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Life cycle and population genetics of bird cherry-oat aphids *Rhopalosiphum padi* in China: an important pest on wheat crops

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Abstract *Rhopalosiphun padi* is a pest that seriously devastates wheat crops. The life cycle, genetic diversity, and genetic structure of *R. padi* populations throughout China remain unclear. In the current study, we collected 17 *R. padi* populations throughout the wheat-growing region of China. Classical standard methods were used to determine the life cycles of 369 clones from the sampled populations. Five polymorphic microsatellite loci were used to genotype individuals from each clone. The results revealed that two populations from spring wheat-growing regions showed cyclical parthenogenesis, whereas 15 populations from winter wheat-growing regions showed obligate parthenogenesis. There was a significant genetic difference between populations with obligate parthenogenesis and populations with cyclical parthenogenesis. Populations with cyclical parthenogenesis did not show significant departures from Hardy–Weinberg equilibrium, whereas all populations with obligate parthenogenesis exhibited significant departures from Hardy–Weinberg equilibrium. Significant genetic structures were found in the populations. Two *R. padi* populations with cyclical parthenogenesis showed similar genetic structures, two populations from a subtropical plateau had similar genetic structures, and the populations sampled in the large winter wheat-growing regions in northern and central China showed similar genetic structures. There was a significant isolation-by-distance effect present among different populations. To the best of our knowledge, this is the first documentation of the life cycle and population genetic of *R. padi* in China. Our results are important for the design and optimization of sustainable pest management strategies.

Keywords *Rhopalosiphum padi* · Microsatellite marker · Reproductive mode · Genetic diversity · Genetic structure · Hemiptera · Aphididae

Key message

- *Rhopalosiphum padi* is one of the most serious insect pests that devastate wheat crops. This species is distributed in all wheat-production areas and causes serious damage to wheat each year in China. The life cycle and population genetics of *R. padi* populations throughout China remain unclear.
- Our analysis of reproductive-mode and population-genetics can reveal the reproduction evolution and ecological adaptation strategies of the aphid species in agroecosystems, and thereby increase our understanding of how sexual and asexual reproduction affects the genetic diversity, genetic structure, and gene flow of different aphid populations.
- Cyclical parthenogenesis and obligate parthenogenesis occur in the *R. padi* populations of China. The life cycle and geography-related climates underlie the genetic diversity and genetic structure of *R. padi* populations. Design and implementation of sustainable pest management strategies could, therefore, be adjusted to the different life cycle and population genetics of this aphid.

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Introduction

The prevalence and maintenance of sexual reproduction remains an important research area in evolutionary and developmental biology. In fact, asexual lineages possess twice the reproductive capacity of sexual lineages because they avoid the cost of producing males (Maynard Smith 1978), which provides an immediate demographic advantage. However, sexual reproduction still dominates, whereas asexual reproduction is rare. Many theories have explained the evolutionary success of sexual reproduction based on the long-term and short-term advantages of sex (Kondrashov 1988; Barton and Charlesworth 1998). The general consensus has held that genetic constraints such as mutation accumulation and plasticity of environmental changes would advance the sexual reproductive mode (Kondrashov 1988; Peck 1994; Hamilton 1980; Doncaster et al. 2000).

Aphids are unusual organisms that show alternative pathways of sexual and asexual development under specific environments and are, therefore, well-suited to research regarding the evolution of sex (Rispe and Pierre 1998; Simon et al. 2002). The coexistence of different reproductive modes within some aphid species offers a clear and direct comparison of the sexual and asexual reproductive modes. Numerous, irreversible transitions between the two reproductive modes within the same species or population provides multiple opportunities to assess the effect of recent and older clonality and the costs and benefits of sex (Dedryver et al. 2001; Delmotte et al. 2001; Simon et al. 2003; Vorburger et al. 2003). Some aphid species that are adapted to a specific environment can use both sexual and asexual forms and can have either or both of the two major life cycles (holocyclic life cycle and anholocyclic life cycle) under different climates. In a holocyclic life cycle, the cyclical parthenogenesis lineages would engage in sexual reproduction once per year under inductive conditions, with generations of asexuality followed by the production of apterous viviparous females (named virginoparae). The males and gynoparous females would then be parthenogenetically produced by virginoparae, and oviparous females (sexual type of female) would be parthenogenetically produced by gynoparous females. At the end of the season, the males would mate with oviparous females, which would produce cold-resistant, diapausing eggs. During the anholocyclic life cycle, the obligate parthenogenesis lineages show continuous parthenogenesis with viviparous females throughout the year. The apterous virginoparae, viviparae, gynoparae, apterous ovipare, and the males are morphologically different, whereas the alate viviparae and gynoparae show similar morphologies. For the bird cherry-oat aphids *Rhopalosiphum padi*, the gynoparae can produce both males and ovipare, but the viviparae can produce only apterous and alate viviparae, and the alate viviparae and gynoparae can be separated based on their preference for either *Prunus* (alate gynoparae) or wheat (alate viviparae) (Blackman 1971; Dedryver et al. 1998; Simon et al. 2002).

In organisms with sexual and asexual taxa such as aphids, the geographical distributions of reproductive modes shows striking differences, and this characteristic is referred to as geographical parthenogenesis (Vandel 1928; Peck et al. 1998). This phenomenon presumably reflects ecological differences in the reproductive modes or historic colonization patterns (Dixon 1998; Paland et al. 2005; van Emden and Harrington 2007). Geographic parthenogenesis occurs at higher latitudes and altitudes or in extreme habitats (Lynch 1984; Peck et al. 1998). Most aphid species, including *Sitobion avenae* (Dedryver et al. 2001), *Macrosiphum rosae* (Wöhrmann and Tomiuk 1988), *Acyrthosiphon pisum* (Kanbe and Akimoto 2009), and *Myzus persicae* (Blackman 1974; Guillemaud et al. 2003), are obligate parthenogens in the lower latitudes with temperate environments. The life cycle of aphids appears to be primarily determined by the winter climate (Rispe and Pierre 1998; Rispe et al. 1998; Dedryver et al. 2001). In regions with cold winters, the cold-resistant, diapausing eggs, which are annually produced by sexual females, are the only life stage surviving in environments with low temperature and without suitable hosts, where the nymph and adult aphids die (Gilabert et al. 2009). In regions with a mild winter climate, there is no diapause for the asexual forms, and the survival of eggs is possibly compromised a higher temperatures (Danjuma et al. 2014), which makes the asexual forms predominate because of being demographically favored (Simon et al. 2002).

