Korean J. Genetics 28 (4) : 325-332 (December 2006)

Genetic Variations of the Golden Orb-web Spider *Nephila clavata* (Araneae: Tetragnathidae) in Korea, using AFLP Markers

Jongwoo Jung, Jin-Won Lee[1], Joo-Pil Kim[1] and Won Kim*

School of Biological Sciences, Seoul National University, Seoul 151-747, Republic of Korea
[1]Department of Biological Sciences, Dongguk University, Seoul 100-715, Republic of Korea

Received June 17, 2006; accepted July 25, 2006

ABSTRACT

In this study, we assessed genetic variations in eight Korean populations and one Japanese population of the golden orb-web spider, *Nephila clavata*, using an amplified fragment length polymorphism (AFLP) marker. Upon the analysis of AFLPs amplified by the ECO-AGG and MSE-ACC primers, we detected/identified 167 loci with sizes ranging from 40 - 703 bp. The Jeonju population of Korea evidenced the closest genetic relationship with the Japanese population. In accordance with a principal component analysis (PCA) in which all of the individuals were assigned to one of three groups, the majority of the Korean genotypes were assigned to GROUP 1, whereas the majority of the Japanese genotypes were assigned to GROUP 2; this group also included nine individuals from the Korean population. The results of AMOVA (analysis of molecular variance) showed that genetic diversity was more pronounced within each population than among the populations. These results indicate that no obvious genetic-geographic association exists among Korean and Japanese *N. clavata* populations; this can be explained primarily on the basis of gene flow via ballooning. Also, the divergence of genetic groups may have been affected by historical processes, including geographical isolation.

Key words: *Nephila clavata*, Ballooning, AFLP, PCA, Korea.

INTRODUCTION

Spiders, of the order Araneae, constitute one of the most diverse known animal groups. A variety of spider species are widely distributed as the consequence of ballooning—a unique dispersal method exploited by spiders (Decae, 1987). During ballooning, a juvenile spider can be lifted far from the hatching site by air currents. In some cases, ballooning spiders have landed on ships distanced more than 300 km from the nearest land surface, and spiderlings have been collected at heights up to approximately 5 km (Gertsch, 1979). Therefore, ballooning ameliorates differentiation in spider populations due to the profound level of gene flow afforded by this technique (Lee et al., 2004). The golden orb-web spider, *Nephila clavata* L. Koch, 1878 is one of the largest spider species in Korea, and can be readily identified by its brilliantly colored abdomen and its characteristic golden-colored web. In late autumn, eggs are laid, and overwinter by attaching to the tree trunks or branches. In May, spiderlings hatch from the egg sacs and are dispersed via ballooning. However, the effects of ballooning on the genetic structure of this

*To whom correspondence should be addressed.
E-mail: wonkim@plaza.snu.ac.kr. This work was supported by the Korea Science and Engineering Foundation (R21-2005-000-10013-0).

Table 1. Information on the specimens used in this study.

Location	No. Inv. [a]	Coordinates	Location	No. Inv.	Coordinates
Yeongcheon	9	35°57′N 128°56′E	Gangneung	11	37°45′N 128°54′E
Jeju	9	33°14′N 126°34′E	Changnyeong	13	35°32′N 128°30′E
Seoul	10	37°30′N 126°55′E	Jeonju	11	35°49′N 127°08′E
Shinan	10	35°04′N 126°06′E	Japan[b]	7	35°38′N 134°35′E
Incheon	18	37°28′N 126°38′E	Total	98	

[a]No. Inv., number of individuals.
[b]San-In Kaigan National Park, Hyogo Prefecture, Japan.

species remain to be clearly elucidated. In this study, we have evaluated the population genetic structure of Korean and Japanese *N. clavata* populations, using amplified fragment length polymorphism (AFLP) markers.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

We collected 98 *Nephila clavata* individuals from eight locations within South Korea, and one location in Japan (Table 1, Fig. 1). The collected samples were then preserved in 80% ethanol, and their morphological characteristics were determined in the laboratory, using a stereomicroscope. We extracted DNA from the walking legs of the spiders, via a standard phenol extraction (Sambrook and Russel, 2001).

