Genetic diversity and genetic differentiation pattern of *Tuta absoluta* across China

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Abstract: *Tuta absoluta* (Meyrick) is a cosmopolitan invader and one of the most serious pests of tomato. This pest has expanded rapidly in China since it initially invaded Xinjiang in the northwest in 2017 and Yunnan in the south in early 2018. More complete knowledge of the migration patterns and genetic structure of this pest is important for implementing effective management strategies. To fill this knowledge gap, the *COI* gene of *T. absoluta* was sequenced based on nationwide routine monitoring. This study showed a new invasion into Gansu and Inner Mongolia, indicating ongoing expansion of this pest. Most populations of *T. absoluta*, both in China and worldwide, have high genetic homogeneity. Nevertheless, some degree of genetic variability was found in populations in southern China, especially in Yunnan. Two distinct haplogroups were distinguished, and clade A was predominant at the global level. The high level of sequence divergence indicated the possible existence of cryptic species. Furthermore, F_{ST} and analysis of molecular variance (AMOVA) revealed weak but significant differentiation between the Xinjiang northwestern group and southern group/subgroups and provides compelling evidence of the different dispersal avenues of *T. absoluta* in northwestern and southern China. *T. absoluta* likely established bridgehead populations in Yunnan and then spread rapidly throughout this province and neighboring territories in southern China. Knowledge regarding genetic diversity, population structure and dispersal pattern is valuable for improving management guidelines for this pest. Strict plant quarantines, local eradication and practical control measures are needed to solve the problem caused by *T. absoluta*.

Keywords: South American tomato leafminer; invasive pest; Gelechiidae; genetic variability; dispersal avenue; population structure; cryptic forms

1 Introduction

The South American tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) is a devastating pest of tomato (*Solanum lycopersicum* L.) worldwide (Biondi et al. 2018). This invasive pest gained notoriety since its spread from the center of origin in Peru to other Latin American countries and subsequently to Europe, Africa, and Asia (Desneux et al. 2010, 2011; Campos et al. 2017; Biondi et al. 2018; Mansour et al. 2018; Han et al. 2019). To date, *T. absoluta* has colonized 88 confirmed and 23 unconfirmed countries and districts worldwide with extraordinary speed (Zhang et al. 2021) and caused severe economic losses to tomato crops. *Tuta absoluta* can attack all developmental stages of the host plant, resulting in up to 100% yield losses without appropriate control (Desneux et al. 2010). In

some production areas, *T. absoluta* remains unmanageable, especially in open fields, and the intensive use of insecticides facilitates increasing problems with insecticide resistance (Biondi et al. 2018; Guedes et al. 2019; Desneux et al. 2022). Although its preferred host is tomato, *T. absoluta* has also been reported to attack other cultivated and wild plants, such as potato, eggplant, pepper, tobacco, black nightshade, and other Solanaceous weeds (Arnó et al. 2019; Zhang et al. 2021). More worryingly, as revealed by recent surveys, *T. absoluta* showed potential host-range expansion (Zhang et al. 2021). The capacity of this pest to colonize a wide range of wild host plants is problematic, as such hosts would act as population reservoirs in the absence of tomato and facilitate successive invasions of *T. absoluta* in new areas (Cifuentes et al. 2011).

In China, this pest was first found in the northwest (i.e., Ili, Xinjiang) in 2017 and southwest (Yunnan) in 2018; afterward, it spread rapidly to other provinces, including Guizhou, Jiangxi, Guangxi, Sichuan, Chongqing and Hunan (Zhang et al. 2020; 2021). China is the major producer of tomato, contributing 648.7 million tonnes of annual production, which accounts for 34% of the total global value. Xinjiang and Yunnan are the two leading tomato-producing areas. The unrestricted movement of tomato fruits and plants associated with the transport of goods and persons is responsible for the large-scale dispersion of *T. absoluta* due to its modest self-mediated spread capabilities (Xian et al. 2017; Biondi et al. 2018). Thus, the rapid expansion of *T. absoluta* is a far-reaching concern for tomato production in China, especially Yunnan and Xinjiang (the principal tomato-producing and exporting regions), considering that its colonization ability is the strongest in Yunnan and Guizhou, followed by Xinjiang (Zhang et al. 2021).

The success of an invasive species may be determined by the different ecological, genetic, and evolutionary features that accelerate its adaptation and spread through a wide range of areas (Prentis et al. 2008). The genetic characteristics of invasive pests have been studied for a long time. Loss of genetic diversity is quite common for invasive species due to founder effects, bottlenecks and bridgehead effects (Hawley et al. 2006). Alternatively, multiple independent introduction events and subsequent introgression, admixture and hybridization can enhance the genetic diversity of the invader and enable successful invasion (Miller et al. 2005). Therefore, understanding the genetic characteristics of *T. absoluta* is essential for gaining insights into its dispersal patterns, as well as the origin of the invasion and population dynamics (Wei et al. 2013). Such knowledge is needed to develop effective management strategies for *T. absoluta* (Liebhold & Tobin 2008). The genetic characteristics of recent invasive populations of *T. absoluta* have rarely been investigated. At the global scale, almost no genetic variation in *T. absoluta* populations throughout the invaded regions, including South America, Europe, the Mediterranean Basin, Africa and Asia,

has been revealed (Cifuentes et al. 2011; Guillemaud et al. 2015; Carvalho et al. 2018; İnak et al. 2021). Nevertheless, Guillemaud et al. (2015) revealed a marked level of differentiation within the native populations of *T. absoluta* in South America by using microsatellite markers.

