Phylogeography of an Australian termite, *Amitermes laurensis* (Isoptera, Termitidae), with special reference to the variety of mound shapes.

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Abstract

In northern Australia, the debris-feeding termite *Amitermes laurensis* builds tall, wedge-shaped mounds in the northern part of Cape York Peninsula and Arnhem Land, where their habitats are seasonally flooded, and small dome shaped mounds in the southeastern part of Cape York Peninsula, where their habitats are well-drained. Phylogeographic analyses were conducted in 238 individuals from 30 populations using the mitochondrial cytochrome oxidase II (COII) gene. DNA sequences of 50 haplotypes were used to construct NJ, MP and ML trees. Phylogenetic trees for 16 *Amitermes* species showed monophyly of *A. laurensis*, and that the variation of *A. laurensis* mounds did not strongly correspond to the intraspecific phylogeny. It was observed that mounds with the same shape were constructed by phylogenetically different groups under similar environmental conditions, and different mounds shapes were built by phylogenetically closely related groups under the different environmental conditions. Thus, phylogenetically close groups of *A. laurensis*, in different habitats, may adapt to environmental conditions by constructing different mound shapes. We also investigated the phylogeographic structure of *A. laurensis*. The significant positive correlation between genetic and geographical distances indicated isolation by distance, reflecting restricted dispersal ability of alates. Although the overall genetic structure of *A. laurensis* showed isolation by distance, we also identified two exceptions: (i) secondary contacts of genetically divergent lineages in southern Cape York Peninsula, and (ii) low genetic differences between geographically separated populations of Cape York Peninsula and Arnhem Land. Therefore, the phylogeography of *A. laurensis* may reflect continuous gene flow restricted to short distances and past changes of gene flow associated with the fluctuation of environmental conditions accompanying the changing sea levels in the Quaternary.

Key words: *Amitermes laurensis*, COII, intraspecific phylogeny, mound shape, termite

1. Introduction

The considerable structural diversity of termite nests is associated with differences of social evolution, colony size and feeding habits, as well as the establishment of a microclimate suitable for termites (Abe and Higashi, 2001). In northern Australia, termite mounds are a characteristic feature of the tropical savanna biome. In this region, several species of termites construct mounds of various shapes and size, from small cones to large cathedrals, sometimes reaching 5m in height including the remarkable north-south aligned wedge-shaped mounds built by species in the genus *Amitermes* (Andersen and Jacklyn, 1993).

Termites of genus *Amitermes*, most species of which feed on grass or vegetable debris, are found all over the continent, but are particularly abundant in northern and western Australia. They are the largest Australian genus with 58 described species. Six *Amitermes* species are known to build mounds, while most have either unknown nesting habits or have subterranean colonies (Smith et al., 1998). One of the most interesting and well known of these mounds is the tall wedge-shaped mound, with an elongated axis that has a striking north-south orientation (referred to hereafter as meridional mounds). The meridional termite mounds in seasonally flooded habitats in northern Australia are constructed by two species; *A. laurensis* and *A. meridionalis* (the latter species constructs only meridional mounds while the former builds more variables mound types). Observational (Spain et al., 1983; Jacklyn, 1991; Korb, 2003) and experimental (Grigg, 1973; Jacklyn, 1992; Jacklyn and Munro, 2002) approaches have been used to study the meridional mounds, mainly to investigate the adaptive values of this unique mound shape.

A. laurensis is widespread in Cape York Peninsula and Arnhem Land, two separated regions in northern Australia, while *A. meridionalis* is restricted to areas south and west of Darwin in the Northern Territory (Watson and Abbey, 1993; Fig. 1). *A. laurensis* constructs meridional mounds on Cape York Peninsula and Arnhem Land in

habitats that are flooded during the austral summer rainy season (known as the 'wet season'). This species is known to construct a diversity of meridional mound types differing in size, degree of elongation and number of buttresses and it also builds smaller dome shaped mounds in the southeastern part of Cape York Peninsula, where the habitats are well-drained (Fig. 2; Spain, 1983; Jacklyn, 1991; Korb, 2003; Fig. 2). Such large variation of mound shape within one species is remarkable. Thus, it is sometimes doubted whether *A. laurensis* is a single species that constructs different mounds or a complex of species responsible for the differences in mound shapes (Jacklyn, 1991). Moreover, the relationship between the intraspecific phylogeography and the different mound shapes is interesting from an evolutionary perspective. It is unclear if differences in mound shapes correspond to the intraspecific phylogeny and/or can vary depending on other factors such as environmental conditions.

