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Investigation of the genetic diversity of an invasive whitefly (*Bemisia tabaci*) in China using both mitochondrial and nuclear DNA markers

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Abstract

It is often considered that reduced genetic variation due to bottlenecks and founder effects limits the capacity for species to establish in new environments and subsequently spread. The recent invasion (during the past five years) of an alien whitefly, one member of *Bemisia tabaci* cryptic species complex, referred to as Mediterranean (herein referred to as Q-type) in Shandong Province, China, provides an ideal opportunity to study the changes in genetic variation between its home range in the Mediterranean region and its invasion range. Using both the mitochondrial cytochrome oxidase I (mtCOI) and nuclear (microsatellite) DNA, we show that Q in Shandong likely originated in the western Mediterranean. We also found that the haplotype diversity was low compared with its presumed geographic origin, whereas microsatellite allele diversity showed no such decline. A key factor in invasions is the establishment of females and so bottleneck and founder events can lead to a very rapid and considerable loss of mitochondrial diversity. The lack of haplotype diversity in Shandong supports the interpretation that, at one or more points between the western Mediterranean and China, the invading Q lost haplotype diversity, most probably through the serial process of establishment and redistribution through trade in ornamental plants. However, the loss in haplotype diversity does not necessarily mean that nuclear allelic diversity should also decline. Provided females can mate freely with whichever males are available, allelic diversity can be maintained or even increased relative to the origin of the invader. Our findings may offer some explanation to the apparent paradox between the concept of reduced genetic variation limiting adaptation to new environments and the observed low diversity in successful invaders.

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Introduction

Biological invasions have considerable combined ecological and economic impacts (Mack *et al*., 2000; Pimentel *et al*., 2000; Perrings *et al*., 2002; Sax *et al*., 2005). It is often argued that one of the key factors affecting the establishment likelihood of species and their subsequent capacity to invade is propagule pressure (Lockwood *et al*., 2007) and the low levels of genetic diversity associated with introductions of small numbers of individuals. Despite this, many introduced species that have experienced bottlenecks and founder effects during initial introduction have become invasive (e.g. Goodisman *et al*., 2001; Meunier *et al*., 2001; Charbonnel *et al*., 2002; Downie, 2002; Golani *et al*., 2007). These suggest that the loss of genetic diversity may not limit the capacity for some species to become invasive (Sakai *et al*., 2001; Golani *et al*., 2007; Miura, 2007; Gammon & Kesseli, 2010).

Molecular genetic approaches are useful in order to explore the ecological and evolutionary aspects of biological invasions and their associated influences on the genetic structure and variation of an invasive species (Miura, 2007). However, in most cases, only one type of marker, such as mitochondrial cytochrome oxidase I (mtCOI) (Bucciarelli *et al*., 2002; Downie, 2002; Facon *et al*., 2003; Kolbe *et al*., 2004; Golani *et al*., 2007) or nuclear (microsatellite) DNA (Tsutsui *et al*., 2000; Meunier *et al*., 2001; Charbonnel *et al*., 2002; Giraud *et al*., 2002; Johnson & Starks, 2004), has been used. This limits our capacity to more fully explore the consequences of genetic variation on invasion potential.

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a cryptic species complex which may contain at least 24 morphologically indistinguishable species (Dinsdale *et al*., 2010). Two members of this complex, referred to by Dinsdale *et al*. (2010) as Middle East - Asia Minor 1 (herein referred to as B-type) and Mediterranean (herein referred to as Q-type) have now invaded well beyond their home ranges as a consequence of trade in ornamental plant species (Cheek & MacDonald, 1994; Dalton, 2006). In the 1980s, the B-type form of *B. tabaci* became a severe outbreak pest in the southwestern USA and is now regarded as one of the most globally damaging pests in open field or protected cropping production (Brown, 2010). The Q-type was originally thought to be restricted to the Iberian Peninsula (Guirao *et al*., 1997). However, later studies have shown that its native range covers the countries bordering the Mediterranean Basin and possibly extending into some Sub-Saharan African countries (Frohlich *et al*., 1999; De Barro *et al*., 2000; Boykin *et al*., 2007; Dinsdale *et al*., 2010).

In China, *B. tabaci* Q-type was first detected in Yunnan Province in 2003 (Chu *et al*., 2005). In the following years, it has been reported in Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hainan, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shandong, Shanxi, Tianjin, Xinjiang and Zhejiang provinces (Chu *et al*., 2006 and Genbank records). In 2005, Q-type was first reported from Shandong Province, one of China's most important agricultural provinces where the B-type had established ten years earlier (Chu *et al*., 2007). Since then, Q has become the predominant whitefly (Chu *et al*., 2010a,b). The recent invasion by Q provides an ideal opportunity to study the changes in genetic variation of the invader during the invasion process.

