

Mitochondrial DNA Sequence Variation of the Mason Bee, *Osmia cornifrons* (Hymenoptera: Apidae)

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In order to understand geographic genetic variation and relationship among populations of the mason bee (*Osmia cornifrons* Radoszkowsky), which is used as pollinator for apple tree, we sequenced a portion of mitochondrial (mt) COI gene, which corresponds to "DNA Barcode" region (658 bp) from 81 *O. cornifrons* individuals collected over eight localities in Korea. The sequence data revealed overall moderate to low genetic diversity within species, with a maximum sequence divergence of 0.76%. Geographically, two haplotypes (BAROC01 and BAROC02) were widespread with a frequency of 82.7%, whereas several haplotypes were found in a locality as a single individual, suggesting that haplotype distribution can be summarized as coexistence of a few widespread haplotypes and several regionally restricted haplotypes. Overall, high rate of per generation female migration ($Nm=1.1\sim\infty$) and low level of geographic subdivision ($F_{ST}=0\sim0.315$) among localities were characteristic. Although two populations ($p<0.026$) were genetically subdivided from the remaining localities, no clear polarity was observed. Taken together, the nature of genetic divergence of the mason bee populations is characterized as one that possessing moderate to low genetic diversity, high gene flow, and wide spread haplotypes with a high frequency, concordant with the capability of dispersal in connection with the lack of historical biogeographic barriers.

Key words: Mitochondrial DNA, COI gene, DNA barcode, Mason bee, *Osmia cornifrons*, Population genetic structure, Genetic diversity, Geographic variation, Hymenoptera

Introduction

Bees including solitary ones are important pollinators for the prosperous of the terrestrial biodiversity including plants. In return, plant diversity is an important source for bee diversity, because bees obtain their resources from plants. Due by several threatening factors such as massive destruction of the habitat, increase in human population, urbanization, greenhouse phenomenon, contamination and so on, bee-plant interactions are seriously affected. These threats not only influence to species diversity but also threaten various levels of diversity, such as subspecies, regional population, and genetic diversity (Soulé, 1986; Wilson, 1992).

Osmia cornifrons Radoszkowsky, known as mason bee which is a bee species belonging to the family Apidae in an insect order Hymenoptera, is distributed in Korea and Japan, ranges in size from 8 to 12 mm in adult, and occur once a year during late March to early April in Korea (Lee, 1998). The mason bee differs from the honey bee in that the species is solitary, all females reproduce, both female and male visit floral apple blossoms, and female carries pollen on the lower surface of their abdomens (Kim, 1994; Kwon *et al.*, 1996). The species are commonly used for commercial apple pollination. In fact, the pollinating ability for the apple flower by the mason bee has been reported to be excellent compared with the honey bee in Korea (Kim, 1994; Huh *et al.*, 1997). Thus, the mason bee is an important genetic source that is being

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utilized as pollinator, particularly in Korea.

Nevertheless, there is no genetic, geographic information of the species at all, except for some phylogenetic relationships among several *Osmia* species (Kwon *et al.*, 2003). Such information can serve as species identification to distinguish it from foreign imported, identical or similar species. Also, such information will provide background geographic knowledge that might be important when the species is utilized for genetic engineering. Further, such information will be utilized as prior knowledge for the establishment of long term conservation strategies in the bee-disappearing circumstances.

We previously studied geographic genetic variation of several insect species such as mushroom pest flies (Bae *et al.*, 2001), firefly species (Kim *et al.*, 2001; Lee *et al.*, 2003), bumble bee (Yoon *et al.*, 2003), diamondback moth (Kim *et al.*, 2003; Li *et al.*, 2006), oriental mole cricket (Kim *et al.*, 2007), and dragon fly (Kim *et al.*, 2007). Nevertheless, no study has been made for the mason bee. In this study, we, thus, investigated genetic diversity and geographic variation of the species for the first time. For the purpose of study, we sequenced a portion of mitochondrial COI gene that is used as "DNA Barcode" region (Hebert *et al.*, 2003). This portion of DNA is known to provide the level of genetic variation that can be utilized for species identification (Hebert *et al.*, 2003) and also for a certain level of population genetic structure, depending on the species history (*i.e.*, biogeography, dispersal capability, speciation event and so on) (Lee *et al.*, 2003; Li *et al.*, 2006).

Materials and Methods

Insects

A total of 81 cocoons of *O. cornifrons* were collected from eight localities in 2007. Sampling locality and individual sex are listed in Table. 1 Although similar effort for the collection of samples was made, cocoons from some localities were not qualified enough for DNA work and not enough samples were obtained from some spots of the localities, resulting in variable numbers of samples from locality to locality.