Termites expand their distribution by dispersal flights of winged reproductives known as alates. In general, alates have poor dispersal ability. Thus, gene flow of termites occurs mainly between nearby locations, and a pattern of genetic isolation with increasing distance is expected. Until now, only a few studies have been conducted to assess the genetic structure of termites, and they demonstrated the genetic isolation by distance over macrogeographic scale (Thompson and Hebert, 1998; Goodisman and Crozier, 2002).

Paleogeographical studies have showed large changes in land-sea distributions associated with the wide continental shelf in northern Australia (e.g. Voris, 2000). For example, such eustasy has seen the transformation of a freshwater lake in the late Pleistocene, the so called ‘Lake Carpentaria’, to the current shallow sea that forms the Gulf of Carpentaria (Smart, 1977; Torgersen et al., 1983; Torgersen et al., 1985; Jones and Torgersen, 1988). There is emerging evidence that such marked changes to coastlines and river drainages must have significantly impacted the distribution of organisms in northern Australia throughout the Quaternary glacial cycles. For example,

phylogeographical studies have highlighted the significant effect of historical environmental fluctuations on fresh water taxa (McGuigan et al., 2000; Bruyn et al., 2004). Over the longer time scale of the Quaternary it has been suggested that the biogeographic barrier of Gulf of Carpentalia may have driven the speciation of three Australian grass finches, *Poephilia* (Jennings and Edwards, 2005). The wide and separated distribution of *A. laurensis* renders it an excellent object for a phylogeographic study that may shed light on the effect of historical environmental changes on northern Australian terrestrial taxa with poor dispersal ability.

In this study, we investigated the phylogeography of *A. laurensis* from northern Australia (1) to determine if *A. laurensis* is really a single species despite constructing different mound types, (2) to look at the intraspecific mound shape polymorphism in a phylogenetic context, (3) to examine whether or not the genetic structure of *A. laurensis* shows genetic patterns of isolation by increasing distance, and (4) to evaluate the inference of historical environmental fluctuations on terrestrial organisms in northern Australia.

2. Materials and methods

2. 1. Insect material

Samples were collected from 30 sites covering the range of *A. laurensis* (Fig. 1) from 2002 to 2004. A total of 238 individuals of *A. laurensis* were used for analyses (Table 1). Samples of *A. meridionalis* and *A. vitiosus*, closely related species to *A. laurensis*, were also collected to be used as outgroups. In order to analyze the monophyletic nature of *A. laurensis*, 17 samples of another 15 *Amitermes* species (*A. accinctus*, *A. arcuatus*, *A. borevs*, *A. darwini*, *A. germanus*, *A. gracilis*, *A. inops*, *A. meridionalis*, *A. obtusidaris*, *A. pavidus*, *A. peramatus*, *A. procervs*, *A. scopulus*, *A. vitiosus*, *A. westraliensis*) were obtained from The Australian National Insect Collection

(ANIC). All samples acquired were placed directly into absolute ethanol and stored at 4 °C until DNA extraction.

2.2. Mound Type Classificaiton

We classified three mound types according to their volume, degree of meridional elongation and amount of buttressing and sculpturing.

Strongly meridional mounds (examples 1-4 in Fig. 2, and A-1 and A-2 in Fig. 3) These mounds have a high degree of meridional elongation i.e. the north-south length of the base of the mound exceeds the east-west width by at least a factor of three. Large mounds may have volumes exceeding 1 m^3 and resemble tomb-stones. East-west buttresses can be found on these mounds but they are almost always a small fraction of the length of the north-south axis. These mounds are characterized by finely sculptured features with buttresses and turrets that can taper to widths of a few millimeters.