AFLP Procedure

DNA digestion and adaptor attachment

The AFLP methods used in this study were adapted from the techniques of Choi et al. (2002) and Park et al. (2006). Genomic DNA was digested in a mixture containing restriction enzymes for 1 hour at 37°C, followed by 3 additional hours of digestion at 16°C. The reaction mixtures (total volume: 40 μL) were composed of 4 μL of genomic DNA, 0.15 μL of *Eco*RI (20 U/μL) (NEB, Massachusetts), 0.3 μL of *Mse*I (10 unit/μL) (NEB), 4 μL of NEB #2 buffer, 0.4 μL of BSA, and 31.15 μL of distilled water (DW). After the digestion reaction, the mixtures were heated for 15 minutes at 70°C in order to inactivate the restriction enzymes. Then, the *Eco*RI adaptor (ECO-F and ECO-R) and the *Mse*I adaptor (MSE-F and MSE-R) were attached to the sticky ends of the restricted fragments using T4 DNA ligase

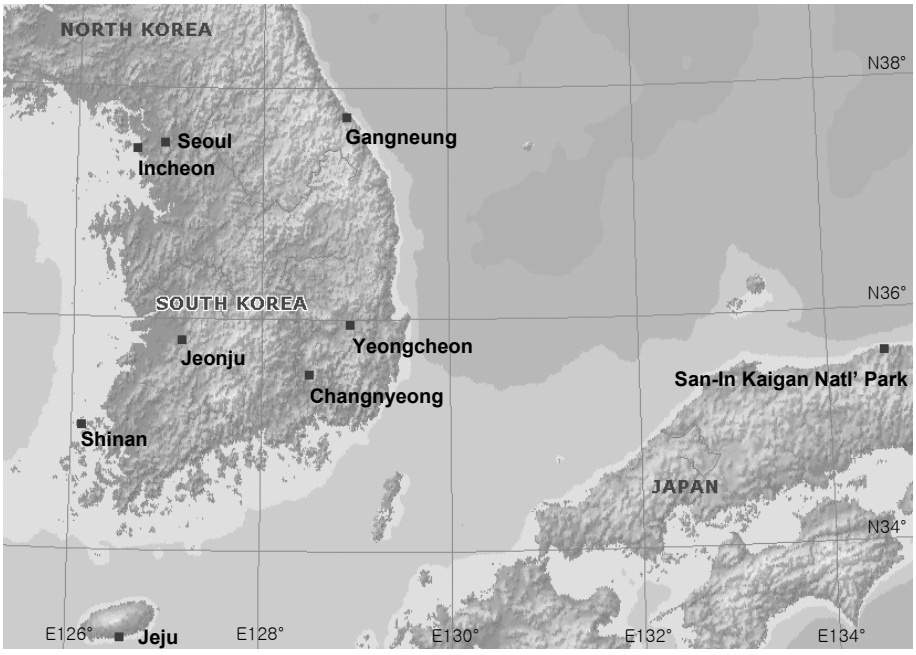

Figure 1. Collection sites of the *Nephila clavata* specimens used in this study.

Table 2. Oligonucleotides used in this study.

Primer	Sequence (5′→3′)	Purpose
ECO-F	CTC-GTA-GAC-TGC-GTA-CC	*Eco*RI-adaptor[a]
ECO-R	AAT-TGG-TAC-GCA-GTC-TAC	*Eco*RI-adaptor[a]
MSE-F	GAC-GAT-GAG-TCC-TGA-G	*Mse*I-adaptor[a]
MSE-R	TAC-TCA-GGA-CTC-AT	*Mse*I-adaptor[a]
ECO-A	GAC-TGC-GTA-CCA-ATT-CA	pre-amplification
MSE-A	GAT-GAG-TCC-TGA-GTA-AA	pre-amplification
ECO-AGG	6FAM-GAC-TGC-GTA-CCA-ATT-CAG-G	selective amplification, fluorescent labeled
MSE-AGG	GAT-GAG-TCC-TGA-GTA-AAG-G	selective amplification
MSE-ACC	GAT-GAG-TCC-TGA-GTA-AAC-C	selective amplification
MSE-ACG	GAT-GAG-TCC-TGA-GTA-AAC-G	selective amplification

[a] Two strands of each adaptor are annealed to each other before use. After mixing 5 μL of each adaptor (100 μM) with 450 μL of TE buffer, the mixture is heated at 95°C for 5 min and is then allowed to cool.

