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Population genetic structure of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) from Yunnan province (China) and nearby sites across the border

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Abstract *Bactrocera dorsalis* (Diptera: Tephritidae) is an important pest for many tropical and subtropical fruits. The fly is probably introduced in Yunnan, a southwestern province of China that shares borders with Vietnam, Laos and Myanmar. Depending on local environmental conditions, this species occurs either only in the most favorable seasons or year-round. To infer the genetic diversity and structure of the fly in the region, and to understand the relationships between the flies of year-round and seasonal areas, we analyzed 304 individuals from 14 populations using the mitochondrial cytochrome oxidase I gene (COI). The sampled populations were structured into four groups, probably isolated by the main natural barriers in Yunnan such as mountain ranges and rivers. Our data suggest either that *B. dorsalis* in Yunnan originated from multiple introductions events, even if the source populations still need to be identified; or that Yunnan is a natural origin of this species (i.e., that it is not invasive there). Finally, we found some evidences that the seasonal populations were colonized from nearby year-round populations.

Keywords *Bactrocera dorsalis* · Phylogeography · Genetic structure · mtDNA

Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is one of the most economically serious insect pests of tropical and subtropical fruits and vegetables (Li et al. 2007). The fly was first recorded in 1912 in Taiwan and expanded throughout most countries or regions in South East Asia and also around the Pacific Ocean over the following years (Hardy 1973). In mainland of China, the appearance of the fly was reported in 1937 (Xie 1937). This insect is highly polyphagous, and is able to infest more than 250 species and varieties of host plants, including a number of commercially grown fruits, such as melon, banana, mango and guava (Li and Ye 2000). *B. dorsalis* is thus regarded as one of the most important quarantine pests listed by many countries (Armstrong 2003; Follett and Armstrong 2004).

In China, *B. dorsalis* occurs mainly in the southeastern and southwestern regions (Wang 1996). Yunnan is one of the major provinces infected by the fly. It is situated in the southwest of China, sharing borders with Vietnam, Laos and Myanmar (21°8′32″–29°15′8″N, 97°31′39″–106°11′47″E) (Liu et al. 2007). In this region, *B. dorsalis* was first reported in 1956 in Jinping, and it is now present in more than 2/3 of the province (Wang 1996; Ye 2001).

Ninety-four percent of the Yunnan territory is mountainous (Wang 2002). The elevations vary from 70 m in the south to 3,000 m in the north, with consequent various climatic zones ranging tropic to frigid. Longitudinally, three mountain ranges (Wumeng, Daliang and Nu) reach 2,900 m above sea level (asl), and three rivers (Nujiang, Lanchangjiang and Jingshajiang) run parallel from northwest to southeast (Wang 2002). The habitat of the oriental fruit fly is thus highly fragmented (Shi et al. 2005). *B. dorsalis* populations are therefore scattered in various areas relatively isolated by these huge mountains, deep valleys, as well as the various climatic zones.

The populations of *B. dorsalis* in Yunnan show two different infestation patterns depending on local environmental

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conditions (Ye 2001). The fly occurs throughout the year in the southernmost regions (latitude lower than 24°N, hereafter "year-round populations"), while it is only found seasonally, from May to November in the northern plateaus located at latitudes 24°–26°N (hereafter "seasonal populations": Shi et al. 2005). The climate in the southern region where the fly occurs annually is tropical to semitropical, and the elevations are at most 1,100 m asl (Ye 2001). On the other hand, the elevations of the northern plateaus are 1,700–2,300 m asl. It has been hypothesized that the localities where *B. dorsalis* occurs only seasonally are re-colonized each year from southern locations (Ye 2001).