Although *T. absoluta* has rapidly spread to several provinces in China, current knowledge of its genetic characteristics is limited. In this study, populations of *T. absoluta* across China were collected during nationwide routine monitoring. The genetic variability and population structure as well as the potential avenues of dispersal of this pest were characterized based on the *mitochondrial cytochrome oxidase subunit I* (*mtCOI*) gene to gain adequate knowledge for the management of this invader.

2   Material and methods

2.1   Insect populations

The South American tomato leafminer was surveyed in its invaded areas of mainland China between 2017 and 2021. A total of 320 individuals were collected from 52 locations, covering the following ten provinces: Xinjiang (in August and September 2017, and in May and August 2018), Yunnan (between April 2018 and July 2019), Guizhou (between September and November 2018), Jiangxi (in March and April 2018), Guangxi, Hunan (in November 2018), Chongqing (in October 2018), Sichuan (in June 2020), Gansu, and Inner Mongolia (in August 2021) (Fig. S1 and Table S1). In consideration of the large intervals of the collecting dates of the populations from Xinjiang, these samples were incorporated into 11 provincial/year-level populations in the subsequent analysis. The male adults of *T. absoluta* were collected by using pheromone traps, and the larvae of *T. absoluta* were obtained from the infested plants (Zhang et al. 2021). All specimens were preserved in 95% ethanol and stored at −20°C until DNA extraction.

2.2   DNA extraction, PCR and sequencing

Genomic DNA was extracted from the whole body of individuals using the DNeasy Blood and Tissue Kit (Qiagen, Duesseldorf, Germany) following the manufacturer's protocol. PCR amplification of the *mtCOI* fragment from the samples collected in 2017 was performed with the primer pair COIF1 (CCTGGTAAAATTAAAATATAAACTTC) and COIR1 (ATTCTACAAATCATAAAGATATTGG). However, the primer pair COIF2 (TGAGCAGGAATAGTAGGA) and COIR2 (AGTCTGAATAACGACGAG) was used for other samples. All PCRs were conducted in a total volume of 25 µL, including 2.5 µL of 10× reaction buffer, 0.5 µL of dNTPs (10 mM), 0.5 µL of *Taq* DNA polymerase (2.5 U µL^{-1}), 0.5 µL of each primer (10 µM), 1.0 µL of DNA and 19.5 µL of ddH$_2$O. PCRs were performed under the following conditions: 5 min at 94°C; 35 cycles of 30 s at 94°C,

45 s at 47°C (56°C for COIF2/R2) and 60 s at 72°C; and 10 min at 72°C. The PCR products were sequenced in both directions by Sangon Biotech Co., Ltd. (Shanghai, China). In addition, the fragments of other two widely used genes, *COII* and *ITS2*, were amplified from the samples collected after 2017 (197 individuals in total) (Table S1).

2.3   Analysis of haplotypes and networks

Sequence alignment and haplotype analysis were performed with MAFFT v7.490 and DnaSP 5.10.01, respectively. The sequences of the 320 *T. absoluta* individuals obtained in this study as well as those available in GenBank (including 706 sequences, Table S2) were included in the study. A 521-bp overlap fragment of *COI* was used for the subsequent analysis. Networks of the haplotypes were depicted by PopART v1.7 with a median-joining method. The sequences of *COI* haplotypes generated herein were deposited in GenBank (accessions OP050188-OP050197).

2.4   Phylogenetic analysis

To identify the relationships among separate haplotypes of *T. absoluta*, the sequences of haplotypes obtained in this study together with those available in GenBank were used for the phylogenetic analysis. The maximum likelihood (ML) phylogenetic tree was constructed using IQ-TREE under the HKY+G4+F model for 100,000 ultrafast bootstraps. *Phthorimaea operculella* (MF121882) and *Scrobipalpa disjectella* (MN805133) were used as the outgroups. An additional ML phylogeny of Gelechiidae was also inferred under the GTR+I+G4+F model to verify the relationship between the two haplogroups. And *Helicoverpa armigera* (accession MF051184) was used as the outgroup at this point.

2.5   Genetic diversity and genetic differentiation

For each population of *T. absoluta*, the number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), the average number of nucleotide differences (k), and the number of polymorphic sites (S) were calculated using DnaSP 5.10.01. To estimate genetic differentiation between populations, pairwise genetic distance (F_{ST}) and gene flow (Nm) were estimated in Arlequin 3.5 (Excoffier & Lischer 2010). In addition, hierarchical analyses of molecular variation (AMOVA) were carried out by separating populations into different ecoregion groups. The northwestern group included the populations of Xinjiang, Gansu, and Inner Mongolia. The southern group included the populations of Yunnan, Guizhou, Jiangxi, Guangxi, Hunan, Sichuan, and Chongqing. Two subgroups (i.e., Yunnan and other southern populations) were considered within the ecoregion of southern China because Yunnan, the most genetically diverse population, was the first invasion region of *T. absoluta* in southern China.

3   Results

3.1   Haplotype network and phylogenetic analysis

The sequences of the 320 *T. absoluta* individuals obtained in the present study as well as the 706 sequences available in GenBank were analyzed to construct a haplotype network. A total of 16 haplotypes were identified across all the sequences of *T. absoluta* collected worldwide. H1 was determined to be the most frequent haplotype (Figs. 1 and 2) and was shared by 1003 individuals (i.e., 97.8% of sequences). The other 15 haplotypes were represented by no more than four individuals (Fig. 1) and considered to be at low frequency. Ten haplotypes (named H1–H10) were detected among the 320 *T. absoluta* individuals collected in China (Fig. 2). Haplotypes H2 to H10 were not reported in individuals from other countries previously (the red and green branches in Fig. 2). Six haplotypes (i.e., H11–H16, the orange branches in Fig. 2) were reported in GenBank from individuals collected in countries other than China.

