Evidence for a complex evolutionary history of mound building in the Australian nasute termites (Nasutitermitinae)

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Termite mounds have intrigued humans for millennia. Despite great interest in their beautiful and often complex structures, the question of why termites acquired mound-building behaviour has received little attention. Here, we focus on two Australian lineages of the Nasutitermitinae (composed primarily of *Nasutitermes* and *Tumulitermes* spp.), which have evolved mound-building behaviour in parallel from arboreal and soil/wood-nesting ancestors, respectively. We used environmental niche modelling and ancestral niche reconstructions to investigate whether abiotic factors, including precipitation, temperature and soil composition, were associated with the repeated acquisition of mound building. Although we found strong evidence for ecological speciation leading to niche divergence in the nasutes, ultimately no abiotic variable was consistently correlated with mound-building behaviour. We also observed no trend in the variables limiting the environmental tolerances of mound builders. This suggests a more complex evolutionary history of mound building that cannot be explained by the abiotic factors we examined. Instead, biotic factors not considered here, e.g. colony expansion and protection, might have played a key role in the acquisition of this trait.

ADDITIONAL KEYWORDS: Australia – Blattodea – climate change – environmental niche modelling – Isoptera – mound building – niche divergence – termites.

INTRODUCTION

Insect architecture has long been a subject of fascination for humans, and perhaps none more so than the impressive mounds built by termites. Termite mounds, defined as epigean structures that rise from the soil, are common in the savannahs of Africa, Asia, Australia and South America. They are constructed largely by members of the Termitidae, one of most recently evolved and diverse termite families (Bourguignon *et al.*, 2017). They are intricate structures that can include a wide range of architectural elements comparable to those in human buildings, such as domes and cathedral-like spires (Noirot & Darlington, 2000). Termite mounds have been used as sources of medicinal products, mosquito repellent and cleansing ceremonies in Australian Aboriginal societies (Andersen *et al.*, 2005) and as shields for game hunting in African societies (Rennie, 1857). They have also acted as inspiration for 'intelligent' buildings with temperature regulation systems that maintain homeostasis in the face of environmental change (Worall, 2011; Dahl, 2013).

The functional aspects of these structures have been studied in detail, from the biological significance of north–south orientations of 'magnetic' termite mounds, to the optimal construction of bends in tunnels (e.g. Korb, 2003; Korb & Linsenmair, 1999; Turner, 2001; Lee *et al.*, 2007, 2008). Less well understood are the factors that drove the evolution of mound-building behaviour. The evolution of any adaptive trait, including complex traits such as nest building, is thought to be driven by a combination of biotic and abiotic selective pressures and conditions (Theokritoff, 1992). For some organisms, abiotic factors have played a relatively large role in shaping the evolution of a trait. For others, the reverse is true, such as in cases of strong sexual selection. The biotic and abiotic factors influencing the evolution of mound building are of particular interest given that nests built by unrelated species often appear similar based on their exterior structure (Hill, 1942).

The earliest termites probably built their nests in rotting wood (Hill, 1942; Inward *et al.*, 2007). The

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construction of mounds and other nesting strategies (i.e. in soil, or arboreally) have evolved multiple times from this ancestral state (Arab *et al.*, 2017; Lee *et al.*, 2017). The trait of foraging away from the nest was presumably an important preadaptation to the evolution of mound building (Higashi *et al.*, 1991; Inward *et al.*, 2007), Abiotic factors, such as ancient temperature changes and certain types of soil, might have favoured mound building over other nest types. Factors including the underlying geology, annual precipitation and hillslope morphology have been shown to influence mound densities and distributions of savannah-dwelling termites (Davies *et al.*, 2014), although whether they influenced the evolution of mound building in the first place is not known. Multiple biotic factors might also have been important in the development of this trait. These include the benefits of a temperature-controlled environment for development of offspring, areas for the storage of food and immatures, and protection of the colony from predators.

Formal studies examining the role of biotic factors in the evolution of mound building within a phylogenetic framework remain challenging. There is a lack of biotic data available for related mound-building and non-mound-building termite taxa, and data on observable interactions (e.g. direct competition or predation between species) are both rarely encountered and difficult to score statistically. In contrast, investigations into the role of abiotic factors in mound evolution are more feasible owing to the availability of extensive environmental data and the development of ancestral niche reconstruction methods. Lee *et al.* (2017) recently performed the first formal analysis of abiotic factors influencing the evolution of mound building. Their study focused on Australian members of the genus *Coptotermes*, the only group outside the family Termitidae that is known to build conspicuous mounds (Lee *et al.*, 2015). Through ancestral niche reconstructions of eight species of this genus, Lee *et al.* (2017) found that all mound-building taxa examined had significantly different environmental niches and that there was no clear relationship between the abiotic factors considered and the incidence of mound-building behaviour.

A group with a larger number of acquisitions of mound-building behaviour is the Australian lineage of the subfamily Nasutitermitinae, also called snouted termites or 'nasutes' (Hare, 1937). This group comprises 13 mound-building species, including *Nasutitermes triodiae*, whose mounds reach over six metres high and are the tallest in the world (Gay & Calaby, 1970). Nasutes boast an impressive variety of life histories, and in addition to mounds, their nesting habitats include decaying logs, under rocks or in the soil, and tree branches (Hill, 1942).

