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ORIGINAL ARTICLE

Biological traits yield divergent phylogeographical patterns between two aphids living on the same host plants

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ABSTRACT

Aim Animals' phylogeographical patterns are frequently explained by Pleistocene glacial fluctuations and topographical environments. However, species-specific biological traits are thought to have profound impacts on distribution patterns, particularly in aphids. We hypothesize that the phylogeographical patterns and/or population dynamics of two sympatric aphids may be different due to their different reproductive modes and feeding sites, even though they share the same hosts and environmental conditions.

Location China.

Methods We explored our hypothesis in *Chaitophorus saliniger* and *Tuberolachnus salignus*, two aphids that share the same host plants (genus *Salix*) but differ biologically. *Chaitophorus saliniger* is characterized by alternating sexual and asexual reproduction and only feeds on willow leaves, whereas *T. salignus* has obligate asexual reproduction and feeds on trunks and branches. The genetic diversity, population structure and demographic history of the aphids were analysed based on both mitochondrial DNA (cytochrome *c* oxidase subunit I and cytochrome *b*) and nuclear DNA (translation elongation factor 1 alpha). Ecological niche models (ENMs) were used to explore historical changes in distribution. The chief environmental variables that discriminate the different haplogroups were identified through multivariate statistical analysis.

Results There were striking differences in the phylogeographical patterns between the species. The sexual *C. saliniger* exhibited higher genetic diversity and population variations than the asexual *T. salignus*. According to genetic analyses and ENMs, both species experienced glacial contraction and post-glacial expansion. Multivariate statistical analysis revealed that the climatic differences between the divergent haplogroups were explained by principal components mainly loaded with temperature and elevation.

Main conclusions Our results suggest that species-specific biological traits and historical climate fluctuations have both shaped the current phylogeographical patterns of both aphid species. Their distinct genetic diversity and population structures highlight the importance of intrinsic biological features in driving phylogeographical patterns.

Keywords
Aphid, biological characteristic, *Chaitophorus saliniger*, ecological modelling, phylogeographical patterns, *Tuberolachnus salignus*

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INTRODUCTION

In evolutionary biology, an important issue concerns the relationships between the phylogeographical structures of different species, their ecological environments and species-specific attributes (Avise, 2000). Comparative phylogeography has therefore emerged as a powerful tool for comparing genetic structure and population dynamics, revealing the

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importance of different species' biological traits in relation to their responses to environmental change (Bertheau *et al.*, 2013; Jezkova *et al.*, 2015).

Co-distributed species with similar or different intrinsic biological traits may have experienced the same historical and geological events (Avise, 2000; Hickerson *et al.*, 2010). Many studies have tried to determine the effects of shared climatic fluctuations on multiple species by comparing species with similar biological traits (Hewitt, 2000; Soltis *et al.*, 2006). Concordant phylogeographical patterns among species can reveal common climatic refugia and post-glacial recolonization routes (Hewitt, 2000; Qu *et al.*, 2010; Morgan *et al.*, 2011; Endo *et al.*, 2015). A recent alternative approach compares co-distributed species with different species-specific traits (such as life histories or ecological requirements) to highlight their effects on genetic structure and variation. Different phylogeographical patterns may reflect the importance of species-specific characteristics on genetic variation and the adaptation to new environments (Peterson & Denno, 1998; Zhang *et al.*, 2012a; Mayer *et al.*, 2015; Phillipsen *et al.*, 2015). This approach has great potential for unravelling current phylogeographical patterns. Although there has been an increase in the number of recent comparative genetic studies in insects, most have focused on host–parasite or herbivore–host interactions (Whiteman *et al.*, 2007; Ren *et al.*, 2008; Borer *et al.*, 2012; Bertheau *et al.*, 2013) or on closely related species (Papadopoulou *et al.*, 2009; Morgan *et al.*, 2011; Triponez *et al.*, 2015), and researchers have rarely examined two sympatric insects with different biological traits.

Aphids are phytophagous insects and are intimately linked to their host plants (Hille Ris Lambers, 1979). Variation in the level of specialization to the host plants has produced monophagous, oligophagous and polyphagous aphids. Most of their divergence and speciation is due to adaptation to the host plants and host-driven speciation (Hawthorne & Via, 2001; von Dohlen *et al.*, 2002; Huang *et al.*, 2012; Zhang *et al.*, 2012b). It has been shown that the current distributions of East Asian plants were primarily shaped by various geological events and climate fluctuations in the Quaternary period (Harrison *et al.*, 2001; López-Pujol *et al.*, 2011; Li *et al.*, 2013). This raises the question of whether these environmental factors have had a similar influence on aphids, particularly for pairs of co-distributed aphids with the same host plants.

To determine the importance of species-specific features under similar environmental conditions, we selected *Chaitophorus saliniger* Shinji, 1924 (Chaitophorinae) and *Tuberolachnus salignus* (Gmelin, 1790) (Lachninae), two cosmopolitan aphid species that belong to different subfamilies but are both significant pests of willow (*Salix*) (Shinji, 1931; Collins *et al.*, 2001a). Even though they infest the same host-plant genus, the two aphids have different biological traits, including their reproductive modes and feeding sites. *Chaitophorus saliniger* is a small, black aphid that infests both sides of willow leaves (Shinji, 1931; Shingleton & Stern, 2003; in our field investigation). It has a complex life cycle with alternating sexual and asexual reproduction phases. In contrast, *T. salignus* lives on willow stems including trunks and branches (Collins *et al.*, 2001b). It is apomictic and reproduces solely by parthenogenesis (Blackman & Spence, 1996).