Two distinct clades (A and B) were identified within the monophyletic group of *T. absoluta* with a high bootstrap value (≥ 96), indicating that the node was well supported (Figs. 2 and S2). Clade A comprised 12 haplotypes (1019 sequences) and was widespread across the invaded areas. Clade B comprised five haplotypes (only seven sequences) and was found only in China (i.e., Yunnan, Guizhou and Hunan). The inferred network of *COI* haplotypes, as expected, was congruent with the ML tree: mutational steps separated the clade A and clade B haplogroups, revealing substantial divergence between them (Fig. 1). Most haplotypes within haplogroups differed by a single mutation, suggesting a population expansion event, especially for clade A (Fig. 1).

3.2   Genetic diversity

Although low genetic diversity has been observed in the populations of *T. absoluta* worldwide, some degree of genetic variation in its invaded areas of China has been found. The genetic diversity indices are summarized in Table 1. The *COI* sequences of 706 samples obtained from GenBank revealed only seven haplotypes. The global *COI* haplotype diversity (Hd) was 0.02, nucleotide diversity (π) was 0.00009, average number of nucleotide differences (k) was 0.045, and number of polymorphic sites (S) was 15. For the ten haplotypes detected in the 320 *T. absoluta* samples collected in China, the Hd, π, k and S values were 0.086, 0.00307, 1.599 and 38, respectively. The most common haplotype, H1, was shared by 306 individuals (i.e., 95.6% of samples) across all 11 populations in China. The relative frequency of H1 in each population ranged from 81.8% to 100% (Figs. 3 and 4). The other nine haplotypes were very rare, occurring in 0–18.2% of the samples in each population.
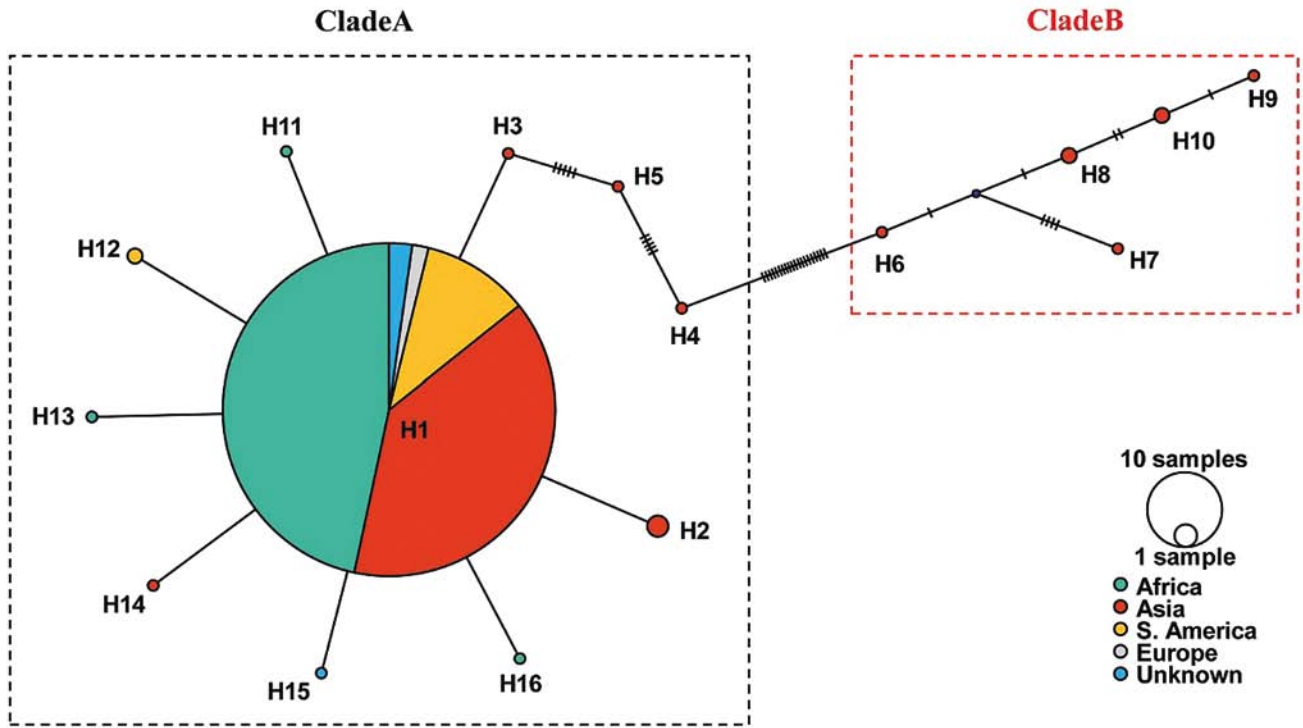
Fig. 1. Haplotype network of *Tuta absoluta* worldwide. The size of the circle is proportional to the frequency of the haplotype. The color of the circle indicates the origin of those sequences. Haplogroups are indicated by the colored squares (black, clade A; red, clade B). Notches between haplotypes indicate the speculative number of mutational steps between those haplotypes.
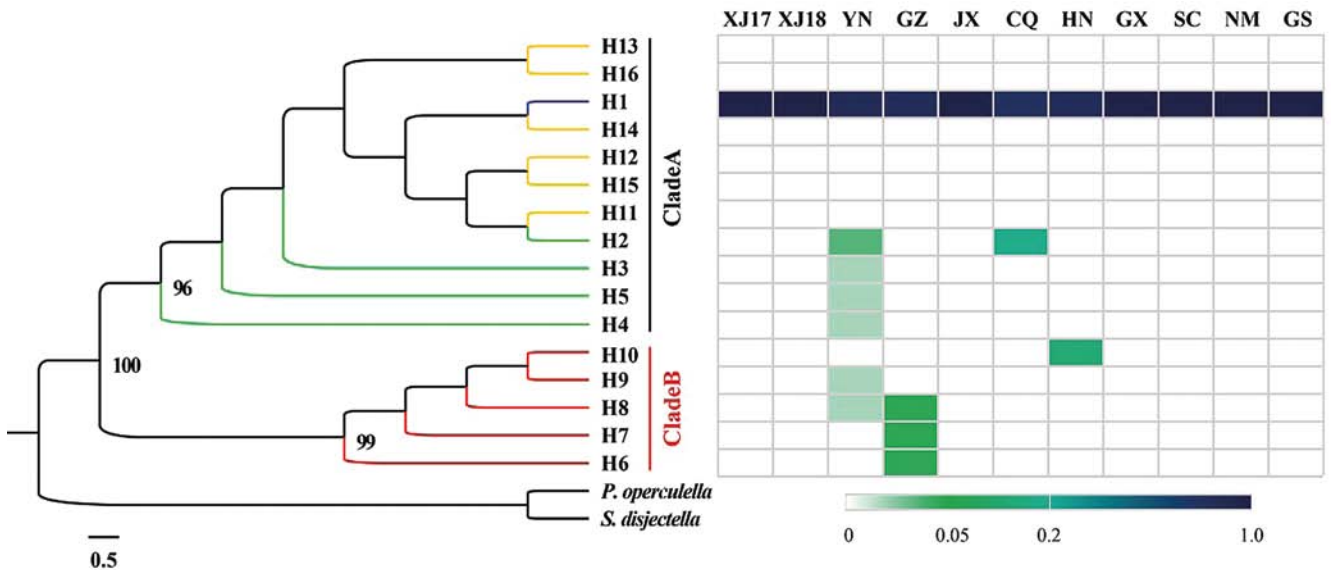
Fig. 2. Maximum likelihood (ML) phylogenetic tree and the positions of *Tuta absoluta* haplotypes. Colored branches indicate the location of the haplotypes (red, belonged to Clade B and only detected in China; green, belonged to Clade A and only detected in China; orange, only detected in other countries; blue, detected in China and other countries). Colored side bars indicate *COI* clades (black, clade A; red, clade B). *Phthorimaea operculella* (MF121882) and *Scrobipalpa disjectella* (MN805133) were used as the outgroups. The nodal numbers indicate the bootstrap values of clades A and B. The heatmap indicates the relative frequency of each haplotype at the 11 provincial/year-level populations in China. XJ17, the populations collected from Xinjiang in 2017; XJ18, the populations collected from Xinjiang in 2018; YN, Yunnan; GZ, Guizhou; JX, Jiangxi; CQ, Chongqing; HN, Hunan; GX, Guangxi; SC, Sichuan; NM, Inner Mongolia; GS, Gansu.