We have recently performed a phylogenetic analysis of 44 species of Australian nasutes (Arab *et al.*, 2017). Our findings demonstrate that mound building has evolved in parallel from wood- or soil-nesting ancestors on up to eight occasions over the past ~20 Myr, a period in which mesic environments shifted to drier biomes as a result of 'bursts' of sustained aridification interspersed by warm and wet periods (Byrne *et al.*, 2011). The appearance of mound building coincident with aridification raises the possibility that abiotic factors played an important role in the evolution of this nesting behaviour. Alternatively, the overlapping of climate change events with the emergence of this behaviour might be purely coincidental, and biotic or other abiotic factors might instead be responsible for the acquisition of mound building.

These hypotheses can be tested using a framework that considers the phylogeny of the group of interest, per-species distribution data and (modern) climate trends from the study site. Environmental niche models (ENMs) predicting the fundamental niche of each species can be constructed from these data and analysed in ancestral niche reconstructions (ANRs). In the present study, we use the above-mentioned framework to examine whether certain abiotic variables were associated with the evolution of mound building in Australian nasutes. We pair this with the most recent phylogenetic framework for the group from Arab *et al.* (2017). We also test for niche divergence vs. conservatism during the diversification of the Australian nasutes. This study represents the most detailed investigation to date on the potential role played by abiotic factors in the evolution of mound building in termites.

MATERIAL AND METHODS

DATA COLLECTION

Distribution data were sourced from Watson & Abbey (1993), Arab (2015) and the Atlas of Living Australia (2018), summarized in Table 1. Species without adequate distribution data (> 15 occurrence points) were not included. We obtained a dated phylogenetic tree from Arab *et al.* (2017) and produced simplified phylogenies of taxa for both lineages of interest (*Nasutitermes* and *Tumulitermes*; Fig. 1). *Nasutitermes centraliensis* was omitted from our study owing to the incorrect placement of its label in the Arab *et al.* (2017) tree (D. A. Arab, pers. comm.). These two reduced phylogenies were used to represent six independent acquisitions of mound building in total, and analyses were conducted on each tree separately given the independent evolution of the taxa they represent. For simplicity, here we classified the nesting habits
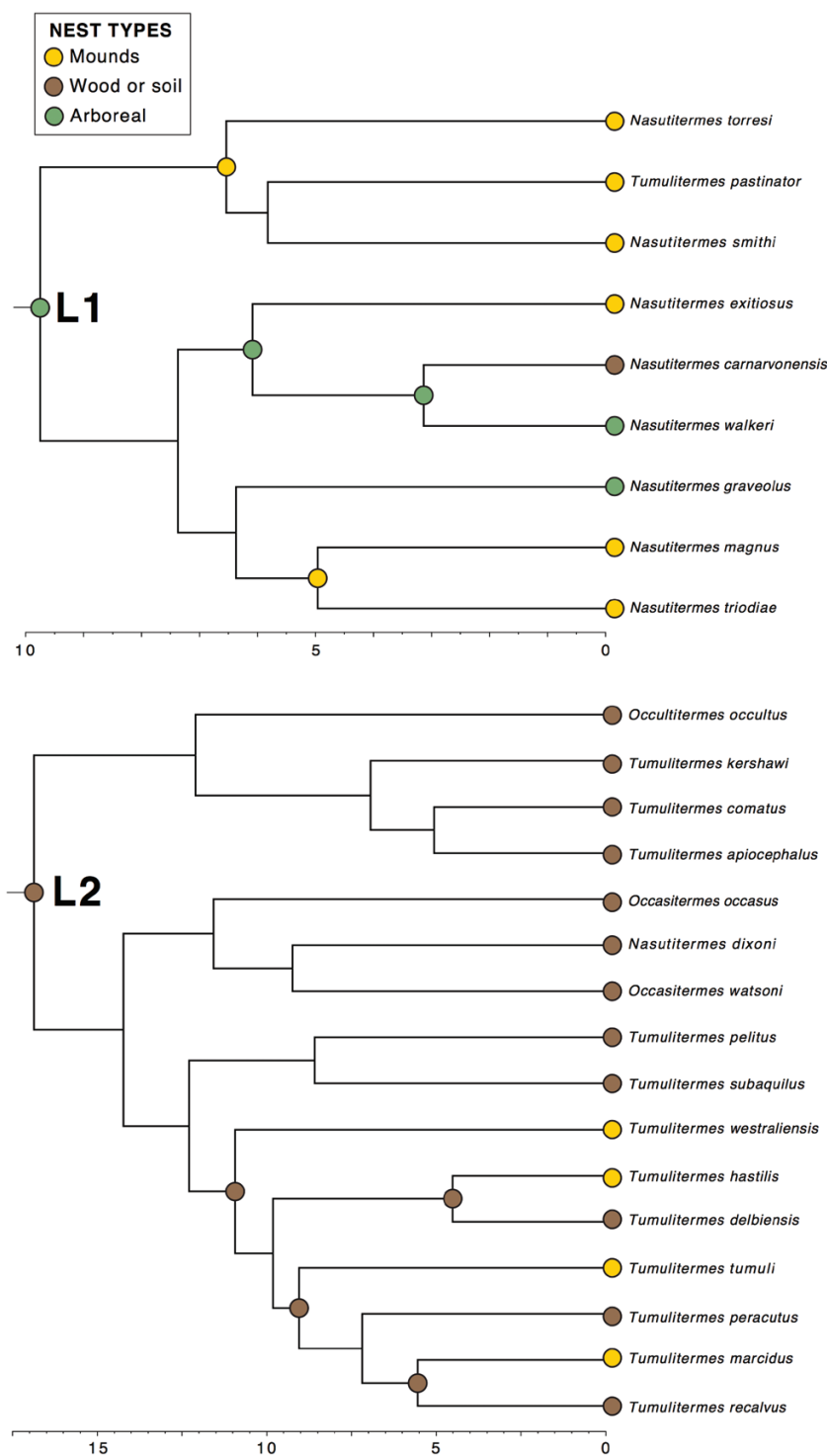
Figure 1. Simplified phylogenetic trees of lineages 1 (L1) and 3 (here coded as L2) from Arab *et al*. (2017). Nesting types are denoted by coloured circles at the tips. Coloured circles at internal nodes are ancestral state reconstructions from Arab *et al*. (2017). The wider nasute phylogeny was estimated using 12S, 16S and *COII* genes using Bayesian inference. Scale bars are in millions of years from the present.

