

Three haplotypes found in populations of the red imported fire ant invading China

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Abstract: The red imported fire ant, *Solenopsis invicta*, is an invasive species of economic concern (primarily an agricultural and ecosystem pest). Such ant was found in some areas of Guangdong and Hong Kong at the end of 2004. By examining genetic variation of the mitochondrial cytochrome oxidase I (CO I) gene, we analyzed the colony introduction of 13 populations of such ant. A 904 bp fragment of the CO I gene was sequenced for 56 individuals from 28 nests. Three haplotypes were obtained. Using the uncorrected pairwise sequence divergence, we found that the divergence between haplotype II and haplotype III was smallest. All three haplotypes were observed in the Hong Kong population, while only one or two haplotypes were observed in the other populations. Such data of the highest genetic diversity in Hong Kong population suggested that Hong Kong may be the original place of the ant invading other areas in China. All of the three haplotypes observed in Hong Kong population were reported in Argentina, and it is so inferred that the Chinese fire ant may come from Argentina or South America.

Key words: *Solenopsis invicta*; invasive species; CO I gene; haplotype

1 INTRODUCTION

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, an original species in South American region, was introduced to Mobile, AL in the United States between 1930 and 1940 (Buren *et al.*, 1974). By 1990, the ant had spread throughout the southern United States. In Texas only, it costs annually over US \$ 1.2 billion in damage and for controlling (Lard *et al.*, 2002). The fire ant also expanded to West Indies (Davis *et al.*, 2001), New Zealand (Pascoe, 2002), and Puerto Rico (Callcott and Collins, 1996) and Australian (Natrass and Vanderwoude, 2001). The current total area that has been treated is 40 000 ha in Australian (Vanderwoude *et al.*, 2004). An extensive 5-year program of control and eradication of such ant is underway in Australia. It takes 100 million \$ a year to annihilate the pest (Vanderwoude *et al.*, 2004).

As an invasive species of economic concern (primarily an agricultural and urban pest), *S. invicta* was first found in China at the end of 2004 (Zeng *et al.*, 2005). In China, as many as 25 provinces, autonomous regions or municipalities were the potential distribution areas of the fire ant (Xue *et al.*, 2005). The fire ant has been very difficult to eradicate wherever it has been established (Henshaw *et al.*, 2005). Several steps of precautions and preventive measures

were proposed or already taken in China (Zhang *et al.*, 2005). While, understanding about the introduction history of the fire ant is important for management in future. In particular, 34 haplotypes have been observed based on the CO I sequences in Argentine populations (Shoemaker *et al.*, 2003). Effective quarantine is the best measure for China to prevent the expansion of *S. invicta* in the long term. This will necessitate identifying the haplotype and most likely sources of introduction. In this study, we used the mitochondrial cytochrome oxidase I (CO I) gene as a genetic marker to test the introduction of fire ant in China.

2 MATERIALS AND METHODS

2.1 Collection and identification of ants

A list of fire ant workers and males analyzed and collected in the study is presented in Table 1. All samples were identified based on the morphology.

2.2 Sequencing

Genomic DNA was extracted from a leg of each worker ant examined (Wen and He, 2003). The CO I fragments were amplified in an MJ Research Minicycler using the following profile: 94°C, 1 min for first cycle; 35 cycles for 94°C, 30 s denature; 50°C (55°C for CO II R), 1 min anneal; 68°C (72°C for CO II R), 2 min extension; 72°C, 5 min final extension. PCR

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Table 1 Collection data and haplotypes of the red imported fire ants from different sites

Locality	Date	Individuals/colonies	Haplotype	Collector
China				
Hong Kong	1/26/2005	8/4	I, II, III	Y. H. STEPHEN
Shenzhen, Guangdong	3/11/2004	2/1	I	HE Xiao-Fang
Zhuhai, Guangdong	21/2/2005	8/4	I	LAI Si-Chun
Zhongshan, Guangdong	24/31/2005	2/1	I	LIU Hao
Dongguan, Guangdong	14/12/2004	8/4	I	LU Yong-Yue
Huizhou, Guangdong	18/11/2004	2/1	I	LU Yong-Yue
Guangzhou, Guangdong	21/10/2004	6/5	I	HE Xiao-Fang
Wuchuan, Guangdong	19/09/2004	2/1	II	LU Yong-Yue
Yangchun, Guangdong	28/1/2005	2/1	II	LU Yong-Yue
Nanning, Guangxi	15/1/2005	4/1	I, II	SHENG Kun
Zhangjiajie, Hunan	20/1/2005	4/1	I, II	ZENG Xing-Nian
Taiwan	29/7/2005	2/1	I	ZENG Ling
Australia				
Brisbane	22/8/2005	2/1	I	ZENG Ling
USA				
Texas	20/11/2004	2/1	I	ZHANG Ming-Ling
Florida	20/12/2005	2/1	III	FAN Yu-Qing

amplifications were carried out using the primer C1-J-2195 (Simon *et al.*, 1994), DDS-CO II -4 (Ross and Shoemaker, 1997) and a newly designed primer (CO II R 5'-GGGGAATTGAAATTTTGAAGAGC-3'). Amplifications were performed in 25 μ L volumes using 1 μ L genomic DNA, 2.5 mmol/L MgCl₂, 13 Biolase reaction buffer, 0.20 mmol/L each dNTP, 0.25 mM each primer, and 5 units of Taq DNA polymerase (DingGuo Ltd.). PCR products were purified using PCR purification kit (TaKaRa Inc.) and sequenced directly from both directions using ABI-3730 DNA sequencer with ABI Prism Big Dye cycle sequencing kit.

