Insights into the evolution of ‘magnetic’ termites: mound shape and population genetics

DISSERTATION ZUR ERLANGUNG DES DOKTORGRADES DER
NATURWISSENSCHAFTEN (DR. RER. NAT.) DER FAKULTÄT FÜR BIOLOGIE UND
VORKLINISCHE MEDIZIN DER UNIVERSITÄT REGENSBURG

vorgelegt von
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aus
Hamburg

im Jahr
2014

Das Promotionsgesuch wurde eingereicht am:
16.06.2014

Die Arbeit wurde angeleitet von:
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Unterschrift:

Summary

Termite mounds are among the most impressive structures in the animal kingdom and exhibit a surprisingly great variety of shapes. The mounds of the Australian ‘magnetic’ or ‘compass’ termites (*Amitermes meridionalis*, *A. laurensis* and an unnamed species) with their elongated north-south aligned axis and thus wall or wedge shape differ fundamentally from the (more or less) spheroidal mounds of other termite species. Since they only occur on seasonally-flooded plains, they have an island-like distribution which could lead to a reduced gene flow and significant genetic differentiation of the (sub-) populations.

The present study set out to test several hypotheses about the adaptive value of the ‘magnetic’ termite mounds of *A. meridionalis* using specific manipulations of, among others, the shape (by amending a wedge shape to an approximate sphere) and different measurements: i) is the shape an adaptation to facilitate gas exchange and/or preservation of food stores during the rainy season or ii) is the shape of thermoregulatory significance?

Another aim was to develop specific genetic markers for *A. meridionalis* to investigate the influence of genetic drift on the island-like distributed (sub-) populations and to be able to answer basic population genetic questions of this species.

It turned out that the rainy season and associated damp conditions reduced the quality and quantity of the stored plant material and temporarily also the gas exchange, but a more spheroidal shape had no significant effect on gas exchange or food availability. The core temperatures of manipulated more spheroidal mounds were more stable than those of natural ‘magnetic’ mounds, so the frequently proposed thermostability cannot be the reason for the unusual shape and orientation of ‘magnetic’ termite mounds. However, the natural meridional mounds had significantly higher mean core temperatures than the manipulated more spheroidal mounds in the seasons in which the development of offspring takes place – a factor that could have played an important role in the evolution of ‘magnetic’ termite mounds.

Using specifically developed microsatellites, i.e. neutral genetic markers, it was possible to determine gene flow, genetic differentiation and genetic diversity of nine different subpopulations. Even between close subpopulations, gene flow was already strongly reduced and thus led to an unusually high genetic differentiation. Nevertheless, the genetic diversity even in small or strongly isolated subpopulations was not reduced. The reason seems to lie in the likewise highly differentiated and very long-lived colonies. Since the colonies are passed on to the so-called replacement reproductives and thereby rarely mix with each other, different alleles are lost by chance in the different colonies. This might result in loss of gene diversity within a colony but maintenance of gene diversity at the subpopulation level.

Zusammenfassung

Termitenhügel gehören zu den eindrucksvollsten Gebilden im Tierreich und weisen eine erstaunlich hohe Formenvielfalt auf. Die Hügel der australischen Kompasstermiten (*Amitermes meridionalis*, *A. laurensis* und eine noch unbenannte Art) unterscheiden sich mit ihrer stark verlängerten nord-süd-ausgerichteten Längsachse und damit wand- bzw. keilförmigen Form fundamental von den (mehr oder weniger) sphärischen Hügel anderer Termitenarten. Da sie nur auf saisonal überfluteten Flächen vorkommen, sind sie außerdem inselhaft verteilt, was zu einem reduzierten Genfluss und einer signifikanten genetischen Differenzierung der einzelnen (Sub-) populationen führen könnte.

In der vorliegenden Studie wurden verschiedene Hypothesen zum adaptiven Wert der Kompasshügel von *A. meridionalis* mithilfe von gezielten Manipulationen u. a. der Hügelform (von einer Keilform zu einer Kegelform) und verschiedenen Messungen getestet: i) ist die Form eine Anpassung, um Gasaustausch oder Nahrungsverfügbarkeit während der Regenzeit zu verbessern oder ii) bietet die Form Vorteile im Hinblick auf bestimmte Temperaturparameter?

Darüber hinaus wurden spezifische genetische Marker für *A. meridionalis* entwickelt, um den Einfluss genetischer Drift auf die inselhaft verteilten (Sub-) populationen zu untersuchen und so grundlegende populationsgenetische Fragen zu dieser Art beantworten zu können.

Es zeigte sich, dass die Regenzeit und die damit verbundenen feuchten Bedingungen die Qualität und Quantität des eingelagerten Pflanzenmaterials und auch den Gasaustausch zeitweise verringerten, eine rundere Form jedoch keinen signifikanten Einfluss auf diese Faktoren hatte. Die Kerntemperaturen von manipulierten runderen Hügeln waren stabiler als die von natürlich geformten Kompasshügeln, daher kann die häufig angeführte Temperaturstabilität nicht der Grund für die außergewöhnliche Form und Ausrichtung von

Kompasshügeln sein. Allerdings zeigten die natürlich geformten Kompasshügel signifikant höhere mittlere Kerntemperaturen als die manipulierten runderen Hügel in den Jahreszeiten, in denen die Entwicklung des Nachwuchses stattfindet – ein Faktor, der in der Evolution von Kompasshügeln durchaus eine wichtige Rolle gespielt haben könnte.

