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Population genetic structure and migration patterns of *Liriomyza sativae* in China: moderate subdivision and no Bridgehead effect revealed by microsatellites

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Abstract

While *Liriomyza sativae* (Diptera: Agromyzidae), an important invasive pest of ornamentals and vegetables has been found in China for the past two decades, few studies have focused on its genetics or route of invasive. In this study, we collected 288 *L. sativae* individuals across 12 provinces to explore its population genetic structure and migration patterns in China using seven microsatellites. We found relatively low levels of genetic diversity but moderate population genetic structure ($0.05 < F_{ST} < 0.15$) in *L. sativae* from China. All populations deviated significantly from the Hardy–Weinberg equilibrium due to heterozygote deficiency. Molecular variance analysis revealed that more than 89% of variation was among samples within populations. A UPGMA dendrogram revealed that SH and GXNN populations formed one cluster separate from the other populations, which is in accordance with STRUCTURE and GENELAND analyses. A Mantel test indicated that genetic distance was not correlated to geographic distance ($r = -0.0814$, $P = 0.7610$), coupled with high levels of gene flow ($M = 40.1$–817.7), suggesting a possible anthropogenic influence on the spread of *L. sativae* in China and on the effect of hosts. The trend of asymmetrical gene flow was from southern to northern populations in general and did not exhibit a Bridgehead effect during the course of invasion, as can be seen by the low genetic diversity of southern populations.

Keywords: *Liriomyza sativae*, microsatellite, population genetic structure, genetic diversity, migration patterns

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Introduction

Three invasive leafminer species, *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard, and *Liriomyza trifolii* (Burgess), are damaging too many ornamental and vegetable crops (Spencer, 1973, 1990; Reitz *et al.*, 1999; Shiao, 2004). In China, *L. sativae* was first found in Hainan in 1993 (Kang, 1996), and then dispersed across most of provinces in mainland China within 5 years (Wang & Zhao, 1998). The *L. sativae* is especially difficult to control due to its polyphagous nature, high reproductive rate, and short development period. In recent decades, many studies have focused on the biology (Petitt & Wietlisbach, 1994; Araujo *et al.*, 2013), ecology (Yildirim *et al.*, 2012; Tian *et al.*, 2013), and control (Saberfar *et al.*, 2012; Saryazdi *et al.*, 2014) of *L. sativae* whether in

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Table 1. Collection site, code, number of studied individuals, and host of *Liriomyza sativae*.

Number	Collection location	Location code	Longitude (°E)	Latitude (°N)	Number of individuals tested	Host
1	Beijing	BJ	116.29	40.02	24	Cowpea
2	Baoding Hebei	HBBD	115.30	39.30	24	Kidney bean
3	Taiyuan Shanxi	SXTY	112.54	37.88	24	Towel gourd
4	Jinan Shandong	SDJN	117.08	36.71	24	Kidney bean
5	Yancheng Jiangsu	JSYC	120.14	33.37	24	Kidney bean
6	Shanghai	SH	121.32	31.22	24	Kidney bean
7	Hangzhou Zhejiang	ZJHZ	120.21	30.25	24	Kidney bean
8	Jinsha Hubei	HUBJS	114.19	29.62	24	Cucumber
9	Rongjiang Guizhou	GZRJ	108.41	26.03	24	Cowpea
10	Nanning Guangxi	GXNN	108.29	22.80	24	Kidney bean
11	Guangzhou Guangdong	GDGZ	113.30	23.11	24	Cowpea
12	Danzhou Hainan	HNDZ	109.41	19.57	24	Towel gourd

China or other countries. As for genetic aspects of *L. sativae*, there are several researches on this topic (Scheffer, 2005; Scheffer & Lewis, 2005; Scheffer *et al.*, 2006; Amin *et al.*, 2014), and we previously reported its complete mitochondrial genome (Yang *et al.*, 2011, 2013), developed microsatellite markers (Ji & Du, 2013) and preliminarily analyzed the genetic differentiation of the host- and geo-populations of *L. sativae* based on mitochondrial DNA (Du *et al.*, 2008, 2014; Wang *et al.*, 2008). Given its economic and ecological importance for agriculture, more comprehensive investigations of genetic structure and variation of *L. sativae* populations throughout its range in China, as well as the mechanism of its dispersion and invasion, are needed.

In this study, we investigated the genetic diversity, population structure, and invasion mechanism of this species, based on 288 individuals from 12 populations of *L. sativae* in China, using polymorphic microsatellite markers we developed previously (Ji & Du, 2013).

Materials and methods

Sampling and DNA extraction

A total of 288 *L. sativae* adults were collected from common vegetables (cowpea, kidney bean and towel gourd) in 12 locations across China (table 1). These individuals were preserved in 100% ethanol at −20°C until DNA extractions were performed. Genomic DNA was extracted from *L. sativae* adults using an AxyPrep Multisource Genomic DNA Miniprep Kit (Axygen, Suzhou, China) as recommended by the manufacturer.

