Genetic Analysis of Population Structure of *Coptotermes gestroi* (Isoptera: Rhinotermitidae) in Native and Introduced Populations

BENG-KEOK YEAP, AHMAD SOFIMAN OTHMAN, AND CHOW-YANG LEE[1]

School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

ABSTRACT   We used microsatellite markers to characterize the population structure of *Coptotermes gestroi* (Wasmann), an economically important termite species in the tropics. Eighty-five colonies were sampled from seven countries (Malaysia, Thailand, Singapore, Indonesia, the Philippines, Taiwan, and the United States [Hawaii]). Ten to 20 workers per colony were genotyped using eight variable microsatellite loci. STRUCTURE analysis partitioned the 13 spatially separated populations into four clusters. Individuals in the Peninsular Malaysia and Singapore population groups displayed admixture clusters. There was low genetic differentiation ($F_{ST} = 0.073$) among the Peninsular Malaysia populations, suggesting moderate gene flow among them. Comparatively, there was moderate genetic differentiation ($F_{ST} = 0.192$) and positive correlation between genetic distance and geographic distance among all of the 13 putative populations studied, which suggests limited gene flow among them. There was no significant isolation by distance within the three largest populations (Penang, Kuala Lumpur, and Singapore), presumably because humans aid the dispersal and fragmentation of colonies in these cities. However, significant isolation by distance was found in introduced populations (Taiwan). Population structure analysis demonstrated that *C. gestroi* populations in Taiwan were likely introduced from the Philippines.

KEY WORDS   *Coptotermes gestroi*, microsatellite markers, population structure, gene flow

The Asian subterranean termite, *Coptotermes gestroi* (Wasmann), is a destructive species that has been introduced from its native region in Indo-Malaya (Scheffrahn and Su 2000, Takematsu et al. 2006) to many parts of the world, including the New World tropics (Brazil and Barbados), southern Mexico, the Southeastern United States, some islands of the West Indies, the Marquesas Islands (Pacific Ocean), Mauritius and Reunion Islands (Indian Ocean) (Yeap et al. 2009a), and more recently Taiwan (Tsai and Chen 2003). It causes serious damage to buildings and structures. In southeast Asia, *C. gestroi* is the most important termite species (Yudin 2002; Acda 2004; Kirton 2005; Yeap et al. 2007, 2009a, 2010, causing approximately $400 million per year in damage (Lee 2007). In Malaysia, Thailand, and Singapore, *C. gestroi* accounts for >85% of the total termite damage in buildings and structures in urban areas (Sornnuwat et al. 1996, Lee 2002, Kirton and Azmi 2005, Lee et al. 2007).

Successful spread of invasive termite species to new habitats mainly depends on the number of reproductives within a colony, its breeding system, modes of colony founding and dispersal, and unicolonial characteristics (Ross 1993, Chapman and Bourke 2001, Dronnet et al. 2005, Vargo and Husseneder 2009). A colony in insect socioecology is often regarded as an important unit of selection and an intermingling group of socially cooperative individuals of a given species (Thorne et al. 1999). A number of distinct colonies in a particular area, each with its own nest, reproductive organization, genetic structure, foraging range and territory, and mechanisms for identifying colony members and nonmembers constitutes a population (Thorne et al. 1999, Vargo and Husseneder 2009).

Variation in the colony and population structure of subterranean termites has been reported in numerous studies (Clément 1986, Reilly 1986, Bulmer et al. 2001, Clément et al. 2001, Vargo 2003, Dronnet et al. 2005, Vargo and Husseneder 2009), and many studies continue to focus on various species to understand the different breeding systems in native and introduced populations. Because of its cryptic nature, little is known about the population structure of *C. gestroi*, and this makes it difficult to investigate colony and population structure, the breeding system, and dispersal behavior of this species.

Polymorphic genetic markers represent an effective tool to elucidate the details of colony organization, population structure, and relationships among introduced and native populations (Ross 2001, Vargo et al. 2003). In this study, we used polymorphic microsatellite markers of *C. gestroi* (Armany et al. 2009, Yeap et al. 2009b) to investigate the population genetic structure of 85 colonies of *C. gestroi* collected from

