Genetic Structure and Colonization History of the Fruit Fly *Bactrocera tau* (Diptera: Tephritidae) in China and Southeast Asia

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ABSTRACT *Bactrocera tau* (Walker), a major invasive pest worldwide, was first described in Fujian (China) in 1849 and has dispersed to tropical and subtropical Asia and the South Pacific region. Few data are available on its colonization history and expansion processes. This pilot study attempted to reconstruct the colonization history and pathways of this pest in China and neighboring Southeast Asian countries based on mitochondrial DNA. Results of the study showed six genetic groups corresponding to geographical characteristics, although the pattern was relatively weak. Homogeneous genetic patterns were observed within southern and central China, and northern Vietnam. Continuous colonization from the coast of southern China to inland regions of China and northern Vietnam was suggested. Strong genetic structure was observed in western China, Thailand, and Laos. The isolation of four of the six groups was most probably attributable to major topographical barriers of western China. Yunnan acted as a contact zone of *B. tau* in China and neighboring Southeast Asia. The absence of isolation by distance and the overall low phylogeographic structure of *B. tau* suggested that long distance dispersal events and human activities could play a major role in the colonization and expansion patterns of *B. tau*. By analyzing the genetic diversity, gene flow, haplotype phylogeny, and demographic history of 23 fly populations, we hypothesized that *B. tau* could have been introduced long ago in southern China, from which it further expanded or that southern China could correspond to the native range of this species.

KEY WORDS gene flow, dispersal, migration, mitochondrial DNA, biological invasion

Biological invasions cause serious threats to human economy, agriculture, health, and natural environment (Bai et al. 2012). Reconstructing colonization history of invasive species, i.e., revealing their origin and genetic composition, understanding the invasion pathways and history, and inferring expansion routes (Wan et al. 2012), are helpful for planning quarantine and control strategies.

Molecular genetics provides powerful tools for invasion studies (Estoup and Guillemaud 2010, Lombaert et al. 2010). Mitochondrial DNA (mtDNA) is a very popular molecular marker, in particular for phylogeography (Wan et al. 2011). Features such as nonrecombinant, high copy numbers, uniparentally inherited, and a lower effective population size when compared with nuclear markers (Wan et al. 2012) make mtDNA more sensitive to the loss of mutation–drift equilibrium and to shifts from neutrality. It has been often used in analyses of earlier phylogeographic events. Even though allelic-based markers are increasingly used to infer invasion pathways and colonization history (Lombaert et al. 2010), mtDNA is still successfully used to study invasive species (Rollins et al. 2011).

Bactrocera tau (Walker) is a worldwide invasive pest belonging to the Tephritidae family (Huang et al. 2005). It has been listed as a quarantine species in many regions and countries (e.g., Japan, the United States, Indonesia, Pakistan; Ohno et al. 2008, Hasyim et al. 2008, Huque 2006). *B. tau* is highly polyphagous and can attack >80 host plants, mostly from the Cucurbitaceae family (Christenson and Foote 1960). It causes damage on many economic cultivated species, such as cucumber, sweet melon, sweet pepper, pumpkin, sponge cucumber, wax apple, and tomato (Wang et al. 2006). Like other Tephritidae fly species, *B. tau* lays eggs under the skin of host fruits, and the larvae subsequently feed in the decaying flesh of melons and fruits (Hasyim et al. 2008).

B. tau was first described by Walker (1849) from the Fujian province in southeastern China. This pest was then reported from the Yunnan and Guangdong provinces around 1912, and from Sichuan in 1934. It has since then spread in most regions of southern, southeastern, and southwestern China (Zhao 1996). In the past decades, *B. tau* has expanded northward in China, where it was sporadically mentioned in Shanxi and Shaanxi, two northern Chinese provinces (Wang et al. 1994, Yang et al. 1994). Currently, *B. tau* is known

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Table 1. Indices of genetic diversity for the 23 studied *B. tau* populations