(adaptor sequences are listed in Table 2). The previously digested DNA mixtures (40 μL) were mixed in 3 μL of *Eco*RI adaptor (5 μM), 3 μL *Mse*I adaptor (50 μM), 1 μL T4 DNA ligase (1-3 unit/μL) (Promega, Wisconsin), and 2.5 μL of DW. They were subsequently ligated overnight at 4°C. AFLP fingerprinting involved two amplification steps: pre-amplification and selective amplification.

Pre-amplification

Pre-amplification was conducted using two AFLP primers (ECO-A and MSE-A) harboring a single selective nucleotide, "A." The reaction mixture (20 μL) was composed of 2 μL of the ligates, 2.8 μL of $MgCl_2$ (25 mM), 2 μL of PCR buffer (10×), 1 μL of dNTP mixture (10 mM), 0.4 μL of primer MSE-A (10 μM), 0.4 μL of ECO-A (10 μM), 0.6 μL of *Taq* polymerase, and 10.8 μL of DW (primer sequences listed in Table 2). The PCR conditions were as follows: initial denaturation for 2 minutes at 72°C and 1 minute at 94°C, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, elongation at 72°C for 1 minute, and a 2-minute final extension at 72°C.

Selective amplification

After verifying the PCR products via agarose gel electrophoresis, we conducted selective amplification using two primers (ECO-AGG and MSE-AXX) to which two additional selective nucleotides had been added. ECO-AGG was labeled with fluorescent dye (6FAM), and three types of MSE-AXX—MSE-ACC, MSE-ACG, and MSE-AGG—were prepared. Among these, the most polymorphic type was selected. The composition of the PCR reaction mixture was identical to that employed in the pre-amplification, except that a 20-fold diluted pre-amplification PCR product was utilized as the template. The selective amplification products were determined using a Genetic Analyzer 3730 (ABI), and the band size and genotype were determined using the GENESCAN 3.7 and GENOTYPER 3.7 systems (ABI).

Analyses

We evaluated independence among the polymorphic loci using AFLPOP (Duchesne and Bernatchez, 2002); this program identifies clusters of redundant loci, and then eliminates all loci except the first. Genetic diversity, the mean number of pairwise differences between populations (π_{XY}), and the linearized F_{ST} were calculated using ARLEQUIN 3.01 software (Excoffier et al., 2005). Genetic relationships among populations were then schematized using MEGA 3.1 (Kumar et al., 2004) via the neighbor joining (NJ) method and minimum evolution (ME), based on the matrix of a corrected average pairwise difference; this was calculated via the subtraction of π_{XY} from the mean of the difference within the compared populations ($\pi_X + \pi_Y/2$). In order to delineate the relationship among individuals using the MINITAB 13.20 program (Minitab Inc.), we conducted principal component analyses (PCA), in which each of the genotypes was represented in the scatterplot on two axes, PC1 and PC2. Analysis of molecular variance (AMOVA) (Weir and Cockerham, 1984) was conducted using ARLEQUIN 3.01 (Excoffier et al., 2005) to measure the distribution of genetic variability over the following hierarchical levels: between groups (South Korea and Japan), within groups, or within populations.

Table 3. Average pairwise differences between populations.

Location	YC	JJ	SE	SA	IC	GN	CN	JE	JP
Yeongcheon (YC)	**20.78**	28.69	22.94	26.21	23.44	25.66	34.20	32.03	36.54
Jeju (JJ)	3.69	**29.22**	28.94	28.63	26.02	29.45	34.15	36.21	41.71
Seoul (SE)	2.52	4.30	**20.07**	24.30	21.21	23.45	32.50	31.15	37.30
Shinan (SA)	4.94	3.14	3.39	**21.76**	21.87	25.45	31.22	32.79	37.70
Incheon (IC)	5.00	3.35	3.12	2.93	**16.12**	21.89	27.77	31.06	38.46
Gangneung (GN)	3.27	2.84	1.41	2.57	1.83	**24.00**	30.59	32.88	40.62
Changnyeong (CN)	8.26	3.98	6.92	4.79	4.16	3.04	**31.10**	36.74	45.37
Jeonju (JE)	6.08	6.04	5.56	6.35	7.44	5.32	5.63	**31.13**	39.39
Japan (JP)	9.29	10.25	10.41	9.97	13.54	11.77	12.97	6.97	**33.71**

The diagonal above represents the average number of pairwise differences between populations (π_{XY}), diagonal elements shaded with boldface represent the average number of pairwise differences within populations (π_X), and the diagonal below represents the corrected average pairwise difference ($\pi_{XY} - (\pi_X + \pi_Y)/2$).