The aim of this study was, thus, to compare the genetic variation of Q in China and the Mediterranean Basin using both mtCOI and nuclear (microsatellite) DNA markers. In doing so, we were interested in identifying the Mediterranean origin of Q in China and the consequences of invasion on genetic diversity.

Materials and methods

DNA methods

Adult Q-type *B. tabaci* were collected from different host plants from seven representative locations (Dezhou, Liaocheng, Jinan, Shouguang, Zibo, Zaozhuang and Linyi) throughout Shandong Province, China between 2005 and 2008 (Chu *et al*., 2010a,b). Each collection involved sampling whitefly from every second available plant until at least 100 adults had been collected (table 1). These were collected live and placed into tubes containing 95% ethanol and then stored at –20°C. The collections of Q-type from the Mediterranean region were made from a number of different countries (table 1). They were likewise collected and stored as above.

DNA was extracted from individual whitefly as described in De Barro & Driver (1997). A total of 91 Q individuals collected in 2008 from the seven aforementioned locations throughout Shandong Province were amplified using the primers C1-J-2195 and L2-N-3014 and then sequenced (Frohlich *et al*., 1999). The sequencing of the mtCOI from the 5′ end yielded a 646-bp fragment.

Because the amplification efficiencies using C1-J-2195 and L2-N-3014 have since been shown to be low and often result in a less than optimal quantity of product (Shatters *et al*., 2009), we also used the designed primers R-BQ-2195 (5′-CTGGTTYTTTGGTCATCCRGARGT-3′) and F-BQ-2819 (5′-CTGAATATCGRCGAGGCATTCC-3′) to obtain additional mtCOI sequences (Chu *et al*., 2010b). A total of 520 mtCOI fragments of Q individuals from Shandong, China (2005–2008) and Mediterranean countries were amplified using these primers to give a 623-bp fragment, which was then sequenced (Chu *et al*., 2010a). Of these sequences, 95 were from the Mediterranean region and 425 were from Shandong Province, China (insect samples collected in 2005–2008) (table 1). These sequences were aligned with Clustal W (Thompson *et al*., 1994); sequences were then checked for indels and numts. The unknown sequences were compared against the consensus sequences for each of the 24 putative species identified by Dinsdale *et al*. (2010). Each unknown sequence is associated with the consensus sequence with which it has the lowest divergence difference, i.e. the match which is closest to 100%. Assignment also requires that divergence from the consensus sequence is <3.5%. These various sequences, produced using the two primer pairs, thereby were used to determine the evolutionary origin of the Q collections in Shandong Province, China.

In addition, a suite of six microsatellites (BEM6, BEM11, BEM18, BEM25, BEM31 and BEM37) was also used to amplify

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Table 1. Data of *Bemisia tabaci* samples used.

Collection site (code)	Collection time	Host plant	MtCOI (646 bp)	Microsatellite
Mediterranean				
Crete, Greece (Greece 1)	2007.5	Cucumber		5
Crete, Greece (Greece 2)	2007.6	Melon		10
Crete, Greece (Greece 3)	2007.6	Melon		
Mansoura, Egypt (Egypt)	1996	Lantana		
Acate, Sicily, Italy (Italy)	2004	Tomato		
Morocco (Morocco 1)	2007	Tomato		15
Souss Valley, Morocco (Morocco 2)	2007	Red bell pepper		7
Almaria, Spain (Spain 1)	2007.5	Tomato		15
Almaria, Spain (Spain 2)	2006.3	Tomato		15
Spain (Spain 3)	1993	–		
Sudan (Sudan)	–	–		
Spilt, Croatia (Croatia 1)	2001.9	Poinsettia		13
Pula, Croatia (Croatia 2)	2001.10	Poinsettia		15
Spilt, Croatia (Croatia 3)	2007.10	Poinsettia		15
Rogoznica, Croatia (Croatia 4)	2001.9	Cucumber		
Komiza, Vis, Croatia (Croatia 5)	2001.9	*Ipomea purpurea*		
Bosnia and Herzegovina (Bosnia and Herzegovina)	2007.10	Solanaceae, Cucurbitaceae		15
Cyprus (Cyprus)	2006	Cotton		15
China (Shandong)				
Jinan (Jinan 1)	2008.8	Cotton	12	15
Jinan (Jinan 2)	2008.8	Japanese Hop		15
Liaocheng (Liaocheng 1)	2008.8	Cotton	10	15
Liaocheng (Liaocheng 2)	2008.8	Japanese Hop		15
Dezhou (Dezhou 1)	2008.8	Cotton	12	15
Dezhou (Dezhou 2)	2008.8	Japanese Hop		15
Shouguang (Shouguang 1)	2008.8	Cotton	10	15
Shouguang (Shouguang 2)	2008.8	Japanese Hop		15
Zibo (Zibo 1)	2008.8	Cotton	12	15
Zibo (Zibo 2)	2008.8	Japanese Hop		15
Zaozhuang (Zaozhuang 1)	2008.8	Cotton	18	15
Zaozhuang (Zaozhuang 2)	2008.8	Japanese Hop		15
Linyi (Linyi)	2008.8	Cotton	17	15

