Journal of Heredity 2004:95(1):46–52
DOI: 10.1093/jhered/esh008
© 2004 The American Genetic Association

Genetic Structure of Asian Populations of *Bombus ignitus* (Hymenoptera: Apidae)

Z.-Y. Shao, H.-X. Mao, W.-J. Fu, M. Ono, D.-S. Wang,
M. Bonizzoni, and Y.-P. Zhang

From the Laboratory of Coevolution, Shanghai Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 20032, P. R. China (Shao, Mao, and Fu), Laboratory of Molecular Biology of Domestic Animals, and Cellular and Molecular Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, P. R. China (Shao and Zhang), Laboratory for Conservation and Utilization of Bioresources, Yunnan University, Kunming 650091, China (Zhang), Laboratory of Insect Technology, Department of Bioresource Sciences, Faculty of Agriculture, Tamagawa University, Machida, Tokyo 194-8610, Japan (Ono), Shanghai Key Laboratory of Protected Horticultural Techniques, Shanghai Academy of Agricultural Sciences, 2901 Beidi Rd., Shanghai 201106, China (Wang), and Department of Animal Biology, University of Pavia, Piazza Botta 9, 27100 Pavia, Italy (Bonizzoni). We are indebted to many friends and colleagues: Myenong-Lyeol Lee (Korea), W.-J. Yu, and M.-F. Yang for sample collection; Y.-G. Yao, J.-F. Pang, H.-R. Luo, K.-X. Qu, D. Pang, G.-S. Wu, Y.-X. Luan, J.-G. Xiangyu, J. Luo, and X.-L. Song for technical and theoretical help; and A. Widmer (Switzerland) and R. Malacrida (Italy) for manuscript revisions. This work was supported by the Chinese Academy of Sciences (KSCX2-1-05), Program for Key International S&T Cooperation Project of China (2001CB711103), and Natural Science Foundation of China (NSFC).

Address correspondence to Ya-Ping Zhang, Laboratory for Conservation and Utilization of Bioresources, Yunnan University, Kunming 650091, China, or e-mail: zhangyp@public.km.yn.cn.

Abstract

The genetic structure of seven mainland and island Asian populations of *Bombus ignitus* was investigated using nine microsatellite markers and the sequences of part of the mitochondrial cytochrome *b* (*cytb*) gene. While microsatellite markers showed high genetic variability, no sequence variation was found in the *cytb* gene fragment analyzed. The number of microsatellite alleles ranged from 9 to 24. Gene diversities per locus per population ranged from 0.378 to 0.992. Analysis of molecular variance (AMOVA) and most pairwise F_{ST} values showed significant genetic differentiation between mainland and island populations. *Cytb* sequences data and microsatellite bottleneck tests indicated that almost all populations were subjected to recent bottlenecks. Our results suggest that *B. ignitus* populations diverged due to recent bottlenecks and geographic isolation.

Bumblebees are widely used as tomato pollinators in greenhouses because they are more efficient than honeybees in low-light conditions. After the refinement of bee-rearing techniques (Eijnde et al. 1991; Pouvreau and Marileau 1980), many companies could provide colonies at any developmental stage. However, there were only a few species merchandized, most commonly *Bombus terrestris*. As it was inevitable that bees were transported between different regions (Ortiz-Sanchez 1992), thus promoting gene exchange, it is urgent to characterize the genetic structure of different geographic populations to avoid the spreading of some dangerous parasites and phenomena analogous to the hybridized European honeybee problem from the African bee invasion (Goka et al. 2001; Visscher 2001).

Bombus ignitus was mainly distributed in China, Japan, and Korea (Williams 1996) and was used in China and Japan as a commercially available pollinator. Many merchandisers were trying to export it to all over the world because of its biology and efficiency for pollinating, similar to *B. terrestris* (Asada and Ono 2000; Bilinski 1999). Two main European bumblebee species, *B. terrestris* and *Bombus pascuorum*, were studied for geographic genetic variation and both of them showed that insular populations differentiated from continental ones (Estoup et al. 1996; Pirounakis et al. 1998;

