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Russian wheat aphids (*Diuraphis noxia*) in China: native range expansion or recent introduction?

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Abstract

In this study, we explore the population genetics of the Russian wheat aphid (RWA) (*Diuraphis noxia*), one of the world's most invasive agricultural pests, in north-western China. We have analysed the data of 10 microsatellite loci and mitochondrial sequences from 27 populations sampled over 2 years in China. The results confirm that the RWAs are holocyclic in China with high genetic diversity indicating widespread sexual reproduction. Distinct differences in microsatellite genetic diversity and distribution revealed clear geographic isolation between RWA populations in northern and southern Xinjiang, China, with gene flow interrupted across extensive desert regions. Despite frequent grain transportation from north to south in this region, little evidence for RWA translocation as a result of human agricultural activities was found. Consequently, frequent gene flow among northern populations most likely resulted from natural dispersal, potentially facilitated by wind currents. We also found evidence for the long-term existence and expansion of RWAs in China, despite local opinion that it is an exotic species only present in China since 1975. Our estimated date of RWA expansion throughout China coincides with the debut of wheat domestication and cultivation practices in western Asia in the Holocene. We conclude that western China represents the limit of the far eastern native range of this species. This study is the most comprehensive molecular genetic investigation of the RWA in its native range undertaken to date and provides valuable insights into the history of the association of this aphid with domesticated cereals and wild grasses.

Keywords: *Diuraphis noxia*, genetic structure, geographic isolation, native range, population expansion, wheat domestication

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Introduction

Biological invasions have occurred in many ecosystems and have evoked concern in evolutionary ecology and biological conservation (Pysek *et al.* 2008), as they are an important factor influencing global change (Bright 1999). Comparative studies to examine an invasive species in both its introduced and native range can improve understanding of how a nonindigenous species shapes its new environment (Scott 2007). Such studies not only provide information on the basic biological characteristics of an invader, but can also provide knowledge of the genetic background of the founding population of an invasive species (Ross *et al.* 2003, 2007; Ross & Shoemaker 2008), the dispersal pattern (Goodisman *et al.* 2001) and the invasion pathway of a species throughout its introduced range (Bonizzoni *et al.* 2004). Data of this kind improve our ability to predict the array of evolutionary responses and impacts that may result, as well as the future distribution of the invasive species.

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In this study, we analyse the population genetics of the Russian wheat aphid (RWA), *Diuraphis noxia* Kurdjumov, one of the world's most invasive agricultural pests, in western China. RWAs infest native grasses and cereal crops; however, they are most noted for their potential to severely damage grains such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare*) and their capacity for rapid population growth (Smith *et al.* 2004; Burd *et al.* 2006; Jyoti *et al.* 2006). The native distribution of RWAs is believed to centre on the Iranian-Turkestanian mountain range and extends to southern Russia, the Middle East and central Asia (Kovalev *et al.* 1991), with the earliest documented record of damage coming from Ukraine in the early 1900s. RWAs gradually spread to most European and North African countries during the early part of the 20th century at which time it gained recognition as an emerging global pest. It was during the 1970s and 1980's that RWAs began to rapidly spread, causing severe crop damage in major grain producing areas in Europe, Africa and the Americas (Kovalev *et al.* 1991; Stary 1999; Smith *et al.* 2004).

Russian wheat aphids were first observed in northwestern China in 1975 at Tacheng in the Xinjiang Uyghur Autonomous Region (Zhang *et al.* 1999a). RWAs have not been detected in any other province in China. There is some dispute as to whether the RWA is an exotic or native species in China, with most Chinese entomologists regarding it as an invasive pest (Zhang *et al.* 1999a,b), possibly because it was around this time that invasive populations of RWAs were first reported in South Africa (1978), Mexico (1980), North America (1986) and South America (1988).

In recent years, most research on RWAs has focused on documenting the biology and genetics of this species in its invasive range (Shufran *et al.* 2007; Shufran & Payton 2009; Liu *et al.* 2010) and much emphasis has been placed on documenting variant biotypes and discovering resistance genes in wheat and barley cultivars (Puterka *et al.* 1992; Basky 2002; Haley *et al.* 2004; Burd *et al.* 2006). Population genetic studies on RWAs from central Asia, including China, have not been undertaken. A significant body of research does exist, however, on the biology of this species in China. RWAs exhibit a holocyclic life cycle in China (Zhang *et al.* 1999a) with parthenogenesis the predominant mode of reproduction in late spring and summer, and sexual reproduction occurring in October. Cold-resistant eggs are laid in late October which over-winter on the basal leaves of the host plants (Zhang *et al.* 1999a). Invasive populations of RWA have been characterized as primarily anholocyclic (obligatory parthenogenetic), although the appearance of sexual females and eggs has been reported recently in North America and Argentina (Kiriac *et al.* 1990; Clua *et al.* 2004).

Host plants of RWA include cultivar crops, such as wheat, barley and oats, and native grasses, wild oats and rye. Variable population growth rates and relative virulence on wheat and barley have been reported amongst invasive populations of RWA (Puterka *et al.* 1992; Basky 2002; Smith *et al.* 2004; Jimoh *et al.* 2011) however little is known about the level of host adaptation in native populations of RWA. Host-based adaptation has been reported in other aphid species (Ferrari *et al.* 2006; Charaabi *et al.* 2008; Peccoud *et al.* 2009), and in the greenbug (*Schizaphis graminum*), another cereal aphid, mitochondrial data suggest that genotypes associated with cultivated cereals have a single origin (Shufran *et al.* 2000). Parthenogenetic reproduction is thought to facilitate sympatric host specialization in aphids (Sunnucks *et al.* 1997); parthenogenesis is also probably a key factor leading to the dominance of single genotypes ('superclones') across space and time (Abbot 2011).

It is not yet clear what biological, genetic and/or ecological factors are responsible for RWA invasiveness, and which factors are limiting its range expansion after establishment. RWAs quickly spread through most of the wheat-growing districts in the western USA soon after its introduction in 1986, but did not expand its range significantly to the east (Smith *et al.* 2004). Large-scale dispersal is important in facilitating the expansion of aphid populations in both their native and invasive ranges (Dolatti *et al.* 2005; Michel *et al.* 2009; Shufran & Payton 2009). Aphid dispersal morphs (alatae) exhibit weak flying ability (Loxdale *et al.* 1993; Zhang *et al.* 2008), with most movement across long distances attributed to wind-aided dispersal (Venette & Ragsdale 2004). Monitoring insect movement using traditional ecological methods is problematic (Roderick 1996). Genetic methods are now used widely to examine the levels of migration among populations and provide answers to a range of questions relating to movement patterns and population demographic history.