2.3 Data analyses

Sequences were arranged and aligned by Dnastar (DNASTAR Inc.). Homologous sequences of the fire ant from GenBank (Accession number: AY249092 – AY249134 for Argentina populations, AY499580 – AY499590 for Brazil populations) were compared and aligned. PAUP* 4.0b10 (Swofford, 1999) was employed for all maximum parsimony (MP) and neighbour-joining (NJ) searches. All trees were rooted with *S. electra*. We used the HKY85 distance measure to construct the NJ tree. Bootstrap support values were generated from 1 000 replicates in both methods with tree bisection and reconnection (TBR) and branch swapping; other settings were the PAUP* defaults.

3 RESULTS

A 904 bp fragment of the CO I gene was obtained from 54 individuals. Fifty polymorphisms were observed, of which 47 sites were informative. None of these 50 polymorphisms results in amino acid change.

The ratio of transition to transversion is 4.1:1. The CO I fragment was highly AT-biased (69.6% AT in average), which was consistent with the findings from other Hymenoptera (Dowton and Austin, 1995).

Three haplotypes based on CO I polymorphisms were found from the Chinese populations. GenBank accession numbers for nucleotide sequences of the three haplotypes for the CO I gene are DQ831670 – DQ831672. Their distribution was showed in Table 1. Samples from Shenzhen, Dongguan, Zhuhai, Guangzhou, Zhongshan and Taiwan populations shared haplotype I. Haplotype II was found in Yangchun and Wuchuan populations. All three haplotypes were observed in the Hong Kong population. The uncorrected pairwise sequence divergence among all haplotypes for the CO I gene ranged from 0 to 7.0%. Between the haplotype II and haplotype III, haplotype I and haplotype II, and haplotype I and haplotype III, the sequence divergences are about 2.8%, 6.5% and 5.8%, respectively.

Parsimony analysis produced most-parsimonious tree of 226 steps in length, with a consistency index (CI) of 0.695 and a retention index (RI) of 0.930. The results of the NJ and MP searches showed the evolutionary relationships of these haplotypes (Fig. 1). The NJ and MP trees were quite similar except the bootstrap proportions. Phylogenetic analysis showed that these populations in China could be divided into three clusters according to the haplotypes. The clade with haplotype II and the clade with haplotype III constitute a sister group with high bootstrap values (97/96). Haplotype I is placed in separate lineages with significant support (100/100) in both arithmetic.