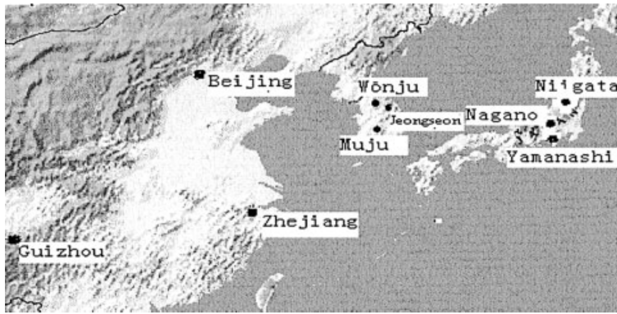

Figure 1. Sample localities (map of East Asia). The sample size for microsatellite analysis (former number), sequencing (latter number), and sampling time for each locality: China: Guizhou, 5, 1, 1999–2000; Zhejiang, 21, 4, 2000–2001; Beijing, 33, 2, 2001; South Korea: Wōnju, 3, 1, 2001; Jeongseon, 3, 0,

Widmer et al. 1998, 1999). However, the population structure of *B. ignitus* has been rarely explored.

Mitochondrial DNA (mtDNA) has been widely used in vertebrates and invertebrates as a molecular genetic marker because of its high mutation rate, haploid maternal inheritance, and its abundance in cells, more than 10,000 copies (Brown et al. 1979; Gillham 1994; Moritz et al. 1987). After the honeybee mtDNA genome was completely sequenced (Crozier and Crozier 1993), mtDNA sequences were used to study the evolutionary relationships among many Hymenoptera taxa (Estoup et al. 1996; Koulianos 1999; Koulianos and Schmid-Hempel 2000; Widmer et al. 1998, 1999). Estoup et al. (1996) found little difference within and between continent and island *B. terrestris* populations based on mitochondrial COII gene sequences, while Widmer et al. (1998) found more in the *cytb* gene. Widmer and Schmid-Hempel (1999) and Pirounakis et al. (1998) also found many variations in the *cytb* sequence in *B. pascuorum*.

Microsatellites are popular genetic markers because of their high polymorphism (Paxton et al. 2001; Queller et al. 1993; Widmer et al. 1998, 1999), and are used for studying bumblebee population structure and paternal identity (Estoup et al. 1995a–c, 1996; Paxton et al. 2001; Widmer et al. 1998, 1999).

In the present study we analyzed the variation in nine microsatellite loci and part of the *cytb* gene sequence to study the genetic structure of different populations of *B. ignitus* from China, South Korea, and Japan. Our aim was to determine whether there was genetic differentiation between different local populations.

Materials and Methods

Materials

Queen and worker bees were collected from three localities in mainland China (Beijing, Zhejiang, and Guizhou), three localities in mainland South Korea (Wōnju, Muju, and Jeongseon), and three localities in Japan (Nagano, Niigata, and Yamanashi) between 1999 and 2001 (Figure 1). Wōnju, Muju, and Jeongseon bees were regarded as one population because of the small sample size. All bees were kept in pure ethanol at room temperature. Individuals were taken more than 500 m apart to decrease the possibility of sampling individuals from the same colony (Estoup et al. 1996).

DNA Extraction

DNA was isolated from about 50 mg of tissue from the head, thorax, or legs using standard phenol/chloroform methods (Garnery et al. 1991; Koulianos 1999).

Microsatellite Analysis

A total of 124 individuals were analyzed at nine microsatellite loci (*B10*, *B11*, *B124*, *B96*, *B100*, *B126*, *B119*, *B118*, and *B132*), previously described in Estoup et al. (1995b, 1996). Polymerase chain reactions (PCRs) were set up with 5–10 ng total DNA, 0.4 pM for each pair of primers, 0.2 mM for each dNTP (including 1/100 florescent dye-labeled dNTP), 0.4 μg/μl BSA, 1× PCR buffer, 0.5–1.0 mM $MgCl_2$, 0.5 U *Taq* polymerase to a final 10 μl volume. PCR was performed at 96°C for 5 min, 35 cycles of 96°C for 50 s, 53–58°C (referring primers as described in Estoup et al. 1995b, 1996) for 40 s, 72°C for 40 s, followed by a 10 min extension at 72°C. The PCR products were scanned with internal standard Mark GS350 [ROX] using an ABI 377 sequencer (Perkin-Elmer). The results were read with Gene Analysis 3.1 and further analyzed with Genotyper 2.0, then checked by eye.