The reproductive mode of aphids can influence the structure of the aphid population (Delmotte et al. 2002). During sexual reproduction, the favorable mutations are recombined and the deleterious mutations are eliminated, which increases the genotypic diversity of the population (Balloux et al. 2003; Bengtsson 2003). The sexual populations show higher polymorphism and genetic variability than the asexual populations of a species, which leads to significantly different population genetics in populations with different life cycles (Delmotte et al. 2002). Compared with sexual populations, asexual aphid populations show significant heterozygote excess (Sunnucks et al. 1997; Simon et al. 1999b). Meanwhile, studies showed that populations with cyclical parthenogenesis differed from those with obligate parthenogenesis in the genetic indices, revealing a specific genetic differentiation and limited gene flow between different reproductive modes (Simon et al. 1999b; Miller 2000; Delmotte et al. 2002; Guillemaud et al. 2003; Papura et al. 2003; Vorburger et al. 2003). Despite

their complex life cycles, aphids have a high population growth rate and different phenotypes that can cause serious damage in temperate countries by ingesting plant sap for nutrition and transmitting viral diseases to many crops (Blackman 1974; Parry et al. 2006; Loxdale 2008; Fenton et al. 2010).

Wheat (*Triticum aestivum* L.) cultivation has a long history in China, and this crop is grown across most of the country (except for four southernmost provinces). Two types of wheat, spring wheat and winter wheat, are used. Spring wheat was grown in northwest and northeast China, where the winter temperature was between −10 °C and −30 °C, whereas winter wheat is grown in temperate areas. *Rhopalosiphum padi* is one of the most serious insect pests affecting wheat in China. This species is distributed in all wheat-production areas and causes serious damage to wheat each year (Chen et al. 2007a, b; Lu and Gao 2009). Similar management methods were used to control wheat aphis in both spring wheat and winter wheat production areas. Because of the massive use of insecticides, *R. padi* had developed resistance to different types of chemical insecticides (Chen et al. 2007a, b; Lu and Gao 2009). Meanwhile, besides direct feeding on wheat, *R. padi* damages wheat seriously by transmitting the Barley yellow dwarf virus (BYDV) throughout China (Zhang et al. 1985). Actually, to keep the quality and yield of wheat crops, the central government promoted area-wide wheat pest control strategies in the whole country in the last decade.

Reproductive mode and population genetics can reveal the evolution of reproduction mode and adaptive strategies of aphid species in agroecosystems, and thereby increase our understanding of how sexual and asexual reproduction affect the genetic diversity and genetic structure of different aphid populations, as well as gene flow between aphid populations, which is important for the design and optimization of sustainable pest management strategies (Delmotte et al. 2002; Wang 2007; Halkett et al. 2008; Kanbe and Akimoto 2009). For example, life cycles can affect the mutation rates and flow of insecticide resistance genes (Fenton et al. 2003; Halkett et al. 2008; Loxdale 2008). Populations with similar genetic structure and genetic diversity should be considered as a management unit for effective control (Chen and Dorn 2010; Zheng et al. 2013; Torriani et al. 2010). In that frame, our objective was to characterize the reproductive mode, genetic diversity, and genetic structure of *R. padi* populations in China. We collected *R. padi* populations from wheat in different regions of China and analyzed the reproductive modes using standard biological methods under induced environmental conditions. The genetic diversity and genetic structure were analyzed based on microsatellite data. We hypothesized that the population genetics of *R. padi* are affected by the reproductive modes of the population and by the widely varied climates of the sampling regions.

Materials and methods

Insect sampling

Apterous adults of *Rhopalosiphum padi* were collected from wheat (*Triticum aestivum* L.) from 17 localities covering 12 provinces in China during the jointing and heading stage of wheat. A minimum of 18 to a maximum of 30 aphids per population were sampled during May–July 2013 (Table 1), and to minimize sibling collection we sampled one aphid every 30 m. Each individual was brought to the laboratory in a tube to set up a clone (parthenogenetic line) on seedlings of wheat cultivar "Xiaoyan 22" at 24 ± 1 °C, 40 % RH, and a 16:8 h (L:D) photoperiod. Aphids from the same locality were referred to as one "population". Two populations (JLB and GSL) were collected from spring wheat-growing areas with cold winters. A total of 15 populations were collected from the temperate winter wheat-growing areas, of which two populations (CQB and SAH) were collected from two neighboring physiographical basins and two populations (GZG and YNK) were collected from the subtropical Yunnan–Guizhou Plateau. All the clones were parthenogenetic under the rearing condition in the laboratory, one individual was randomly taken from the first generation of each clone and preserved in absolute ethanol for DNA extraction and PCR analysis. Genotype of the randomly-taken individual of the first generation in the parthenogenetic line was considered to represent that of the field sample.

Determination of reproductive modes

To determine the reproductive mode of the populations, the *R. padi* clones were induced using standard methods in program-controlled incubators with a light intensity of 20,000 lx at 50 cm from the light source under the long-night (L:D 8:16 h) and low-temperature (12 °C) inductive condition (Simon et al. 1991; Dedryver et al. 1998; Delmotte et al. 2001; Margaritopoulos et al. 2002; Vorburger et al. 2003). Briefly, 15 adult aphids (G_0) from each clone were randomly chosen, transferred to inducing conditions, and allowed to reproduce for 3 days. Three apterous females from these offspring (G_1) were randomly selected and maintained separately under the same temperature and photoperiod regime. All G_1 progeny (G_2) were reared on wheat seedlings. The morph of each of the G_2 aphids was identified when they reached adulthood. Males were

Table 1 Sampling information for 17 *Rhopalosiphum padi* populations in China

Province	Locality	Population code	Coordinates	Collection date	Wheat type	*N*
Jilin	Baicheng	JLB	122°52′ 45°39′	10 Jul 13	SW	18
Gansu	Lanzhou	GSL	103°41′ 36°05′	13 Jun 13	SW	21
Shaanxi	Hanzhong	SAH	107°27′ 33°11′	8 Apr 13	WW	21
	Xianyang	SAX	108°05′ 34°17′	18 Jul 13	WW	23
Chongqing	Beibei	CQB	106°25′ 29°49′	2 Apr 13	WW	24
Hebei	Baoding	HBB	115°26′ 38°49′	7 Jun 13	WW	20
Shanxi	Taigu	SXT	112°34′ 37°25′	27 May 13	WW	20
	Hongtong	SXH	111°41′ 36°13′	28 May 13	WW	24
Shandong	Zibo	SDZ	118°02′ 37°06′	10 May 13	WW	20
	Taian	SDT	117°14′ 36°06′	6 May 13	WW	24
	Heze	SDH	115°29′ 35°10′	4 May 13	WW	18
Anhui	Chuzhou	AHC	118°20′ 32°21′	21 Apr 13	WW	22
Henan	Nanyang	HNN	112°36′ 33°14′	18 Apr 13	WW	18
Hubei	Zaoyang	HUZ	112°47′ 32°08′	16 Apr 13	WW	20
	Wuhan	HUW	114°19′ 30°29′	14 Apr 13	WW	22
Guizhou	Guiyang	GZG	106°32′ 26°28′	20 Apr 13	WW	24
Yunnan	Kunming	YNK	103°06′ 25°21′	27 Mar 14	WW	30