The combination of sexual and asexual reproduction not only ensures a larger population via short and rapid breeding in stable conditions (Wang, 2011), but also enhances the genetic diversity in harsh environments by genetic recombination via sexual reproduction (Kondrashov, 1988; Hamilton *et al.*, 1990; West *et al.*, 1999). Asexual individuals, in contrast, have a superior colonizing ability (Lynch, 1984), and asexual reproduction in aphids can ensure identity between mother and daughter, generating a high frequency of identical genotypes and keeping levels of genetic diversity low even over large distances (Wilson *et al.*, 1999; Delmotte *et al.*, 2002; Aradottir *et al.*, 2012; Piffaretti *et al.*, 2013; Nibouche *et al.*, 2014). We therefore predict that, due to its parthenogenesis, *T. salignus* will exhibit lower genetic diversity than *C. saliniger*. Furthermore, it has been revealed that species that use fewer feeding sites require more appropriate habitat (Fang *et al.*, 2006). *Chaitophorus saliniger* is likely to be more sensitive to environmental fluctuations than *T. salignus*, because the reduced number of feeding sites for *C. saliniger*, which feeds only on leaves, requires more appropriate habitat than *T. salignus,* which feeds on trunks and branches (Fang *et al.*, 2006). It has been observed that asexual aphids cannot survive harsh winters and are restricted even by mild winters (Vorburger *et al.*, 2003; Piffaretti *et al.*, 2013). We predict that *T. salignus* survived the Pleistocene glaciations in refugia, because the complex topography and mountain network in China could provide relatively stable micro-ecosystems as glacial refugia for both plants and animals (Flanders *et al.*, 2011; López-Pujol *et al.*, 2011; Qu *et al.*, 2012; Fang *et al.*, 2013; Shi *et al.*, 2014). Considering their differences in feeding sites and the potential requirement for suitable conditions, there might be some differences in these two aphids' responses to glaciation. We presume that if the contrasting reproductive modes and feeding sites and climatic conditions determine the genetic variation and distribution patterns, the two aphids will exhibit two contrasting phylogeographical patterns.

To test our predictions, we conducted comparative phylogeographical analysis using both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) markers. We also used ecological niche models (ENMs) to predict the changes in the species' distribution from the past – both the last interglacial maximum (LIG) and Last Glacial Maximum (LGM) – to the present. The aims of this study were: (1) to examine the genetic diversity, population structure and demographic history of both aphids; (2) to determine through comparative analysis whether these two aphids have distinct phylogeographical patterns and (3) to assess our predictions and identify whether the aphids' species-specific biological traits have acted as important drivers in shaping the current phylogeographical patterns.

MATERIALS AND METHODS

Taxon sampling

Both species were extensively sampled over the majority of their distributions in China to quantify their genetic diversity, structure and demographic history. A total of 350 *C. saliniger* and 135 *T. salignus* aphid colonies were collected from *Salix* plants between 2002 and 2014. All samples were preserved in 95% ethanol at −20 °C for DNA extraction. *Periphyllus koelreuteriae* and *Chaitophorus populialbae* were selected as outgroups for *C. saliniger*, and *Cinara formosana* and *Stomaphis sinisalicis* as outgroups for *T. salignus*. All samples and voucher specimens have been deposited in the National Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences, Beijing, China. (See Table S1 in Appendix S1 of the Supporting Information for collecting information and voucher numbers).

DNA extraction, amplification and sequencing

Aphid DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The mitochondrial genes cytochrome oxidase subunit I (*COI*) and cytochrome *b* (*cytb*), and the nuclear gene elongation factor 1α (*EF-1α*) were amplified (see Table S2 in Appendix S1 for primers and PCR conditions). Polymerase chain reaction (PCR) amplification was performed in 30-μL reaction mixtures containing 2.4 μL of dNTPs (TransGen Biotech, Beijing, China), 3 μL of 10× Buffer ($+Mg^{2+}$) (TransGen Biotech), 0.4 U of Taq DNA Polymerase (TransGen Biotech), 0.6 μL each of 10 μM forward and reverse primer, 1 μL of DNA extract and 22 μL of ddH_2O. The products were examined by electrophoresis on a 1% agarose gel, and suitable products were sequenced on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

PCR sequences had to be cloned when *EF-1α* chromatograms of the two species displayed numerous double peaks. PCR products were purified and ligated into the pMD19-T vector (TaKaRa, Dalian, China) and transformed into Trans5α competent cells (TransGen Biotech) according to the manufacturer's instructions. Plasmid DNA from 3 to 10 clones per individual was sequenced using the universal M13 forward primer. Sequences were assembled using the SeqManII module of lasergene 5.0 (DNASTAR, Madison, WI, USA) and aligned using mega 6.0 (Tamura *et al.*, 2013). No insertions, deletions or stop codons were present in the alignment. The introns in the *EF-1α* gene were included in later analyses. All sequences have been submitted to GenBank (accession numbers KT236456–KT236922 and KT237402–KT238343; see Table S1). Details of the data analyses are given in Appendix S2.

RESULTS

Genetic diversity

We obtained a 1355-bp combined fragment of the *cytb* (697 bp) and *COI* (658 bp) genes for both species, and 939-bp and 922-bp *EF-1α* sequences (including introns) for *C. saliniger* and *T. salignus* respectively. *Tuberolachnus salignus* showed considerably less haplotype and nucleotide diversity than *C. saliniger* in both mtDNA and nDNA (Table 1).

Phylogeographical structure

We reconstructed phylogenetic trees (Figs 1b, 2b, 3b & 4b) and haplotype networks (Figs 1c, 2c, 3c & 4c) for *C. saliniger* and *T. salignus* based on mitochondrial and nuclear markers. The two species showed very different phylogeographical patterns (Figs 1a, 2a, 3a & 4a).

For *C. saliniger*, the mtDNA trees revealed no deep lineage splits, but instead comprised multiple divergent haplogroups (HG1–HG5; Fig. 1). The basalmost haplogroup was from Heilongjiang Province (marked in blue in Fig. 1a), and this haplogroup formed an independent lineage (HG1, Fig. 1b,c, marked in blue), which was the sister to a group containing four separate haplogroups (HG2–HG5, Fig. 1b,c). All HG3 haplotypes (green) were found in north-eastern China. The HG2 (yellow) and HG5 (purple) haplotypes were located in south-western China, and the HG4 (brown) haplotypes were dispersed throughout eastern China (east and south of the Taihang Mountains and Qinling Mountains). The remaining HG6 (grey) haplotypes were primarily concentrated in the central regions of China.

The more slowly evolving nuclear DNA distinguished two well-supported haplotype lineages: L1, a north-eastern China lineage composed of all of individuals from Heilongjiang and

Table 1 Genetic diversity of *C. saliniger* and *T. salignus* in mtDNA and nuclear DNA.