Lineage 1
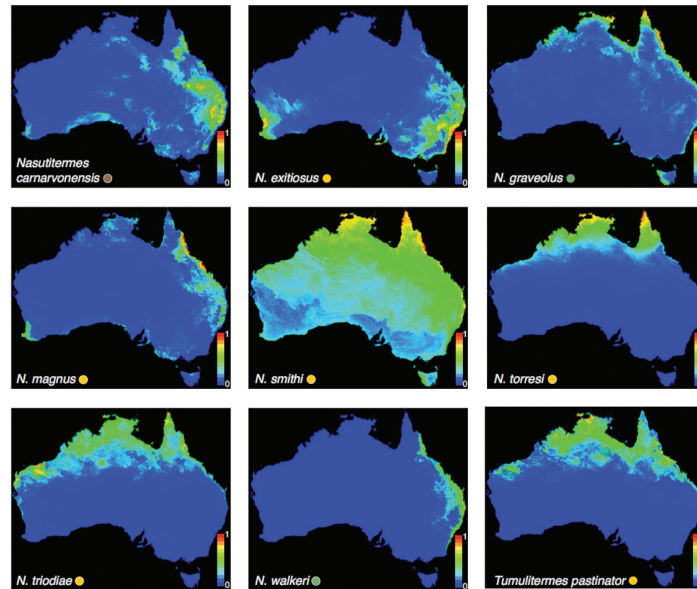
Lineage 2
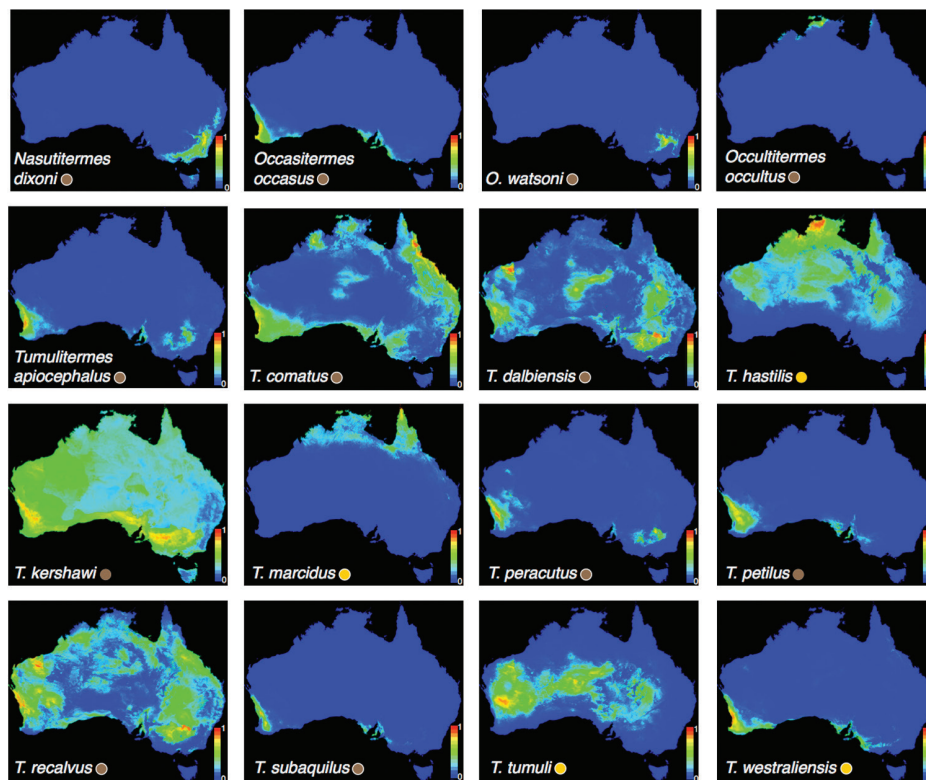
Figure 2. Environmental niche models (ENMs) produced using MaxEnt for the 25 nasute species examined in this study. Environmental niche models were constructed using distribution data and the 11 bioclimatic variables listed in the Supporting Information (Fig. S1). Warmer colours in the heat maps represent a higher probability of occurrence within the constraints of this analysis, whereas cooler colours represent a lower probability of occurrence. The ENMs here represent only the predicted fundamental niches of species as opposed to their realized niches. An interactive map containing the distribution data used to create these ENMs is available in the Supporting Information (Figure S3).