Molecular markers have been widely used to infer the population genetic structure, to identify sources of origin and to understand the processes of invasion and expansion for other tephritid fruit flies, such as the medfly *Ceratitis capitata* (Bonizzoni et al. 2001, 2004; Gasperi et al. 2002; Meixner et al. 2002; Baliraine et al. 2004), the olive fly *Bactrocera oleae* (Ochando and Reyes 2000; Augustinos et al. 2005; Nardi et al. 2003, 2005), the pumpkin fruit fly *Bactrocera depressa* (Mun et al. 2003) and the melon fruit fly *Bactrocera cucurbitae* (Hu et al. 2008). Some genetic data are now also available for the oriental fruit fly (Dai et al. 2004; Shi et al. 2005; Aketarawong et al. 2007). In particular, a previous study suggested that *B. dorsalis* shows a relatively high genetic diversity in Yunnan even though it is introduced there, with a low overall genetic differentiation (Shi and Ye 2003; Shi et al. 2005). However, the limited sampling scheme did not permit to describe precisely the distribution of genetic diversity in Yunnan and surrounding countries nor to test the origin of the seasonal populations and their relationship with the stable, year-round populations. In the present study, based on analysis of maternally inherited mitochondrial sequences, we sampled more populations and more individuals within populations to determine (1) the distribution and structure of the genetic diversity of the fly; (2) the effect of geographical barriers on gene flow; (3) the genetic origins of the populations that only occur seasonally. All the information will provide us an essential clue to understand invasion and expansion rules for this species, and will be helpful to develop appropriate strategies for the fruit fly control in Yunnan.

Materials and methods

Sample collection

The specimens of *B. dorsalis* were collected in August 2005 and 2006 from 14 sampling locations, including 11 sites in Yunnan province (China), and three nearby sites across the borders (Bhamo in Myanmar, Muang Khua in Laos and Yên Bái in Vietnam) (Table 1). Four Yunnan

Table 1 Sampling information and haplotypes found in each population

Localities	Number	Haplotypes	Elevation (m)	Longitude (E)	Latitude (N)	Collection date
Kunming (Yunnan)	25	H1(2), H2(2), H3(8), H4(3), H5(3), H6(2), H7(3), H8(2)	1,892	102°41′	25°01′	August 2005
Qujing (Yunnan)	20	H2(3), H4(9), H5(4), H7(4)	1,857	103°48′	25°30′	August 2006
Dali (Yunnan)	20	H1(1), H2(13), H4(2), H5(2), H30(2)	1,991	100°72′	25°42′	August 2005
Yuanjiang (Yunnan)	23	H1(3), H2(4), H4(6), H6(3), H7(2), H8(2), H30(3)	364	101°59′	23°36′	August 2005
Huanian (Yunnan)	24	H1(2), H2(2), H3(3), H4(2), H5(8), H6(1), H7(2), H8(1), H9(2), H29(1)	1,134	102°12′	24°03′	August 2006
Liuku (Yunnan)	16	H6(10), H25(2),H27(1), H28(3)	1,654	98°51′	25°52′	August 2006
Ruili (Yunnan)	25	H6(5), H23(2), H24(3), H25(1), H26(3), H27(1), H28(8), H41(2)	776	97°51′	24°01′	August 2006
Jinghong (Yunnan)	21	H10(2), H11(3), H12(4), H13(3), H14(3), H15(1), H16(2), H29(3)	553	100°48′	21°59′	August 2005
Mengzi (Yunnan)	18	H17(3), H20(5), H34(6), H35(4)	1,301	103°23′	23°23′	August 2006
Wenshan (Yunnan)	20	H17(5), H18(3), H20(4), H22(4), H34(4)	1,272	104°15′	23°23′	August 2006
Hekou (Yunnan)	23	H17(3), H18(2), H19(3), H20(5), H21(2), H22(2), H33(3), H34(3)	137	103°57′	22°30′	August 2006
Bhamo (Myanmar)	28	H6(7),H23(1), H24(1), H25(1), H26(1), H28(2), H37(1), H38(1), H39(1), H40(2), H41(8), H42(2)	744	97°17′	24°16′	August 2006
Yên Bái (Vietnam)	21	H17(2), H18(3), H22(9), H34(3), H36(4)	1,108	104°86′	21°70′	August 2006
Muang Khua (Laos)	20	H10(6), H11(4), H16(4), H29(3), H31(2), H32(1)	679	102°50′	21°08′	August 2006