DNA extraction, primer, PCR, and sequencing

The total DNA was extracted using the Wizard Genomic DNA Purification Kit, in accordance with the manufacturer's instructions (Promega, USA). For the amplification of a portion of mt COI gene, corresponding to "DNA Barcode" region being utilized for global animal identification (Hebert *et al.*, 2003) a pair of primer was designed based on Folmer *et al.* (1994): LCO1490, 5'-GGTCAA-

CAAATCATAAAGATATTGG-3' and HCO2198, 5'-TAAACTTCAGGGTGACCAAAAATAAC-3'. After an initial denaturation step at 94°C for 7 min, a 35-cycle amplification (94°C for 1 min, 50~55°C for 1 min, and 72°C for 1 min) was conducted. The final extension step was continued for 7 min and 45 s at 72°C. To confirm the successful DNA amplification, electrophoresis was carried out using 0.5 × TAE buffer on 0.5% agarose gel. The PCR product was then purified using PCR purification Kit (QIAGEN, Germany). The COI gene fragments were directly sequenced from PCR products. DNA sequencing was performed using the ABI PRISM[®] BigDye[®] Terminator v3.0 Cycle Sequencing Kit under the ABI 377 Genetic Analyzer (PE Applied Biosystems, USA). All products were sequenced from both strands. Sequence alignment was performed using CLUSTAL X programs (ver. 1.8; Thompson *et al.*, 1997). When homologous sequences from two individuals differed by ≥ one nucleotide base, the sequences were considered as different haplotypes. Haplotype designations were applied to new sequences as they were discovered (*i.e.*, BAROC01, BAROC02, PBAROC3 and so forth).

Phylogenetic analysis using PAUP and networks

Phylogenetic analysis was performed by maximum-parsimony (MP) method (Fitch, 1971) using PAUP* (Phylogenetic Analysis Using Parsimony and Other Method*) ver. 4.0b10 (Swofford, 2002). To root trees, the homologous region of *Osmia taurus* was sequenced and utilized as an outgroup. The analysis was performed using an equal weighting of transitions and transversions by heuristic search. The reliability of the trees was tested by 1,000 iterations of bootstrapping (Felsenstein, 1985). With intraspecific mtDNA sequence data it often happens that parsimony analyses provide limited resolution because of polytomies, possibly caused by back mutations and parallel mutations. One solution, which we employed, is to prepare one-step median networks, which provide insight into probable relationships among closely related lineages (Bandelt *et al.*, 1995).

Genetic diversity and distance

Genetic diversity estimates, such as haplotype diversity and nucleotide diversity within each locality were obtained using Arlequin ver. 3.0 (Excoffier *et al.*, 2005). On the other hand, maximum sequence divergence within population was obtained by extracting the estimate of unrooted pairwise distance within population from PAUP (Swofford, 2002).

Genetic distance and migration estimate

Genetic distance and migration rate were estimated from

Table 1. A list of trapping localities, animal numbers, and mitochondrial COI haplotypes of *Osmia cornifrons*

Collecting locality (no. of individuals)	Animal number	Sex	COI haplotype	Gen Bank number
1. Milyang Gyungsoangnamdo Province (15)	OC0573	F	BAROC01	EU726555
	OC0574	F	BAROC02	EU726601
	OC0575	F	BAROC01	EU726590
	OC0576	F	BAROC01	EU726564
	OC0577	F	BAROC01	EU726594
	OC0578	F	BAROC01	EU726595
	OC0579	F	BAROC02	EU726605
	OC0580	M	BAROC01	EU726589
	OC0581	M	BAROC02	EU726610
	OC0582	M	BAROC01	EU726574
	OC0583	M	BAROC03	EU726615
	OC0584	M	BAROC03	EU726617
	OC0585	M	BAROC01	EU726596
	OC0586	M	BAROC03	EU726613
	OC0587	M	BAROC01	EU726578
2. Gokseong Jellanamdo Province (13)	OC0590	F	BAROC01	EU726565
	OC0591	F	BAROC01	EU726568
	OC0592	F	BAROC01	EU726569
	OC0593	F	BAROC02	EU726606
	OC0594	F	BAROC01	EU726558
	OC0595	M	BAROC02	EU726607
	OC0596	M	BAROC01	EU726566
	OC0597	M	BAROC01	EU726600
	OC0598	M	BAROC01	EU726567
	OC0599	M	BAROC02	EU726602
	OC0601	M	BAROC04	EU726623
	OC0602	M	BAROC05	EU726624
	OC0603	M	BAROC05	EU726625
3. Icheon Gyunggido Province (11)	OC0606	F	BAROC06	EU726626
	OC0607	F	BAROC07	EU726627
	OC0608	F	BAROC01	EU726557
	OC0609	F	BAROC01	EU726575
	OC0610	F	BAROC01	EU726597
	OC0611	M	BAROC01	EU726588
	OC0612	M	BAROC03	EU726614
	OC0613	M	BAROC01	EU726587
	OC0614	M	BAROC01	EU726576
	OC0615	M	BAROC01	EU726570
	OC0616	M	BAROC01	EU726598
4. Chuncheon Gangwondo Province (1)	OC0617	F	BAROC03	EU726618
5. Dangjin Chungsoangnamdo Province (12)	OC0624	F	BAROC01	EU726556
	OC0625	F	BAROC01	EU726559
	OC0628	F	BAROC02	EU726609
	OC0629	F	BAROC02	EU726604

