–KX827367). With regard to the geographical distribution of

COI haplotypes in China, H2, the most common haplotype, was shared by 186 individuals distributed in nearly all 15 geographical populations (except XZL). This haplotype was dominant in 11 *R. padi* geographical populations (Table 2). H1 was dominant in XZL, with an occupancy of 89.5% (17 of 19 individuals), while H3 was detected in 14 *R. padi* samples from QHX, with one individual characterized as haplotype H2. Moreover, the three common haplotypes H1, H2, and H3 shared only one or two polymorphic sites among them. The remaining haplotypes, i.e. H4, H5, H6, and H7 were found in single populations at low frequencies (one or two individuals). Except for H4 (one individual in XZL) with eight polymorphic sites compared with H1, there were few differences (Figure 1-A).

For the *Cytb* gene, H1 and H3 were dominant in the samples, with H1 detected in all *R. padi* populations and H3 in all 15 populations, except for XZL. H3 was the main haplotype in 11 populations. The main haplotype in populations JLB and XZL was H1, while haplotypes H2 and H6 were dominant in populations CQB and QHX, respectively, although each was only detected in its single population. Nucleotide comparisons of these dominant haplotypes (H1, H2, H3, and H6) in different *R. padi* populations showed only one or two mutation sites (Table 3). In addition, except for H9, which was found in three individuals from CQB and QHX, the remaining nine haplotypes were rare, represented by only 10 of the total 275 samples (Table 3).

Genetic Diversity

The average H_d values of COI, Cytb and the combined COI and Cytb partial sequences in the entire sample were 0.491, 0.607 and 0.689, respectively (data not shown). Among the populations of *R. padi*, the H_d of COI ranged from 0.000 (SXT) to 0.552 (SAH) (Table 4). For the *Cytb* gene, HBB had the lowest H_d (0.125), while QHX had the highest H_d (0.629) (Table 4). The haplotype diversity of the combined COI and Cytb partial sequences varied from 0.125 (SXT) to 0.743 (SAH) (Table 4). Compared with Cytb, the *COI* gene had a relatively lower H_d in both the total sample and in different geographical populations.

For π, COI varied from 0.000 (SXT) to 0.217% (SDH), Cytb from 0.019% (SXT) to 0.258% (SXH), and the combined COI and Cytb partial sequences ranged from 0.010% (SXT) to 0.218% (SXH) (Table 4). At the whole population level, the π values of COI, Cytb and the combined COI and Cytb partial sequences were 0.147%, 0.160% and 0.154%, respectively (data not shown). The various populations and mitochondrial markers both showed low levels of π.

Network of Mitochondrial Gene Haplotypes

The MJ network of COI haplotypes (Figure 1-A) revealed no apparent clades, and all haplotypes were grouped together.

Table 2. Distribution of the *COI* gene haplotypes in different *R. padi* georgraphical populations.[a]

H	Number of individuals from each population														
	CQB	HNN	AHC	HBW	HBZ	SAX	SAH	SDH	SDZ	SXT	SXH	HBB	JLB	QHX	XZL
H1	1	1	5	2		6	6	4	4		8	1	3		17
H2	17	15	15	21	15	18	13	5	11	16	12	14	13	1	
H3	8			1			2							14	1
H4															1
H5												1			
H6					2										
H7								1							

[a] Population codes are explained in Table 1. H: Haplotype.

