

## Is Central Mountain Range a Geographic Barrier to the Giant Wood Spider *Nephila pilipes* (Araneae: Tetragnathidae) in Taiwan? A Population Genetic Approach

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(Accepted October 6, 2003)

**Jun-Wei Lee, Ling Jiang, Yong-Chao Su and I-Min Tso (2004)** Is the Central Mountain Range a geographic barrier to the giant wood spider *Nephila pilipes* (Araneae: Tetragnathidae) in Taiwan? A population genetic approach. *Zoological Studies* 43(1): 112-122. Most phylogeographic studies in Taiwan show that the Central Mountain Range is a major geographic barrier to vertebrates inhabiting low-elevation areas. In this study, we choose to investigate the widely distributed giant wood spider *Nephila pilipes* (Fabricius, 1793) to determine if their population genetic structure also shows an east-west differentiation pattern resembling those of terrestrial vertebrates studied so far. Mitochondrial cytochrome oxidase I (*COI*) was used as a genetic marker, and its partial sequence was determined in 189 specimens collected from 24 localities in the Ryukyu Islands, Taiwan, and mainland China. The 617-base partial sequence of *COI* was determined from DNA extracted from the leg muscle, and 11 haplotypes were identified from all specimens examined. Neighbor-joining (NJ) and maximum parsimony (MP) methods were used to construct phylogenetic trees using *N. clavata* Koch, 1877 and *N. antipodiana* (Walckenaer, 1841) as outgroups. Results from both methods indicate that *N. pilipes* populations can be separated into 3 major lineages: group A (haplotypes EA, RK, CN2, CN4, CN5, and CN6), B (haplotypes TW1, TW3, CN1, and CN3), and C (TW2). Group A consists of most specimens from 23 localities. Group B consists of specimens from southeastern China and northwestern Taiwan. Group C consists of a few specimens from a single locality in northeastern Taiwan. The percentage sequence differences from pairwise comparisons of all haplotypes ranged between 0.2% and 3.5%. Within-region nucleotide diversity ( $\pi$ ) ranged between 0.0% and 0.57%. The EA haplotype was the main component of all populations, and haplotypes in different Taiwanese populations were not structured geographically. Haplotypes TW1, 2, or 3 were sporadically distributed and could only be found within a few populations. These results indicate that a high level of gene flow exists among different populations of *N. pilipes* in Taiwan, and therefore the Central Mountain Range does not seem to be a major geographic barrier to this spider. <http://www.sinica.edu.tw/zool/zoolstud/43.1/112.pdf>

**Key words:** *Nephila pilipes*, Population genetic structure, Mitochondrial DNA, Cytochrome oxidase I, Taiwan.

Historical vicariance events during glacial periods and the geographic barrier provided by the Central Mountain Range (CMR) have long been considered to be 2 major factors affecting the genetic structures of several species of vertebrates in Taiwan (Lue 1998). Taiwan was connected with and separated from the Asian continent several times due to sea-level changes during glacial and interglacial periods (Lin 1963 1966). Each connection and isolation provided terrestrial animals on the Asian mainland an opportunity to

disperse into Taiwan and to separate from ancestral populations (Shen 1997). The CMR, which was formed under the effect of tectonic dynamics and runs along the north-south longitudinal axis of the island, has been suggested to be a geographic barrier to many species of terrestrial vertebrates inhabiting low-elevation areas. This geographic barrier further facilitates the differentiation of invading populations. For example, on the basis of PCR-RFLP data, Yeh (1997) found that Moltrecht's treefrog *Rhacophorus moltrehti* in Taiwan could

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be divided into 2 distinct (eastern and western) lineages. Liu (1995) also found a similar pattern in Swinhoe's tree lizard *Japalura swinhonis* by restriction fragment length polymorphism. Using allozyme electrophoresis, Toda et al. (1998) found substantial differences in allele frequencies at several loci between the eastern and remaining populations of the Indian rice frog *Rana limnocharis*. These studies demonstrate that many terrestrial vertebrates can be divided into eastern and western groups according to their population genetic structures. The barrier provided by the steep CMR might be responsible for these shared differentiation patterns.

Although the population genetic structures of several terrestrial vertebrates in Taiwan are well understood, those of terrestrial arthropods are still largely unknown. It is not clear whether or not historical vicariance events and the CMR have affected terrestrial arthropods in the same way as those events affected land vertebrates. The population genetic structure of *Psechrus* spiders (Araneae: Psechridae) inhabiting low-elevation forests of Taiwan was examined by Lin et al. (1999). Based on RAPD data, Lin et al. (1999) reported that Taiwanese *Psechrus* populations can be separated into northern/central and southern/eastern lineages, a pattern similar to those of vertebrates investigated so far. However, the *Psechrus* specimens examined in that study exhibited great variation in genital structures. The authors could not be certain whether the specimens used in the phylogenetic analysis were members of a single species or several species. Therefore, the conclusions of Lin et al. (1999) remain controversial, and similar studies using taxa with confirmed taxonomic status are still needed.