Table 1. Molecular diversity of *Tuta absoluta* populations. N, number of sequences; h, number of haplotypes; Hd, haplotype diversity; π, nucleotide diversity; S, number of polymorphic sites; k, average number of nucleotide differences; N/A, no diversity detected in this population. XJ17, the populations collected from Xinjiang in 2017; XJ18, the populations collected from Xinjiang in 2018; YN, Yunnan; GZ, Guizhou; JX, Jiangxi; CQ, Chongqing; HN, Hunan; GX, Guangxi; SC, Sichuan; NM, Inner Mongolia; GS, Gansu.

| Population ID	Province	*COI* clade	N	h	Hd	π (×10⁻²)	S	k
XJ17	Xinjiang	A	123	1	N/A	N/A	N/A	N/A
XJ18	Xinjiang	A	15	1	N/A	N/A	N/A	N/A
YN	Yunnan	A & B	56	7	0.236	0.617	38	3.212
GZ	Guizhou	A & B	21	4	0.271	1.525	33	7.943
JX	Jiangxi	A	14	1	N/A	N/A	N/A	N/A
CQ	Chongqing	A	11	2	0.327	0.188	3	0.982
HN	Hunan	A & B	14	2	0.264	1.772	35	9.231
GX	Guangxi	A	15	1	N/A	N/A	N/A	N/A
SC	Sichuan	A	21	1	N/A	N/A	N/A	N/A
NM	Inner Mongolia	A	15	1	N/A	N/A	N/A	N/A
GS	Gansu	A	15	1	N/A	N/A	N/A	N/A
NCBI		A	706	7	0.020	0.009	15	0.045
China		A & B	320	10	0.086	0.307	38	1.599
Clade A of China			313	5	0.044	0.037	14	0.191
Clade B of China			7	5	0.905	0.640	9	3.333

There was considerable variability in the genetic diversity indices between clades A and B, as shown in Table 1. Overall, clade B was recorded to have higher genetic diversity than clade A, including for the indices Hd (0.905 vs. 0.044), π (0.00640 vs. 0.00037), and k (3.333 vs. 0.191). Clear predominance of clade A was found across all populations in China (Figs. 3 and 4), with 97.8% of the samples belonging to this clade. In contrast, the five haplotypes of clade B were observed in only the southern ecoregion: Yunnan (3.57% of samples), Guizhou (14.29% of samples) and Hunan (14.29% of samples) (Figs. 2 and 3).