Roughly meridional mounds (examples 5-8 in Fig. 2, and B-1 and B-2 in Fig. 3) These mounds are meridionally elongated but to a lesser degree: i.e. the north-south length of the base of the mound exceeds the east-west width only slightly - often by less than a factor of two. Sometimes there are many buttress oriented both north-south and east-west and these buttresses may rival the main axis of the mound in size. Sometimes the buttresses appear to have become separated and resemble a cluster of columns. Roughly meridional mounds may resemble tall pyramids (example 5 in Fig. 2 and B-1 in Fig. 3), wedges or clusters of columns. These mounds can attain a large size and may exceed 1 or $2m^3$ in volume. These mounds are also characterized by finely sculptured features with buttresses and turrets that can taper to widths of a few millimeters.

Small dome mounds (examples 9-11 in Fig. 2 and C in Fig. 3) These mounds grows to around 0.5m high and resemble irregular domes with no consistent orientation. These mounds can overlap with very small examples of the meridional mound types in

elongation and volume (a large dome mound may occassionaly be slightly meridionally elongated) however these mounds are quite distinct in other characters: they do not feature buttresses and the sculpturing is coarse - their surfaces are smooth undulations and are never drawn out into thin features.

2. 2. DNA extraction, PCR and direct sequencing

DNA was extracted from the termite head using standard SDS/proteinase K protocol and phenol chloroform extractions (e.g., Sambrook and Russell, 2001). The primers A-tLEU, 5'-ATG GCA GAT TAG TGC AAT GG-3' (forward); and B-tLYS, 5'-GTT TAA GAG ACC AGT ACT TG-3' (reverse) (Liu and Beckenbach, 1992) were used to amplify the cytochrome oxidase II (COII) gene fragments. PCR was performed in a thermal cycler (GeneAmp PCR System 2700, Applied Biosystems, Foster City, CA, USA) under the following conditions: initial denaturation at 95 °C for 1 min; followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 70 °C for 2 min; and final extension at 70 °C for 7 min. The reaction was performed in a 30 μl final volume of the following solutions: 20.35 μl of distilled water, 0.75 U of *TaKaRa Ex Taq* (Takara Shuzo Co., Otsu, Japan), 3 μl of 10 × *Ex Taq* buffer (Mg_2^+ free), 2.4 μl of $MgCl_2$ (25 mM), 3.0 μl of dNTP Mixture (2.5 mM each), 0.3 μl of each primer (50 pM), and 0.5 μl of template DNA.

The PCR products were purified with a High Pure PCR Product Purification Kit (Roche Applied Science, Nonnenwald Penzberg, Germany). Purified products were used as templates for sequencing. Sequencing reactions were performed with ABI BigDye™ Terminators Cycle Sequencing Kit version 3.1 (Applied Biosystems, Foster City, CA, USA) and a GeneAmp 2700 thermal cycler. Electrophoresis and data collection were performed using an ABI PRISM3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. Both strands of the amplified PCR

product were sequenced.

2.3. Sequence alignment and phylogeographic analyses

Sequencher 3.1.1 software (Gene Codes Corp., Ann Arbor, MI, USA) was used to edit individual electropherograms. Nucleotide diversity, π (Nei, 1987), overall and within individual populations, was calculated using DNASP 4.10. (Rozas et al., 2003). There were no indels among the DNA sequences and consensus sequences were aligned manually.