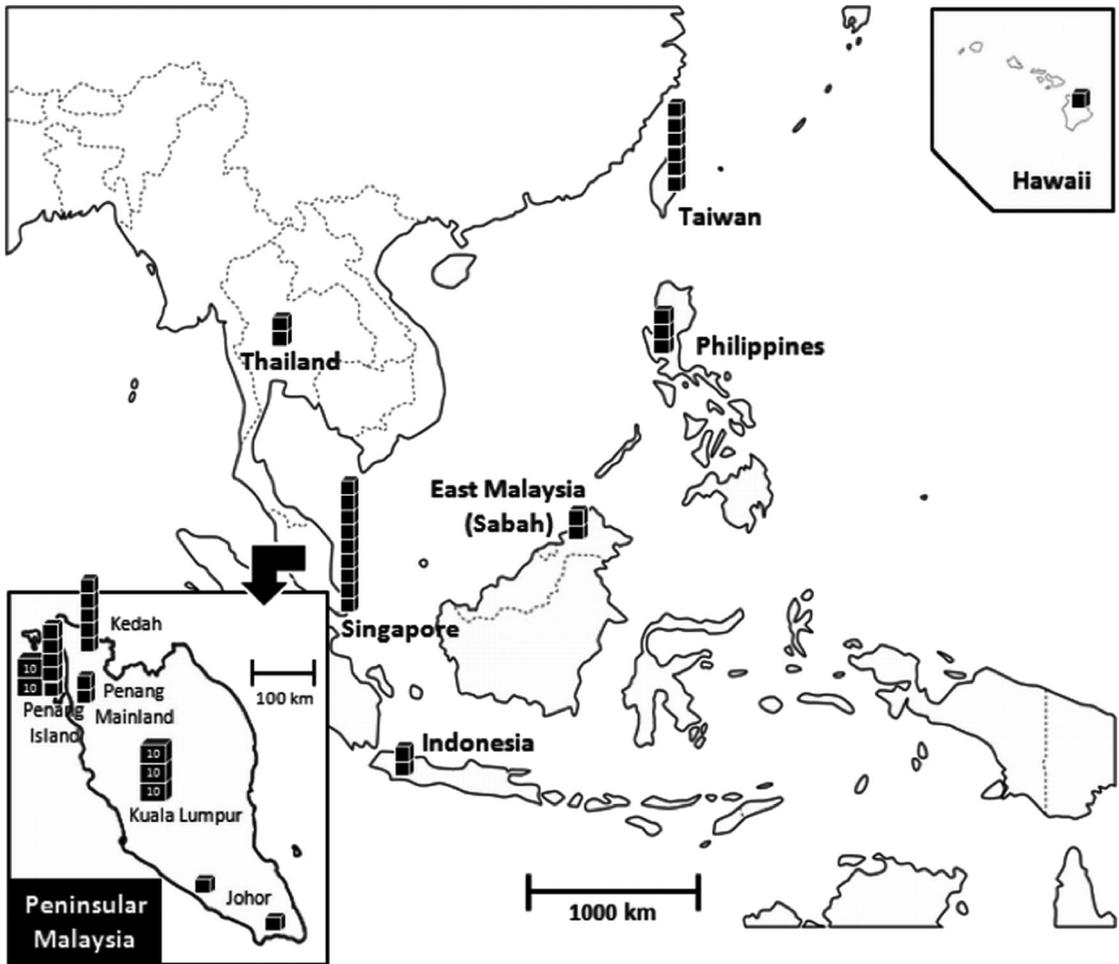[1] Corresponding author, email: chowyang@usm.my.

Fig. 1. Map of sample locations in Asia.

various parts of Asia. We determined the relationship between genetic variation and geographical distribution. Finally, we discerned the gene flow among the various populations that we studied. Understanding on the biology and ecology of *C. gestroi* are essential to provide effective management of this species.

Materials and Methods

Sites and Sample Collection. *C. gestroi* samples were collected from infested buildings and trees at various localities in Peninsular Malaysia, East Malaysia, Singapore, Thailand, Indonesia, the Philippines, Taiwan, and Hawaii (Fig. 1). Eighty-five colonies were collected from 13 spatially separated populations (Kedah, Penang Island, Penang mainland, Kuala Lumpur, Johor, Sabah, Singapore, Thailand, Indonesia, the Philippines, Tainan, south Taiwan, and Hawaii) (Table 1). The maximum distance between colonies that were assigned to the putative populations was 25 km. These populations were located at least 100 km apart or were separated by water. Su and Scheffrahn (1988) reported the maximum linear distance of *Coptotermes*

formosanus Shiraki foragers to be 115 m. Therefore, termites were sampled at a minimum distance of 0.7 km between structures and trees to ensure that each of the collection points represented a different colony. All termites were preserved in 100% ethanol for subsequent microsatellite analysis. Voucher specimens from each colony were deposited at the Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia.