The sampled populations contained one to seven haplotypes, with an average of two haplotypes in each population (Table 1). A unique haplotype, H1, was found in the populations in Xinjiang, Jiangxi, Guangxi, Sichuan, Inner Mongolia, and Gansu (Figs. 2 and 3). However, seven haplotypes were recognized in samples from Yunnan, followed by Guizhou (four haplotypes), Chongqing (two haplotypes) and Hunan (two haplotypes), which indicated high genetic diversity (Table 1). In addition, interannual and ecological differences were recorded for the genetic diversity indices. Overall, no *COI* diversity was detected in the populations from the northwestern region (including Xinjiang, Inner Mongolia and Gansu) or the first invading population in China in 2017 (i.e., Xinjiang2017) (Table 1 and Fig. 3). In contrast, some degree of genetic variation (e.g., haplotypes H2-10) was found in the populations from the southern region (especially those mentioned above: Yunnan, Guizhou, Chongqing and Hunan), which did not invade before early 2018.

3.3   Population genetic differentiation

The pairwise F_{ST} values between populations ranged from −0.0552 to 0.5621 (with an average much lower at 0.0596) and indicated very limited genetic differentiation between these populations (Fig. 4). Comparatively, three populations from the southern regions (including Guizhou, Chongqing and Hunan) exhibited significant genetic differentiation from the first invading population (Xinjiang 2017), with F_{ST} estimates varying from 0.3365 to 0.5621 ($F_{ST} > 0.25$, $p < 0.05$). This genetic divergence is likely to occur due to genetic drift, confirmed by limited gene flow (Nm) between those populations (ranging between 0.1947 and 0.4929) (Table S3). Complete and expedited gene flow was observed between populations from Yunnan and all other populations (Table S3), with a lower average F_{ST} value of 0.0069 (Fig. 4).

In all seven cases (northwestern vs. southern, northwestern vs. Yunnan vs. other southern, northwestern vs. Yunnan, northwestern vs. other southern, Yunnan vs. other southern, Gansu and Inner Mongolia vs. southern, and Xinjiang vs. southern), distinct structuring among populations within regions (FSC, $p > 0.05$) was not observed, and the vast majority of variation (> 70%) occurred within populations (Table 2). Generally, consistent significant regional structure was detected between the northwestern group and southern group/subgroups (FCT, $p < 0.05$) and within populations (FST, $p < 0.05$) in those cases, including northwestern vs. southern, northwestern vs. Yunnan vs. other southern, northwestern vs. Yunnan, and northwestern vs. southern. A separate AMOVA for Xinjiang vs. southern also revealed
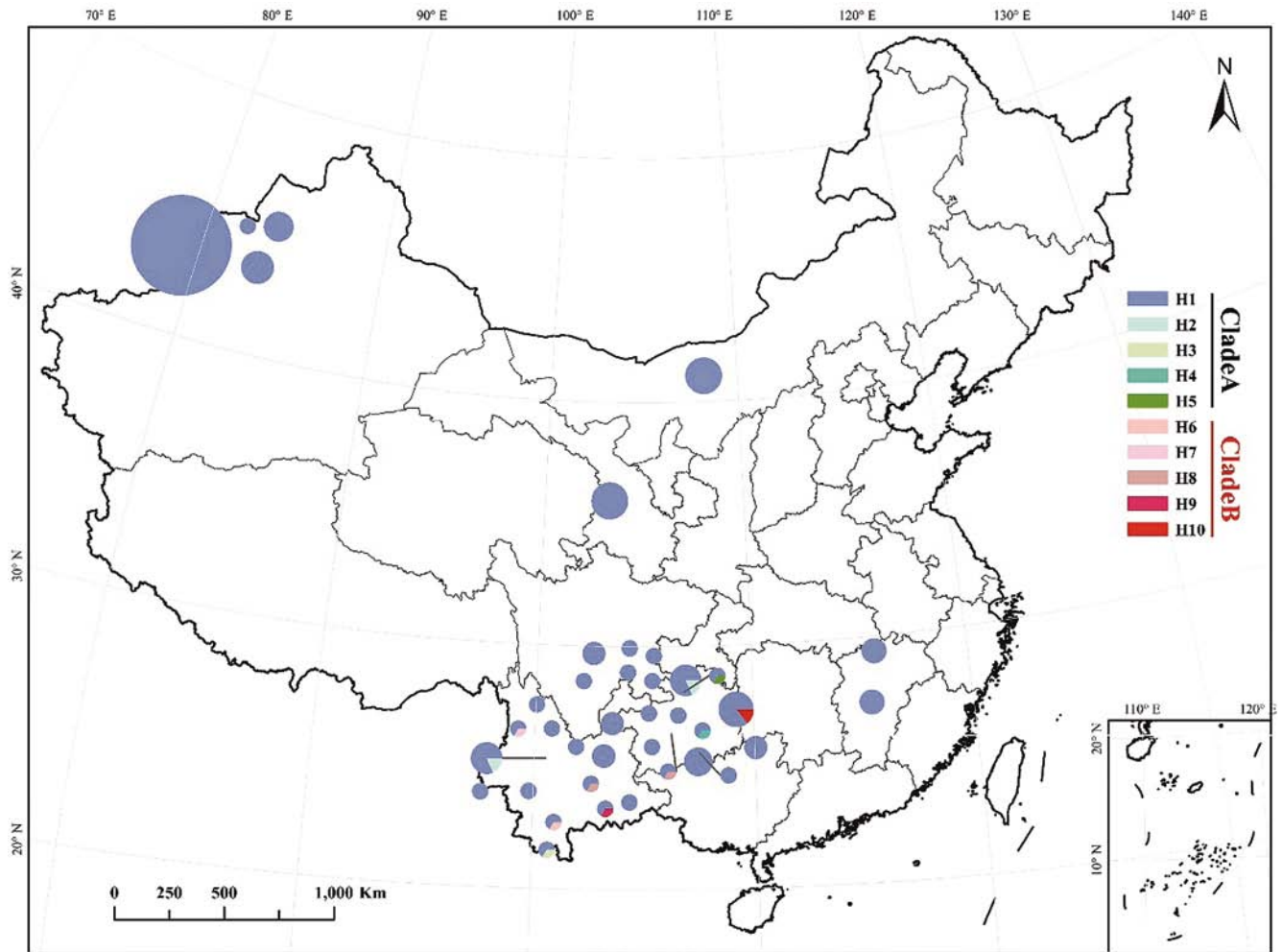
Fig. 3. The spatial distribution of the haplotypes of *Tuta absoluta*. Pie charts indicate the frequency of each haplotype in the prefectural/year-level populations. The size of the pie charts indicates the number of samples used.