Table 1. Continued

Collecting locality (no. of individuals)	Animal number	Sex	COI haplotype	Gen Bank number
	OC0630	M	BAROC01	EU726586
	OC0631	M	BAROC01	EU726585
	OC0632	M	BAROC02	EU726608
	OC0633	M	BAROC01	EU726599
	OC0634	M	BAROC02	EU726612
	OC0635	M	BAROC01	EU726584
	OC0636	M	BAROC01	EU726593
	OC0637	M	BAROC01	EU726583
6. Yesan	OC0638	F	BAROC01	EU726560
Chungcheongnamdo (9)	OC0639	F	BAROC02	EU726611
	OC0641	F	BAROC02	EU726603
	OC0644	F	BAROC01	EU726573
	OC0645	M	BAROC01	EU726577
	OC0646	M	BAROC01	EU726579
	OC0647	M	BAROC01	EU726582
	OC0649	M	BAROC01	EU726592
	OC0650	M	BAROC01	EU726591
7. Yeongju	OC0651	F	BAROC08	EU726628
Gyeongsangbukdo Province (13)	OC0652	F	BAROC09	EU726629
	OC0653	F	BAROC04	EU726622
	OC0654	F	BAROC01	EU726561
	OC0655	F	BAROC01	EU726562
	OC0656	F	BAROC01	EU726549
	OC0657	F	BAROC01	EU726554
	OC0659	M	BAROC01	EU726553
	OC0660	M	BAROC01	EU726552
	OC0661	M	BAROC01	EU726551
	OC0662	M	BAROC04	EU726621
	OC0664	M	BAROC01	EU726563
	OC0665	M	BAROC01	EU726550
8. Jecheon	OC0666	F	BAROC03	EU726616
Chungcheongbukdo Province (7)	OC0667	F	BAROC03	EU726619
	OC0669	F	BAROC03	EU726620
	OC0670	F	BAROC01	EU726572
	OC0675	M	BAROC01	EU726581
	OC0676	M	BAROC01	EU726580
	OC0677	M	BAROC01	EU726571

M, male; and F, female.

mtDNA sequences and subroutines in the Arlequin version 3.0 (Excoffier *et al.*, 2005). Population pairwise genetic distance (F_{ST}) and a permutation test of the significant differentiation of the pairs of localities (1,000 bootstraps) were obtained following the approach described in Excoffier *et al.* (1992) and the distance

between DNA sequences were calculated by the Kimura 2-parameters method (Kimura, 1980). Pairwise F_{ST} values were used to estimate per generation migration rate, Nm (the product of the effective population size N_e and migration rate, m) based upon the equilibrium relationship: $F_{ST} = 1/(2Nm + 1)$.

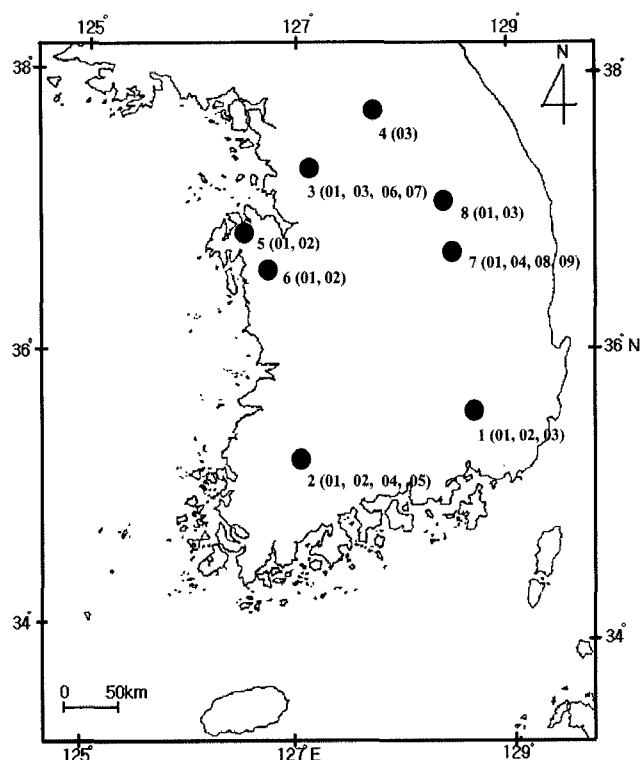


Fig. 1. Sampling location of *Osmia cornifrons* in Korea. General locality names are as follows: 1, Milyang, Gyung-sangnam-do Province; 2, Gokseong, Jellanam-do Province; 3, Icheon, Gyung-gido Province; 4, Chuncheon, Gangwondo Province; 5, Dangjin, Chungcheongnam-do Province; 6, Yesan, Chungcheongnam-do Province; 7, Yeongju, Gyeongsangbuk-do Province; and 8, Jecheon, Chungcheongbuk-do Province. Within parenthesis denotes haplotype name, omitting the antecedent alphabets, BAROC.