Country, Province	Sample site	Population code	Coordinates	π	Haplotype no.	Private haplotype	Haplotype distribution
China, Yunnan	Kunming	KM	25° 01′ N, 102° 41′ E	0.0094	3	0	H1 (11), H2 (5), H3 (4)
China, Yunnan	Ruili	RL	24° 01′ N, 97° 51′ E	0.0072	3	0	H1 (6), H4 (6), H5 (8)
China, Yunnan	Hekou	HK	22° 30′ N, 103° 57′ E	0.0092	3	0	H2 (6), H6 (7), H7 (7)
China, Yunnan	Wenshan	WS	23° 23′ N, 104° 15′ E	0.0083	3	0	H2 (8), H3 (2), H8 (10)
China, Guizhou	Jianhe	GZ	26° 20′ N,108° 17′ E	0.0087	3	0	H9 (8), H10 (6), H11 (6)
China, Guangxi	Nanning	GX	22° 47′ N, 108° 21′ E	0.0143	4	1	H9 (5), H10 (4), H11 (6), H13 (5)
China, Chongqing	Wulong	CQ	29° 29′ N, 108° 72′ E	0.0083	5	0	H2 (2), H8 (3), H14 (3), H15 (5), H16 (7)
China, Sichuan	Chengdu	SC	30° 67′ N, 104° 06′ E	0.0081	4	0	H2 (6), H14 (4), H15 (4), H16 (6)
China, Henan	Zhengzhou	EN	34° 44′ N, 113° 42′ E	0.0085	4	0	H3 (5), H8 (2), H10 (6), H17 (7)
China, Fujian	Fuzhou	FJ	26° 05′ N, 119° 18′ E	0.0153	9	3	H3 (2), H8 (1), H9 (2), H10 (5), H11 (2), H18 (2), H19 (2), H20 (3), H21 (1)
China, Guangdong	Guangzhou	GD	23° 06′ N, 113° 15′ E	0.0109	5	1	H9 (3), H10 (7), H11 (4), H18 (3), H22 (3)
China, Hubei	Xiangyang	HB	31° 14′ N, 110° 45′ E	0.0042	3	0	H8 (7), H10 (7), H17 (6)
China, Hunan	Zhuzhou	HN	26° 46′ N, 113° 45′ E	0.0054	4	1	H8 (6), H10 (4), H17 (5), H23 (5)
China, Jiangxi	Nanchang	JX	28° 13′ N, 115° 29′ E	0.0121	5	0	H3 (6), H9 (3), H10 (3), H11 (6), H18 (2)
China, Zhejiang	Hangzhou	HZ	30° 03′ N, 120° 02′ E	0.0100	4	0	H9 (6), H10 (6), H11 (4), H18 (4)
China, Hainan	Haikou	NN	18° 25′ N, 109° 05′ E	0.0045	3	0	H8 (3), H10 (12), H11 (5)
Myanmar	Bagan	MB	21° 10′ N, 94° 53′ E	0.0088	5	0	H4 (4), H5 (4), H24 (4), H25 (4), H26 (4)
Myanmar	Mandala	MM	21° 58′ N, 96° 04′ E	0.0079	5	1	H4 (6), H12 (2), H24 (4), H25 (3), H26 (5)
Vietnam	Tuyên Quang	VT	21° 21′ N, 105° 22′ E	0.0092	5	0	H9 (8), H10 (2), H18 (4), H27 (3), H28 (3)
Vietnam	Phú Thọ	VU	20° 55′ N, 104° 50′ E	0.0070	4	0	H9 (7), H18 (5), H27 (3), H28 (5)
Laos	Luang Prabang	LL	19° 53′ N, 102° 09′ E	0.0054	3	0	H6 (8), H7 (6), H29 (6)
Thailand	ChiangMai	TC	18° 47′ N, 98° 59′ E	0.0117	6	2	H1 (5), H30 (4), H31 (3), H32 (3), H33 (3), H34 (2)
Thailand	Bangkok	TB	13° 45′ N, 100° 30′ E	0.0120	4	0	H3 (6), H30 (6), H31 (3), H34 (5)

throughout tropical and subtropical Asia and the South Pacific region (Singh et al. 2010); however, the evolutionary history of different populations is still unclear because historical data are scarce. The dates when *B. tau* was reported from a given region do not necessarily correspond to the chronology of the invasion because the fly may have remained undetected until it caused noticeable damage. Most of the molecular genetic studies concerning *B. tau* focused on the molecular characterization of the species complex it belongs to (Baimai et al. 2000, Sujinda and Urusa 2003, Saelee et al. 2006, Wang et al. 2009, Kitthawee and Dujardin 2010) and on phylogeny and evolutionary history of the *Bactrocera* genus (Jamnongluk et al. 2003, Wang 2007). The population genetic structure and diversity patterns of *B. tau* at a regional scale have not been studied so far to decipher its recent population history.

In this study, we collected samples of *B. tau* over its distribution range in mainland China and southeast Asian countries and sequenced one mitochondrial gene to 1) determine the patterns of genetic structure and spatial gene diversity of the sampled populations, and 2) reconstruct the colonization history of the fly in mainland China. The expected results will provide important information for control and quarantine of this pest.

Materials and Methods

Samples Collection. Samples of *B. tau* were collected from ripened melons or from traps baited with cue-lure (i.e., 4-(p-acetoxyphenyl)-2-butanone) in 23 sites covering 13 provinces of China and 4 Southeast Asian countries (namely Myanmar, Thailand, Vietnam, and Laos) between 2009 and 2011 (Table 1; Fig. 1). All samples were obtained as adults and preserved in 95% ethanol at 4°C.

DNA Extraction and Sequences. Total DNA was extracted from 20 individuals per sampling site using the commercial tissue/cell DNA Mini Kit (Watson Biotechnologies, Shanghai, China). The DNA extracted from each individual was eluted in 60 μl of Tris-EDTA and stored at −20°C.

Polymerase Chain Reaction (PCR) was used to amplify 574 bp of the first subunit of the mitochondrial NADH dehydrogenase gene (ND1) using the primers ND1-F (5′-TTTAGTTGCTTGGTTGTGTATTCC-3′) and ND1-R (5′-GAAAAAGGTAAAAAACTCTTTCA AGC-3′; Nardi et al. 2005). PCR was performed in 20-μl reactions and carried out for 35 cycles of 30 s at 96°C, 30 s at 58°C, and 30 s at 72°C, with an initial denaturation step of 5 min at 95°C and a final extension of 7 min at 72°C. Finally, PCR products were purified and sequenced by GeneCore Biotechnology Co. (Shanghai, China) on both strands using PCR primers.