The aforementioned historical events and geographic barrier might have affected terrestrial arthropods, especially those inhabiting low-elevation forests, in ways quite different from vertebrates. Take the giant wood spider *Nephila pilipes* (Fabricius, 1793) (Araneae: Tetragnathidae) as an example. *Nephila pilipes* is abundant in low-elevation subtropical and tropical forests of Taiwan (Lee 1964). The mean annual temperature of the current northern most distribution range of *N. pilipes* in East Asia (in southern Japan; Yaginuma 1986) is about 18°C (Shen 1996). During glacial periods of the Pleistocene, however, the average temperature of most regions in Taiwan ranged from 8.0 to 11.0°C (Lin and Lin 1983). Furthermore, most areas were occupied by either savanna (Tsukada 1966) or pine forests (Shen 1996), which were

unsuitable for *N. pilipes*. It is quite possible that *N. pilipes* became extirpated from Taiwan during the glacial periods of the Pleistocene. Current populations might have entered Taiwan during the latest glacial period, which occurred 15 000 to 10 000 yr ago. Individuals entering Taiwan after the last glacial period might have rapidly reoccupied and eventually have become distributed throughout the island. Such a short period of time may not have allowed differentiation of populations inhabiting western and eastern sides of the CMR to have been built up. Therefore, for *N. pilipes* or terrestrial arthropods inhabiting low-elevation areas in general, a more-homogenous population genetic structure should be expected.

This study examines the genetic structure of the giant wood spider *Nephila pilipes* to determine if populations in Taiwan exhibit an east-west differentiation pattern similar to those of vertebrates, or a homogenous pattern according to our hypothesis. In this study, in addition to specimens from Taiwan, we also obtained a limited number of specimens from the southeastern coast of mainland China. Although phylogeographic studies of terrestrial animals in Taiwan are abundant, most of them have only examined Taiwanese materials (Tzeng 1986, Yang et al. 1994, Tsai 1999, Yang 1999, Hsu 2000 2001, Wang et al. 2000). Only a very few of them incorporated specimens from mainland China in the analysis (e.g., Toda et al. 1997). Evidence illustrating how historical vicariance events determine phylogenetic relationships of terrestrial animals co-inhabiting southeastern China and Taiwan is still quite limited. Therefore, another objective of this study was to compare the haplotypes of Taiwanese and mainland China populations to gain a preliminary assessment of their phylogeographic relationship.

## MATERIALS AND METHODS

### Sampling localities

One hundred eighty-nine female specimens of *Nephila pilipes* were obtained from 18 localities in Taiwan and nearby coastal islands, 2 localities from the Ryukyu Is., and 4 localities from southwestern mainland China (Table 1; Fig. 1). More than 50 km separated each locality. In every locality, spiders were sampled from an area greater than 2 km<sup>2</sup> to avoid obtaining offspring of the same breeding individuals. Female *N. clavata* Koch, 1877 (GenBank accession no. AY052586) collect-

ed from Maolin, Kaohsiung County, Taiwan and female *N. antipodiana* (Walckenaer, 1841) (GenBank accession no. AY052587) collected from Singapore were both used as outgroups in the subsequent analyses.

### DNA extraction, amplification, and sequencing

**Table 1.** Collection localities, haplotypes, and GenBank accession numbers of *Nephila pilipes* examined in this study. Numbers in boldface indicate the number of specimens examined in each population

Code	Locality	Sample size	Haplotypes	Accession no.
1	Iheya I. Japan	<b>4</b>	RK	AY052595
2	Iriomote I. Japan	<b>2</b>	RK	
3	Guangdong, China	<b>10</b>		
		3	EA	AY052594
		3	CN1	AY052588
		1	CN2	AY052589
		3	CN5	AY052592
4	Guangxi, China	<b>1</b>	CN3	AY052590
5	Yunnan, China	<b>6</b>		
		3	EA	
		2	CN1	
		1	CN4	AY052591
6	Fujian, China	<b>13</b>		
		10	EA	
		3	CN6	AY052593
7	Shuangshi, Taiwan	<b>9</b>	EA	
8	Nankang, Taiwan	<b>6</b>	EA	
9	Keelung, Taiwan	<b>9</b>		
		7	EA	
		2	TW1	AY052596
10	Foyanshan, Taiwan	<b>21</b>		
		16	EA	
		5	TW1	
11	Huisunlinchang, Taiwan	<b>4</b>	EA	
12	Lianshan, Taiwan	<b>5</b>	EA	
13	Dajin, Taiwan	<b>8</b>	EA	
14	Kenting, Taiwan	<b>4</b>	EA	
15	Nanjenshan, Taiwan	<b>6</b>	EA	
16	Shuanglianpi, Taiwan	<b>23</b>		
		19	EA	
		3	TW2	AY052597
		1	TW3	AY052598
17	Gululindao, Taiwan	<b>10</b>	EA	
18	Kueishan I., Taiwan	<b>2</b>	EA	
19	Fuyuan, Taiwan	<b>6</b>	EA	
20	Baqi, Taiwan	<b>5</b>	EA	
21	Shenmigu, Taiwan	<b>5</b>	EA	
22	Chipon, Taiwan	<b>9</b>	EA	
23	Green I., Taiwan	<b>10</b>	EA	
24	Orchid I., Taiwan	<b>11</b>	EA	