Microsatellite analysis

Variable microsatellite loci previously identified for *L. sativae* by Ji & Du (2013) were examined and seven loci with strong, unambiguous banding patterns were selected for use in this study (Supplementary Table S1). These primers were attached to FAM, HEX and TAMRA fluorophores at the 5′ ends for genotyping. All PCR amplifications were performed in 25 µl reaction volumes containing 5 µl of 10 × PCR buffer, 50 ng of template DNA, 1.5 mM of $MgCl_2$, 2.5 µl of dNTPs (2.5 mM each), 1 µl each of the primer (10 µM), and 0.75 U of rTaq DNA polymerase (Takara, Dalian, China). PCR amplifications were performed, which included an initial denaturation at 94°C for 4 min, followed by 42 cycles of 50 s at 94°C, 50 s at 54–63°C depending on the primer pair (Supplementary Table S1), 1 min at 72°C, and a final extension for 10 min at 72°C. PCR products were analyzed using an ABI 3730XL DNA sequencer. Electropherograms were derived using Gene Scan 4.0 and used to deduce DNA fragment sizes using Gene Mapper 4.0 (Sangon Biotech, Shanghai).

Data analysis

Genetic polymorphism for each population was assessed by calculating the number of alleles (A), the effective number of alleles (Ae), and observed (H_O) and expected heterozygosities (H_E), as well as Nei's genetic distance (1978) using POPGENE version 1.31 (Yeh *et al.*, 1999).

In addition, the number of private alleles (Ap) was calculated by CONVERT 1.31 (Glaubitz, 2004). F_{ST} (Differentiation index) was performed by ARLEQUIN 2.0 (Excoffier *et al.*, 2005). For each population-locus combination, departure from Hardy–Weinberg expectation was assessed using exact tests (Guo & Thompson, 1992), with unbiased *P*-values estimated through a Markov-chain method (Guo & Thompson, 1992). The null allele frequency was calculated using Micro-Checker (Van Oosterhout *et al.*, 2004). Nei's genetic distance (1978) was then imported into PHYLIP computer package version 3.66 (Felsenstein, 1995) from which a neighbor joining phenogram was generated using the program NEIGHBOR using the unweighted pair-group with the arithmetic mean (UPGMA) method. Bootstrap values were calculated using 1000 replicates.

A Mantel test for isolation by distance was tested using the IBDWS web service (Jensen *et al.*, 2005) with 10,000 randomizations. In addition, STRUCTURE 2.3.3 (Pritchard *et al.*, 2000; Falush *et al.*, 2003) was used to determine a reasonable number of partitions (*K*) for the studied populations. Clustering results were then visualized by the program CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007). In this clustering analysis, we specified an initial range of potential genotype clusters (*K*) from 1 to 10 under the admixed model and the assumption of correlated allele frequencies among populations. For each value of *K*, ten runs were performed with 1,000,000 iterations discarded as burn-in, followed by an additional 1 million iterations. The most probable number of *K* values in the data was detected both by comparing the log probability of the data lnP (D) for each value of *K* across all ten runs of Structure and by examining the standardized second-order change of lnP (D), Δ*K* (Evanno *et al.*, 2005). We also carried out an independent

Table 2. Characterization of seven polymorphic microsatellite loci of *Liriomyza sativae*.