The initial two letters of the population code refer to the province, and the third letter refers to the sampling location

N number of individuals genotyped in each population, *SW* spring wheat-growing regions, *WW* winter wheat-growing regions

recorded and then discarded. The same process was followed to ascertain aphid morphs of the G_2–G_5 generations. Under the induced conditions, clones that could produce gynoparae, ovipare, and males were classified as characterized by cyclical parthenogenesis, and clones that could only produce parthenogenetic viviparae were classified as characterized by obligate parthenogenesis.

Microsatellite genotyping

One individual from each reared clone of the populations was used for genomic DNA extraction. Genomic DNA of each individual was extracted using the EasyPure™ Genomic DNA Kit (TransGen Biotech Co., Ltd. China). Extraction was performed according to the bench protocol for animal tissues. DNA was eluted in 30 μL of ultra-pure water and stored at −20 °C.

All individuals were genotyped at five microsatellite loci isolated from *R. padi* (Simon et al. 2001; Table 2). Three primers, which included the forward primer with an M13 (−21) at the 5′ end, the reverse primer and the FAM fluorescent dye labeled M13 (−21) primer (Schuelke 2000), were used for amplification of each microsatellite locus in a polymerase chain reactions (PCR). PCR were performed using a C1000 Thermal Cycler (BIO-RAD, Hercules, CA, USA) in a total volume of 25 μL, containing 12.5 μL 2 × *Taq* Master Mix (containing 0.05 U/μL *Taq* DNA Polymerase, 2 × *Taq* PCR Buffer, 3 mM $MgCl_2$ and 400 μM dNTP mix) (Beijing CoWin Biotech Co., China), 0.5 μL each forward primer (10 μM), 2 μL each reverse primer (10 μM), 2 μL M13 primer (10 μM), and 1.5 μL genomic DNA (10–30 ng/μL). PCR amplification was employed with denaturation at 95 °C for 2 min, followed by 30 amplification cycles consisting of 95 °C for 20 s, 20 s at the primer-specific annealing temperature (Table 2), 72 °C for 20 s. This was followed by eight cycles including 95 °C for 30 s, 53 °C for 45 s, and 72 °C for 45 s, and a final step at 72 °C for 10 min. To examine the length of the amplified PCR products, an ABI3730XL automated DNA sequencer (Applied Biosystems, Foster City, CA, USA) were used and all genotypes were called by GENEMAPER version 4.0 (Applied Biosystems, Foster City, CA, USA).

Data analysis

The null allele frequency of each microsatellite locus was estimated using MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004). The observed heterozygosity (H_O) and expected heterozygosity (H_E), number of alleles (N_A), and allelic richness (A_R) were calculated using FSTAT Version 2.9.3.2 (Goudet 2002). Hardy–Weinberg equilibrium (HWE) and HWE *P* values were calculated using GENEPOP v4.0 (Rousset 2008). Based on the Bayesian

Table 2 Polymorphic microsatellite loci used for genotyping *Rhopalosiphum padi* populations

Microsatellite loci	Numbers of alleles			Size range		
	China	Australia	France	China	Australia	France
R2.73	24	8	6	243–317	246–286	262–285
R3.171	21	6	15	229–292	227–294	214–252
R5.10	14	8	8	253–292	256–268	256–274
R5.138	31	13	20	221–292	211–263	211–287
R5.50	12	16	25	292–333	303–343	297–403

Number of alleles and size range information for *R. padi* population of China is obtained from the current study, for *R. padi* population of French was cited from Simon et al. (2001), and for *R. padi* population of Australia was cited from Valenzuela et al. (2010)

clustering approach, the population structure was analyzed using STRUCTURE version 2.3.3 (Pritchard et al. 2000) with the admixture ancestry model and the correlated allele frequency model. The number of clusters (K) was set from 1 to 10 and repeated 20 times. Each repetition consisted of a burn-in period of 50,000 iterations and one million Markov Chain Monte Carlo (MCMC) repetitions. The online program STRUCTURE HARVESTER (Earl and Vonholdt 2012) was used to calculate the most probable value of genetic clusters (K) using the Evanno method (Evanno et al. 2005). The graphical display of genetic structure was produced using DISTRUCT (Rosenberg 2004). The genetic distances were calculated using the program MICROSATELLITE ANALYSER (MSA) version 3.15 (Dieringer and Schlotterer 2003). The neighbor-joining (NJ) was performed using MEGA 5 (Tamura et al. 2011) based on Nei's standard genetic distance (D_S) and Cavalli-Sforza's chord distance (D_C). Dc distance-based tree topology is generally more robust for gene frequency data (Takezaki and Nei 1996), and it is insensitive to null alleles (Chapuis and Estoup 2007; Zhang et al. 2009). The molecular variance (AMOVA) between different groups was ascertained using ARLEQUIN version 3.5.1.2 (Excoffier and Lischer 2010), as well as by calculating the inbreeding coefficients (F_{IS}) and pair-fixation indices (F_{ST}), and the significance levels were assessed (P values) with 10,000 permutations (Weir and Cockerham 1984).

In terms of AMOVA, samples were arbitrarily grouped according to the sexual or asexual mode of reproduction. A principal component analysis (PCA) was used to visualize the patterns of genetic relationships among populations based on the covariance matrix of gene frequencies using PCAGEN version 1.2 (http://www2.unil.ch/popgen/softwares/). The analysis of isolation by distance (IBD) was performed using the ZT software package (Bonnet and Van der Peer 2002). For the Mantel test, the matrices of genetic distance $F_{ST}/(1 - F_{ST})$ and the geographic distance (in) between different populations were compared using the Mantel test with 10,000 permutations (Mantel 1967).