Data Analysis. All obtained sequences were aligned manually using ClustalX as implemented in BIOEDIT 7.0 (Hall 2004). Spatial analysis of molecular variance (SAMOVA) was performed using SAMOVA 1.0 (Dupanloup et al. 2002) to identify groups of populations that were phylogeographically homogeneous and maximally differentiated from each other, taking into account the geographic distances. This analysis permits the identification of maximally differentiated groups that correspond to predefined genetic barriers by maximizing the proportion of total genetic variance due to differences between groups (Crawford 2007). To select the optimal number of groups (K), two criteria must be considered. First, F_{CT} values should reach a maximum or a plateau. Second, the configurations where at least one group comprises only one population should be excluded because this indicates that the group structure is disappearing (Magri et al. 2006). The most supported number of groups was

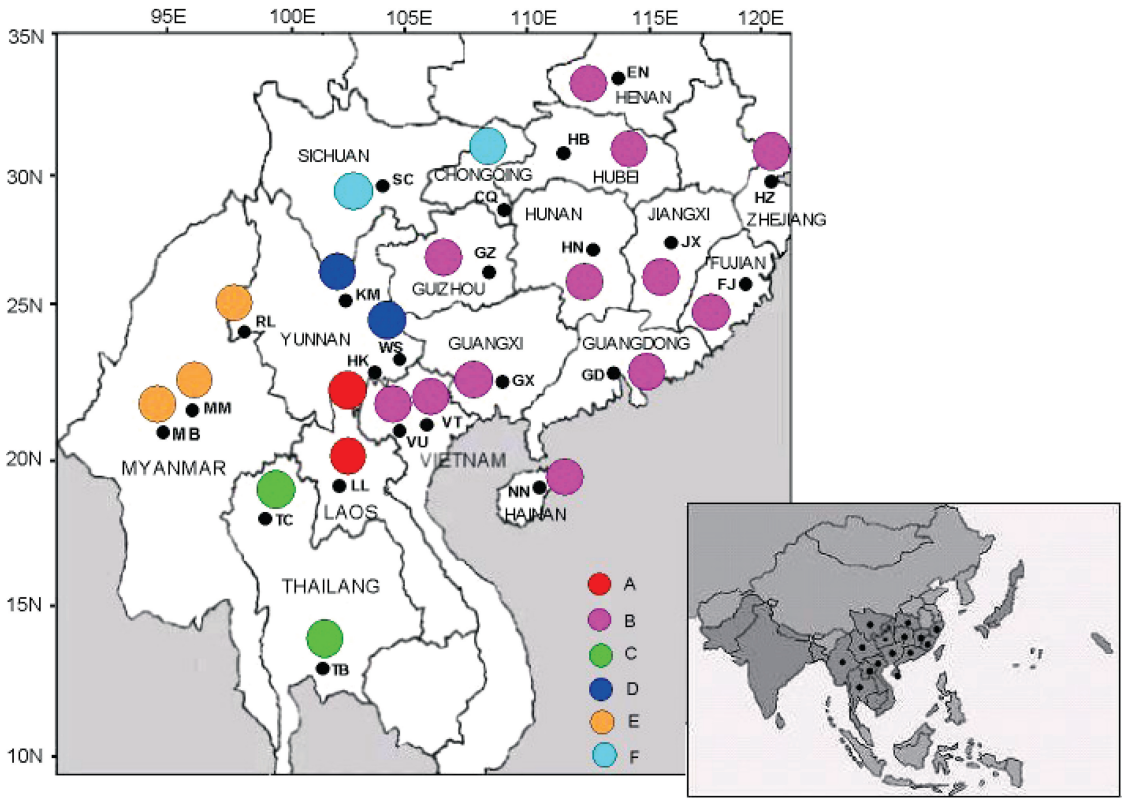

Fig. 1. Sampling sites (codes for localities are given in Table 1) and geographical distribution of the six groups (K = 6) identified by the SAMOVA (see text for details). The map in the low left corner is the known distribution range of *B. tau* in Asia (in dark gray), where ● shows the countries and provinces where we sampled the pest during the current study.

determined by repeating the analysis with K (the number of subpopulations) ranging from 2 to 10 (5 simulations repeated for each K) and selecting the subdivision scheme associated with the highest F_{CT}. Analyses of molecular variance (AMOVA) (Excoffier et al. 1992) were then performed to test the genetic relationships between the different groups defined by the SAMOVA.

Genealogical relationships between mitochondrial haplotypes were reconstructed using TCS 1.21 (Clement et al. 2000) with the method described by Templeton et al. (1992). A population phylogenetic tree was constructed using the neighbor-joining method in PHYLIP (Felsenstein 2005) based on average genetic distances between populations (Kimura 2-parameter, K2P). A population of 20 individuals of *Bactrocera dorsalis* (Hendel) (GenBank accession numbers JF521167–JF521176, mitochondrial ND1 gene), which is phylogenetically close to *B. tau* (Jamnongluk et al. 2003), was used as an outgroup.

Numbers and distribution of haplotypes per locality, numbers of private haplotypes, polymorphic sites, and nucleotide diversity (π) were assessed using ARLEQUIN 3.5 (Excoffier and Lischer 2010). The three diversity indices were compared among the identified SAMOVA groups (see results) using the Mann–Whitney *U* test (Mendenhall and Beaver 1991) for the most plausible partitioning (K = 6, see Fig. 1). ARLEQUIN 3.5 was also used to calculate pairwise F_{ST} values to measure the genetic differentiation among *B. tau* populations. To detect isolation by distance, the matrix of pairwise estimates of genetic differentiation (F_{ST} values) was compared with the matrix of geographic distances by means of a simple Mantel test (Legendre and Legendre 1998). The Mantel test was also performed using ARLEQUIN.

The extent of gene flow between population pairs was tested by the coalescent-based strategy implemented in MIGRATE 3.2.17 (Beerli 2006). This strategy tests for the existence of asymmetrical gene flow between populations. The mutation scaled effective immigration rate ($M = m/\mu$) ingoing and outgoing per population and per generation was estimated by applying the Bayesian search strategy. We set one long chain of 100,000,000 generations with the initial 10,000 excluded as burn-in.