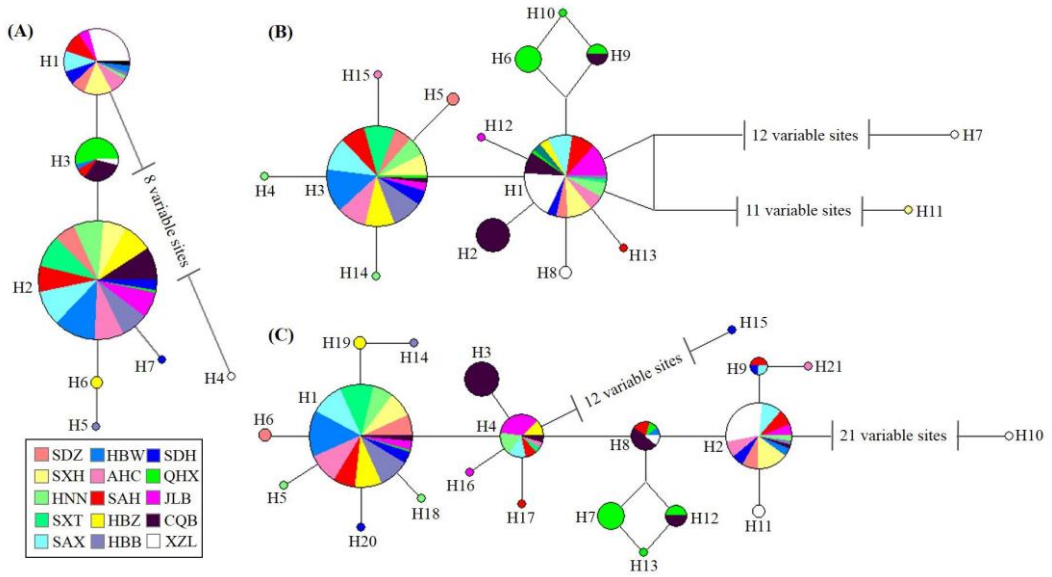

Figure 1. Median-joining network based on mitochondrial gene haplotypes. (A) Statistical parsimony network of seven *R. padi* COI haplotypes. (B) Statistical parsimony network of 15 *R. padi* Cytb haplotypes. (C) Statistical parsimony network of 21 *R. padi* haplotypes for the combined COI and Cytb partial sequences. Each circle represents a haplotype, and the area of the circle is proportional to the number of observed individuals. Colors within the nodes refer to *R. padi* sampling regions. Each line indicates a single variable site or is given when there is more than one variable site.

Table 3. Distribution of the *Cytb* gene haplotypes in different *R. padi* geographical populations.[a]

H	Number of individuals from each population														
	CQB	HNN	AHC	HBW	HBZ	SAX	SAH	SDH	SDZ	SXT	SXH	HBB	JLB	QHX	XZL
H1	7	5	5	3	3	8	8	3	4	1	8	1	11	1	16
H2	14														
H3	2	9	14	21	14	16	12	7	9	15	11	15	4	1	
H4		1													
H5									2						
H6														9	
H7															1
H8															2
H9	3													3	
H10														1	
H11											1				
H12													1		
H13							1								
H14		1													
H15			1												

[a] Population codes are explained in Table 1. H: Haplotype.

H2 was dominant in all populations, while the other haplotypes, which showed few genetic differences compared with H2, indicated no correlation with geographical distribution. There was only one haplotype (H4) with any genetic distance, but it was only detected in a single sample from the

Table 4. Genetic diversity of 15 *R. padi* populations as revealed by mitochondrial *COI* and *Cytb* genes.[a]

PC[a]	N[b]	*COI*				*Cytb*				Combined gene[g]			
		H[c]	*V*[d]	*Hd*[e]	π (%)[f]	*H*	*V*	*Hd*	π (%)	*H*	*V*	*Hd*	π (%)
CQB	26	3	2	0.495	0.094	4	4	0.643	0.164	6	6	0.677	0.131
HNN	16	2	2	0.125	0.043	4	3	0.617	0.106	5	5	0.650	0.077
AHC	20	2	2	0.395	0.135	3	2	0.468	0.074	4	4	0.489	0.103
HUW	24	3	2	0.236	0.066	2	1	0.228	0.034	3	3	0.236	0.049
HUZ	17	2	1	0.221	0.038	2	1	0.309	0.046	3	2	0.485	0.042
SAX	24	2	2	0.391	0.134	2	1	0.464	0.070	4	3	0.572	0.100
SAH	21	3	2	0.552	0.158	3	2	0.552	0.092	6	4	0.743	0.123
SDH	10	3	3	0.644	0.217	2	1	0.467	0.070	4	4	0.711	0.139
SDZ	15	2	2	0.419	0.143	3	2	0.590	0.100	3	4	0.590	0.120
SXT	16	1	0	0.000	0.000	2	1	0.125	0.019	2	1	0.125	0.010
SXH	20	2	2	0.505	0.173	3	13	0.563	0.258	3	15	0.563	0.218
HBB	16	3	4	0.242	0.085	2	1	0.125	0.019	3	5	0.228	0.047
JLB	16	2	2	0.325	0.111	3	2	0.492	0.079	4	4	0.692	0.094
QHX	15	2	1	0.133	0.023	5	4	0.629	0.192	5	5	0.629	0.113
XZL	19	3	9	0.205	0.162	3	14	0.292	0.235	4	23	0.380	0.201

[a] Population Code; [b] Sample size of each population; [c] Number of haplotypes; [d] Number of polymorphic sites; [e] Haplotypes diversity; [f] Nucleotide diversity, [g] the combination of COI and Cytb partial sequences.