The clone diversity was evaluated by a previously reported as *G/N* ratio, with G equal to the number of MLG (multilocus genotype) revealing by molecular markers and N equal to the sample size of each population (Vorwerk and Forneck 2006). The *G/N* ratio ranges from 0 (all individuals share the same genotype, in strict clonality) to 1 (all individuals have distinct genotypes, under sexual reproduction; Ivey and Richards 2001). We calculated the number of distinct MLG detected per population (G) by the computer programs GENCLONE (Arnaud-Haond and Belkhir 2007). Meanwhile, the MLGsim program was used to calculate the likelihood of MLGs originating from clonal reproduction (Stenberg et al. 2003). The P_{sex} values were estimated for each repeated genotypes from each population by Monte Carlo simulation method as reported by Halkett et al. (2005). The significant P_{sex} value indicated the clone production existing in the population, and non-significant P_{sex} value suggested the sexual reproduction (Halkett et al. 2005; Vorwerk and Forneck 2006).

Finally, POWSIM ver. 4.1 (Ryman and Palm 2006) was used to evaluate the sensitivity of the statistical power of microsatellite markers for detecting genetic differentiation at various levels of F_{ST}. Simulations were carried out for an effective population size of $N_e = 1000$ to yield F_{ST} values of 0.001, 0.0025, 0.005, 0.01, 0.02, 0.025, and 0.05 with 1000 replicates (Ryman and Palm 2006). The power of the analysis was indicated by the proportion of tests that were significant at $P < 0.05$ based on Chi square tests and Fisher's exact tests using the respective allele number and frequency at five microsatellite loci studied (Ryman and Palm 2006; Lyons et al. 2012; Provan et al. 2013).

Results

Reproductive mode

A total of 369 clones were established for the *R. padi* samples collected from 17 different populations. Each

individual of a clone was genotyped with five microsatellite loci. Life cycles of each clone were determined using standard methods.

Under the inductive conditions, only alate and apterous viviparous females were observed in all clones of the 15 populations (CQB, SAH, SAX, HNN, HUZ, HUW, HBB, SXT, SXH, SDZ, SDH, SDT, AHC, GZG, and YNK) collected from winter wheat-growing regions with mild winters, meaning the life cycle of these populations are anholocyclic. All clones from two populations (JLB and GSL) were collected in the spring wheat-growing area of northeastern China and were able to produce gynoparae, males, and ovipare, indicating that these populations were holocyclic.

Genetic variation and diversity

All five microsatellite loci were polymorphic in these *R. padi* populations. A total of 110 alleles were detected across five microsatellite loci, the number of allele at each locus was ranging from 13 to 35, and an average of 22 alleles per locus (Table 2). The Chinese populations showed a higher alleles number of five loci than the observed allelic number in similar French (Simon et al. 2001) and Australia study (Valenzuela et al. 2010). The average number of alleles ranged (N_A) from 2.6 to 10.0 per population, and the allelic richness (A_R) ranged from 2.52 to 10.0 (Table 3). Four populations showed a high number of alleles, considerable allelic richness, two of which populations (JLB and GSL) were from the spring wheat-growing regions and two of which (CQB and SAH) were from two respective physiographic basins. The mean value of observed heterozygosity (H_O) was between 0.600 and 0.825, whereas the expected heterozygosity (H_E) was between 0.434 and 0.830. Two populations (JLB and GSL) determined to be characterized by cyclical parthenogenesis did not show significant departures from HWE, whereas the other 15 populations with obligate parthenogenesis showed significant departures from HWE equilibrium. All populations from winter and spring wheat areas showed similar genetic diversity across the five microsatellite loci, with the exception of the populations from the two physiographic basins. The frequency of null alleles ranged from 0 to 0.175, which was similar to previous reports of *R. padi* (Gilabert et al. 2009).

We found 169 MLGs in all the individuals at the five microsatellite loci, 24 of which were shared among populations, and 145 genotypes were unique in one population. Most of the shared MLGs showed significant P_{sex} value, indicating the MLGs were generated by clonal reproduction (Table 4). The number of MLGs for each population was from three to 22, and the *G/N* ratio for clone diversity

Table 3 Indices of genetic diversity and the reproductive mode of the 17 *Rhopalosiphum padi* populations

Pop	N_A	A_R	H_O	H_E	F_{IS}	HWE-*P*	MLGs	*G/N*	*RG*	P_{sex}-*S*	*LC*
JLB	10.0	10.0	0.811	0.830	0.023	0.060	18	1	0	0	CP
GSL	7.4	6.99	0.600	0.656	0.087	0.214	21	1	0	0	CP
CQB	8.8	8.41	0.775	0.802	0.035	*	22	0.92	4	4	OP
SAH	7.8	7.54	0.714	0.757	0.058	*	20	0.95	3	3	OP
HBB	2.6	2.52	0.780	0.434	−0.838	*	3	0.15	2	2	OP
SXT	3.0	2.92	0.760	0.448	−0.727	*	5	0.25	1	1	OP
SXH	4.0	3.75	0.792	0.560	−0.426	*	5	0.21	4	4	OP
SAX	5.4	5.08	0.757	0.588	−0.294	*	10	0.43	5	5	OP
SDZ	3.8	3.76	0.730	0.565	−0.301	*	12	0.60	6	5	OP
SDT	3.8	3.49	0.792	0.499	−0.608	*	6	0.25	2	2	OP
SDH	4.0	4.00	0.733	0.579	−0.277	*	12	0.67	5	4	OP
AHC	3.8	3.71	0.800	0.570	−0.417	*	9	0.41	5	5	OP
HNN	5.4	5.40	0.744	0.570	−0.318	*	9	0.50	5	5	OP
HUZ	3.6	3.54	0.780	0.487	−0.628	*	6	0.30	3	2	OP
HUW	5.2	4.88	0.736	0.555	−0.336	*	16	0.73	4	4	OP
GZG	5.2	4.87	0.825	0.597	−0.394	*	13	0.54	6	6	OP
YNK	4.8	4.47	0.813	0.695	−0.174	*	17	0.57	4	4	OP

The codes for populations are explained in Table 1

N_A mean numbers of alleles per locus, A_R allelic richness based on seventeen samples per population, *Ho* observed heterozygosity, H_E expected heterozygosity, F_{IS} the inbreeding index, *HWE-P P* value for Hardy–Weinberg equilibrium, * Significant departures from HW equilibrium, $P < 0.05$ MLGs, number of multilocus genotypes; *G/N*, index of reproduction diversity (MLGs/N), *RG* repeated genotype, $P_{sex} - S$ number of MLG with significant P_{sex}, *LC* life cycle, *CP* cyclical parthenogenesis, *OP* obligate parthenogenesis

Table 4 Information of repeated multilocus genotypes of *Rhopalosiphum padi* populations in China

