Genetic variation among Asian populations of rice planthoppers, *Nilaparvata lugens* and *Sogatella furcifera* (Hemiptera: Delphacidae): mitochondrial DNA sequences

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Abstract

Many species of insects associated with cultivated rice do not over-winter in Korea and Japan, but migrate into these areas each year. To understand better the origins of these immigrations as well as the geographic structure of rice pests in Asian rice growing regions, intraspecific variation in two species of delphacid planthoppers, *Nilaparvata lugens* (Stål) and *Sogatella furcifera* Horvath, was examined. An 850 base pair region of mitochondrial DNA *cytochrome oxidase*-I (CO-I) was sequenced from a total of 71 individuals collected from 11 localities in seven countries: Korea, Philippines, China, Bangladesh, Malaysia, Vietnam and Thailand. In *N. lugens*, three haplotypes were found and all populations sampled shared a dominant haplotype. Localities in Korea contained two haplotypes and localities in China and the Philippines contained three. However, in samples from the Indochina peninsula no variation was detected either within or between populations, consistent with a hypothesis of regular migration and gene flow. These populations did not contain some haplotypes found in Korea, suggesting they were not the source of yearly immigration into Korea and, by extension, Japan. Populations from China did share haplotypes with Korea, which was consistent with the hypothesis that China was the source for yearly immigration into Korea. There was insufficient resolution to distinguish among populations in China. For *N. lugens*, the data suggested that populations south of the Red River Valley in Vietnam experienced regular mixing and were distinct from populations to the north which contributed to yearly immigrations. In *S. furcifera*, there was less differentiation among populations. Two haplotypes were found in all populations except Malaysia. The results for both species were consistent with seasonal weather data and indicated that more detailed analysis of DNA sequence data will be fruitful.

Introduction

The rice planthoppers, *Nilaparvata lugens* (Stål) and *Sogatella furcifera* Horvath (Hemiptera: Delphacidae) are among the most serious insect pests of rice and are widely distributed in South, Southeast and East Asia, the South Pacific islands and Australia (Dyck & Thomas, 1979). These insects are found throughout the year in tropical regions. In temperate areas such as in middle China, Korea and Japan, however, they do not survive in the winter season, and migrate into these countries each year with the new rice

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season (Park, 1973; Kisimoto, 1976; Asahina & Tsruoka, 1986; Uhm *et al.*, 1988). In Korea, these pests first appear each year in mid-June, with most of the immigration occurring during mid- to late-July. Population development of the newly arrived planthoppers in the rice fields is influenced by many factors associated with the migration, such as timing, size, pattern, route and origin. For these reasons, a better understanding of the patterns migration and especially their origins, is considered necessary for more accurate prediction and better management of planthopper outbreaks in temperate rice regions (Uhm *et al.*, 1988; Denno & Roderick, 1990; Song, 1996).

Previous studies of the origins of migration of rice pests have focused primarily on synoptic weather conditions (Kisimoto, 1976, 1979; Rosenberg & Magor, 1983; Asahina & Tsruoka, 1986; Uhm *et al.*, 1988). Although much circumstantial evidence has been published that the migration of the planthoppers follows north-easterly flowing air currents, especially from the south-east of China through the Korean peninsula and Japan (Kisimoto & Sogawa, 1995), the primary source of the yearly migration of rice insects remains a matter of debate (Sogawa, 1997b). Also unresolved is whether distinct populations of these insects exist in Asia, or whether the Asian populations actually represent one large panmictic population. One approach to address such questions concerning the origins of exotic insect pests, as well as patterns of migration and population structure, is through the use of genetic markers (Roderick, 1996; Gasparich *et al.*, 1997; Roderick *et al.*, 1998; Villablanca *et al.*, 1998; Davies *et al.*, 1999).