Locus		BJ $n = 24$	HBBD $n = 24$	SXTY $n = 24$	SDJN $n = 24$	JSYC $n = 24$	SH $n = 24$	ZJHZ $n = 24$	HUBJS $n = 24$	GZRJ $n = 24$	GXNN $n = 24$	GDGZ $n = 24$	HNDZ $n = 24$	$n = 288$
	A	10	13	8	11	9	13	7	8	11	10	10	9	28
	Ae	5.5823	9.3659	3.6233	9.4902	5.9077	9.5207	4.1439	4.9689	6.9150	6.1656	5.5823	6.1266	10.1710
JY4	Ho	0.6667	0.5833	0.5652	0.5455	0.5000	0.5417	0.5833	0.6000	0.5217	0.5000	0.4762	0.6818	0.5647
	H_E	0.8409	0.9122	0.7401	0.9154	0.8484	0.9140	0.7748	0.8192	0.8744	0.8573	0.8409	0.8562	0.9033
	P-HW	*	**	*	**	**	**	**	*	**	**	**	**	/
	N. f.	0.1098	0.2079	0.0918	0.2498	0.2480	0.2486	0.1352	0.1556	0.2390	0.2194	0.2819	0.1033	/
	A	4	6	3	8	8	9	5	6	6	6	5	5	13
	Ae	1.8927	3.9185	2.2851	3.0839	3.6993	4.5214	2.8918	2.0942	3.0633	3.2190	2.8544	2.6667	3.7763
JY14	Ho	0.3952	0.3913	0.0435	0.3810	0.3043	0.3478	0.3333	0.2500	0.2727	0.1429	0.3333	0.2273	0.2639
	H_E	0.4832	0.7614	0.5749	0.6922	0.7459	0.7961	0.6702	0.5359	0.6892	0.7062	0.6655	0.6395	0.7366
	P-HW	**	**	**	**	**	**	**	**	**	**	**	**	/
	N. f.	0.6785	0.3326	0.8597	0.3399	0.4201	0.3965	0.3161	0.3977	0.4401	0.6926	0.2595	0.4938	/
	A	7	6	7	6	9	8	6	8	10	8	9	8	12
	Ae	3.8146	4.3146	4.4651	5.1882	4.9442	4.9000	5.3088	5.0690	4.4641	4.1023	3.4326	4.0563	5.5604
JY15	Ho	0.7500	0.6667	0.7083	0.6190	0.7500	0.6667	0.7917	0.9524	0.5652	0.5714	0.5909	0.7083	0.6975
	H_E	0.7535	0.7846	0.7926	0.8269	0.8147	0.8153	0.8289	0.8223	0.7932	0.7747	0.7252	0.7695	0.8216
	P-HW	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	/
	N. f.	−0.0092	0.0628	0.0387	0.1033	0.0374	0.0889	0.0106	−0.0646	0.1713	0.1188	0.1014	0.0325	/
	A	5	8	5	9	8	11	4	7	10	10	7	6	16
	Ae	1.8529	4.5000	3.4388	4.0502	3.7921	7.9448	1.9692	4.5198	3.4239	6.6301	2.5813	2.4757	4.5528
JY46	Ho	0.4286	0.3333	0.5417	0.5909	0.5652	0.2917	0.5417	0.4500	0.5217	0.5455	0.4545	0.6364	0.4946
	H_E	0.4715	0.7943	0.7243	0.7706	0.7527	0.8927	0.5027	0.7987	0.7237	0.8689	0.6268	0.6099	0.7818
	P-HW	n.s.	**	*	**	n.s.	**	n.s.	**	*	**	*	n.s.	/
	N. f.	0.0352	0.4108	0.1179	0.0672	0.1394	0.5027	−0.0407	0.2581	0.1462	0.2188	0.1258	−0.0419	/
	A	6	4	6	4	9	4	4	7	7	3	7	6	12
	Ae	4.1143	3.0236	3.4491	2.9963	4.1439	2.6362	3.5556	5.1765	3.3391	2.1851	3.0440	3.5888	4.0234
JY60	Ho	0.7917	0.6667	0.7083	0.6500	0.7500	0.8333	0.7917	0.9091	0.5417	0.5909	0.6364	0.7083	0.7203
	H_E	0.7730	0.6835	0.7252	0.6833	0.7748	0.6339	0.7340	0.8256	0.7154	0.5550	0.6871	0.7367	0.7528
	P-HW	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	**	n.s.	n.s.	n.s.	/
	N. f.	−0.0303	−0.0048	−0.0055	0.0063	0.0069	−0.1767	−0.0431	−0.0759	0.1372	−0.0765	0.0442	0.0042	/
	A	5	5	4	6	5	8	4	6	6	7	7	6	13
	Ae	3.5446	3.5446	2.2948	2.7586	3.8755	4.0000	2.0140	3.4054	3.3481	3.3611	3.6528	3.2914	4.2204
JY70B	Ho	0.6667	0.5417	0.5417	0.4500	0.6957	0.5000	0.5000	0.7143	0.6522	0.4091	0.6818	0.7500	0.5866
	H_E	0.7332	0.7332	0.5762	0.6538	0.7585	0.7660	0.5142	0.7236	0.7169	0.7188	0.7431	0.7110	0.7644
	P-HW	*	n.s.	n.s.	*	n.s.	**	n.s.	n.s.	n.s.	**	n.s.	n.s.	/
	N. f.	0.0523	0.1468	0.0208	0.2087	0.0290	0.2117	−0.0235	−0.0143	0.0376	0.3266	0.0712	−0.0358	/
	A	8	10	7	10	9	8	8	9	11	9	8	7	20
	Ae	3.6456	6.0114	2.4668	2.9697	4.8889	2.8800	6.2951	6.2051	4.8608	4.1545	3.9190	2.1255	4.9286
JY72	Ho	0.5417	0.7391	0.5833	0.4286	0.5455	0.2917	0.6250	0.6364	0.4167	0.3182	0.6818	0.4583	0.5176
	H_E	0.7411	0.8522	0.6073	0.6794	0.8140	0.6667	0.8590	0.8584	0.8112	0.7770	0.7622	0.5408	0.7985
	P-HW	*	n.s	n.s	*	**	**	n.s	**	**	**	*	n.s	/
	N. f.	0.1436	0.0641	0.0390	0.1924	0.1937	0.4109	0.1427	0.1493	0.3044	0.4551	0.0384	0.0577	/

A, mean number of alleles; Ae, effective number of alleles; H_O, observed heterozygosities; H_E, expected heterozygosities; P-HW, test for Hardy–Weinberg equilibrium; n.s., denotes no significant deviation from Hardy–Weinberg equilibrium.
N. f., null allele frequence.
*denotes a significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$).
**denotes a significant deviation from Hardy–Weinberg equilibrium ($P < 0.01$).

Table 3. Genetic diversity of 12 *Liriomyza sativae* populations based on seven microsatellite loci.

Populations	Sample size	A	Ap	Ae(SD)	H_O(SD)	H_E(SD)
BJ	24	6.429	1	3.492 ± 1.299	0.606 ± 0.514	0.685 ± 0.146
HBBD	24	7.429	4	4.954 ± 2.160	0.560 ± 0.150	0.789 ± 0.076
SXTY	24	5.714	0	3.146 ± 0.824	0.527 ± 0.225	0.677 ± 0.089
SDJN	24	7.714	1	4.362 ± 2.418	0.524 ± 0.104	0.746 ± 0.096
JSYC	24	8.143	2	4.465 ± 0.814	0.587 ± 0.161	0.787 ± 0.039
SH	24	8.714	8	5.200 ± 2.587	0.496 ± 0.204	0.784 ± 0.105
ZJHZ	24	5.429	0	3.740 ± 1.634	0.595 ± 0.163	0.698 ± 0.143
HUBJS	24	7.286	3	4.491 ± 1.374	0.645 ± 0.247	0.769 ± 0.111
GZRJ	24	8.714	4	4.202 ± 1.369	0.499 ± 0.122	0.761 ± 0.067
GXNN	24	7.571	2	4.260 ± 1.606	0.440 ± 0.163	0.751 ± 0.106
GDGZ	24	7.571	3	3.581 ± 0.997	0.551 ± 0.133	0.722 ± 0.070
HNDZ	24	6.714	0	3.476 ± 1.347	0.596 ± 0.188	0.695 ± 0.106

A, mean number of alleles; Ap, private alleles; Ae, effective number of alleles; H_O, observed heterozygosities; H_E, expected heterozygosities.