significant regional structure (FCT = 0.0329, $p < 0.05$); however, Gansu and Inner Mongolia vs. southern showed the opposite result (FCT = −0.00180, $p > 0.05$). In addition, a separate AMOVA for the southern region revealed no significant genetic variation among groups (FCT = −0.00343, $p > 0.05$) or within populations (FST = 0.00085, $p > 0.05$). Taken collectively, these results indicate that the populations of *T. absoluta* from Xinjiang in the northwestern ecoregion were significantly differentiated from those in the southern ecoregion.

4　Discussion

This study constitutes the first attempt to understand the pattern of genetic variability in this pest as a consequence of its introduction and expansion in China. In fact, this study described the first recorded observation of this invasive pest

in Gansu and Inner Mongolia, indicating that the expansion of the pest in China is ongoing. This may be attributed to the rapid invasion of mainland China by *T. absoluta*. A similar phenomenon has been reported for other invasive insect pests, such as the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Arias et al. 2019).

Mitochondrial DNA (e. g., *COI* and *COII*) and nuclear loci (e. g., *ITS1* and *ITS2*) have been widely used to improve the understanding of the invasion source, genetic diversity, population genetic structure and demographic history of invasive insects. In this study, *COII* and *ITS2* genes have also been used to illustrate the relationship between Clade A and B. *ITS2* gene is a nuclear-encoded gene, and has been used for the genetic diversity analysis of *T. absoluta* in previous studies (Cifuentes et al. 2011; İnak et al. 2021). Like *COI* gene, *COII* is also a mixed region of highly conserved and variable fragments in mitochondrial genome, and particularly useful for evolutionary studies. Despite clustering on
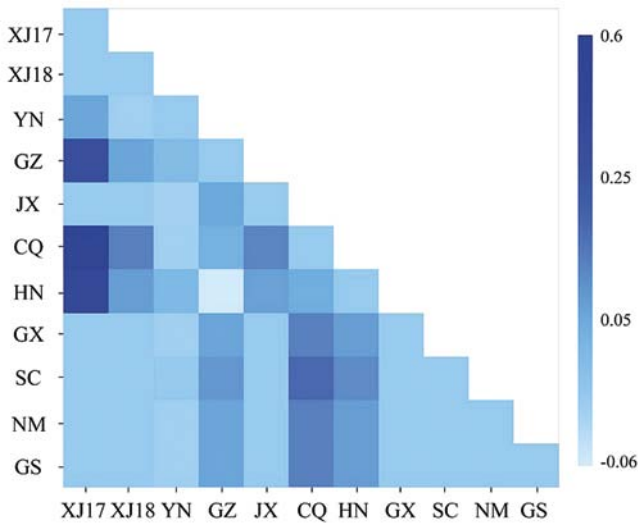
Fig. 4. Pairwise F_{ST} between different *Tuta absoluta* populations. XJ17, the populations collected from Xinjiang in 2017; XJ18, the populations collected from Xinjiang in 2018; YN, Yunnan; GZ, Guizhou; JX, Jiangxi; CQ, Chongqing; HN, Hunan; GX, Guangxi; SC, Sichuan; NM, Inner Mongolia; GS, Gansu.