Here we report results of the most comprehensive population genetic study yet undertaken on RWAs. We investigate the patterns of spatial and temporal genetic differentiation among sampled populations and infer possible dispersal mechanisms. We provide evidences for historical demographic population expansion throughout western China and predict the potential for future expansion of this species in other wheat-growing districts with similar geographic features in China.

Materials and methods

Aphid sampling

Russian wheat aphids were collected from wheat fields (*Triticum aestivum* L.) in northern and southern Xinjiang

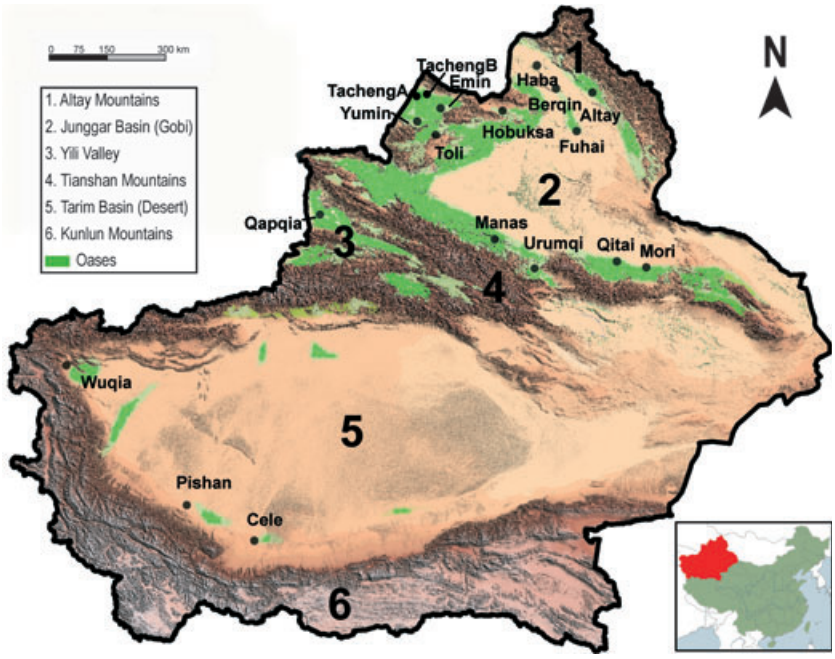

Fig. 1 Topographical map of north-western China, Xinjiang, with the sample localities represented by black dots.

including desert, oasis and mountain foothill regions. In total, 18 sites were sampled including 15 in the north and three in south, from May to June of 2009 and 2010 (Fig. 1 and Appendix S1, Supporting information). Nine sites were sampled in both years to provide a temporal comparison. Up to fifty colonies were identified at each site and one parthenogenetic, wingless female aphid was collected from each plant. Consecutive samples at a location were collected a minimum of 50 m apart, or in different fields, to minimize the chance of sampling aphids from the same colony. RWA specimens were preserved in 100% ethanol until DNA extraction.

DNA extraction and amplification

Total genomic DNA was extracted from single adult aphids using a salting-out method (Sunnucks & Hales 1996). All RWAs were screened for 12 microsatellite loci, including three cross-species loci developed from *Sitobion* aphids (Sa4Σ—Simon *et al.* 1999; Sm11—Wilson *et al.* 1997; Sm23—Wilson *et al.* 2004), and nine loci newly developed from RWAs. Microsatellite loci were amplified in a total volume of 10 μL containing 10 nmol of fluorescent-labelled primers (Sangong Company, China), 0.5 U Taq, 1× PCR Buffer, 0.3 mM each dNTP, 2 mM $MgCl_2$ (TaKaRa Taq™; Takara Biomedical) and 20 ng of aphid DNA. PCR cycling conditions followed Shufran & Payton (2009), except that different annealing temperatures were used. Electrophoresis of the amplification products was conducted in a capillary sequencer ABI3730×1 (Applied Biosystems), with an internal size ladder (500 LIZ). Allele sizes were analysed using GeneMapper (version 3.0; Applied Biosystems) and allele designation was confirmed following visual examination.

We also sequenced two mitochondrial DNA regions: partial cytochrome oxidase I (CO1) and a continuous fragment centered on NADH dehydrogenase subunit 6 (ND6), including partial NADH subunit 4 L, two tRNA genes, total ND6 and partial cytochrome B. The 436 bp CO1 gene was amplified using the primers C1-J-1718 and C1-N-2191 (Simon *et al.* 1994), and the ND6 fragment (837 bp) was amplified using the primers N4L-J9648 and CB-N10608 (Simon *et al.* 2006). The PCR protocol and cycling conditions followed Shufran & Payton (2009), except that ExTaq (TaKaRa Taq™; Takara Biomedical) was used. PCR products were purified using an ABgene Ultra PCR Clean-Up Kit (Thermo Scientific) and run on an ABI3130 sequencer.

Genetic diversity

Genetic diversity estimates were calculated using FSTATv2.9.3. (Goudet 2001) and included observed and expected heterozygosity (H_O & H_E), allele size range, number of alleles (Na), allelic richness (Ar) and the *f* estimator of F_{IS} and significance values (Weir & Cockerham 1984). Allele frequencies, Hardy–Weinberg equilibrium (HWE), and linkage disequilibrium tests were calculated using GENEPOP v4.0 with 1000 iterations

and 100 Markov Chain approximations (Raymond & Rousset 1995; Rousset 2008). Significance was assessed following Bonferroni correction (Rice 1989). MICRO-CHECKER v2.2.3 was used to test for large allele dropout (Van Oosterhout *et al.* 2004). Null allele frequencies for each locus were estimated using CERVUS v3.0 (Marshall *et al.* 1998). All individuals were also classified according to multilocus genotype (MLG) in GENCLONE v2.0 (Arnaud-Haond & Belkhir 2007). Genetic diversity was analysed based on gross genotypic diversity, which was calculated as G/N, with G equal to the number of MLGs and N equal to the sample size (Llewellyn *et al.* 2003).

Mitochondrial DNA sequences were aligned and edited using BIOEDIT v7.0.0 (Hall 2004) and MEGA v4.1 (Tamura *et al.* 2007). The number and frequency of haplotypes were calculated using DNASP v5 (Librado & Rozas 2009), and a phylogeographic network was inferred using TCS (Clement *et al.* 2000). We also calculated Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) implemented in ARLEQUIN v3.5.1.2 (Excoffier *et al.* 2005) to infer deviations from neutrality and to detect demographic changes or selection (Fu & Li 1993).

