

Genetic Analysis of the Diamondback Moth, *Plutella xylostella*, Collected from China Using Mitochondrial COI Gene Sequence

Jianhong Li[†], Yong Soo Choi, Iksoo Kim¹, Hung Dae Sohn and Byung Rae Jin*

College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea.

¹Department of Agricultural Biology, The National Institute of Agricultural Science & Technology, Suwon 441-100, Korea.

(Received 10 July 2004; Accepted 20 August 2004)

The diamondback moth, *Plutella xylostella*, is notorious because of its extensive potential and actual dispersal ability. Previously, the Korean populations of *P. xylostella* was extensively collected and analyzed for their genetic population structure using a portion of mitochondrial DNA (mtDNA). One of the postulated characteristics on population genetic structure of the species includes the presence of heterogeneous haplotypes, possibly possessed by some dispersed ones from neighboring countries. In this study, we sequenced ten *P. xylostella* collected from China (~2,000 km away from the middle part of Korea) to know the genetic relationships of these to the Korean *P. xylostella*. Sequence analysis of the identical portion of COI gene resulted in five haplotypes with the sequence divergence ranging from 0.5% (two nucleotides) to 1.1% (five nucleotides) among them and from 0.7% (three) to 2.5% (11) to the pre-existing 52 Korean haplotypes. Phylogenetic analysis showed that the Chinese *P. xylostella* were neither clearly separated from the Korean haplotypes nor clustered with one heterogeneous Korean haplotype. This result reinforces the significance of gene flow in this species and suggests to exclude the possibility that the heterogeneous Korean haplotypes may have emigrated from China, where our samples were obtained, although further extensive investigation is required.

Key words: Diamondback moths, *Plutella xylostella*,

MtDNA, COI gene, Gene flow

Introduction

The diamondback moth, *Plutella xylostella*, is a world-widely distributed insect pest of cruciferous plant. This species is notorious for its high number of generations per year in the tropical region, long dispersal distance, and high dispersal ratio (Lorimer, 1981; Caprio and Tabashnik, 1992a). Researches on genetic aspect of the species using allozyme (Caprio and Tabashnik, 1992b) and mitochondrial DNA (mtDNA) (Chang *et al.*, 1997) have been conducted to better understand the biology of the species and to establish management strategy against the species, and concluded that the *P. xylostella* is found to have a high rate of gene flow enough to prevent populations from genetic divergence.

Previously, Kim *et al.* (2000a, b, 2003) sequenced a portion of mtDNA from a total of 86 individuals collected from 11 localities in Korea to investigate the magnitude and nature of genetic variation of this species. They found that the Korean *P. xylostella* populations were not genetically differentiated, contains high number of haplotypes (total of 52 among 86 individuals), and characterized to have a high gene flow ratio among populations (Kim *et al.*, 2000a, b, 2003). Further, they found one haplotype (DB 24), which is genetically and/or phylogenetically differentiated obviously from the remaining haplotypes and suggested these as one that may have immigrated to Korea from neighboring countries (Kim *et al.*, 2003).

In this study, we sequenced ten *P. xylostella* collected at one locality from China to know the genetic relationships between Chinese and Korean *P. xylostella*. In particular, we wanted to know whether or not one heterogeneous Korean haplotype (DB 24) is genetically similar to newly included Chinese samples.

*To whom correspondence should be addressed.

College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea. Tel: +82-51-200-7594; Fax: +82-51-200-7594; E-mail: brjin@daunet.donga.ac.kr

[†]Present address: Department of Plant Protection, Huazhong Agricultural University, Wuhan 430070, P. R. China.

Materials and Methods

Insects

Adult diamondback moths, *Plutella xylostella*, were sampled at Wuhan, Hubei, China on July 2003. This location is about 2,000 km away from Seoul, Korea. Samples were frozen at -20°C for transportation to Korea after capture.

Amplification of mitochondrial COI gene

Total DNA was extracted by following the standard Proteinase K method (Kocher *et al.*, 1989). Primer information and PCR condition for the amplification of a partial COI gene (438 base pairs) are in detail described in Kim *et al.* (2000a). To ascertain successful DNA replication, electrophoresis was carried out for 40 min using $0.5\times$ TAE buffer in 0.7% agarose gel. The PCR product was then purified using PCR purification Kit (Qiagen, USA) by following manufacturers instruction. DNA sequencing was performed with an automatic DNA sequencer (PE Applied Biosystems, 310). Sequence alignment was performed using IBI MacVector (ver. 6.0). When homologous sequences from two individuals differed by \geq one nucleotide base, the sequences were considered as different haplotypes. Haplotype designations were applied to new sequences as they were discovered (DBM1, DBM2, DBM3, DBM4 and DBM5) and GenBank accession numbers of each individual is listed in Table 1.

Phylogenetic analysis

Phylogenetic analysis was performed by neighbor-joining (NJ) method incorporated in PAUP* (Phylogenetic Analysis Using Parsimony and Other Method*) ver 4.0b10 (Swofford, 2002). NJ analysis was performed with the distance measure using Kimuras 2-parameter method

(Kimura, 1980). Tree was evaluated using the bootstrap test (Felsenstein, 1985) by 1,000 iterations. One homologous mtDNA sequence of the southern pestworm, *Zerynthia rumina* (GenBank accession no. AF170870), belonging to the same Lepidoptera was incorporated in the analysis to root the tree.