loci from 335 individuals collected in 2008 from the Mediterranean region and Shandong as described by De Barro *et al*. (2003). The PCR reaction was mixed with 6× loading buffer, denatured for 3 min at 96°C, loaded on a 7% SDS (sodium dodecyl sulfate) polyacrylamide gel and analysed electrophoretically.

Data analysis

As of 3 Feb 2010, we have retrieved 1490 mtCOI sequences in Genbank. Of these, 96 were found to be unique haplotypes that fall within the Mediterranean putative species group (Q) based on the 3.5% pairwise genetic divergence bounds identified by Dinsdale *et al*. (2010) as being the boundary separating different species. This boundary is supported by evidence for either complete or partial mating isolation between a number of the putative *B. tabaci* 'species' (Xu *et al*., 2010). The work of Dinsdale *et al*. (2010) suggests that the home range of the Mediterranean putative species group extends from East and West Africa, through Sudan to the countries bordering the Mediterranean Basin. We, therefore, compared the four haplotypes found in Shandong Province with those from the presumed home range.

In order to compare the genetic structure using mtCOI and microsatellite markers, only Q-type individuals collected in 2008 from Shandong Province and the Mediterranean (Bosnia and Herzegovina, Croatia, Cyprus, Egypt, Greece, Italy, Morocco, Spain and Sudan) were used. A series of genetic parameters for mtCOI were estimated for 91 individuals from Shandong Province and 95 from the Mediterranean countries using DnaSP 5.0 (Librado & Rozas, 2009). We computed the number of polymorphic (segregating) sites (S), the total number of mutations (η) (Watterson, 1975), the average number of nucleotide differences (K) (Tajima, 1983), the number of haplotypes (H), the haplotype diversity (Hd) (Nei, 1987) and the nucleotide diversity (π), defined as the average number of pairwise nucleotide differences per site (Nei, 1987), the nucleotide diversity with Jukes and Cantor correction [π(JC)] (Lynch & Crease, 1990) within each population and region. To assess the possibility of population change or selection, we also calculated Tajima's D Fu's F from the sequences within each sampling site and region. All analyses were performed for all collections with the exception of Egypt, Italy, Sudan and Croatia 3, Croatia 4 and Croatia 5 where the number is less than five.

For microsatellites, the alleles for each locus among collections (from China, Greece, Egypt, Italy, Morocco,

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Table 2. Statistics of variation in mtCOI sequences from the presumed origin in Morocco and Spain and those from different locations in Shandong, China.