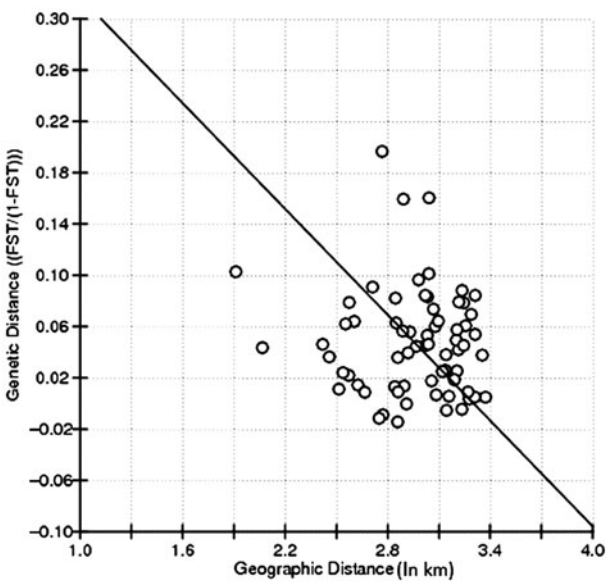

Fig. 1. Scatter plots of genetic distance versus geographical distance for pairwise population comparisons.

analysis of spatial structure using the R package GENELAND 3.1.4 (Guillot *et al.*, 2005*a*, *b*), which has been explicitly tested for robustness in the presence of null alleles. Like STRUCTURE, the software uses a markov chain monte carlo (MCMC) strategy to determine the most likely number of populations (K), and assigns individuals to the most appropriate population based on individual multi-locus genotypes. We carried out ten independent MCMC simulations (10,000,000 iterations, thinnings of 10,000 iterations) using the spatial model, null allele model and correlated allele frequency model to analyze, during which we allowed K to vary between 1 and 12. Analysis of molecular variance (AMOVA) analyses were performed using ARLEQUIN 2.0 (Excoffier *et al.*, 2005). We used MIGRATE version 3.6 (Beerli, 2006) to estimate the effective number of migrants ($M = m\,\mu^{-1}$) entering and leaving each population per generation to verify if there was asymmetrical gene flow between populations. We relied on Bayesian search strategy and used ten short chains, three long chains with 10,000 trees discarded as initial 'burn-in', replicates = YES: 5, randomtree = YES, heating = ADAPTIVE: 1{1 1.2 1.5 3.0}, and ran migrate four times, verifying consistency in our results. The estimates from the final run are reported here. The method described Wilcoxon's signed-rank test (Piry *et al.*, 1999), which were performed using the Bottleneck version 1.2.02 (Piry *et al.*, 1999), to evaluate whether the natural populations examined here had experienced recent bottlenecks under three mutational models: an infinite allele model (IAM), a stepwise mutation model (SMM) and a two-phase model (TPM).

Results

Genetic diversity and Hardy–Weinberg equilibrium (HWE)

All of the seven microsatellite markers (Supplementary Table S1) proved to be polymorphic and informative. In detail, the number of alleles per locus ranged from 12 (JY60) to 28 (JY4) with an average of 16.3. The expected heterozygosities (H_E) ranged from 0.7366 (JY14) to 0.9033 (JY4), while the observed heterozygosities (H_O) ranged from 0.2639 (JY14) to 0.7203 (JY60) (table 2). In addition, there were significant deviations from HWE at multiple loci from all sampling locations. For example, BJ and SXTY showed significant departure from HWE for only one locus after sequential Bonferroni's correction ($P < 0.01$), but five loci for SH, GZRJ and GXNN populations ($P < 0.01$). Of these loci, JY60 showed no significant departure from HWE (table 2), while JY4 showed significant departure from HWE in nine populations ($P < 0.01$), which may be the result of heterozygote deficiencies in all cases. What's more, null allele frequencies varied from −0.1767 (JY60) to 0.8507 (JY14), and three of the seven loci (JY15, JY60, and JY70B) showed low levels of estimated null alleles (table 2).

The average number of alleles (A) per population varied from 5.429 (ZJHZ) to 8.714 (SH and GZRJ), and SH population also possessed the most effective number of alleles (Ae), whereas SXTY had the least. Private alleles were distributed in most of the populations except SXTY, ZJHZ, and HNDZ with the most (8) in the SH population (table 3). H_E and H_O values were moderate, ranging from 0.677 to 0.789 and 0.440–0.645, respectively. All values of H_O were less than that of H_E.

Table 4. Pairwise F_{ST} (below the diagonal) and P value (above the diagonal) of *Liriomyza sativae*.