PCR Amplification and Sequencing

Sixteen individuals selected from all the populations (Figure 1) were amplified and sequenced for part of the *cytb* gene by using the CB1 and CB2 primers of Simon et al. (1994). These primers locate to 11,400–11,425 bp and 11,859–11,884 bp in the honeybee mtDNA sequence (Crozier and Crozier 1993). Sequencing was performed using a Bigdye Terminator cycle sequencing kit (PE Biosystems) on an ABI 377 sequencer. Each individual was sequenced in both directions. Sequences were submitted to GenBank with the accession number AF498398.

Data Analyses

The *cytb* sequences were edited by Editseq and aligned by Segman in the DNAStar package. Unless specified, microsatellite data were gathered using Arlequin 2.0 (Schneider et al. 2000). Pairwise linkage disequilibrium and the Hardy-Weinberg equilibrium (HWE) were checked to analyze the association between the microsatellite loci and population states (Guo and Thompson 1992; Lewontin and Kojima 1960; Nei 1978; Slatkin and Excoffier 1996). The pairwise F_{ST} and R_{ST} could be used as short-term genetic distances between populations, the former fitting for the infinite alleles model (IAM) and the latter for single stepwise mutation model (SMM; Balloux and Lugon-Moulin 2002; Schneider et al. 2000; Slatkin 1995). We tested the relationship between local populations by calculating pairwise F_{ST} because *B. ignitus* mainly performed IAM based on previous studies (discussed in the results of the bottleneck test). To estimate the degree of population differentiation, an analysis of