Index	*C. saliniger* *COI–cytb*	*EF-1α* (intron)	*T. salignus* *COI–cytb*	*EF-1α* (intron)
Number of ID	350	350 (350)	125	123 (123)
Length (bp)	1355	939 (158)	1355	922 (141)
Polymorphic sites	117	33 (12)	34	7 (0)
Nucleotide diversity	0.00759	0.00251 (0.00554)	0.00046	0.00069 (0)
Number of haplotypes	82	23 (11)	13	6 (1)
Haplotype diversity	0.942	0.576 (0.483)	0.24	0.419 (0)

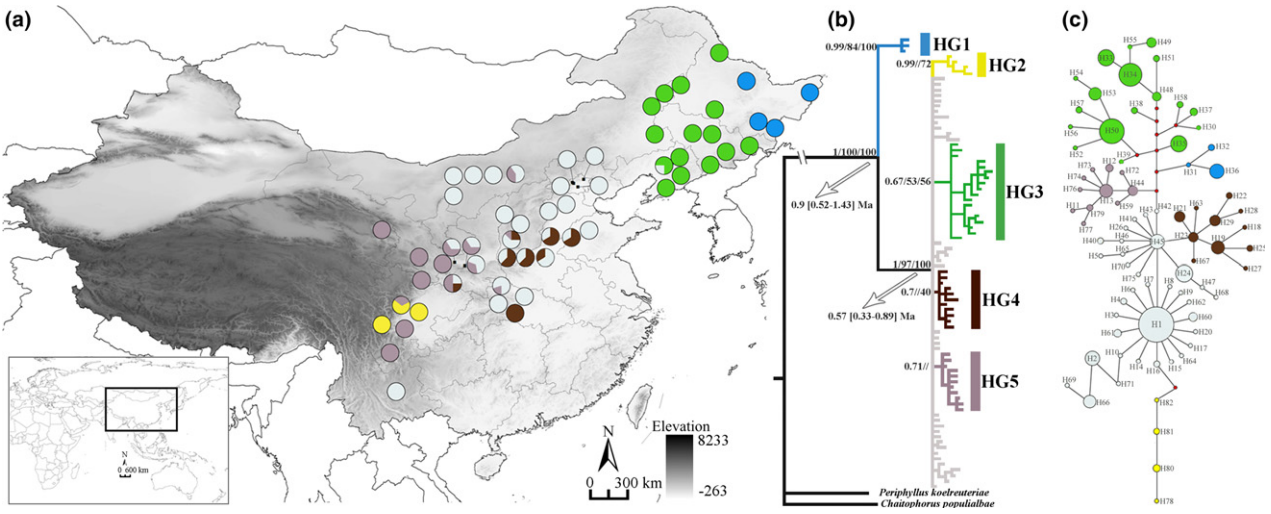

Figure 1 Geographical distribution, phylogenetic trees and networks of mitochondrial (*COI–cytb*) haplotypes for *Chaitophorus saliniger* in China. The haplogroups are coloured according to the phylogenetic structure. The rectangular box in the insert shows the location of the study area in Asia. (a) Geographical mapping; (b) phylogenetic tree (the numbers above the tree branches are the support values under Bayesian inference, maximum likelihood and maximum parsimony respectively; the white arrows represent divergence times); (c) network map, where each circle corresponds to one haplotype, as detailed in Table S1, and the circle size is proportional to the number of individuals with that haplotype. Each link between circles indicates one mutational event [Colour figure can be viewed at wileyonlinelibrary.com].

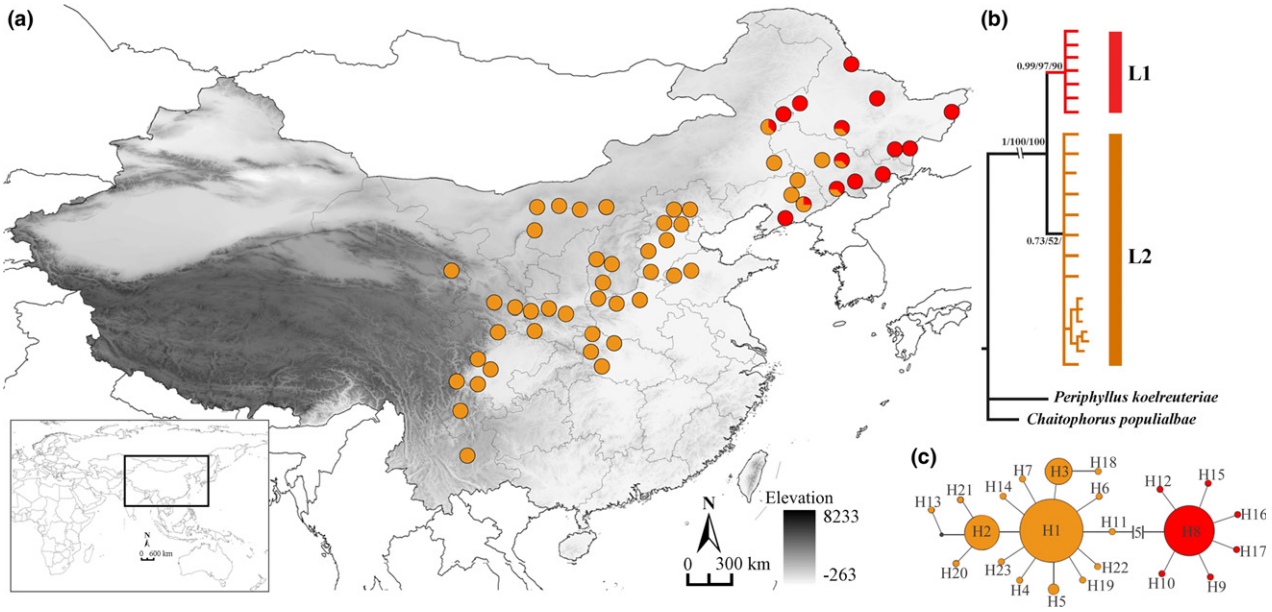

Figure 2 Geographical distribution, phylogenetic trees and networks of the nuclear alleles (*EF-1α*) for *Chaitophorus saliniger* in China. The lineages are coloured according to the phylogenetic structure. The rectangular box in the insert shows the location of the study area in Asia. (a) Geographical mapping; (b) phylogenetic tree (the numbers above the tree branches are the support values under Bayesian inference, maximum likelihood and maximum parsimony respectively); (c) network map, where each circle corresponds to one haplotype, as detailed in Table S1, and the circle size is proportional to the number of individuals with that haplotype. Each link between circles indicates one mutational event [Colour figure can be viewed at wileyonlinelibrary.com].

Jilin provinces (marked in red on Fig. 2) and L2, including the remaining populations from central, eastern and south-western China (marked in orange on Fig. 2).