sites, namely Kunming, Dali, Qujing and Liuku, were located in regions where the fly occurs seasonally, whereas all other sampling sites were in regions of year-round occurrence (Fig. 1). The sampling locations cover the major distributional districts of *B. dorsalis* in Yunnan.

In each sampling spot, two to three fruit orchards located ca. 10 km from each others were chosen. The sampling procedure is precisely described in Shi et al. (2005). We used both adults emerging from host fruits and lured into traps (Methyl Eugenol, Zhejiang, China). All samples were preserved in 99.5% ethanol at 4°C.

Amplification and sequencing

DNA was extracted individually for each specimen using the commercial tissue/cell DNA Mini Kit (Watson Biotechnologies, Shanghai, China). The DNA extracted from each individual was suspended in 50 µl of Tris–EDTA (10 mM Tris–HCl, 1 mM EDTA, pH = 8.0).

A portion of the mitochondrial COI gene (601 bp) was amplified by PCR using the primer pair P1 (5′-CGTGCCT ATTTCACTTCAGC-3′) and P2 (5′-CAGCTGGAGGGGT ATTTTGA-3′). All details for amplification and sequencing are described elsewhere (Shi et al. 2005).

Data analyses

All obtained sequences from the fourteen studied populations were aligned using Clustal X as implemented in BIOEDIT 7.0 (Hall 2004) and these sequences were deposited in Genbank (accession numbers GQ414975-GQ414988).

A statistical parsimony network of haplotypes (Templeton et al. 1992) was constructed using the 95% parsimony criterion with the program TCS 1.21 (Clement et al. 2000). Haplotype numbers and distribution, polymorphic sites, within-population mean number of pairwise differences and nucleotide diversity were assessed using ARLEQUIN 3.11 (Excoffier et al. 2005). Nei's average numbers of pairwise differences between populations (Nei and Li 1979) as well as pairwise FST estimates were also calculated using ARLEQUIN. Differences in both Nei's average numbers of pairwise differences within population and nucleotide diversity were tested between the year-round and the seasonal regions using a Mann–Whitney *U*-test (Mendenhall and Beaver 1991) by SPSS ver.15.0 (SPSS 2007).

Spatial analysis of molecular variance (SAMOVA) was performed using SAMOVA 1.0 (Dupanloup et al. 2002) to identify groups of populations that are phylogeographically homogeneous and maximally differentiated from each other. This analysis permits to identify the maximally