Results and Discussion

COI gene sequence analysis and sequence divergence among haplotypes

A total of five haplotypes (DBM1 – DBM5) was obtained by sequencing 438-bp of COI gene from ten adult diamondback moths (Fig. 1). Previously, Kim *et al.* (2003) found a total of 52 haplotypes from 83 *P. xylostella* individuals, corresponding that new haplotype was found in every 1.65 individuals sequenced (new haplotype per 2.0 individuals in the Chinese samples). In terms of haplotype diversity ($H = \text{number of haplotypes}/\text{total number of individual sequenced}$), the Chinese sample ($H = 0.5$) was lower limit found in several Korean populations, where values ranged from 0.5 ~ 1.0 (see Table 3 in Kim *et al.*, 2003), although this comparison is far less to the conclusion due to limited sample size.

Uncorrected pairwise distance between pairs of haplotypes showed that sequence divergence among five haplotypes ranged from 0.5% (two nucleotides) to 1.1% (five nucleotides), where the minimum sequence divergence was observed in several comparisons, but the maximum divergence was observed when DBM4 was compared with DBM5 only (Table 2).

When the five Chinese *P. xylostella* were compared to the pre-existing 52 Korean haplotypes sequence divergence ranged from a minimum of 0.7% (three nucleotides) to a maximum of 2.5% (11 nucleotides), whereas among-Korean haplotypes ranged from 0.2% (one) to 2.7% (12) (Table 2; Kim *et al.*, 2003). Thus, the maximum sequence divergence of between-Korean and Chinese samples is almost as same as within-Korean samples, suggesting that genetic divergence of the *P. xylostella* between the two distant geographic regions (~2,000 km away) is not obvious. The similar situation was also found when only Korean samples were considered, although the geographic distance within Korean localities is much shorter. For example, an island population of Jangjeon-lee on Jeju Island and one mainland population from Yeongwol-up in Gangwon Province showed essentially zero genetic distance (Kim *et al.*, 2003). The geographic distance between these two localities is ~ 500 km and this pair is one of the most distant localities investigated by Kim *et al.* (2003). Similar situation also was found when

Table 1. A list of *P. xylostella* collected from China with COI haplotypes and GenBank accession number

Collecting locality	Collection date	Animal number	COI haplotype	GenBank number
Wuhan, Hubei, China (10)	2003. 7. 16	DC1	DBM1	AY722697
		DC2	DBM2	AY722698
		DC3	DBM1	AY722699
		DC4	DBM3	AY722700
		DC5	DBM4	AY722701
		DC6	DBM5	AY722702
		DC7	DBM2	AY722703
		DC8	DBM2	AY722704
		DC9	DBM3	AY722705
		DC10	DBM5	AY722706

			30		60	
DBM1 (DC1)	CGAATAAATA	ATATAAGATT	TTGACTACTT	CCCCCCTCAT	TAACCTTATT	TAACCTTATT
DBM2 (DC2)
DBM3 (DC9)
DBM4 (DC5)
DBM5 (DC6)
			90		120	
DBM1 (DC1)	AGAATTGTTG	AAAATGGGGC	AGGTACTGGA	TGAACTGTTT	ATCCTCCTTT	ATCTTCAAAT
DBM2 (DC2)A..
DBM3 (DC9)
DBM4 (DC5)A..C.
DBM5 (DC6)
			150		180	
DBM1 (DC1)	ATCGCTCATA	GAGGAAGATC	TGTCGATTTA	GCTATTTTTT	CTCTTCATTT	AGCTGGTATT
DBM2 (DC2)
DBM3 (DC9)
DBM4 (DC5)
DBM5 (DC6)
			210		240	
DBM1 (DC1)	TCTTCAATT	TAGGGGCAAT	TAATTTTATT	ACAACTATTA	TTAATATAAA	AAGAAATGGA
DBM2 (DC2)
DBM3 (DC9)
DBM4 (DC5)
DBM5 (DC6)
			270		300	
DBM1 (DC1)	ATGTCATTTG	ATCGTATAAC	TTTATTTGTT	TGAGCTGTAG	GAATTACAGC	TATTTTATTA
DBM2 (DC2)
DBM3 (DC9)
DBM4 (DC5)
DBM5 (DC6)
			330		360	
DBM1 (DC1)	TTGTTATCTC	TACCAGTTTT	AGCAGGAGCT	ATTACTATAT	TATTAACAGA	TCGAAACTTA
DBM2 (DC2)
DBM3 (DC9)	...A.....
DBM4 (DC5)A.....
DBM5 (DC6)T.
			390		420	
DBM1 (DC1)	AATACTTCCT	TCTTTGATCC	TGCAGGAGGG	GGAGATCCTA	TTTTATACCA	ACATTTATTT
DBM2 (DC2)
DBM3 (DC9)
DBM4 (DC5)
DBM5 (DC6)	..C.....
		438				
DBM1 (DC1)	TGATTTTTTG	GCCATCCT				
DBM2 (DC2)C...				
DBM3 (DC9)C...				
DBM4 (DC5)C...				
DBM5 (DC6)C...				

Fig. 1. Sequence alignment of five mitochondrial haplotypes obtained from 438-bp COI sequences of *P. xylostella* collected in Wuhan, China. Within-parenthesis indicates animal number corresponding to each haplotype.

one Hawaiian *P. xylostella* haplotype (Chang *et al.*, 1997) was compared to the Korean samples (ranging in sequence divergence from 0.2% to 2.1%), indicating that some Korean haplotypes are very close even to the Hawaiian haplotype (Kim *et al.*, 2000a). Collectively, these results

suggest that the *P. xylostella* is cosmopolitan pest even in the genetic aspects. Detailed cause for high genetic relatedness was extensively discussed in several studies (Caprio and Tabashnik, 1992a, b; Kim *et al.*, 2001; Kim *et al.*, 2003).