The mitochondrial network revealed that haplotype H1 is abundant (64 individuals) in central China and has radiated many other haplotypes (Fig. 1c). The nuclear network also demonstrated a similar haplotype, H1, in central China (Fig. 2c).

For *T. salignus*, neither mtDNA-based or nDNA-based phylogenetic analysis showed genetic divergence (Figs 3b & 4b).

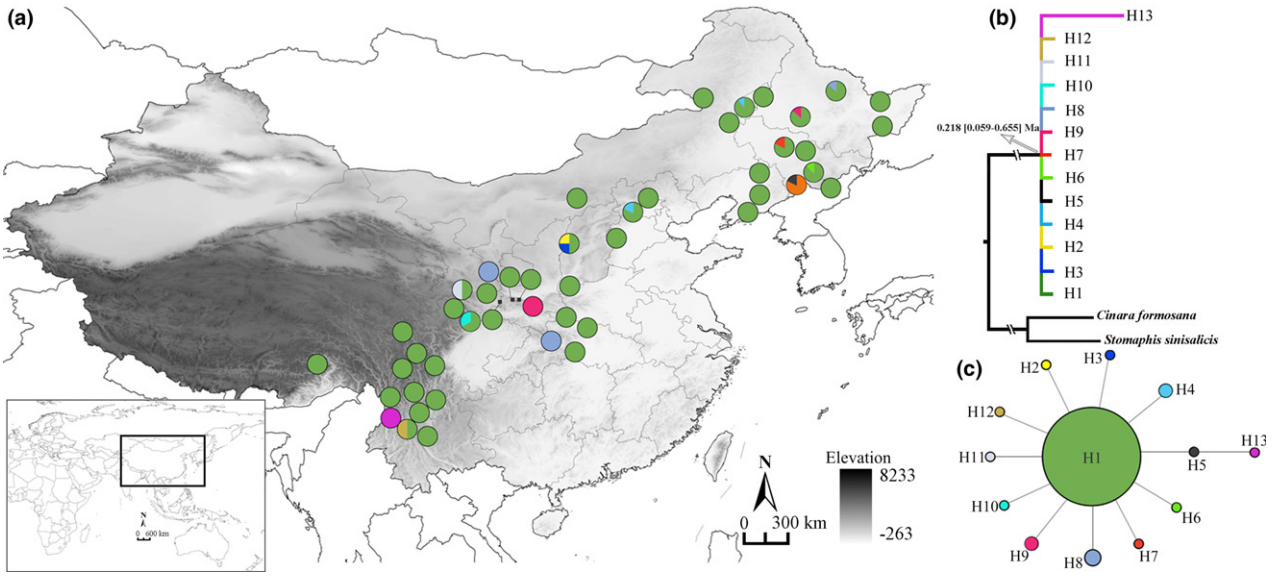

Figure 3 Geographical distribution, phylogenetic trees and networks of the mitochondrial (*COI–cytb*) haplotype for *Tuberolachnus salignus* in China. The haplotypes are coloured according to the phylogenetic structure. The rectangular box in the insert shows the location of the study area in Asia. (a) Geographical mapping; (b) consensus tree based on Bayesian inference, maximum likelihood and maximum parsimony, where the white arrows represent divergence times; (c) network map, where each circle corresponds to one haplotype, as detailed in Table S1, and the circle size gives the proportion of individuals with that haplotype. Each link between circles indicates one mutational event [Colour figure can be viewed at wileyonlinelibrary.com].

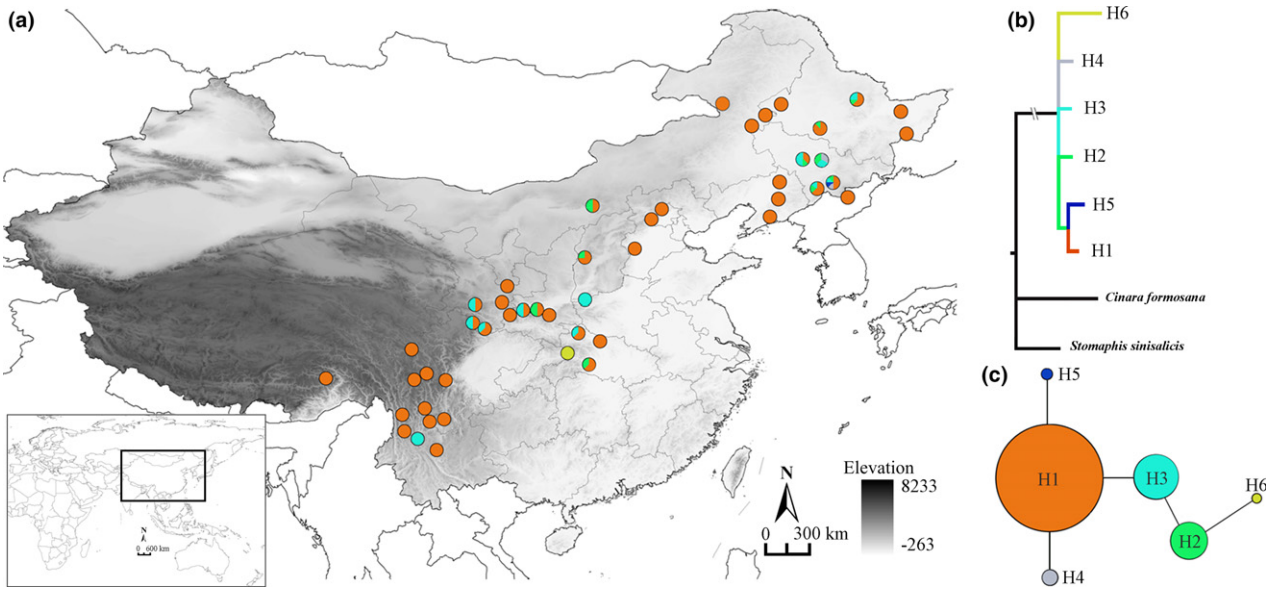

Figure 4 Geographical distribution, phylogenetic trees and networks of the nuclear alleles (*EF-1α*) for *Tuberolachnus salignus* in China. The haplotypes are coloured according to the phylogenetic structure. The rectangular box in the insert shows the location of the study area in Asia. (a) Geographical mapping; (b) consensus tree based on Bayesian inference, maximum likelihood and maximum parsimony; (c) network map, each circle corresponds to one haplotype, as detailed in Table S1, and the circle size gives the proportion of individuals with that haplotype. Each link between circles indicates one mutational event [Colour figure can be viewed at wileyonlinelibrary.com].