Table 1. The 25 nasute termite species considered in this study, with their taxonomic authorities, total occurrence points used in environmental niche model construction (*N*), nesting types and localities also denoted

Species name and authority	*N*	Nesting type	Locality*†
Lineage 1			
Nasutitermes carnarvonensis (Hill, 1942)	14	Wood or soil	Carnarvon Range (QLD)
Nasutitermes exitiosus (Hill, 1925)	30	Mounds	Southern Australia broadly; SW WA
Nasutitermes graveolus (Hill, 1925)	10	Arboreal	Darwin and Tiwi Islands region of NT; N QLD
Nasutitermes magnus (Froggatt, 1898)	20	Mounds	N QLD
Nasutitermes smithi (Hill, 1942)	13	Mounds	Central Desert northward to Birdum (NT)
Nasutitermes torresi (Hill, 1942)	12	Mounds	N QLD; Thursday Island (Torres Strait)
Nasutitermes triodiae (Hill, 1942)	174	Mounds	N WA; Central Desert northward to Darwin (NT); N QLD
Nasutitermes walkeri (Hill, 1942)	114	Arboreal	SE coast of QLD; E coast of NSW
Tumulitermes pastinator (Hill, 1915)	192	Mounds	Northern Australia generally
Lineage 2			
Nasutitermes dixoni (Hill, 1932)	57	Wood or soil	Mid North and South Coast regions of NSW; VIC broadly
Occasitermes occasus (Silvestri, 1909)	145	Wood or soil	W and SW WA; Eyre Peninsula region of SA
Occultitermes occultus (Hill, 1927)	23	Wood or soil	N NT; Kimberley region of WA; N QLD
Occasitermes watsoni Gay, 1974	17	Wood or soil	Riverina region of NSW
Tumulitermes apiocephalus (Silvestri, 1909)	120	Wood or soil	SW regions of WA; Fitzroy region of QLD; central NSW
Tumulitermes comatus (Hill, 1942)	106	Wood or soil	SW regions of WA; Central Desert region of NT; QLD broadly; Northern Inland and Riverina regions of NSW
Tumulitermes dalbiensis (Hill, 1942)	99	Wood or soil	S WA; Alice Springs and Darwin regions of NT; QLD broadly; Orana and Riverina regions of NSW
Tumulitermes hastilis (Froggatt, 1898)	144	Mounds	Central and northern Australia broadly
Tumulitermes kershawi (Hill, 1942)	12	Wood or soil	Goldfields–Esperance and W WA; Charters Towers (QLD); Angeldool and Binya SF (NSW)
Tumulitermes marcidus (Hill, 1942)	39	Mounds	NW and N QLD, Barcaldine region (QLD)
Tumulitermes peracutus (Hill, 1925)	89	Wood or soil	SW WA; Riverina and Orana regions of NSW
Tumulitermes petilus (Hill, 1942)	46	Wood or soil	SW WA; Eyre Peninsula region of SA
Tumulitermes recalvus (Hill, 1942)	87	Wood or soil	Australian mainland broadly
Tumulitermes subaquilus (Hill, 1942)	35	Wood or soil	SW WA
Tumulitermes tumuli (Froggatt, 1898)	132	Mounds	WA broadly; Central Desert region of NT; Outback region of SA; SW QLD
Tumulitermes westraliensis (Hill, 1921)	57	Mounds	Ngaanyatjarraku Shire and southern WA

Species authorities without brackets represent a reclassification.
Locality abbreviations are as follows: E, east; N, north; NSW, New South Wales; NT, Northern Territory; NW, northwest; QLD, Queensland; S, south; SA, South Australia; SE, southeast; SF, state forest; SW, southwest; VIC, Victoria; W, west; WA, Western Australia.
*Watson & Abbey (1993). †Hill (1942).

of nasutes into three categories: arboreal, dwellers in wood/soil substrates or mound building.

Environmental data were retrieved from the CliMond Archive (Hutchinson *et al.*, 2009; Kriticos *et al.*, 2012) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian Soil Resource Information System (ASRIS, 2011) to a total of 26 raster layers (summarised in Table S1). In all, our chosen rasters pertained to temperature, precipitation, soil composition and plant water capacity at three varying soil depths. Rasters sourced from ASRIS were edited using the *gdalwarp* and *gdal_translate* functions in GDAL v.1.11.5 (GDAL,

2016) to ensure that their file formats and resolutions were consistent with the CliMond data. To ensure the independence of our variables, correlations between them were assessed using Pearson's correlation coefficient in the R v.3.3.3 package *raster* (Hijmans, 2017; R Core Team, 2017). Variables with positive or negative correlations > 75% were discarded using a random number generator (Table S2), and the following were used for the construction of ENMs: BIO1, BIO2, BIO3, BIO8, BIO9, BIO11, BIO12 and BIO17 (sourced from CliMond), soil clay content, soil sand content, available water capacity (AWC) at 0–5 cm and AWC at 100–200 cm (sourced from ASRIS).

ENVIRONMENTAL NICHE MODELLING

Environmental niche models predicting potential distributions for each species were created using MaxEnt v.3.3.3k (Phillips *et al.*, 2006). Default model training settings were used, except that duplicate occurrence points were permitted to account for many of our collection records being termites from different mounds sourced from almost identical locations. Ten jackknife replicates were created for each species, using 75% of the data for training and the remaining 25% as a test dataset to produce probability values. We used area-under-the-curve (AUC) values to assess the model performance of our ENMs. Area-under-the-curve values > 0.5 suggest that the model fits better than one generated at random, and values close to 1.0 indicate that presence points are almost always more informative than random background locations, i.e. a 'perfect' fit (Baldwin, 2009; Lee *et al.*, 2017).