To date, genetic variation among populations of *N. lugens* have been examined using allozyme electrophoresis and using nuclear and mitochondrial DNA. Demayo *et al.* (1990) used starch gels to examine variation among populations in the Philippines and found little population structure. More recently, Hoshizaki (1994) used isoelectric focusing to investigate a range of laboratory populations from Asia (including Sri Lanka, Java, Malaysia, Philippines, Taiwan and Ishigaki Island, but not China nor Vietnam) and several Japanese populations from the field (Kyushu and Honshu Islands). This study showed significant variation among the laboratory populations from Asia, but little variation within the main Japanese islands. The Japanese populations could not be traced to any of the Asian laboratory colonies sampled. Jones *et al.* (1996) inferred molecular phylogenies of *N. lugens* and related species using amplification and restriction site analysis of both mitochondrial and nuclear DNA. In this study, the mitochondrial DNA (16srNA, COI, II) showed some, but limited, variability between populations of *N. lugens* and related species. The limited variation observed in the nuclear DNA (ITSI) strongly supported a division between the *N. lugens* populations in Asia and Australia, regardless of host plant associations. To our knowledge, there have been no previous reports on the origins of migratory rice insects using mitochondrial DNA sequence data from field caught populations of *N. lugens* and no population genetic studies of *S. furcifera.*

By virtue of its simple structure, maternal inheritance and relatively rapid evolutionary rates, mitochondrial DNA has become a widely used marker for understanding population structure and phylogenetic relationships of insects (Simon *et al.*, 1994; Roderick, 1996). One of the most well known mitochondrial genes is *cytochrome oxidase*-I, which is highly variable at silent sites, yet conserved at the amino acid level. For this reason, this gene has been used both in evolutionary studies at several levels including intra-specific variation and species identification, as well as higher level systematics (Brown *et al.*, 1994; Simon *et al.*, 1994; Sperling & Hickey, 1994; Funk *et al.*, 1995a,b; Jones *et al.*, 1996; Chang *et al.*, 1997).

In this study, we examined intraspecific variation in two species of rice planthoppers (*N. lugens* and *S. furcifera*) using DNA sequences of mitochondrial *cytochrome oxidase*-I. We examined individuals from seven Asian rice growing regions, including Korea, China, Philippines, Bangladesh, Malaysia, Vietnam and Thailand. We addressed two questions. First, does geographic structure exist among rice planthopper populations in Southeast and East Asia, or alternatively, do these Asian populations represent one large panmictic population? Second, if genetic structure does exist, is mitochondrial DNA a good candidate for examining sources of yearly migrations? If these planthoppers are genetically differentiated among geographical localities as suggested by earlier allozyme studies (e.g. Hoshizaki, 1994) and restriction site analysis of nuclear and mitochondrial DNA (Jones *et al.*, 1996), then we would expect to find differentiation among geographic populations with a marker that exhibits higher resolution, such as sequences of mitochondrial DNA. Here, we report not only that there is a pattern to population differentiation, in particular a distinction between northern and southern continental populations, but also that mitochondrial DNA is a good candidate for further work on the question of origins of yearly migrations of rice insects. Such data will be critical for better prediction and management of planthopper outbreaks in Korea and elsewhere in East and Southeast Asia.

Materials and methods

Insect species and collections

Individuals of the rice brown planthopper, *N. lugens*, were collected from 11 localities in seven countries: Korea, Philippines, China, Bangladesh, Malaysia, Vietnam and Thailand (see table 1 for specific locations and dates of collection). All populations were collected in the field in 1997 and placed directly in 95% ethyl alcohol with the following exceptions. The population from Chinju, Korea was collected in 1996 and had been reared in the laboratory for ten generations. The population from the International Rice Research Institute (IRRI), Los Baños, Philippines was collected in Laguna, Philippines, in 1983 and has since been reared in culture at IRRI.

Individuals of the whitebacked planthopper, *S. furcifera*, were collected from five localities in Korea, the Philippines, China, Malaysia and Vietnam (table 1). All populations were collected in rice fields in 1997.

Total DNA extraction, polymerase chain reaction (PCR) and sequencing

DNA was extracted by the phenol/chloroform method (Palumbi *et al.*, 1991; Palumbi, 1996). The DNA was ethanol-precipitated, and resuspended in 25 μl double-distilled (dd) H_2O. Two microlitres of this total extracted DNA were diluted in 8 μl of dd H_2O. Two microlitres of the diluted

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Table 1. Collection localities of the rice brown planthopper (BPH), *Nilaparvata lugens* and white-backed planthopper (WBPH), *Sogatella furcifera*.