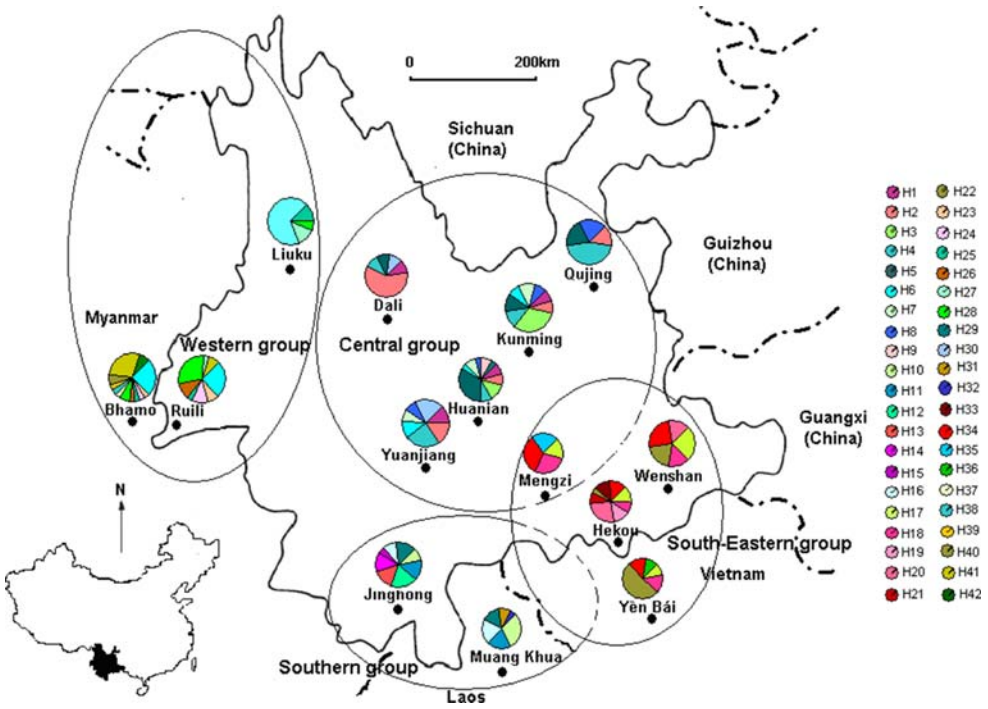

Fig. 1 Geographical distributions of the 42 haplotypes of *B. dorsalis* among the four groups defined by the SAMOVA analysis (see text for details). The four population groups are enclosed in separate circles. Pie charts indicate the frequency of haplotypes in each location. Liuku, Dali, Kunming and Qujing are seasonal populations, while Ruili, Hekou, Mengzi, Wenshan, Jinghong, Yuanjiang and Huanian are year-round populations

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, FCT values should reach a maxim or a plateau. Second, the configurations with one or more single-population groups should be excluded, because this indicates that the group structure is disappearing (Magri et al. 2006). We performed analyses for K = 2–10 groups to identify the most likely number of groups. AMOVA analyses (Excoffier et al. 1992) were then performed to test the genetic relationships between the different groups defined by SAMOVA.

In addition, occurrence a significant phylogeographic structure was estimated by testing if GST (coefficient of genetic variation over all populations) was significantly lower than NST (equivalent coefficient taking into account the similarities between haplotypes) by using PERMUT (Pons and Petit 1996).

Results

We merged the sequences obtained in the present study with previously published sequences (accession numbers DQ06 0280-DQ060304, DQ100468, DQ100470, DQ100471) from five locations in Yunnan province (Ruili, Kunming, Yuanjiang, Huanian and Hekou, see Shi et al. 2005) to obtain a final alignment of 304 fly individuals. The total length of the aligned sequences was 514 bp and no insertions or deletions were recorded. Of the 514 characters, forty-seven were polymorphic, including 16 singletons and 31 parsimony informative sites.

Fourty-two haplotypes were observed in the fourteen *B. dorsalis* populations. Only five haplotypes (H15, H32, H37, H38 and H39) were found in one population, while the other 37 haplotypes were shared by at least two populations. The geographical distribution of the 42 haplotypes is shown in Fig. 1. Table 1 lists the haplotypes present in each of the 14 studied populations. Figure 2 shows the 95% parsimony network of all haplotypes. The network was not clearly structured. It contained many loops, and no haplogroup could be identified. The haplotypes presented in any given locality or region were spread all over the network and were not phylogenetically related (see below). There were 48 missing haplotypes.