The mtDNA network was star-shaped and did not reveal any phylogenetic structure (Fig. 3c). It was characterized by one main haplotype, H1, shared by 109 individuals sampled all over the region. All other haplotypes were separated from H1 by at most two mutation steps. The nuclear networks demonstrated that the species consisted of ancestral and derived haplotypes (Fig. 4c).

For *C. saliniger*, analyses of molecular variance (AMOVA) showed that the largest variation was distributed among populations (80.39% and 71.55%, depending on the marker;

Table 2 Results of hierarchical AMOVA for the two aphids based on mtDNA.

Species	Groups	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation index
Chaitophorus saliniger	Population groups	Among populations	68	1537.332	4.2671	80.39%	$F_{ST} = 0.8039 P = 0.000$
		Within populations	281	292.468	1.0408	19.61%	
		Total	349	1829.800	5.3079		
	Six haplogroups	Among haplogroups	5	1300.74	5.381	77.77%	$F_{ST} = 0.7777 P = 0.000$
		Within haplogroups	344	529.064	1.538	22.23%	
		Total	349	1829.8	6.919		
Tuberolachnus salignus	Population groups	Among populations	50	30.422	0.2038	63.77%	$F_{ST} = 0.6377 P = 0.05$
		Within populations	74	8.568	0.1158	36.23%	
		Total	124	38.990	0.3196		

Table 3 Results of hierarchical AMOVA for the two aphids based on nDNA.

Species	Groups	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation index
Chaitophorus saliniger	Population groups	Among populations	68	323.929	0.858	71.55%	$F_{ST} = 0.7155 P = 0.000$
		Within populations	288	98.237	0.341	28.45%	
		Total	356	422.166	1.199		
	Two lineages	Among lineages	1	337.78	2.938	92.59%	$F_{ST} = 0.9259 P = 0.000$
		Within lineages	354	83.25	0.235	7.41%	
		Total	355	421.03	3.174		
Tuberolachnus salignus	Population groups	Among populations	49	17.687	0.028	8.71%	$F_{ST} = 0.0872 P = 0.2317$
		Within populations	73	21.396	0.293	91.29%	
		Total	122	39.082	0.321		

Tables 2 & 3), indicating strong population divergence. Furthermore, we found significant genetic variance between multiple haplogroups (77.77% of mtDNA variation among the six haplogroups; 92.59% of nDNA variation between the two lineages; see Tables 2 & 3). In contrast, we found 91.29% variation in nDNA within *T. salignus* populations (Table 3) and only 63.77% mtDNA variation among populations (Table 2), indicating no significant divergence at the population level.

Divergence time and demographic histories

Analyses of *C. saliniger* using BEAST (Drummond *et al.*, 2012) produced a mean estimate of the divergence time of haplogroup HG1 from the other populations of *c.* 0.9 Ma (Fig. 1b). Multiple haplogroups (HG2–HG5) have diverged since 0.57 Ma (Fig. 1b), corresponding to the middle–late Pleistocene, although the estimates suggested a more recent genetic differentiation within *T. salignus* (0.218 Ma, late Pleistocene glaciation; Fig. 3b) than within *C. saliniger*. The coalescence time within species, usually used to evaluate the effective population size, N_e (Lynch & Conery, 2003), was earlier in *C. saliniger* than in *T. salignus*, indicating a larger effective population in the former.

With the exception of the *T. salignus* nuclear network, the haplotype networks of the two aphids exhibited star-like structures radiating from a central haplotype, suggesting a recent population expansion (Figs 1c, 2c, 3c & 4c). The pairwise comparisons of both aphids with their respective outgroup species found no significant deviation from neutrality ($P > 0.05$) in the McDonald–Kreitman neutrality test (McDonald & Kreitman, 1991). The significantly negative values of Tajima's *D* and Fu's *Fs* (see Table S3, Appendix S1) support a recent demographic expansions of both species. Bayesian skyline plots indicated that *C. saliniger* maintained a relatively stable population size from 0.3 Ma to 0.017 Ma, after which its population size rapidly increased (Fig. 5a). *Tuberolachnus salignus* has undergone a slow population decline since 0.02 Ma, followed by a considerable expansion – smaller than that of *C. saliniger* – at *c.* 0.0065 Ma, until it stabilized (Fig. 5b). The total effective population size of *C. saliniger* was always larger than *T. salignus* (Fig. 5), which is consistent with the inference from the coalescence time.

Isolation by distance

The mtDNA analysis revealed a clear pattern of isolation by distance (IBD) for *C. saliniger*, and variance in F_{ST} increased with geographical distance (Mantel test, $r^2 = 0.2604$; $P < 0.001$; Fig. 6a). In *T. salignus*, there was no significant IBD relationship between genetic variation and Euclidean distances ($r^2 = 0.0006$; $P = 0.292$; Fig. 6b), indicating a greater dispersal ability than *C. saliniger*. The pattern of