MLG	*N*	*P* value and significant of P_{sex}	Microsatellite allele size					Distribution and number of MLG
			R2.73	R3.171	R5.10	R5.138	R5.50	
RP007	2	0.009*	248/279	244/244	272/276	254/264	321/331	YNK2
RP017	2	0.063^{ns}	279/279	244/244	272/276	254/264	321/331	HBB1, GZG1
RP022	3	0.012^{*}	279/284	244/246	272/272	268/274	319/321	SDH3
RP028	2	0.006*	279/285	243/245	272/272	268/274	319/321	HUW1, HUZ1
RP031	2	0.040*	279/285	244/244	272/276	264/264	321/331	SDH1, SDZ1
RP035	3	0.026*	279/285	244/246	272/272	268/274	292/319	SDZ3
RP037	46	0.000*	279/285	244/246	272/272	268/274	319/321	SDZ6, SDT2, SDH4, AHC2, HNN9, HUZ14, HUW6, SAH1, CQB2
RP038	61	0.000*	279/285	244/246	272/272	268/276	319/321	HBB18, SXT16, SXH15, SAX12
RP042	4	0.003*	279/285	244/246	272/272	269/274	319/321	AHC1, HUW2, HNN1
RP045	2	0.017*	279/285	244/246	272/272	292/292	292/319	SAH1, SDH1
RP048	2	0.189^{ns}	279/285	244/253	272/272	268/274	319/321	HUZ2
RP053	30	0.000*	279/285	246/248	272/272	268/274	319/321	SDT18, AHC12
RP064	25	0.000*	279/300	244/244	272/276	254/264	321/331	GZG7, YNK9, AHC2, HNN1, SAX2, SAH1
RP066	5	0.000*	279/300	244/244	272/276	254/292	321/331	GZG4, HNN1
RP068	4	0.000*	279/300	244/244	272/276	264/264	321/331	SDZ2, SDH1, HUW1
RP071	3	0.309^{ns}	279/300	244/246	272/272	268/274	319/321	SDH2, SDZ1
RP079	8	0.000*	279/301	244/244	272/276	254/264	321/331	SXH4, SAX1, AHC1, GZG2
RP110	2	0.000*	283/285	242/242	272/272	260/260	319/319	YNK2
RP121	6	0.000*	283/300	242/246	268/272	259/265	319/321	GZG2, YNK2
RP126	2	0.000*	283/301	242/246	268/272	259/265	319/321	GZG1, CQB1
RP137	2	0.000*	298/300	238/242	272/274	252/252	321/323	SAH2
RP138	4	0.000*	298/303	238/240	272/272	261/268	321/321	HNN2, SAX2
RP140	2	0.000*	299/303	238/240	272/272	261/268	321/321	SXH1, SAX1
RP149	2	0.000*	300/303	240/242	272/276	256/263	311/323	CQB2

Arabic numerals after the population codes in the last column indicated the number of sample with same MLG, the codes for populations are explained in Table 1

MLG name of multilocus genotypes, *N* number of sample with same MLG, * Significant of P_{sex} value of MLG, *ns* non-significant P_{sex} value

was between 0.15 and 1 (Table 3). Population from two spring wheat areas (GSL and JLB) had the maximal value of *G/N* ratio, indicating the populations were sexual reproductive. Four MLGs (RP037, RP038, RP053, and RP064) were most common and widely spread in winter wheat-growing regions (Table 4).

Genetic structure

According to the Bayesian analysis performed with the software STRUCTURE 2.3.3, the mostly likely value of *K* was 3 (Fig. 1), indicating that the 17 populations could be assigned to three genetic clusters. The proportions of each population that contributed to each of the three clusters are shown in Fig. 2. Cluster 1 (in blue) mainly included individuals from two populations from spring wheat-growing area and two populations from the physiographic basin in northwestern China (JLB: 0.975, implying that 97.5 % of individuals from JLB contributed

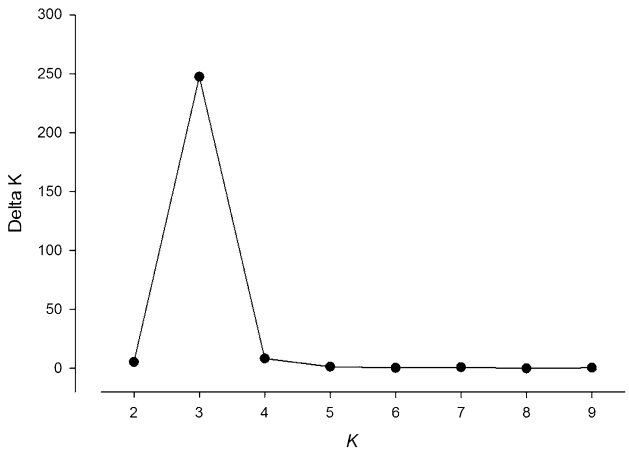

Fig. 1 ΔK values for different *K* calculated using the Evanno method

to this cluster; GSL: 0.934; CQB: 0.749; and SAH: 0.428). Cluster 2 (in green) consisted primarily of individuals sampled in two subtropical regions on the

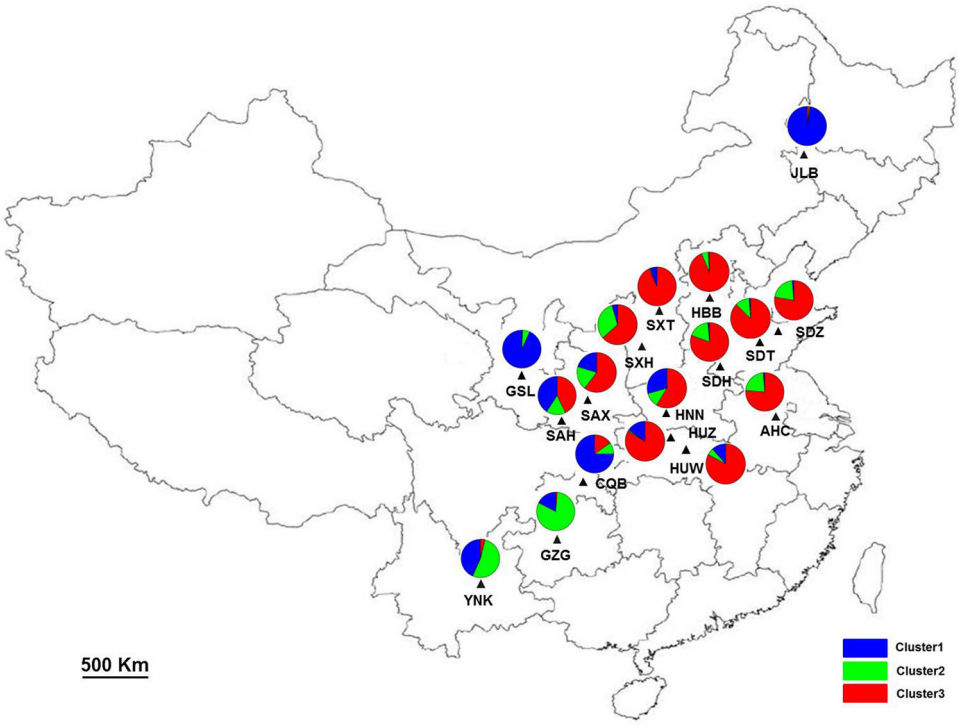

Fig. 2 Sampling locations of *Rhopalosiphum padi* populations and Bayesian clustering analysis using STRUCTURE. The codes for populations are explained in Table 1