Genotype Analysis and Marker Evaluation. Genomic DNA of individual worker was extracted using the CTB Tissue Extraction Kit (Intron, Seongnam-Si, Gyeonggi-do, Korea) after grinding the whole body in liquid nitrogen. Ten to 20 termites from each colony were genotyped at 10 microsatellite loci that were identified previously from *C. gestroi* (Yeap et al. 2009b) (Table 2). For each primer pair, forward primers were labeled and primer pairs were multiplexed into three groups: 1) CG2, CG6, CG19, and CG21; 2) CG23, CG26, and CG29; and 3) CG31, CG33, and CG38. Polymerase chain reaction amplifications were conducted according to methods described in Yeap et al. (2009b). Amplified fluorescence-labeled products

Table 1. Populations, locations, number of colonies, and number of *C. gestroi* workers that were analyzed

Region	Pop	N^a	Locale	n^a
Malaysia	Kedah	1	Alor Setar, Pokok Sena	10
		1	Alor Setar, Jalan Langgar	10
		1	Alor Setar, Changloon	10
		1	Alor Setar, Pulai	10
	Penang Island	1	Minden, USM	20
		1	Teluk Kumbar	12
		1	Island Glades	10
		1	Island Glades, Solok Tembaga4	10
		1	Balik Pulau	20
		1	Pulau Betong	18
		1	Bayan Lepas	10
		1	Bayan Baru, Lintang Mashuri	10
		1	Maybank Union St	16
		1	Jalan Taylor	10
		1	Jalan Vermont	12
		1	2 Derai Pinang 10	15
		1	Jelutong, Jalan Perusahan	14
		1	34, Jalan Phuah Hin Leong	11
		1	LHK IC2	10
		1	5 Lorong Macang Bubuk 6	10
		1	Lengkok, Kg. Jawa	16
		1	Air Itam	15
		1	Botanical Garden	20
		1	Permatang Damai Laut	10
		1	Tanjung Bunga	10
		1	Batu Feringgi	10
		1	Tanjung Tokong	14
	Penang Mainland	1	Bukit Mertajam, Taman Kaiki Bukit	10
		1	Kulim, Taman Penaga	10
	Kuala Lumpur	10	Selangor, Petaling Jaya	100
		4	Selangor, Puchong	40
		4	Selangor, Subang Jaya	40
		2	Selangor, Sunway Jaya	20
		1	Selangor, Sunway Damansara	15
		1	Selangor, Bukit Jelutong	10
		1	Selangor, Kota Kemunting	10
		2	Selangor, Sungai Buloh	20
		2	Shah Alam	20
		2	Kuala Lumpur, Bangsar	20
	Johor	1	Muar	10
		1	Johor Bahru	10
	Sabah	1	Kota Kinabalu, Taman Fortuna	10
		1	Kota Kinabalu, Taman Somerset	10
Singapore	Singapore	1	John Rd.	10
		1	Dunean Rd.	10
		1	Upper Changi Rd.	10
		1	Ocean Dr.	10
		1	22G Lowland Rd.	10
		1	Sentosa Island	10
		1	106 Yunnan Crescent	10
		1	6 Pandan Valey	10
		1	Pandan Rd.	10
Thailand	Thailand	2	Bangkok	26
Indonesia	Indonesia	1	Bogor	16
		1	Cibinong	10
Philippines	Philippines	3	Laguna	30
Taiwan	Tainan	3	Tainan	30
	South Taiwan	2	Pingtung	20
		1	Kaohshiung	10
United States	Hawaii	1	Oahu	20
Total		85		950

[a] *N*, number of colonies; and *n*, number of *C. gestroi* workers that were analyzed.

were sent to Macrogen Inc. (Seoul, South Korea) for fragment analysis, which was conducted using an ABI3730 sequencer and a GeneScan-500 LIZ size standard (Applied Biosystems, Foster City, CA). Microsatellite allele sizes were scored using the Peak Scanner v1.0 (Applied Biosystems, Foster City, CA).

The neutrality and independence of the microsatellite loci were analyzed. Deviation from Hardy-Weinberg equilibrium (HWE) for each locus and tests for linkage disequilibrium between all pairs of loci were conducted with the exact test using the program GENETIC DATA ANALYSIS version 1.1 (GDA; Lewis and Zaykin 2000) with 3,200 shufflings. Individuals within colonies are related and thus genotypes within colonies were not independent. Therefore, only a single individual per colony was used for these tests. As suggested by Vargo et al. (2003), a resampling procedure with one individual randomly selected from every colony for a total of 20 replications was performed.