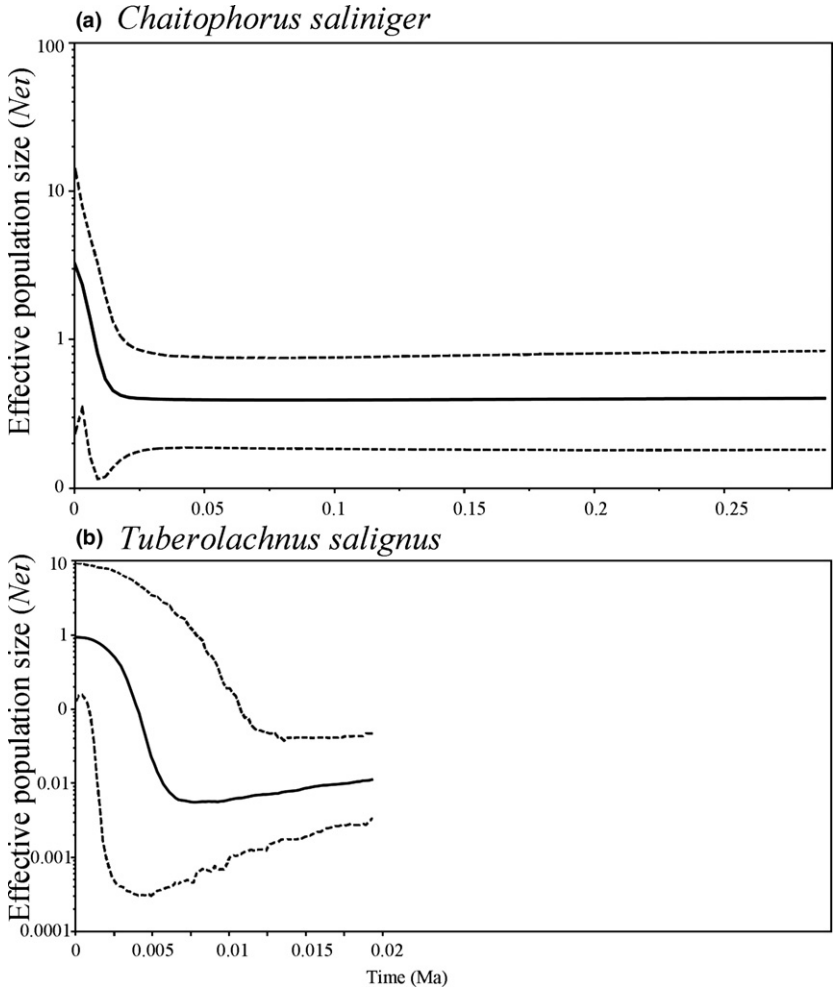

Figure 5 Bayesian skyline plots (BSP) showing the changes in the effective population size over time in (a) *Chaitophorus saliniger*, and (b) *Tuberolachnus salignus*, in China; *x*-axis, time in millions of years ago, Ma; *y*-axis (on a logarithmic scale), estimated population size, in units of $N_e\tau$, the product of the effective population size and the generation length in years. Solid lines indicate the median value of the effective population size; dashed lines denote the 95% highest posterior probability interval. The time on the *x*-axis is based on a mutation rate of 1.77% lineage^{-1} Myr^{-1}.

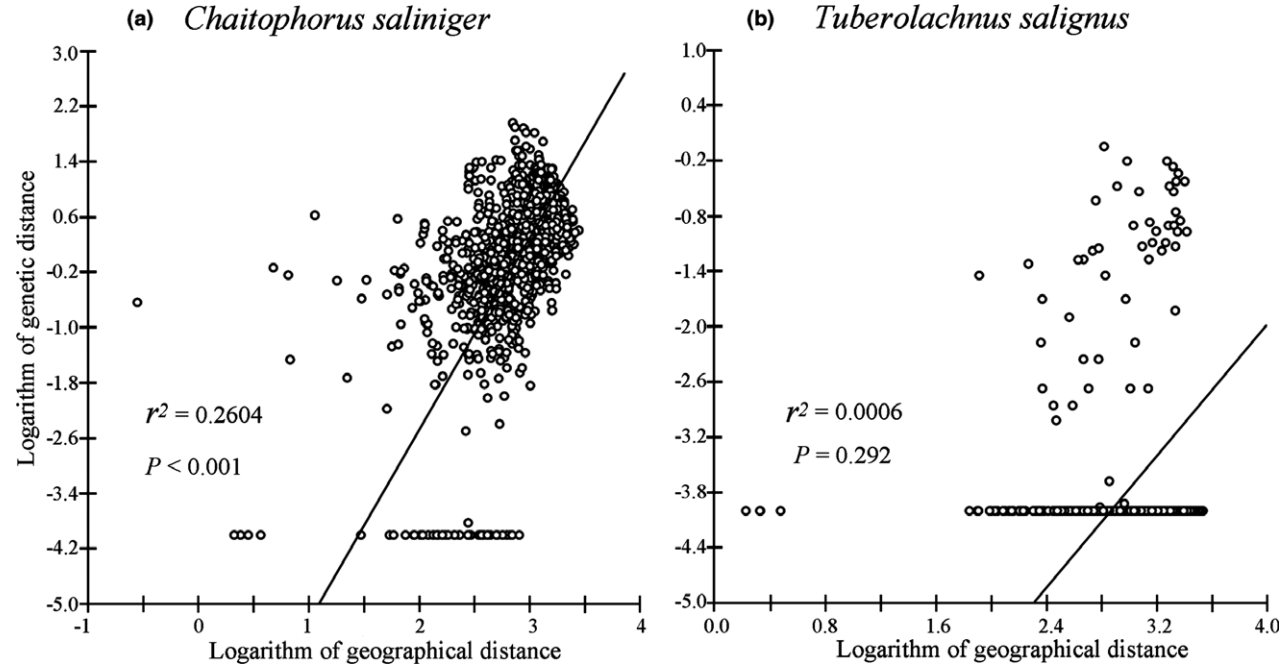

Figure 6 Results of ISOLATION BY DISTANCE analyses of the mtDNA of (a) *Chaitophorus saliniger* and (b) *Tuberolachnus salignus* in China, including a reduced-major-axis regression plot that shows the pairwise logarithm of the genetic distance against the logarithm of geographical distance between each population, as well as correlation coefficients and *P*-values from a Mantel test.

isolation by distance for both species based on nDNA data was similar to that based on mtDNA. Although not strong, we found a significant IBD relationship in *C. saliniger* ($r^2 = 0.0094$; $P < 0.05$; see Fig. S1a, Appendix S3), whereas there was no such relationship in *T. salignus* ($r^2 = 0.00001$; $P = 0.462$; see Fig. S1b, Appendix S3). Overall F_{ST} values for *C. saliniger* (mtDNA, 0.8039; nDNA, 0.7542) were higher than those for *T. salignus* (mtDNA, 0.6377; nDNA, 0.03195).

Ecological niche models and multivariate statistical analysis

The ecological niche models based on present-day climate conditions (see Fig. S2, Appendix S3) captured the known distribution of both species, as shown by AUC (area under the curve) values of 0.99 and 0.988 for *C. saliniger* and *T. salignus* respectively; 10 replicate runs for each species generated the same AUC values. The LIG models (see Fig. S2) indicated that the aphids lived in areas similar to its current distribution. In contrast, only small areas were projected as suitable for both species during the LGM. Moreover, *C. saliniger* showed considerably smaller distribution than *T. salignus* in glacial periods (see Fig. S2). The modelling predicted a region east of the Taihang Mountains to the Qinling Mountains, as well as China's south-western mountains for *T. salignus*, whereas the distribution of *C. saliniger* was restricted to a small area south of the Qinling Mountains.