To study the demographic history of the groups of populations identified previously, we studied mismatch distributions (Rogers and Harpending 1992). We calculated Tajima's *D* and Fu's F_S, which can be used to detect past expansions, for the six identified SAMOVA groups (see Fig. 1) and each population as well as in the whole data set using ARLEQUIN.

Results

Sequence Characteristics. Partial sequences of the mitochondrial ND1 gene were obtained for 460 individuals of *B. tau* from the 23 sampled populations. The total length of the aligned sequences was 549 bp and no insertions or deletions were observed. As nuclear copies of mitochondrial genes can generate doubtful results, we double-checked all obtained chromatograms to ensure that double peaks did not occur and that the haplotypes were functional coding genes. Of the 549 characters, 45 were polymorphic, including 35 parsimony informative sites. Thirty-six transitions and nine transversions were found. In total, 34 unique haplotypes were detected (GenBank accession numbers KN450034–KN450067) in all studied populations and their geographical distribution is given in Table 1.

Population Structure. Table 2 lists all mitochondrial pairwise F_{ST} values of the 23 *B. tau* populations, which ranged from 0.02 (SC–CQ) to 0.39 (EN–MM and HB–VT). The Mantel test based on the whole data set showed that the correlation between geographic distances and pairwise F_{ST} was not significant (r_M = 0.244; P = 0.157).

SAMOVA was performed to identify groups of populations. The F_{CT} value was highest for K = 6; in this case, each of the six groups, named A, B, C, D, E and F (Table 3), contained at least two populations. The six groups were geographically consistent and corresponded to regions (Fig. 1). The AMOVA performed using this particular grouping of populations revealed that only 10.31% molecular variance was among populations within groups. Still, 28.49% of total variance was found among groups, and this partitioning was significant ($P < 0.001$). Most of the molecular variance was actually found within populations (61.20%; $P <$ 0.001). The Mantel tests done within each of the six SAMOVA groups showed that correlation between geographic distances and F_{ST} was significant only in the B group (r_M = 0.2287; P = 0.04). When we considered the SAMOVA results for K = 7 (because the NJ tree revealed 7 clusters, see below, even though the F_{CT} value was slightly lower), the main difference with K = 6 was that group B was split in two, with B1 located along the coast of the South China Sea (i.e., VT + VU + FJ + GD + GX + JX + HZ) and B2 composed of the most inland sites and the island of Hainan (i.e., HB + HN + NN + EN + GZ).

An NJ tree (Fig. 2) was constructed based on average mitochondrial genetic distances (K2P) between populations using *B. dorsalis* as an outgroup. Seven clades could be identified, which were similar to the SAMOVA groups identified for K = 7 (group B was also divided into B1 and B2), except that population GZ fell close to group F (CQ + SC), and that two populations were placed in group B2 rather than B1 (HZ and JX). Group B1 was the sister group of all other *B. tau* populations, and was placed close to the outgroup.

The parsimony network obtained for the 34 mitochondrial haplotypes is shown in Fig. 3. Forty-eight missing haplotypes were detected. The median joining network showed limited structure and no phylogenetic haplogroup could be identified. Moreover, the haplotypes present in any given locality or region were not phylogenetically related, but scattered over the whole network. Most haplotypes were found exclusively in one of the SAMOVA groups, except H1, H2, H3, and H8.

Genetic Diversity. We used three indices, namely, π, number of haplotypes, and number of private haplotypes, to measure genetic diversity within populations (Table 1). Nucleotide diversity (π) ranged from 0.004 to 0.018, haplotype numbers from three to nine and the numbers of private haplotypes was three at most. Among the 23 studied *B. tau* populations, the three indices (π, haplotype number, and the number of private haplotypes) were slightly highest in the FJ population. The three values were also high in three other Chinese populations (JX, GX, and GD) and two Thai populations (TC and TB). Conversely, they were low in HB and in NN. Mann–Whitney *U* tests between SAMOVA groups showed that π was significantly higher in SAMOVA group B than in groups C, D, E, and F. Concerning the numbers of haplotypes, no significant difference was found among the six SAMOVA groups, but the number of private haplotypes was significant higher in group B than in D, F, and A.

Migration Rate Estimates. Table 4 shows the values of mutation-scaled immigration rate in both directions estimated using MIGRATE. Levels of migration rate ranged from 33.2 (from CQ to GD) to 632.2 (from FJ to HZ). Preferential migration values (i.e., value >200 in one direction only) were found from FJ to 12 populations in China, including sites belonging to groups A, D, and F and the two populations from Vietnam. No preferential migration was observed from FJ to other populations of Southeast Asia. The two populations from Myanmar showed asymmetric migration toward western China (RL), while populations from Thailand (TB and TC) and Laos (LL) had preferential migration values toward southern Yunnan (HK and WS).

Demographic History. Neutrality tests (Tajima's *D* and Fu's *Fs*) were first performed for each of the six groups identified by the SAMOVA. The two indices were significantly different from zero in the group B (Table 3), indicating that this group did not fit a simple model of neutral evolution. Neutral evolution could not be rejected for the other five SAMOVA groups. Consistently, mismatch distributions were compatible with the sudden expansion model for the group B only, with P_{SSD} = 0.2. In all other groups, and in the whole data set, this model was rejected.