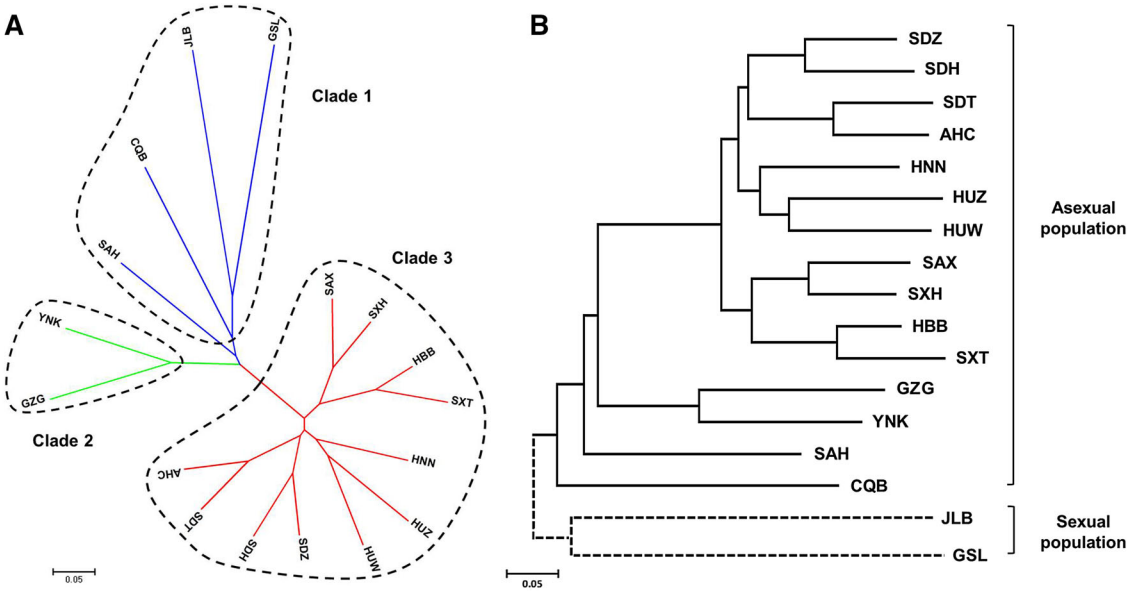

Fig. 3 The neighbor-joining (NJ) tree based on Cavalli-Sforza's chord distance (Dc) at five microsatellite loci. **a** Unrooted NJ tree showing that a total of 17 *Rhopalosiphum padi* populations were assigned to three clades indicated by I, II, and III in correspondence to clusters 1–3 of the Bayesian clustering analysis. The tree based on Nei's standard genetic distance (Ds) has highly concordant topology and thus is not shown. **b** Rooted tree showing two sexual populations (*dashed lines*) and 15 asexual populations (*solid lines*) of *Rhopalosiphum padi*

Yunnan–Guizhou Plateau located in southwestern China (GZG, 0.811 and YNK, 0.530), and Cluster 3 (in red) consisted primarily of individuals from 11 populations collected from large areas of winter wheat areas distributed in central and northern China (SAX: 0.605, HNN: 0.589, HUZ: 0.836, HUW: 0.828, HBB: 0.938, SXT: 0.932, SXH: 0.635, SDZ: 0.776, SDT: 0.871, SDH: 0.806, and AHC: 0.762).

The NJ tree based on Cavalli-Sforza and Edwards chord distance (Dc) is shown in Fig. 3 (tree-based Nei's standard

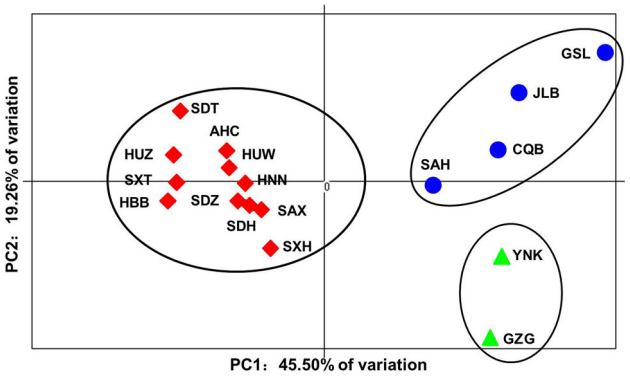

Fig. 4 Principal component analysis of 17 *Rhopalosiphum padi* populations. The same shapes indicate the populations in the same cluster and the *circles* indicate the populations that are clustered together

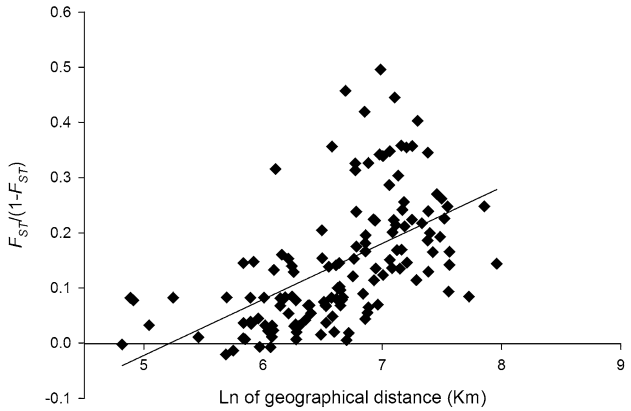

Fig. 5 Isolation-by-distance *plot* of $F_{ST}/(1 - F_{ST})$ plotted against the natural log of geographic distance (km). The *solid line* represents the best-fit linear regression based on all points

genetic distances, Ds, showed highly concordant topology). The unrooted NJ tree (Fig. 3a) resulted in three major clades, which were consistent with the Bayesian clustering results using STRUCTURE. Two populations (JLB and GSL) from a spring wheat area and two populations (CQB and SAH) from the northwestern physiographic basin were included in Clade 1. Two populations (YNK and GZG) from the subtropical region of the Yunnan–Guizhou Plateau in southwestern China were found in Clade 2. A total of 11 populations sampled in the large areas of winter wheat areas formed Clade 3. In the rooted NJ tree (Fig. 3b), the two sexual populations were included in a subbranch, whereas all asexual population were in another subbranch. PCA analysis showed that all populations formed three clusters, which was consistent with the patterns observed in Bayesian clustering and NJ trees (Fig. 4).

