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Source: Zoological Science, 19(10) : 1141-1146

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.19.1141

ZOOLOGICAL SCIENCE **19**: 1141–1146 (2002)

Population Structure and Genetic Diversity in Insular Populations of *Nasutitermes takasagoensis* (Isoptera: Termitidae) Analyzed by AFLP Markers

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ABSTRACT—Dispersal ability and degree of inbreeding in a population can indirectly be assessed using genetic markers. In general, it was suggested that winged termites are not able to fly distances greater than several hundred meters. Here, amplified fragment length polymorphism (AFLP) was used to analyze genetic diversity, population substructure, and gene flow among insular populations of the termite *Nasutitermes takasagoensis* (Isoptera: Termitidae) in the Yaeyama Islands, Okinawa, Japan. Samples were collected from 77 nests on seven islands of the Yaeyama Group. Using three primer combinations a total of 155 bands were generated with 78 (50%) polymorphic bands. Genetic distance and G_{st} values among insular populations were calculated. Relatively high genetic diversity and low values of G_{st}, suggest there is moderate subpopulation structure. Based on these results, we discussed two possibilities; first, winged termites are able to fly over distances of several kilometers, and second, these results were obtained because insular populations share a recent common origin.

Key words: AFLP, *Nasutitermes takasagoensis*, Okinawa Japan, population structure, termites

INTRODUCTION

Termites are eusocial insects whose colonies are founded by winged adults, formed by budding (isolation of parts of a colony) or by sociotomy (migration of complete units of a mature colony) (Nutting, 1969). In general, it was suggested that termite winged adults (alates) have a poor dispersal ability. Nutting (1969) reported that distances of termite flight can vary several meters to some hundreds, depending on the species and on the climatic conditions. Bodot (1967) showed that alates of *Allodontotermes giffardi* flew several meters and established a new colony in the proximity of their mother nest. Abe (1984) and Gathorne–Hardy *et al.* (2000) in their studies of recolonization of the Krakatau Islands suggested that winged termites were unlikely to be able to cross distances of around 2 km. Ikehara (1966) in his study on insular populations of termites in southwestern Japan, showed that the longest distance recorded for alate flights of *Coptotermes formosanus* and *Odontotermes formosanus* was 1 km.

Because this suggested low dispersal ability, it has been proposed that in termite populations inbreeding is the general rule since low dispersal favors mating among relatives (Bartz, 1979; Pamilo, 1984; Williams and Williams, 1957). Dispersal ability and degree of inbreeding in a population can indirectly be assessed using genetic markers. Until now, genetic population structure, genetic diversity and gene flow in termites have been poorly studied (Reilly, 1987; Thompson and Hebert, 1998).

In this study we used the amplified fragment length polymorphism (AFLP) technique (Vos *et al.*,1995) to analyze the population genetic structure, genetic diversity and gene flow of *Nasutitermes takasagoensis* on the Yaeyama Islands, Southern Japan (Fig. 1). AFLP was chosen over other techniques because it has been demonstrated to be a powerful method for the characterization of infraspecific polymorphism among populations, and because of its high reproducibility (Krauss, 1999; Qamaruz-Zaman *et al.,* 1998; Semblat *et al.,* 1998; Winfield *et al*., 1998; Yan *et al*., 1999).

N. takasagoensis distributes in the Yaeyama Islands, Taiwan and southeastern mainland China, living in arboreal carton nests with two to several reproductives in a colony (Miura and Matsumoto, 1996). Islands of the Yaeyama group are small and separated from each other by 200 meters to several kilometers; they might have been isolated from China mainland since the late Late Pleistocene (*ca.* 30,000–10,000 years BP) (Otsuka and Takahashi, 2000). The isolation of *N. takasagoensis* on the Yaeyama Islands

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offers a good opportunity to apply molecular techniques to analyze genetic diversity, population structure and gene flow in a natural population of termites. If the dispersal ability of *N. takasagoensis* was low, little genetic variation within islands, high population substructure, and high genetic distance between insular populations would be expected.

MATERIALS AND METHODS

Sampling

Samples of *N. takasagoensis* were collected from 77 nests on seven islands of the Yaeyama Islands, Ryukyu Archipelago, Japan (Iriomote, Ishigaki, Kohama, Taketomi, Kuro, Uchibanari and Hateruma) (Fig. 1) during three surveys in May, 1995, April, 2000 and July, 2001. Termites were preserved in 100 percent acetone.