Specimens used for DNA extractions were either frozen at  $-80^{\circ}\text{C}$  or were preserved in 95% ethanol. Crude DNA was extracted from the muscle of legs or pedipalps using the phenol/proteinase K/sodium dodecyl sulfate method (Sambrook et al. 1989). The mtDNA cytochrome oxidase I (*COI*) partial sequence was amplified using the polymerase chain reaction (PCR), with a set of universal primers (Folmer et al. 1994) modified on the basis of consensus sequences of several arthropods (Crustacea X69067, Araneae X74571, and Insect NC001322) retrieved from NCBI GenBank (Bethesda, Maryland, USA): L CO 1490 5'-**GGTCAACAAATCATAAAGATATTGG** -3'; H CO 2198 5'-TAAACTTCAGGGTGACCAAAAA-ATCA -3'; with L standing for light and H standing for heavy DNA strands, CO referring to cytochrome oxidase, and the numbers (1490 and 2198) representing the position of *Drosophila yakuba*'s mitochondrial genome (Folmer et al. 1994). Bold and italicized letters indicate modifications from the original sequences.

Amplification was carried out for 30 cycles as follows: 70 s of denaturation at  $95^{\circ}\text{C}$ , 90 s of annealing at  $58^{\circ}\text{C}$ , and 2 min of extension at  $72^{\circ}\text{C}$ , followed by a final 10-min extension at  $72^{\circ}\text{C}$ . PCR products were separated and eluted using agarose gel purification (Gene-Spin™ Gel Extraction Kit, Protech Technology, Taiwan). Cycle sequencing with *Taq* polymerase was carried out with an ABI PRISM *BigDye* Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, U. S. A.), and it was directly sequenced using an automated DNA sequencer (ABI PRISM™ 377 DNA Sequencer, Perkin Elmer, U. S. A.). For sequencing, the same PCR primers were used. The sequence of each sample was verified through a comparison of light and heavy strands.

### Sequence alignment and phylogenetic analyses

All sequences were aligned using the Clustal W program (Thompson et al. 1994) in the BioEdit vers. 4.7.8 (Hall 1999) computer software and corrected manually using the GeneDoc (Nicholas et al. 1997) computer software. Phylogenetic trees were constructed by both the distance matrix method (Neighbor-joining (NJ) method; Saitou and Nei 1987) and the character state (maximum parsimony; MP) method. The NJ analysis was performed by calculating Kimura's (1980) 2-parameter distance with the transition/transversion ratio estimated by the MEGA vers. 2.0 (Kumar et al. 2001), and clades were evaluated by bootstrapping with

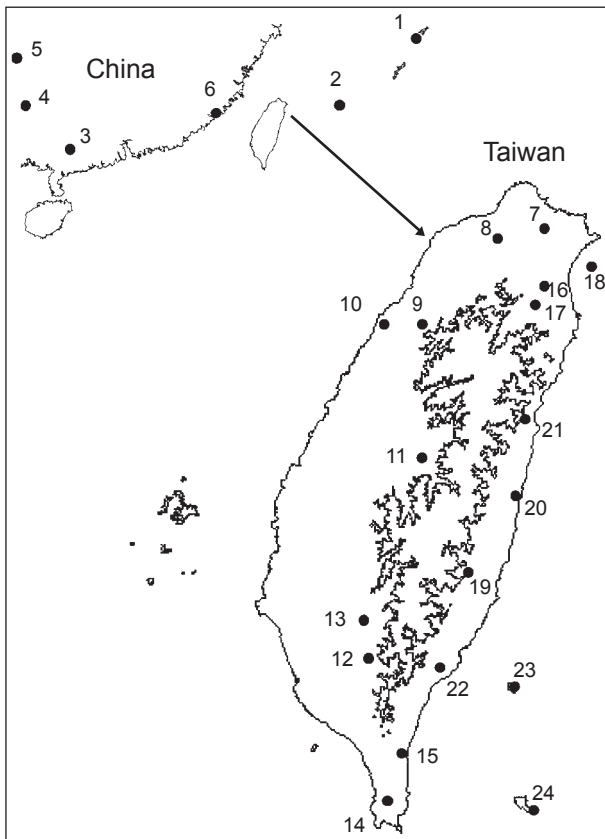
1000 replicates using unweighted characters. The MP analysis was performed by bootstrapping (Felsenstein 1985) with 1000 replicates using unweighted characters, with ACCTRAN, MULPARS, and TBR options, and a heuristic search with 1 random entry of data by stepwise addition options with the 50% majority-rule consensus in PAUP vers. 3.1 (Swofford 1993).