Hierarchical genetic structure

Genetic relationships among populations and sets of populations were assessed by the Holsinger and Mason-Gamer (H-MG) method (1996). A detailed rationale of this method is described in the original study of Holsinger and Mason-Gamer (1996) and other reports, which utilized this method (Kim *et al.*, 1998). Unlike other variance analyses, this approach generated the hierarchical relationships of the groups without specifying the hierarchical structure of the populations before the analysis (Holsinger and Mason-Gamer, 1996).

Results and Discussion

COI gene sequence analysis

A total of nine haplotypes (BAROC01 ~ BARNP09) was obtained by sequencing 658-bp of COI gene from 81 *O. cornifrons* (Table 1; Fig. 1). Sequence alignment revealed eight variable nucleotides: four transitions (each two TC and

GA), three transversions (one CG and two AC), and one polymorphic site (CTA). Among these five nucleotide substitutions replaced amino acids: serin into tyrosin and phenylalanine, respectively, by nucleotide substitution in position 30, serin into phenylalanine by nucleotide substitution in position 99, alanine into glycine by nucleotide substitution in position 131, valine into isoleucine by nucleotide substitution in position 380, and threonine into asparagine by nucleotide substitution in position 525 (Fig. 2).

Sequence divergence among haplotypes

Pairwise comparison between pairs of haplotypes was performed to know about the divergence and relationships among haplotypes (Table 2). The sequence divergence among nine haplotypes ranged from 0.152% to 0.760% (1 ~ 5 bp). Comparison of BAROC06 to several haplotypes such as BAROC02, BAROC03, BAROC05, BAROC08, and BAROC09 provided the highest estimate, 0.760% (5 bp) (Table 2). Considering other similar studies, which utilized same region of mitochondrial genome, the estimate of *O. cornifrons* was overall moderate to low. For example, the estimate was 0.2% for domestic silkworm (Kim *et al.*, 2000), 0.2% and 1.2% for two species of mushroom flies (Bae *et al.*, 2001), ~0.23% and 0.12% for two species of the rice planthoppers (Mun *et al.*, 1999), 0.4% for spruce budworm species (Sperling and Hickey, 1994), 0.45% for tiny dragonfly (Kim *et al.*, 2007), 0.5% for *Heliconius* butterflies (Brower, 1994), 0.9% for the diamondback moth (Li *et al.*, 2006), 4.0% for the firefly, *Luciola lateralis* (Kim *et al.*, 2001), 5.0% for another firefly *Pyrocoelia rufa* (Lee *et al.*, 2003). Excluding two firefly species, which have been reported to have a taxonomic implication (Kim *et al.*, 2001; Lee *et al.*, 2003) it was approximately $\leq 1.0\%$ in the insect mitochondrial COI gene, although exact comparison may not be possible, at least because of the areas covered in each study. In the case of another bee species such as *Bombus ardens*, the maximum sequence divergence was 0.5% among four haplotypes in the 44 samples collected over seven Korean localities (Yoon *et al.*, 2003). Considering these findings, it seems that the magnitude of sequence divergence of the *O. cornifrons* is moderate to low compared with other insect species occurring in Korea.

Regional distribution of haplotype

Geographic distribution and frequency of haplotypes are listed in Tables 3. Among nine haplotypes five haplotypes (BAROC05, BAROC06, BAROC07, BAROC08, and BAROC09) were found only in one locality as a single or two individuals. On the other hand, BAROC01 was found in all localities, except for locality 4 (Chuncheon), where only a single individual was obtained (Table 3).

				30		60
				F		
				Y	S	
	I L Y	M I F A	L W S	G M I	G S A M	S I I
BAROC01 (OC0656)	AATTCTATAT	ATAATTTTTG	CTTTATGATC	TGGAATAATT	GGATCAGCAA	TAAGAATTAT
BAROC02 (OC0574)
BAROC03 (OC0586)
BAROC04 (OC0662)T
BAROC05 (OC0602)T
BAROC06 (OC0606)C.....A	.A.....
BAROC07 (OC0607)T
BAROC08 (OC0651)
BAROC09 (OC0652)
				90		120
					F	
	I R M	E L S I	P G S	W I S	N D Q I	Y N S
BAROC01 (OC0656)	TATTCGAATA	GAATTAAGTA	TCCCAGGATC	ATGAATTCT	AATGATCAAA	TTTATAATTC
BAROC02 (OC0574)
BAROC03 (OC0586)
BAROC04 (OC0662)
BAROC05 (OC0602)
BAROC06 (OC0606)T
BAROC07 (OC0607)T
BAROC08 (OC0651)
BAROC09 (OC0652)
				150		180
		G				
	L V T	A H A F	L M I	F F L	V M P F	L I G
BAROC01 (OC0656)	TTTAGTAACT	GCTCATGCCT	TTTAAATAAT	TTTTTTTCTT	GTCATACCAT	TTTTAATTGG
BAROC02 (OC0574)
BAROC03 (OC0586)
BAROC04 (OC0662)
BAROC05 (OC0602)
BAROC06 (OC0606)
BAROC07 (OC0607)
BAROC08 (OC0651)G.....
BAROC09 (OC0652)
				210		240
	G F G	N W L I	P L M	L G I	P D M A	F P R
BAROC01 (OC0656)	AGGATTTGGA	AATTGATTAA	TTCCATTAAT	ATTAGGAATT	CCAGATATAG	CTTTTCCTCG
BAROC02 (OC0574)
BAROC03 (OC0586)
BAROC04 (OC0662)
BAROC05 (OC0602)
BAROC06 (OC0606)
BAROC07 (OC0607)
BAROC08 (OC0651)
BAROC09 (OC0652)
				270		300
	M N N	I S F W	L L P	P S L	M L L L	L S N
BAROC01 (OC0656)	AATAAATAAT	ATTAGATTTT	GACTTTTACC	ACCATCCTTA	ATATTATTAC	TTTTAAGAAA
BAROC02 (OC0574)	C.....
BAROC03 (OC0586)
BAROC04 (OC0662)
BAROC05 (OC0602)	C.....
BAROC06 (OC0606)
BAROC07 (OC0607)
BAROC08 (OC0651)
BAROC09 (OC0652)