Based on the DNA sequences, intraspecific phylogenetic analyses were performed using maximum likelihood (ML) criteria (Felsenstein, 1981) following the methods of Tsubota et al. (2004). Prior to phylogenetic reconstruction, MrModeltest 2.2 (Nylander, 2004) was implemented in hierarchical likelihood ratio tests and the Akaike information criterion (AIC; Akaike, 1974) to make a rational decision regarding the nucleotide-based substitution model that best fitted our data, and the best model was used for some adaptable analyses and AU test in the final stage of the analysis scheme. Phylogenetic trees were constructed using the following six program packages to obtain the candidate topologies: (1) MEGA 3.1 (Kumar et al., 2004) with neighbor-joining (NJ) method (Saitou and Nei, 1987) using TN93 model (Tamura and Nei, 1993) with a gamma distribution for rates among sites; (2) PAUP*4b10 (Swofford, 2002) with maximum parsimony (MP) method (Fitch, 1971) using a heuristic search of 1,000 random addition analyses with tree bisection-reconnection (TBR) branch-swapping under the assumption of weighting transversions at 2; (3) PAUPRat (Sikes and Lews, 2001) over PAUP* with MP method to implement Parsimony Ratchet searches (Nixon, 1999) using the Parsimony Ratchet search strategy with random weighting of each characters in thirty 200 iteration runs; (4) MOLPHY 2.3b3 (Adachi and Hasegawa, 1996) with the maximum-likelihood (ML) method (Felsenstein, 1981) using HKY85 model (Hasegawa et al., 1985) and TN93

model; (5) PHYML 2.4.4 (Guindon and Gascuel, 2003) with ML method using GTR (REV; Lanave et al. 1984; Tavaré, 1986; Rodriguez et al. 1990) + proportion invariant + gamma (GTR + I + G) model; and (6) MrBayes 3 (Ronquist and Huelsenbeck, 2003) with Bayesian inference (BI) method using GTR + I + G model with 10,000,000 generations. Based on the ML criteria, a likelihood value was re-calculated for each topology obtained by NJ, MP, ML and BI methods using the program packages PAML 3.15 (Yang, 1997; updated March, 2006) with GTR + G model and CONSEL 0.1i (Shimodaira and Hasegawa, 2001; updated Sept. 26, 2005). This involved the calculation of *p*-values of confidence for candidate topologies with the approximately unbiased (AU) test (Shimodaira, 2002) using the multiscale bootstrap technique to assess the significance of the difference between the likelihood values of the best and the other topologies. A 50% majority-rule condensed tree for the topologies with high ranking log-likelihood values that passed the AU test was also computed by PHYLIP 3.65 (Felsenstein, 1989, 2005). Supporting values (calculated probabilities or the consensus of the resulting topologies) more than 50% were overlaid to assess the robustness of each branch of the condensed topology: local bootstrap probabilities (LBP; Adachi and Hasegawa, 1996) using ML method by MOLPHY with HKY85 model, classical bootstrap probabilities (BP; Efron, 1979; Felsenstein, 1985) based on 10,000 replications using NJ method by MEGA, Bayesian posterior probabilities by MrBayes (PPB) and CONSEL (PPC), and the values of percentage of supported topologies with high ranking log-likelihood values that passed the AU test (AU) at 0.05 significance level are shown on or near each branch (LBP/BP/*PPB*/PPC/**AU**; in %). Local bootstrap probability is a relative bootstrap frequency obtained from a topology search by local rearrangements of MOLPHY. The value is comparable with that of Felsenstein's (1985) bootstrap probability, and is a little larger than the classical bootstrap probability.

To infer the genetic structure of *A. laurensis*, pairwise comparison between

genetic and geographical distances was conducted. When multiple samples with an identical haplotype were found at one site, one representative sample was chosen. On the other hand, when samples that had an identical haplotype were found in different sites, each sample was used. The genetic distance between individuals was estimated following the Kimura's 2-parameter model (Kimura, 1980) with MEGA. The geographical distance was calculated from latitudes and longitudes of sampling sites. The significance of correlation between genetic and geographical distances was assigned by the Mantel test (Mantel, 1967) with 5,000 permutations, calculated using R-package (Casgrain and Legendre, 2000).

3. Results

3. 1. Mound variations

The strongly meridional and roughly meridional mound types shared many characteristics and both types were always found in seasonally waterlogged habitats. They may represent examples from a continuum of mound types found in these habitats. There was a sharp dichotomy, however, between the characteristics of the meridional mound types and those of the small dome type mounds which were always found in well-drained habitats (Fig. 3). Meridional mounds were found from northern part of seasonally flooded habitats and small dome shaped mounds were restricted to southeastern part of well-drained habitats (Fig. 1).