The same analysis was then performed for each population. Significant negative Tajima's *D* and Fu's *Fs* were found in five localities, namely EN (Tajima's D = −1.94; Fu's Fs = −14.66), HZ (D = −1.94; Fs = −16.73), VT (D = −2.28; Fs = −22.9), VU (D = −2.35; F = −15.12), and HB (D = −1.98; Fs = −23.77). These populations, which all belonged to group B, therefore did not fit the model of neutral evolution. No significant negative values were found in the whole data set

Table 2. Pairwise F_{ST} values of 23 *B. tau* populations

Population	KM	RL	HK	WS	GZ	GX	CQ	SC	EN	FJ	GD	HB	HN	JX	HZ	NN	MB	MM	VT	VU	LL	TC	TB
KM																							
RL	0.13																						
HK	0.20	0.18																					
WS	0.22	0.16	0.23																				
GZ	0.30	0.27	0.37	0.26																			
GX	0.30	0.23	0.21	0.27	0.14																		
CQ	0.27	0.27	0.14	0.22	0.15	0.19																	
SC	0.26	0.31	0.15	0.20	0.12	0.26	0.02ns																
EN	0.30	0.26	0.24	0.24	0.24	0.18	0.24	0.29															
FJ	0.27	0.25	0.24	0.21	0.03ns	0.09ns	0.23	0.29	0.12														
GD	0.32	0.26	0.26	0.28	0.04ns	0.14	0.36	0.32	0.25	0.07ns													
HB	0.31	0.21	0.26	0.25	0.14	0.20	0.35	0.25	0.08ns	0.08ns	0.25												
HN	0.30	0.28	0.21	0.14	0.15	0.17	0.27	0.31	0.14	0.08ns	0.26	0.02ns											
JX	0.24	0.29	0.23	0.17	0.06ns	0.13	0.21	0.25	0.09ns	0.01ns	0.10	0.04ns	0.21										
HZ	0.31	0.28	0.28	0.19	0.03	0.14	0.37	0.33	0.27	0.07ns	0.04ns	0.28	0.28	0.09ns									
NN	0.30	0.30	0.24	0.14	0.17	0.17	0.34	0.31	0.14	0.06ns	0.22	0.06ns	0.08ns	0.14	0.24								
MB	0.18	0.03ns	0.22	0.23	0.24	0.34	0.31	0.30	0.32	0.32	0.34	0.32	0.31	0.35	0.31	0.30							
MM	0.18	0.07ns	0.23	0.19	0.21	0.32	0.38	0.32	0.39	0.29	0.31	0.34	0.32	0.32	0.32	0.32	0.06ns						
VT	0.31	0.33	0.27	0.27	0.16	0.13	0.37	0.33	0.33	0.32	0.19	0.39	0.38	0.31	0.15	0.38	0.31	0.35					
VU	0.32	0.28	0.23	0.22	0.15	0.16	0.33	0.30	0.31	0.39	0.26	0.37	0.30	0.38	0.23	0.36	0.35	0.33	0.07ns				
LL	0.30	0.27	0.09ns	0.2	0.19	0.28	0.31	0.31	0.31	0.35	0.32	0.30	0.31	0.38	0.31	0.31	0.33	0.30	0.37	0.32			
TC	0.17	0.24	0.11	0.24	0.16	0.09ns	0.14	0.24	0.16	0.18	0.19	0.16	0.10	0.12	0.19	0.12	0.33	0.20	0.32	0.31	0.22		
TB	0.26	0.33	0.13	0.23	0.22	0.13	0.10	0.16	0.15	0.15	0.22	0.29	0.24	0.08	0.22	0.27	0.35	0.36	0.38	0.30	0.30	0.09ns	

Nonsignificant estimates are followed by "ns."

Table 3. Results of AMOVA and the neutrality tests for the six SAMOVA groups

Groups	Group names	Group index					
		Tajima's *D*	Fu's F_S	SSD	Source of variation	Percentage of variation	Fixation indices
1. LL + HK	A	1.6438	4.1972	0.0856**	Among groups	28.49	F_{CT} = 0.284**
2. EN + FJ + HN + JX + GZ + HZ + HB + GD + GX + NN + VU + VT	B	−0.0118*	−24.457**	0.0118			
3. TC + TB	C	0.892	3.2339	0.0363**	Among populations within groups	10.31	F_{SC} = 0.388**
4. KM + WS	D	2.7334	5.2231	0.1386**			
5. MB + MM + RL	E	2.6445	0.582	0.0523**	Within populations	61.2	F_{ST} = 0.353**
6. CQ + SC	F	2.3691	1.6135	0.0917**			

**, $P < 0.001$.

(Tajima's $D = -0.974$, $P_{\text{Tajima's } D} = 0.156$; $Fs = -3.78$, $P_{Fs} = 0.252$).

Discussion

In this study, using mtDNA, we inferred for the first time the genetic structure of the pest *B. tau*, and we analyzed its colonization history based on a large-scale sampling.

Patterns of Population Genetic Structure of *B. tau* in China and Southeastern Asia Countries. The ranges of genetic diversity indices found in the current study (π, numbers of haplotypes, and private haplotypes) were in line with previous results found in another *Bactrocera* pest in the same region (Shi et al. 2012). The results of the SAMOVA and of the phylogenetic NJ tree both suggest the occurrence of genetic clusters corresponding to a geographic pattern among the 23 studied *B. tau* populations (Myanmar and western Yunnan; Laos and southern Yunnan; central Yunnan; Thailand; southern China, central China and northern Vietnam; and southwestern China). It seemed that topography and environmental factors within the studied areas played an important role in shaping the genetic structure of *B. tau.* Some genetic structures could be attributed to geographic barriers, whereas others were associated with smooth topography.