Fig. 2. Sequence alignment of nine mitochondrial haplotypes (designated as BAROC01~BAROC9) obtained from 658-bp COI gene sequences of *Osmia cornifrons*. Only nucleotide positions that differ from haplotype BAROC01 are indicated. Corresponding amino acid sequences are also indicated on the top of nucleotide sequence of haplotype BAROC01.

				330			360
	F L N	P S P G	T G W	T I Y	P P L S	S N L	
BAROC01 (OC0656)	TTTTTTAAAT	CCAAGACCTG	GAACAGGATG	AACAATTTAT	CCACCTTTAT	CATCAAATTT	
BAROC02 (OC0574)	
BAROC03 (OC0586)	
BAROC04 (OC0662)	
BAROC05 (OC0602)	
BAROC06 (OC0606)	
BAROC07 (OC0607)	
BAROC08 (OC0651)	
BAROC09 (OC0652)	
				390			420
	F H S	S P S V	D L A	I F S	L H I S	G L S	
BAROC01 (OC0656)	ATTCATTCT	TCTCCTTCAG	TTGATTAGC	AATTTTTTCT	TTACATATTT	CAGGTTTATC	
BAROC02 (OC0574)	
BAROC03 (OC0586)A	
BAROC04 (OC0662)	
BAROC05 (OC0602)	
BAROC06 (OC0606)	
BAROC07 (OC0607)	
BAROC08 (OC0651)	
BAROC09 (OC0652)	
				450			480
	S I M	G S L N	F I V	T I I	M M K N	I S L	
BAROC01 (OC0656)	TTCTATTATA	GGTTCATTA	ATTTTATTGT	TACAATTATT	ATAATAAAAA	ACATTTTCCTT	
BAROC02 (OC0574)	
BAROC03 (OC0586)	
BAROC04 (OC0662)	
BAROC05 (OC0602)	
BAROC06 (OC0606)	
BAROC07 (OC0607)	
BAROC08 (OC0651)	
BAROC09 (OC0652)	
				510			540
	K Y I	Q L S L	F P W	S V F	I T T I	L L L	
BAROC01 (OC0656)	AAAATATATT	CAATTATCCT	TATTCCTTGT	ATCTGTATT	ATTACTACTA	TTCTTTTACT	
BAROC02 (OC0574)	
BAROC03 (OC0586)	
BAROC04 (OC0662)	
BAROC05 (OC0602)	
BAROC06 (OC0606)	
BAROC07 (OC0607)	
BAROC08 (OC0651)	
BAROC09 (OC0652)A.....	
				570			600
	F S L	P V L A	G A I	T M L	L F D R	N F N	
BAROC01 (OC0656)	TTTTTCTTTA	CCTGTATTAG	CTGGAGCAAT	TACTATATTA	TTATTTGATC	GAAATTTTAA	
BAROC02 (OC0574)	
BAROC03 (OC0586)	
BAROC04 (OC0662)	
BAROC05 (OC0602)	
BAROC06 (OC0606)	
BAROC07 (OC0607)	
BAROC08 (OC0651)	
BAROC09 (OC0652)	

Fig. 2. Continued.

	T	S	F	F	D	P	T	630			658								
	T	S	F	F	D	P	T	G	G	G	D	P	I	L	Y	Q	H	L	F
BAROC01 (OC0656)	TACATCTTTT	TTTGATCCAA	CAGGAGGTGG	AGATCCAATT	CTTTATCAAC	ATTTATTT													
BAROC02 (OC0574)
BAROC03 (OC0586)
BAROC04 (OC0662)
BAROC05 (OC0602)
BAROC06 (OC0606)
BAROC07 (OC0607)
BAROC08 (OC0651)
BAROC09 (OC0652)

Fig. 2. Continued.