NICHE OVERLAP

Fundamental niche overlap using our ENMs was calculated using ENMTools v.1.0 (Warren *et al.*, 2008, 2010), a program that relies on *D* and *I* statistics as a percentage measure of the similarity between niches. We chose these statistics in combination to compensate for a lack of absence data in our study. The degree of niche overlap as a function of the time since divergence was plotted using the *age.range. correlation* function in the R package *phyloclim* (Heibl & Calenge, 2013). Additional logistic regression analyses were performed in JMP v.13 (JMP, 2016) to evaluate statistically significant differences between realized niches, i.e. our abiotic data paired with our distribution points. In these analyses, abiotic variables were reduced to principal components accounting for ≥ 95% of cumulative variation. The niches of mound builders, arboreal nesters and wood/soil nesters (excluding phylogenetic relationships) were then compared, in addition to all possible sister taxa.

PHYLOGENETIC SIGNAL

Phylogenetic signal, as measured by Blomberg's *K* (Blomberg *et al.*, 2003) and Pagel's λ (Pagel, 1999), was calculated in R using the package *phytools* (Revell, 2012). For both measures, a value close to or greater than one indicates a higher than expected degree of signal under a Brownian motion model of evolution. Evidence of strong phylogenetic signal would suggest that niches had been preserved over evolutionary time, whereas weak signal would suggest niche divergence as a prevailing method of ecological speciation.

ANCESTRAL NICHE RECONSTRUCTIONS

In order to assess whether any abiotic variables are consistently associated with the parallel evolution of mound building, we performed ancestral niche reconstructions (ANRs) on all 26 variables considered in this study. The ANRs were produced using the R package *phyloclim*, following the methods of Evans *et al.* (2009). Ancestral niche reconstructions make use of the mean climatic tolerance for each species per abiotic variable and the 80% central density of tolerance. These values are combined with a phylogenetic framework that is transformed to match the mean tolerance of each species for the abiotic variable of interest. Patterns can then be observed in sister species or clades, e.g. a group sharing the same nesting type might all have elevated tolerances compared with the ancestral state present in their sisters. This would indicate that this variable might have played a role in the evolution of the trait.

RESULTS

ENVIRONMENTAL NICHE MODELLING

Our AUC values were generally high (Table 2). All of the species examined had model performance far better than that computed from random (i.e. uninformative) data, and all but two had AUC values of < 0.80. The exceptions were *Tumulitermes kershawi* and *Nasutitermes smithi*, which also had very broad predicted fundamental niches (Fig. 2). It might be the case that the distribution data for these two taxa were uninformative (i.e. they did not contain a sufficient number of occurrence points) or that they spanned many different niche types, meaning that a more precise distribution could not be resolved by MaxEnt in either case. Niche overlap was variable in both lineages; for example, ranging from $D = 0.09$, $I = 0.30$ (*Tumulitermes pastinator* × *Nasutitermes exitiosus*) to $D = 0.73$, $I = 0.93$ (*T. pastinator* × *Nasutitermes triodiae*) in lineage 1 (Table S3). Our age range correlation plots produced from these niche overlap values show no overall directionality regarding niche overlap significantly increasing or decreasing over evolutionary time (Fig. 3). Subsequent logistic regression analyses found that niches of all possible sister taxa differed significantly from one another, although the degree of difference as measured by the χ^2 statistic was not consistent, nor were the variables influencing these differences. (see also Supporting Information, Tables S4, S5).

The importance of each variable in our ENMs (i.e. the environmental tolerances of each species based on their predicted fundamental niche) was assessed using

Table 2. Area-under-the-curve values for the environmental niche models constructed in this study

Species	AUC value
Lineage 1	
Nasutitermes carnarvonensis	0.904
Nasutitermes exitiosus	0.934
Nasutitermes graveolus	0.977
Nasutitermes magnus	0.918
Nasutitermes smithi	0.802
Nasutitermes torresi	0.964
Nasutitermes triodiae	0.909
Nasutitermes walkeri	0.980
Tumulitermes pastinator	0.914
Lineage 2	
Nasutitermes dixoni	0.978
Occasitermes occasus	0.981
Occultitermes occultus	0.996
Occasitermes watsoni	0.989
Tumulitermes apiocephalus	0.964
Tumulitermes comatus	0.848
Tumulitermes dalbiensis	0.845
Tumulitermes hastilis	0.828
Tumulitermes kershawi	0.747
Tumulitermes marcidus	0.943
Tumulitermes peracutus	0.956
Tumulitermes petilus	0.966
Tumulitermes recalvus	0.782
Tumulitermes subaquilus	0.992
Tumulitermes tumuli	0.902
Tumulitermes westraliensis	0.961

Values have been averaged across the ten replicates of test data for each species.
Abbreviation: AUC, area under the curve.

percentage contribution analyses from the MaxEnt output. There was no clear trend in the types of variables influencing our models (grouped as related to rainfall, temperature, water capacity or soil composition) in lineage 1, although the majority of species in lineage 2 were constrained by temperate-related variables above all others aside from *Tumulitermes comatus* and *Nasutitermes dixoni* (see also Supporting Information, Fig. S1).