Population Genetic Structure. Genetic relationships among the population groups were investigated using STRUCTURE program version 2.2 (Pritchard et al. 2000). A single individual per colony from all populations was included in the analysis to determine a reasonable number of partitions (*K*) for the studied populations to identify distinct genetic populations, migrants, and admixed individuals. For this clustering analysis, the admixture model and correlated allele frequencies among populations were chosen. Twenty runs for each *K* from 2 to 15 with a burn-in length and Markov chain Monte Carlo of 20,000 each were performed. The true *K* then was determined by comparing posterior probabilities of data under different *K* settings.

To assess isolation by distance within each population group (only tested on the four largest population groups: Penang Island, Kuala Lumpur, Singapore, and Taiwan) and among populations, F_{ST} values between pairs of colonies and between pairs of population groups, respectively, were obtained. These values were converted to $F_{ST}/(1-F_{ST})$ and correlated to the logarithm (ln) of geographical distance using Pearson's correlation coefficient. The significance of the correlation coefficients was assessed using a Mantel test with 10,000 replications (Slatkin 1993, Rousset 1997) as implemented in the program GENEPOP on the Web version 3.1c (http://genepop.curtin.edu.au/; Raymond and Rousset 1995). Genetic differentiation among populations was analyzed in a hierarchical manner with the population level above the colony level, as implemented in GENETIC DATA ANALYSIS version 1.1 (GDA; Lewis and Zaykin 2000). The confidence intervals of the F_{ST} among populations were determined by bootstrapping over loci.

Results

Basic Genetic Data. Table 2 shows the allele number and the frequency of the most common allele of the 10 variable microsatellite loci. Allele frequencies were calculated from the entire genotype of 950 individual

Table 2. Number of alleles and frequency of the most common allele at 10 microsatellite loci for the three populations with the highest number of alleles and for the sum of the 13 putative population groups

Locus	Penang Island pop		Kuala Lumpur pop		Singapore pop		Overall (13 populations)	
	No. of alleles	Freq. off most common allele	No. of alleles	Freq. of most common allele	No. of alleles	Freq. of most common allele	No. of alleles	Freq. of most common allele
CG2	9	0.46	7	0.47	7	0.67	14	0.22
CG6	16	0.44	21	0.17	9	0.42	31	0.22
CG19	4	0.72	2	0.66	3	0.59	6	0.50
CG21	10	0.40	10	0.21	4	0.43	14	0.45
CG23[a]	2	0.82	3	0.45	2	0.82	6	0.61
CG26	8	0.25	8	0.23	4	0.69	13	0.25
CG29	15	0.17	19	0.19	9	0.33	24	0.15
CG31[a]	2	0.81	3	0.68	3	0.79	4	0.77
CG33	10	0.33	10	0.27	8	0.3	13	0.25
CG38	5	0.65	2	0.74	2	0.58	8	0.36
Mean	9.6		9.9		5.75		15.4	

[a] Deviated significantly from Hardy-Weinberg equilibrium.

workers. Two loci, CG23 and CG31, showed significant deviations from HWE in >50% of the 480 tests performed (20 resampled data sets × 13 populations × 2 loci), and these deviations occurred frequently across nine of the population groups. Thus, these two loci were excluded from the analysis. Pairs of the remaining eight loci did not show significant linkage disequilibrium in any of the resampled datasets. Consequently, the eight microsatellite loci used were considered to be independently assorting markers and suitable for population genetic structure analysis. Overall, there were four to ten alleles per locus, with a mean of 15 alleles per locus for the eight loci retained for subsequent analyses. Means calculated in Table 2 were based on the eight loci used in the analyses.

Clustering Analysis. Clustering analyses performed by the STRUCTURE program were used to study the relationships among populations. Figure 2 shows the assignment of a single individual from each colony into the predefined population groups. Output posterior probabilities ($InPr[X/K]$) from different batches suggested $K = 4$ as the most appropriate configuration according to the method of Evanno et al. (2005). Because only one colony was sampled in Hawaii, STRUCTURE could not assign this colony to a population. Colonies within Penang Island, Johor, Kuala Lumpur, Sabah, Singapore, and Indonesia populations displayed an admixture of clusters. In contrast, colonies from Thailand, Kedah, and Penang mainland populations were closely related, which is consistent with their geographical location. The Malaysia population groups and the Singapore population group showed continuous gene flow among them. Both the Tainan and south Taiwan populations were clustered together, whereas the Indonesian population was distant from all others populations.