one branch (Fig. S2), clade A and clade B showed a genetic distance (5.6% in terms of the *COI* gene) that was surprisingly high for an intraspecific comparison, but they could not be separated by the supplementary *COII* (Fig. S3) and *ITS2* (Fig. S4) markers. Sequences of *COII* or *ITS2* genes from different populations showed complete or almost complete homogeneity (Figs. S3 and S4). It is noticeable that the selection pressures on different gene sites may be discrepant. This phenomenon has already been found in *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Thus, the glasshouse strain and lupin strain of this pest also could not be defined by using *COII* and *ITS2* genes (Tian et al. 2018). Given that a threshold of 2%–3% DNA divergence in the *COI* gene has been recommended for invertebrate species delineation (Andrews et al. 2020), the high level of sequence divergence indicated the possible existence of cryptic species within *T. absoluta*. The same situation has already been found in other invasive insects, such as *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae) (Wang et al. 2019). Guillemaud et al. (2015) also detected distinct clusters among *T. absoluta* samples collected in South America using microsatellite markers. Due to unavailable sequences for related samples in GenBank, this study did not have sufficient evidence to characterize the genetic relationship between clades A and B. The genetic relationship between the two clades should be clarified with further morphological and genetic studies. Additionally, clade A was observed at an extremely high frequency worldwide (Figs. 2 and 3), whereas clade B was represented by five haplotypes and seven individuals exclusively in southern China (including

Yunnan, Guizhou and Hunan). Therefore, the clade B was difficult to detected based on no enough specimens of previous sequencing because of the extremely rare frequency. And of course, the contribution of new genetic variations of *T. absoluta*, which occurred in the later invasion areas that without specimens (e.g., Southeast Asia), could not be rule out. This speculation is discussed below.

In terms of genetic diversity, high genetic homogeneity was observed, with an almost uniform haplotype pattern present throughout the distinct geographic surroundings in the northwestern ecoregion (i.e., Xinjiang, Inner Mongolia, and Gansu) and parts of the southern ecoregion (i.e., Jiangxi, Guangxi, and Sichuan) (Table 1 and Fig. 3). At the global scale, this absence of genetic variability and genetic structuring is congruent with previous reports of *T. absoluta* populations from South America, Europe, the Mediterranean Basin, Africa and Asia (Cifuentes et al. 2011; Guillemaud et al. 2015; Carvalho et al. 2018; İnak et al. 2021) based on *COI* or *ITS* markers, which was verified by reanalysis of available data in the NCBI database (Table 1 and Fig. 1). The absence of genetic variability detected in the populations of *T. absoluta* is not unusual for invasive species. Analogous situations have been observed in other insect invasions, such as that of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Koca et al. 2022) and *Linepithema humile* Mayr (Hymenoptera: Formicidae) (Tsutsui et al. 2000). Introduced populations contain only a subset of the genetic variations of the native populations. They are often genetically impoverished or less variable than the native populations due to the limited number of founders and a bottleneck effect (Hawley et al. 2006). Occasionally, this could contribute to successful invasion (Tsutsui et al. 2000). Considering the low genetic diversity of *T. absoluta* over space, including native populations sampled in previous studies using *COI* markers, this hypothesis could not account for the results of the present study.

As expected, some degree of genetic variation (e.g., haplotypes H2–10, including clade B mentioned above) was present in the invasive populations collected from the southern regions in China (especially Yunnan, Guizhou, Chongqing and Hunan) and was further supported by the haplotype diversity, nucleotide diversity, average number of nucleotide differences and number of polymorphic sites (Table 1 and Fig. 3). Overall, genetic diversity estimates in China, especially in Yunnan, are far higher than those in other regions based on *COI* markers (Table 1). The high genetic diversity is similar to that reported for populations of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in newly invaded areas in South America (Gonçalves et al. 2019). Given the adjacent time points (only several months) in the first detection of *T. absoluta* (Zhang et al. 2020) and the long distance (~4,500 km) between the northwestern region (i.e., Xinjiang) and the southern region (i.e., Yunnan), a previous study speculated that this pest was probably introduced to China via two potential avenues of dispersal, from

Table 2. Analysis of molecular variance of *Tuta absoluta* according to ecoregion divisions. The northwestern group included the populations of Xinjiang, Gansu, and Inner Mongolia. The southern group included the populations of Yunnan, Guizhou, Jiangxi, Guangxi, Hunan, Sichuan, and Chongqing. * $P < 0.05$.

Ecoregions	Source of variation	Sum of squares	Variance components	Percentage of variation explained	Statistic
Northwestern vs. southern					
	Among groups	6.861	0.03236 Va	3.92	FCT: 0.03917*
	Among populations within groups	60.811	0.08985 Vb	10.88	FSC: 0.11320
	Within populations	187.242	0.70392 Vc	85.21	FST: 0.14793*
Northwestern vs. Yunnan vs. other southern					
	Among groups	8.165	0.02782 Va	3.39	FCT: 0.03389*
	Among populations within groups	59.508	0.08920 Vb	10.87	FSC: 0.11247
	Within populations	187.242	0.70392 Vc	85.75	FST: 0.14255*
Northwestern vs. Yunnan					
	Among groups	6.978	0.06817 Va	10.58	FCT: 0.10579*
	Among populations within groups	36.696	0.11265 Vb	17.48	FSC: 0.19551
	Within populations	89.000	0.46354 Vc	71.94	FST: 0.28062*
Northwest vs. other southern					
	Among groups	3.184	0.01927 Va	3.93	FCT: 0.03930*
	Among populations within groups	22.811	0.04015 Vb	8.19	FSC: 0.08523
	Within populations	98.242	0.43089 Vc	87.88	FST: 0.12119*
Yunnan vs. other southern					
	Among groups	1.303	−0.00574 Va	−0.34	FCT: −0.00343
	Among populations within groups	59.508	0.00716 Vb	0.43	FSC: 0.00427
	Within populations	187.242	1.67181 Vc	99.91	FST: 0.00085
Gansu and Inner Mongolia vs. southern					
	Among groups	2.170	−0.00253 Va	−0.18	FCT: −0.00180
	Among populations within groups	60.811	0.07147 Vb	5.08	FSC: 0.05073
	Within populations	187.242	1.33745 Vc	95.10	FST: 0.04902*
Xinjiang vs. southern					
	Among groups	6.225	0.02990 Va	3.29	FCT: 0.03295*
	Among populations within groups	60.811	0.09092 Vb	10.02	FSC: 0.10360
	Within populations	187.242	0.78673 Vc	86.69	FST: 0.13313*