XZL population and was not very informative.

Similarly, the Cytb haplotype network (Figure 1-B), based on statistical parsimony, supported the existence of only one clade. The two most common haplotypes (H1 and H3) were shared by most populations and possessed only one polymorphic site. Four restricted haplotypes (H4, H5, H14, and H15) evolved from H3 with one polymorphic site each. Several haplotypes also evolved from H1, and four restricted haplotypes (H2, H8, H12, and H13) each shared only one polymorphic site with H1. In addition, a small haplotype group (H6, H9, and H10) found mostly in QHX, also evolved from H1 with several polymorphic sites ($\leq$ 3 sites). Two restricted haplotypes (H7 and H11) were genetically distant from H1, with 13 and 12 polymorphic sites, respectively, and despite being similar to haplotype H4 in terms of the *COI* gene, they were found only in a single sample and were not very informative. For the haplotypes of the combined COI and Cytb partial sequences, all the haplotypes gathered together (Figure 1-C).

DISCUSSION

In this study, we explored the genetic diversity of 15 *R. padi* populations from most of the wheat-growing areas in China using mtDNA markers. The low level of genetic variation in *R. padi* was corroborated by the low degree of H_d and π based on the COI and Cytb partial sequences. Moreover, we found that the seven COI haplotypes and 15 Cytb haplotypes from the 275 examined individuals were clustered together for the most part, with no clear clade structure. Using the combined results for COI and Cytb partial sequences, we can infer that the mitochondrial haplotypes of *R. padi* do not show obvious geographical distribution patterns. Every shared haplotype was distributed in various populations, rather than one population predominantly.

The π values of the *COI* and *Cytb* genes were 0.147% and 0.160%, respectively.

This provides powerful evidence of low mitochondrial polymorphism. There are several scenarios that may account for the low level of genetic variation in aphids. First, the mtDNA of aphids may be highly conserved with low divergence. A divergence of only 0.4% was found in the *COI* gene of the pea aphid, *Acyrthosiphon pisum* (Boulding, 1998), and a divergence of only 1.5% was found in the *COI* genes of *Sitobion miscanthi* and *S. avenae* (Sunnucks and Hales, 1996). Furthermore, phylogenetic studies based on barcoding sequences also revealed limited intraspecific genetic divergence among *Aphidinae* species (Lee *et al*., 2011; Wang *et al*., 2011). For *R. padi*, the average intraspecific divergence was only 0.61% among individuals collected from 11 countries (Rakauskas *et al*., 2014).

Based on studies of genetic diversity in other aphids, low mitochondrial variability is common in most aphid species. Xu *et al.* (2011) sequenced part of the *COI* gene from 269 *S. avenae* individuals, collected from 17 geographical populations, and defined 16 haplotypes. Phylogenetic analysis also showed that all of these haplotypes were highly related to each other, with an absence of phylogeographical structure. Moreover, zero variation was found in a 332 bp sequence of the *COI* gene from 83 Russian wheat aphid species collected in the US between 1986 and 2006. Surprisingly, no new mtDNA haplotypes were found in the US over a 20-year period (Shufran *et al*., 2007). Zhao (2014) found no differences in the mitochondrial *COI/II* genes from 27 *M. persicae* populations in China. Simon *et al*. (1996) found only three mtDNA haplotypes among 176 *R. padi* clones, while Martinez-Torres *et al.* (1996) found four mtDNA haplotypes in *R. padi*. Rakauskas *et al*. (2014) found four COI haplotypes in *R. padi* populations from the entire eastern Baltic region. In our study of two mitochondrial genes, *COI* and *Cytb*, only seven and 15 haplotypes, respectively, were found in 275 individuals collected from 15 geographical populations. Moreover, as in a study of *S. avenae* (Xu *et al*., 2011), all haplotypes indicated little genetic difference with no obvious geographical pattern. The very low genetic diversity among the samples does not allow for resolution of the genetic structure of *R. padi*.