The statistical significance of AMOVA was then evaluated over 10,000 permutations. Mantel's test (Mantel, 1967) was conducted using ARLEQUIN 3.01 to evaluate any possible significant relationships between the geographic distance and the population genetic distance (linearized $F_{ST} = F_{ST} / 1 - F_{ST}$) (Excoffier et al., 2005). The P-value was determined via permutation with 10,000 repeats. MINITAB 13.20 (Minitab Inc.) was utilized in order to compute simple correlation and regression analyses between the geographic distance and the population genetic distance (corrected π_{XY}).

RESULTS

AFLP Results

Among the MSE-AXX primers, MSE-ACC was selected due to the fact that it evidenced the highest levels of polymorphism. The AFLP results of ECO-AGG and MSE-ACC verified 167 loci (band) with sizes ranging from 40 to 703 bp. AFLPOP detected no such potentially linked loci. The mean number of pairwise differences between the individuals was 29.3 ± 12.9, and the average gene diversity over the loci was 0.175 ± 0.086.

Genetic Distance Matrix and Population Tree

Among the Korean populations, Incheon evidenced the largest distance from the Japanese population (corrected π_{XY} = 13.54), followed by the Changnyeong population (π_{XY} = 12.97) (Table 3). The genetic distance between the Seoul and Gangneung populations was smaller than any other population pair (1.41). With regard to the average differences among genotypes within these populations, the Japanese population evidenced the highest values (π_X = 33.71), whereas Incheon evidenced the lowest values (π_X = 16.12). The NJ tree was identical to that of ME, except that Incheon was the nearest neighbor to the Changnyeong population (Fig. 2). The Jeonju population, followed by Changnyeong, was the most genetically distant from the majority of the Korean populations.

Principal Component Analysis

In accordance with the PCA plot, the Korean and Japanese *N. clavata* individuals were classified into three groups (Fig. 3): GROUP 1 (77 individuals) was comprised of one Japanese individual and the majority of the Korean genotypes; GROUP 2 (15 individuals) contained six Japanese individuals; five from Jeonju, two from Jeju, and one each from Changnyeong and Yeongcheon; and GROUP 3 (six individuals) composed of five individuals from Changnyeong and one from Jeju. The Korean individuals in GROUP 2 were distributed only throughout the southern regions of Korea, including Changnyeong, Jeju, Jeonju, and Yeongcheon, unlike the GROUP 1 individuals, which were distributed evenly throughout Korea. This result was consistent with the results of the population tree, wherein the Jeonju population was the nearest Korean neighbor to the Japanese population ; this may be attributed to the fact that the Jeonju population evidenced the highest number of members of GROUP 2.