Collection sites Country (Locality)	Latitude and longitude	Collection date	Species
Korea (Chinju)	35°N, 128°E	Sep. 1996	BPH
Korea (Cheju)	33°N, 127°E	30 Aug. 1997	BPH, WBPH
China (Nanjing)	32°N,117°E	18 Jul. 1997	BPH
China (Nanning)	18°N, 109°E	18 Jul. 1997	BPH, WBPH
China (Changsa)	27°N, 125°E	23 Jul. 1997	BPH
Philippines (IRRI Los Baños*)	16°N, 120°E	1983	BPH
Philippines (Central Luzon)	16°N, 122°E	8 Aug. 1997	BPH, WBPH
Vietnam (Mekong Delta)	12°N, 109°E	23 Jul. 1997	BPH, WBPH
Malaysia (Penang)	6.1°N, 100°E	25 Aug. 1997	BPH, WBPH
Bangladesh (Dakka)	20°N, 90°E	28 Aug. 1997	BPH
Thailand(Bangkok)	15°N, 100°E	14 Aug. 1997	BPH

* In cultivation with likely origins near Los Baños.

DNA were used for 50 μl PCR mixture containing 33 μl of dd H_2O, 5 μl of 10 μlTaq reaction buffer, 5 μl of 8 mM dNTPs, 2.5 μl each of the 10 μM oligonucleotide primer and 0.12 μl Taq polymerase (Perkin Elmer). Amplifications were performed using a GeneAmp PCR 9600 thermocycler for 35 cycles under the following conditions: denaturing at 94°C for 1 min, annealing at 48°C for 30 seconds, and extension at 72°C for 45 seconds. The primers used for PCR were CI-J-2183 (alias 'Jerry' 23mer. 5′-CAACATTTATTTTGATTTTTTGG-3′) and TL2-N-3014 (alias 'Pat' 25mer. 5′-TCCAATGCAC-TAATCTGCCATATTA-3′) (developed in the R. Harrison laboratory, Simon *et al*., 1994). The resulting PCR product was purified with the GeneClean Kit (BIO 101).

Seven microlitres of gene clean DNA and 4 μl of 1 μM of the same primer as in the original amplification were used for cycle sequencing using the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (ABI). Automatic sequencing was carried out with a DNA sequencer (ABI 377). To verify sequences, PCR products were sequenced in both directions for each individual. This yielded reliable sequences of approximately 850 base pairs including primer sequences. A total of 71 sequences were obtained from at least four individuals from each population except one: 48 from *N. lugens* and 23 from *S. furcifera*. All DNA sequences were inspected and aligned using Sequencher 3.1. Since *cytochrome oxidase*-I is a protein coding region, all sequences aligned easily and there were no insertions and deletions within species. Alignments were used to identify unique haplotypes and the frequencies of haplotypes were scored for each population.

Data analysis

We addressed two questions for each species. First, is mitochondrial DNA variable (i) within populations and (ii) within regions in Asia? That is, does more than one mitochondrial haplotype exist? An obvious prerequisite for the use of mitochondrial DNA in the study of population structure and origins is heritable variation. We asked this question at two spatial scales: within populations and within regions. Second, if mitochondria are found to be variable, is there a geographic pattern to the variation? That is, does significant population structure exist? We were particularly interested in variation within the regions north and south of the Red River Valley in Vietnam, because earlier weather data had predicted a possible division in weather patterns at this point.

The number of individuals sampled to address the question of whether a population is variable is determined by the variation present within each population. The probability, p, of not detecting an allele present in a population at a frequency, f, with a sample size, n, can be calculated by

$$p = (1 - f)^n. \quad [1]$$

Many statistics exist for quantifying population structure using DNA sequence data and other markers (Weir & Cockerham, 1984; Hudson *et al*., 1992a,b; Slatkin, 1994; Follett & Roderick, 1996; Roderick, 1996). The advantage of DNA sequence data over other types of genetic markers is that genetic differences at the DNA sequence level among alleles or haplotypes contain useful information in addition to the frequencies of haplotypes or genotypes. Here, we report genetic variation within and among populations with both haplotype and sequence statistics.