3. 2. DNA sequence variations

PCR amplification yielded a fragment of 662 base pairs of COII gene with no insertions and deletions from 238 individuals (excluding outgroups) of *A. laurensis* collected from 30 sites (Table 1 and Fig. 1). The nucleotide composition was A/T rich (A = 38.5%, T = 26.5, C = 21.4%, G = 13.6%). There were 75 nucleotide sites observed to

vary, 52 of which were parsimony informative, of which 7 (13.5%) and 45 (86.5%) were found at the first and third codon positions, respectively. The overall estimate of nucleotide diversity, π, was 0.0185 with a standard error (SE) of 0.0007. From these sequences, 50 unique haplotypes were obtained, of which 40 haplotypes were unique to one site, while 10 other were shared among several sites. In each site, from 1 to 4 haplolypes were found, and the nucleotide diversity (π) within each population ranged from 0 to 0.0161 (Table1). All sequences of *A. laurensis* were deposited in the DDBJ database under Accession nos. **AB240384** - **AB240435**.

3. 3. Phylogeographical analyses

The results of both methods by MrModeltest indicated the GTR + I + G substitution model as the best fitted model for the dataset. A total of 181 distinct topologies were obtained in the NJ, MP, ML and BI analyses, of which 164 topologies passed the AU test: Fig. 4 shows the consensus tree of these 164 topologies. All the analyses, and all the supporting values (LBP/BP/*PPB*/PPC/**AU**) supported the monophyly of all the haplotypes of *A. laurensis* (supporting values = 99 – 100; Fig. 4). The intraspecific phylogeny of *A. laurensis* produced six robust clades (clades A - F; Fig. 4).

The structure of intraspecific trees did not strongly correspond to the variations in mound shapes. For example, clades B and D contained both meridional and small dome type mounds (Fig. 4). In addition, identical haplotypes were sometimes shared among several populations that built different mound types; haplotypes A-9, A-12, A-13 and E-1 were shared among populations constructing strongly meridional mounds and those constructing roughly meridional mounds (Table 1). However, it is possible that there is some genetic differentiation between meridional and dome mounds types because clades D and F were dominated by small dome mound builders and no small dome mound builders were found in clades A, C and E (Fig. 4).

Fig. 5 shows the geographical distribution of the six clades and the frequency of haplotypes in each sampling site. The haplotypes of clade A were collected from the northern to central part of Cape York Peninsula, clade B from central Cape York Peninsula to the east coast, clades D and F from the southern part of Cape York Peninsula, and clade E was restricted to the northern part of Arnhem Land. Thus, the basic structure of the cladogram demonstrates geographical clustering. However, in southern Cape York Peninsula, the distributions of several clades overlapped, and haplotypes belonging to two different clades were found in one sampling site; Dunbar (DU) and Dorunda (DO-2) had haplotypes belonging to clades C and D, and Maitland Downs (MD) had haplotypes of clades B and D (Fig. 5).

A. laurensis is distributed in two geographically separated regions of Cape York Peninsula and Arnhem Land, but this species is known to be absent between these two regions (Watson and Abbey, 1993; personal observations) probably due to the drier climate and associated distinctive vegetation (Fox et al. 2001). Haplotypes belonging to clade C were found in southern Cape York Peninsula and Arnhem Land, although in each region there was geographical clustering amongst these clades: C-1 to C-6 in southern Cape York Peninsula and C-7 to C-14 in Arnhem Land (Figs. 4 and 5).

3. 4. Correlation between genetic and geographical distances

A pairwise comparison of genetic and geographical distances was conducted using 67 samples of *A. laurensis* that were selected to capture differences amongst mtDNA haplotypes and sampling locations. The scatter diagram of geographical distance against genetic difference of Kimura's 2-parameter distance is shown in Fig. 6. There was a significant correlation between geographical distance and genetic distance (Mantel test, $r = 0.340$, $P < 0.05$, Fig. 6). Thus, the hypothesis of genetic isolation by distance in *A. laurensis* is supported.

4. Discussion

4.1. Phylogeography and mound shape

The phylogenetic analyses showed monophyly in *A. laurensis* (Fig. 4) among 16 *Amitermes* species, suggesting that *A. laurensis* is a single species that constructs different shaped mounds. In the intraspecific phylogenies of *A. laurensis*, we identified 6 distinct clades (Fig. 4). The variation of mound shape in *A. laurensis* did not strongly correspond to the structure of clades.