PHYLOGENETIC SIGNAL

Statistically significant *K* values were produced for seven variables in lineage 1 and a single variable in lineage 2, but when combined with Pagel's λ no variables showed both high and statistically significant values for the two metrics (see also Supporting Information, Table S6). We therefore observe no strong evidence for phylogenetic signal in either lineage, and this is consistent with a scenario of climatic tolerances not being preserved over evolutionary time (ecological speciation causing niche divergence).

ANCESTRAL NICHE RECONSTRUCTIONS

Ancestral niche reconstructions were performed on internal nodes and the 25 species listed in Table 1 for all abiotic variables (Fig. 4; see also Supporting Information, Fig. S2). For all variables examined, we find no clear grouping of mound-building species with respect to climatic tolerances of any variable, indicating a complex evolutionary history of this trait beyond abiotic influence. Mound builders did not display either a positive or a negative bias of tolerance compared with their sister taxa for any variable, nor did their tolerances group independently of arboreal or wood/soil nesting. Such biases or groupings would suggest some degree of environmental influence on the evolution of mound-building behaviour (Lee *et al.*, 2017), but they were not recovered in our analyses.

DISCUSSION

CLIMATE, SOIL AND THE EVOLUTION OF MOUND-BUILDING BEHAVIOUR

We found no clear evidence that the abiotic variables considered here contributed to the acquisition of mound building, indicating a more complex evolutionary history for this trait than can be explained solely by the factors we examined. There are a variety of additional abiotic factors not considered in this study (e.g. elevation and the presence of water bodies) that might have had a large impact upon determining the distributions of nasutes, and thus on our ability to infer the effect of abiotic factors on mound evolution. Although it would be impossible to account for every abiotic factor shaping the distributions of termites, the incorporation of additional information could improve the accuracy of ENMs inferred for each species.

ROLE OF BIOTIC FACTORS

It might be the case that abiotic factors, no matter how many are considered, are inadequate for resolving any link between the evolution of mound building and external factors. Biotic factors might therefore have been the key selective pressure that led to mound building within the nasutes. Living in a mound affords a colony a suite of benefits, including the ability to support larger population sizes compared with single-piece wood nesting, and the storage of food. A further benefit of mound construction could be enhanced defence from (or less exposure to) predators. Predation pressure
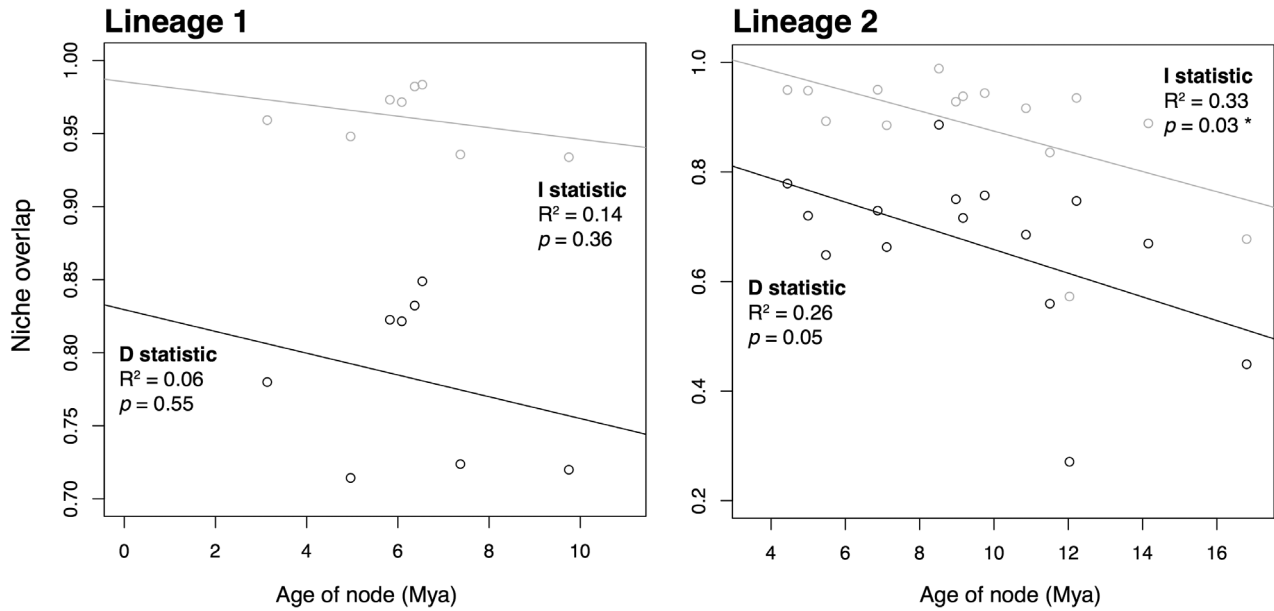
Figure 3. Niche overlap, quantified using *D* and *I* statistics, and median ages of nodes (shown as circles) of the Australian nasute phylogeny shown in Figure 1. The *D* statistic assumes that probability distributions reflect abundance, whereas the *I* statistic does not. This plot was produced using the *phytools* function *age.range.correlation* in R.
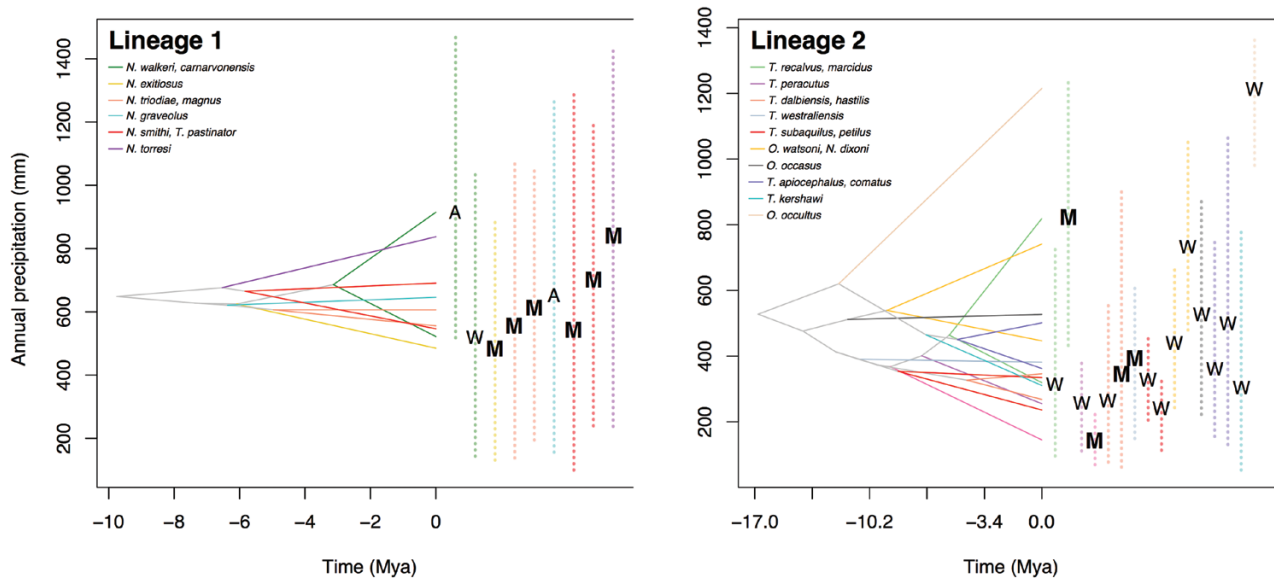
Figure 4. Example ancestral niche reconstructions (ANRs) of Australian nasute lineages 1 and 2 concerning annual precipitation ('BIO12'). We find no evidence for any of the abiotic variables considered here being correlated with the parallel acquisition of mound building. To the left of each panel is the same phylogenetic framework presented in Figure 1, but the tip positions have been transformed to reflect the mean climatic tolerance of each species for the abiotic variable on the *y*-axis. The 80% central densities of climatic tolerance are denoted by dotted vertical lines. The nesting types for the species are coded at the tips as arboreal (A), wood/soil/other (W) or mound building (**M**). Species sharing the same coloured branches are sister to one another.