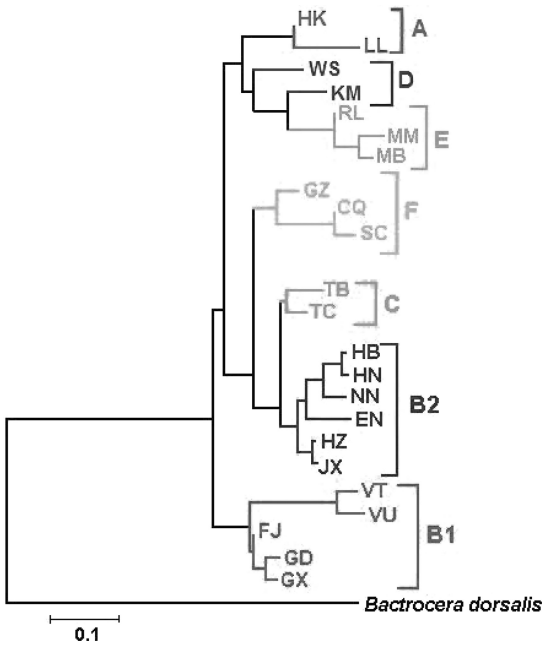

Fig. 2. NJ tree based on K2P distances between 23 populations and *B. dorsalis* as outgroup. The branches are labeled with colors corresponding to the SAMOVA groups defined by K = 7.

The strong genetic structure found in western China, Thailand, and Laos is the isolated case, which was most probably attributed to natural barriers such as mountains, rivers, or gorges. For example, the SAMOVA group E that clusters Myanmar (MM and MB) and Ruili (RL) is isolated from others by the Henduan mountains and a few big rivers (namely, Jingsha and Lancang rivers) of western Yunnan (Wang 2002). Another isolated cluster was formed by the populations of Hekou (HK) and Laos (LL), which are both located on a natural route created by south–north oriented mountains. These localities are isolated from the rest of the studied range by the Yuanjiang and Lancang rivers and by the Wuliang Mountain in western Yunnan (Wang 2002). The third isolated grouping Kunming (KM) and Wenshan (WS) was identified in eastern Yunnan. They occur on a relatively smooth plateau, and are separated from the rest of the studied region by Wumeng Mountains (average altitude 2,900 m) in the east and Henduan Mountains in the west (Fu 1994). According to these evidences, Yunnan appears to be a contact zone between several genetic groups identified in *B. tau*, which is consistent with its topography. A very similar geographical pattern of genetic structure was found for the congeneric *B. dorsalis* (Shi et al. 2010, 2012; Wan et al. 2012). Both fly species most probably experience the same barriers to gene flow that impacted their genetic structure in a similar way.

The last identified genetic cluster corresponds to the two northernmost localities, namely, Sichuan (SC) and Chongqing (CQ). They are both located in the Sichuan Basin and are surrounded by high mountains (>1,000 m in altitude) and plateaus, which probably prevented ongoing gene flow to or from southeast China.