In three sampling lacations (Maitland Downs (MD), Dunbar (DU) and Dorunda (DO-2)), haplotypes belonging to two different clades were found to occur at the same sampling site (Fig. 5) yet all the mounds had the same shape: small dome mounds were constructed at MD and roughly meridional mounds were constructed at DU and DO-2 (Table 1). These results demonstrate that under the same environmental conditions phylogenetically different groups can construct the same shaped mounds. In addition, four identical haplotypes (A-9, A-12, A-13, E-1) were found in populations that produced strongly meridional mounds (Low Lake, Sandy Creek and Tomkinson River-1) and roughly meridional mounds (Nifold Plain, Orange Plains and Tomkinson River-2) (Table 1). These results suggest that phylogenetically close groups can construct different shaped mounds under different environmental conditions.

A. laurensis is known to build meridional mounds in seasonally flooded habitats, and small dome mounds in well-drained habitats (Spain, 1983; Jacklyn, 1991, 1992; Korb, 2003). It seems one likely explanation of the meridional orientation is the maintenance of mound temperature. Termite mounds are affected by fluctuation of daily temperatures in the dry season, particularly because flooded habitats are low lying landscape features they pond cold air at night during the cool dry season (Jacklyn, 1992; Korb, 2003). Theoretical modeling of solar irradiance and measurement of mound temperatures has demonstrated

the thermo-stability of the eastern face of mounds (Jacklyn, 1992). Consistent with this finding is the observation that termites aggregate on the eastern face in the morning, when ambient temperature is low, during the dry season (Korb, 2003). In contrast, in non-flooded habitats many termites avoid thermal stress by moving to subterranean chambers (Bouillon, 1970; Noirot, 1970). Korb (2003) has also suggested that the elongated shape of *Amitermes* mounds might be an adaptation to flooding during the rainy season and proposed the following hypotheses: elongated mounds with a high ratio of surface to volume, and thin walls create a stable microclimate, by ensuring the rapid drying mounds following soaking rains and thereby, facilitating gas exchange by increasing the porosity of the walls. Thus, the observed variation, of mound shape (that is not phylogenetically constrained) in *A. laurensis* is probably determined by environmental conditions, in particular to whether the habitat is seasonally-flooded or well-drained.

The restricted distribution of small dome mounds to south-eastern part of the Cape York Peninsula (Fig. 1) and geographical clustering of each clade (Fig. 5) make it difficult to deduce the capacity of each clade to build the three types of mounds. The inclusion of some meridional mound builders to clades B, D and F that includes all the dome mound builders (Fig. 4) suggests that each clade, including clades A, C, E, has the capacity to build the three types of mounds. A single population made up of a single haplotype may be able to build both strongly meridional and roughly meridional mounds given that identical haplotypes were found in populations that build both types of mounds. However, it remains uncertain whether colonies that build dome type mounds can build meridional mounds in direct response to changing environmental cues and vice versa or whether this can only occur through natural selection. The evidence for the latter view is the failure to observe populations of the same haplotypes colonizing both waterlogged and well-drained habitats by building both dome mounds and meridional mounds in areas (such as Gamboola) where both mound types and habitats occur (see Table 1).

4. 2. Phylogeography

Assuming a stepping-stone model of population structure where dispersal is limited by distance (Kimura and Weiss, 1964), a positive correlation between genetic and geographical distances suggests that a population has existed for sufficient time to establish equilibrium of genetic drift and gene flow (Hutchison and Templeton, 1999). The dispersal of termites depends on the flight ability of the winged reproductives. Previous studies have reported their poor dispersal ability. In general, alates are especially suited to dispersal in calm air near the ground, and dispersal distances are limited to a few kilometers (Garcia et al., 2002). A few studies have investigated the macrogeographic patterns of the genetic structure of termite populations using DNA markers, and demonstrated positive correlations between genetic and geographical distances (Thompson and Hebert, 1998; Goodisman and Crozier, 2002). The poor dispersal ability of alates and the small fractions of alates that go on to found new colonies were thought to account for these genetic structures. Our demonstration of a positive correlation between genetic and geographical distances (Fig. 6), and geographical clustering of phylogenetic clades (Fig. 5) support the idea of isolation by distance (IBD).