Principal components analysis (PCA) reduced the 11 bioclimatic variables to four principal components (PCs) with an eigenvalue ≥ 1 (Kaiser's criterion) (see Table S4, Appendix S1), which together explained 91.3% of the variance (PC1, 45.2%; PC2, 20.0%; PC3, 16.1%; PC4, 10.0%). Annual mean temperature, mean diurnal range, the minimum temperature of the coldest month and the mean temperature of the coldest quarter were loaded onto PC1. Elevation and annual precipitation were loaded onto PC2. Mean temperature of the wettest quarter, precipitation of the wettest month and precipitation seasonality were loaded onto PC3. Isothermality and precipitation of the driest quarter were loaded onto PC4. Based on factorial discriminant analysis (DA) and multivariate analysis of variance (MANOVA), environmental conditions associated with the four PCs differed significantly among the six divergent haplogroups ($P < 0.001$, see Fig. S3, Appendix S3). Tukey's honestly significant difference (HSD) test revealed that the largest climatic difference among the divergent haplogroups resulted from PC1 and PC2, mainly loading in temperature and elevation (see Table S5 in Appendix S1).

DISCUSSION

Genetic variation and the influence of species-specific biological traits

We conducted a comparative assessment of phylogeographical structure in two sympatric but biologically distinct aphids using mitochondrial and nuclear markers. A striking difference was observed in the phylogeographical patterns of the two species, which highlights the importance of species-special biological traits, such as reproductive mode and feeding sites, on genetic variation and distribution patterns. Previous comparisons of the phylogeography in co-distributed organisms, including mosquitos, bees, rats and birds, have revealed the influence of species-specific features on the genetic variation of populations (Morgan *et al.*, 2011; Zhang *et al.*, 2012a; Jezkova *et al.*, 2015; Triponez *et al.*, 2015). The comparisons of the two aphids in this study confirmed our prediction that the differences in the aphids' intrinsic biological features would drive their different phylogeographical patterns, even under the same environmental conditions.

In this study, *Tuberolachnus salignus* exhibited considerably lower genetic diversity than *Chaitophorus saliniger* (Table 1), supporting our first prediction that parthenogenesis would contribute to the lower genetic diversity of *T. salignus* compared to *C. saliniger*, which exhibits bisexual reproduction. Although the much larger sample sizes of *C. saliniger* may have increased the number of detected haplotypes, *C. saliniger* showed a much greater difference in diversity between mtDNA and nDNA than *T. salignus* (mtDNA haplotypes: 82 vs. 13; nDNA haplotypes: 23 vs. 6, Table 1). The coalescent time and N_e in the Bayesian skyline plots revealed a larger effective population size in *C. saliniger* than in *T. salignus*, in line with Orive's (1993) assertion that sexual reproduction tends to increase the effective population size, whereas asexuality decreases it.

In addition, the phylogenetic trees and network maps uncovered clear population divergence in *C. saliniger* (Figs 1 & 2), but no divergence in *T. salignus* in either the mtDNA or nDNA data (Figs 3 & 4). We further confirmed this pattern by AMOVA, which showed the largest variation component to be among populations (mtDNA, 80.39%; nDNA, 71.55%) and haplogroups (mtDNA, 77.77%; nDNA, 92.59%) for *C. saliniger*, whereas the largest variation in the nDNA occurred within populations for *T. salignus* (91.29%), with only 63.77% of variation in the mtDNA among populations (Tables 2 & 3). A similar pattern for *T. salignus* has been observed in other countries using microsatellite markers, and has been attributed to the species' parthenogenetic reproduction (Aradottir *et al.*, 2012).

Although we observed more genetic variation in *C. saliniger* than in *T. salignus*, some differences between the mitochondrial and nuclear patterns were still present in *C. saliniger*. The analyses of nDNA revealed two highly supported lineages in *C. saliniger*: L1, the north-eastern populations and L2, the populations from central, eastern and south-western China (marked red and orange, respectively, in Fig. 2). The mtDNA analyses identified several relatively well-supported haplogroups: HG1 and HG3 (north-eastern populations), HG2 (south-western populations), HG4 (eastern populations) and HG5 (western populations) (Fig. 1). Moreover, *C. saliniger* exhibited much higher genetic diversity in mtDNA than in nDNA. The most likely explanation

for this cytonuclear discordance is that the mitochondrial genome has a higher substitution rate and smaller effective population size than the nuclear genome (Dawid, 1972; Brown *et al.*, 1979; Canestrelli *et al.*, 2006; Singhal & Moritz, 2012), thus leading to faster genetic drift in the former. The nuclear genome, in contrast, still showed shared polymorphisms.

The factors that may have affected the population variations of the aphids include their life histories and powers of dispersal, the temporal and spatial instability of the hosts, and patterns of selection (Hales *et al.*, 1997). Various attempts have been made to link the genetic variation patterns with these biological features of aphids (Wöhrmann & Hales, 1989; Simon & Hebert, 1995). As we predicted, the low genetic diversity in *T. salignus* was mainly attributed to its reproduction by parthenogenesis (Blackman & Spence, 1996; Aradottir *et al.*, 2012), whereas the effective recombination that occurs during sexual reproduction increases the genetic diversity in *C. saliniger* (Kondrashov, 1988; Hamilton *et al.*, 1990; West *et al.*, 1999). The differing patterns of population variation within each species are probably related to their different dispersal abilities and host ranges. The lack of isolation by distance (Fig. 6, see Fig. S1) and the sharing of haplotype H1 across the sampling range (Fig. 3a) both reflect the greater dispersal ability of *T. salignus* than *C. saliniger*. Moreover, the wider host range of *T. salignus* (Zhang & Zhong, 1983) could facilitate the species' dispersal, resulting in lower levels of population variation. These phenomena, involving highly effective dispersal and a subsequent lack of strong genetic divergence across large distances, have been documented in aphids (Michel *et al.*, 2009; Xu *et al.*, 2011; Jun *et al.*, 2013) and a bark beetle (Sallé *et al.*, 2007; Mayer *et al.*, 2015). Although our study found no link between host plants and haplotypes in either aphid (the common haplotypes are mostly found in aphids that feed on several of the five willow species available in this study; see Table S1), we could not rule out the role of host-plant association in aphid speciation and population divergence, because genetic divergence between host-associated populations generally occurs at the level of the host family or genus (Peccoud *et al.*, 2010; Zhang *et al.*, 2012b). *Tuberolachnus salignus* is almost the only species in subfamily Lachninae that feeds on *Salix*, whereas species in the genus *Chaitophorus* exploit a range of host plants in the genera *Salix* and *Populus* (Salicaceae) (Zhang & Zhong, 1983), meaning that *Chaitophorus* has probably experienced host-associated diversification. Future work is necessary to focus on species divergence associated with host plants, especially at the level of families or genera, which will benefit from detailed host information.

