

Genetic Homogeneity of the Korean Native Bumble Bee, *Bombus ardens* (Hymenoptera: Apidae), Detected by Mitochondrial COI Gene Sequences

Hyung Joo Yoon^{1*}, Sam Eun Kim¹, Myeong Lyeol Lee¹, Iksoo Kim¹, Jin Sik Bae², Hung Dae Sohn² and Byung Rae Jin²

¹Department of Sericulture and Entomology, The National Institute of Agricultural Science & Technology, Rural Development Administration, Suwon 441-100, Korea.

²College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea.

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We investigated the sequence divergence of the geographic samples of the queen bumble bee (*Bombus ardens*) in Korea. A portion of mitochondrial COI gene sequences (423 bp) was analyzed for 44 individuals collected from seven localities. Sequence analysis resulted in four COI haplotypes with the maximum nucleotide divergence of only 0.5% (two bp). One haplotype (BA1) was dominant in all localities surveyed (86.4%). The finding of low sequence divergence and dominance of one haplotype appear to reflect, although limited, the life history of the *B. ardens* queens subjected to active dispersal and seasonal fluctuation in queen number.

Key words: Bumble bee, *Bombus ardens*, mtDNA, COI gene, Genetic homogeneity, Dispersal.

Introduction

The use of bumble bees to greenhouses for pollination has become widespread in recent years, and is increasing every year. The immense popularity of bumble bees in greenhouses is due to their own merits as pollinators. In recent years, some bumblebee species are successfully used as an efficient pollinator in greenhouses, but others are not (Iwasaki, 1995; Hannan *et al.*, 1998). Among them, *Bombus terrestris* has been extensively studied and

is commercially available as a pollinator (Iwasaki, 1995; Mitsuhashi, 2000).

The bumble bee is a social insect, working harmoniously for the common good. The workers are infertile daughters of one female, queen, and they appear to be altruistic in adding queens reproductive output (Allen *et al.*, 1978).

The queens that have mated with males in late summer hibernate and emerge in spring (Heinrich, 1979). The queen builds up a store of pollen and lays 8–10 eggs into the pollen for the first time after searching a suitable site to found a colony. Sometimes this may take all day, a few days or possibly two weeks or longer. The eggs are hatched into milky white larvae and the larvae pupate and emerge as adults. The queen usually lays another batch of eggs while the first batch is still in the larval stage. After workers from the first batch emerge the queen can spend more time on oviposition because the workers start to forage two- or three-days after emerging. In the late summer, many males and new queens are produced and only mated queens hibernate and emerge in spring.

Bumble bees are one of the most widely distributed species group and approximately 300 species of bumblebees are known throughout the world (Williams, 1994). Bumble bee classification is heavily depended on color patterns (Lavery and Harder, 1988; Labougle, 1990; Starr, 1992), and some on male genitalia (Krüger, 1917) and behavioral information, such as nest architecture (Sakagami, 1976). Twenty-one species are known to occur in Korea and *B. ardens* is known to occur in China, Japan, and Korea and found in abundantly at the time of blooming (Lee and Dumouhel, 1999). Although the field data is not much available for the species, the biological information as a pollinator for mass-production has been investigated substantially (Kim *et al.*, 2002; Yoon *et al.*, 1998,

*To whom correspondence should be addressed.

Department of Sericulture and Entomology, The National Institute of Agricultural Science & Technology, Rural Development Administration, Suwon 441-100, Korea. Tel: +82-31-290-8541; E-mail: YoonHJ@rda.go.kr

1999a, b, 2002).

To utilize the wild bumble bees for pollination they are seasonally captured and adjusted for indoor-rearing conditions. During this process some bumble bees are better adapted to indoor conditions (*e.g.*, produce more queens and workers), but the others are not. Although slight, this trend appears to reflect the place of geographic origin (Yoon *et al.*, personal observation). For example, the bumble bees captured from an isolated islet named Namhe tend to tolerate somewhat larger temperature fluctuation and produce more queens than those from other areas (Yoon *et al.*, personal observation). Although this observation may reflect more likely the difference in the physiological requirement of queens rather than genetics, no study, however, tested such hypothesis. Also, understanding the magnitude of genetic divergence and, if any, population genetic structure might be important knowledge in utilizing the species as a pollinator, because the strategy for domestication of the bumble bees should be more subdivided if there are a large genetic divergence and systematic implication among geographic populations.