Table 2. Pairwise comparison among nucleotide sequences of COI genes obtained from this study and published by Kim *et al.* (2003)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 DB1	-	0.007	0.002	0.011	0.011	0.007	0.014	0.005	0.009	0.007	0.007	0.007	0.009	0.009	0.007	0.007	0.014	0.007	0.009	0.007
2 DB2	3	-	0.009	0.005	0.009	0.005	0.011	0.002	0.002	0.005	0.005	0.005	0.007	0.007	0.005	0.009	0.011	0.005	0.002	0.005
3 DB3	1	4	-	0.014	0.009	0.005	0.011	0.007	0.011	0.009	0.009	0.009	0.011	0.007	0.009	0.005	0.011	0.009	0.011	0.009
4 DB4	5	2	6	-	0.014	0.009	0.011	0.007	0.007	0.009	0.009	0.009	0.007	0.007	0.009	0.014	0.016	0.005	0.002	0.009
5 DB5	5	4	4	6	-	0.005	0.007	0.007	0.011	0.009	0.009	0.009	0.011	0.007	0.009	0.009	0.007	0.009	0.011	0.009
6 DB6	3	2	2	4	2	-	0.007	0.002	0.007	0.005	0.005	0.005	0.007	0.002	0.005	0.005	0.007	0.005	0.007	0.005
7 DB7	6	5	5	5	3	3	-	0.009	0.014	0.011	0.011	0.011	0.009	0.005	0.011	0.011	0.009	0.007	0.009	0.011
8 DB8	2	1	3	3	3	1	4	-	0.005	0.002	0.002	0.002	0.005	0.005	0.002	0.007	0.009	0.002	0.005	0.002
9 DB9	4	1	5	3	5	3	6	2	-	0.007	0.007	0.007	0.009	0.009	0.002	0.011	0.014	0.007	0.005	0.007
10 DB10	3	2	4	4	4	2	5	1	3	-	0.005	0.005	0.007	0.007	0.005	0.009	0.011	0.005	0.007	0.005
11 DB11	3	2	4	4	4	2	5	1	3	2	-	0.005	0.002	0.007	0.005	0.009	0.011	0.005	0.007	0.005
12 DB12	3	2	4	4	4	2	5	1	3	2	2	-	0.007	0.007	0.005	0.009	0.011	0.005	0.007	0.005
13 DB13	4	3	5	3	5	3	4	2	4	3	1	3	-	0.005	0.007	0.011	0.014	0.002	0.005	0.007
14 DB14	4	3	3	3	3	1	2	2	4	3	3	2	3	-	0.007	0.007	0.009	0.002	0.005	0.007
15 DB15	3	2	4	4	4	2	5	1	1	2	2	2	3	3	-	0.009	0.011	0.005	0.007	0.005
16 DB16	3	4	2	6	4	2	5	3	5	4	4	4	5	3	4	-	0.011	0.009	0.011	0.009
17 DB17	6	5	5	7	3	3	4	4	6	5	5	5	6	4	5	5	-	0.011	0.014	0.011
18 DB18	3	2	4	2	4	2	3	1	3	2	2	2	1	1	2	4	5	-	0.002	0.005
19 DB19	4	1	5	1	5	3	4	2	2	3	3	3	2	2	3	5	6	1	-	0.007
20 DB20	3	2	4	4	4	2	5	1	3	2	2	2	3	3	2	4	5	2	3	-
21 DB21	3	2	4	2	4	2	5	1	3	2	2	2	3	3	2	4	5	2	3	2
22 DB22	4	3	5	3	5	3	4	2	4	3	3	3	2	2	3	5	6	1	2	3
23 DB23	4	1	5	3	5	3	6	2	2	3	3	3	4	4	3	5	6	3	2	3
24 DB24	12	11	11	11	11	9	10	10	10	11	11	11	10	8	9	11	12	9	10	11
25 DB25	4	1	5	3	5	3	6	2	2	3	3	3	4	4	3	5	6	3	2	3
26 DB26	2	3	3	5	5	3	6	2	4	3	3	3	4	4	3	3	6	3	4	3
27 DB27	2	3	1	5	3	1	4	2	4	3	3	3	4	2	3	3	4	3	4	3
28 DB28	2	3	3	5	5	3	6	2	4	3	3	3	4	4	3	3	6	3	4	3
29 DB29	5	2	6	4	6	4	7	3	3	4	4	4	5	5	4	6	7	4	3	4
30 DB30	5	4	4	6	2	2	3	3	5	4	4	4	5	3	4	4	3	4	5	4
31 DB31	4	3	5	5	5	3	6	2	4	3	3	3	4	4	3	5	6	3	4	3
32 DB32	2	3	1	5	3	1	4	2	4	3	3	3	4	2	3	1	4	3	4	3
33 DB33	3	2	4	4	4	2	5	1	3	2	2	2	3	3	2	4	5	2	3	2
34 DB34	4	1	5	3	5	3	6	2	2	3	3	3	4	4	3	5	6	3	2	3
35 DB35	3	2	4	4	4	2	5	1	3	2	2	2	3	3	2	4	5	2	3	2
36 DB36	2	3	3	5	5	3	6	2	4	3	3	3	4	4	3	3	6	3	4	3
37 DB37	1	2	2	4	4	2	5	1	3	2	2	2	3	3	2	2	5	2	3	2
38 DB38	4	1	5	3	3	3	6	2	2	3	3	3	4	4	3	5	6	3	2	3
39 DB39	4	3	5	5	5	3	6	2	4	3	3	3	4	4	3	5	6	3	4	3
40 DB40	3	2	4	4	4	2	5	1	3	2	2	2	3	3	2	4	5	2	3	2
41 DB41	4	3	5	3	5	3	4	2	4	3	3	3	2	2	3	5	6	1	2	3
42 DB42	7	6	6	6	6	4	7	5	7	6	6	6	7	5	6	6	7	6	7	6
43 DB43	3	4	2	6	4	2	5	3	5	4	4	4	5	3	4	2	5	4	5	4
44 DB44	3	2	4	4	4	2	5	1	3	2	2	2	3	3	2	4	5	2	3	2
45 DB45	4	3	5	3	5	3	4	2	4	3	3	3	2	2	3	5	6	1	2	3
46 DB46	5	4	6	4	6	4	5	3	5	4	4	4	3	3	4	6	7	2	3	4
47 DB47	5	4	4	4	4	2	3	3	5	4	4	4	3	1	4	4	5	2	3	4
48 DB48	4	1	5	3	5	3	6	2	2	3	3	3	4	4	3	5	6	3	2	3
49 DB49	6	5	7	7	7	5	8	4	6	5	5	5	6	6	5	7	8	5	6	5
50 DB50	2	3	3	5	5	3	6	2	4	3	3	3	4	4	3	3	6	3	4	3
51 DB52	5	2	6	2	6	4	5	3	3	4	4	4	3	3	4	6	7	2	1	4
52 DB52	3	2	4	4	4	2	5	1	3	2	2	2	3	3	2	4	5	2	3	2
53 DBM1	6	7	5	9	7	5	8	6	6	7	7	7	8	6	5	7	8	7	8	7
54 DBM2	6	5	5	7	5	3	6	4	4	5	5	5	6	4	3	5	6	5	6	5
55 DBM3	7	6	6	8	6	4	7	5	5	6	6	6	7	5	4	6	7	6	7	6
56 DBM4	8	7	7	9	7	5	8	6	6	7	7	7	8	6	5	7	8	7	8	7
57 DBM5	9	8	8	10	8	6	9	7	7	8	8	8	9	7	6	8	9	8	9	8