Genetic differentiation

AMOVA results (Table 5) indicated significant genetic differentiation at the three hierarchical levels, with populations grouped according to the two reproductive modes. The results indicated that 12.31 % of the overall molecular variation was explained by reproductive modes, revealing that significant variances existed among populations with different life cycles. The Mantel test for 17 *R. padi* populations throughout China revealed a significant positive relationship between the genetic distances and the geographic distance ($r = 0.5504$, $P < 0.001$), indicating that a significant isolation-by-distance effect among different populations (Fig. 5).

Power analysis

Both the Chi square test (Fig. 6a) and the Fisher's exact test (Fig. 6b) for power calculations showed that F_{ST} values as low as 0.0032 can be detected with more than 80 % probability, indicating that the number of individuals and the numbers of loci used in this study provide sufficient statistical power to detect significant population differentiation and to identify genetic structure at very low levels of F_{ST} values as low as 0.0032 (Ryman and Palm 2006; Lyons et al. 2012; Provan et al. 2013).

Discussion

In the present study, we found that all *R. padi* clones from spring wheat-growing regions were characterized by cyclical parthenogenesis, whereas clones from winter wheat-growing regions were characterized by obligate parthenogenesis. Populations with cyclical parthenogenesis showed different genetic diversity and structures than all populations with obligate parthenogenesis excluding two physiographical basins in Western China. We observed

Table 5 AMOVA results of microsatellite data comparing genetic variation of *Rhopalosiphum padi* populations with two different life cycles

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P* value
Among groups	1	41.377	0.2358	12.31	$F_{CT} = 0.1231$	$P < 0.05$
Among populations within groups	15	137.08	0.1751	9.14	$F_{SC} = 0.1042$	$P < 0.001$
Within populations	721	1085.082	1.5050	78.55	$F_{ST} = 0.2145$	$P < 0.001$

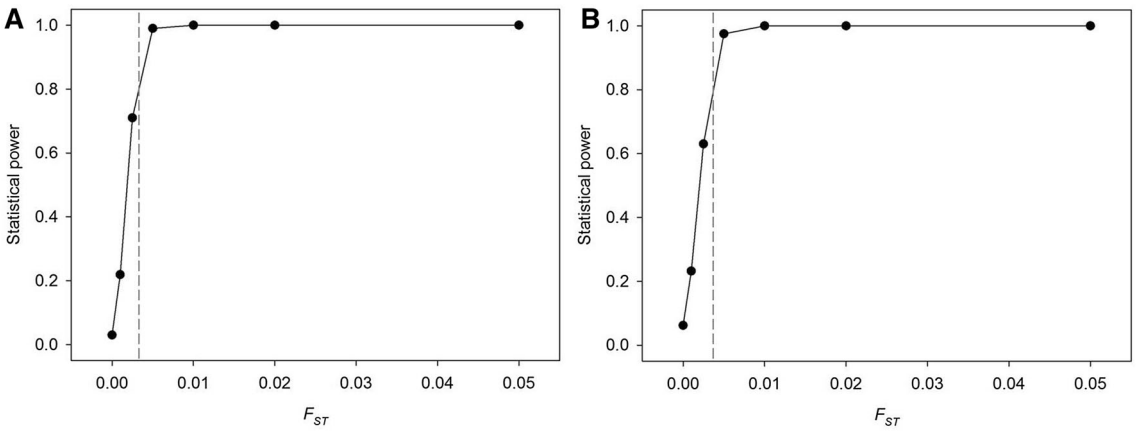

Fig. 6 POWSIM analyses for testing power to detect genetic differentiation at a range of F_{ST} values for five microsatellite loci. Vertical dashed lines in Chi square test (**a**) and Fisher's exact test (**b**) indicate that F_{ST} values as low as 0.0032 can be detected with more than 80 % probability

significant genetic differentiation between anholocyclic populations and holocyclic populations. A significant isolation-by-distance effect was observed among different populations (Table 5).

Life cycles of *R. padi* in China

In this study, we collected *R. padi* populations throughout the large wheat-growing areas of China. The present data showed strong patterns of the climate partitioning of life-cycle polymorphisms. We found that clones from regions with severe winters, where spring wheat can be cultivated, had a cyclical parthenogenesis life cycle, whereas clones from regions with mild winters showed obligate parthenogenesis life cycles. The climate may account for the different life cycles of different *R. padi* clones. Similarly, the French *R. padi* populations collected from the regions with cold winters were largely recruited from holocyclic clones, whereas in areas with mild winters, *R. padi* populations were mainly derived from anholocyclic clones (Simon et al. 1999a). The clones of *R. padi* with cyclical parthenogenesis could produce diapausing eggs on primary host plants for overwintering when the parthenogenetic females could survive the severe winters, whereas the obligate parthenogenesis clones could survive with viviparous females on its secondary hosts (wheat cultivars) throughout the entire year (Simon et al. 2002). Eggs, males, and sexual females were observed in *Prunus* trees of northeastern regions of China in late autumn (He et al. 1994), but not found in the eastern China with mild winter (Luo et al. 1994). Our findings are consistent with previous reports on aphid reproductive modes (Rispe and Pierre 1998; Rispe et al. 1998), and similar with findings from other aphid species such as *A. fabae* (Sandrock et al. 2011), *S. avenae* (Dedryver et al. 2001; Papura et al. 2003), *M. persicae* (Guillemaud et al. 2003), and *A. pisum* (Kanbe and Akimoto 2009). In addition to the climate, the primary host plants form another factor that influences the distribution of the cyclical parthenogenesis clones of an aphid species (Sandrock et al. 2011). *Prunus* trees are the primary host plants of *R. paid* (Blackman and Eastop 2000). Several economic *Prunus* fruit plants, which include *P. simonii* Carrière, *P. spinosa* L., *P. insititia L.*, *P. Domestica* L., *P. cerasifera* Ehrh., *P. salicina Lindl.*, and *P. ussuriensis* Kovalev & Kostina are grown in the regions from 21°00′N to 47°50′N, which covered nearly the whole mainland of China (20°13′N to 53°33′N) (Zhang, 1990; China Flora database, http://frps.eflora.cn/). As an important ornamental plant, *Prunus padus* is widely grown in northern, southern, and central China, especially abundant in the northeastern and northwestern regions where winter is regularly cold (China Flora database, http://frps.eflora.cn/). *Prunus* trees grows widely in all the sampling regions of the current study, the absence of the first primary host plants did not affect the sexual reproduction mode of *R. padi*.