There was a greater frequency of polymorphisms in Cytb than COI, based on the number of haplotypes and H_d. Thirteen insect mitochondrial protein-coding genes can be classified into three groups of good (ND4, ND5, ND2, Cytb, and COI), medium (COB, COIII, NDl, and ND6), and poor phylogenetic performers (ATPase6, ND3, ATPase 8, and ND4L) in terms of recovering the expected trees among phylogenetically distant relatives (Mandal *et al*., 2014). COI is the best molecular marker for evolutionary studies in most insects, although in this study, Cytb performed slightly better than did COI in *R. padi*. The mitochondrial *COI* gene appears to be among the most conserved protein-coding genes in the mitochondrial genome, which may make it more suitable for DNA barcoding rather than genetic evolution in some insects, including aphids. Studies of *S. avenae* and *Diuraphis noxia* also demonstrated this. The Cytb sequence may be more effective and informative than the COI sequence in the study of aphid population genetics, especially within populations, as shown for the Lachninae (Chen *et al*., 2012).

The haplotype network analysis of COI and Cytb all revealed no distinguishable cluster and no obvious geographical distribution among the *R. padi* haplotypes, similar to the distribution pattern of *Schlechtendalia chinensis* in China (Li, 2009). This type of haplotype distribution pattern is characterized as "phylogenetic continuity, lack of spatial separation", with relatively extensive and recent historical interconnections through gene flow (Avise *et al*., 1987).

Migration may be one factor explaining the relatively low mitochondrial genetic diversity in *R. padi*. Radar detection of mass migration of aphids in Finland showed that *R. padi* was capable of long-range

migration, and this seasonal migration may affect the genetic structure of long-distance geographical populations (Nieminen *et al*., 2000). With regard to flight behavior, many factors need to be considered, including winter temperatures, density of insects in the air, wind conditions, among others (Llewellyn *et al*., 2003). Different environmental conditions also influence population differentiation, such as complex topography (mountains, unpopulated areas, and deserts), climate (arid or frigid), and different agricultural landscapes (Cardé and Minks, 1995), which may influence the differentiation of local *R. padi* populations.

The reproductive mode of aphids may also be a factor contributing to the low polymorphism frequency. Aphids have alternate pathways of adaptation to specific environments between sexual and asexual forms. The different lifecycles and fast rate of reproduction contribute to high rates of population increase and the success of aphids as a very successful group of organisms and as the most destructive insect pests (Afshari *et al.*, 2009; Loxdale, 2008; Kaldeh *et al*., 2012). We previously showed that most *R. padi* populations in China are anholocyclic with only parthenogenic females, and a few populations were able to produce gynoparae, males, or eggs (Duan *et al*., 2017). mtDNA exhibits strict maternal transmission with a limited repair system, and natural hybridization and introgression may result in the formation of new hybrid mitochondrial haplotypes (Shearer *et al*., 2002). The reproductive mode can influence genetic diversity and genetic structure and lead to significant genetic differentiation. In France, significant genetic differentiation was found between sexual and asexual *R. padi* populations, with multilocus F_{ST} estimates ranging from 0.103 for allozymes to 0.144 for microsatellites (Delmotte *et al*., 2002). The genetic variation in fitness of *R. padi* was higher in asexual genotypes compared with sexual genotypes (Carter *et al*., 2012).

Symbionts can also influence the mtDNA diversity of the host. Hurst and Jiggins (2005) reviewed the extent of symbiont shaping of mtDNA evolution, and most cases (17/19) indicated symbiont-driven decreases in mtDNA diversity, symbiont-driven increases in diversity, symbiont-driven changes in mtDNA variation over geographical areas, and symbiont-associated paraphyly of mtDNA. Once the host populations acquire one or more symbionts, patterns of mitochondrial polymorphism may be altered by natural selection. The selective sweeps of symbionts running through the population reduce mtDNA diversity, which is similar to the pattern produced by population bottlenecks (Hurst and Jiggins, 2005). *Wolbachia*-uninfected species were found to harbor more diverse mitochondria than host individuals of *Solenopsis invicta*, *S. richteri*, *Acraea encedon*, and *Drosophila recens* (Shoemaker *et al*., 1999, 2004; Jiggins, 2003). Aphids, as a model system for the study of insect–bacterium interactions, have been particularly well studied with regard to an obligate symbiont (*Buchnera aphidicola*) and various facultative symbionts, including several commonly studied species: *Hamiltonella defensa*, *Regiella insecticola*, *Serratia symbiotica*, *Rickettsia*, *Spiroplasma*, X-type, *Arsenophonus*, and *Wolbachia*. We detected *S. symbiotica* and *Wolbachia* in some *R. padi* populations used in this study (Liu, 2014). Such single infections or co-infections may facilitate host-parasite coevolution, resulting in strong directional selection.