Table 2. Pairwise comparisons among nine haplotypes obtained from the partial sequences of mitochondrial COI gene of *Osmia cornifrons*

	1	2	3	4	5	6	7	8	9	10
1. BAROC01	-	0.00152	0.00152	0.00152	0.00304	0.00608	0.00304	0.00152	0.00152	0.04711
2. BAROC02	1	-	0.00304	0.00304	0.00152	0.0076	0.00456	0.00304	0.00304	0.04711
3. BAROC03	1	2	-	0.00304	0.00456	0.0076	0.00456	0.00304	0.00304	0.04863
4. BAROC04	1	2	2	-	0.00152	0.00608	0.00152	0.00304	0.00304	0.04863
5. BAROC05	2	1	3	1	-	0.0076	0.00304	0.00456	0.00456	0.04863
6. BAROC06	4	5	5	4	5	-	0.00456	0.0076	0.0076	0.05319
7. BAROC07	2	3	3	1	2	3	-	0.00456	0.00456	0.05015
8. BAROC08	1	2	2	2	3	5	3	-	0.00304	0.04863
9. BAROC09	1	2	2	2	3	5	3	2	-	0.04863
10. <i>Osmia taurus</i>	31	31	32	32	32	35	33	32	32	-

Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute distance values.

Table 3. Relative frequencies of mtDNA COI haplotypes through the populations of *Osmia cornifrons*

Haplotype	Locality							
	1. Milyang (15)	2. Gokseong (13)	3. Icheon (11)	4. Chuncheon (1)	5. Dangjin (12)	6. Yesan (9)	7. Yeongju (13)	8. Jecheon (7)
BAROC01	0.6 (9)	0.538 (7)	0.727 (8)		0.667 (8)	0.778 (7)	0.692 (9)	0.571 (4)
BAROC02	0.2 (3)	0.231 (3)			0.333 (4)	0.222 (2)		
BAROC03	0.2 (3)		0.091 (1)	1 (1)				
BAROC04		0.077 (1)					0.154 (2)	
BAROC05		0.154 (2)						
BAROC06			0.091 (1)					
BAROC07			0.091 (1)					
BAROC08							0.077 (1)	
BAROC09							0.077 (1)	

Numbers in parentheses indicate sample size at each population.

This haplotype accounts for 64.2% of the samples utilized in this study (52 among 81 individuals). In a similar fashion, BAROC02 was found in five localities and accounts for 18.5% (12 individuals). Thus, distribution of *O. cornifrons* haplotypes can be summarized as a wide distribution of a few haplotypes and very restricted distribution in most haplotypes.

Phylogenetic analysis

Phylogenetic analysis was performed to investigate relationships among *O. cornifrons* haplotypes (Fig. 3). All haplotypes were weakly associated or unresolved, due mainly to moderate to low genetic divergence among them. To further illustrate the genetic relationships among *O. cornifrons* haplotypes, we used an unrooted one-step median network, which visualizes a possible evolutionary

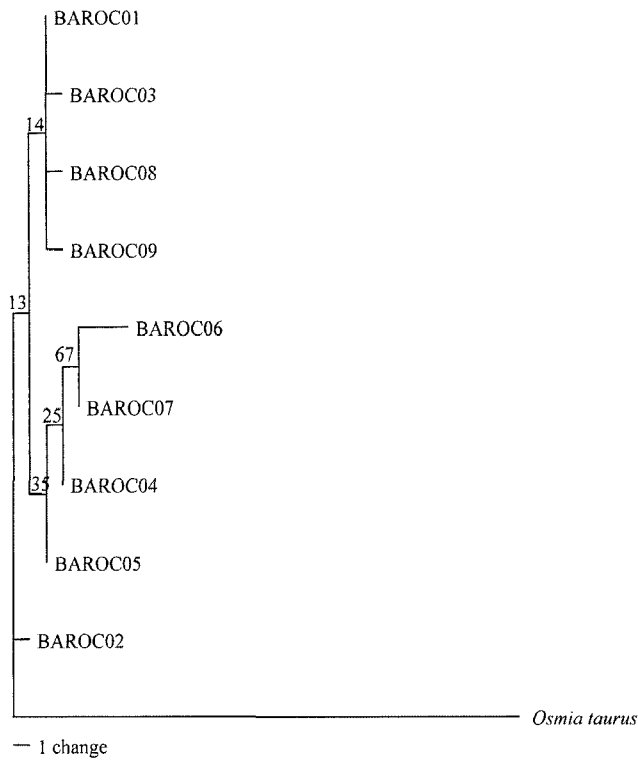


Fig. 3. Phylogenetic analysis of nine mitochondrial haplotypes of *Osmia cornifrons*. The tree was acquired via the Maximum Parsimony method incorporated in the PAUP (Phylogenetic Analysis Using Parsimony and Other) ver. 4.0b10 software (Swofford, 2002). The tree length is 41 steps, Consistency Index is 0.976, Retention Index is 0.750, and Homoplasy Index is 0.027. *Osmia taurus* was used to an outgroup in order to root tree. The numbers on the branches represent bootstrap values of 1,000 replications.