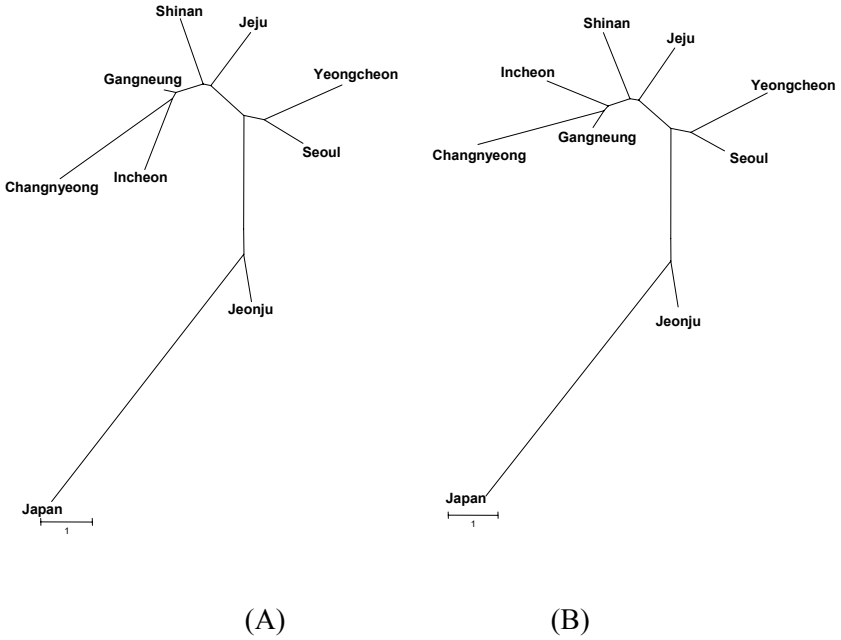

Figure 2. An unrooted phylogram of the genetic relationship among Korean and Japanese *Nephila clavata* populations, inferred via neighbor joining (A) and minimum evolution (B) methods predicated on the matrix of corrected average pairwise distance from Table 3. Scale bars indicate one unit of corrected average pairwise distance of AFLP loci.

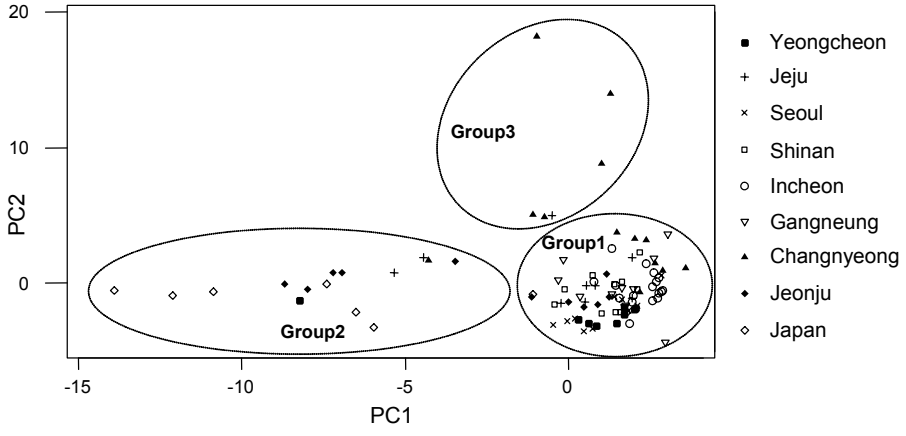

Figure 3. A scatter plot representing individual genotypes in accordance with two-axis principal component analysis (PCA). The first two components account for 13.7% of the total variation.

Individuals from Changnyeong, which evidenced the second largest distance (after Jeonju) from other Korean populations, constituted the majority of the GROUP 3 individuals.

AMOVA Analysis and Mantel's Test

AMOVA revealed that 67.8% of the variance remained within the populations and was significant. However, in between groups (Korean and Japanese), we detected relatively low genetic variance (20.2%) with no significance (Table 3). Our results indicate that genetic variance was more prominent within populations than among populations, thereby indicating that genetic variability was hardly structured among the Korean populations. We detected no significant association between the genetic and geographic distances (P = 0.114) by Mantel's test. The results of the Mantel's tests were corroborated by the regression analysis between the linearized F_{ST} and the geographic distance, in which the majority of the values lay outside the 95% confidence interval (Fig. 4).

Table 4. Results of AMOVA (analysis of molecular variance).

Source of variation	d.f.[a]	Sum of squares	Variance components	Percentage of variation	Fixation index
Among groups	1	75.53	3.64	20.2	0.20184
Among populations within groups	7	256.81	2.17	12.0	0.15059*** [b]
Within populations	89	1088.59	12.23	67.8	0.32202***
Total	97	1420.93	18.04		

[a]d.f., degree of freedom.
[b]***, P < 0.0001.