Genetic diversity of populations with cyclical and obligate parthenogenesis

It is believed that the asexual lineages of an aphid species are derived from sexual ancestors (Delmotte et al. 2001, 2002). During sexual reproduction, the gene would be spread and reconstructed to significantly enrich the genetic diversity (Balloux et al. 2003; Bengtsson 2003). In contrast to the cyclical parthenogenesis populations, the obligate cyclical parthenogenesis populations of an aphid species may have less genetic diversity and show no significant deviation from *HWE* (Delmotte et al. 2002; Simon et al. 2002). In the present study, microsatellite data revealed that the cyclical parthenogenesis *R. padi* populations (JLB and GSL) from spring wheat areas have a higher number of

alleles (N_A), greater allelic richness (A_R), and high genotypic diversity compared with most of those with obligate parthenogenesis. High genetic diversity with many different combinations of the same alleles would be the result of genetic recombination in populations with sexual generations in the life cycle; however, genetic recombination is unlikely to occur during asexual reproduction in aphid populations with obligate parthenogenesis (Sunnucks et al. 1997), which explains our data showing the relatively higher genetic diversity of the two populations with sexual generations. Moreover, the two cyclical parthenogenesis *R. padi* populations showed heterozygotic deficiency and no significant deviation from *HWE*, but most obligate parthenogenesis populations showed heterozygotic excess and significant deviation from *HWE*. Similar results were observed in other aphid species such as *M. persicae* (Fenton et al. 2003), *S. avenae* (Simon et al. 1999b; Papura et al. 2003), *D. vitifoliae* (Vorwerk and Forneck 2006; Sandrock et al. 2011), and the French populations of *R. padi* (Delmotte et al. 2002). Null alleles, selection, inbreeding of sexual generations, and accumulated mutations in asexual populations may affect the heterozygosity of aphids, but their effect remains unclear (Sunnucks et al. 1996; Normark 1999; Wilson et al. 1999; Delmotte et al. 2002; Fenton et al. 2003). Using two models suited for different time scales, Bengtsson (2003) analyzed the genetic variation of species with both sexual and asexual reproduction models and found that different reproductive modes of a species influence the genetic diversity and genetic structure of populations and cause significant genetic differentiation between sexual and asexual populations. Consistently, the AMOVA results of the present study showed significant genetic differentiation between populations of *R. padi* with different reproductive modes. It was interesting that the rooted NJ tree based on the Nei's standard genetic distance was split by sexual and asexual populations, and the tree structure showed that asexual populations of *R. padi* were possibly derived from sexual ancestors, which was similar to the previous reports of the species (Delmotte et al. 2001, 2002). Compared with France (Simon et al. 2001) and Australia (Valenzuela et al. 2010) *R. padi* populations, Chinese populations showed higher number of alleles per locus (Table 2). As we sampled aphids in relatively larger range, the different topography, climates, or landscapes may cause locally differentiated populations with higher genetic diversity (Cardé and Minks 1995; Simon et al. 2001; Valenzuela et al. 2010). Four MLGs (RP037, RP038, RP053, and RP064) with significant P_{sex} value were widely spread in winter wheat-growing regions, indicating continuous parthenogenetic reproduction in theses regions (Valenzuela et al. 2010). The *R. padi* population in the current study showed different size range of microsatellite alleles with those reported in French and Australian populations, which indicated different populations of a same species often varied in mutations under different micro-evolutionary environments (Schlotterer 2000).

Genetic structure of *R. padi* populations

We observed significant genetic structure in the *R. padi* populations sampled throughout China. The similar climate, wheat phenology, and reproduction mode may explain the similar genetic structure of the two cyclical parthenogenesis populations from the spring wheat-growing regions. Eleven *R. padi* populations from the large winter wheat-growing regions in central and northern China formed the same cluster in the PCA, Bayesian clustering, and NJ tree analyses. These populations were mainly collected in the North China Plain, which is the most important winter wheat-growing region in China. The North China Plain is an area with low altitude and flat terrain, where wheat has nearly the same planting and harvest time, which allows the wheat aphids to develop similarly. Actually, the seasonal monsoon in this region may facilitate the migration of aphids in the absence of geographical barriers (Zhang et al. 1985), which could promote the mixing of the *R. padi* populations, thus resulting in the similar genetic structures of the populations (Shabani et al. 2013; Krascsenitsová et al. 2013). In fact, the three most common repeated MLGs (RP037, RP038, and RP053) were mainly found in individuals of the 11 populations, which were sampled in a large neighboring area, and were structured together in the Bayesian clustering analysis. Another common MLG (RP064) was mainly found in two populations (GZG and YNK) with similar genetic structures from southwestern China. There two populations sampled in the Yunnan–Kweichow Plateau which is a subtropical region where wheat is grown only sporadically in some high mountain regions.

It was interesting that two *R. padi* populations (SAH and CQB) collected from Sichuan and Hanzhong Basins showed similar population genetics based on similar genetic structures, heterozygotic deficiency, and high genetic diversity. These two basins are surrounded by high mountains and have mild winters; however, the neighboring regions of the two basins have harsh winters with temperatures that usually under −10 °C. The respective *G/N* ratio of CQB and SAH population was 0.92 and 0.95, indicating that the ancestors of the *R. padi* populations in the two basins may originate from the adjacent cold regions and lost the sexual reproduction mode over geological time, but they still maintain some of the genetic background of their ancestors. Further analysis is required to characterize the genetic background of the *R. padi* populations in the two basins.

Conclusions

Cyclical parthenogenesis and obligate parthenogenesis occur in the *R. padi* populations of China. Populations collected from the spring wheat-growing regions with rigid cold winters were characterized by cyclical parthenogenesis, whereas populations from winter wheat-growing areas were characterized by obligate parthenogenesis. The life cycle and geography-related climates underlie the genetic diversity and genetic structure of *R. padi* populations. Hence, design and implementation of sustainable pest management strategies could therefore be adjusted to the different life cycle and population genetics of this aphid.

Author contribution

XD, XP, and MC conceived and designed the experiments. XD and XP performed the experiments. XD, XP, and XQ analyzed the data. XD, XP, and MC wrote the paper. All authors read and approved the manuscript.

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