### Population genetic analyses

Populations of *Nephila pilipes* in Taiwan were comprehensively and systematically sampled to determine their genetic structures. Specimens collected from 15 populations in Taiwan were grouped into 6 regions: northern (N, localities 7 and 8), western (W, localities 9-11), southern (S, localities 12-14), southeastern (SE, localities 15 and 22), eastern (E, localities 19-21), and northeastern (NE, localities 16-18). However, since the sample size from mainland China was small, those spiders were not included in the population genetic analy-

sis. Genetic polymorphism and diversity of regions and populations were quantified by values of haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) (Nei 1987). Haplotype diversity was calculated by the equation,  $h = n(1 - \sum f_i^2) / (n-1)$ , where  $f_i$  is the frequency of the  $i$ th haplotype, and  $n$  is the sample size. Nucleotide diversity ( $\pi$ ) was estimated by the equation,  $\pi = n \sum f_i f_j p_{ij}^2 / (n-1)$ , where  $f_i$  is the frequency of the  $i$ th haplotype,  $f_j$  is the frequency of the  $j$ th haplotype,  $p_{ij}$  is the sequence divergence between the  $i$ th of  $j$ th haplotypes, and  $n$  is the sample size. The calculation used DNA SP vers. 3.14 (Rozas and Rozas 1999) among geographical regions and among populations. The Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) program in ARLEQUIN vers. 2.0 (Schneider et al. 2000) was used to deduce the significance of geographical divisions both between western and eastern groups, among and within populations. The statistics of molecular variance,  $\Phi_{CT}$  (between western (populations: 7-10 and 12-14) and eastern groups (populations: 15-22),  $\Phi_{ST}$  (among populations within groups), and  $\Phi_{SC}$  (within populations) were estimated using ARLEQUIN. In addition, analysis of the frequency distribution of pairwise differences in mtDNA sequences, known as mismatch distributions, among Taiwanese *N. pilipes* specimens was calculated using ARLEQUIN, vers. 2.0 (Schneider et al. 2000). Slatkin and Hudson (1991) showed that the mismatch distribution of stable populations has a smooth unimodal distribution when a population has experienced a recent demographic expansion. In contrast, the distribution is usually multimodal in samples drawn from populations at demographic equilibrium.

Nucleotide differentiation between populations was computed using  $F$ -statistics (Wright 1951), which estimates the proportion of total variance distributed among populations.  $F_{ST}$  values were estimated by the equation,  $F_{ST} = 1 - (H_w / H_b)$ , where  $H_w$  and  $H_b$  are the average numbers of pair-wise differences (polymorphic DNA sites) within and between populations (Hudson et al. 1992). Gene flow was estimated as  $N_{fm}$ , which represents the effective population size of females times the fraction of migrating individuals. In Wright's  $F_{ST}$  based models,  $N_{fm}$  is estimated as  $[(1/F_{ST}) - 1] / 2$  (for mtDNA).



**Fig. 1.** Sampling localities of *Nephila pilipes* examined in this study. Locality codes are the same as those in table 1. The Central Mountain Range is schematically illustrated along the north-south longitudinal axis of Taiwan.

## RESULTS

### Divergence of the mitochondrial cytochrome oxidase I partial sequence





treated as a single population, there was no genetic differentiation among different regions.  $N_{fm}$  ranged from 0 to infinity ( $\infty$ ), and  $F_{ST}$  ranged from 0 to 0.21.