The indices of population structure GST and NST were 0.172 and 0.201, respectively and NST was not significantly higher than GST, suggesting that the phylogeographical structure was either non-existing or very weak. SAMOVA 1.0 was used to identify the optimal grouping of populations. As the FCT values reached a plateau at K = 4, and single-population groups were formed when K > 4, we used four as the optimal number of population groups. The four groups found by the SAMOVA are geographically consistent and correspond to regions (Table 2). The first group contains Huanian, Yuanjiang, Kunming, Qujing and Dali and will be referred to as "Central group"; the second

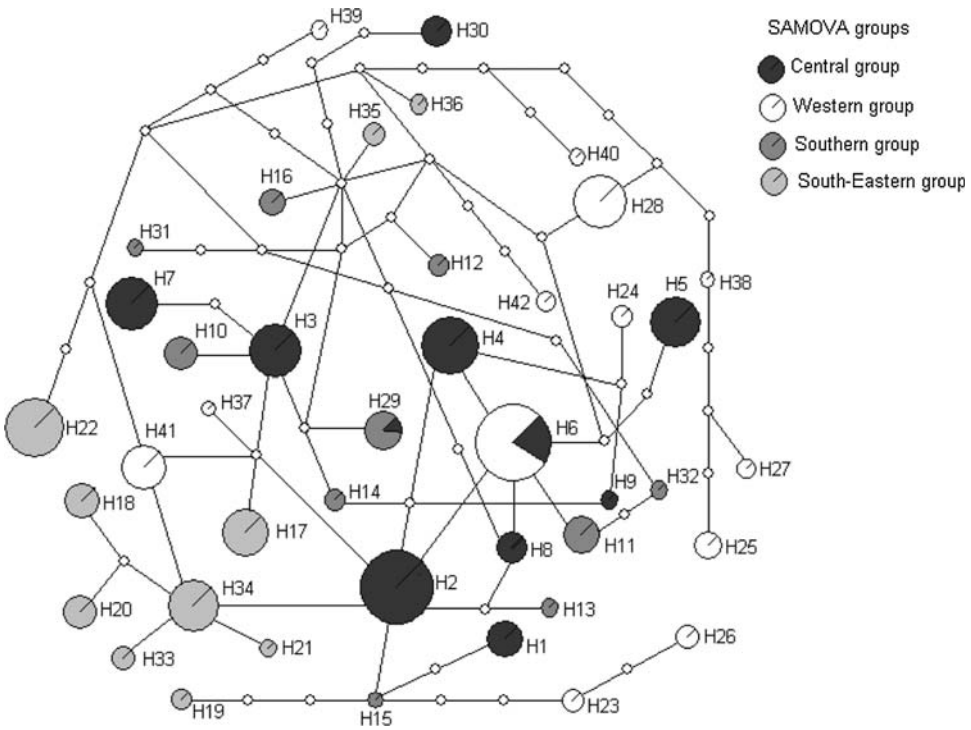

Fig. 2 Haplotype network of the haplotypes showing the proportion of individuals belonging to each of the four groups. The size of circles is proportional to the number of individuals having that haplotypes. Each line corresponds to a mutational step and the empty circles correspond to missing intermediate haplotype

Table 2 Analysis of molecular variance based on the four groups defined by SAMOVA

Group	Source of variation	Percentage of variance	Fixation index
Central group	Among groups	18.09	FCT = 0.18086**
Western group	Among populations	4.62	FSC = 0.05645**
Southern group	Within groups		
South-eastern group	Within populations	77.29	FST = 0.22710**

The group composition (see "Results")

** $P < 0.001$

group clusters Ruili, Liuku and Bhamo ("Western group"), the third one gathers Jinghong and Muang Khua ("Southern group") and the fourth is Wenshan, Mengzi, Hekou and Yên Bái ("South-Eastern group"). These four groups of populations are shown in Fig. 1. Moreover, for each haplotype, the proportion of individuals belonging to each group is shown on the haplotype network (Fig. 2), which illustrates that haplotypes occurring in the same geographical regions are not genetically related.