Populations	BJ	HBBD	SXTY	SDJN	JSYC	SH	ZJHZ	HUBJS	GZRJ	GXNN	GDGZ	HNDZ
BJ		0.04688	0.00000	**0.18457**	**0.27051**	0.00000	0.00000	0.00391	**0.10156**	0.00000	0.02344	0.02344
HBBD	0.02043		0.00000	0.03906	0.04102	0.00000	0.00000	0.02734	0.01953	0.00000	0.00000	0.00000
SXTY	**0.08074**	**0.05724**		0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
SDJN	0.01010	0.02429	**0.06498**		0.00684	0.00195	0.00000	0.01074	**0.32227**	0.00000	**0.06250**	**0.26270**
JSYC	0.00647	0.02129	**0.07580**	0.03951		0.00000	0.00000	0.00000	0.00977	0.00000	0.00000	0.00000
SH	**0.09764**	**0.08639**	**0.13075**	**0.07688**	**0.10985**		0.00000	0.00098	0.00195	0.02148	0.00000	0.00000
ZJHZ	**0.05114**	**0.06539**	**0.16366**	0.04960	**0.07555**	**0.11127**		0.00000	0.00000	0.00000	0.00000	0.00000
HUBJS	0.03246	0.02377	**0.05073**	0.02758	0.04491	**0.06728**	**0.07168**		0.01270	0.00000	0.00000	0.00000
GZRJ	0.01802	0.03054	**0.09679**	0.00717	0.03276	**0.08158**	0.04604	0.02534		0.00000	**0.93359**	**0.27930**
GXNN	**0.11467**	**0.11180**	**0.14493**	**0.10388**	**0.13076**	0.03580	**0.13877**	**0.08108**	**0.10814**		0.00000	0.00000
GDGZ	0.02365	**0.06040**	**0.11341**	0.01881	0.04781	**0.10465**	**0.06087**	**0.05111**	−0.00644	**0.13538**		**0.39844**
HNDZ	0.02251	**0.06205**	**0.11424**	0.00718	**0.05174**	**0.08831**	**0.07143**	0.04889	0.00693	**0.10770**	0.00311	

Bold indicates $F_{ST} > 0.05$ (below the diagonal) and $P > 0.05$ (above the diagonal).

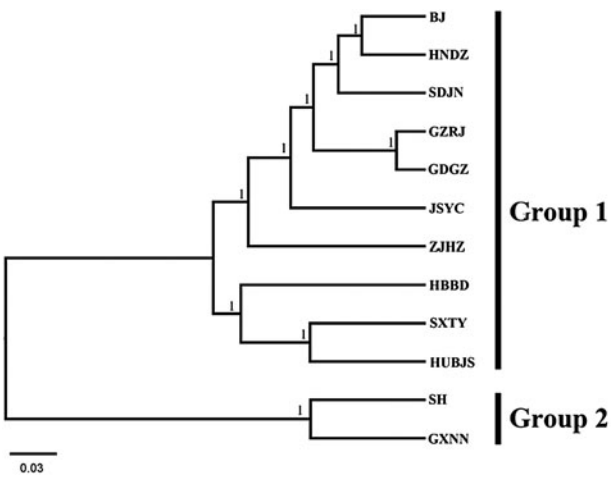

Fig. 2. UPGMA dendrogram between 12 *Liriomyza sativae* populations.

Mantel test for matrices of different loci

To explore if the dispersal of *L. sativae* was limited by distance, a Mantel test for isolation by distance was constructed. Our results showed that the isolation by distance correlation was insignificant and slightly negative ($Z = 9.1746$, $r = -0.0814$, $P = 0.7610$; fig. 1), suggesting a possible anthropogenic influence on the spread of the *L. sativae* in China, possibly the transportation of vegetables.

Population genetic structure

The genetic differentiation among 12 *L. sativae* populations in China was estimated by pairwise F_{ST} values between each pair. All population pairwise comparisons were no more than 0.15, and most of them ranged from 0.05 to 0.15 (table 4), revealing only moderate genetic differentiation, since pairwise F_{ST} is usually regarded as a standard measurement of population differentiation, and Wright (1938) assumed that $0.05 < F_{ST} < 0.15$ exhibited moderate level of genetic diversity. We also found no genetic differentiation between GZRJ and GDGZ populations ($F_{ST} = -0.00644$). Conversely, the F_{ST} value of SXTY was significantly higher ($F_{ST} = 0.16366$) than that of ZJHZ (table 4). In addition, the molecular diversity between most of the populations was significant ($P < 0.05$) (table 4).

The UPGMA tree generated from the genetic distance matrix (Nei) grouped the 12 *L. sativae* populations into two major groups. Unexpected, SH and GXNN populations formed one cluster and the remaining ten populations were assigned to the other cluster, which we named Group 1 (fig. 2).

Further STRUCTURE analysis of these 12 populations showed the same major patterns as those detected by the analysis of the UPGMA dendrogram. The highest ΔK value was obtained for $K = 2$ ($\Delta K = 469.25$; fig. 3A). Fig. 3B shows the proportion of each population that contributed to each of the two clusters. Likewise, most individuals from the SH and GXNN populations were grouped into one group.

The most likely number of inferred populations was $K = 9$ based on analysis in GENELAND. Although the results from GENELAND indicated a greater degree of population structure compared to the STRUCTURE results ($K = 2$), there were no qualitative disagreements between the two analyses. For example, SH and GXNN clustered together based on the GENELAND analysis (fig. 4) also clustered together in the STRUCTURE analysis (fig. 3), whereas, there were no obvious clusters when $K = 9$ in STRUCTURE (fig. 3C).

The AMOVA analysis revealed that more than 89% of variation was among individuals within populations when $K = 2$. However, only a small portion of the variation attributed among populations was within groups (4.27%) and among groups (6.73). In addition, our results revealed significant genetic differentiation between *L. sativae* both among populations ($F_{SC} = 0.04579$, $P < 0.0001$) and among groups ($F_{CT} = 0.06726$, $P < 0.05$).