Genetic differentiation

Pairwise F_{ST} estimates were calculated from the microsatellite data using Arlequin, and exact G tests of allelic differentiation were calculated using Genepop. The datasets were analysed by year, and one site, Fuhai, was excluded because of low sample size. A Mantel test implemented in Genepop (using 10 000 permutations) was used to examine whether there was a relationship between F_{ST} and geographic distance. The sampling coordinates were recorded in GPS, and the straight-line distance between each pairwise locality was calculated using Google Earth (Google Inc., Mountain View, CA, USA).

Three clustering methods were used to identify population structure. Firstly, a Bayesian Markov Chain Monte Carlo (MCMC) method implemented in STRUCTURE v2.1 (Pritchard *et al.* 2000) was used. An admixture model was assigned by assuming independent allelic frequencies with 100 000 iterations of MCMC after a 20 000 burn-in period, and 10 independent runs for each K were evaluated. To select the most likely K value, we adopted two criteria: first, the K reached a plateau in the Ln(K) plot, and $\triangle K$ attained its maximal value (Evanno *et al.* 2005); and second, a parsimony method was used in which the lowest K is selected that captures most differentiation among populations (DiLeo *et al.* 2010). We then used DISTRUCT v1.1 (Rosenberg 2004) to display the bar plot under the most likely K value. Secondly, factorial correspondence analysis (FCA) was carried out in GENETIX v4.05 (Belkhir *et al.* 1996–2004) to examine the three-dimensional spatial distribution of genetic variation for each individual. Finally, an analysis of molecular variance (AMOVA) was conducted in Arlequin to confirm population clusters and to differentiate the variation component among populations and years.

We used the microsatellite data to examine evolutionary scenarios of expansion and gene flow among sites using DIYABC v 0.7 (Cornuet *et al.* 2008), MIGRATE v3.2.7 (Beerli 2008) and BAYESASS 1.3 (Wilson & Rannala 2003). DIYABC estimates the posterior distributions of different evolutionary scenarios by generating simulated data and comparing selected simulated data that are closest to the observed data (Cornuet *et al.* 2008). Five scenarios of simultaneous expansion were examined using four geographically widespread sites—Qapqia (Yili Valley, north-west Xinjiang), Yumin (north-west Xinjiang), Mori (north-east Xinjiang) and Wuqia (south Xinjiang)—and an unsampled site as the origin of expansion. We assumed a stable effective population size (N_e), a transitory bottleneck (db = 5) and a generalized stepwise model of mutation. 250 000 simulated datasets were produced for each scenario, and the 15 000 closest simulations to the observed data were compared using logistic regression.

MIGRATE detects gene flow over historical timescales—up to $4N_e$ generations in the past. It is implemented using a maximum likelihood model with two long chains, followed by 10 short chains recorded at the sampling increment of 100 iterations, and with a burn-in of 10 000 iterations. The programme was run five times using different random seeds. BayesAss estimates recent migration rates with 95% confidence intervals. Five independent runs with different initial random seeds were undertaken using 20 million iterations and a 10 million burn-in chain to check the congruence.

Demographic changes in population size

Changes in demographic history are known to affect the frequency of alleles, the distribution of mutations and the coalescent times of gene copies. Two tests were used to determine whether the microsatellite data displayed any signature for past population expansion or contraction. Firstly, using the program BOTTLENECK v1.2.02 (Cornuet & Luikart 1996), observed and expected heterozygosity were compared to detect any heterozygote excess (Piry *et al.* 1999). We also used Bottleneck to test for mode shift. Secondly, k and g tests were used to detect any signal of population expansion in the ancestral generations (Reich & Goldstein 1998; Reich *et al.* 1999; Bilgin 2007). Negative k values at each locus indicate population expansion. A low value of g (under 1) can be interpreted as evidence of population expansion.

The mitochondrial data were also examined for evidence of population expansion using a pairwise mismatch distribution implemented in Arlequin. The goodness-of-fit of the observed data to a simulated model of expansion was tested with the sum of squared deviations (SSD) and raggedness index. The age of expansion was estimated with the formula ô = 2ìt, where ì equals the aggregate mutation rate across all nucleotides per generation and t is the expansion time in generations. We also adopted Ramos-Onsins and Rosas's *R2* test (Ramos-Onsins & Rozas 2002) in DnaSP to complement the power of the pairwise mismatch distribution. The *R2* test was conducted using coalescent simulations with 1000 replicates and 95% confidence intervals.

Results

Genetic diversity

Twelve microsatellite loci were screened for 1040 RWA colonies sampled in western China in 2009 and 2010. Two of the cross-species loci (Sm11 and Sm23) were discarded as a high number of scoring errors were detected. The remaining 10 loci were polymorphic (Appendix S2, Supporting information) and could be confidently scored (i.e. no large allele dropout or scoring errors were detected using Micro-Checker). Only one locus (Dn1) was potentially affected by null alleles, having a null allele frequency >0.1; however, no significant departure from HWE was found for this locus. Significant deviation from HWE was identified in five of the 27 tests as a result of heterozygote deficit or excess. Although a small proportion of linkage disequilibrium tests indicated significant linkage, no consistent pattern between any particular pair of loci was evident therefore the 10 loci are providing independent assessments of genetic variation.

Within each site, the highest allelic number and richness was found in Haba, with 11.1 and 6.08, respectively (Table 1). In contrast, the lowest allelic number was found in Pishan with 2.7 and lowest allelic richness in Cele with 2.51 (Table 2). Sites located in northern Xinjiang, including the regions surrounding Tacheng, Altay and Urumqi, presented similar average gene diversities during both years. An ANOVA revealed that sites in the south had significantly reduced gene diversity (F = 3.68, d.f. = 3,22, P = 0.027) and allelic richness (F = 5.36, d.f. = 3,21, P = 0.007) compared with the north.

A total of 928 MLGs were identified from 1040 RWAs based on the data from 10 microsatellite loci (Tables 1 and 2). The number of MLGs shared within a site ranged from 0 to 8, with the highest sharing occurring in Cele. Four sites were entirely composed of unique MLGs. Interestingly only one MLG was shared among sites (between two individuals from Pishan and Cele). No MLGs were shared among years at any site.