The fixation indices of the AMOVA run with the grouping of populations suggested by the SAMOVA results are shown in Table 2. Most of the molecular variance is found within populations (77.29%, $P < 0.001$), but ca.18.09% of variance is still found among groups ($P < 0.001$). Pairwise FST estimates and Nei's average number of pairwise differences were calculated to measure the genetic differentiation among the 14 populations (Table 3). Pairwise FST values ranged from 0.010 to 0.367 and Nei's average number of pairwise differences between populations varied from 3.124 to 6.016. When populations were grouped according to the SAMOVA results, pairwise FST and Nei's average number of pairwise differences between groups varied from 0.121 to 0.282 and from 4.205 to 5.453, respectively (Table 4). The average number of pairwise differences within groups is shown in Table 4.

Nei's average number of pairwise differences within population and nucleotide diversity are shown in Table 3. The average value of Nei's average numbers of pairwise differences within population and nucleotide diversity were 3.858 and 0.0075, respectively for the 7 year-round populations, while they were 3.025 and 0.0059, respectively for the four seasonal populations. Both the average value of nucleotide diversity and Nei's average numbers of pairwise differences within population were all significantly higher in the year-round regions than in the seasonal regions (two-tailed Mann–Whitney *U*-test corrected for ties, $P < 0.05$).

Discussion

Genetic structure and natural barriers to gene flow

The fourteen *B. dorsalis* populations sampled from Yunnan province and nearby sites across the border (Bhamo, Muang Khua, and Yên Bái in Myanmar, Laos and Vietnam, respectively) were structured into four groups, located in the west, the center, the south and the southeast of Yunnan. All results concerning genetic structure suggest that genetic relationship may be low between regions sampled in the present study. It is possible that the environmental features of Yunnan contributed to low genetic relationship. Overall, Yunnan is a province with several mountain ranges and rivers (Wang 2002; Shi et al. 2005 Hu et al. 2008). Three big rivers and mountain ranges run parallel from northwest to southeast (see "Introduction") (Chao 1987). These mountains and rivers most probably act as natural barriers and significantly limit gene flow between groups. For instance, the Western group is isolated from the others by Daliang, Nu and Wumeng mountains and by Jingsha, Nu and Lanchang rivers. Moreover, Yunnan is dominated by southwestern monsoon currents that originate from the Bengal fjord (Chao 1987) and blow somewhat anti-parallel (from southwest to northeast) to the main mountains and rivers. The currents could facilitate the dispersing of the fly populations from south to north while dispersion from west to east is probably strongly limited (Wang 2002; Shi et al. 2005). It seemed that geographic features combined with air currents probably play a major role in the genetic structure of the 14 fly populations. In addition, *B. dorsalis* movements are probably favored and increased by human activities and fruit transportations. The growing trade of exotic fruits, as well as the tourist industry, have been recognized as important factors influencing fly dispersal in many studies, such as: *Ceratitis capitata* and *B. oleae* (Malacrida et al. 2007).

Evidence for multiple introductions, or natural origin of the fly in Yunnan?

In South-East Asia, *B. dorsalis* was first recorded in Taiwan, and subsequently found in most countries of the Asian-Pacific region over the following 90 years (Hardy 1973). The fly may be considered as introduced in Yunnan province of China (Wang 1996). The routes and modes of colonization in this region are still unknown. Our first hypothesis could be that the colonization of Yunnan is a recent event, based on the fact that *B. dorsalis* is a well-known species that develops on many cultivated plants (Li

Table 3 Average number of pairwise differences between populations (above diagonal), within populations (diagonal elements), pairwise FST values (below diagonal) and nucleotide diversity (last line) for 14 populations of *B. dorsalis*