Genetic Differentiation Within and Among Populations. Results from clustering analysis showed that colonies from Taiwan and south Taiwan were closely related. Thus, in the assessment of isolation by distance, they were grouped together as the Taiwan population. Because of the small sample size for most
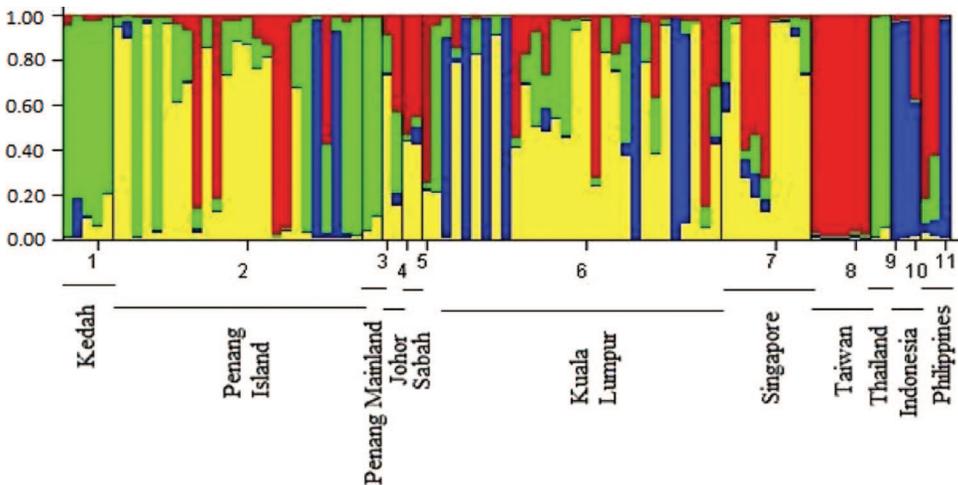

Fig. 2. Estimated membership proportions for $K = 4$. Each individual is plotted in a single vertical line that is separated into K colored segments representing the proportion of membership (Q) in each of the K clusters. Black lines separate individuals from two different predefined populations.
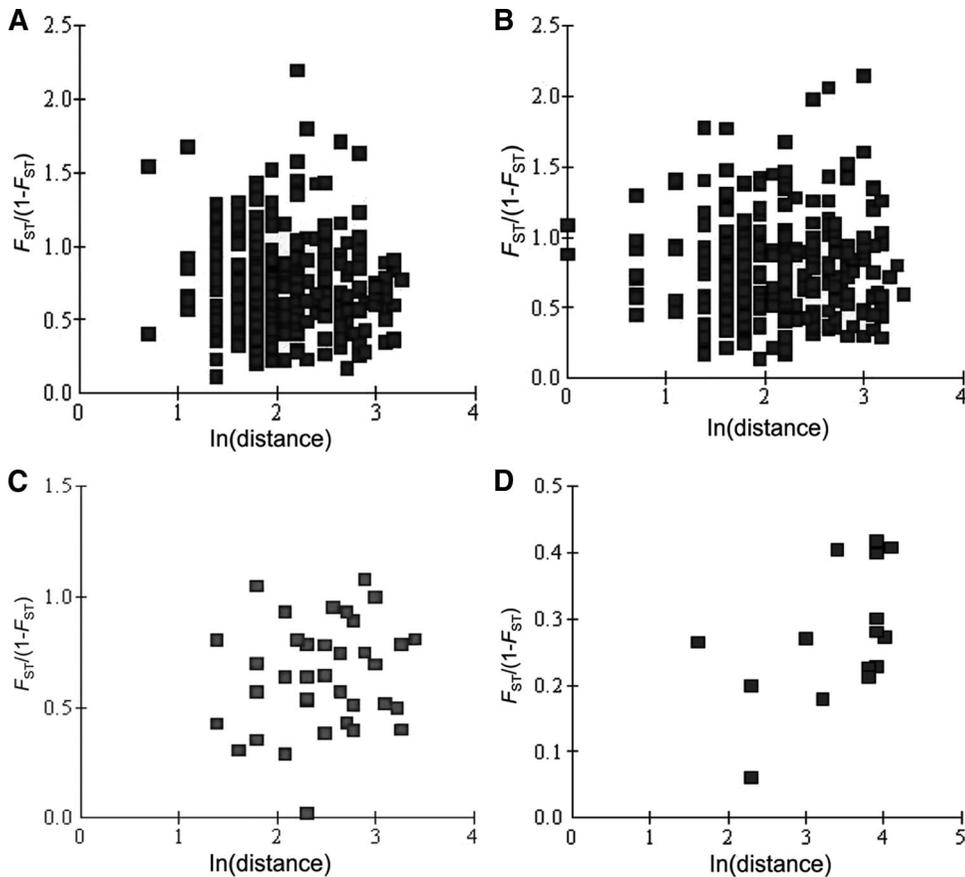
Fig. 3. Relationship between pairwise estimates of $F_{ST}/$ (one $- F_{ST}$) and geographical distance of *C. gestroi* colonies within each of the four largest population groups. A: Penang Island, B: Kuala Lumpur, C: Singapore, and D: Taiwan.

populations, isolation by distance was tested only for the four largest population groups: Penang Island, Kuala Lumpur, Singapore, and Taiwan. The correlation coefficients were not significant for Penang Island ($r = -0.05$, $P = 0.46$); Kuala Lumpur ($r = 0.02$, $P = 0.34$); and Singapore ($r = 0.16$, $P = 0.25$) (Fig. 3A–C), but they were significant in Taiwan ($r = 0.49$, $P = 0.03$) (Fig. 3D).