Concatenated 1272 bp of mitochondrial DNA was obtained from 178 RWAs. Eighteen haplotypes were identified, with one common haplotype found at all sites (relative frequency: 88.8%), and 17 rare haplotypes found at low frequencies (0.5–1.1%). Three haplotypes were shared among sites: Hap1 (universal), Hap3 (found at Yumin and Qapqia) and Hap7 (found at Haba and Toli). Hap10 was found in two individuals from Wuqia (Fig. 2). The remaining 14 haplotypes were unique to one site. Mori in north-east Xinjiang had the

Table 1 Indices of genetic diversity for the 13 sites sampled in 2009

2009	TCA	TCB	TL	EM	YM	BR	UR	QT	ML	HF	AL	FH	HB
N	49	31	50	50	31	44	16	50	42	40	10	6	50
H_O	0.62	0.61	0.62	0.62	0.64	0.67	0.65	0.60	0.62	0.59	0.81	0.78	0.61
H_E	0.65	0.65	0.67	0.66	0.68	0.67	0.64	0.61	0.64	0.64	0.76	0.70	0.68
H_S	0.65	0.65	0.67	0.66	0.68	0.67	0.58	0.61	0.64	0.64	0.68	0.62	0.68
Na	9.6	8.1	9.7	9.7	8.2	8.8	4.9	8	9.4	6.8	5.5	4.5	11.1
Ar	5.42	5.47	5.7	5.68	5.63	5.56	4.23	4.75	5.49	4.73	5.30	–	6.08
MLGs	48	31	49	36	30	36	15	44	42	25	9	5	48
#within	2	0	1	6	1	6	1	5	0	6	1	1	2
#among	0	0	0	0	0	0	0	0	0	0	0	0	0
GGD	0.96	1	0.98	0.82	0.97	0.82	0.94	0.88	1	0.63	0.9	0.83	0.96
F_{IS}	0.036	0.065	0.081	0.06	0.059	0.007	−0.002	0.008	0.037	0.072	−0.077	−0.12	0.097*

H_O, observed heterozygosity; H_E, expected heterozygosity; H_S, gene diversity; Na, numbers of alleles; Ar, allelic richness based on nine samples per population; MLGs, number of multilocus genotypes; #within, number of MLGs shared within a population; # among, number of MLGs shared among populations; GGD, index of global genotypic diversity (MLGs/*N*); F_{IS}, the inbreeding index, the asterisks indicate significance after Bonferroni correction at 0.05 level. TCA, TachengA; TCB, TachengB; TL, Toli; EM, Emin; YM, Yumin; HF, Hobuksa; AL, Altay; FH, Fuhai; HB, Haba; BR, Berqin; UR, Urumqi; QT, Qitai; ML, Mori.

Table 2 Indices of genetic diversity for the13 sites sampled in 2010

2010	TCA	TCB	TL	EM	YM	BR	UR	QT	ML	QP	MS	WQ	CL	PS
N	41	22	50	31	50	11	50	50	50	53	50	52	52	9
H_O	0.61	0.76	0.64	0.71	0.62	0.74	0.62	0.57	0. 60	0.65	0.62	0.59	0.60	0.59
H_E	0.63	0.73	0.65	0.71	0.67	0.60	0.60	0.64	0.59	0.65	0.65	0.71	0.42	0.52
H_S	0.63	0.65	0.65	0.64	0.67	0.47	0.60	0.64	0.59	0.65	0.59	0.71	0.33	0.47
Na	7.5	6.7	10	7.9	10.5	3.5	7.7	8.7	8.6	9.8	7.9	8.1	3.9	2.7
Ar	4.99	5.27	5.57	5.382	5.85	3.3	4.51	5.29	5.00	5.50	4.76	5.4	2.52	2.7
MLGs	30	30	49	30	50	5	34	48	46	51	43	52	41	7
#within	4	3	1	1	0	2	7	2	2	2	5	0	8	1
#among	0	0	0	0	0	0	0	0	0	0	0	0	1	1
GGD	0.73	0.77	0.98	0.97	1	0.45	0.68	0.96	0.92	0.96	0.86	1	0.79	0.78
F_{IS}	0.03	−0.044	0.004	0.011	0.079*	−0.26	−0.032	0.12*	−0.004	0.003	0.046	0.17*	−0.43*	−0.15

The abbreviations are the same as indicated in Table 1. QP, Qapqia; MS, Manas; WQ, Wuqia; CL, Cele; PS, Pishan.

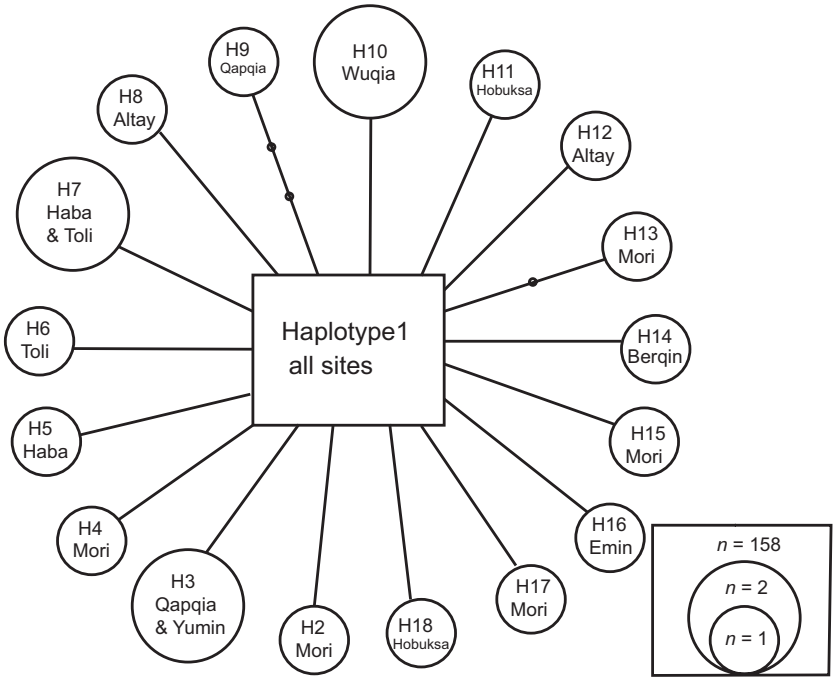

Fig. 2 Estimated mitochondrial DNA network with 95% plausible set of haplotype connections. Each haplotype (1–18) is shown as a circle or square. The size of the circle or square relates to the number of individuals sampled (scale shown at base of figure). Small black circles represent putative haplotypes that were not sampled (not labelled). Lines between circles represent a single base pair mutation.

highest nucleotide diversity as well as significant Tajima's *D* and significant Fu's *Fs* values (Appendix S3, Supporting information). Twenty variable sites were found and although 18 of these occurred among protein coding regions, the majority of single base pair mutations were transitions (12/18) and synonymous mutations (13/18).