Regions and populations (Individual no.)	S	η	H	H_d(SD)	π(SD)	K	π(JC)	D_{Taj}(P)	Fu's F(P)
Western Mediterranean (38)	4	5	7	0.777(0.045)	0.002969(0.002098)	1.327	0.003	0.29674(ns)	0.20390(ns)
Morocco (16)	4	5	5	0.783(0.056)	0.003654(0.002547)	1.633	0.004	0.27667(ns)	0.46967(ns)
Morocco 1 (7)	3	3	3	0.667(0.160)	0.003409(0.002649)	1.524	0.003	1.10686(ns)	1.35330(ns)
Morocco 2 (9)	4	5	4	0.694(0.147)	0.003480(0.002592)	1.556	0.003	−0.65421(ns)	−0.13592(ns)
Spain (22)	2	2	4	0.714(0.064)	0.002266(0.001763)	1.013	0.002	1.91752(ns)	1.31254(ns)
Spain 1 (9)	0	0	1	0.000(0.000)	0.000000(0.000000)	0.000	0.000	na	na
Spain 2 (7)	2	2	3	0.714(0.127)	0.002557(0.002141)	1.143	0.003	1.64955(ns)	1.37408(ns)
Spain 3 (6)	0	0	1	0.000(0.000)	0.000000(0.000000)	0.000	0.000	na	na
China (91)	1	1	2	0.180(0.050)	0.000403(0.000587)	0.180	0.000	−0.09821(ns)	0.49834(ns)
Dezhou 1 (12)	1	1	2	0.167(0.134)	0.000373(0.000605)	0.167	0.000	−1.14053(ns)	−1.32974(ns)
Jinan 1 (12)	1	1	2	0.303(0.147)	0.000678(0.000849)	0.303	0.001	−0.19492(ns)	0.75202(ns)
Liaocheng 1 (10)	1	1	2	0.200(0.154)	0.000447(0.000681)	0.200	0.000	−1.11173(ns)	−1.24341(ns)
Shouguang 1 (10)	1	1	2	0.200(0.154)	0.000447(0.000681)	0.200	0.000	−1.11173(ns)	−1.24341(ns)
Zibo 1 (12)	1	1	2	0.167(0.134)	0.000373(0.000605)	0.167	0.000	−1.14053(ns)	−1.32974(ns)
Zaozhuang 1 (18)	1	1	2	0.294(0.119)	0.000658(0.000810)	0.294	0.001	0.02193(ns)	0.66689(ns)
Linyi (17)	0	0	1	0.000(0.000)	0.000000(0.000000)	0.000	0.000	na	na

S, number of polymorphic (segregating) sites; η, total number of mutations; H, number of haplotypes; H_d, haplotype diversity; π, nucleotide diversity; K, average number of nucleotide differences; π (JC), nucleotide diversity with Jukes and Cantor correction; D_{Taj}, Tajima's D statistic; Fu's F, Fu and Li's F test statistic.

Spain, Sudan, Croatia, Bosnia and Herzegovina, and Cyprus) were calculated and the allele-sharing among populations estimated. Genetic diversity for the 13 collections from Shandong Province (Dezhou, Liaocheng, Jinan, Shouguang, Zibo, Zaozhuang and Linyi) and the two collections from both Morocco and Spain were calculated using POPGENE version 1.31 (Yeh *et al*., 1997). The average number of alleles per locus (N_a), the effective number of alleles (N_e), the observed heterozygosity (H_o), the expected heterozygosity (H_e) and Nei's expected heterozygosity (Nei, 1973) were calculated.

Results

The evolutionary origin of Q-type B. tabaci *in Shandong, China based on mitochondrial haplotype analysis*

Of the 425 Shandong individuals sequenced from the 2005–2008 collections, we recovered only four different haplotypes (GU472435, GU472434, GU472429, GU472426). When compared against 96 unique haplotypes, haplotype 1 accounted for 86.3% (367 individuals) of individuals, haplotype 2 for 11.8% (50 individuals), haplotype 3 for 1.2% (five individuals) and haplotype 4 for 0.7% (three individuals). Haplotype 1 was found in all collection locations within Shandong (Dezhou, Jinan, Liaocheng, Linyi, Shouguang, Zaozhuang and Zibo), haplotype 2 in all except Linyi while haplotype 3 was recovered from only Liaocheng and Shouguang and haplotype 4 from Shouguang only. None matched any of the haplotypes from West and East Africa, Algeria, Croatia, Egypt, Greece (mainland), Israel, Italy, Sudan, Syria or Turkey. Haplotypes 1–3 (99.3%) matched those from Morocco, Spain, Portugal, France and Crete. In the case of haplotype 4 (0.7%), no match could be found with any location within the presumed home range of Q. The haplotype data supports the conclusion that Q-type *B. tabaci* in Shandong originated in the western Mediterranean.

Studies by Guirao *et al*. (1997) and Dalmon *et al*. (2008) suggest that *B. tabaci* is a relatively new invader to France. Similarly, of the four haplotypes in Shandong, only Haplotype1 was detected in Crete in 2008 but was present in only 1/28 locations in 2002–2004 (Tsagkarakou *et al*., 2007) and is genetically more related to those from the western Mediterranean than to those in Crete. This haplotype is one of the most commonly encountered globally and is known to be invasive, having been detected in Guatemala, South Korea, Taiwan and USA (P. De Barro, unpublished data). It, therefore, was concluded that the western Mediterranean region encompassed by Morocco, Portugal and Spain was the likely evolutionary origin of the four Shandong haplotypes. As a consequence, a comparison of mtCOI diversity between Shandong Province, and Morocco and Spain was then undertaken.