The statistic ϕ estimates differentiation among populations based on haplotype frequencies and is calculated as

$$\phi = 1 - \frac{\overline{H}_W}{\overline{H}_B} \quad [2]$$

where $\overline{H}_w$ is the mean number of base differences between different sequences sampled from the same population and $\overline{H}_B$ is the mean number of base differences between sequences sampled from the two different populations sampled (Hudson *et al*., 1992b). Note that here localities are considered populations, but that in the literature, subpopulations are sometimes used to refer to localities, while the population refers to the set of subpopulations. When the differences between sequences are replaced by 0 for identical sequences and 1 for different sequences then equation 2 can be written as

$$\theta = 1 - \frac{\overline{H}_W}{\overline{H}_B} \quad [3]$$

with θ as a measure of population differentiation equivalent to an unbiased fixation index (Weir & Cockerham, 1984; Weir, 1996). Population genetic structure can be estimated for haplotype data using both statistics

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A. *Nilaparvata lugens*

TCACCCAGGAAGTTATACATCCTTATTCTTCCAGGATTTGGATTAATTTCTCAT
ATTATTATACAAGAAAGAGGAAAAAGGGAAACTTTCGGATCTATTGGAATAA
TTTATGCAATAATTGCAATTGGAATTTTAGGTTTTATTGTTTGAGCTCACCATA
TTTACTGTAGGTATAGATATTGATACCCGAGCCTATTTTACGTCAGCTACTAT
AATTATTGCGGTCCCCACCGGAATCAAAATTTTTAGATGATCGCAACAATTTA
CGGTTCCAAAATGAACTTTTCCCCCCAAATAATTTGATCATTAGGATTCATTT
TACTTTTTACTATTGGAGGATT**A***ACAGGTGTAATATTATCAAATTCCTCAATT
GATATTATTCTACATGATACCTATTATGTAGTGGCTCATTTTCACTATGTCCTT
TCCATGGGAGCAGTATTCACCATTATCGCTAGATTTATCCATTGATACCC**C****T
TATTTACAGGTAGAAACATAAATAATAAATGACTAAAAATTCAATTTTATTCC
ATATTTCTAGGAGTAAATTTAACATTTTTTCCCCAACATTTTTTAGGATTAACT
GGTATACCACGACGATACTCTGACTATCCAGATATATACACCCTGTGAAACCT
TTTTTCTTCTATGGGTTCATTCATTTCCTTAATTAGAATTTTAATATTAATGTTT
ATTATATGAGAAAGATTAAGATTTAAACGAAAAATGGTGTTTAAAACCAATC
AACCTCAATCAATTGAATGAAAAATAAATTTACCCCCTAGAGAACACTCCTTT
AATGAAATTCCTATATTAATTAAGTTC

B. *Sogatella furcifera*

TCACCCAGGAAGTTTATATCCTGATTCTCCCCGGATTTGGATTAATTTCCCAT
ATCATTATACAACAAAGAGGTAAACGTGAAACCTTTGGATCAATTGGTATAA
TCTACGCCATACTAGCTATTGGAATCCTAGGATTTATCGTTTGAGCACACCAT
ATATTTACAGTAGGAATAGATATTGATACACGAGCGTACTTTACTTCAGCGAC
AATAATTATTGCTGTACCTACAGGAATTAAAATTTTTAGATGGATCGCCACCA
TTTACGGATCAAAAATTAATTTTTCCCCCCAAATAATTTGGTCTATAGGATTC
ATTTTGCTTTTTACAATTGGTGGTCTAACAGGAGTAATACTAGCAAACTCCTC
AATCGATGTTGTTCTTCATGATACCTACTATGTAGTTGCTCACTTTCACTATGT
TTTGTCTATAGGAGCCGTTTTTACAATTGTTGCCAGTTT**C***ATCCACTGGTAAC
CAATTTTTACTGGAGTTGCCTTAAACAATAAATGACTAAAAATTCAATTTTTT
TCTATATTTTTAGGAGTAAATTTAACATTTTTTCCGCAACATTTTCTAGGGCTT
ACAGGTATACCACGTCGATATTCTGATTACCCTGATATATACACCCTATGAAA
CTTAACGTCTTCAATCGGATCCATAATTTCATTAATTAGAATTTTACTATTAAC
ATTTATTACTTGAGAAAGATTAGTTTATAAACGAAAAATTCTTTTTAAAACAA
ATATAGCGCAATCTTTAGAATGAAAAATAAATCTACCCCCGTCTGAACATGC
ATTTAATAATCCCATTCTAGCAAGTCC