The TCS network revealed a star-like pattern centred on the widely distributed Hap1 (Fig. 2). From the central haplotype (Hap1), 15 haplotypes diverged by one mutation, one haplotype (Hap13) diverged by two mutations and another haplotype (Hap9) diverged by three mutations.

Genetic differentiation (nDNA)

Population differentiation was analysed using pairwise F_{ST} values and exact tests of allelic differentiation. In 2009, pairwise F_{ST} values among northern sites were generally low (0.0055–0.1129), but majority of pairwise comparisons were significant (Table 3A). In 2010, the majority of F_{ST} were significant among northern sites

Table 3 F_{ST} values and significance of pairwise comparisons among (A) 2009 and (B) 2010 populations

(A) 2009	EM	TCA	TCB	UR	HB	TL	QT	BR	HF	YM	ML	AL		
Emin	–													
TachengA	0.0061	–												
TachengB	**0.0251**	**0.0318**	–											
Urumqi	**0.0917**	**0.1045**	**0.0770**	–										
Haba	0.0089	0.0086	**0.0336**	**0.0778**	–									
Toli	**0.0186**	**0.0200**	**0.0206**	**0.0710**	**0.0143**	–								
Qitai	**0.0444**	**0.0401**	**0.0466**	**0.1071**	**0.0366**	**0.0141**	–							
Berqin	**0.0338**	**0.0354**	**0.0264**	**0.0774**	**0.0275**	0.0134	**0.0223**	–						
Hobuksa	**0.0552**	**0.0639**	**0.0626**	**0.0868**	**0.0490**	**0.0575**	**0.0808**	**0.0569**	–					
Yumin	0.0091	0.0058	**0.0265**	**0.0981**	0.0113	0.0055	**0.0270**	**0.0237**	**0.0553**	–				
Mori	**0.0210**	**0.0234**	**0.0299**	**0.0943**	**0.0196**	0.0096	0.0057	**0.0167**	**0.0533**	0.0088	–			
Altay	0.0248	0.0204	**0.0525**	0.1129	0.0214	0.0271	**0.0665**	0.0422	**0.0768**	0.0195	**0.049**	–		
(B) 2010	WQ	CL	PS	QP	MS	UR	QT	ML	YM	TL	TCA	TCB	EM	BR
Wuqia	–													
Cele	**0.2689**	–												
Pishan	**0.1304**	**0.1203**	–											
Qapqia	**0.0989**	**0.2214**	**0.1394**	–										
Manas	**0.1192**	**0.2525**	**0.1539**	**0.0682**	–									
Urumqi	**0.1085**	**0.2772**	**0.1648**	**0.0671**	**0.0653**	–								
Qitai	**0.1141**	**0.2673**	**0.1829**	**0.0294**	**0.0654**	**0.0527**	–							
Mori	**0.1346**	**0.2363**	**0.1812**	**0.0403**	**0.0735**	**0.0664**	**0.0167**	–						
Yumin	**0.0843**	**0.2097**	**0.1211**	**0.0212**	**0.0452**	**0.0440**	**0.0163**	**0.0262**	–					
Toli	**0.1116**	**0.2637**	**0.1715**	**0.0363**	**0.0479**	**0.0500**	**0.0125**	**0.0344**	0.0081	–				
TachengA	**0.0957**	**0.2375**	**0.1157**	**0.0440**	**0.0640**	**0.0374**	**0.0422**	**0.0676**	**0.0210**	**0.0393**	–			
TachengB	**0.1117**	**0.3004**	**0.1541**	**0.0743**	**0.0809**	**0.0666**	**0.0647**	**0.0906**	**0.0520**	**0.0615**	**0.0465**	–		
Emin	**0.0793**	**0.2794**	**0.1422**	**0.0307**	**0.0427**	0.0220	**0.0301**	**0.0517**	**0.0177**	**0.0214**	0.0219	**0.0407**	–	
Berqin	**0.1761**	**0.3768**	**0.2402**	**0.1210**	**0.1204**	**0.1233**	**0.1362**	**0.1620**	**0.1068**	**0.1156**	**0.1187**	**0.1521**	**0.1003**	–

The abbreviated names were the same as the localities in Table 1. Bold values indicate significance after Bonferroni correction at 0.05 level. The grey cells highlight the F_{ST} between southern and northern populations.

again; however, a much higher level of differentiation was detected between northern and southern sites (Table 3B). The average F_{ST} values of southern sites (Wuqia, Pishan and Cele) to the other eleven northern sites were 0.112, 0.16 and 0.266, respectively. Furthermore, the pairwise F_{ST} value between the two southern sites, Wuqia and Cele was also very large 0.27. These data indicated that gene flow is considerably restricted among southern sites and between northern and southern sites.

Mantel tests based on the 2009 data (only northern sites were sampled) did not reveal a significant correlation between F_{ST} and geographic distance ($r = 0.25$, $P = 0.17$). However in 2010, both northern and southern sites were sampled and a strong pattern of isolation by distance was detected ($r = 0.57$, $P < 0.0001$).

An AMOVA was conducted using 2010 data and separating sites into three groups (1. Wuqia, 2. Cele and Pishan, and 3. northern sites). The proportion of variance among groups (12.42%) was larger than that found among sites within groups (4.43%), and the fixation index ($F_{CT} = 0.124$) was significant, indicating extremely restricted gene flow among the three groups (Appendix S4, Supporting information). We also analysed temporal differentiation among the nine sites that were sampled in both 2009 and 2010. Pairwise F_{ST} and exact tests revealed significant differentiation between years in all populations except Emin (Appendix S5, Supporting information). Genetic variation between years resulted in a fixation index ($F_{SC} = 0.028$) greater than that for among sites ($F_{CT} = 0.007$), suggesting that more structure exists within a site when sampled from 1 year to the next than among sites sampled within a single year.

Population structure

Similar patterns of hierarchical structure were obtained using individual-based clustering in structure and three-dimensional FCA. Both methods revealed three clusters (k = 3) among northern sites sampled in 2009 (Fig. 3A, Appendix S6A, Supporting information).