Table 2. Continued

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1 DB1	0.007	0.009	0.009	0.027	0.009	0.005	0.005	0.005	0.011	0.011	0.009	0.005	0.007	0.009	0.007	0.005	0.002	0.009	0.009	0.007
2 DB2	0.005	0.007	0.002	0.025	0.002	0.007	0.007	0.007	0.005	0.009	0.007	0.007	0.005	0.002	0.005	0.007	0.005	0.002	0.007	0.005
3 DB3	0.009	0.011	0.011	0.025	0.011	0.007	0.002	0.007	0.014	0.009	0.011	0.002	0.009	0.011	0.009	0.007	0.005	0.011	0.011	0.009
4 DB4	0.005	0.007	0.007	0.025	0.007	0.011	0.011	0.011	0.009	0.014	0.011	0.011	0.009	0.007	0.009	0.011	0.009	0.007	0.011	0.009
5 DB5	0.009	0.011	0.011	0.025	0.011	0.011	0.007	0.011	0.014	0.005	0.011	0.007	0.009	0.011	0.009	0.011	0.009	0.007	0.011	0.009
6 DB6	0.005	0.007	0.007	0.021	0.007	0.007	0.002	0.007	0.009	0.005	0.007	0.002	0.005	0.007	0.005	0.007	0.005	0.007	0.007	0.005
7 DB7	0.011	0.009	0.014	0.023	0.014	0.014	0.009	0.014	0.016	0.007	0.014	0.009	0.011	0.014	0.011	0.014	0.011	0.014	0.014	0.011
8 DB8	0.002	0.005	0.005	0.023	0.005	0.005	0.005	0.005	0.007	0.007	0.005	0.005	0.002	0.005	0.002	0.005	0.002	0.005	0.005	0.002
9 DB9	0.007	0.009	0.005	0.023	0.005	0.009	0.009	0.009	0.007	0.011	0.009	0.009	0.007	0.005	0.007	0.009	0.007	0.005	0.009	0.007
10 DB10	0.005	0.007	0.007	0.025	0.007	0.007	0.007	0.007	0.009	0.009	0.007	0.007	0.005	0.007	0.005	0.007	0.005	0.007	0.007	0.005
11 DB11	0.005	0.007	0.007	0.025	0.007	0.007	0.007	0.007	0.009	0.009	0.007	0.007	0.005	0.007	0.005	0.007	0.005	0.007	0.007	0.005
12 DB12	0.005	0.007	0.007	0.025	0.007	0.007	0.007	0.007	0.009	0.009	0.007	0.007	0.005	0.007	0.005	0.007	0.005	0.007	0.007	0.005
13 DB13	0.007	0.005	0.009	0.023	0.009	0.009	0.009	0.009	0.011	0.011	0.009	0.009	0.007	0.009	0.007	0.009	0.007	0.009	0.009	0.007
14 DB14	0.007	0.005	0.009	0.018	0.009	0.009	0.005	0.009	0.011	0.007	0.009	0.005	0.007	0.009	0.007	0.009	0.007	0.009	0.009	0.007
15 DB15	0.005	0.007	0.007	0.021	0.007	0.007	0.007	0.007	0.009	0.009	0.007	0.007	0.005	0.007	0.005	0.007	0.005	0.007	0.007	0.005
16 DB16	0.009	0.011	0.011	0.025	0.011	0.007	0.007	0.007	0.014	0.009	0.011	0.002	0.009	0.011	0.009	0.007	0.005	0.011	0.011	0.009
17 DB17	0.011	0.014	0.014	0.027	0.014	0.014	0.009	0.014	0.016	0.007	0.014	0.009	0.011	0.014	0.011	0.014	0.011	0.014	0.014	0.011
18 DB18	0.005	0.002	0.007	0.021	0.007	0.007	0.007	0.007	0.009	0.009	0.007	0.007	0.005	0.007	0.005	0.007	0.005	0.007	0.007	0.005
19 DB19	0.007	0.005	0.005	0.023	0.005	0.009	0.009	0.009	0.007	0.011	0.009	0.009	0.007	0.005	0.007	0.009	0.007	0.005	0.009	0.007
20 DB20	0.005	0.007	0.007	0.025	0.007	0.007	0.007	0.007	0.009	0.009	0.007	0.007	0.005	0.007	0.005	0.007	0.005	0.007	0.007	0.005
21 DB21	-	0.007	0.007	0.025	0.007	0.007	0.007	0.007	0.009	0.009	0.007	0.007	0.005	0.007	0.005	0.007	0.005	0.007	0.007	0.005
22 DB22	3	-	0.009	0.023	0.009	0.009	0.009	0.009	0.011	0.011	0.009	0.009	0.007	0.009	0.007	0.009	0.007	0.009	0.009	0.007
23 DB23	3	4	-	0.023	0.005	0.009	0.009	0.009	0.007	0.011	0.009	0.009	0.007	0.005	0.007	0.009	0.007	0.005	0.009	0.007
24 DB24	11	10	10	-	0.027	0.025	0.023	0.027	0.025	0.025	0.023	0.023	0.025	0.027	0.025	0.023	0.025	0.027	0.023	0.025
25 DB25	3	4	2	12	-	0.009	0.009	0.009	0.007	0.011	0.009	0.009	0.007	0.005	0.007	0.009	0.007	0.005	0.009	0.007
26 DB26	3	4	4	11	4	-	0.009	0.005	0.011	0.011	0.009	0.005	0.007	0.009	0.007	0.005	0.002	0.009	0.009	0.007
27 DB27	3	4	4	10	4	4	-	0.009	0.011	0.007	0.009	0.005	0.007	0.009	0.007	0.009	0.007	0.009	0.009	0.007
28 DB28	3	4	4	12	4	2	4	-	0.011	0.011	0.009	0.005	0.007	0.009	0.007	0.005	0.002	0.009	0.009	0.007
29 DB29	4	5	3	11	3	5	5	5	-	0.014	0.011	0.011	0.009	0.007	0.009	0.011	0.009	0.007	0.007	0.009
30 DB30	4	5	5	11	5	5	3	5	6	-	0.011	0.007	0.009	0.011	0.009	0.011	0.009	0.011	0.011	0.009
31 DB31	3	4	4	10	4	4	4	4	5	5	-	0.009	0.007	0.009	0.007	0.005	0.007	0.009	0.009	0.007
32 DB32	3	4	4	10	4	2	2	2	5	3	4	-	0.007	0.009	0.007	0.005	0.002	0.009	0.009	0.007
33 DB33	2	3	3	11	3	3	3	3	4	4	3	3	-	0.007	0.005	0.007	0.005	0.007	0.007	0.005
34 DB34	3	4	2	12	2	4	4	4	3	5	4	4	3	-	0.007	0.009	0.007	0.005	0.009	0.007
35 DB35	2	3	3	11	3	3	3	3	4	4	3	3	2	3	-	0.007	0.005	0.007	0.007	0.005
36 DB36	3	4	4	10	4	2	4	2	5	5	2	2	3	4	3	-	0.002	0.009	0.009	0.007
37 DB37	2	3	3	11	3	1	3	1	4	4	3	1	2	3	2	1	-	0.007	0.007	0.005
38 DB38	3	4	2	12	2	4	4	4	3	5	4	4	3	2	3	4	3	-	0.009	0.007
39 DB39	3	4	4	10	4	4	4	4	3	5	4	4	3	4	3	4	3	4	-	0.007
40 DB40	2	3	3	11	3	3	3	3	4	4	3	3	2	3	2	3	2	3	3	-
41 DB41	3	2	4	10	4	4	4	4	5	5	4	4	3	4	3	4	3	4	4	3
42 DB42	4	7	7	12	7	5	5	7	8	6	7	5	6	7	6	7	6	7	7	6
43 DB43	4	5	5	11	3	3	3	3	6	4	5	1	4	5	4	3	2	5	5	4
44 DB44	2	3	1	9	3	3	3	3	4	4	3	3	2	3	2	3	2	3	3	2
45 DB45	3	2	2	8	4	4	4	4	5	5	4	4	3	4	3	4	3	4	4	3
46 DB46	4	3	5	11	5	5	5	5	6	6	5	5	4	5	4	5	4	5	5	4
47 DB47	4	3	5	7	5	5	3	5	6	4	3	3	4	5	4	3	4	5	5	4
48 DB48	3	4	2	10	2	3	4	4	3	5	4	4	3	2	3	4	3	2	4	3
49 DB49	5	6	6	10	6	6	6	6	7	7	4	6	5	6	5	4	5	6	6	5
50 DB50	3	4	2	10	4	2	4	2	5	5	4	2	3	4	3	2	1	4	4	3
51 DB51	4	3	3	11	3	5	5	5	4	6	5	5	4	3	4	5	4	3	5	4
52 DB52	2	3	3	9	3	3	3	3	2	4	3	3	2	3	2	3	2	3	1	2
53 DBM1	7	8	8	10	6	8	4	8	9	7	8	6	7	6	7	8	7	8	8	7
54 DBM2	5	6	6	8	6	6	4	6	7	5	6	4	5	4	5	6	5	6	6	5
55 DBM3	6	7	7	10	5	7	5	7	8	6	7	5	6	5	6	7	6	7	7	6
56 DBM4	7	8	8	10	8	8	6	8	9	7	8	6	7	6	7	8	7	8	8	7
57 DBM5	8	9	9	11	7	9	7	9	10	8	9	7	8	7	8	9	8	9	9	8