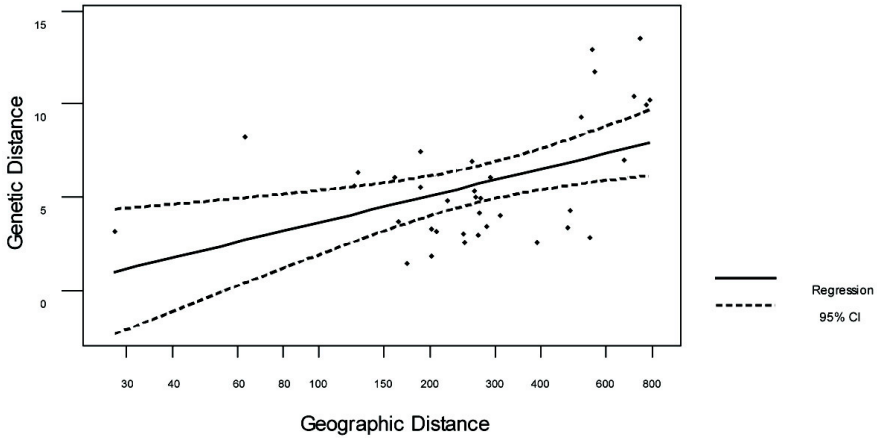

Figure 4. Fitted line plots of regression analysis between genetic distance and geographic distance of pairwise comparisons of populations. The axis of geographic distance is shown by a log scale. Statistical values are as follows: standard error = 2.88354; R-square values = 21.0%; adjusted R-square values = 18.7%; genetic distance = -5.91265 + 4.77766 log (geographic distance).

DISCUSSION

Spider populations that were incapable of ballooning, for instance, Eresid spiders, were determined to be highly differentiated; this phenomenon may be attributable either to their slow dispersal and/or their characteristic social behaviors (Johannesen et al., 1998; Johannesen and Veith, 2001). The features of highly structured spider populations were as follows. Genetic variance is normally concentrated among populations rather than within populations, and genealogy tends to be consistent with geographical distribution (Johannesen et al., 2002, 2005). By way of contrast, spiders capable of ballooning evidenced only a minimally genetically structured population, which was attributed to significant gene flow (Lee et al., 2004). The dispersal of these types of spiders does not appear to be limited by geographical barriers, such as mountain ranges, seas, and rivers (Lee et al., 2004).

Our results were diametrically opposed to what has been noted with highly structured populations: the genetic variance of *N. clavata* resided principally within populations, rather than among populations or between groups (Korea and Japan), and we did not observe any nonrandom associations between genetic distance and geographical distance, as PCA and Mantel's test commonly suggest. Therefore, our results clearly indicate high levels of gene flow; this may be attributed principally to an effective dispersal mechanism, namely ballooning, among the *N. clavata* populations evaluated in our study. Tetragnathid spiders are famed for their long-distance migratory abilities, by which they are able to cross even oceanic expanses (Okuma and Kisimoto, 1981). Therefore, it was not particularly surprising that we concluded that the low degree of differentiation inherent to the structures of the *N. clavata* populations in this study was primarily attributable to the efficient dispersal afforded by the ballooning behavior. This also explains why the GROUP 1 and 2 genotypes were detected throughout both the Korean and Japanese populations.

Although ballooning may be sufficient to explain these genetic patterns, other possibilities may also be considered. Principal among these may be introduction

as the result of human activities. Thousands of plant and animal species have expanded their distributional ranges —whether intentionally or unintentionally—as the result of human behavior (Vitousek et al., 1997). Considering that *N. clavata* attaches its egg sacs to tree branches and trunks, this species could have been shuttled between the two countries via the lumber trade. However, this seems unlikely, as Korea and Japan are both primarily lumber importers, and there is only minimal lumber trade being engaged in between the two countries. Secondly, the possibility should be considered that some sort of ecological factor may favor or constrain specific genotypes in accordance with the characteristic genetic pattern that GROUP 2 individuals were distributed only over relatively southerly regions, below lat 35°57′N, as compared to GROUP 1 members, which evidenced an even distribution. This notion is bolstered by the fact that multi-locus molecular markers such as AFLP and RFLP, which represent genetic variation over the genomic scale, are usually employed to evaluate the degree to which a species is adapted to its environment (Enjalbert et al., 2005; Marmiroli et al., 1999; Zeng, 2005). However, this consideration remains mere speculation until conclusive evidence can be collected regarding the relationship between ecological factors and genetic diversity in these species. Finally, the possibility must be considered that historical processes may have had some effect on *N. clavata* populations. If several genetically different groups could be detected within a spider species employing an effective dispersal mechanism such as ballooning, historical processes may have affected the differentiation of the AFLP genotype groups. It might be inferred that historical processes, such as geographical isolation, may have proven sufficient for the divergence of genotypes, after which a high level of gene flow could occur between genotypes within the relevant geographical range.