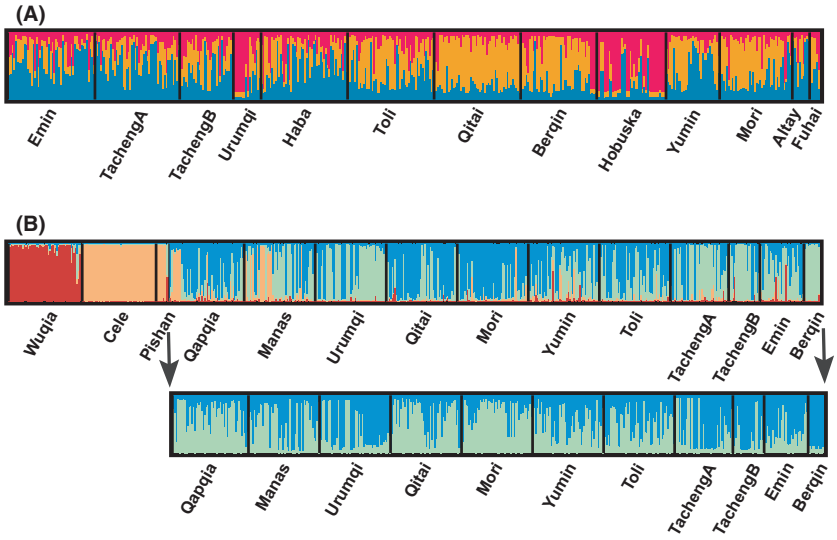

Fig. 3 Structure bar plot of Chinese Russian wheat aphids sampled in 2009 (A, k = 3) and 2010 (B, k = 4). The 2010 data are also presented following removal of the three southern populations and reanalysis (k = 2). Each individual is shown as a vertical bar representing ancestry.

However, no distinct groups could be discerned that corresponded to any of the 13 sites, indicating that all individuals sampled were of mixed ancestry. Further increasing k in structure did not reveal any distinct subdivisions. An analysis of 2010 data using structure revealed four clusters corresponding to three regions with distinctive population groups: (1) Wuqia, (2) Cele and Pishan, and (3) all other northern sites (Fig. 3B). The FCA analysis also identified the three southern sites as distinct from the northern sites, with Pishan genetically intermediate between Cele and Wuqia (Fig. 4). The three axes explained over 50% of the variation among the sites. Structure (k = 2) and FCA identified a varying degree of admixture amongst the northern populations in 2010 (Fig. 3B and Appendix S6B, Supporting information).

Evolutionary scenario testing using DIYABC revealed higher posterior probabilities for simultaneous expansion from the three northern sites analysed (Qapqia: 0.370, 95% CI 0.283–0.456; Yumin: 0.365, 95% CI 0.279–0.451; Mori: 0.235, 95% CI 0.169–0.302) than from southern Xinjiang (Wuqia: 0.005, 95% CI 0.002–0.007) or an unsampled alternative (0.025, 95% CI 0.014–0.0037). Yumin and Qapqia about the border with Kazakhstan and showed slightly higher posterior probabilities than Mori (north-east Xinjiang) as being the expansion origin. Similarly, MIGRATE estimates of long-term gene flow were significantly asymmetric based on nonoverlapping 95% confidence intervals (Appendix S7, Supporting information), indicating that Yumin and Qapqia may be expansion origins. Additionally, the most divergent mitochondrial haplotype was found at Qapqia further suggesting that this site may represent the ancestral origin of RWAs in China. Given the low level of haplotype sharing detected (only three haplotypes shared out of 18), it is interesting to note that Yumin and Qapqia shared haplotype 3 (Fig. 2). However, when we used BayesAss to look for evidence of recent gene flow between north and south Xinjiang, no trace of migration was detected among Yumin, Qapqia, Mori and Wuqia (nonmigration rate: 0.833, 95% CI 0.675–0.992).

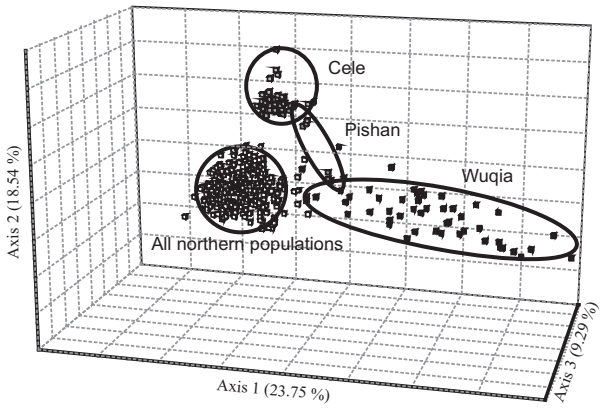

Fig. 4 Three-dimensional factorial correspondence analysis of Chinese Russian wheat aphids sampled in 2010. The circles indicate populations that cluster according to geography.

RWA population demographic history

Population demographic history examined using BOTTLENECK and KGTEST displayed little evidence for past popu-

Table 4 Tests for Chinese Russian wheat aphids demographic fluctuation under bottleneck or expansion calculated using BOTTLENECK and KGTEST

	2009	Hobuksa	Altay	Fuhai	Haba	Berqin	Urumqi	Qitai	Mori	Yumin	Toli	TachengA	TachengB	Emin	
BOTTLENECK	TPM	1.000	0.734	–	0.160	0.432	0.820	0.105	0.01855*	0.432	0.557	0.232	0.193	0.432	
	Shape Mode	L	L	–	L	L	L	L	L	L	L	L	L	L	
KGTEST	k	5	5	7	6	5	4	6	7	6	6	8*	6	5	
	g	1.445	0.754	0.809	0.861	0.884	2.515	1.140	1.124	0.910	0.815	0.803	0.771	0.935	
	2010	Wuqia	Cele	Pishan	Qapqia	Berqin	Urumqi	Qitai	Mori	Yumin	Toli	TachengA	TachengB	Emin	Manas
BOTTLENECK	TPM	0.922	1.000	–	0.01855*	0.844	0.275	0.322	0.131	0.084	0.00488**	0.232	0.570	0.426	0.129
	Shape Mode	L	L	–	L	L	L	L	L	L	L	L	L	L	L
KGTEST	k	6	6	5	8*	7	6	6	6	8*	8*	6	3	6	9**
	g	0.546	3.397	1.859	0.851	2.813	1.088	1.377	1.320	0.743	0.784	1.095	1.160	0.781	1.149

*$P < 0.05$; **$P < 0.01$. Dash (–) indicates that the test was not performed because the sample size was too low.

lation fluctuation (Table 4). Significant heterozygote deficits were only detected at three sites. Therefore, the reduction in allele number within populations was probably due to founder events rather than rapid decline in population size. Likewise, the L-shaped mode of allele frequency distribution suggests a long-term stable population size. Furthermore, the *k* test was not significant for most sites indicating that the allele length distribution was not significantly different from a binomial distribution and that the population size has been steady. The *g* tests were also not significant providing further evidence of stable population size. However, when considering all 18 sites as one population, the *k* test indicated significant population expansion. Although the *g* test value was not significant, it was less than one, thus supporting the conclusion of past population expansion in western China.