Table 2. Continued

	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
1 DB1	0.009	0.016	0.007	0.007	0.009	0.011	0.011	0.009	0.014	0.005	0.011	0.007	0.014	0.014	0.016	0.018	0.021
2 DB2	0.007	0.014	0.009	0.005	0.007	0.009	0.009	0.002	0.011	0.007	0.005	0.005	0.016	0.011	0.014	0.016	0.018
3 DB3	0.011	0.014	0.005	0.009	0.011	0.014	0.009	0.011	0.016	0.007	0.014	0.009	0.011	0.011	0.014	0.016	0.018
4 DB4	0.007	0.014	0.014	0.009	0.007	0.009	0.009	0.007	0.016	0.011	0.005	0.009	0.021	0.016	0.018	0.021	0.023
5 DB5	0.011	0.014	0.009	0.009	0.011	0.014	0.009	0.011	0.016	0.011	0.014	0.009	0.016	0.011	0.014	0.016	0.018
6 DB6	0.007	0.009	0.005	0.005	0.007	0.009	0.005	0.007	0.011	0.007	0.009	0.005	0.011	0.007	0.009	0.011	0.014
7 DB7	0.009	0.016	0.011	0.011	0.009	0.011	0.007	0.014	0.018	0.014	0.011	0.011	0.018	0.014	0.016	0.018	0.021
8 DB8	0.005	0.011	0.007	0.002	0.005	0.007	0.007	0.005	0.009	0.005	0.007	0.002	0.014	0.009	0.011	0.014	0.016
9 DB9	0.009	0.016	0.011	0.007	0.009	0.011	0.011	0.005	0.014	0.009	0.007	0.007	0.014	0.009	0.011	0.014	0.016
10 DB10	0.007	0.014	0.009	0.005	0.007	0.009	0.009	0.007	0.011	0.007	0.009	0.005	0.016	0.011	0.014	0.016	0.018
11 DB11	0.007	0.014	0.009	0.005	0.007	0.009	0.009	0.007	0.011	0.007	0.009	0.005	0.016	0.011	0.014	0.016	0.018
12 DB12	0.007	0.014	0.009	0.005	0.007	0.009	0.009	0.007	0.011	0.007	0.009	0.005	0.016	0.011	0.014	0.016	0.018
13 DB13	0.005	0.016	0.011	0.007	0.005	0.007	0.007	0.009	0.014	0.009	0.007	0.007	0.018	0.014	0.016	0.018	0.021
14 DB14	0.005	0.011	0.007	0.007	0.005	0.007	0.002	0.009	0.014	0.009	0.007	0.007	0.014	0.009	0.011	0.014	0.016
15 DB15	0.007	0.014	0.009	0.005	0.007	0.009	0.009	0.007	0.011	0.007	0.009	0.005	0.011	0.007	0.009	0.011	0.014
16 DB16	0.011	0.014	0.005	0.009	0.011	0.014	0.009	0.011	0.016	0.007	0.014	0.009	0.016	0.011	0.014	0.016	0.018
17 DB17	0.014	0.016	0.011	0.011	0.014	0.016	0.011	0.014	0.018	0.014	0.016	0.011	0.018	0.014	0.016	0.018	0.021
18 DB18	0.002	0.014	0.009	0.005	0.002	0.005	0.005	0.007	0.011	0.007	0.005	0.005	0.016	0.011	0.014	0.016	0.018
19 DB19	0.005	0.016	0.011	0.007	0.005	0.007	0.007	0.005	0.014	0.009	0.002	0.007	0.018	0.014	0.016	0.018	0.021
20 DB20	0.007	0.014	0.009	0.005	0.007	0.009	0.009	0.007	0.011	0.007	0.009	0.005	0.016	0.011	0.014	0.016	0.018
21 DB21	0.007	0.009	0.009	0.005	0.007	0.009	0.009	0.007	0.011	0.007	0.009	0.005	0.016	0.011	0.014	0.016	0.018
22 DB22	0.005	0.016	0.011	0.007	0.005	0.007	0.007	0.009	0.014	0.009	0.007	0.007	0.018	0.014	0.016	0.018	0.021
23 DB23	0.009	0.016	0.011	0.002	0.005	0.011	0.011	0.005	0.014	0.005	0.007	0.007	0.018	0.014	0.016	0.018	0.021
24 DB24	0.023	0.027	0.025	0.021	0.018	0.025	0.016	0.023	0.023	0.023	0.025	0.021	0.023	0.018	0.023	0.023	0.025
25 DB25	0.009	0.016	0.007	0.007	0.009	0.011	0.011	0.005	0.014	0.009	0.007	0.007	0.014	0.014	0.011	0.018	0.016