It has been proposed that climatic variables have a direct effect on population divergence in aphids (Blackman *et al.*, 1990; Puterka *et al.*, 1993; Martinez-Torres *et al.*, 1997). It has been suggested, for example, that temperature is an important driver of aphid activity and abundance (Dixon, 1977; Collins & Leather, 2001). This is consistent with our finding that temperature was mainly loaded onto PC1, with 45.2% of the total climatic variation. In addition, we identified statistically significant differences in the environmental variables among the divergent *C. saliniger* haplogroups (see Table S5), reflecting the effects of ecological conditions on genetic variation. Niche differentiation and local adaptation could further promote genetic differentiation among the various haplogroups of *C. saliniger*. We saw no such outcome in *T. salignus* samples from different climatic conditions, however, which may be due to that species' more recent population variation (*C. saliniger*: 0.9 Ma, middle–late Pleistocene; *T. salignus*: 0.218 Ma, late Pleistocene glaciation). It would require a much longer time for *T. salignus* to acquire further genetic divergence and develop local adaptation. Another possibility is that the transportation of cut trees by humans, such as occurs in the timber trade, could play a role in homogenizing the population structure of *T. salignus*, which feeds primarily on willow trunks and branches (Collins *et al.*, 2001b). Therefore, the distinct genetic patterns between *C. saliniger* and *T. salignus* support the hypothesis that different species' phylogeographical patterns are primarily related to species-specific biological traits, including modes of reproduction, effective dispersal and ecological niches, as well as their history of population differentiation and human activities.

Influence of climatic fluctuations

Previous phylogeographical studies have emphasized the influence of Pleistocene climatic fluctuations and topographical environments on intraspecific divergence, genetic structure and demographic history in many organisms (e.g. Flanders *et al.*, 2011; Zhou *et al.*, 2013; Lei *et al.*, 2014, 2015; Shi *et al.*, 2014; Qu *et al.*, 2015). The present distributions were primarily derived from multiple post-glacial expansions from refugia (Flanders *et al.*, 2011; Qu *et al.*, 2012; Shi *et al.*, 2014). In this study, the range of both aphid species were revealed to have contracted around the LGM and expanded after the glaciation, as inferred from both the ecological niche modelling and phylogeographical analyses. During glacial periods, the suitable area for *C. saliniger* was concentrated south of the Qinling Mountains in central China (see Fig. S2a, in LGM), an area which has been documented as a potential refugium for other plants and animals (López-Pujol *et al.*, 2011; Wang *et al.*, 2012, 2013; Fang *et al.*, 2013; Zhao *et al.*, 2013). Intriguingly, the potential refugium for *C. saliniger* was smaller than that for *T. salignus* (see Fig. S2a,b, in LGM), which could indicate that *C. saliniger* is more sensitive to cold conditions than *T. salignus*. This is in accord with our second prediction. Because species that use fewer feeding sites require an appropriate habitat (Fang *et al.*, 2006), *C. saliniger*, which only feeds on willow leaves, would have to contract into limited microenvironments to survive, whereas *T. salignus* has more diverse feeding sites (such as the willow trunks and branches) than *C. saliniger*, which could promote its survival in more ecological niches. In our field investigations, for example, *T. salignus* was often found

in cracks in the bark of trunks, which suggests that *T. salignus* could survive better in harsh conditions. The reconstructed distributions for both species in the present day and the LIG (see Fig. S2a,b) suggested large suitable environmental conditions for post-glacial expansion. The recent population expansions in both species were supported by the increase in the effective population size revealed in the Bayesian skyline plots (Fig. 5) and by Tajima's *D* and Fu's *Fs* statistical tests (see Table S3).

Genetic analyses revealed a star-like network for both species. For *C. saliniger*, both the mtDNA and nDNA networks exhibited a star-like form (Figs 1 & 2). The most frequent and central haplotype, H1, is the most likely ancestral haplotype (Avise, 2000; Hewitt, 2000; Jing *et al.*, 2014), and was shared by multiple populations in central China. Haplotype H1 was connected by few mutational steps to many lower frequency haplotypes in north-eastern and south-western China, which supports the proposed population expansion and refugium hypothesis. It is likely that the distribution of *C. saliniger* contracted into an area south of the Qinling Mountains during glaciations, and then expanded to north-eastern and south-western China after glaciation. The population expanded at *c.* 0.017 Ma (Fig. 5a), corresponding to a period after the LGM. Although it exhibited different levels of contraction and colonization to *C. saliniger*, *T. salignus* underwent a similar migration route to the north-east and south-west, resulting in the sympatric distribution of both aphids. This finding highlights the important influences of the Pleistocene climatic oscillations on the distribution of these two aphids.

The comprehensive approach of a multispecies, multifaceted analysis used in this study allowed us to compare the phylogeographical patterns of insects with distinct biological traits. The current distribution patterns of the two aphids can be attributed to a combined effect of species-specific biological traits and climatic fluctuation. Their distinct genetic diversity and population structure highlight the importance of species' intrinsic features in shaping their phylogeographical patterns. An understanding of how the biological traits of these insects influence their phylogeographical structure under the same historical environments provides us with a deeper insight into the relationships between phylogeographical patterns, species-specific attributes and environmental interference (Avise, 2000).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supporting tables (Tables S1–S5).
Appendix S2 Data analysis.
Appendix S3 Supporting figures (Figs S1–S3).

DATA ACCESSIBILITY

All complete mitochondrial and nuclear sequences have been deposited in GenBank (accession numbers KT236456–KT236922, KT237402–KT238343).

Sample information and GenBank accession numbers for all sequenced individuals of both species uploaded as online supporting material, Table S1 (Appendix S1).

Variables used in ecological niche modelling uploaded as online supplemental material, Appendix S2.

BIOSKETCH

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Author contributions: G.-X.Q. and F.F. conceived and designed the research and wrote the manuscript. G.-X.Q., F.F., J.C. and L.-Y.J. organized the collection of the specimens and species identification. F.F. and R.C. performed laboratory experiments. F.F. analysed the data. All authors read and approved the final manuscript.

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