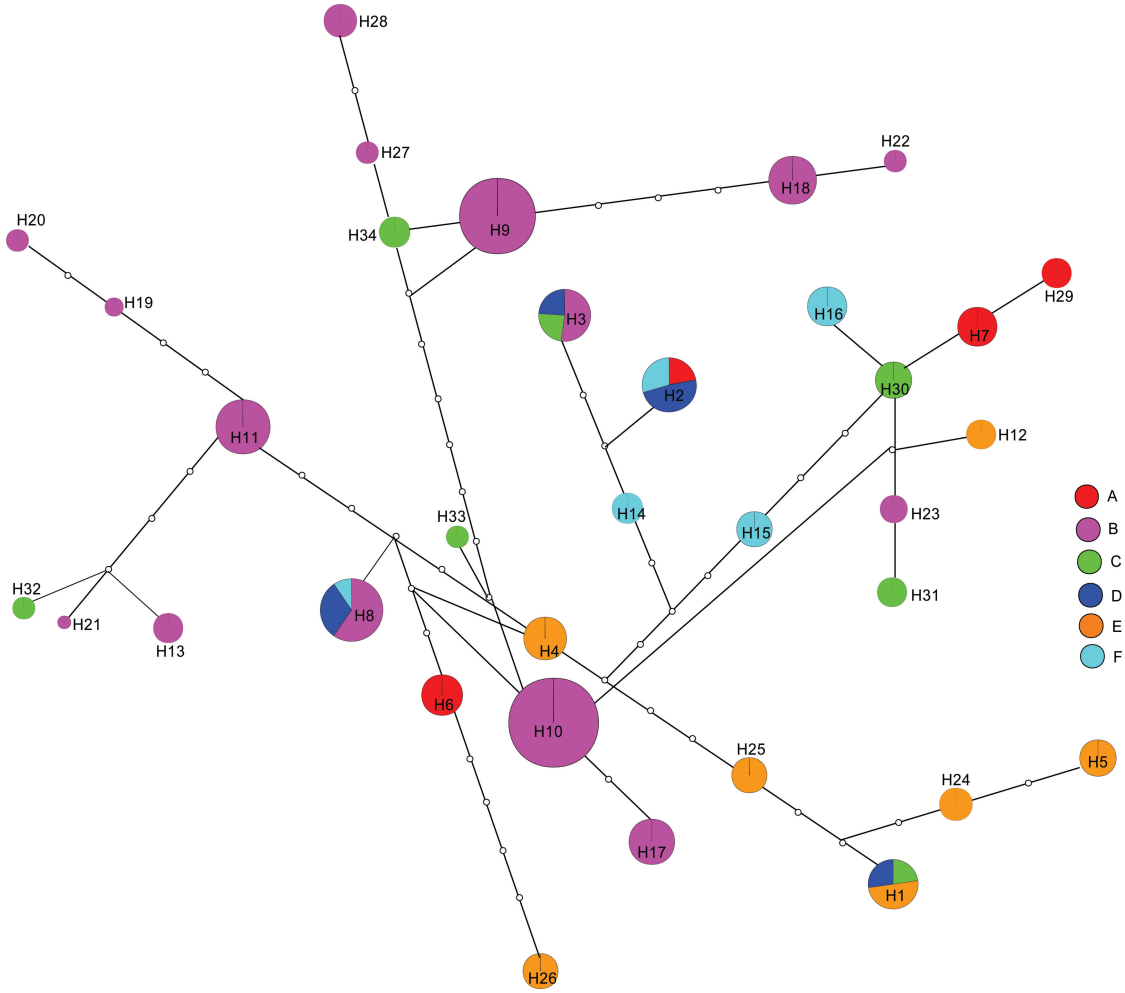

Fig. 3. Parsimony network of the 34 haplotypes identified in this study. Each pie shows the proportion of individuals belonging to each of the six SAMOVA groups.

The geographically largest group corresponded to southern and central China as well as northern Vietnam. Interestingly, in this region, the topography is smooth and there are wide-range cultivations of potential host plants, which probably facilitate the dispersal and the continuous expansion of *B. tau* in these areas. The data are fully consistent with a dispersal pattern from the coast of southern China to the inland regions of China and northern Vietnam. Such an expansion can be aided by the southeast monsoon originating from the Pacific Ocean (Fu 1994). Evidence of this dispersal pattern was well-illustrated by continuous gene flow from FJ to other Chinese populations and significant isolation by distance within group B.

Although population genetic pattern correlated with geographic pattern, this pattern is rather weak, with 28.49% of the molecular variance found among groups. As suggested above, the low phylogeographic structure suggests that human-induced movements can be an important mode of dispersion in *B. tau*, which would blur the natural genetic patterns. Human activities provide vectors for long-distance dispersion of the fly (air traffic, train networks, canals, etc.), thereby promoting introductions into remote areas and favoring spatial expansions (Estoup and Guillemaud 2010). Human-mediated expansion were also found in other Tephritidae flies, such as *B. dorsalis* (Shi et al. 2010, 2012), *B. cucurbitae* (Hu et al. 2008) or *Anastrepha fraterculus* (Wiedemann) (Alberti et al. 2008), for which a weak phylogeographical structure was also found.

The Colonization History and Plausible Expansion Pathways of *B. tau*. Except for the fact that *B. tau* was first described in Fujian province in China (Walker 1849), detailed historical information is lacking concerning the most plausible natural origin of this species. It may have been detected after a geographical or demographical expansion from its native range, after an introduction event, or it could be native from southeast China. The actual colonization history of this species is still unknown.

Table 4. Effective immigration rate between all sampled *B. tau* population pairs