Comparison of mtCOI diversity between Shandong Province and Morocco and Spain

The comparison of mtCOI haplotype diversity between Shandong Province, Morocco and Spain showed that diversity was generally lower in China (table 2) with diversity values from Shandong 63.3–74.8% lower than those from Morocco and Spain (fig. 1a). The difference of the H_d value between Chinese populations and Morocco and Spain was significant using independent-samples t-test ($P<0.05$), which suggested that the mtDNA of Q in Shandong had experienced either severe bottleneck effects or founder effects.

Neither Tajima's D nor Fu's F were significantly different from zero, suggesting neither recent population expansion or purifying selection in these populations. Non-significant, positive Tajima's D and Fu's F for Morocco, Spain and China suggests no change in population size or selection in those regions (table 2).

Microsatellite gene diversity

Analysis of the six microsatellite loci revealed the presence of 33 alleles across the Mediterranean region, 31 in the western and 26 in the eastern Mediterranean. In Shandong, there were

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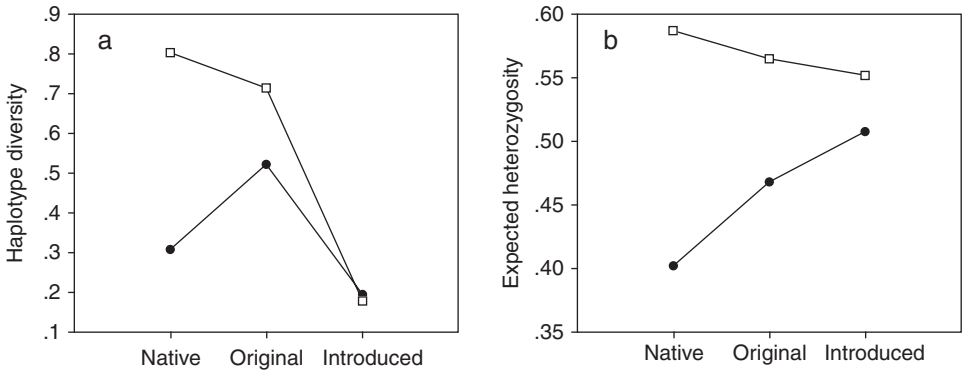

Fig. 1. Changes in genetic diversity associated with introduction. (a) A plot of haplotype diversity (H_d) of mtCOI sequences across populations and that calculated for each region as a whole. (b) Plot of expected heterozygosity (H_e) of microsatellite across populations and that calculated for each region as a whole (—●—, Average of populations; —□—, Regional level).

30 alleles of which 29 could be found in the western Mediterranean (Morocco and Spain) while only 24 occurred in the eastern Mediterranean. The greatest number of the alleles shared between Shandong and Mediterranean populations was with Morocco (24 alleles) followed by Spain (22 alleles) (table 3). The populations in Shandong shared 80.0% and 73.3% alleles with Moroccan and Spanish populations, respectively. In addition, there were two private alleles in the Shandong collections, one found in the Spanish populations and both in the Moroccan populations. These data are consistent with the conclusion drawn from the mtCOI data that Shandong Q was most related to those from the western Mediterranean.

The percentage of the shared alleles between collections from Shandong and Morocco ranged from 81.0% to 95.7% and between Shandong and Spain ranged from 65.0% to 81.0%. In total, the number of alleles in Shandong (30) is higher than these in Morocco (25) and Spain (24). The number of alleles in Shandong (30) was similar to the number in the either the western Mediterranean (31) or the Mediterranean as a whole (33). The data above suggest that the Q-type populations in Shandong might have multiple origins, which has resulted in a greater number of alleles being present in Shandong than in individual countries in the presumed origin, but a very similar number when compared with the region as a whole. Multiple origins of Shandong Q might be a consequence of multiple introductions or perhaps the secondary introduction from an invaded region.

The comparison of genetic diversity values (table 4) for the introduced populations across Shandong did not show any marked decrease, suggesting that the bottleneck and founder effects had no severe impact on genetic diversity of the nuclear DNA markers. For example, the expected heterozygosity (H_e) in western Mediterranean populations ranged from 0.3594 to 0.6124 and 0.3897 to 0.5886 for the Shandong collections. H_e values for the western Mediterranean and Shandong, at the regional level, was 0.5658 and 0.5527, respectively, and were not significantly different ($P > 0.05$) using independent-samples *t*-test (fig. 1b).