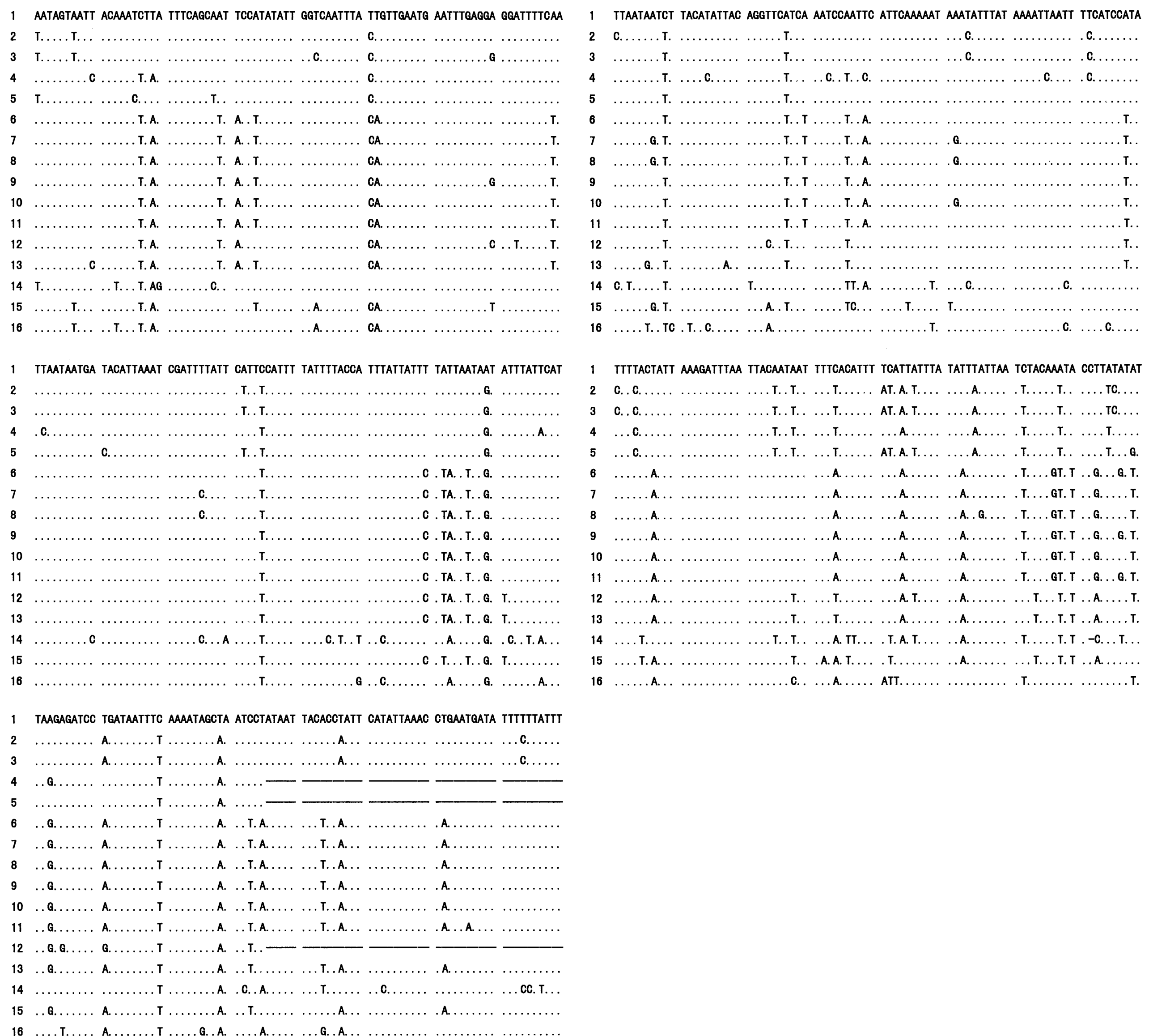

Figure 2. *Cytb* gene sequence variation between bumblebee species. The species name and sequence accession numbers are as follows: 1, *B. ignitus*, AF498398; 2, *B. patagiatus*, AF498399; 3, *B. hypocrita*, AF498397; 4, *B. terrestris*, AF002721; 5, *B. lucorum*, AF002722; 6–11, *B. pascuorum*, AF017511–AF017516; 12, *B. ruderarius*, AF002723; 13, *B. humilis*, AF017517; 14, *B. avinoviellus*, AF181610; 15, *B. pennsylvanicus*, AF181611; 16, *B. impatiens*, AF281169. Notes: "." indicates the same nucleotide as corresponding residues of the first line; "-" means gap.

molecular variance (AMOVA) was performed (Excoffier et al. 1992; Schneider et al. 2000; Slatkin 1991; Weir 1996; Weir and Cockerham 1984; Wright 1931). For this analysis, the populations analyzed were divided into two groups: a group of island populations and a group of mainland populations. Because there was no definite boundary separating individuals and the population definition might be not appropriate, the distribution of the assignment criterion for individuals of a given population was used to confirm the probability that an individual belonged to the population with the software GeneClass (Cornuet et al. 1999). This analysis was done with the Bayesian likelihood method, with individuals assigned to the population in which the likelihood of their genotype was highest, which was better on the basis of Cornuet's description (Cornuet et al. 1999).

When a population was subjected to a bottleneck or expansion event, the microsatellite observed heterozygosity would be larger or smaller than the expected heterozygosity (Cornuet and Luikart 1996). The bottleneck test was performed with Bottleneck version 1.2.02 (Cornuet and Luikart 1996). The significance of the test was assessed with the Sign and Wilcoxon tests. The first test suffers from low statistical power; the second test provides relatively high power and can be used with as few as four polymorphic loci and any number of individuals (Cornuet and Luikart 1996). The test was run with the IAM mutation model because it