On a larger scale, there was significant isolation by distance in the Malaysia populations (Fig. 4A; Mantel test: $n = 6$, $r = 0.674$, $P = 0.019$) and in populations across Asia (Fig. 4B; Mantel test: $n = 13$, $r = 0.6563$, $P = 0.020$).

Genetic differentiation was lower among the populations in Peninsular Malaysia ($F_{ST} = 0.073$, 95% CIs $= 0.053–0.092$) than when Sabah was added ($F_{ST} = 0.082$, 95% CIs $= 0.062–0.101$), indicating higher gene flow among the Peninsular Malaysian populations than with East Malaysia (Sabah). Moderate gene flow among the 13 putative population groups ($F_{ST} = 0.166$, 95% CIs $= 0.126–0.202$) may be because populations that were separated by great distances (i.e., Malaysia and Hawaii, geographic distance range $\approx 11,140$ km).

Discussion

Colonies in Taiwan populations showed significant isolation by distance, which suggested that there is population viscosity within these populations. This result was in contrast with findings for *C. formosanus* in Japan; New Orleans, LA; and Oahu, HI, which suggested a general lack of strong population viscosity in introduced populations (Vargo et al. 2003). Most introduced populations have low genetic diversity (Vargo et al. 2003, 2006, Husseneder et al. 2006). These two populations also exhibited low genetic variability, with a maximum of three alleles per locus. Similar results of reduced genetic variation in introduced populations compared with native populations were found in French populations of *R. flavipes* and *C. formosanus* (Vargo 2003, Vargo et al. 2003, Dronnet et al. 2005, Husseneder et al. 2005). Based on the histogram generated by STRUCTURE (Fig. 2), the phylogenetic relationship among *C. gestroi* in southern Taiwan, the Philippines, and Hawaii (Yeap et al. 2009a), geographical location, and the extensive trade between countries, it is likely that *C. gestroi* was introduced into Taiwan from a single introduction from the Philippines that likely originated from the same population source.
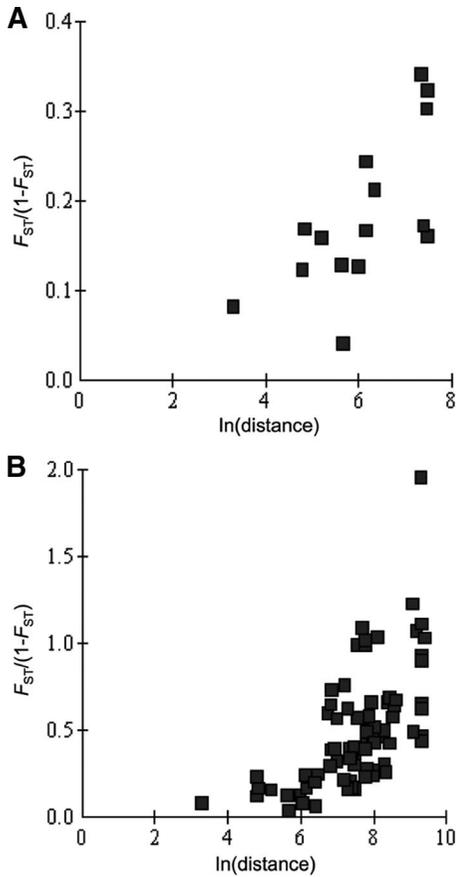
Fig. 4. Relationship between pairwise estimates of $F_{ST}/$ (one – F_{ST}) and geographical distance among *C. gestroi* populations. The correlation coefficients were significant for A: among Malaysia populations and B: all 13 populations in the study.

When we examined the Penang Island, Kuala Lumpur, and Singapore populations, there was no significant isolation by distance among colonies at the spatial scale studied in either population. This was further supported by the high number of alleles in both populations, suggesting human aid transportation is high in the cities.