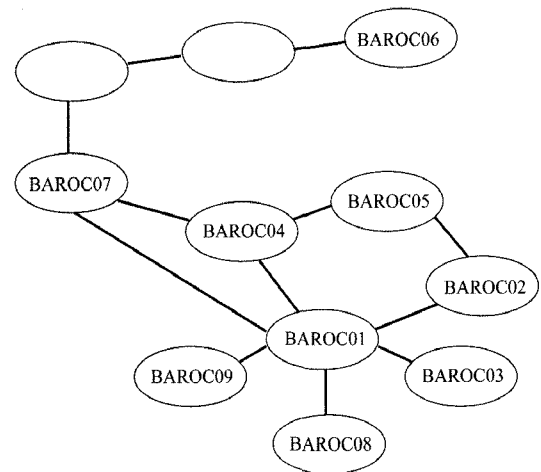


Fig. 4. Parsimonious one-step median networks analysis among nine mitochondrial COI haplotypes of *Osmia cornifrons*. Each bar indicates one nucleotide difference from the neighboring haplotype, and the empty circle indicates the hypothetical haplotype, which was not found in this study.

pathway among closely related haplotypes (Fig. 4). Although we expected more resolution in the closely

related haplotypes, it provided us the limited information. Nevertheless, the network showed that all haplotypes were very closely related to BAROC01, with a minimum sequence divergence, showing “star phylogeny”. However, BAROC06 showed somewhat distant relationship to BAROC01. Neigel and Avise (1993) have reported that the variance of an mtDNA lineage’s geographic distribution is expected be proportional to its age under a simple isolation by distance model. If this theory is applied to *O. cornifrons* the BAROC01 that is most widely distributed (all, but one localities, from which a single individual was collected) with the highest frequency (64.2% of the samples utilized in this study) seems to be ancestral.

Table 4. Within-locality diversity estimates of *Osmia cornifrons*

Locality	SS ^{a)}	NH ^{b)}	H ^{c)}	NP ^{d)}	MSD ^{e)} (%)	MPD ^{f)}	p ^{g)}
1. Milyang	15	3	0.6000	2	0.304	0.685714	0.001042
2. Gokseong	13	4	0.6795	2	0.304	0.897436	0.001364
3. Icheon	11	4	0.4909	5	0.608	1.218182	0.001851
5. Dangjin	12	2	0.4848	1	0.152	0.484848	0.000737
6. Yesan	9	2	0.3889	1	0.152	0.388889	0.000591
7. Yeongju	13	4	0.5256	3	0.304	0.589744	0.000896
8. Jecheon	7	2	0.5714	1	0.152	0.571429	0.000868

^{a)}Sample size

^{b)}Number of haplotypes

^{c)}Haplotype diversity

^{d)}Number of polymorphic sites

^{e)}Maximum sequence divergence

^{f)}Mean number of pairwise differences

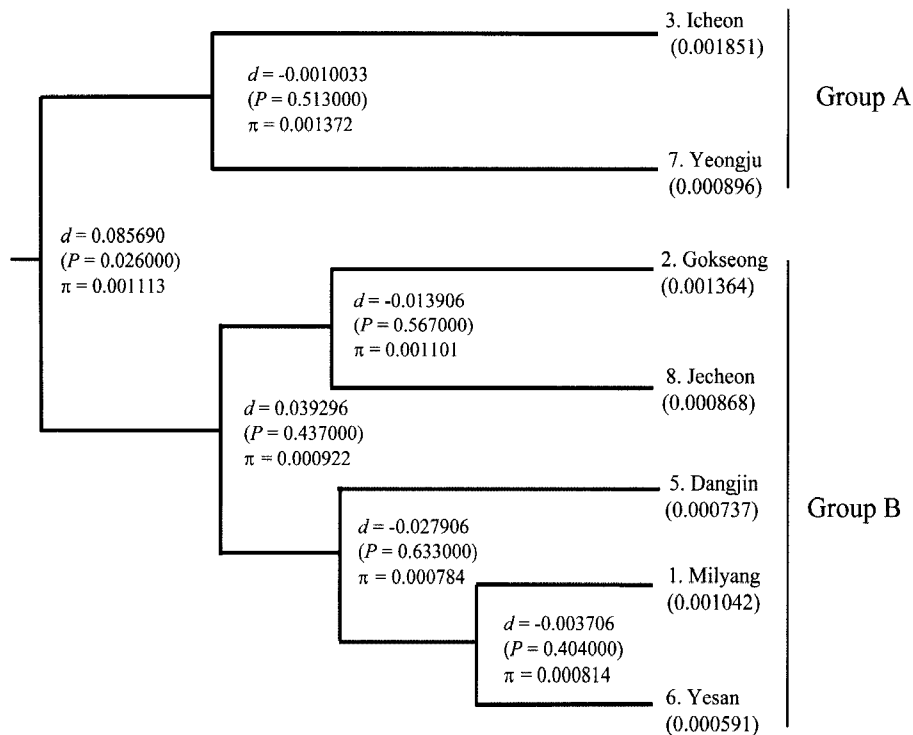
^{g)}Nucleotide diversity

Table 5. Fixation indices (F_{ST}) and migration rate (Nm) between pairs of populations of *Osmia cornifrons*