The plasticity of genetic diversity is also influenced by the host plant or family (Charaabi *et al*., 2008; Carletto *et al*., 2009). Unitary host selective pressure is not beneficial for genetic differentiation or diversity (Valentine, 1976). Genetic variability of the green citrus aphid *Aphis spiraecola* assessed by Random Amplified Polymorphic DNA (RAPD) and COI revealed that the host plant had a significant effect on the pattern of genetic diversity (Mezghani-Khemakhem *et al*., 2012). Generally, the host plant of *R. padi* is wheat, although this species may use alternative

hosts in autumn. In fact, all *R. padi* individuals collected from wheat showed low host selection pressure on genetic variation. Recently, anthropogenic selective pressures have been taken into account, such as the development of resistant plants and/or the use of insecticides, which kill most individuals in the field, thereby reducing the genetic diversity of *Aphis gossypii* and *Myzus persicae* (Brévault *et al*., 2008; Zamoum *et al*., 2005). Genetic hitchhiking by advantageous genotypes could reduce π (Martínez-Torres *et al*., 1997). Meanwhile, the impact of human activities, such as agricultural activities, variety selection, and pest management techniques, on genetic differentiation cannot be ignored (Chen *et al*., 2007a, b; Lu and Gao, 2009). Human-aided dispersal may also accelerate differentiation. The global presence of the melon fly *Bactrocera cucurbitae* is associated with human-mediated dispersal, and the very low genetic variation may be related to large-scale management techniques (Prabhakar *et al*., 2012).

R. padi is a notorious pest insect widely distributed throughout the wheat-growing regions of China. We analyzed the partial sequences of the mitochondrial *COI* and *Cytb* genes of *R. padi* to determine the extent and nature of the genetic variation in this species in China. We observed low levels of polymorphism in *R. padi* field populations. This phenomenon was also found in other aphids, such as *Aphis spiraecola*, *Diuraphis noxia*, and *Sitobion avenae*, indicating that mtDNA is not an effective or informative molecular marker. Evaluation of microsatellite markers in nuclear genes and single nucleotide polymorphisms at the genome level, together with next generation sequencing, may yield more promising molecular markers for population genetics.

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تنوع ژنتیکی در میتوکوندری شته Bird Cherry-Oat Aphid *Rhopalosiphum padi* (L.) در چین

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چکیده

دی.ان.ای میتوکندریا (mtDNA)نشانگر ژنتیکی مهمی در زیست شناسی جمعیتی و تکاملی به شمار می رود. به منظور آزمون امکان سنجی از دو نشانگر ژن میتوکندریایی (COIو Cytb) برای *Rhopalosiphum padi*، ما ۲۷۵ نمونه از این گونه حشره را از ۱۵ محل که شامل بیشتر مناطق وگستره پراکنش تحت پوشش این حشره در چین بود جمع آوری کردیم و تنوع ژن های مزبور را تجزیه وتحلیل کردیم. درنتیجه، ۷ هپلوتیپ(تک جور) *COI* و۱۵ هپلوتیپ *Cytb*با، به ترتیب،۱۳ و

۳۶ مکان پلی مورفیک شناسایی شد. در همه نمونه ها میانگین تنوع هپلوتیپ (H_d) مربوط به COIو Cytbبه ترتیب ۰/۴۹۱ و ۰/۶۰۷ بود و برای تنوع نوکلئوتید (π) مربوط به COIو Cytbبرابر ۰/۱۴۷٪ و ۰/۱۶۰٪ بود. میتوان گفت که بر مبنای این دو ژن، در میان همه جامعه *R. padi*سطح تنوع ژنتیکی و تمایز ژنتیکی نسبتا پایین بود. افزون بر این، شبکه های parsimonyهپلوتیپ های COIو Cytbدر *R. padi*همگی از یک هم نیای واحد (single clade) بود. هرچند تغییرات نوکلئوتید ژن های میتوکوندریایی در حشرات دیگر به کار برده شده، با بررسی منابع علمی جدید در مورد تنوع میتوکوندری در گونه شته آشکار شد که زیست شناسی جمعیتی و تکاملی شته ها منجمله *R. padi*را نمی توان تنها با تحلیل mtDNAروشن ساخت و این امر بیشتر به علت کم بودن تنوع ژنتیکی مربوط به نشانگر های ژنتیکی میتوکوندری در جامعه است. به این قرار، پیشنهاد می شود که در بررسی های تکاملی روی جمعیت های شته، برای جبران مشکل کمبود اطلاعات ژنتیکی به دست آمده از mtDNA، علاوه بر mtDNAاز نشانگر های دیگر مانند ریزماهواره ها نیز استفاده شود.