We found low levels of genetic differentiation among the Malaysian population groups ($F_{ST} = 0.082$) and among population groups in Peninsular Malaysia ($F_{ST} = 0.073$), but they exhibited a positive correlation with geographic distance. This indicates that gene flow in *C. gestroi* is considerably high at large spatial scales (ranging from 100 to 600 km) and even more extensive at smaller spatial scales. Jenkins et al. (1999) reported weak differentiation in *R. flavipes* (Kollar) across the state of Georgia. In contrast, in populations of *C. formosanus* from Japan, distances of ≥100 km over land or water are sufficient to warrant a certain degree of genetic differentiation among populations ($F_{ST} = 0.12$) (Vargo et al. 2003). At larger spatial scales ranging from 100 km (between Kedah and Penang) to 11,200 km (between Penang and Hawaii), a greater degree of genetic differentiation was detected ($F_{ST} = 0.19$). Genetic exchange through natural dispersal may be impossible for populations that are too far apart. Thus, the most likely route of migrants connecting the populations is the occasional transportation of infested materials by humans.

The results of this study provide some basic information about the genetic structure of *C. gestroi*. Further studies with more complete colony census data, termite sampling at a finer scale, and more colonies in the population groups across a wider geographic area would be required for a better understanding of the variation of the breeding system and the extent and direction of gene flow among populations. The diverse and flexible breeding system may enable *C. gestroi* to successfully invade new or marginal habitats, thus facilitating the expansion of this species worldwide.

Acknowledgments

References Cited

Acda, M. N. 2004. Economically important termites (Isoptera) of the Philippines and their control. Sociobiology 43: 159–167.

Armany, G. R., M. P. De Arruda, W. Arthofer, Z. K. Atallah, M. W. Bruford, C. Burdine, J. W. Busch, N. R. Campbell, D. Carey, B. C. Carstens, et al. 2009. Permanent genetic resources added to Molecular Ecology Resources Database 1 May 2009 – 31 July 2009. Mol. Ecol. Resour. 9: 1460–1559.

Bulmer, M. S., E. S. Adams, and J.F.A. Traniello. 2001. Variation in colony structure in the subterranean termite *Reticulitermes flavipes*. Behav. Ecol. Sociobiol. 49: 236–243.

Chapman, R. E., and A.F.G. Bourke. 2001. The influence of sociality on the conservation biology of social insects. Ecol. Lett. 4: 650–662.

Clément, J.-L. 1986. Open and closed societies in *Reticulitermes* termites (Isoptera, Rhinotermitidae): geographic and seasonal variations. Sociobiology. 11: 311–323.

Clément, J.-L., A.-G. Bagnères, P. Uva, L. Wilfert, A. Quintana, J. Rienhard, and S. Dronnet. 2001. Biosystematics of *Reticulitermes* termites in Europe: morphological, chemical and molecular data. Insectes. Soc. 48: 202–215.

Dronnet, S., M. Chauisat, E. L. Vargo, C. Lohou, and A.-G. Bagnères. 2005. Genetic analysis of the breeding system of an invasive subterranean termite, *Reticulitermes san-*

tonensis, in urban and natural habitats. Mol. Ecol. 14: 1311–1320.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14: 2611–2620.

Husseneder, C., M. T. Messenger, N.-Y. Su, J. K. Grace, and E. L. Vargo. 2005. Colony social organization and population genetic structure of an introduced population of Formosan subterranean termite from New Orleans, Louisiana. J. Econ. Entomol. 98: 1421– 434.

Husseneder, C., D. M. Simms, and D. R. Ring. 2006. Genetic diversity and genotype differentiation between the sexes in swarm aggregations decrease inbreeding in the Formosan subterranean termite. Insectes. Soc. 53: 212–219.

Jenkins, T. M., C. J. Bastern, and R. Dean. 1999. Matriarchal genetic structure of *Reticulitermes* (Isoptera: Rhinotermitidae) populations. Sociobiology 33: 239–263.

Kirton, L. G. 2005. The importance of accurate termite taxonomy in the broader perspective of termite management, pp. 1–7. *In* C.-Y. Lee and W. H. Robinson (eds.), Proceedings of the Fifth International Conference on Urban Pests. P&Y Design Network, Penang, Malaysia.

Kirton, L. G., and M. Azmi. 2005. Patterns in the relative incidence of subterranean termite species infesting buildings in peninsular Malaysia. Sociobiology 46: 1–15.

Lee, C.-Y. 2002. Subterranean termite pests and their control in the urban environment in Malaysia. Sociobiology 40: 3–9.

Lee, C.-Y. 2007. Perspective in urban insect pest management in Malaysia. Vector Control Research Unit, Universiti Sains Malaysia. 104 pp.

Lee, C.-Y., C. Vongkaluang, and M. Lenz. 2007. Challenges to subterranean termite management of multi-genera faunas in southeast Asia and Australia. Sociobiology 50: 213–221.