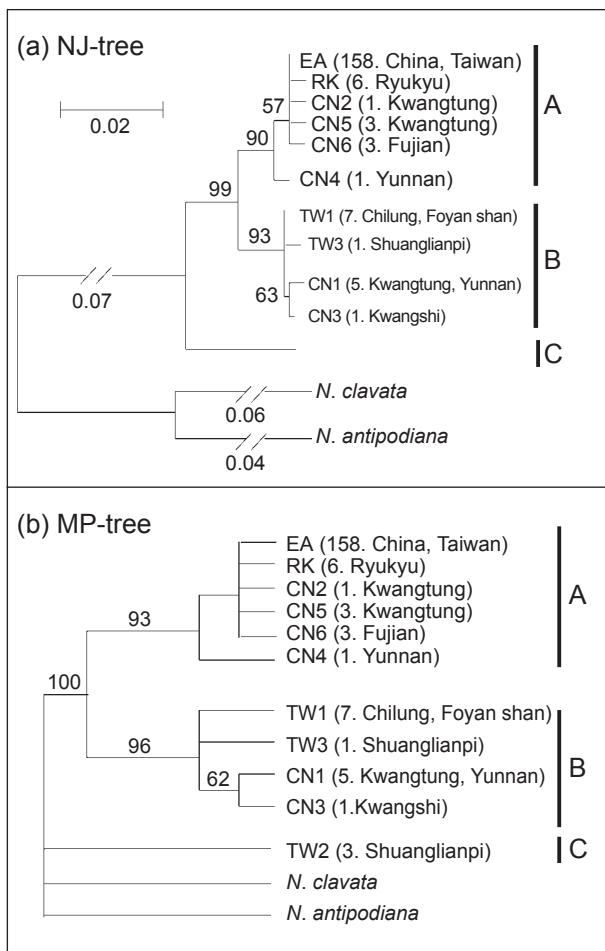
## DISCUSSION

### Population genetic structure of *Nephila pilipes* in Taiwan

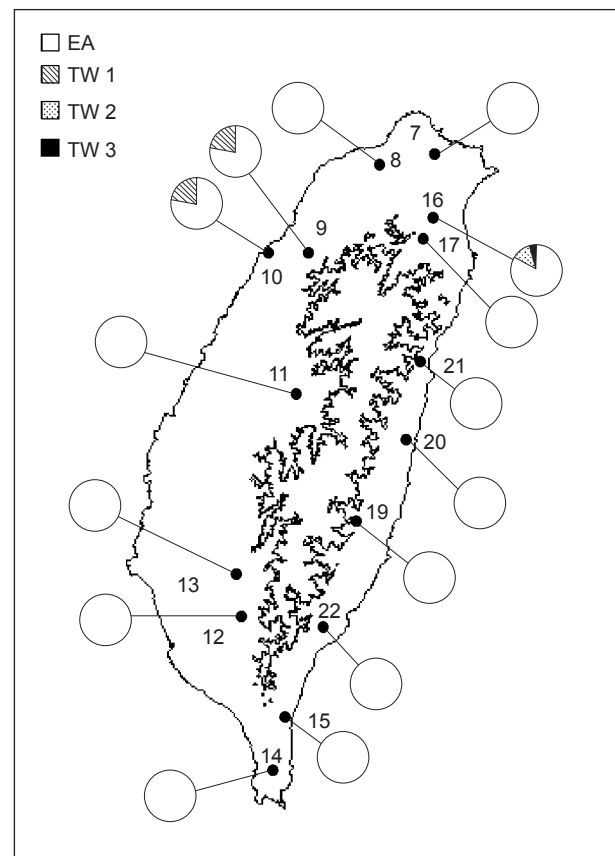
Results of various analyses indicate that populations of *N. pilipes* in Taiwan exhibit little genetic

differentiation. The EA haplotype was the only type in 12 of 15 populations sampled in Taiwan which represented a fixed haplotype in most areas of Taiwan. The fixation of a single haplotype across populations usually does not allow discrimination between historical and current gene flow. However, populations in the western and north-eastern regions exhibited haplotype polymorphism. The level of estimated gene flow between these regions and other regions were all considerably high, ranging from 1.92 to 6.09. Therefore, there seems to be no isolation between populations on both sides of Central Mountain CMR. Therefore, while CMR does seem to be responsible for differentiation patterns of many organisms in Taiwan, it does not seem to be a major geographic barrier for *N. pilipes*.

Why CMR represents a major geographic barrier to many terrestrial animals in Taiwan but not to *N. pilipes* may be a result of this organism's unusual dispersal ability. In terrestrial arthropods, some groups such as insects, mites, and spiders have developed distinctive dispersal methods by aerial travel. For insects (Johnson 1960), aerial



**Fig. 2.** Phylogenetic relationships constructed by the (a) Neighbor-joining (NJ) and (b) maximum parsimony (MP) methods based on the mitochondrial cytochrome oxidase I partial sequence of *Nephila pilipes*. Scale bar for the NJ tree represents branch lengths in terms of nucleotide substitutions per site. Numbers under branches in the NJ tree represent branch lengths. Numbers at nodes higher than 50% were derived from 1000 replications for both the NJ and MP trees. Numbers in parentheses correspond to the sample size. (MP tree: CI = 0.975, RI = 0.958, RC = 0.934).