Discussion

Study of population diversity using molecular markers can be very informative in providing clues to the likely origin of invading organisms. For example, in order to investigate whether there were repeated introductions of *Fallopia* spp. (knotweeds) introduced into the USA from Asia, Gammon & Kesseli (2010) compared 21 Japanese haplotypes with 46 USA samples from 11 States, two Canadian samples, and six European samples using 800 bp of the non-coding chloroplast marker accD–rbcL. Their results supported the hypothesis of multiple introductions into the USA. Thus, comparison of the haplotypes between invasion populations and native populations can help determine the origin of the invasive species.

Based on the work of Chu *et al*. (2006), we speculated that Q-type individuals from Yunnan Province, China may have originated in Spain or other nearby locations. In order to determine where Q in Shandong evolved exactly, we compared the mtCOI haplotypes found in Shandong Province, China with those in its home range. We concluded that the western Mediterranean and, more precisely, the region encompassed by Morocco, Portugal and Spain was the evolutionary origin of this form of *B. tabaci*. While the haplotypes found in China were also found in France and Greece (Crete), these did not form part of the 'evolutionary home range'. In the case of France, surveys in 1995 showed Q was not present (Guirao *et al*., 1997) and the first records of Q in France date back to 2003 (Dalmon *et al*., 2008). Similarly for Crete, only one haplotype of presumed western Mediterranean origin was found in Crete as part of this study. A previous study (Tsagkarakou *et al*., 2007) showed that none of the four Shandong haplotypes were present in mainland Greece and only one was present in only one of the 14 sites surveyed in Crete between 2002 and 2004. The earliest record of *B. tabaci* in Crete is from 1992 (Kirk *et al*., 1993), although records from the mainland date back to the 1880s (Mound & Halsey, 1978). In Genbank as of 11 Feb 2010, there were 95 unique haplotypes belonging to the Mediterranean putative species; of these, four are known to have invaded countries outside of the presumed home range of this species (P. De Barro, unpublished data). One of these is the haplotype found in Crete and Shandong, as well as Morocco and Spain, which has also invaded China, France, Guatemala, South Korea and Taiwan. We argue that it is more likely that this haplotype has recently invaded Crete from the Western Mediterranean.

The microsatellite data also supported a western Mediterranean origin of Q in Shandong, as 96.7% of the

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Table 3. The allele-sharing among populations.