Lewis, P. O., and D. Zaykin. 2000. GENETIC DATA ANALYSIS: computer program for the analysis of allelic data. Version 1.1.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): a population genetics software for exact tests and ecumenism. J. Heredity 86: 248.

Reilly, L. M. 1986. Measurements of population structure in the termite *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) and comparisons with the eusocial Hymenoptera. Ph.D. dissertation, Vanderbilt University, Nashville, TN.

Ross, K. G. 1993. The breeding system of the fire ant *Solenopsis invicta:* effects on colony genetic structure. Am. Nat. 141: 554–576.

Ross, K. G. 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. Mol. Ecol. 10: 265–284.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145: 1219–1228.

Scheffrahn, R. H., and N.-Y. Su. 2000. Asian subterranean termite, *Coptotermes gestroi* (=*havilandi*) (Wasmann) (Insecta: Isoptera: Rhinotermitidae). University of Florida IFAS Extension document. EENY-128. 5 pp.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47: 264–279.

Sornnuwat, Y. C., C. Vongkaluang, M. Takahashi, K. Tsunoda, and T. Yoshimura. 1996. Survey and observation on damaged houses and causal termite species in Thailand. Jpn. J. Entomol. Zool. 7: 191–2 00.

Su, N.-Y., and R. H. Scheffrahn. 1988. Foraging population and territory of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in an urban environment. Sociobiology 14: 353–360.

Takematsu, Y., T. Yoshimura, S. Yusuf, Y. Yanase, K. Kambara, A. Tashiro, S. Doi, M. Takahashi, P. Sukartana, T. Inoue, et al. 2006. Termite assemblages in urban areas of south east Asia – Diversity and economic impacts, pp. 84–91. *In* Y. Imamura, T. Umezawa, and T. Hata, (eds.), Sustainable development and utilization of tropical forest resources RISH, Kyoto University, Japan.

Thorne, B. L., J.F.A. Traniello, E. S. Adams, and M. Bulmer. 1999. Reproductive dynamics and colony structure of subterranean termites of the genus *Reticulitermes* (Isoptera: Rhinotermitidae): a review of the evidence from behavioral, ecological, and genetic studies. Ethol. Ecol. Evol. 11: 149–169.

Tsai, C. C., and C. S. Chen. 2003. First record of *Coptotermes gestroi* (Isoptera: Rhinotermitidae) from Taiwan. Formosan Entomol. 23: 157–161.

Vargo, E. L. 2003. Hierarchical analysis of colony and population genetic structure in the eastern subterranean termite, *Reticulitermes flavipes*, using two classes of molecular markers. Evolution 57: 2805–2818.

Vargo, E. L., and C. Husseneder. 2009. Biology of subterranean termites: insights from molecular studies of *Reticulitermes* and *Coptotermes.* Annu. Rev. Entomol. 54: 379– 403.

Vargo, E. L., C. Husseneder, and J. K. Grace. 2003. Colony and population genetic structure of the Formosan subterranean termite, *Coptotermes formosanus*, in Japan. Mol. Ecol. 12: 2599–2608.

Vargo, E. L., C. Husseneder, D. Woodson, M. G. Waldvogel, and J. K. Grace. 2006. Genetic analysis of colony and population structure of three introduced populations of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the continental United States. Environ. Entomol. 35: 151–166.

Yeap, B. K., A. S. Othman, V. S. Lee, and C. Y. Lee. 2007. Genetic relationship between *Coptotermes gestroi* and *Coptotermes vastator* (Isoptera: Rhinotermitidae). J. Econ. Entomol. 100: 467– 474.

Yeap, B. K., A. S. Othman, and C. Y. Lee. 2009a. Molecular systematics of *Coptotermes* (Isoptera: Rhinotermitidae) from east Asia and Australia. Ann. Entomol. Soc. Am. 102: 1077–1090.

Yeap, B. K., A. S. Othman, and C. Y. Lee. 2009b. Identification of polymorphic microsatellite markers for the Asian subterranean termite *Coptotermes gestroi* (Wasmann) (Blattodea: Rhinotermitidae). (http://tomato.biol.trinity.edu/manuscripts/9–6/mer-09–0192.pdf).

Yeap, B. K., F. M. Dugal, A. S. Othman, and C. Y. Lee. 2010. Genetic relationship between *Coptotermes heimi* and *Coptotermes gestroi* (Isoptera: Rhinotermitidae). Sociobiology 56: 291–312.

Yudin, L. 2002. Termites of Mariana Islands and Philippines, their damage and control. Sociobiology 40: 71–74.