In conclusion, the genetic variation patterns detected in our *N. clavata* studies may be due primarily to the high level of gene flow afforded by ballooning, a unique dispersal mechanism exploited by the majority of spider species. Also, several distinct groups of AFLP genotypes may have been affected by recent historical processes. This particular possibility can be assessed via the application of diverse molecular markers to populations encompassing the entire distributional range of this species.

ACKNOWLEDGEMENTS

We are grateful to Hyun Sook Kim for obtaining the raw data, and to Young Seok Cho for providing us with the specimens.

REFERENCES

Choi I-K, Lee JK, Goo HJ, Park JH, Kim N-S, Park C-H and Chang KJ (2002) Genetic diversity and relationships among *Dioscorea alata* L., and related *Dioscorea* species revealed by AFLP analysis. Korean J. Genetics 24: 305-312.

Duchesne P and Bernatchez L (2002) AFLPOP: a computer program for simulated and real population allocation, based on AFLP data. Mol. Ecol. Notes 2: 380-383.

Decae AE (1987) Dispersal: Ballooning and other mechanisms. In Ecophysiology of spiders, W. Nentwig, ed., Springer-Verlag, New York, pp. 348-356.

Enjalbert J, Duan X, Leconte M, Hovmoller S and De Vallavieille-Pope C (2005) Genetic evidence of local adaptation of wheat yellow rust (*Puccinia striiformis* f. sp. *tritici*) within France. Mol. Ecol. 14: 2065-2073.

Excoffier L, Laval G and Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinform. Online 1: 47-50.

Foelix RF (1996) Biology of spiders, 2nd edn. Oxford University Press, New York, 330 pp.

Gertsch WJ (1979) American spiders, 2nd edn. Van Nostrand Reinhold, New York, 274 pp.

Johannesen J, Baumann T, Seitz A and Veith M (1998) The significance of relatedness and gene flow on population genetic structure in the subsocial spider *Eresus cinnaberinus* (Araneae: Eresidae). Biol. J. Linn. Soc. 63: 81-98.

Johannesen J and Veith M (2001) Population history of *Eresus cinnaberinus* (Araneae: Eresidae) colour variants at putative species transition. Heredity 87: 114-124.

Johannesen J, Hennig A, Dommermuth B and Schneider JM (2002) Mitochondrial DNA distributions indicate colony propagation by single matri-lineages in the social spider *Stegodyphus dumicola* (Eresidae). Biol. J. Linn. Soc. 76: 591-600.

Johannesen J, Kiefer A, Veith M and Kral J (2005) Genetic cohesion of *Eresus walckennaeri* (Araneae, Eresidae) in the eastern Mediterranean. Biol. J. Linn. Soc. 86: 1-9.

Kumar S, Tamura K and Nei M (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief Bioinform. 5: 150-163.

Lee JW, Jiang L, Su YC and Tso IM (2004) Is Central Mountain Range a geographic barrier to the giant wood spider *Nephila pilipes* (Araneae: Tetragnathidae) in Taiwan? A population genetic approach. Zool. Stud. 43: 112-122.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209-220.

Marmiroli N, Maestri E, Liviero L, Malcevschi A and Monciardini P (1999) Application of genomics in assessing biodiversity in wild and cultivated barley. Mol. Ecol. 8: S95-S106.

Okuma C and Kisimoto R (1981) Airborne spiders collected over the East China Sea. Jpn. J. Appl. Entomol. Zool. 25: 296-298.

Park BJ, Lee JK, Shin J-H, Park J-I, Kim N-S and Park C-H (2006) Genetic diversity of cultivated and wild Tartary buchwheat (*Fagopyrum tataricum* Gaert.) accessions by AFLP analyses. Korean J. Genetics 28: 91-98.

Sambrook J and Russel DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York, 999 pp.

Vitousek PM, D'Antonio CM, Loope LL, Rejmanek M and Westbrooks R (1997) Introduced species: a significant component of human-caused global change. New Zeal. J. Ecol. 21: 1-16.

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

Zeng ZB (2005) QTL mapping and the genetic basis of adaptation: recent developments. Genetica 123: 25-37.