Patterns of gene flow

Estimates of gene flow calculated by MIGRATE indicated that the levels of gene flow between populations of *L. sativae* are generally high. Unidirectional estimates of M ranged from 40.1 (HNDZ→BJ) to 817.7 (SH→ZJHZ) (Supplementary Table S2). Of the 66 pairwise comparisons, 14 had asymmetrical gene flow, as indicated by non-overlapping 95% confidence intervals (CI) around the estimate of M into each population (indicated in bold in Supplementary Table S2). We also marked asymmetrical gene flow on the map (fig. 5). Overall, the trend of asymmetrical gene flow was from south to north, with southern populations having an asymmetric migration toward northern populations, especially from HNDZ

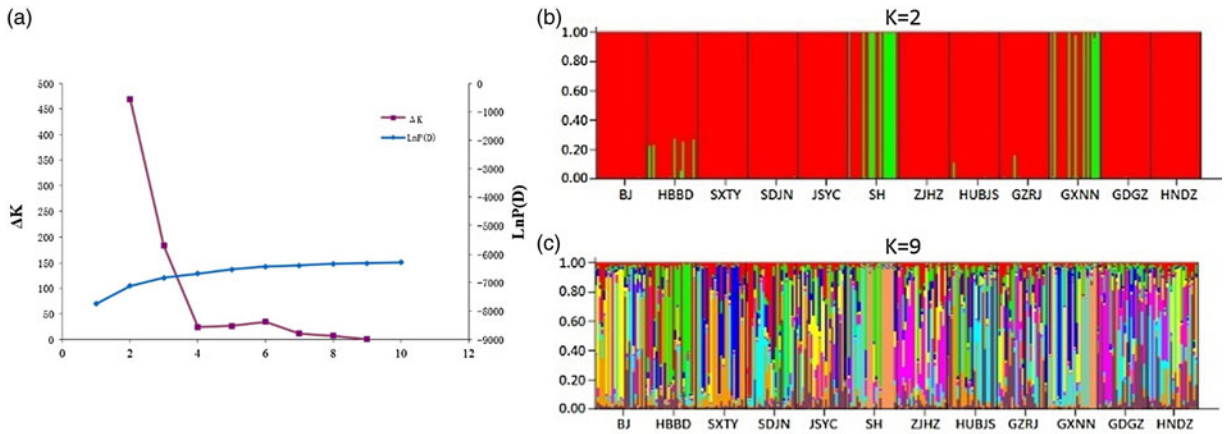

Fig. 3. Clustering analysis by STRUCTURE for full-loci dataset. (A) Inference of the number of genetic cluster (*K*) for *Liriomyza sativae* populations. (B) Proportion of the genome of each individual assigned to each of the two clusters. Each individual is represented by a vertical bar. (C) Proportion of the genome of each individual assigned to each of the nine clusters. Each individual is represented by a vertical bar.

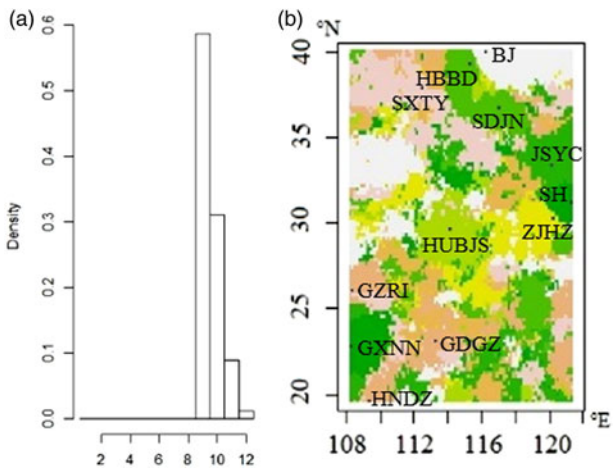

Fig. 4. (A) Posterior distribution of the estimated number of populations using GENELAND. (B) Population structure inferred in GENELAND at $K = 9$. The black dots indicate the sampling locations. The abscissa and ordinate show the coordinates of sampling locations.

to GZRJ and HUBJS, then to SXTY and HBBD, respectively (fig. 5).

Bottleneck test

Bottleneck analysis with 12 populations of *L. sativae* across China showed that none of these populations exhibited excess heterozygosity under the SMM. Only two populations (HBBD and ZJHZ) had a statistically significant excess of heterozygotes under the TPM. However, heterozygosity excess was observed in all populations except five (HNDZ, GDGZ, BJ, GZRJ, and SDJN) under the IAM (table 5), suggesting that most *L. sativae* populations that we studied had not undergone a genetic bottleneck.

Discussion

Low genetic diversity and moderate genetic structure

All of the microsatellite markers exhibited high polymorphism (table 2), whereas A, Ap, and Ae values (table 3) suggests that the genetic diversity within each population of *L. sativae* in China is relatively low. The loss of genetic diversity is consistent with another research which showed that mitochondrial variation of *L. sativae* across sampled New World populations was higher than that from Old World populations (Scheffer & Lewis, 2005). Indeed, most successful invasive insect species show a reduction in genetic diversity from the native to invaded areas (Ahern *et al.*, 2009; Lozier *et al.*, 2009; Chu *et al.*, 2011; Yang *et al.*, 2012). The most likely reason for reduced variation of *L. sativae* is that initial introductions to other places such as South and Southeast Asia involved bottlenecks and *L. sativae* has then spread with reduced variation from those locations. What's more, genetic drift (Baker *et al.*, 2003; Schmitt *et al.*, 2005) and selection pressure (Suarez & Tsutsui, 2008) may not be the reasons for that as this pest typically reached outbreak proportions shortly after they arrive (Kang, 1996; Wang & Zhao, 1998). In addition, the reduced variation character is distinct from native species such as *Sesamia inferens* (Walker) (Tang *et al.*, 2014*a*) or another exceptional invasive species, *Bactrocera dorsalis* (Hendel) (Wan *et al.*, 2011), which exhibited fairly high levels of genetic diversity.