The mtDNA data also provided evidence of rapid demographic expansion, with the universal haplotype at the centre of a star-like cluster formed by the 17 rare haplotypes (Fig. 2). Furthermore, the pairwise mismatch distribution was unimodal, with a strong peak evident at zero, which steeply declined from zero to one base pair. The goodness-of-fit tests were not significant [*P*(SSD) = 0.52 and *p*(Harpending's RI) = 0.68], and evidence for highly significant population expansion was detected in the *R2* statistic (*R2* = 0.08347, $P = 0.002$), Tajima's *D* ($D = -2.39352$, $P < 0.01$) and Fu's *Fs* (*Fs* = −28.395, $P < 0.0001$). The estimated generation time since expansion for Chinese populations was approximately 3200 years, based on ô value of 0.146 and 1.77%/MY as mutation rate based on the rate given by Papadopoulou *et al.* (2010) for beetle mtDNA.

Discussion

This study has investigated the population genetics, demographic history and evolutionary adaptation of the RWA in its rarely investigated, far eastern native range in China. We have also rejected the hypothesis that this invasive pest had been introduced into Western China in the last couple of decades. An understanding of the levels and patterns of genetic variation in native populations can provide valuable insights into the factors that have facilitated the recent global invasion by this damaging pest species.

Genetic diversity of RWAs in China

The microsatellite data revealed high genetic diversity and large numbers of MLGs. No MLGs were shared between two consecutive sampling years at any single site, and very few MLGs were shared within and among sites in the Xinjiang region, strongly supporting

previous research that sexual reproduction is prevalent in China (Zhang *et al.* 1999a). High population densities of RWAs in China, together with little, recent migration among sites, may have also contributed to the high genetic diversity found in this study.

Consistently, our findings revealed significantly higher genetic diversity of RWAs in northern sites compared with southern, suggesting limited gene flow among and possible founder events in southern sites. A gradual reduction in genetic diversity and gene flow was evident, declining from Wuqia, the most northerly of the southern sites, to Pishan and Cele (the most southerly located site). Of all the sites sampled, Cele was the least diverse having the lowest allelic richness and a number of MLGs shared among individuals within the population. From this, we surmise that the population in Cele was probably founded by very few RWAs—possibly colonizing from Pishan. In contrast, the northern sites exhibited roughly equivalent levels of microsatellite variation. While the mtDNA data were generally less informative because of low levels of variation, one site in the north-east (Mori) displayed the highest diversity.

Genetic diversity within a site was correlated with geographic location and latitude; northern sites had higher diversity than southern sites. One possible explanation is that different patterns of introduction and establishment of RWAs occurred in the two regions. Given that ecological and environmental conditions in the north and south are quite different, RWAs would have experienced different selection pressures, potentially on different hosts and different ecological conditions influenced by climate and geography. In southern Xinjiang, microclimatic variation will have a strong effect on RWA populations as they occur in mountain regions above 2000 m elevation (even above 3300 m in Taxkorgan; Du 2000). In northern Xinjiang, RWAs occur at elevations ranging from 700 to 1000 m, mostly on plains or flat areas. Broad (or macro) scale fluctuations in climate will have a greater influence in the north, and elevation is less likely to be a barrier to insect dispersal or migration compared with the south. Furthermore, grain fields in the south are predominantly cultivated in small patches (i.e. oases) that are discontinuously located along the edge of the Taklamakam Desert and the Tarim River basin. Conversely, cultivated fields and wild grasslands are continuously distributed along the northern slope of the Tianshan Mountain range, offering RWAs a selection of host plants on which they can live or use as stepping stones to migrate. Finally, in southern Xinjiang, farmers plant only winter wheat and have one wheat-growing season per year, while in northern Xinjiang, farmers plant both winter and spring wheat each year, with an overlapping growth season from April to June. As a result, RWAs can persist over longer time periods in the north and because of plentiful food resources, their survival and reproductive success may be enhanced.

The high genetic diversity observed at microsatellite loci contrasted markedly with the low level of mtDNA genetic diversity that we observed in the Chinese RWA populations. Only 18 haplotypes were identified from 178 RWA individuals, and 17 of these were rare and found at very low frequency. This level of mtDNA diversity is still much higher than that found in invasive RWA populations, which have no mtDNA variation (Shufran *et al.* 2007; Shufran & Payton 2009). In other aphid species, anholocyclic populations have mitochondrial haplotypes that are distinct from holocyclic populations, and often exhibit reduced mtDNA diversity (Martinez-Torres *et al.* 1997).

Gene flow among RWA populations in Xinjiang

All methods of population structure analysis used in this study provided unequivocal support for strong differentiation among Chinese RWA populations relative to geography. Little evidence of gene flow between northern and southern regions was found. The Tianshan Mountain range segregates Xinjiang into northern and southern regions and the dominant wind direction is from west (Siberia) to east (China). The wind from north to south across the mountain range is weak and unlikely to facilitate passive RWA dispersal and although not conclusive evidence, RWAs have not been found along the southern slope of the Tianshan Mountains. However, aphids have been found suspended in air currents and are thought to be capable of long distance (100's of kilometers) flight (Dixon 1998; Delmotte *et al.* 2002). In this study, the low level of gene flow between northern and southern Xinjiang suggests that RWAs probably have a low active flying capacity, and this may be due to demographic or behavioural factors.