26 DB26	0.009	0.011	0.007	0.007	0.009	0.011	0.011	0.007	0.014	0.005	0.011	0.007	0.018	0.014	0.016	0.018	0.021
27 DB27	0.009	0.011	0.007	0.007	0.009	0.011	0.007	0.009	0.014	0.009	0.011	0.007	0.009	0.009	0.011	0.014	0.016
28 DB28	0.009	0.016	0.007	0.007	0.009	0.011	0.011	0.009	0.014	0.005	0.011	0.007	0.018	0.014	0.016	0.018	0.021
29 DB29	0.011	0.018	0.014	0.009	0.011	0.014	0.014	0.007	0.016	0.011	0.009	0.005	0.021	0.016	0.018	0.021	0.023
30 DB30	0.011	0.014	0.009	0.009	0.011	0.014	0.009	0.011	0.016	0.011	0.014	0.009	0.016	0.011	0.014	0.016	0.018
31 DB31	0.009	0.016	0.011	0.007	0.009	0.011	0.007	0.009	0.009	0.009	0.011	0.007	0.018	0.014	0.016	0.018	0.021
32 DB32	0.009	0.011	0.002	0.007	0.009	0.011	0.007	0.009	0.014	0.005	0.011	0.007	0.014	0.009	0.011	0.014	0.016
33 DB33	0.007	0.014	0.009	0.005	0.007	0.009	0.009	0.007	0.011	0.007	0.009	0.005	0.016	0.011	0.014	0.016	0.018
34 DB34	0.009	0.016	0.011	0.007	0.009	0.011	0.011	0.005	0.014	0.009	0.007	0.007	0.014	0.009	0.011	0.014	0.016
35 DB35	0.007	0.014	0.009	0.005	0.007	0.009	0.009	0.007	0.011	0.007	0.009	0.005	0.016	0.011	0.014	0.016	0.018
36 DB36	0.009	0.016	0.007	0.007	0.009	0.011	0.007	0.009	0.009	0.005	0.011	0.007	0.018	0.014	0.016	0.018	0.021
37 DB37	0.007	0.014	0.005	0.005	0.007	0.009	0.009	0.007	0.011	0.002	0.009	0.005	0.016	0.011	0.014	0.016	0.018
38 DB38	0.009	0.016	0.011	0.007	0.009	0.011	0.011	0.005	0.014	0.009	0.007	0.007	0.018	0.014	0.016	0.018	0.021
39 DB39	0.009	0.016	0.011	0.007	0.009	0.011	0.011	0.009	0.014	0.009	0.011	0.002	0.018	0.014	0.016	0.018	0.021
40 DB40	0.007	0.014	0.009	0.005	0.007	0.009	0.009	0.007	0.011	0.007	0.009	0.005	0.016	0.011	0.014	0.016	0.018
41 DB41	-	0.016	0.011	0.007	0.005	0.007	0.007	0.009	0.014	0.009	0.007	0.007	0.018	0.014	0.016	0.018	0.021
42 DB42	7	-	0.014	0.014	0.016	0.018	0.014	0.014	0.021	0.016	0.018	0.014	0.021	0.016	0.018	0.021	0.023
43 DB43	5	6	-	0.009	0.011	0.014	0.009	0.011	0.016	0.007	0.014	0.009	0.011	0.011	0.009	0.016	0.014
44 DB44	3	6	4	-	0.002	0.009	0.009	0.007	0.011	0.002	0.009	0.005	0.016	0.011	0.014	0.016	0.018
45 DB45	2	7	5	1	-	0.007	0.007	0.009	0.014	0.005	0.007	0.007	0.018	0.014	0.016	0.018	0.021
46 DB46	3	8	6	4	3	-	0.009	0.011	0.016	0.011	0.009	0.009	0.021	0.016	0.018	0.021	0.023
47 DB47	3	6	4	4	3	4	-	0.011	0.011	0.011	0.009	0.009	0.016	0.011	0.014	0.016	0.018
48 DB48	4	6	5	3	4	5	5	-	0.014	0.009	0.007	0.007	0.018	0.014	0.016	0.018	0.021
49 DB49	6	9	7	5	6	7	5	6	-	0.014	0.016	0.011	0.018	0.014	0.018	0.018	0.021
50 DB50	4	7	3	1	2	5	5	4	6	-	0.011	0.007	0.018	0.014	0.016	0.018	0.021
51 DB52	3	8	6	4	3	4	4	3	7	5	-	0.009	0.021	0.016	0.018	0.021	0.023
52 DB52	3	6	4	2	3	4	4	3	5	3	4	-	0.016	0.011	0.014	0.016	0.018
53 DBM1	8	9	5	7	8	9	7	8	8	8	9	7	-	0.005	0.005	0.009	0.007
54 DBM2	6	7	5	5	6	7	5	6	6	6	7	5	2	-	0.005	0.005	0.007
55 DBM3	7	8	4	6	7	8	6	7	8	7	8	6	2	2	-	0.009	0.007
56 DBM4	8	9	7	7	8	9	7	8	8	8	9	7	4	2	4	-	0.011
57 DBM5	9	10	6	8	9	10	8	9	9	9	10	8	3	3	3	5	-