Table 1. Population diversity and HWE test based on nine microsatellite markers

	Microsatellite loci								
	B124	*B11*	*B96*	*B100*	*B126*	*B132*	*B10*	*B118*	*B119*
Zhejiang									
Number of alleles	11	11	6	16	12	10	5	11	3
Size[a]	242–272	195–217	236–248	142–190	147–177	167–193	181–189	217–237	128–132
H_e	0.876	0.919	0.775	0.938	0.905	0.890	0.706	0.869	0.664
H_o	0.619	0.619	0.381	0.857	0.905	0.714	0.524	0.762	0.191
P	SN	**	**	SN	SN	*	SN	SN	**
Beijing									
Number of alleles	14	14	5	20	16	10	9	14	8
Size[a]	242–276	189–221	238–248	156–194	149–185	165–191	177–197	217–255	122–138
H_e	0.883	0.915	0.773	0.937	0.893	0.858	0.748	0.897	0.787
H_o	0.788	0.879	0.273	0.879	0.879	0.546	0.727	0.818	0.364
P	*	SN	**	**	SN	**	SN	**	**
Guizhou									
Number of alleles	7	5	2	5	6	7	3	6	2
Size[a]	248–268	201–213	240–242	154–176	157–179	173–185	181–187	221–237	128–130
H_e	0.94	0.756	0.378	0.800	0.844	0.62	0.711	0.889	0.533
H_o	0.400	0.800	0.200	0.600	1.000	0.400	0.600	0.800	0.000
P	*	SN	SN	SN	SN	**	SN	SN	SN
Nagano									
Number of alleles	8	6	8	11	9	10	4	10	8
Size[a]	250–268	199–215	218–248	160–182	159–179	175–199	181–187	217–239	126–140
H_e	0.843	0.772	0.818	0.894	0.872	0.894	0.628	0.896	0.854
H_o	0.808	0.808	0.346	0.615	0.731	0.808	0.423	1.000	0.731
P	*	*	**	**	**	SN	SN	*	**
Niigata									
Number of alleles	10	11	6	11	10	9	3	10	5
Size[a]	250–270	199–223	218–266	160–192	157–179	175–191	183–187	219–239	128–136
H_e	0.875	0.844	0.757	0.885	0.898	0.822	0.637	0.876	0.751
H_o	0.737	0.684	0.579	0.789	0.895	0.895	0.579	0.789	0.842
P	*	SN	*	*	SN	SN	SN	*	*
Yamanashi									
Number of alleles	9	7	5	8	10	8	3	7	5
Size[a]	252–274	199–215	218–246	164–192	157–181	177–193	183–187	223–237	126–132
H_e	0.918	0.862	0.667	0.818	0.888	0.913	0.606	0.857	0.805
H_o	0.727	0.818	0.364	0.818	0.909	0.546	0.727	0.909	0.727
P	SN	SN	*	SN	SN	*	SN	SN	*
South Korea									
Number of alleles	8	8	4	11	9	8	4	8	4
Size[a]	254–272	175–223	238–246	148–194	155–183	175–201	183–189	221–239	126–132
H_e	0.850	0.850	0.758	0.954	0.915	0.882	0.730	0.992	0.660
H_o	0.444	0.778	0.333	0.667	1.000	0.778	0.778	0.889	0.444
P	**	NS	*	SN	SN	SN	SN	SN	SN

[a] The range of allele sizes (in bp).

H_e, unbiased estimates of expected heterozygosity; H_o, observed heterozygosity; P, probability for the HWE test.

* $P < .05$; ** $P < .01$; NS, not significant.

was the fittest model for this species based on present results (outlined in the results section).

Results

mtDNA Variation

A segment of 450 bp from the *cytb* gene was sequenced from 16 individuals chosen from the eight bee populations. We did not observe any variation in this region in *B. ignitus*. However, extensive variation has been observed between bumblebee species (Figure 2).

Gene Diversity, HWE, and Linkage Disequilibrium

Table 1 shows the polymorphism results at nine microsatellite loci in the eight populations of *B. ignitus*. The number of microsatellite alleles ranged from 9 to 24. Gene diversities per locus per population ranged from 0.378 to 0.992.

Table 2. Pairwise F_{ST} distance comparisons between *B. ignitus* populations

	Zhejiang	Beijing	Guizhou	Nagano	Niigata	Yamanashi
Beijing	0.016*					
Guizhou	0.020	0.021				
Nagano	0.091**	0.066**	0.099**			
Niigata	0.098**	0.069**	0.99**	0.066**		
Yamanashi	0.099**	0.067**	0.112**	0.068**	0.012	
South Korea	−0.001	0.002	0.003	0.085**	0.059**	0.061**

* $P < .05$; ** $P < .01$.

Departure from HWE was found in 29 of 72 cases, most commonly in the *B96* and *B119* loci (Table 1). The reason for the deviations from HWE might be high selective stress (bottleneck; discussed later) and small sample size. No departure was found when the exact test was performed in each population. Linkage disequilibrium was found in 35 of 252 cases among the analyzed loci and populations, but no common pair of loci deviated from equilibrium in all the populations (data not showed).

Population Differentiation

Population differentiation was tested first by F_{ST} distance. In addition to the fact that all the mainland-island comparisons were significant, F_{ST} comparisons were also significant between the two mainland populations (Zhejiang and Beijing) and between the island populations (Nagano and Niigata, and Nagano and Yamanashi) (Table 2). Gene diversity per population per locus was similar for island and mainland populations, which is in contrast with the results of Estoup et al. (1996).