Location (allele no.)	Shandong, China (30)	Jinan 1 (24)	Jinan 2 (23)	Liaocheng 1 (23)	Liaocheng 2 (22)	Dezhou 1 (22)	Dezhou 2 (21)	Shouguang 1 (22)	Shouguang 2 (22)	Zibo 1 (21)	Zibo 2 (19)	Zaozhuang 1 (19)	Zaozhuang 2 (17)	Linyi (20)
Western Mediterranean (31)	29(96.7)*	24(100.0)	23(100.0)	23(100.0)	22(100.0)	22(100.0)	21(100.0)	22(100.0)	22(100.0)	21(100.0)	19(100.0)	19(100.0)	17(100.0)	19(95.0)
Morocco (25)	24(80.0)	22(91.7)	22(95.7)	20(87.0)	19(86.4)	21(95.5)	17(81.0)	19(86.4)	18(81.8)	19(90.5)	17(89.5)	16(84.2)	16(94.1)	18(90.0)
Morocco 1 (24)	23(76.7)	20(83.3)	19(82.6)	18(78.3)	18(81.8)	19(86.4)	16(76.2)	17(77.3)	17(77.3)	16(76.2)	14(73.7)	15(78.9)	14(82.4)	15(75.0)
Morocco 2 (16)	15(50.0)	13(54.2)	12(52.2)	13(56.5)	12(54.5)	13(59.1)	10(47.6)	10(45.5)	9(40.9)	12(57.1)	12(63.2)	9(47.4)	8(47.1)	9(45.0)
Spain (24)	22(73.3)	17(70.8)	18(78.3)	18(78.3)	17(77.3)	16(72.7)	16(76.2)	17(77.3)	17(77.3)	17(81.0)	14(73.7)	15(78.9)	12(70.6)	13(65.0)
Spain 1 (15)	15(50.0)	14(58.3)	15(65.2)	14(60.9)	12(54.5)	13(59.1)	12(57.1)	12(54.5)	12(54.5)	13(61.9)	11(57.9)	12(63.2)	10(58.8)	11(55.0)
Spain 2 (21)	19(63.3)	16(66.7)	15(65.2)	15(65.2)	15(68.2)	16(72.7)	15(71.4)	16(72.7)	15(68.2)	15(71.4)	14(73.7)	13(68.4)	11(64.7)	11(55.0)
Eastern Mediterranean (26)	24(80.0)	19(79.2)	20(87.0)	19(82.6)	17(77.3)	18(81.8)	16(76.2)	18(81.8)	17(77.3)	18(85.7)	16(84.2)	18(94.7)	16(94.1)	18(90.0)
Greece (16)	15(50.0)	13(54.2)	14(60.9)	13(56.5)	12(54.5)	11(50.0)	11(52.4)	11(50.0)	11(50.0)	12(57.1)	10(52.6)	11(57.9)	11(64.7)	13(65.0)
Greece 1 (13)	12(40.0)	10(41.7)	11(47.8)	10(43.5)	9(40.9)	8(36.4)	10(47.6)	9(40.9)	9(40.9)	9(42.9)	8(42.1)	9(47.4)	9(52.9)	10(50.0)
Greece 2 (13)	12(40.0)	11(45.8)	11(47.8)	10(43.5)	10(45.5)	10(45.5)	10(47.6)	10(45.5)	9(40.9)	10(47.6)	9(47.4)	9(47.4)	8(47.1)	11(55.0)
Bosnia and Herzegovina (19)	18(60.0)	16(66.7)	16(69.6)	15(65.2)	15(68.2)	15(68.2)	12(57.1)	16(72.7)	12(54.5)	14(66.7)	13(68.4)	14(73.7)	15(88.2)	15(75.0)
Croatia (21)	20(66.7)	16(66.7)	17(73.9)	17(73.9)	15(68.2)	15(68.2)	12(57.1)	14(63.6)	13(59.1)	15(71.4)	15(78.9)	16(84.2)	13(76.5)	15(75.0)
Croatia 1 (10)	10(33.3)	8(33.3)	8(34.8)	8(34.8)	7(31.8)	8(36.4)	8(38.1)	8(36.4)	9(40.9)	7(33.3)	9(47.4)	10(52.6)	8(47.1)	9(45.0)
Croatia 2 (11)	11(36.7)	11(45.8)	11(47.8)	11(47.8)	11(50.0)	10(45.5)	7(33.3)	10(45.5)	7(31.8)	11(52.4)	9(47.4)	9(47.4)	9(52.9)	8(40.0)
Croatia 3 (16)	15(50.0)	12(50.0)	13(56.5)	13(56.5)	11(50.0)	12(54.5)	11(52.4)	12(54.5)	10(45.5)	12(57.1)	12(63.2)	12(63.2)	11(64.7)	12(60.0)
Cyprus (16)	15(50.0)	11(45.8)	12(52.2)	12(52.2)	11(50.0)	11(50.0)	10(47.6)	12(54.5)	10(45.5)	11(52.4)	11(57.9)	11(57.9)	10(58.8)	11(55.0)
Mediterranean (33)	30(100.0)	24(100.0)	23(100.0)	23(100.0)	22(100.0)	22(100.0)	21(100.0)	22(100.0)	22(100.0)	21(100.0)	19(100.0)	19(100.0)	17(100.0)	20(100.0)

* The percentage (%) of the sharing alleles is in parenthesis.

Table 4. Estimates of diversity in Q populations based on six microsatellite loci.

Regions and populations (individual no.)	N_a	N_e	H_o	H_e	*Nei*
Western Mediterranean (52)	5.1667	2.8871	0.1575	0.5658	0.5603
Morocco (22)	4.1667	2.8264	0.2273	0.6185	0.6043
Morocco 1 (15)	4.0000	2.7553	0.2667	0.6124	0.5917
Morocco 2 (7)	2.6667	2.0016	0.1429	0.4890	0.4541
Spain (30)	4.0000	2.1073	0.1057	0.4447	0.4371
Spain 1 (15)	2.5000	1.8900	0.1119	0.3594	0.3466
Spain 2 (15)	3.5000	1.9536	0.1000	0.4096	0.3959
Shandong (195)	5.0000	2.7692	0.1666	0.5527	0.5512
Jinan (30)	4.1667	2.6137	0.1702	0.5542	0.5449
Jinan 1 (15)	4.0000	2.3833	0.1385	0.5213	0.5035
Jinan 2 (15)	3.8333	2.6331	0.2000	0.5736	0.5544
Liaocheng (30)	4.0000	2.9701	0.2833	0.5837	0.5739
Liaocheng 1 (15)	3.8333	2.9510	0.2556	0.5801	0.5607
Liaocheng 2 (15)	3.6667	2.8103	0.3111	0.5886	0.5687
Dezhou (30)	4.1667	2.5104	0.2167	0.5319	0.5229
Dezhou 1 (15)	3.6667	2.3100	0.2556	0.5229	0.5052
Dezhou 2 (15)	3.5000	2.3596	0.1778	0.5157	0.4982
Shouguang (30)	4.1667	2.3507	0.1611	0.5008	0.4923
Shouguang 1 (15)	3.6667	2.3218	0.1222	0.4659	0.4497
Shouguang 2 (15)	3.6667	2.2428	0.2000	0.5218	0.5044
Zibo (30)	4.0000	2.5354	0.1111	0.5377	0.5286
Zibo 1 (15)	3.5000	2.3687	0.1000	0.5046	0.4878
Zibo 2 (15)	3.1667	2.3328	0.1222	0.5027	0.4854
Zaozhuang (30)	3.3333	2.1638	0.1025	0.4277	0.4205
Zaozhuang 1 (15)	3.1667	2.2700	0.0714	0.4656	0.4498
Zaozhuang 2 (15)	2.8333	2.0344	0.1333	0.3897	0.3767
Linyi (15)	3.3333	2.0841	0.0778	0.4510	0.4359