Population	KM	RL	HK	WS	GZ	GX	CQ	SC	EN	FJ	GD	HB	HN	JX	HZ	NN	MB	MM	VT	VU	LL	TC	TB
KM		126.7	110.8	**476.6**	67.8	74.9	165.2	77.2	43.4	**295.7**	48.8	41.1	66.1	92.2	60.6	46.7	65.3	80.4	52.2	48.1	41.4	53	42.4
RL	72		50.9	**204.4**	67.4	61.3	99.1	73.6	56.7	96.1	62.4	47.4	67.4	61.5	62.3	69.5	**374.5**	**366.7**	43	54.4	44.6	54.7	68
HK	**299.4**	72.1		143.1	58	85.4	105.9	50.3	57.6	**220.3**	47.4	99.5	71.6	83.2	62.9	51.3	53.2	63.5	191	41.6	**283.2**	196.5	189.6
WS	**549.6**	73.8	**371.5**		65.6	55.3	69	93.7	74	70.4	64	55.5	45.8	81.8	75.9	49.4	50.6	76.7	163	39.3	196.2	**217.9**	**227.1**
GZ	87.1	65	67.5	56.2		**247.4**	142.1	142.4	85.4	**287.7**	61.4	50.5	55.2	82.1	42.1	54.1	46.7	45.3	70.2	47.1	52	42.8	94.3
GX	42.7	41.9	63.8	64.1	56.9		51	66.6	58	**211.6**	**216.3**	66.6	60.4	66.6	90.4	**266.2**	45.8	50.6	48.7	41.6	46.2	56.7	74.2
CQ	106.4	41.3	42.7	66.9	52.8	93.2		150.8	73.9	97.8	58.4	44.5	55.4	55.2	75.6	80.2	49.2	56.1	49.5	61.1	46.3	55.9	69.1
SC	143.2	60.8	34.4	57.7	66.3	48.7	**548.4**		45.7	**290.4**	53.7	54.5	93.8	54.2	78.9	82.5	51.7	59.4	44.7	41.7	43.3	72.9	83.9
EN	70.9	76.4	70.6	77.4	49.9	154.8	45	92.1		**354.5**	114.8	43.5	46.3	52.7	147.2	156.7	60.7	73.1	49.1	93.1	42.3	80.8	80.4
FJ	40.3	96	95.2	63.7	86	186.3	65.3	55.2	179.2		79.5	42.2	71.5	51.3	146.4	165.8	68.7	66.7	67.1	185.3	57.1	50.4	68.1
GD	66.7	72.3	40	47.7	68.8	176.1	33.2	43.9	140.7	**377.4**		58.7	62.1	48.2	178.8	**308.1**	60.8	41.5	57.1	174.6	63.1	103.7	83.9
HB	84.7	56.7	44.3	89.5	76.7	146.9	57.3	44.8	221	**240.4**	68		95.9	56.8	97.8	123.7	79	40.9	83.5	99.7	50.2	71.4	77.5
HN	85.4	43.9	88	66.6	89.3	151.9	69.6	51.2	**232.8**	**540.1**	193.3	70.5		48.4	63.3	199.6	88.3	40.1	60.6	75.8	69.3	73.9	68.1
JX	64.6	68.8	86.4	96.8	74.4	136.2	51.4	46.2	145.6	**555.2**	166.7	68.4	62.5		178	150.2	47.8	48.1	51.8	98.1	49.3	172.3	64.8
HZ	99.9	92.7	56.6	57.5	64.3	129.9	84.2	65.7	97.3	**632.2**	162.3	81.4	49.5	51.7		95.4	50.3	58.6	65.1	71.9	57.6	78.3	72.1
NN	68.8	43.6	90.3	54.5	47.7	98	39.7	49.2	49.3	**417.6**	194.4	57.1	51.9	75.1	149.9		41.9	50.6	94.7	129.4	57.5	99.9	73
MB	81	151.1	52.1	86.9	45	74.9	98.5	54.4	53.9	58.3	66.9	69.5	41.9	88.8	46.5	41.6		42.1	47.2	51.8	61.6	86.7	42.7
MM	89.6	188.2	88.4	98.6	41.4	49.3	41	57.8	45.8	45.4	48.8	47.4	40.7	41.3	52.4	60.8	34.8		68.1	55.1	50.4	74.8	48.4
VT	50.2	48.2	51.4	78	60.4	135.3	71.2	41.1	62.8	**370**	167.2	44.4	45.2	60.5	47.3	49	46.3	45.2		62.8	61.1	80.7	59.4
VU	57.9	50.5	47.4	58.4	84.9	114.4	61.3	57.7	58	**273.9**	94.4	35.8	58.9	58.8	51.7	66.1	71.5	75.5	60.4		47.8	75.6	68.6
LL	58.3	41.3	69.7	41	92.2	50.6	34.1	75.9	48.7	84.1	62.3	57.9	74.8	48.3	55.2	44.6	43.1	77.2	64.9	51.7		53.1	42.3
TC	42	42.5	54.1	72.4	47.8	92.7	44.6	68.9	55.4	112.6	46.9	47.4	69.5	97.2	49	50.1	44	48.4	51.7	45.9	41.9		**524.6**
TB	81.7	59.9	64.4	79.6	54.6	75.2	59.7	84.2	43.1	96.5	44.7	46.1	57.7	50.2	54.6	47.3	53.8	45.1	54.8	87.1	43.7	144.9	

Instances of asymmetrical gene flow are indicated in bold (values >200). The source populations is indicated in columns, the target populations in row.

Based on our results, southern Chinese populations (FJ, GD, and GX) could be considered as source populations that continuously colonize inland China and southern Vietnam. This pattern is evidenced by the observed preferential migration from Fujian to several, sometimes remote, Chinese provinces and to northern Vietnam (VT and VU). Moreover, signs of recent expansions were found in these southern populations (mismatch distributions and neutrality tests), and a high number of haplotypes were shared between Fujian and the above-mentioned populations.

Two hypotheses could then be suggested to explain the mitochondrial genetic patterns found in our study. One would be that southern China would correspond to the native range of *B. tau.* This would be consistent with the fact that the species was first reported and described from this region (Fujian province, 1894), and that populations from southern China lie at the root of the population phylogenetic tree. Higher diversity indices (numbers of haplotypes and unique haplotypes, and π) found in southern China (SAMOVA group B) could also be a sign of a local origin of the fly. Preferential gene flow from southern China to Vietnam and other regions of China suggest that eastward and northward expansions are still in process. The alternative hypothesis would be that *B. tau* was introduced in Fujian from a still unknown origin. After its discovery in 1894, *B. tau* would have colonized the central and northern regions of China. Such a colonization process would match the historical records, as the fly was reported in the different Chinese provinces well after its first mention in Fujian (in Yunnan after 1912, in Sichuan after 1934 [Zhao 1996], and in Shanxi after 1994 [Yang et al. 1994]). A rapid expansion from Fujian after the accidental introduction of the fly in this region could be explained by intense human activity, as Fujian is the oldest trading port in China, and that the exchanges with other countries date back to the 16th century at least (Wang 2009). Both hypotheses need to be tested by a range-wide study of this species, as samples from south Asia and the South Pacific region are still lacking. Including informative nuclear markers would also be useful to decipher the origin of *B. tau.*

However, a relatively high genetic diversity was observed in Thailand (TC and TB). However, these populations seem to be genetically isolated, and only exchange gene flow with the neighboring region of RuiLi (RL). We did not find any evidence of migration from Thai populations to other populations or vice versa (Table 4). Thai populations of *B. tau* could originate from a divergent but still undetected source.

The current study revealed the colonization history and expansion routes of *B. tau* in its major distributional regions. Furthermore, the study will lead us to pay attention to its potential expansion tendency. Sporadic appearance of *B. tau* was mentioned in some northern area of China such as Shanxi (Yang et al. 1994). In this study, some northern Chinese populations (HZ, HB, and EN) were found to have experienced recent expansion. Improved quarantine and sanitary control measures should be implemented to prevent new long-distance, human-aided dispersal of the fly.

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