Mitochondrial DNA (mtDNA) has a high evolutionary rate compared to the functional counterpart of nuclear DNA. In addition, it is inherited maternally, lacking genetic recombination, and is easy to handle (Brown *et al.*, 1982; Harrison, 1989). These characteristics make the mtDNA molecule as particularly appropriate marker, suitable for tracing the recent evolutionary history of animals (Wilson *et al.*, 1985). It is known that the cytochrome oxidase subunit I (COI) gene of mtDNA is highly variable in its DNA sequence. This sequence from several insects was determined to infer their intraspecific genetic variation (Bae *et al.*, 2001; Kim *et al.*, 2000a, b, c, d; Kim *et al.*, 2001; Lee *et al.*, 2000; Simon *et al.*, 1994; Yoon *et al.*, 2001).

In this study, we determined the sequences of the COI gene of mtDNA from the Korean native bumble bee, *B. ardens*, collected from seven localities in Korea and analyzed the magnitude and nature of genetic divergence to see if any geographic variation exists among Korean populations.

Materials and Methods

Insects

Queens of the Korean native bumble bee, *Bombus ardens*, was collected from seven localities in Korea. Collection information of the samples is listed in Fig. 1 and Table 1. The collected samples were frozen at -70°C for molecular analysis after a brief morphological examination and taking of picture for future reference.

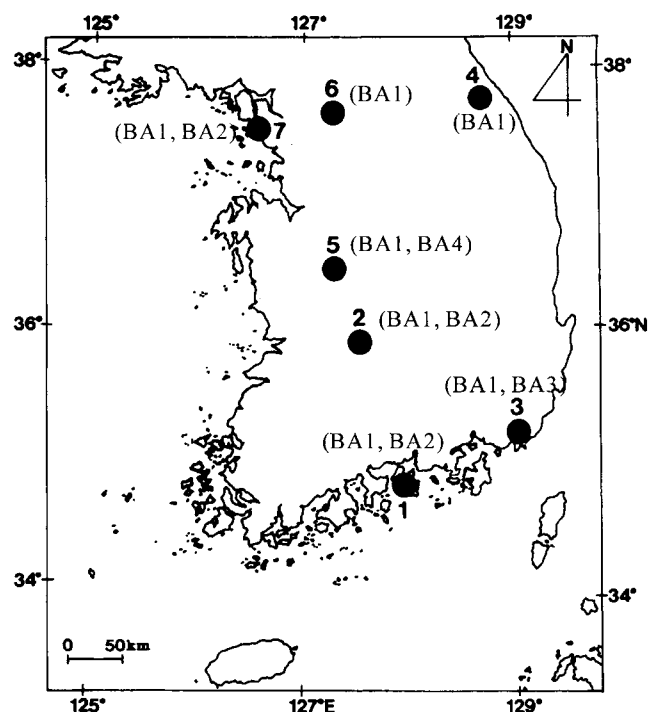


Fig. 1. Sampling locations of the bumblebee, *B. ardens*, in Korea. General locality names in the text are as follows: 1, Sangga-ri, Nam-myeon, Namhae-gun, Gyeongsangnam-do; 2, Samgong-ri, Seolcheon-myeon, Muju-gun, Jeonlabug-do; 3, Hadan-dong, Saha-gu, Busan-si; 4, Songeumgang [River], Yeongog-myeon, Ganleung-si, Gangwon-do; 5, Gabsa [Temple], Junggang-ri, Gyeolyong-myeon, Gongju-si, Chungcheongnam-do; 6, Cheonmasan [Mt.], Hwado-eub, Namyangju-si, Gyeonggi-do; 7, Jag-yagdo [Island], Manseog-dong, Dong-gu, Incheon-si.

MtDNA amplification and sequencing

Total DNA was extracted from the tissue samples by using the WizardTM Genomic DNA Purification Kit, according to the manufacturer's instructions (Promega, USA). The primers used for amplification of the sequences of the COI gene were primer CI-J-1718 (forward), 5-GGAG CTCCTGACATAGCATTCCC-3, and primer CI-N-2191 (reverse), 5-CCCGGTAAAATTTAAAATATAAACTTC-3 (Simon *et al.*, 1994; Yoon *et al.*, 2001). The PCR amplification condition consisted of an initial denaturation step of 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 50°C for 40 sec and 72°C for 45 sec, and a final extension step of 72°C for 10 min. To ascertain successful DNA amplification, electrophoresis was carried out on 1.0% agarose gel. The amplified products were purified with Qiaquick PCR Purification kit (QIAGEN), and then used directly for sequencing. DNA sequencing was performed using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, CA). Each strand was sequenced twice for accuracy, aligned using

Table 1. A list of trapping localities, animal numbers, mitochondrial COI haplotypes, and GenBank accession numbers