However, two patterns of clade distribution cannot be explained by the IBD model with equilibrium conditions under restricted gene flow due to poor alate dispersal. (1) The wide and divided distribution of clade C. Although there is a biogeographic barrier for *A. laurensis* in the southern Gulf of Carpentaria due to soil dryness, haplotypes belonging to clade C were found in the geographically separated regions of southern Cape York Peninsula and Arnhem Land (Fig. 5). It is implausible that these patterns have arisen due to the migration of alates between southern Cape York Peninsula and Arnhem Land (more than 500 km). Thus, the distribution of clade C is best explained as being due to historical processes rather than as a consequence of contemporary gene flows under the

current environmental conditions. (2) Three sampling sites (Maitland Downs (MD), Dunbar (DU) and Dorunda (DO-2)) at the base of Cape York showed high nucleotide diversity (π) within populations (0.98 – 1.6%; Table 1) where one population had haplotypes belonging to two genetically different clades (Fig. 5). These results also seem to demonstrate the secondary contacts of genetically divergent lineages in the past.

Previous paleogeographical studies have documented that the northern part of Australia, particularly around the Gulf of Carpentaria, underwent large eustasy in the last glacial cycle, and therefore this probably occurred throughout the Quaternary (Smart, 1977; Torgersen et al., 1983; Torgersen et al., 1985; Jones and Torgersen, 1988). These environmental fluctuations undoubtedly had a major impact on the gene flow and historical distributions of the organisms distributed over northern Australia and the pattern of gene flow between populations. During the Quaternary, the sea level of the Gulf Carpentaria fell to form a freshwater lake (Smart, 1977; Torgersen et al., 1983; Torgersen et al., 1985; Jones and Torgersen, 1988). Intraspecific phylogeographical studies have demonstrated that this environmental fluctuation changed the direction and amount of gene flow of the taxa distributed in this region. A phylogeographic study of freshwater fish *Melanotaenia* collected from Australia and New Guinea was performed based on mtDNA sequences (McGuigan et al., 2000). They showed that fish from southern New Guinea and northern Australia form monophyletic clades. Bruyn et al. (2004) investigated the intraspecific phylogeography of giant freshwater prawns (*Macrobrachium rosenbergii*) collected from northern Australia, New Guinea and Irian Jaya. In that study, specimens collected from Australian rivers that discharge into the Gulf of Carpentaria formed a monophyletic clade. These results suggested that the fresh to brackish water of Lake Carpentaria, formed approximately 80000 - 8500 years ago, provided a habitat for fresh water taxa, and acted as a conduit for gene flow.

The exposure of the continental shelf with falling sea levels could have changed

the environmental conditions of the terrestrial area, and also changed the historical distribution of terrestrial taxa. Formation of the lake Carpentaria as a result of marine regression would have provided continuous moist habitats for *A. laurensis* between Arnhem land and Cape York Peninsula. The remarkable distribution of clade C suggests significant changes in the amount and direction of gene flow between populations of *A. laurensis* around the Gulf of Carpentaria/Lake Carpentaria accompanied the changing coastlines. However, it must be admitted that the palaeo-ecology of the habitats that surrounded Lake Carpentaria is unknown.

Our work provides an initial framework for the phylogeography of terrestrial taxa in northern Australia. It is to be expected that the opening and closing of the Gulf of Carpentaria biogeographic barrier had a profound effect on the historical distribution and gene flow of both terrestrial taxa and aquatic taxa (McGuigan et al., 2000; Bruyn et al., 2004). Elsewhere comparative phylogeographic approaches using multiple genetic markers and several taxa have provided a vital framework for investigating historical geographic events in biogeography (e.g., Bernatchez and Wilson, 1998; Schneider et al., 1998). Subsequent phylogeographic analyses of *A. laurensis* using other genetic markers (e.g., sequence data of other mitochondrial genes and nuclear genes, AFLP data) and other widespread taxa are needed to more fully understand the historical biogeography of northern Australia.