Analysis of molecular variance is a method of estimating population differentiation directly from molecular data and testing hypotheses about differentiation (Excoffier et al. 1992). The result of the AMOVA test showed that the island group was differentiated from the mainland group ($P < .05$, data not shown).

An individual assignment test showed 64.52% of those individuals were correctly identified, and only 5 of the 44 wrongly assigned individuals were interchanged between the mainland and island populations. Thus samples from those localities were, to some extent, typical (data not shown).

Bottleneck Test

The bottleneck test with the IAM model showed deviations from mutation equilibrium for three populations (Beijing, Zhejiang, and Nagano) with the Sign test, and only two populations (Guizhou and South Korea) showed no deviation with the Wilcoxon test (Table 3). All the deviations were due to excess observed heterozygosity.

Three models of evolution have been proposed for microsatellites: SMM, IAM, and TPM (Cornuet and Luikart 1996; Di Rienzo et al. 1994; Kimura and Crow 1964; Kimura and Otha 1978). Estoup (1995a,c) found that, for honeybees and bumblebees, microsatellites did not fit the SMM, but did fit the IAM. Most recent studies have shown that dinucleotides evolved in several steps (Huang et al. 2002). Although there was no definite model for *B. ignitus*, the nine microsatellite loci we studied were dinucleotide repeats. Thus we followed the IAM. Under this model, excess heterozygosity was found for most populations, which may be a response to recent bottlenecks. The small sample size of Guizhou and South Korea possibly contributed to their results of no significance.

Discussion

Genetic Variation

The analysis of nine microsatellite markers and part of the *cytb* sequences of 124 individuals from seven local populations of *B. ignitus* showed that while microsatellite markers were polymorphic, no variation was found in the *cytb* sequences. Previous studies of *B. pascuorum* showed six haplotypes in the same part of the *cytb* sequence (Figure 2), which suggests that genetic diversity of mtDNA in *B. pascuorum* is at least several times higher than that in *B. ignitus* (Pirounakis et al. 1998). Another kind of bumblebee, *B. terrestris*, also showed high diversity in this part of *cytb* (Widmer et al. 1998). A high level of *cytb* sequence variation among different bumblebee species has also been observed (Figure 2).

There were three possible explanations for the lack of *cytb* sequence variation in *B. ignitus*. First, *B. ignitus* diverged later than the other two bee species. Second, the *B. ignitus cytb* sequence evolved slower than that of the other two bee species. Third, *B. ignitus* populations experienced a recent bottleneck. Our previous phylogenetic analysis made us reject the first explanation because *B. ignitus* diverged earlier than *B. terrestris* (Shao et al. 2002). The similar branch length of *B. ignitus* and *B. terrestris* (Shao et al. 2002) and the results of the relative rate test did not support that the *B. ignitus cytb* gene evolved slower. However, the third explanation was supported by the results of the bottleneck test, which found that most populations were subject to bottlenecks. Further studies are required to verify our explanation.

Table 3. P value for the bottleneck test by comparing H_e and H_o under the IAM mutation process (Cornuet and Luikart 1996)

Test	Guizhou	Zhejiang	Beijing	South Korea	Niigata	Nagano	Yamanashi
Sign	NS	**	*	NS	NS	**	NS
Wilcoxon	NS	**	**	NS	*	**	**

* $P < .05$; ** $P < .01$; NS, not significant.

Population Differentiation

Genetic variability of *B. ignitus* was high for microsatellites. Most of the variation was present within populations rather than among populations, as was previously found in *B. pascuorum* (Widmer and Schmid-Hempel 1999). Most pairwise F_{ST} comparisons were not significant between pairs of mainland populations or between pairs of island populations, which might result from long-time gene pool homogenizing. Although no migration of *B. ignitus* has so far been reported, bumblebees are capable of flying long distances, and migration of *B. terrestris*, *B. lucorum*, and *B. lapidarius* have been observed (Mikkola 1978; Philip 1957). Moreover, a small number of migrants can be enough to homogenize different populations (Hartl and Clark 1989; Ibrahim et al. 1996).