	1. Milyang	2. Gokseong	3. Icheon	5. Dangjin	6. Yesan	7. Yeongju	8. Jecheon
1. Milyang		$F_{ST}=0.08319$ $Nm=5.51007$	$F_{ST}=0.05692$ $Nm=8.28458$	$F_{ST}=0.19675^*$ $Nm=2.04131$	$F_{ST}=-0.01916$ $Nm=inf$	$F_{ST}=0.09770^*$ $Nm=4.61770$	$F_{ST}=0.03553$ $Nm=13.57143$
2. Gokseong			$F_{ST}=0.11333^*$ $Nm=3.91170$	$F_{ST}=0.12754$ $Nm=3.42019$	$F_{ST}=0.02029$ $Nm=24.13720$	$F_{ST}=0.12731$ $Nm=3.42727$	$F_{ST}=-0.04158$ $Nm=inf$
3. Icheon				$F_{ST}=0.03492$ $Nm=13.81673$	$F_{ST}=0.5158$ $Nm=9.19320$	$F_{ST}=0.00311$ $Nm=160.35098$	$F_{ST}=0.13501$ $Nm=3.20345$
5. Dangjin					$F_{ST}=0.20879^*$ $Nm=1.89474$	$F_{ST}=0.00151$ $Nm=331.15789$	$F_{ST}=0.31453^*$ $Nm=1.08968$
6. Yesan						$F_{ST}=0.06770$ $Nm=6.88507$	$F_{ST}=-0.03716$ $Nm=inf$
7. Yeongju							$F_{ST}=0.20989^*$ $Nm=1.88222$
8. Jecheon							

* $p < 0.05$.

inf, infinite.

**Fig. 5.** Hierarchical relationships among localities analyzed using Holsinger and Mason-Gamer method (1996). The value at each node is the distance (d) between its two daughter nodes and the P value is the significance of differentiation (based on 10,000 random resamplings). π is the diversity estimate of the node or locality.**Genetic diversity indices**

Within-locality diversity was estimated in terms of haplotype diversity (H), maximum sequence divergence (MSD), mean number of pairwise differences (MPD), and nucleotide diversity (π), except for locality 4, where a single individual was obtained (Table 4). In a range of 0~1 in H , locality 2 (Gokseong) and locality 1 (Milyang) were

comparatively high ($H=0.6795$ and 0.6000 , respectively). In terms of π , the estimates were highest in the order of locality 3 (Icheon) as 0.00185 , locality 2 (Gokseong) as 0.001364 , and locality 1 (Milyang) as 0.001042 . On the other hand, the remaining four localities were relatively low in π , with the range of $0.000591 \sim 0.000896$ (Table 4). Considering geographic location of Icheon, Gokseong,

and Milyang, no regional bias in local genetic diversity was observed.

Gene flow

Genetic distance (F_{ST}) and per-generation migration rates (Nm) between pairs of populations are shown in Table 5. Test of statistical significance of pairwise F_{ST} estimates were found in six cases: between Milyang and Dangjin, between Milyang and Jecheon, between Gokseong and Icheon, between Dangjin and Yesan, between Dangjin and Jecheon, and between Yeongju and Jecheon (Table 5). Consistent result was obtained from gene flow estimate (Nm), showing the least gene flow estimate between Dangjin and Yesan ($Nm=1.895$). Overall, no obvious geographic subdivision between localities was obtained. Considering the results of gene flow and genetic diversity estimates, it seems that the *O. cornifrons* populations in the Korean peninsula are very well connected to each other and the different genetic diversity found among populations are probably seasonal fluctuation, rather than actual genetic population size. To have further solid data redundant collection might be required.

Hierarchical population genetic structure

The hierarchical relationships among localities, analyzed by Holsinger and Mason-Gamer (HM-G) method (1996) are presented in Fig. 5. The seven Korean localities were structured into two groups ($p=0.039$): Icheon+Yeongju (termed group A) and Gokseong+Jecheon+Dangjin+Milyang+Yesan (termed group B). Although the geographic populations of *O. cornifrons* showed subdivision into two groups, it seems that there is no immediate relationship between geographic distance and genetic distance. This result probably reflects a high potential/actual dispersal ability of the species without long term zoogeographic barrier against gene flow.

Summarized, the sequence analysis of the mason bee, *O. cornifrons*, provided overall moderate to low genetic diversity. In terms of geography, no bias in regionally higher genetic diversity and no particular geographic partition was observed. For more information, further investigation with further hypervariable DNA portion and repetitive sample collection may provide more detailed sketch of the genetic diversity on this valuable species.

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