To have a more representative sampling, in almost all islands samples were collected from points as far as possible from each other, except for some samples collected in Iriomote (Fig. 1).

Genetic distance among samples was calculated at two spatial scales, one within Iriomote (local scale) and the other between pairs of islands (inter-island scale). In the local scale, geographic distance was measured among 21 nests collected in a relatively small area on Iriomote Island (Fig. 1). In the inter-island scale, geographic distances were measured between the two nearest coastal points among all islands. Distances at the local scale varied from 1 to 855 m whereas inter-island scale distances ranged from 0.2 to 46 km.

Genetic analyses

To maximize the sampling efficiency, we extracted DNA from only one individual per nest, since termites from the same colony are expected to be genetically close. To confirm this assumption, ten or nine individuals from the same colony in three different nests were genetically compared.

Total genomic DNA was obtained from the head and thorax by standard chloroform extraction and isopropanol precipitation (Miura *et al.*, 1998). DNA was digested by two restriction endonucleases *Eco*RI and *Mse*I (New England Biolabs). The AFLP Amplification Core Mix (Applied Biosystems) was used as recommended by the manufacturer. Using two fluorescent-labeled *Eco*RI primers and three non-labeled *Mse*I primers (Applied Biosystems), we produced a total of three primer combinations (Table 1).

Samples were run on a 6 percent polyacrylamide gel at 1,800 V and 35 W for 1:30 hr. After electrophoresis, gels were scanned using a Hitachi's FMBIO II Fluorescence Imaging System. Findings were scored in the form of the presence or absence of each fragment within each individual and then pooled over all fragments and primer combinations.

By this method we genotyped a total of 77 samples from seven island populations (37 from Iriomote, 12 from Ishigaki, 6 from Kohama, 6 from Taketomi, 4 from Kuro, 2 from Uchibanari, and 10 from Hateruma), plus 29 individuals from three different nests for analysis of genetic distance within and between nests.

Statistical Analyses

Two types of polymorphisms are detected with AFLPs: (a) substitutions in the restriction sites or in primer elongation binding sites which result in the loss of the band, and (b) insertions/deletions within the restriction fragment which results in different sized bands (Qamaruz-Zaman, 1998). Since AFLP loci segregate as dominant markers, we made the following assumptions to estimate population heterozygosity (Yan *et al.*, 1999).

First, AFLP fragments segregate according to Mendelian expectations. Second, amplified fragments of the same size (dominant alleles) are identical in state among and between populations.