We noted that three of the 12 populations (SH, GZRJ, and GXNN) in this study deviated significantly from HWE (table 2), which is obviously associated with significant heterozygote deficiency. The H_O of these populations was much lower than H_E at less than 0.500 (table 3). This may arise from the Wahlund effect (http://www.dorak.info/genetics/popgen.html) related to recurrent inbreeding and subpopulation structure. It is reasonable to conclude that inbreeding has a major impact on heterozygote deficiency since sampling mainly from greenhouses restricts the randomness of mating, and since this species produces multiple generations in a year, unlike some other species with special reproductive strategies such as *Aleurocanthus spiniferus* (Quaintance) (Tang *et al.*, 2014*b*, 2015).

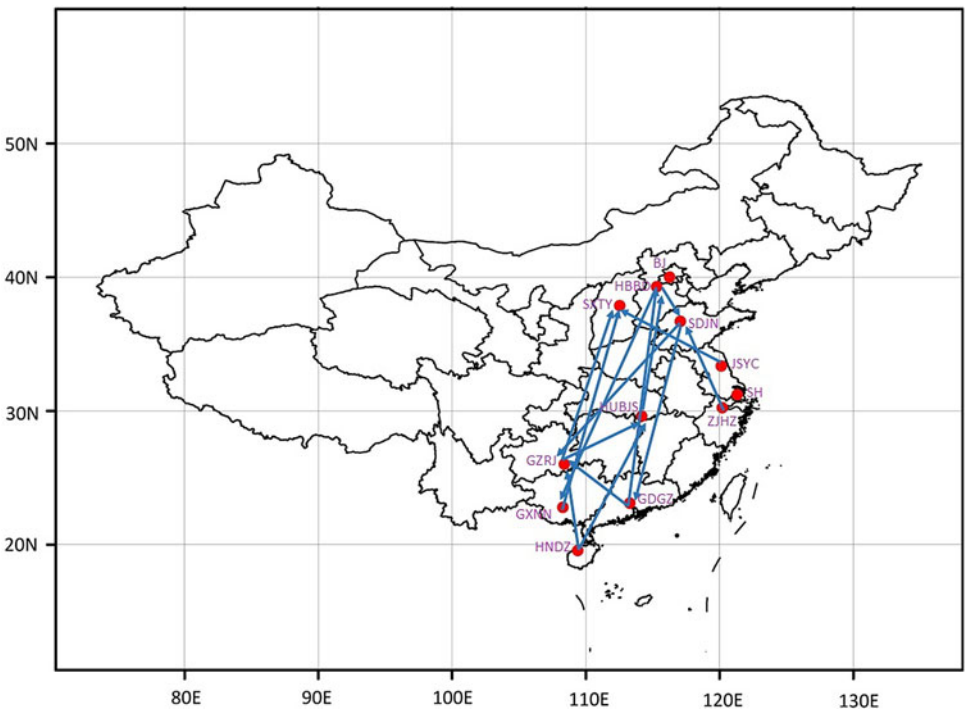

Fig. 5. Asymmetric gene flow between *Liriomyza sativae* populations. Values of gene flow (*M*) were showed in Supplementary Table S2. Maps were created using Esri's ArcGIS platform (http://www.esri.com/software/arcgis). Arrows represent asymmetric gene flow.

Table 5. Bottleneck test for *Liriomyza sativae* populations.

Population	IAM		TPM		SMM	
	Hde/Hex	Wilcoxon sign-rank test	Hde/Hex	Wilcoxon sign-rank test	Hde/Hex	Wilcoxon sign-rank test
BJ	2/5	0.05469	4/3	0.59375	5/2	0.98828
HBBD	0/7	0.00391	0/7	0.00391	2/5	0.14844
SXTY	1/6	0.02734	2/5	0.40625	4/3	0.81250
SDJN	2/5	0.28906	4/3	0.65625	4/3	0.85156
JSYC	0/7	0.00391	4/3	0.46875	5/2	0.98047
SH	1/7	0.01953	3/4	0.28906	4/3	0.81250
ZJHZ	0/7	0.00391	2/5	0.01953	4/3	0.40625
HUBJS	1/6	0.00781	1/6	0.14844	3/4	0.65625
GZRJ	3/4	0.28906	5/2	0.96094	7/0	1.00000
GXNN	0/7	0.00391	2/5	0.14844	5/2	0.98047
GDGZ	2/5	0.34375	6/1	0.99219	7/0	1.00000
HNDZ	2/5	0.18750	3/4	0.59375	6/1	0.99219

Hde, heterozygote deficiency; Hex, heterozygote excess.