Fig. 1. Sequences of mitochondrial DNA *cytochrome oxidase*-I from A. *Nilaparvata lugens* and B. *Sogatella furcifera*. In A, * and ** refer to nucleotide base positions in Table 2. Primers are not shown because they are expected to be identical as a result of the amplification procedure.

through the framework of an analysis of molecular variance (Excoffier *et al.*, 1992). Here, variation was examined among geographical regions, among populations within regions, and within populations using the implementation in the computer program *Arlequin* (Schneider *et al.*, 1997). For *N. lugens*, regions were defined as north and south of the Red River of Vietnam, and the Philippines. For *S. furcifer*a, the Philippines population was pooled with the southern populations.

Genetic variation within populations was estimated by two statistics. The first, H_S, is similar to a heterozygosity for diploid data and is estimated as

$$H_s = 1 - \sum_i p_i^2 \qquad [4]$$

where P_i is the frequency of the *i*th haplotype within a population. The second statistic, $\hat{\pi}$, takes into account sequence variation, and is calculated as the average pairwise sequence difference among haplotypes within populations.

Results

Genetic variation within populations of Nilaparvata lugens

A total of 48 sequences each of 874 base pairs were obtained for individuals of *N. lugens*. Two nucleotide sites were found to be variable in the region sequenced (fig. 1A) resulting in three distinct haplotypes (table 2). One haplotype, *AA* differed by one base from both *AC* and *GA*, while *AC* and *AG* differed by two bases. Haplotypes *AC* and *GA* were found in Korea (fig. 2). Individuals from Chinju, Korea, all shared the *AC* haplotype but in Cheju, Korea, both the *AC* haplotype and the *GA* haplotype were observed. Three haplotypes were detected within localities in China. Populations from the Philippines contained three haplotypes. Surprisingly, all individuals sampled from the

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Table 2. Haplotype frequencies for DNA sequences of *cytochrome oxidase*-I among localities for *Nilaparvata lugens* and *Sogatella furcifera*.

Collection site	*Nilaparvata lugens*						
	No. individuals	Haplotype frequencies			No. individuals	Haplotype frequencies	
		A*C**	A*A**	G*A**		C	T
Korea (Chinju)	4	1.000	–	–			
Korea (Cheju)	6	0.833	–	0.167	6	0.667	0.333
China (Nanjing)	8	0.875	0.125	–			
China (Nanning)	4	0.500	0.250	0.250	5	0.800	0.200
China (Changsa)	4	0.750	–	0.250			
Philippines (IRRI)	4	–	–	1.000			
Philippines (Central Luzon)	2	0.500	0.500	–	4	0.750	0.250
Vietnam (Mekong Delta)	4	1.000	–	–	4	0.750	0.250
Malaysia (Penang)	4	1.000	–	–	4	1.000	–
Bangladesh (Dakka)	4	1.000	–	–			
Thailand (Bangkok)	4	1.000	–	–			

*, ** Denote nucleotide base positions in fig. 1A.