5. Conclusion

To return to our original questions, we conclude that *Amitermes laurensis* is a species that constructs a range of differently shaped mounds. Mound shape was not closely coupled to intraspecific phylogeny but was consistently related to local environmental conditions showing the remarkable capacity of these termites to modify their mound types to suit these conditions. The mechanism by which this is achieved is

unclear. *A. laurensis* shows genetic patterns of isolation by increasing distance but the spatial pattern of the distribution of mound types suggests that populations have had time to make secondary contact after genetic differentiation. The phylogeography of *Amitermes laurensis* also indicates that its distribution has been affected by eustacy during the Quaternary and therefore *Amitermes laurensis* phylogeography provides insights into the historical biogeography of northern Australia, particularly the opening and closing of the Gulf of Carpentaria biogeographic barrier.

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Figure legends

Figure 1 (a) The distribution of *A. laurensis* and *A. meridionalis* based on Watson and Abbey (1993). (b) Map of North Australia depicting *A. laurensis* sample sites. The symbols denote mound shapes. Squares, triangles and circles show strongly meridional, roughly meridional and small dome shaped mounds, respectively.

Figure 2 Variation of the mound shapes in *A. laurensis*. Based on measurements and observation (P. Jacklyn unpub. data) *A. laurensis* mounds can be classed into two broad types based on mound volume, degree of meridional elongation (based on the ratio of the N-S axis to the E-W axis) and the nature of mound construction. Meridional mounds vary considerably in shape and size as shown by the eight representative mounds (1-8) but the volume of mature mounds usually exceeds 0.5 m^3 and there is almost always some degree of meridional elongation, often to a marked degree. Meridional mounds are also characterized by fine sculptured features often drawn out into thin buttresses. Meridional mounds can be devided into two sub categories: strongly meridional (1 - 4) and roughly reridional (5 - 8) mounds. For detail description see text. Dome mounds (9-11) rarely exceed 0.5 m^3, are not strongly elongated in any particular direction and do not have buttresses.

Figure 3 Mounds of *A. laurensis*. (A) Strongly meridional mounds corresponding to the mound type 2 in Figure 2, showing the long N-S axis (A-1) and short E-W axis (A-2). (B) Roughly meridional mounds corresponding to mound type 5 in Figure 2 (B-1) and type 6 in Figure 2 (B-2). The strongly and roughly meridional mounds occurred in seasonally flooded habitats. (C) Smaller dome mounds corresponding to mound type 11 in Figure 1, being found in well-drained sites.

Figure 4 The intraspecific phylogeny of *A. laurensis* based on COII haplotypes depicted by a 50% majority-rule condensed tree for the topologies with high ranking log-likelihood values that passed the AU test by CONSEL. Supporting values (calculated probabilities or the consensus of the resulting topologies) more than 50% were overlaid (less than 50%, but the most support in each method, were indicated as a plus mark): local bootstrap probabilities (LBP) using ML method by MOLPHY with HKY85 model, classical bootstrap probabilities (BP) based on 10,000 replications using NJ method by MEGA, Bayesian posterior probabilities by MrBayes (PPB) and CONSEL (PPC), and the values of percentage of supported topologies with high ranking log-likelihood values that passed the AU test are shown on or near each branch (LBP/BP/*PPB*/PPC/**AU**; in %). The root is arbitrarily placed on the branch leading to the *A. inops* and *A. perarmatus*. The marks in brackets indicate mound shapes; squares, triangles and circles show strongly meridional, roughly meridional and small dome shaped mound, respectively.

Figure 5 Geographical distribution of six clades and frequency of mitochondrial DNA haplotypes observed in *A. laurensis*. The clades and haplotype names correspond to Table 1 and Fig. 4. The frequency is represented in a pie-chart for each population.

Figure 6 A scatter diagram of the genetic difference in *A. laurensis* (Kimura 2-parameter distance) plotted against geographical distances between sampling locations. Mantel test was used to calculate the significance of the correlation by using 5000 permutations.