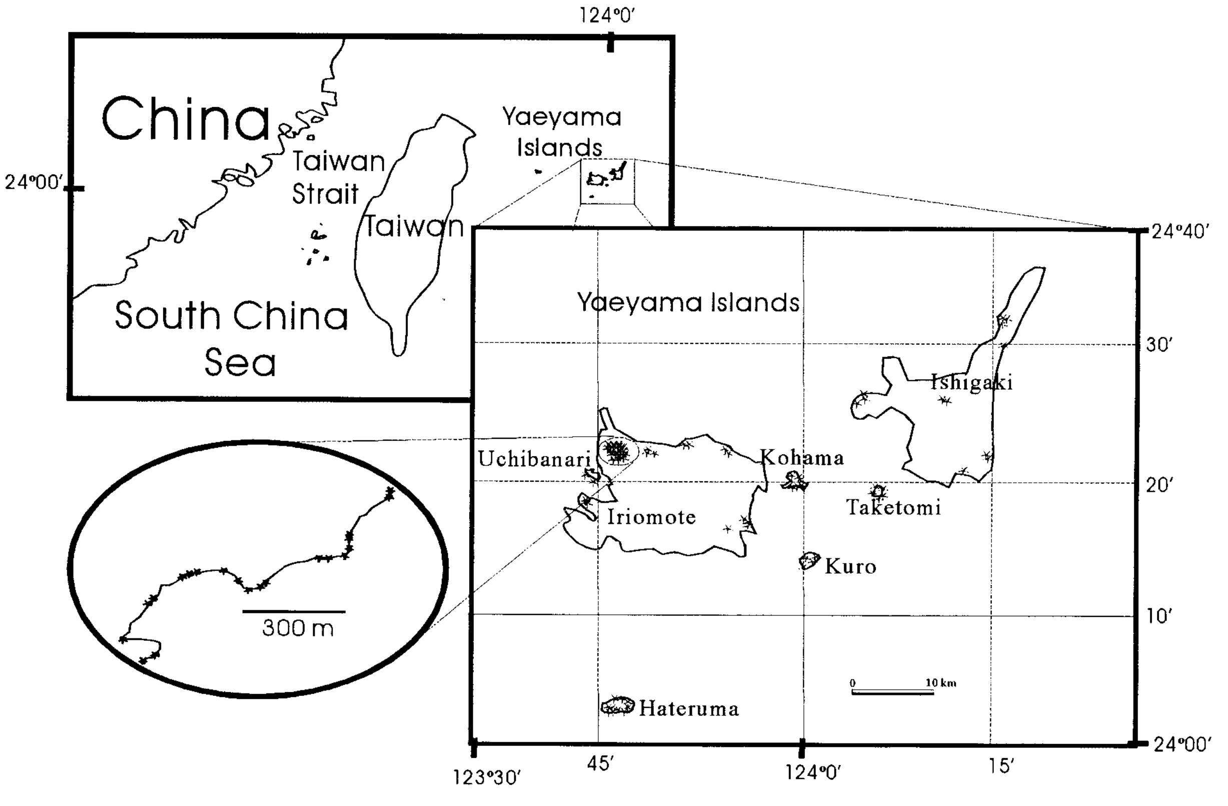

Fig. 1. Sampling area, the Yaeyama Islands. Asterisks show sampling places. The figure into the oval shows the sampling area at local scale.

Third, unamplified fragments (recessive alleles) of a locus are identical in state among and between populations. Finally it is also assumed that genotypes at all AFLP loci are in Hardy-Weinberg equilibrium.

Using the program TFPGA (Miller, 1997), genetic diversity within populations was estimated on the basis of Nei's (1978) average heterozygosities, and percentages of polymorphic loci. In addition Nei's (1973) gene diversity was calculated using the program POPGENE Version 1.31 (Yeh *et al.*, 1997). The same program was used to calculate Nei's (1973) G_{st} (which is equivalent to Wright's F_{st},) for pairs of insular populations. Nei's (1978) genetic distance was calculated for an estimate of genetic distance between insular populations and between the 21 samples at local scale. To estimate migration between islands, gene flow, *Nm* (Slatkin and Barton, 1989) was estimated from G_{st}.

To clarify if there is a significant correlation between geographic and genetic distances, Mantel tests were conducted at both local and inter-island scales.

To compare genetic distances between and within nests, genetic distance was calculated as total pairwise differences between several individuals from three nests (ten individuals from each of two nests and nine individuals from the remaining one) and compared, by a one-tailed Mann-Whitney test, to pairwise differences between single individuals from ten different nests collected in the same locality. Pairwise comparisons were done by using the program PAUP*4.0b8 (Swofford, 2000).

RESULTS

A total of 155 AFLP fragments were obtained for the three primer combinations used, with 78 (50%) polymorphic

Table 1. Primer combinations (showing the three selective nucleotides only) used to produce AFLPs in the final selective amplification, and the number of loci and percentage of polymorphisms produced from each.

Restriction site	Primer Combination			Totals
	1	2	3	
*Eco*RI	ACT	ACA	ACA	
*Mse*I	CTC	CTA	CTG	
Number of loci	39	54	62	155
No. of polymorphic loci	24	22	32	78
Percentage of polymorphism	61%	40%	51%	50%

Table 2. Geographic distance (km), genetic distance according to Nei (1978), G_{st} (Nei 1973), and gene flow *Nm* (Slatkin & Barton 1989) between island populations.

Population Comparisons	Geographic Distance	Genetic Distance	G_{st}	Gene Flow (*Nm*)
Iriomote / Ishigaki	16	0.0162	0.057	8.22
Iriomote / Kohama	2.4	0.0282	0.120	3.66
Iriomote / Taketomi	15	0.0186	0.085	5.37
Iriomote / Kuro	9.4	0.0331	0.138	3.09
Iriomote / Uchibanari	0.2	0.0276	0.160	2.62
Iriomote / Hateruma	21.6	0.0467	0.182	2.24
Ishigaki / Kohama	11.4	0.0419	0.154	2.73
Ishigaki / Taketomi	3.8	0.0308	0.119	3.69
Ishigaki / Kuro	16.4	0.0485	0.172	2.39
Ishigaki / Uchibanari	34	0.0442	0.204	1.94
Ishigaki / Hateruma	46	0.0729	0.237	1.60
Kohama / Taketomi	7.2	0.0445	0.213	1.84
Kohama / Kuro	8	0.0272	0.171	2.42
Kohama / Uchibanari	21	0.0555	0.348	0.93
Kohama / Hateruma	34	0.0443	0.244	1.54
Taketomi / Kuro	9	0.0393	0.199	2.00
Taketomi / Uchibanari	33.4	0.0357	0.251	1.48
Taketomi / Hateruma	38.8	0.0611	0.279	1.29
Kuro / Uchibanari	17.4	0.0637	0.370	0.85
Kuro / Hateruma	26.2	0.0461	0.251	1.48
Uchibanari / Hateruma	30.6	0.0928	0.494	0.51