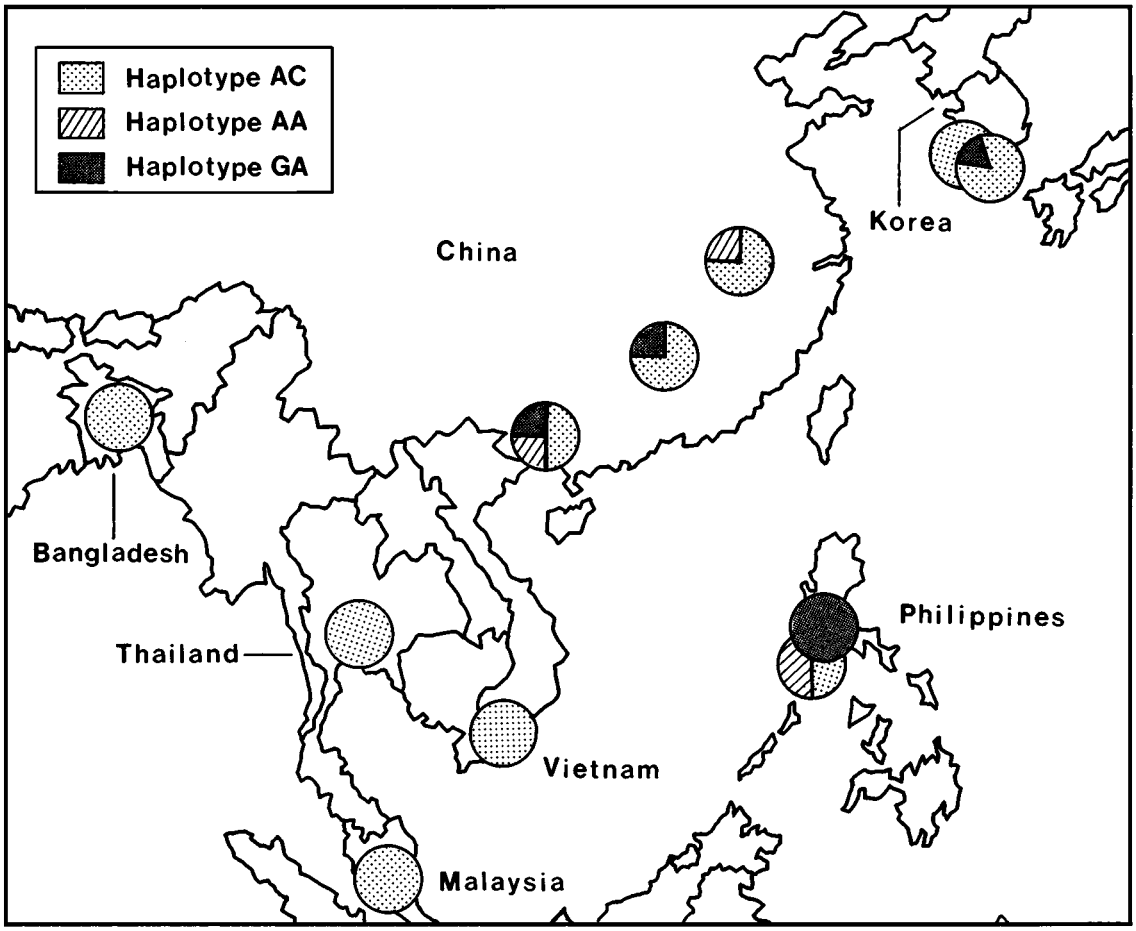

Fig. 2. Geographical distribution of mitochondrial haplotypes in East and Southeast Asia for *Nilaparvata lugens*.

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Indochina Peninsula (Vietnam, Malaysia, Thailand and Bangladesh) shared the same haplotype. The total sample size for the populations examined from the Indochina Peninsula (n = 16) was large enough to detect with some degree of certainty haplotypes other than *AC* occurring at a frequency of 17 or 18% if they did exist (from equation 1). The frequency of haplotypes other than *AC* within populations outside of the Indochina Peninsula was 36% and for populations in China and Korea, 21%.

Genetic variation among populations of N. lugens

Whether genetic differentiation exists among *N. lugens* populations in Asia was tested with a nested analysis: among regions, among populations within regions, and within populations (table 3). Significant population differentiation existed at all levels. However, among the five populations north of the Red River in Vietnam, population differentiation was not significant, nor was it significant for the four populations south of the Red River. Negative values for both ϕ and θ indicated that population differentiation not different from zero.

The amount of genetic variation within populations differed between these two regions of Vietnam. Populations north of the Red River were found to be more variable in both haplotype diversity, H_S, and pairwise sequence difference, $\hat{\pi}$, than those south of the Red River, which showed no variation. This difference between regions was found to be significantly different for both statistics (Mann-Whitney U = 18.0, P = 0.032).