**Fig. 3.** Haplotype frequencies of *Nephila pilipes* populations examined in Taiwan.

dispersal is primarily limited to adult forms. But in spiders, except in some rare cases, only juveniles perform aerial travel. Especially in the more-advanced Araneomorphae, the spread of individuals can be achieved by ballooning (Decae 1987). Ballooning behaviors exist among species of 22 families of the Araneomorphae, including the Tetragnathidae (Dean and Sterling 1985, Decae 1987, Greenstone et al. 1987). Ballooning spiders were reported to be found on ships 300 km from the nearest land and from heights of up to approximately 5 km (Gertsch 1979). Therefore, it seems that few geographical barriers can effectively prevent the dispersal of ballooning spiders.

Grant and Bowen (1998) reviewed several approaches to the general demographic history of a population from genetic data and proposed that a population with low haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) may have experienced an extended or severe demographic bottleneck in recent times. All populations of *N. pilipes* in Taiwan exhibit low  $h$  (0.370) and low  $\pi$  (0.362%) values, which correspond to the low haplotype and nucleotide diversity situation proposed by Grant and Bowen (1998). How can this observed low differentiated pattern be interpreted in terms of geographical events which occurred during glacial periods? Why have the same historical vicariance events during glacial periods generated considerable differentiation in vertebrate populations, but have generated no such pattern in *N. pilipes*? In addition to *N. pilipes*' aerial dispersal ability, this difference may also result from these tropical/sub-

tropical spiders' differential adaptability to the climate and vegetation existing during glacial periods. During glacial periods, there were savannas or pine forests in low-elevation areas of Taiwan (Tsukada 1966). However, *N. pilipes* can only inhabit low-elevation tropical or subtropical broadleaf forests, so the environment in Taiwan during the peak of glacial periods was not suitable for *N. pilipes*. In the Quaternary, at the interface between glacial and interglacial periods when the climate became warmer and broadleaf forests began to form while Taiwan was still partially connected with the Asian mainland, a small number of *N. pilipes* might have been able to disperse from China into Taiwan again. Because *N. pilipes* exhibits strong dispersal ability, the CMR in Taiwan has

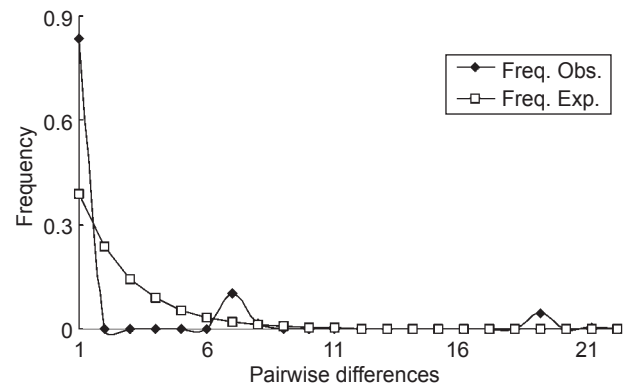


Fig. 4. Frequency distributions of pairwise sequences between individual haplotypes for *Nephila pilipes* populations in Taiwan.

**Table 3.** Pairwise comparison of mitochondrial cytochrome oxidase I partial sequences (617 bases) between 11 haplotypes. Numbers above the diagonal represent percentage differences corrected by Kimura's two-parameter model (Kimura 1980). Data below the diagonal are the numbers of nucleotide substitutions (transition/ transversion)

	EA	CN1	CN2	CN3	CN4	CN5	CN6	TW1	TW2	TW3	RK	<i>N. clavata</i>	<i>N. antipodiana</i>
EA		1.3	0.2	1.1	0.2	0.2	0.2	1.0	3.0	1.1	0.2	16.8	15.8
CN1	8/0		1.5	0.2	1.5	1.5	1.5	0.3	3.3	0.5	1.5	17.0	16.2
CN2	1/0	9/0		1.3	0.3	0.3	0.3	1.1	3.2	1.3	0.3	16.8	15.8
CN3	7/0	1/0	8/0		1.3	1.3	1.3	0.2	3.5	0.3	1.3	17.2	16.4
CN4	0/1	8/1	1/1	7/1		0.3	0.3	1.1	3.2	1.3	0.3	16.6	15.6
CN5	1/0	9/0	2/0	8/0	1/1		0.3	1.1	3.2	1.3	0.3	17.0	16.0
CN6	1/0	9/0	2/0	8/0	1/1	2/0		1.1	3.2	1.3	0.3	17.0	16.0
TW1	6/0	2/0	7/0	1/0	6/1	7/0	7/0		3.3	0.2	1.1	17.0	16.2
TW2	16/2	18/2	17/2	19/2	16/3	17/2	17/2	18/2		3.5	3.2	17.7	15.6
TW3	7/0	3/0	8/0	2/0	7/1	8/0	8/0	1/0	19/2		1.3	17.0	16.4
RK	0/1	8/1	1/1	7/1	0/2	1/1	1/1	6/1	16/3	7/1		16.8	16.0
<i>N. clavata</i>	36/57	37/57	36/57	38/57	36/56	37/57	37/57	37/57	40/57	37/57	35/58		10.5
<i>N. antipodiana</i>	34/54	36/54	34/54	37/54	34/53	35/54	35/54	36/54	33/54	37/54	34/55	39/21	