Group	Population	Central group					Western group			Southern group		South-eastern group			
		Huanian	Yuanjiang	Kunming	Qujing	Dali	Bhamo (Myanmar)	Ruili	Liuku	Jinghong	Muang Khua (Laos)	Hekou	YênBái (Vietnam)	Wenshan	Mengzi
Central group	Huanian	**3.888**	4.216*	3.618	3.500	3.646*	4.985**	4.885**	4.260**	4.288**	4.613	5.432**	5.482**	4.950**	4.690**
	Yuanjiang	0.064*	**4.008**	3.696	3.617	3.567	4.958**	4.946**	4.114**	4.402**	4.517**	5.461**	5.491**	5.050**	4.698**
	Kunming	0.021	0.025	**3.200**	3.124	3.294**	4.467**	4.597**	3.975**	3.789**	3.872**	4.964**	4.931**	4.454**	4.122**
	Qujing	0.018	0.033	0.010	**2.979**	3.320*	4.579**	4.540**	3.775**	3.962**	4.065**	5.204**	5.095**	4.710**	4.311**
	Dali	0.060*	0.023	0.065*	0.107*	**2.953**	4.518**	4.522**	3.694**	4.131**	4.455**	4.472**	5.031**	4.140**	3.928**
Western group	Bhamo (Myanmar)	0.125**	0.108**	0.100**	0.146**	0.132**	**4.823**	4.804	4.263**	5.216**	5.179**	5.387**	5.039**	4.875**	4.802**
	Ruili	0.147**	0.145**	0.168**	0.178**	0.177**	0.035	**4.447**	3.865	5.333**	5.508**	6.016**	5.684**	5.478**	5.344**
	Liuku	0.189**	0.147**	0.222**	0.212**	0.199**	0.076**	0.0352	**2.967**	4.845**	4.975**	5.424**	5.131**	4.875**	4.722**
Southern group	Jinghong	0.102**	0.112**	0.076*	0.143**	0.181**	0.169**	0.224**	0.296**	**3.810**	3.933	5.754**	5.692**	5.298**	4.939**
	Muang Khua (Laos)	0.170**	0.139**	0.101**	0.170**	0.246**	0.166**	0.252**	0.319**	0.037	**3.768**	5.930**	5.510**	5.345**	4.922**
South-eastern group	Hekou	0.289**	0.282**	0.292**	0.343**	0.239**	0.194**	0.311**	0.367**	0.336**	0.359**	**3.834**	4.615	3.659	3.867
	YênBái (Vietnam)	0.299**	0.289**	0.292**	0.333**	0.328**	0.141**	0.273**	0.336**	0.332**	0.313**	0.173**	**3.800**	4.017*	4.463
	Wenshan	0.262**	0.265**	0.259**	0.322**	0.232**	0.151**	0.278**	0.344**	0.319**	0.329**	0.010	0.103*	**3.405**	3.550
	Mengzi	0.199**	0.187**	0.176**	0.237**	0.165**	0.116**	0.242**	0.302**	0.248**	0.250**	0.037	0.169**	0.012	**3.614**
Nucleotide diversity		0.0076	0.0078	0.0062	0.0058	0.0057	0.0094	0.0087	0.0058	0.0074	0.0073	0.0075	0.0074	0.0066	0.0070

* $P < 0.05$, ** $P < 0.01$

Table 4 Average number of pairwise differences between groups (above diagonal), within groups (diagonal elements), and the FST value (below diagonal) for the four groups obtained from the SAMOVA (see text for details)

Group	Central group	Western group	Southern group	South-eastern group
Central group	**3.550**	4.538**	4.205**	4.868**
Western group	0.127**	**4.404**	5.211**	5.269**
Southern group	0.121**	0.204**	**3.963**	5.453**
South-eastern group	0.231**	0.208**	0.282**	**3.955**