Kim *et al.* (2003) previously found one heterogeneous haplotype (DB24), possessed by two individuals in a locality. Although the sequence divergence among Korean *P. xylostella* haplotypes excluding this haplotype ranged from 0.2% to 2.1% that including this haplotype sharply increased to 2.7% (Table 2). Furthermore, the mean uncorrected pairwise distance of DB24 against others is largest (10 nucleotides, 2.3%), whereas others ranged from 0.2% (one nucleotide) to 1.48% (6.5 nucleotides) (Kim *et al.*, 2003). Thus, this peculiar haplotype was speculated to be one of the immigrants possibly dispersed from neighboring countries including China. The theoretical background on this assumption stems from the notion that mitochondrial lineages of two independent clades are very unlikely to survive within a single population for a long time, and one of the most plausible interpretations on the presence of two independent clades includes a secondary admixture of once geographically isolated populations (Avise *et al.*, 1987). However, our uncorrected pairwise distance of DB24 to the Chinese haplotypes ranged from 1.8% (eight nucleotides) to 2.5% (11 nucleotides) and this values is almost equidistant when this haplotype was compared to the remaining 51 Korean haplotypes, where values ranging from 1.6% (7) to 2.7% (12). This result indicates that DB24 may not be an immediately related one to the Chinese samples we utilized in this study and may have originated elsewhere that we did not cover. To have firm knowledge more samples covering a diverse Chinese region are essential.

Phylogenetic analysis

Phylogenetic analysis was performed to investigate the relationship between Korean and Chinese haplotypes (Fig. 2). Most haplotypes were weakly associated or unresolved due to small nucleotide difference among them (Table 2). This feature is similar to the previous study by Kim *et al.* (2003). The newly added Chinese five haplotypes all grouped together, but the bootstrap value supporting this group was not high (62%), emphasizing that this group cannot be regarded as an independent phylogenetic group.