not constituted a significant geological barrier for this species. This may explain why the current *N. pilipes* population in Taiwan exhibits such low levels of haplotype diversity and nucleotide diversity.

### Genetic structure of *Nephila pilipes* and *Rana limnocharis*: a comparison

Similar to the giant wood spider, the Indian rice frog *R. limnocharis* is also quite abundant in low-elevation areas of Taiwan. Toda et al. (1997) examined the genetic structure of *R. limnocharis* populations in Taiwan using allozyme electrophoresis and found an east-west differentiation pattern across CMR. In another study, Toda et al. (1998) incorporated specimens from southeastern mainland China in their analysis and found western Taiwanese populations to exhibit similar genetic structures with the mainland populations. Why do sympatric Taiwanese *N. pilipes* and *R. limnocharis* populations exhibit such striking differences in genetic structures? The variation in population differentiation patterns may have resulted from these 2 organisms' differential temperature tolerance and dispersal abilities. Similar to *N. pilipes*, *R. limnocharis* is also widely distributed in East Asia (Frost 1985). However, while *N. pilipes* can only be found as far north as southern Japan, *R. limnocharis* is abundant even in central Japan

(Toda et al. 1998). The average annual temperature of central Japan is generally about 14°C, but that of southern Japan is about 18°C (Shen 1996). Differences in distribution patterns of these 2 organisms suggest that *R. limnocharis* might be more tolerant to low temperatures. Therefore, it is possible that during peak glacial periods, *R. limnocharis* in Taiwan was able to maintain a viable density, but *N. pilipes* in general could not survive the severe glacial climatic conditions. As the CMR gradually arose due to the collision of tectonic plates, *R. limnocharis* in eastern Taiwan might have gradually been isolated from western populations and eventually evolved unique alleles. However, there might have been no isolated *N. pilipes* population in eastern Taiwan during glacial periods, and they only reentered Taiwan at the end of each glacial period. On the other hand, CMR may have been an effective geographic barrier to *R. limnocharis*, so the gene flow between populations on the 2 sides of the island was severely limited. However, invading *N. pilipes* could easily bypass CMR via ballooning and spread to the eastern part of the island, thus generating a more-homogenous genetic structure.

### The origin of *Nephila pilipes* in Taiwan: a preliminary assessment

**Table 4.** Sample size, haplotype diversity ( $h$ ), and nucleotide diversity ( $\pi$ ) of *Nephila pilipes* in 6 regions of Taiwan

Region (locality code)	Sample size	Haplotype diversity ( $h$ )	Nucleotide diversity ( $\pi$ )
1. Northern region (N) (7, 8)	15	0.00	0.00
2. Western region (W) (9, 10, 11)	34	0.37	0.003
3. Southern region (S) (12, 13, 14)	17	0.00	0.00
4. Southeastern region (SE) (15, 22)	15	0.00	0.00
5. Eastern region (E) (19, 20, 21)	16	0.00	0.00
6. Northeastern region (NE) (16, 17)	35	0.23	0.006

See text for populations included in each region.

**Table 5.** Results of the AMOVA test examining the distribution of genetic variance among 14 populations of *Nephila pilipes* in Taiwan

Source of variation	Variance	Percent (%)	$p$	$\Phi_{st}$
Between western and eastern groups	0.03	3.19	0.28	0.03 ( $\Phi_{ct}$ )
Among populations within groups	0.01	1.16	0.29	0.04 ( $\Phi_{st}$ )
Within populations	0.78	95.65	0.18	0.01 ( $\Phi_{sc}$ )