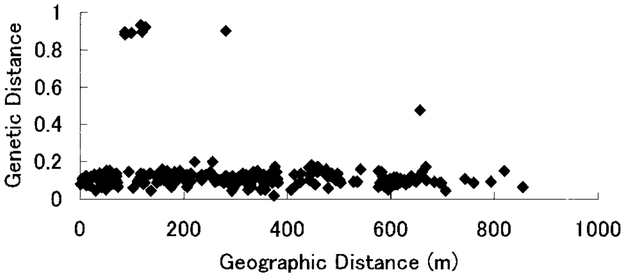

Fig. 2. Geographic distance vs. observed Nei's (1978) genetic distance at local scale.

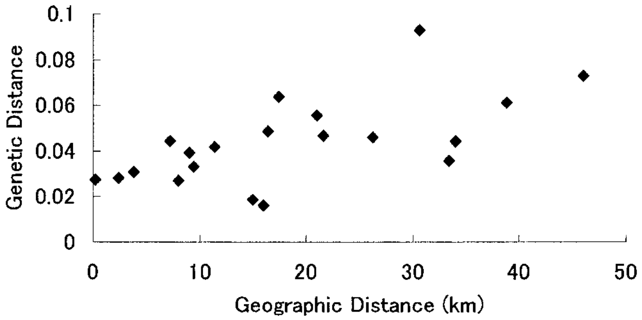

Fig. 3. Geographic distance vs. observed Nei's (1978) genetic distance at inter-island scale.

Table 3. Genetic diversity of *N. takasagoensis* on Yaeyama Islands as a percentage of the polymorphic loci, Nei's (1973) gene diversity, Nei's (1978) unbiased average heterozygosity. Sample size is also shown.

Population	Polymorphic loci (percentage)	Gene diversity	Average Heterozygosity	Sample size (n)
Iriomote	60 (39%)	0.1376	0.1395	37
Ishigaki	61 (39%)	0.1602	0.1672	12
Kohama	30 (19%)	0.0798	0.0871	6
Taketomi	35 (22%)	0.0951	0.1037	6
Kuro	31 (20%)	0.0857	0.0979	4
Uchibanari	12 (8%)	0.0321	0.0428	2
Hateruma	24 (15%)	0.0608	0.0640	10

bands (Table 1).

The smallest Nei's (1978) genetic distance value was obtained between the populations from Iriomote and Ishigaki (0.0162), followed by Iriomote-Taketomi with 0.0186. The largest distance was obtained between Uchibanari and Hateruma (0.0928) (Table 2).

Gene flow ranged from 0.51 (between Uchibanari and Hateruma) to 8.22 (Iriomote and Ishigaki) (Table 2).

At the local scale the Mantel test did not show a clear relation (r^2=0.04; P=0.323; Fig 2). Nevertheless, at the inter-island scale, a stronger relation was found between geographic and genetic distances (r^2=0.6; P=0.011; Fig. 3).

The highest diversity values were obtained in the samples from Ishigaki with 39 percent of polymorphism, Nei's (1973) gene diversity was 0.1602, and unbiased heterozygosity was 0.1672 (Table 3).

The one-tailed Mann-Whitney test showed that pairwise differences were higher between nests than within them (P <0.0001 for the three cases), corroborating the assumption that individuals in the same colony are genetically closer to each other than to individuals in a different colony.

DISCUSSION

It has been suggested that values of F_{st} in the range 0.05 to 0.15 indicate *moderate* genetic differentiation, values in the range 0.15 to 0.25 indicate *great* genetic differentiation and values above 0.25 indicate *very great* genetic differentiation (Hartl and Clark, 1989). Applying this rule of thumb to our results, it can be said that the G_{st} (which is equivalent to F_{st}) values obtained between the two large islands, Iriomote and Ishigaki, (G_{st} = 0.057; genetic distance =0.0162) show that genetic differentiation is moderate. This moderate genetic differentiation between these two islands could indicate that both populations are not completely isolated.