Most island populations displayed significant genetic differentiation from mainland populations. Geographic isolation might be the cause of this genetic differentiation. An analogous situation of geographic isolation was found in two other bee species, *B. terrestris* and *B. pascuorum* (Estoup et al. 1996; Pirounakis et al. 1998; Widmer and Schmid-Hempel 1999; Widmer et al. 1998).

Another reason for the homogenization within island or mainland populations and differentiation between populations may be recent bottlenecks. Indeed, the lack of mtDNA diversity and the results of the bottleneck test on microsatellite data support the theory that those populations were subjected to recent bottlenecks.

Potential Influence of Human Activity

As *B. ignitus* is now used for pollination in China and Japan and may be commercially transported all over the world, one may have concern about the ecological effect. Our survey indicates that there is little differentiation in continental populations, so there is no need to worry about the impact of *B. ignitus* within isolated fields. However, the Japanese bees showed remarkable differentiation from continental populations, so there is a need to avoid homogenizing both of the separated gene pools when using this bumblebee as pollinator.

References

Asada S and Ono M, 2000. Difference in colony development of two Japanese bumblebees, *Bombus hypocrita* and *Bombus ignitus* (Hymenoptera: Apidae). Appl Entomol Zool 35:597–603.

Balloux F and Lugon-Moulin N, 2002. The estimation of population differentiation with microsatellite markers. Mol Ecol 11:155–165.

Bilinski M, 1999. Ecological characteristics of *Bombus ignitus* (Smith) in Korea. Korean J Appl Entomol 38:101–108.

Brown WM, Matthew Geórge JR, and Wilson AC, 1979. Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA 76:1967–1971.

Cornuet JM and Luikart G, 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Cornuet JM, Piry S, Luikart G, Estoup A, and Soliganc M, 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989–2000.

Crozier RH and Crozier YC, 1993. The mitochondrial genome of honeybee *Apis mellifera*: complete sequence and genome organization. Genetics 133:97–117.

Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, and Freimer NB, 1994. Mutational processes of simple sequence repeat loci in human populations. Proc Natl Acad Sci USA 91:3166–3170.

Eijnde J, de Ruijter A, and Van den Steen J, 1991. Method of rearing *Bombus terrestris* continuously and the production of bumble bee colonies for pollinating purposes. Acta Hort 288:154–158.

Estoup A, Garnery L, Solignac M, and Cornuet JM, 1995a. Microsatellite variation in honeybee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. Genetics 140:679–95.

Estoup A, Scholl A, Pouvreau A, and Solignac M, 1995b. Monoandry and polyandry in bumble bees (Hymenoptera; Bombinae) as evidenced by highly variable microsatellites. Mol Ecol 4:89–93.

Estoup A, Solignac M, Cornuet JM, Goudet J, and Scholl A, 1996. Genetic differentiation of continental and island population of *Bombus terrestris* (Hymenopera Apidae) in Europe. Mol Ecol 5:19–31.

Estoup A, Tailliez C, Cornuet JM, and Solignac M, 1995c. Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). Mol Biol Evol 12:1074–1084.

Excoffier L, Smouse P, and Quattro J, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotype: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Garnery L, Vautrin D, Cornuet JM, and Solignac M, 1991. Phylogenetic relationships in the genus *Apis* inferred from mitochondrial DNA sequence data. Apidologie 22:87–92.

Gillham NW, 1994. Organelle genes and genomes. New York: Oxford University Press.

Goka K, Okabe K, Yoneda M, and Niwa S, 2001. Bumblebee commercialization will cause worldwide migration of parasitic mites. Mol Ecol 10:2095–2099.

Guo S and Thompson E, 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372.

Hartl DL and Clark AG, 1989. Principles of population genetics. Sunderland, MA: Sinauer Associates.

Huang QY, Xu FH, Shen H, Deng HY, Liu YJ, Liu YZ, Li JL, Recker RR, and Deng HW, 2002. Mutation pattern at dinucleotide microsatellite loci in humans. Am J Hum Genet 70:625–634.

Ibrahim KM, Nichols RA, and Hewitt GM, 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity 77:282–291.

Kimura M and Crow JF, 1964. The number of alleles that can be maintained in a finite populations. Genetics 49:725–738.