Kazakhstan into neighboring Xinjiang and from Myanmar into neighboring Yunnan (Zhang et al. 2021). The analysis of population structure revealed weak but significant differentiation between the northwestern group (with the Xinjiang subgroup being especially evident) and southern group (FCT, $p < 0.05$) (Table 2), which confirmed the hypothesis of two different potential dispersal avenues of *T. absoluta*. This result is consistent with the highest comprehensive genetic diversity in Yunnan (Table 1). Yunnan had more private and novel haplotypes, yet it seemed to show more considerable directional gene flow than the other southern populations (Table S3 and Fig. 4). The other southern populations are genetically similar to the Yunnan population and from a nondifferential ecoregion group, which suggests coancestry.

Together, these analyses provide compelling evidence that the invasion events of southern China started in Yunnan, where *T. absoluta* was initially found.

As corroborated by recent surveys, the observed damage level in southern China significantly decreased with increased distance from the first discovery site of *T. absoluta* (Zhang et al. 2021). Thus, *T. absoluta* appears to represent a new case of the bridgehead effect, which was predicted to be common and has been broadly documented in the range expansion of invasive insects (Floerl et al. 2009). The global invasion history of the fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae) indicated that this pest established bridgehead populations in the southern United States, which were the source of later invasions of California, China, Australia, New Zealand and the Caribbean (Ascunce et al. 2011). The invasion of China by the western flower thrips *F. occidentalis* also followed the establishment of a bridgehead population in Yunnan (Yang et al. 2012). This notion corresponds to the fact that Yunnan is a center for the production, transportation and international import of tomatoes and vegetables in China. Indeed, the rapid dispersion of this pest could primarily occur through city-to-city agricultural trade, especially that of tomato fruits and vegetables (McNitt et al. 2019). This is probably also the case in China. The high gene flow over long distances should homogenize the populations of this pest within China, which is represented graphically by low genetic differentiation among populations. Consequently, Yunnan and Xinjiang are probably the ports of entry of *T. absoluta* in southern and northwestern China, respectively. This is especially likely for southern China, in which *T. absoluta* spread rapidly throughout Yunnan and into the neighboring provinces of Guizhou, Jiangxi, Guangxi, Sichuan, Chongqing and Hunan. Of course, given the lack of significant regional structure from the AMOVA of Gansu and Inner Mongolia vs. southern, the sources of these two populations are not restricted to Xinjiang. The truck transport of domestic and international markets increases the likelihood of large-scale dispersal of *T. absoluta* in southern China, which is far beyond the capability in northwestern China. Of specific interest, Myanmar, which exported large amounts of vegetables to Yunnan, was invaded by *T. absoluta* as early as 2016 and is suspected of acting as the source of this pest in southern China (Zhang et al. 2021). Given the short time span between the initial discovery of the invasion and the survey of this study, it must be true that some degree of genetic variation revealed in southern China could also be present in its source populations, namely, populations in Myanmar or other countries in Southeast Asia. This may also explain the genetic differentiation of these private mitochondrial haplotypes. In a global context, NCBI sequences of *T. absoluta* displayed very weak genetic structure, and there were no available specimens of the suspected source populations (i.e., Myanmar or other countries in Southeast Asia), so it was not possible to precisely infer the origin of genetic variations at a finer geographical scale. The results

are nonetheless intriguing, and further studies should be conducted with an extensive sampling scheme to clarify the evolutionary processes occurring in southern China and the ecological niche adaptation and biological attributes of the two aforementioned distinct haplogroups (clades A and B). This knowledge is crucial for understanding the impact of evolution on invasive species and the integrated control of this pest.

Tuta absoluta has rapidly expanded its spatial distribution in China since it was inadvertently introduced to Xinjiang in 2017. In this study, the first confirmed occurrence of this pest in Gansu and Inner Mongolia in China was described. The present study sheds light on the dynamic aspects of the invasion process and the patterns of genetic diversity and population structure soon after the introduction. Although high genetic homogeneity was noticeable throughout most populations worldwide, some degree of genetic variation and regional structuring of *T. absoluta* was revealed in southern China. Of specific interest, two distinct haplogroups of *T. absoluta* were detected, and clade A was predominant globally. As discussed throughout, these analyses provide compelling evidence that the successful invasions of *T. absoluta* in northwestern China and southern China started in Xinjiang and Yunnan, respectively, which were probably the bridgehead populations of *T. absoluta* in China. This is especially the case for southern China. This information offers new insights into the impact of evolution on invasive species and the dispersal pattern and integrated control of this pest. Strict quarantines, local eradication and sustainable management approaches are needed now more than ever to prevent the further spread of this cosmopolitan invasive pest. Future studies will be indispensable for filling the knowledge gap of the evolutionary processes occurring in southern China and the differences in ecological niche adaptation and biological attributes of the distinct haplogroups.

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The pdf version (Adobe JavaScript must be enabled) of this paper includes an electronic supplement:
Table S1, S2, S3, S4, S5, Figure S1, S2, S3, S4