is thought to be ameliorated as a colony becomes less reliant on the soil–nest interface (Abe, 1987).

Foraging away from the nest is also likely to have been a key trait permitting the evolution of mound building.

Within lineage 1, the ancestors of mound builders were arboreal and foraged away from their nests (i.e. separate-piece nesting). In the case of lineage 2, the nesting biology of most non-mound-building

species is not well understood, but most are likely to be separate-piece nesters (Abe, 1987). Intermediate nesters, the precursors to single-piece nesters, notably do not possess a 'true' sterile worker caste that is specialized for foraging. Higashi *et al.* (1991) proposed that workers foraging further away from their nests in intermediate nesting increases colony stability as the nest is consumed less, favouring food–nest separation and the evolution of more specialized foragers ('true' workers) in a cyclical fashion. This might have led to the eventual acquisition of separate-piece nesting.

The transition from one separate-piece nesting strategy to another, as in our lineage 1, might also have increased colony stability. Epigeal mounds might provide more stable habitats than other nest types, enabling termites to persist in harsh environments. For instance, lineage 1 mound-builders, such as *N. triodiae*, *N. smithi* and *T. pastinator*, tend to have larger predicted fundamental niches and are less restricted to areas proximate to the eastern and northern coastlines compared with their tree-dwelling sister species (Fig. 2). Thus, although arboreal nests are likely to offer similar advantages to epigeal mounds, it appears that they are more suited to areas proximate to the coast and do not facilitate colonization of more arid areas. The emergence of mound building might therefore have allowed termites in lineage 1 to colonize a wider variety of niches and/or a larger area of the Australian continent.

Unlike the case for the non-mound-building taxa *Nasutitermes walkeri* and *Nasutitermes graveolus* from lineage 1, non-mound-building species within lineage 2 are collectively found across a wider area of the continent (Fig. 2). The evolution of mound building within lineage 2 therefore does not appear to have been necessary for the colonization of arid habitats by these taxa in Central Australia. At the lineage level, the earlier (~18 Mya) arrival of lineage 2 compared with lineage 1 (~12 Mya) might have permitted its colonization of Central and Southern Australia. This could be attributable to a longer persistence on the continent before a burst of aridification ~15 Mya (Byrne *et al.*, 2011), during which time Australia would have been more forested. The rarity of lineage 1 in these areas could be attributable to competitive exclusion between the two lineages or to the inability of this lineage to colonize Central Australia once arid conditions had spread in this area.