The distribution of haplotypes in Taiwan and China provide some preliminary hypotheses regarding the origin of *N. pilipes* in Taiwan. In this study, a limited number of specimens from China exhibited high haplotype polymorphism. From 30 individuals collected, 7 haplotypes were found. However, in 153 individuals collected from Taiwan only 4 haplotypes were identified. Both NJ and MP phylogenetic trees demonstrated that TW2 is most closely related to the outgroup, followed by group B (haplotypes TW1, TW3, CN1, and CN3) and group A (haplotypes EA, RK, CN2, CN4, CN5, and CN6). Since TW2 is distantly related to lineages A and B and is more-closely related to the outgroups, it is possible that it represents an ancient haplotype. Since EA is the most-dominant haplotype in Taiwan and is also the most-abundant haplotype in southeastern mainland China, it may represent the most recently evolved haplotype that entered Taiwan at the end of most-recent glacial period (about 10 000 to 15 000 yr ago.) If after intensive sampling we are still unable to find haplotypes TW1, TW2, and TW3 in mainland China, then it is possible that these haplotypes represent new mutations from ancestral haplotypes of the Asian mainland and which are isolated from the original mainland populations. However, in addition to the fixation of new mutations in isolated subpopulations, the observed differences between lineages could also have resulted from ancient polymorphisms (Hayes and Harrison 1992). If the TW1, TW2, and TW3 haplotypes are found in mainland China after intensive sampling, then it is possible that the current population genetic structure of *N. pilipes* in Taiwan reflects ancient polymorphisms. Although a comprehensive collection from mainland China has not yet been conducted, results from mismatch distribution analysis of *N. pilipes* in Taiwan suggest that ancient polymorphisms might be responsible for the observed pattern. When a population has recently gone

through a rapid expansion, its mismatch distribution tends to be unimodal. In contrast, populations experiencing long periods of equilibrium usually exhibit a multimodal distribution (Slatkin and Hudson 1991). The mismatch distribution of *N. pilipes* populations in Taiwan is multimodal (Fig. 4), suggesting that this population has not experienced a recent rapid expansion but has properties of a stable population. According to these properties, it is reasonable to infer that *N. pilipes* entered Taiwan after the most-recent glacial period and reoccupied this island from a metapopulation in southeastern mainland China or the nearby southeastern Asian land mass and thus carried the properties of this relatively stable metapopulation. According to this scenario, Taiwanese populations should be regarded as the same population as in mainland China instead of an isolated population which expanded from a few ancestral individuals.

On the other hand, although almost all relevant studies have demonstrated that terrestrial organisms in Taiwan migrated from mainland China during glacial periods, it is possible that *N. pilipes* in Taiwan could have also migrated from areas such as the Philippines during interglacial periods. In addition to dispersing through land bridges formed during glacial periods, small terrestrial animals such as arthropods may also enter Taiwan by ocean currents. For example, the northernmost island of the Philippine archipelago, Batan Is. about 90 km from southern Taiwan, and the speed of the Kuroshio Current is about 0.9 m/s (Veron and Minchin 1992). Transportation of organisms could thus easily occur from the northern Philippines to southern Taiwan in less than 2 d. From the co-occurrence of many unique plant species between the northern Philippines and southern Taiwan, it is possible that a branch of the Kuroshio Current provides a connection between these 2 areas. In addition, during summer the South China Current, driven by the southwestern monsoon, flows northwards into the Taiwan Strait through the Penghu Channel (see reviews in Chen 1999). Therefore, both the Kuroshio and South China Currents may provide opportunities for animals to disperse into Taiwan. Since the tropical flora of Kenting is quite similar to that of the northern Philippines (Shen 1996), it is quite possible that the forest-dwelling *N. pilipes* could have been transported with plants by ocean currents. Because specimens from the Philippines were currently not available in this study, the role ocean currents have played in the origin of *N. pilipes* in Taiwan could not be evaluated. In future studies, a

**Table 6.** Pairwise  $F_{ST}$  and  $N_{fm}$  estimates between geographical regions of *Nephila pilipes* based on sequence variations

	1. N	2. W	3. S	4. SE	5. E	6. NE
1. N		0.21	0.00	0.00	0.00	0.06
2. W	1.92		0.21	0.21	0.21	0.21
3. S	∞	1.92		0.00	0.00	0.06
4. SE	∞	1.92	∞		0.00	0.06
5. E	∞	1.92	∞	∞		0.06
6. NE	7.63	6.09	7.63	7.63	7.63	

systematic sampling of populations in the Philippines and mainland China will help further assess the origin of *N. pilipes* in Taiwan.

**Acknowledgments:** We thank M. S. Zhu, J. Chen, X. J. Peng, and W. H. Chou for providing specimens from mainland China and the Ryukyu Is. We are grateful to comments provided by C. H. Kuo, S. H. Lee, C. A. Chen, D. Q. Li, and T. Oshida. Special thanks are given to all the members of the Behavioral Ecology Laboratory, Tunghai Univ., Taichung, Taiwan for assistance in logistics, field collection, molecular techniques, and genetic data analysis. This study was supported by the National Science Council of the R.O.C. (NSC-89-2311-B-029-002, NSC-89-2311-B-029-008) to I. M. Tso.

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