N_a, the average number of alleles per locus; N_e, the effective number of alleles; H_o, the observed heterozygosity; H_e, the expected heterozygosity; *Nei*, Nei's (*1973*) expected heterozygosity.

alleles found in Q from Shandong can also be found in western Mediterranean populations, whereas only 80.0% are common with the eastern Mediterranean populations. These data strongly support the conclusion that Q in Shandong originated in the western Mediterranean.

Changes of genetic diversity in mitochondrial and nuclear (microsatellite) DNA

Many studies have been performed to compare the genetic diversity between the home range populations and invading populations of invasive species (Tsutsui *et al.*, 2000; Meunier *et al.*, 2001; Bucciarelli *et al.*, 2002; Charbonnel *et al.*, 2002; Downie, 2002; Giraud *et al.*, 2002; Facon *et al.*, 2003; Johnson & Starks, 2004; Kolbe *et al.*, 2004; Golani *et al.*, 2007; Miura, 2007). Some studies (e.g. Bucciarelli *et al.*, 2002; Giraud *et al.*, 2002; Facon *et al.*, 2003; Stepien *et al.*, 2002; Johnson & Starks, 2004; Kolbe *et al.*, 2004) have shown that invasive species do not always experience severe population bottlenecks and the founder events. Rather, they imply that the number of individuals, the number of invasion events, multiple origins of introduction, subsequent introductions from multiply introduced populations, invasion pathways or timings of invasions may overcome the loss of genetic diversity (Kolbe *et al.*, 2004; Frankham, 2005; Miura, 2007). Other studies have concluded that the loss of genetic variation can have a positive effect on the subsequent population growth of an invasive species (Tsutsui *et al.*, 2000).

Our study considered the question of genetic diversity through the combined use of both mitochondrial and nuclear DNA markers and, so, differs from many previous studies, which have considered this question through the application of only a single molecular marker. We found that the haplotype diversity of Q in Shandong was low compared with its presumed origin, whereas microsatellite allele diversity showed no such decline. A key factor in invasions is the establishment of females; and, so, bottleneck and founder events can lead to a very rapid and considerable loss of mitochondrial diversity. The lack of haplotype diversity in Shandong supports the view that at one or more points between the western Mediterranean and China, the invading Q-type insects lost haplotype diversity, most probably through the serial process of establishment and redistribution through trade in ornamental plants. However, the loss in haplotype diversity does not necessarily mean that nuclear allelic diversity should also decline. Provided females can mate freely with all available males, allelic diversity can be maintained or even increased relative to the origin of the invader. An example is seen in studies of the population genetics of a polygynous ant species where males come from a much wider geographic range than the female reproductives, which do not disperse long distances (Berghoff *et al.*, 2008). In such situations, the mtDNA signature shows a low level of diversity, whereas the nuclear DNA shows considerably greater diversity.

Multiple invasion events by a species has been proposed as a means through which the loss of genetic diversity is overcome (Frankham, 2005; Miura, 2007). Our results support the contention that the level of diversity in mitochondrial DNA may be no guide to the level of diversity in the nuclear DNA (Shao *et al.*, 2004; DeHeer & Vargo, 2008). As such, our findings offer some explanation to the apparent paradox

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between the concept of reduced genetic variation limiting adaptation to new environments and the observed low diversity in successful invaders.

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