DB4, which showed the highest genetic distance to the remaining Korean haplotypes, still was excluded from others in the analysis, and no evidence of an association of DB24 with the Chinese haplotype was found (Fig. 2). Thus, as with the evidence of uncorrected pairwise distance (Table 2), the phylogenetic analysis does not support the inference that DB24 may have originated from China or at least from the sampled area, Wuhan, Hubei, China with the current limited samples.

In summary, we newly included COI nucleotide sequences of ten Chinese *P. xylostella* to infer genetic

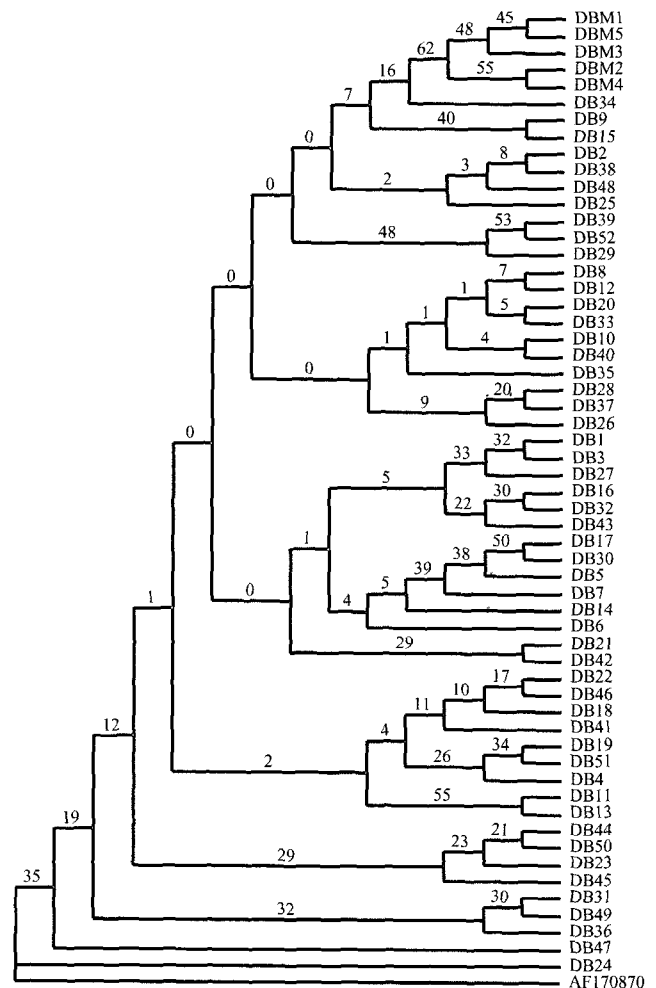


Fig. 2. Phylogenetic analysis of mitochondrial COI sequences of *P. xylostella*. The tree was obtained using a subprogram neighbor-joining method incorporated in PAUP* (Phylogenetic Analysis Using Parsimony and Other Method*) ver 4.0b10 (Swofford, 2002) with the option of Kimura's 2-parameter method (1980). The swallow tail butterfly, *Zerynthia rumina* (GenBank accession AF170870), belonging to the same Lepidoptera was incorporated in the analysis to root tree. The numbers on the branches represent bootstrap values for 1,000 replications.

relationship of these to the pre-existing Korean haplotype data and found an evidence of overall similar genetic relationship of the Chinese samples to the Korean ones, but did not find any specific correlation between one heterogeneous haplotype DB24 and the Chinese haplotypes.

Acknowledgements

This paper was supported by the Dong-A University Research Fund.

References

- Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb and N. C. Saunders (1987) Intra-specific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Ann. Rev. Ecol. Sys.* **18**, 489-522.
- Caprio, M. A. and B. E. Tabashnik (1992a) Gene flow accelerates local adaptation among finite populations: simulating the evolution of insecticide resistance. *J. Econ. Entomol.* **85**, 611-620.
- Caprio, M. A. and B. E. Tabashnik (1992b) Allozymes used to estimate gene flow among populations of diamondback moth (Lepidoptera: Plutellidae) in Hawaii. *Environ. Entomol.* **21**, 808-815.
- Chang, W. X. Z., B. E. Tabashnik, B. Artelt, T. Malvar, V. Bal-
lester, J. Ferré and G. K. Roderick (1997) Mitochondrial DNA sequence variation among geographic strains of diamondback moth (Lepidoptera: Plutellidae). *Ann. Entomol. Soc. Am.* **90**, 590-595.
- Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **29**, 783-791.
- Kim, I., J. S. Bae, K. H. Choi, B. R. Jin, K. R. Lee and H. D. Sohn (2000a) Haplotype diversity and gene flow of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae), in Korea. *Korean J. Appl. Entomol.* **39**, 43-52.
- Kim, I., J. S. Bae, K. H. Choi, S. R. Kim, B. R. Jin, K. R. Lee and H. D. Sohn (2000b) Mitochondrial DNA polymorphism, gene flow, and population genetic structure of the diamondback moths, *Plutella xylostella*, (Lepidoptera: Yponomeutidae), in Southern Korea. *Korean J. Entomol.* **30**, 21-32.
- Kim, I., J. S. Bae, K. S. Lee, E. S. Kim, H. S. Lee, K. S. Ryu, H. J. Yoon, B. R. Jin, B. J. Moon and H. D. Sohn (2003) Mitochondrial COI gene sequence-based population genetic structure of the diamondback moth, *Plutella xylostella*, in Korea. *Korean J. Genetics* **25**, 155-170.
- Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* **116**, 111-120.
- Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, F. X. Villablanca and A. C. Wilson (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86**, 6196-6200.
- Lorimer, R. I. (1981) Lepidoptera immigrants to Orkney in 1890. *Proc. Trans. Br. Entomol. Nat. Hist. Soc.* **14**, 108-109.
- Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.10. Sinauer Associates, Sunderland. MA (on disk).