Kimura M and Otha T, 1978. Stepwise mutation model and distribution of allelic frequencies in finite populations. Proc Natl Acad Sci USA 75:2868–2872.

Koulianos S, 1999. Phylogenetic relationship of the bumblebee subgenus *Pyrobombus* (Hymenoptera: Apidae) inferred from mitochondrial cytochrome *b* and cytochrome oxidase I sequence. Ann Entomol Soc Am 92:355–358.

Koulianos S and Schmid-Hempel P, 2000. Phylogenetic relationship among bumble bees (*Bombus latreille*) inferred from mitochondrial cytochrome *b* and cytochrome oxidase I sequence. Mol Phylogenet Evol 14:335–341.

Lewontin RC and Kojima K, 1960. The evolutionary dynamics of complex polymorphisms. Evolution 14:450–472.

Mikkola K, 1978. Spring migrations of wasps and bumble bees on southern coast of Finland (Hymenoptera Vespidae and Apidae). Ann Entomol Fennici 44:10–26.

Moritz C, Dowling TE, and Brown WM, 1987. Evolution of animal mitochondrial: relevance for population biology and systematics. Annu Rev Ecol Syst 187:269–292.

Nei M, 1978. Estimation of average heterozygosity and genetic distances from a small number of individuals. Genetics 89:583–590.

Ortiz-Sanchez FJ, 1992. Introduction de *Bombus terristris terristris* (Linnaeus, 1758) en el Sur de Espana para la polizacion de cultivos en invernado (Hymenoptera: Apidae). Bol Asoc Espan Entomol 16:247–248.

Paxton RJ, Thoren PA, Estoup A, and Tengo J, 2001. Queen-worker conflict over male production and the sex ratio in a facultatively polyandrous bumblebee, *Bombus hypnorum*: the consequences of nest usurpation. Mol Ecol 10:2489–2498.

Philip EG, 1957. Migrating bumble bee. Entomol Month Mag 93:104.

Pirounakis K, Koulianos S, and Schmid-Hempel P, 1998. Genetic variation among European populations of *Bombus pascuorum* (Hymenoptera: Apidae). Eur J Entomol 95:27–33.

Pouvreau A and Marileau R, 1980. L'élevage des bourdons: leur utilisation pour la pollinisation des plantes Cahier de Liaison OPIE 1–20.

Queller DC, Strassmann JE, and Huges CR, 1993. Microsatellite and kinship. Trends Ecol Evol 8:285–288.

Schneider S, Roessli D, and Excoffier L, 2000. Arlequin version 2.000: a software for population genetics data analysis. Geneva, Switzerland: University of Geneva.

Shao Z, Mao H, Fu W, and Zhang Y-P, 2002. Phylogenetic relationships of 11 bumblebee species (Hymenoptera: Apidae) based on mitochondrial cytochrome *b* gene sequences. Zool Res 23:361–366.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, and Flook P, 1994. Evolution weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651–701.

Slatkin M, 1991. Inbreed coefficients and coalescence times. Genet Res 58:167–175.

Slatkin M, 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462.

Slatkin M and Excoffier L, 1996. Testing for linkage disequilibrium in genotypic data using the EM algorithm. Heredity 76:377–383.

Visscher PK, 2001. Africanized bees in California. Available at http://bees.ucr.edu/ahb-facts.html.

Weir BS, 1996. Genetic data analysis, II: methods for discrete population genetic data. Sunderland, MA: Sinauer Associates.

Weir BS, and Cockerham CC, 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Widmer A and Schmid-Hempel P, 1999. The population genetic structure of a large temperate pollinator species, *Bombus pascuorum* (Scopoli) (Hymenoptera: Apidae). Mol Ecol 8:387–398.

Widmer A, Schmid-Hempel P, Estoup A, and Scholl A, 1998. Population genetic structure and colonization history of *Bombus terrestris* s.l. (Hymenoptera: Apidae) from the Canary Island and Madeira. Heredity 81:563–572.

Williams PH, 1996. Mapping variations in the strength and breadth of biogeographic transition zones using species turnover. Proc R Soc Lond B 263:579–588.

Wright S, 1931. Evolution in Mendelian populations. Genetics 16:97–159.

Recieved October 4, 2002
Accepted August 15, 2003

Corresponding Editor: R. C. Woodruff