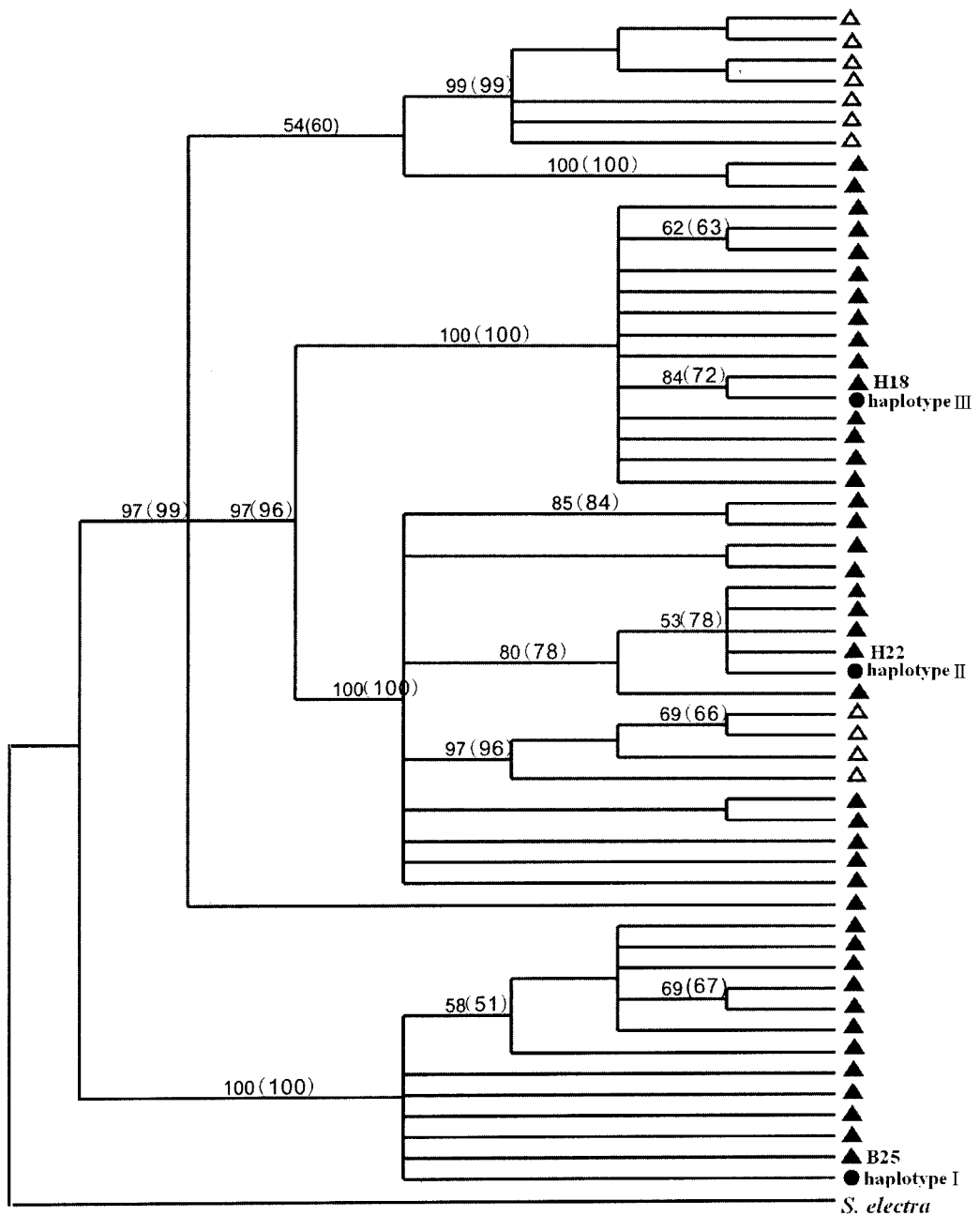


Fig. 1 Neighbour-joining (NJ) tree for mtDNA haplotypes of *Solenopsis invicta* based on sequences from a fragment of the cytochrome oxidase I (CO I) gene

Bootstrap support values for NJ (Maximum parsimony, MP) greater than 50% are shown with percentages above branches for each node. In the most parsimony tree, the consistency index (CI)=0.695, and retention index (RI)=0.930. ▲: *S. invicta* from Argentina; △: *S. invicta* from Brazil; ●: *S. invicta* from China.

4 DISCUSSION

Haplotype I is identical to the haplotypes B25, H13, H5 in Argentina and Brisbane and Texas populations (Shoemaker *et al.*, 2003); haplotype II is identical to the haplotype H22 of Argentina population (Shoemaker *et al.*, 2003); haplotype III is identical to the H18 and Florida population (Shoemaker *et al.*, 2003). These haplotypes would be helpful for

quarantine of imports and exports, developing and implementing rules and regulations to ensure an acceptable level of protection in China. All the three haplotypes have been reported in the Argentina population (Shoemaker *et al.*, 2003). It is so inferred that the Chinese *S. invicta* may come from the Argentina or South American populations.

Hong Kong population showed the highest genetic diversity among all populations we examined. All the three haplotypes found in this study were present in the

Hong Kong population. Therefore, it is most likely that Hong Kong is the original source of the red ant in China. Only haplotype II was found in populations of Yangchun and Wuchuan, while only haplotype I was found in populations of Shenzhen, Dongguan, Zhuhai, Guangzhou, Zhongshan and Taiwan. Distribution of these two haplotypes is irrelevant. On the other hand, multiple haplotypes can be found from the same sample in the populations of Hong Kong, Nanning and Zhangjiajie. The populations sharing one haplotype at the same region indicated one invasive event to large extent, but multi-invasion occasionally. Multiple haplotypes at the same region were complicate and one invasive event or multi-invasion would happen.

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入侵我国红火蚁的三种单倍型

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摘要: 红火蚁 *Solenopsis invicta* Buren 是重要的经济害虫, 特别是在农业和生态系统危害方面。2004 年底在广东和香港的部分地区发现红火蚁入侵。本文用线粒体细胞色素氧化酶 I (CO I) 基因对境内的 13 个种群进行了入侵蚁巢是否独立传入进行了分析。通过对共计 28 个蚁巢 56 个个体的包含 904 个碱基的 CO I 基因分析, 发现入侵中国的红火蚁中存在 3 种单倍型。基于未修正的配对序列变异分析表明, 单倍型 II 与单倍型 III 之间的变异最小。而香港种群同时具有这三种单倍型, 是入侵中国的红火蚁种群中多样性最丰富的地区。这三种单倍型都分别在阿根廷红火蚁种群中有记录。同源性分析显示, 中国的红火蚁可能起源于阿根廷或南美洲, 而香港可能是所研究种群的第一入侵地点。

关键词: 红火蚁; CO I 基因; 单倍型; 入侵物种

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