Collecting locality (no. of individuals)	Collection date	Animal number	COI haplotype	GenBank accession number
1. Sangga-ri, Nam-myeon Namhae-gun, Gyeongsangnam-do (6)	2000. 4. 4	B108	BA2	AY216393
	2000. 4. 4	B109	BA1	AY216394
	2000. 4. 4	B111	BA2	AY216395
	2000. 4. 5	B113	BA1	AY216396
	2000. 4. 5	B114	BA1	AY216397
	2000. 4. 5	B115	BA1	AY216398
2. Samgong-ri, Seolcheon-myeon Muju-gun, Jeonlabug-do (6)	2000. 4. 20	B146	BA1	AY216399
	2000. 4. 29	B148	BA1	AY216400
	2000. 4. 29	B150	BA2	AY216401
	2000. 4. 29	B151	BA1	AY216402
	2000. 4. 29	B152	BA1	AY216403
	2000. 5. 6	B153	BA1	AY216404
3. Hadan-dong, Saha-gu, Busan-si (6)	2000. 4. 6	B159	BA1	AY216405
	2000. 4. 6	B160	BA3	AY216406
	2000. 4. 6	B162	BA1	AY216407
	2000. 4. 6	B164	BA1	AY216408
	2000. 4. 6	B165	BA1	AY216409
	2000. 4. 6	B174	BA1	AY216410
4. Sogeumgang [River], Yeongog-myeon, Gangleung-si, Gangwon-do (6)	2000. 4. 7	B208	BA1	AY216411
	2000. 4. 7	B209	BA1	AY216412
	2000. 4. 7	B210	BA1	AY216413
	2000. 4. 7	B212	BA1	AY216414
	2000. 4. 7	B213	BA1	AY216415
	2000. 4. 7	B214	BA1	AY216416
5. Gabsa [Temple], Junggang-ri, Gyeolyong-myeon, Gongju-si, Chungcheongnam-do (7)	2000. 4. 9	B218	BA4	AY216417
	2000. 4. 9	B219	BA1	AY216418
	2000. 4. 9	B220	BA1	AY216419
	2000. 4. 9	B221	BA1	AY216420
	2000. 4. 9	B222	BA1	AY216421
	2000. 4. 9	B223	BA1	AY216422
	2000. 4. 9	B224	BA1	AY216423
6. Cheonmasan [Mt.], Hwado-eub, Namyangju-si, Gyeonggi-do (6)	2000. 4.12	B259	BA1	AY216424
	2000. 4.12	B260	BA1	AY216425
	2000. 4.12	B261	BA1	AY216426
	2000. 4.12	B262	BA1	AY216427
	2000. 4.12	B263	BA1	AY216428
	2000. 4.12	B265	BA1	AY216429
7. Jag-yagdo [Island], Manseog-dong, Dong-gu, Incheon-si (7)	2000. 4. 11	B279	BA1	AY216430
	2000. 4. 11	B281	BA1	AY216431
	2000. 4. 11	B282	BA1	AY216432
	2000. 4. 11	B283	BA1	AY216433
	2000. 4. 11	B284	BA1	AY216434
	2000. 4. 11	B285	BA2	AY216435
	2000. 4. 11	B286	BA1	AY216436

IBI MacVector (ver. 6.5), and decided for a final individual sequence. When homologous sequences from two individuals differed by \geq one nucleotide base, the

sequences were considered as different haplotypes. Haplotype designations (BA1, BA2, BA3, and BA4) were applied to new sequences as they were discovered.

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          30                                60
BA1 CGAATAAATA ATATTAGATT TTGATTACTT CCTCCATCTC TTTTATATT AATTATAAGA
BA2 .....
BA3 .....
BA4 .....

          90                                120
BA1 AATTTATTTA CACCAAAATGT AGGAACAGGA TGAACCTGTT ATCCACCATT ATCATCTTAT
BA2 .....
BA3 .....
BA4 .....

          150                                180
BA1 ATATTTTCATT CATCACCTTC AATTGATATT GCTATTTTTT CATTACATAT AACAGGTATT
BA2 .....
BA3 .....
BA4 .....

          210                                240
BA1 TCTTCAATTA TTGGACTCTT AAATTTTATT GTAACCTATTA TATTAATAAA AAATTTTTCGA
BA2 .....
BA3 .....
BA4 .....

          270                                300
BA1 TTAATTTATG ATCAAAATTA TTTATTTTCT TGATCAGTTT GTATTACAGT AATTTTATTA
BA2 .....
BA3 .....
BA4 .....

          330                                360
BA1 ATTTTATCAT TACCAGTTTT AGCAGGAGCA ATTACTATAC TTCTTTTGA TCGAAATTTT
BA2 .....
BA3 .....
BA4 .....

          390                                420
BA1 AATACATCAT TTTTGTATCC AATAGGGGGA GGAGATCCAA TCTTTATCA ACATTTATTT
BA2 .....
BA3 .....
BA4 .....