Genetic variation among geographic populations of S. furcifera

A total of 23 sequences for an 823 bp region were obtained for individuals of *S. furcifera*. Only one nucleotide site was found to vary in the *S. furcifera* samples examined to date (fig. 2B), accounting for two different haplotypes, *C* and *T* (table 2). The populations from Korea, China, Philippines and Vietnam contained both haplotypes. Individuals from Malaysia had only the *T* haplotype (table 2). In contrast to the pattern for *N. lugens*, which showed no variability in the Indochina Peninsula, a second haplotype was found in this region at a frequency of 12.5%.

Genetic variation among populations S. furcifera

Genetic differentiation among *S. furcifera* populations in Asia was tested with a nested analysis, first for all populations, then for populations north and south of the Red River in Vietnam (table 3). In contrast to the results for *N. lugens*, no significant differences were found among populations at any scale, either among regions or among populations within regions. The amount of genetic variation within populations did not differ between these two regions (Mann-Whitney U = 3.0, P = 0.439).

Discussion

Mitochondrial DNA markers for population structure and history

Mitochondrial genes are well suited for tracing the history of populations, as well as for estimation of migration and gene flow (Avise, 1994). The mitochondrion is haploid and maternally inherited in most insects, thus the mitochondrial genome is inherited clonally. By contrast, the nuclear genome is diploid and biparentally inherited. Theory suggests that for these reasons, effective population size of the mitochondrial genome (N_f) should be one quarter that of the nuclear genome (N_e), and therefore mitochondrial DNA haplotypes are expected to be more subject to random processes such as random genetic drift and genetic bottlenecks than nuclear loci.

Within a population, on average, monophyly (one haplotype) is predicted in $N_e\mu$ and $4N_e\mu$ generations for mitochondrial and nuclear genes respectively, where μ is the rate of mutation and $N_e = 4 \times 1/4\ N_e = 4 \times N_f$ in the case of mitochondrial genomes (Birky *et al.*, 1989; Kreitman, 1991; Moore, 1995). Two populations will reach monophyly 95% of the time by $2N_e\mu$ and $8N_e\mu$ generations for mitochondrial and nuclear DNA respectively (Nei, 1987; Neigel & Avise, 1985; Tajima, 1983). Thus, if sufficient variation exists, variation in the mitochondrial DNA should track more precisely recent histories in populations than nuclear loci (Moore, 1995). Other factors may reduce this difference between mitochondrial and nuclear DNA including variation in reproductive success (Hoelzer, 1997).

A part of mitochondrial CO-I was sequenced to estimate genetic variation among geographic populations of *N. lugens* and *S. furcifera*. In *N. lugens*, the *AC* haplotype was dominant in all populations except in the Philippines, where the haplotype was found in only one individual. The occurrence of only one haplotype in the other Philippine population may be in part a result of the long history in culture of this one population. Individuals from all localities in the Indochina Peninsula had only the *AC* haplotype, while populations from Korea, China and Philippines localities shared the *AA* and *GA* haplotypes (table 2). The entire sample of individuals collected in the Indochina Peninsula was large enough so that other haplotypes at a high frequency should have been detected, if they did indeed exist. The similarity of mitochondrial DNA haplotypes within continental Southeast Asia is consistent with regular migration and gene flow. By contrast, populations of *S. furcifera* were not found to be differentiated at any level despite genetic variability in nearly all populations. Two haplotypes were found in Korea, China, Philippines and Vietnam, while the locality in Malaysia had only the one dominant haplotype.

Both haplotype and sequence diversity were relatively high within populations, and variation was observed within most populations, even with small sample sizes. The total sequence difference between the two species of planthoppers was 18%. Uncorrected mtDNA sequences variation was 0 to 0.23% for within and among the geographic locations of *N. lugens* and 0 to 0.12% for *S. furcifera*. Within species of both *N. lugens* and *S. furcifera* sequence differentiation was less than 1%, consistent with results from other insects. For example, intraspecific variation within CO-I was 0.4% for the spruce budworm, *Choristoneura fumiferana* Clemens species complex (Lepidoptera: Tortricidae) (Sperling & Hickey, 1994), 0.5% for *Heliconius* butterflies (Brower, 1994), 0.27% for diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae) (Chang *et al.*, 1997) and 1 to 5.7% for *Greya* species (Lepidoptera: Prodoxidae) (Brown *et al.*, 1994).