** $P < 0.01$

and Ye 2000; Aketarawong et al. 2007), and that it would not have remained undetected for a long time (Xie 1937). Yet, the possibility still exists that *B. dorsalis* is native in Yunnan but remained undetected there until it started to cause economical damages to cultures. The genetic data can help determine whether the species is more likely to be invasive or native in this region; moreover, if it is introduced, one can question (1) whether the species most probably entered the region in a single point and was introduced from a single source and a limited number of founder individuals, or (2) if it was introduced from a single region but with a large number of founders, or (3) if it was introduced from multiple points and a large number of migrants. Hypothesis (1) implies that *B. dorsalis* would show a very limited amount of genetic diversity (especially for a mitochondrial marker), with one or a few major haplotypes shared by most individuals in the introduction range like *B. curcurbitae* (Hu et al. 2008) and *B. depressa* (Mun et al. 2003). Obviously, our data do not support this hypothesis as no one haplotype was shared by more than 9.2% of the sampled individuals, and as many different haplotypes were found (42 haplotypes for 304 individuals although the sequenced fragment was only ca. 500 bp). We can confidently suppose that the populations present in the Yunnan region originated from a high number of founders which would explain the high genetic diversity found locally. To distinguish between hypotheses (2) and (3) would require extensive genetic data from the potential source populations and from the supposed native range of the species. If we assume that, as in most species, a phylogeographical structure exists for *B. dorsalis* in its native range, then we can assume that phylogenetically related haplotypes co-occur regionally in the native range and that divergent haplotypes would originate from different regions. If this is true, then our data would suggest that the flies present in Yunnan originate from different regions of the native range, or from a region that was colonized earlier in which many different haplotypes already occur. The geographic structure of genetic diversity found in our data could suggest that each of the four regions was colonized separately, or that limited gene flow between regions (see below) shaped the distribution of genetic diversity after the fly's introduction in the country. Yet, the fact that divergent haplotypes co-occur in each group defined by the SAMOVA analysis supposed that unrelated individuals were introduced in each region. This would also explain why 77.29% of genetic variance is actually found within populations. This scenario now needs to be confirmed both by a range-wide sampling and phylogeographical study, and by using complementary markers such as microsatellite data and assignment analyses. Yet, based on our results, we cannot rule out the hypothesis that the fly is native in the Yunnan region, as we already suggested in a previous work (Shi et al. 2005). It should be noted that Nardi et al. (2005) only found 22 mitochondrial haplotypes during an extensive sampling of the closely related *Bactrocera oleae*, while we obtained almost twice as many mitochondrial haplotypes in Yunnan and nearby populations. This unexpected genetic diversity could well be explained by a long-lasting, natural occurrence of the fly in the region. Interestingly, Aketarawong and collaborators (2007) proposed that China could be a source population for *B. dorsalis*, and suggested an East–West colonization route to other regions of Asia. An extensive, worldwide sampling scheme would now be necessary to fully understand the evolution of this pest's populations.

Relationship between seasonal and year-round populations

Liuku, Dali, Kunming and Qujing populations are seasonal populations, where the flies occur only from May to November each year (Ye 2001). Ye (2001) proposed that seasonal populations can not survive in winter and must re-establish from nearby year-round populations annually. If this hypothesis is true, seasonal populations should possess a reduced genetic diversity as compared to year-round population (Liu et al. 2007), because they are regularly subjected to founder effects and genetic drift following a colonization process (Bonizzoni et al. 2004). Our data revealed that the average gene diversity was significantly lower in seasonal populations than in year-round populations ($P < 0.05$). Moreover, within each of the regional groups found by the SAMOVA analysis, the haplotypes found in the seasonal population are consistently also present in the closest

year-round populations of the same group, and the genetic differentiation between the seasonal locality and its closest year-round neighbor was not significant. For instance, the haplotypes found in Kunming, Dali and Qujing were also present in Huanian or in Yuanjiang. Haplotype composition and genetic differentiation suggested that Qujing population originates from Hunian, Liuku from Ruili and Dali from Yuanjiang. This interpretation needs to be confirmed by ecological investigations and other multilocus genetic studies. Moreover, sampling insects from the seasonal regions in successive year will allow to test whether they are consistently re-colonized from the same sources each year. The genetic interpretation was thus consistent with the hypothesis Ye proposed, following which seasonal populations are recurrently re-established each year from neighboring year-round populations.

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