Finally, we find strong evidence for ecological speciation via niche divergence in the Australian nasutes. Our age range correlation plots, logistic regression analyses and phylogenetic signal data all suggest that niches have not been conserved over evolutionary time in any of these species. This is supported by an additional lack of any pattern regarding climatic tolerance in our ANRs. These data suggest that the nasutes were easily able to colonize new niches, which eventually led to cessation of gene flow from their parent lineage, potentially owing to the great variation in nesting modes mentioned previously.

CONCLUSION

Our study is the first of its kind to assess comprehensively the effect of abiotic influences on the evolution of mound building in Australian nasute termites. Our data suggest a complex evolutionary history of this trait that cannot be explained by the abiotic factors we examined alone. Biotic factors are likely to have played a key role in the acquisition of mound building in the Nasutitermitinae. The transition from arboreal nesting to epigeal nesting in our first lineage might have afforded the nasutes the ability to colonize a wider geographical area. We also observe strong evidence for niche divergence in the Australian nasutes, in agreement with the findings of Lee *et al.* (2017). Ultimately, the analysis of additional variables relevant to the biology of termites, such as the availability of nest substrates or biotic interactions with other species, might provide more resolution in future work. An updated classification of termite nesting types might also be necessary to reassess the ancestral states of nest-building behaviour in these insects.

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SUPPORTING INFORMATION

Table S1. Description of environmental variables used in this study. Variables with a BIO prefix are from the BioClim database; soil and plant-related variables are from the ASRIS database. Variables in grey were used to construct environmental niche models. Remaining variables were discarded owing to correlations > 75%.

Table S2. Pearson's correlation coefficient values between all environmental variables used for the construction of environmental niche models in this study. Correlations ≥ 75% are in bold.

Table S3. Degree of niche similarity, as measured by *D* and *I* statistics, between Australian *Nasutitermitinae* lineages 1 and 2. '0' indicates no similarity and '1' indicates that the niches of the two taxa are identical. *D* statistics are shown in the upper triangle and *I* statistics in the lower triangle. Niche models were produced using MaxEnt with the bioclimatic variables described in the Supporting Information (Table S1).

Table S4. Loading matrix for principal components (PCs) 1, 2 and 3 based on 24 climate variables from the BioClim and ASRIS databases. Principal components were included only if they contributed to ≥ 5% variation. Abbreviation: AWC, available water capacity.

Table S5. Effect likelihood-ratio tests for all logistic regression comparisons of niches of all possible sister pairs, indicating the degree of difference between two taxa and the associated χ^2 values of principal components (PCs). Clades were grouped into a single set to facilitate analysis against a single species. There are three degrees of freedom in all cases. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table S6. Phylogenetic signal for all species considered in this study, as measured by Blomberg's *K* and Pagel's λ statistic. *K* values of > 1 and λ values closer to 1 indicate greater than expected 'clumping' of variables with respect to phylogenetic relationships. Bold values have significant *P*-values and indicate evidence of phylogenetic signal. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Figure S1. Average percentage permutation importance of temperature (yellow), rainfall (blue), available water capacity (green) and soil-related variables (orange) to the environmental niche model of each taxon, i.e. how limited the environmental tolerance of the taxon is by a certain variable.

Figure S2. Twenty-three ancestral niche reconstructions used in this study for lineages 1 and 2, respectively. Phylogenetic trees are shown to the left of each panel. Tips of the tree are positioned at the mean climatic tolerance for a given variable on the *y*-axis for each taxon. The 80% central density of climatic tolerance is denoted by a vertical line. Descriptions of variables are listed in the Supporting Information (Table S1). Species abbreviations for lineage 1: *api*, *T. apiocephalus*; *car*, *N. carnarvonensis*; *com*, *T. comatus*; *dix*, *N. dixoni*; *exi*, *N. exitiosus*; *gra*, *N. graveolus*; *mag*, *N. magnus*; *pas*, *T. pastinator*; *smi*, *N. smithi*; *tor*, *N. torresi*; *tri*, *N. triodiae*; *wal*, *N. walkeri*. Species abbreviations for lineage 2: *dal*, *T. dalbiensis*; *has*, *T. hastilis*; *ker*, *T. kershawi*; *mar*, *T. marcidus*; *occ* (in black), *O. occasus*; *occ* (in beige), *O. occultus*; *per*, *T. peracutus*; *pet*, *T. petilus*; *rec*, *T. recalvus*; *sub*, *T. subaquilus*; *tum*, *T. tumuli*; *wat*, *O. watsoni*; *wes*, *T. westraliensis*. The 46 ancestral niche reconstructions are available in PDF format at https://www.dropbox.com/s/yt26w7hb4619bxl/Lineage%201%20ancestral%20niche%20reconstructions. zip?dl=0 (lineage 1 and https://www.dropbox.com/s/da3jnwfog8ip7s4/Lineage%202%20ancestral%20niche%20 reconstructions.zip?dl=0 (lineage 2).

Figure S3. Distribution data for the 25 nasutes species considered in the present study. Points on the maps refer to individual occurrence points used to construct ENMs as per Table 1 in the main text. The 25 maps of our distribution data, one for each species, are available in PNG format at https://www.dropbox.com/s/du4x1c6wj7z0o0f/ Nasute%20distribution%20data.zip?dl=0&file_subpath=%2FLocality+maps+for+supp+material.