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Table 3. Nested analysis of haplotype frequencies within and among Asian rice populations of *Nilaparvata lugens* and *Sogatella furcifera*.

Comparison		θ	ϕ	ρ	$\bar{H}_S$	$\hat{\pi}$
Nilaparvata lugens	Df					
Among regions	3	0.374	0.437	0.001		
Among populations within regions	8	0.141	0.169	0.001		
Within populations	37	0.462	0.531	0.001		
	N					
N. of Red River, Vietnam	5				0.299	0.617
S. of Red River, Vietnam	4				0.000	0.000
Sogatella furcifera	Df					
Among regions	1	0.001	0.001	0.561		
Among populations within regions	3	−0.167	−0.167	0.664		
Within populations	18	−0.166	−0.166	0.952		
	N					
N. of Red River, Vietnam	2				0.382	0.467
S. of Red River, Vietnam	2				0.188	0.250

See text for explanation of genetic population variation parameters.

The role of weather

Weather conditions have been studied extensively in relation to long distance migration of the planthoppers (Kisimoto, 1976, 1991; Cheng, 1991, 1997; Sogawa, 1997b; R.W. Turner, 1997 unpublished; Uhm *et al*., 1988). According to these studies, the source region for early season migrants was most likely south-eastern China (i.e. south of 25°N and east of 115°E). However, the source region for a major influx of migrants in early July was likely to be further north (between 25°N and 30°N) than that for the early season migrants (R.W. Turner, 1997 unpublished; Uhm *et al*., 1988). Recently, Sogawa (1997a) suggested that because of the limited possibility of over-wintering, it may be that planthoppers in South China actually originate farther south. In this scenario, the primary northward migration takes place from the tropics, where the planthoppers breed throughout the year, first to subtropical regions. A secondary migration then takes place from the subtropical region to the more temperate regions or within temperate regions. If so, *N. lugens* entering Korea each year may only come secondarily from China, with the initial yearly source being farther south. Since *S. furcifera* is the smaller of the two species, it is likely that it may migrate more and farther each year.

The molecular genetic results presented here are consistent with the migration of planthoppers into Korea originating from the south-eastern part of China, as predicted by weather data. The difference in genetic diversity between populations north and south of the Red River region in Vietnam may suggest that populations to the south experience regular gene flow and mixing; this observation warrants further study. Also, it should be noted that for *N. lugens*, the localities from Korea exhibited one of a minor haplotype which was shared not only with individuals from China but also with individuals from the Philippines. Also, nearly all populations shared a dominant haplotype. In the other species, *S. furcifera*, populations did not differ genetically, despite ample variation within populations. For both species, there was insufficient resolution to distinguish among populations in China that may contribute to yearly migrations in Korea and Japan and more data will be needed to address this issue. Finally, the genetic differences observed among major regions, despite small sample sizes, demonstrates the power of markers using DNA sequence data, and suggests that this approach will be valuable in determining the history of populations for these insects.

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Tsetse Biology and Ecology: Their Role in the Epidemiology and Control of Trypanosomosis

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Domestic livestock in Africa are of importance not only as a source of milk and meat but also as a source of animal traction enabling farmers to cultivate larger areas, with crops providing the staple foods. Trypanosomosis, a parasitic disease transmitted cyclically by the tsetse fly (*Glossina* spp.), is arguably still the main constraint to livestock production on the continent, preventing full use of the land to feed the rapidly increasing human population. Sleeping sickness, the disease caused in humans by species of *Trypanosoma*, is an important and neglected disease posing a threat to millions of people in tsetse-infested areas. Often wrongly thought of as a disease of the past, the prevalence of human sleeping sickness is increasing in many areas. Although alternative methods to control the disease are being investigated, such as immunological approaches, use of chemotherapy or exploitation of the trypanotolerance trait, it is only control or eradication of the tsetse fly vector which will remove the threat of the disease rather than providing a better means of 'living' with it. As a result of the economic impact of tsetse-transmitted Trypanosomosis, a large amount of research literature has been produced. This book provides a comprehensive review of this literature. The text is divided into four parts: tsetse biology and ecology, epidemiology, vector control and control of trypanosomosis. The book is invaluable for medical and veterinary entomologists, parasitologists and epidemiologists.

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