Mutation/drift equilibrium of populations usually leads to an equal rate of heterozygosity excess or deficiency (Maruyama & Fuerst, 1985). However, natural populations might not remain in a steady state, and when a population has contracted in size there is a transient deficiency in the number of alleles present in the population compared with that expected in a population in equilibrium that has an equivalent heterozygosity (Maruyama & Fuerst, 1985). Thus, we could explore population dynamics by assessing heterozygosity excess or deficiency (Cornuet & Luikart, 1996). It is well known that the most useful markers for detecting bottlenecks are those evolving under IAM, TPM, and SMM (Di Rienzo *et al.*, 1994; Cornuet & Luikart, 1996; Primmer *et al.*, 1998; Estoup & Cornuet, 2000). Of these, the strict SMM is obviously the most conservative model. Our results showed that heterozygosity excess was not obvious under the SMM for all the populations, although two and seven populations had a statistically significant excess of heterozygotes under the TPM and IAM, respectively. Considering the TPM is thought to more closely simulate microsatellite mutation (Primmer *et al.*, 1998;

Table 6. Results of AMOVA test of *Liriomyza sativae* based on seven microsatellite loci.

Group numbers	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P*-value
$K = 2$	Among groups	1.00	32.098	0.15756 Va	6.73	$F_{CT} = 0.06726$	0.01271 ± 0.00273
	Among populations within groups	10.00	68.873	0.10005 Vb	4.27	$F_{SC} = 0.04579$	0.00000 ± 0.00000
	Within populations	564.00	1175.854	2.08485 Vc	89.00	$F_{ST} = 0.10998$	0.00000 ± 0.00000
	Total	575.00	1276.825	2.34			

Estoup & Cornuet, 2000), coupled with the low genetic diversity, we may conclude that only a few populations experienced a population bottleneck (HBBD and ZJHZ) (table 5). These bottlenecks could be due to two potential factors. The first is interspecific competition among these three *Liriomyza* species. Recent surveys have found that *L. trifolii* has successfully replaced *L. sativae* and become the dominant population in most regions in China (Gao *et al.*, 2012; Xiang *et al.*, 2012; Wang, 2013; Yi, 2014). The second factor is the effects of mortality such as natural enemies or pesticides. Previous studies have shown that natural enemies or pesticides are effective in regulating *Liriomyza* populations whether in native or invaded regions (Johnson, 1993; Murphy & LaSalle, 1999; Rauf *et al.*, 2000; Chen *et al.*, 2003; Bai *et al.*, 2009), which may reduce the population size of *L. sativae* even though resistance to pesticides has become more prevalent.

Analysis of *L. sativae* population structure in China based on F_{ST} and AMOVA analysis revealed moderate ($0.05 < F_{ST} < 0.15$) but significant differentiation (global $F_{ST} = 0.10998$, $P < 0.001$; $F_{ST} = 0.08008$, $P < 0.001$) (tables 4 and 6). Such genetic diversity may be due to the relatively high number of generations of this species each year (for example, 14–17 generations per year in Guangdong Province), which may increase its genetic diversity to some extent. Moderate population subdivision exists despite the species' capacity for long-distance dispersal, which mainly occurs through wind transport of adults or through transfer of infested host plants, especially Solanaceae, Cucurbitaceae, and Leguminosae. The SH population, with more private alleles (Ap = 8) seems to be an isolated population with a significantly F_{ST} value than the other populations except GXNN, which is consistent with phylogenetic analysis (figs 2–4) but at odds with the great geographic distance between the two populations. This suggests that anthropogenic transport is the most likely explanation for the large-scale dispersion of *L. sativae*, as there was no isolation-by-distance effect found in the populations we studied. The moderate differentiation and the absence of isolation by distance may be due to the relatively high gene flow, especially over long distance. Another explanation for such moderate differentiation is the effect of host. On the basis of our previous studies, the trend of genetic differentiation in the host populations was consistent with the preference of *L. sativae* to the plant hosts based on mitochondrial cytochrome oxidase subunit I (mtDNA-COI) gene, the ribosomal internal transcribed spacer 1 (rDNA-ITS1) gene (Wang *et al.*, 2008) and *β-tubulin* gene (Du *et al.*, 2008).

Migration patterns

The direction of gene flow was asymmetrical, generally from southern to northern populations (fig. 5), which potentially reveals the invasion route of *L. sativae*. This hypothesis is supported by the fact that *L. sativae* was first found in Hainan in 1993 (Kang, 1996), and then dispersed across most of provinces in mainland China (Wang & Zhao, 1998). More importantly, we noticed that the Hainan population displayed two asymmetric migration routes north to the GZRJ and HUBJS populations, which then reached the SXTY and HBBD populations, respectively (fig. 5). Furthermore, we also found that southern populations of *L. sativae* did not show higher genetic diversity (table 3). There appears to be no Bridgehead effect for the *L. sativae* invasion, as the leafminer expanded quickly in mainland China and dispersed to 21 provinces in only 5 years (Wang & Zhao, 1998). This is supported by the high levels of gene flow (40.1–817.7, Supplementary Table S2). Above all, unlike other invasive species such as *Frankliniella occidentalis* (Yang *et al.*, 2012) and *Solenopsis invicta* Buren (Ascunce *et al.*, 2011), the leafminer did not show an established a bridgehead population in Hainan Province, possibly because one of the main means of spreading of *L. sativae* is anthropogenic transport through the movement of horticultural products. Of course, we cannot rule out wind as a factor as well. Our study is the first to examine the migration patterns of *L. sativae* in China, which might help to improve management strategies for *L. sativae*, preventing contaminated plants from being transported to other regions.

Supplementary Material

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S0007485315000905.

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