Experiments have shown that live adult RWAs can survive and produce a viable colony after 3 days without food and water (J. Vitou and O. Edwards, unpublished data). Therefore, it cannot be discounted that live adult RWAs may be transported on seedlings or human artefacts over long distances. In fact, wheat seeds are transferred frequently between northern and southern Xinjiang, as Yili and Tacheng have wheat breeding centres that provide on an annual basis, high-quality improved seeds to wheat growers located throughout Xinjiang ('Greater Mekong Subregion Agricultural Information Network', http://www.gms-ain.org/Z_Show.asp?ArticleID=1703). Because of high shipping costs, forage grass species or wheat seedlings are not transferred between northern

and southern regions. Consequently, as we detected little evidence of short-term gene flow from north to south, RWAs are probably not frequently transported by human agricultural activities. As more wheat fields are planted, the possibility remains however, that over time, aphid populations may expand into new areas via natural pathways (flight or wind currents).

Historical expansion of RWAs in China

The accepted opinion is that the original native eastern distribution of RWAs included northern Kazakhstan (Kovalev *et al.* 1991), and therefore, it is logical to suppose that RWAs could have been present along mountain ranges from central Asia (i.e. Kazakhstan) to western China before they were first detected in the 1970s. Our study has provided strong evidence for a long-term association of RWAs with wheat and possibly other cereals in western China.

Our mtDNA data indicate a relatively recent population expansion of Chinese RWAs during the last 3000 years. Although this estimate only provides an approximation, it is concordant with historical climate change events in central Asia and the spread of cereal domestication and cultivation practices. During the last 11 000 years, the warm wet climate of the Holocene (Richerson *et al.* 2001) provided a relatively stable, warm and CO_2-rich environment facilitating rapid plant growth. During this time, plant domestication and associated cultivation spread rapidly. Wheat domestication was first recorded in the Fertile Crescent (including the modern day Turkey, Iran, Iraq, Syria, Lebanon, Jordan, Palestine and Israel) in 9500–7500BC (Bellwood 2001; Diamond 2002) and spread eastward to central Asia by 7000–6000BC, to north-western China by 4600–2000BC (Thornton & Schurr 2004; Li *et al.* 2007) and then to the Indian subcontinent by 3500–3000BC (An *et al.* 2005). The earliest published record of wheat in Xinjiang comes from 2000BC (Thornton & Schurr 2004), a point in time when the Silk Road first became an active conduit for trade and agriculture between western and eastern Asia. We hypothesize that the expansion of RWAs in western China suggested by our mtDNA results was facilitated by agricultural activities associated with the human domestication of wheat.

Our microsatellite data also revealed a signal of population expansion when all sites were combined. Most sites displayed a very slight growth trend, indicating long-term co-evolution of the RWA with its host in natural habitats. Thus, our data are consistent with the theory that long-term effective population size should be in general, closer to the actual size during the remission period than that in the initial expansion and growth period (Motro & Thomson 1982). In addition, high gene flow among populations of RWA in the north during the expansion and growth period probably enhanced the homogenizing effect, as has been found during an outbreak event of the migratory locust, *Locusta migratoria* (Chapuis *et al.* 2009).

Our results from the mtDNA and microsatellite data are difficult to reconcile. The high gene flow we observed among northern Xinjiang RWA populations indicates that there should also be gene flow with populations in neighbouring Kazakhstan, which all available evidence suggests is within the native range of RWAs (Kovalev *et al.* 1991). If so, why would the mtDNA point to a recent population expansion? It is possible that RWAs did not exist in Xinjiang before the arrival of domesticated wheat. However, an alternative explanation is that the widespread planting of domesticated wheat changed the population structure of RWAs across their entire native range by selecting for wheat-adapted genotypes. Exclusively parthenogenetic reproduction during the wheat-growing season would facilitate the fixation of a single wheat-adapted maternal lineage (a 'superclone'), as has been observed in other aphid species (Vorburger 2006; Abbot 2011; Harrison & Mondor 2011). Under this hypothesis, all existing RWAs in Xinjiang and elsewhere in its native range would be descendents from this original wheat-adapted haplotype—the dominant Haplotype 1 in our study. Additional samples throughout the native distribution of RWA should be analysed to further test this hypothesis.

Given the potential capacity of RWAs to invade provinces other than Xinjiang, it is interesting that the most easterly site in Xinjiang where RWAs have been detected in the past is Qincheng, located near the border of Gansu province (Zhang *et al.* 1999a; Du 2000). Why have RWAs failed to establish in more Eastern wheat-growing districts in China, when the climate is predicted to be conducive (Liang *et al.* 1999)? Although a geographic barrier (e.g. Gobi desert) may be responsible, it is also possible that the same environmental factors are limiting range expansion eastward in both China and the USA, which may be an obligate ecological association with high altitudes in areas where an overwintering stage is required (J. Burd, personal communication).

Finally, it is important to consider that in this study, we have only sampled RWAs from wheat and thus, we may have examined the genetic structure of only a subsample of the RWAs in the region. Without sampling on other hosts, particularly perennial native hosts, we cannot discount the possibility that we have missed additional unsampled genotypes in the region. In addition, this study has examined the genetic differentiation of RWAs from only a relatively small part of their native range in Asia. However, our results will be critical in

guiding future studies of patterns of invasion not only of RWAs, but also of other invasive insect herbivores.

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This work forms part of B.Z.'s PhD thesis on *Diuraphis noxia* population genetics. B.Z. conducted the molecular laboratory research under the guidance of L.K. in CAS, and genetic analyses with the help of S.J.F. in QUT. O.R.E. has research interests in invertebrate genomics and in particular, aphid genetics and aphid-plant interactions. S.J.F. is a population geneticist whose research integrates field-based population studies with molecular techniques to deliver ecological management outcomes. L.K. is an entomologist studying the ecogenomics of the migratory locust and the plasticity of phase transition and gene expression modulation.

Data accessibility

Mitochondrial sequences: Genbank accessions JN204386–JN204421.

Microsatellite sequences: Genbank accessions JN204377–JN204385.

Sample locations: Uploaded as Supporting information.

Microsatellite data and mitochondrial haplotypes: DRYAD doi:10.5061/dryad.42sh717m.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Sampling information for RWAs in Xinjiang.

Appendix S2 Primer details and indices of genetic variation for the 10 microsatellite loci used in this study.

Appendix S3 Mitochondrial genetic diversity in each population.

Appendix S4 AMOVA for RWAs sampled in 2010 and analysed in three groups: Wuqia, Cele and Pishan, and northern populations.

Appendix S5 Pairwise F_{ST} and Exact *G* test for each site sampled in 2009 and 2010, and AMOVA analysis of nine groups for temporal comparison.

Appendix S6 FCA of Chinese RWAs from northern populations sampled in 2009 and 2010.

Appendix S7 Gene flow patterns of RWAs in far eastern ranges based on long-term estimates of gene flow.

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