423
BA1 TGA
BA2 ...
BA3 ...
BA4 ...

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Fig. 2. DNA sequence (423 bp) for a region of mitochondrial COI gene in four haplotypes isolated from *B. ardens*. Only nucleotides that differ from BA1 haplotype are indicated.

Table 2. Pairwise Comparisons Among Four Haplotypes Obtained From the Partial Sequences of Mitochondrial COI Gene of *B. ardens*

	1	2	3	4
1 BA1	–	0.002	0.002	0.002
2 BA2	1	–	0.005	0.005
3 BA3	1	2	–	0.005
4 BA4	1	2	2	–

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

Results and Discussion

Sequence analysis of *B. ardens* resulted in four haplotypes (BA1, BA2, BA3, and BA4) by a partial sequence of COI gene (423 bp) of 44 individuals from seven localities in Korea (Fig. 2). Sequence alignment revealed three variable nucleotide positions (97, 281, and 294), and these positions were all transitional substitutions ($G \leftrightarrow A$ and $T \leftrightarrow C$).

Distribution of haplotypes, number of individuals and GenBank accession numbers are listed in Table 1. Among the 44 individuals collected from seven localities, 38 indi-

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          30                                60
BA1 RMNISEFWLL PPSLFMLIMS NLFTPNVGTG WTVYPLPSSY MFHSSPSIDI AIFSLHMTGI
BA2 .....
BA3 .....
BA4 .....

          90                                120
BA1 SSIIGSLNFI VTIMLMKNFS LNYDQINLFS WSVCTIVILL ILSLPVLAGA ITMLLFDRNF
BA2 .....
BA3 .....
BA4 .....

          141
BA1 NTSFFDFMGG GDPILYQHLF W
BA2 .....
BA3 .....
BA4 .....

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Fig. 3. The deduced amino acid sequences of DNA sequence (423 bp) for a region of mitochondrial COI gene in four haplotypes isolated from *B. ardens*. Only amino acid sequences that differ from BA1 haplotype are indicated.

viduals are haplotype BA1 (86.4% of all samples), and this haplotype are found in all localities sampled (Table 1). Except for BA1, others are very rare: four individuals from a total of three localities were BA2 (9.1%) and each one individual possessed BA3 (2.3%) and BA4 (2.3%), respectively. Pairwise comparisons showed that the maximum nucleotide sequence divergence was only 0.5% among haplotypes (two nucleotide bases), and BA1 differed only one nucleotide base from other haplotypes, respectively. Furthermore, the deduced amino acid sequence of BA1 was identical to BA2 and differed only one amino acid sequence from BA3 and BA4, respectively (Fig. 3). From these results, the nucleotide/amino acid sequence divergence of *B. ardens* was revealed to be extremely low.

Such a low genetic divergence was previously reported in other insect species. For example, the sequence divergences were ~ 0.2% for *Bombyx mori* (Kim *et al.*, 2000c), 0.2% for *Lycoriella mali* (Bae *et al.*, 2001), ~ 0.4% for spruce budworm species (Sperling and Hickey, 1994), 0.23% and 0.12% for two species of the rice planthoppers (Mun *et al.*, 1999), and 0.5% for *Heliconius* butterflies (Brower, 1994).

It has been suggested that many insects and birds, which tend to be vigil organisms, show significantly lower levels of mean population structure than do relatively sedentary creatures such as some amphibians (Avice, 1994). The queen bumble bees have a capability of traveling several kilometers to find out suitable site and to found a colony and of attacking other nests to take the brood of other queens in far distance (Heinrich, 1979).

Maruyama and Kimura (1980) furthermore found a fact that frequent extermination and remigration of population decrease the substantial genetic size of species. If this theory is applied to *B. ardens*, we can regard *B. ardens* as a species that undergoes a frequent fluctuation in population size. In a successful year, more than hundred of new bumble bee queens are produced from one colony, but it drops

down to dozens in poor years (Heinrich, 1979). When overwintering success is poor, a few of the surviving queens tend to be successful in establishing large colonies by utilizing nearby resources. But when overwintering success is high, the new queens from a single colony will be amounted to hundreds and they will quickly utilize the available food resources. In this case, an inevitable dispersal in search of new resources will compensate for the small population size of the neighboring subpopulations experienced near and/or complete extermination during harsh winter.

In conclusion, *B. ardens* collected from seven localities in Korea shows very low nucleotide sequence divergence (0.2% to 0.5%), and one haplotype (BA1) was dominant over entire populations surveyed with a high frequency (a total of 86.4%). Considering these data, *B. ardens* found in Korea appear to be genetically similar, at least in the mitochondrial CO I genome.

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