Migration of termites between islands can occur in several ways. Ikehara (1966), Abe (1984) and Gathorne-Hardy *et al.* (2000) have suggested that rafting of pieces of wood containing reproductives could be an effective means of dispersal for some termite species. Strong typhoons hit Yaeyama islands several times a year; which could facilitate transportation of infested pieces of wood to the sea via the inland temporal and permanent rivers, and carry them to the nearby islands. Nevertheless, Ikehara (1966), using filter papers damped with seawater, demonstrated that the tolerance of *N. takasagoensis* against sea water is quite low. Therefore, it is unlikely that *N. takasagoensis* could succeed in rafting from one island to another.

Blowing out of alates by typhoon strong winds could be considered as another possible mode overseas dispersals. Nevertheless, it is also unlikely, since emergence of alates from their mother nests occurs during May, and the typhoon season in the area is from July to November.

Anthropogenic dispersal is considered to be another possible means of putative dispersion for *N. takasagoensis*. However, this species infests only decayed wood, and it has not been recorded that it lives in human-manufactured wood items (Ikehara, 1966). Thus, this dispersal mode does not seem to be responsible to the putative overseas dispersals.

It is expected that in order to achieve this moderate genetic differentiation between both Iriomote and Ishigaki, frequent migration among populations is necessary. Such frequency might not be easily acquired by rafting, which must be a very stochastic process, or by anthropogenic dispersal, which is improbable; but, by a more reliable means like alate flight. Without ruling out rafting and anthropogenic dispersal, it is suggested that the most frequent kind of migration must be by flight of alates. The relatively low G_{st} and genetic distance values between Ishigaki-Taketomi (G_{st} =0.119; genetic distance=0.0308) and Iriomote-Taketomi (G_{st}=0.085; genetic distance=0.0186), could indicate that gene flow occurs between these small islands and the larger ones.

Nevertheless, values of *Gst* of some island combinations indicate higher genetic differentiation. For example, Ishigaki-Uchibanari (G_{st}=0.204; genetic distance=0.0442), Ishigaki-Hateruma (G_{st}=0.237; genetic distance=0.0729), Uchibanari-Hateruma (G_{st}=0.494; genetic distance=0.0. 0928). Islands of these combinations are separated from each other by longer distances, suggesting isolation by distance. Nevertheless, in the comparisons between Uchibanari and the other islands, genetic distance is in general high. This could probably be the result of the small sample size of Uchibanari (just two samples) since small sample sizes yield high values of Nei's (1978) genetic distance (Nei, 1978).

Another possible explanation for the low genetic differentiation among some of the insular populations is based on the fact that the islands included in this study conformed from 30,000 to 10,000 years ago a sole island, which after the last glacial era and rising of sea level divided into the actual small islands (Otsuka and Takahashi, 2000); thus, it could be suggested that this island splitting could have produced populations which have not had adequate time to derive genetically. Also, since there are some relatively shallow waters around these islands, some dry connections should have remained between some of them, after the separation of the whole Yaeyama Group from the continent. Consequently, the genetic similarity observed at present could be the result of their recently shared common origin, and not a result of actual high migration rates.

However, the association found by the Mantel test between genetic distance and geographic distance at the inter-island scale suggests that geographically close island populations are genetically closer to each other than to more distant island populations. For this reason, it could be suggested that the most plausible explanation for the moderate genetic differentiation among insular populations is dispersal of termites, although, further studies are needed to validate this hypothesis.

Genetic distance values at local scale are higher than the values at inter-island scale (Figs. 2 and 3). This is the result of the small sample size used in the local scale since as demonstrated by Nei (1978) small sample sizes yield high values of Nei's (1978) genetic distance. Due to this difference in sample size comparisons between both scales cannot be done. The almost no association found by the Mantel test between geographic and genetic distances at the local scale, suggests that winged termites are able to disperse over the distances considered in the local scale of at least 800 m.

ACKNOWLEDGMENTS

We thank H. Katoh, S. Sameshima, S. Koshikawa, Dr. M. Machida and Dr. K. Araya for their help during field and laboratory work. Also we thank to two anonymous reviewers and the editor this journal for their time and valuable comments. This study was supported by Grant-in-Aid Scientific Research Program (no. 11691174 and 13440228) from the Ministry of Education Science and Culture of Japan. We thank Dr. T. Takaso and the staff members of Iriomote Institute, Tropical Biosphere Research Centre, University of Ryukus for their help.

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(Received January 25, 2002 / Accepted August 1, 2002)