Anhand von eigens für diese Art entwickelten Mikrosatelliten, also neutralen genetischen Markern, war es möglich, Genfluss, genetische Differenzierung und genetische Diversität von neun verschiedenen Subpopulationen zu bestimmen. Sogar zwischen nah gelegenen Subpopulationen war der Genfluss bereits stark eingeschränkt und führte so zu einer außergewöhnlich hohen genetischen Differenzierung. Trotzdem war die genetische Diversität auch von kleinen oder stark isolierten Subpopulationen nicht vermindert. Der Grund hierfür scheint in den ebenfalls stark differenzierten und sehr langlebigen Kolonien zu liegen. Da die Kolonien an sog. Ersatzgeschlechtstiere weitervererbt werden und sich dadurch selten untereinander mischen, bleiben durch Zufall verschiedene Allele in den unterschiedlichen Kolonien erhalten. Dieser Umstand kann zwar zu einem Verlust an genetischer Diversität innerhalb einer Kolonie führen, die genetische Diversität innerhalb einer Subpopulation aber erhalten.

List of publications

This thesis incorporates the following manuscripts:

Schmidt A. M., Jacklyn P. & Korb J., 2014: 'Magnetic' termite mounds: is their unique shape an adaptation to facilitate gas exchange and improve food storage? *Insectes Sociaux* 61 (1), 41-49 (**chapter 2**).

Schmidt A. M., Jacklyn P. & Korb J., 2014: The unique shape of 'magnetic' termite mounds: what role does temperature play? Submitted to *Behavioral Ecology* (**chapter 3**).

Author's contributions (chapter 2 and 3): A.M.S. set up the manipulations and sampled the data in the field, computed statistical analyses, created the figures and wrote the text of the articles as a first version; J.K. designed the project, received funding, computed statistical analyses and cowrote the article; P.J. assisted with data sampling and cowrote the article.

Schmidt A. M., Trindl A. & Korb J., 2007: Isolation and characterization of 10 microsatellite loci in the magnetic termite, *Amitermes meridionalis* (Isoptera: Termitidae). *Molecular Ecology Notes*, 7: 1045-1047 (**chapter 4**).

Author's contributions: A.M.S. developed the microsatellites, performed the genetic analyses and wrote the article; J.K. designed the project and received funding; A.T. assisted developing the microsatellites in the lab.

Schmidt A. M., Jacklyn P. & Korb J., 2013: Isolated in an ocean of grass: low levels of gene flow between termite subpopulations. *Molecular Ecology* 22, 2096-2105 (**chapter 5**).

Author's contributions: A.M.S. sampled material, performed microsatellite analyses, computed population genetic statistics, created the figures and wrote the text of the article as a first version; J.K. designed the project, received funding, sampled material and cowrote the article; P.J. sampled material and cowrote the article.

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CHAPTER 1

General introduction

Building as a behavioural trait is widespread throughout the animal kingdom and, although resulting from simple building rules, can yield astonishing architectural structures. Such structures can be divided into three broad categories according to their function: homes, traps and displays. All of these structures are expected to increase the fitness of their builders by increasing survival probability and/or reproductive success (Hansell 2005). Therefore, the building itself can be viewed in two different ways: as manifested behaviour and as an extended phenotype that are both subject to natural selection (Turner 2000b; Hansell 2005).

Protective homes increase the builders' fitness by serving as nursery, protection against predators as well as unfavourable climatic conditions and sometimes food storage. In order to fulfil one or more of these functions simultaneously, home buildings can reach sophisticated complexity. Famous examples for the complex result of such functional adaptations are the nest buildings of a group of social insects: the mounds of termites (reviewed in Korb 2011).

Despite their famousness, only few termite species have been extensively studied in regard to the functional and adaptive significance of their mounds (e.g. *Macrotermes bellicosus* and *M. michaelseni*; reviewed in Korb 2011). These studies showed that “a mature living *Macrotermes* mound is arguably the most complex colonial organism in nature” (Eggleton 2011), owing to its symbiotic relationship with a specialized fungus and the associated structural adaptations to achieve homeostatic conditions. Much less complex, but nonetheless remarkable mounds are built by *Amitermes meridionalis*, one of Australia's ‘magnetic’ termite species (Fig. 1.1; Hill 1942; Gay & Calaby 1970; Duelli & Duelli-Klein 1978).

Figure 1.1

Elongated meridional mounds of *A. meridionalis* in dry (left) and flooded (right) conditions

The study system *Amitermes meridionalis*

Amitermes meridionalis, also called ‘magnetic’, ‘meridional’ or ‘compass termite’, belongs to the family Termitidae in the insect order Blattodea (Inward et al. 2007) and is classified as a separate-piece nester (reviewed in Eggleton 2011). This means that the nesting and feeding substrate are not the same, and material from outside the colony area is needed to construct the nest mounds (Eggleton 2011). The mounds are mostly constructed with grey soil from the surrounding habitat which gives the mounds their typical appearance (Fig 1.1; Hill 1942). Additionally, feeding material is needed to sustain the colony inside the mound. *A. meridionalis* is a debris-feeder and collects dead grass and other vegetable debris that is stored in the outermost cells in the upper half of the mound (Fig. 1.2; Hill 1942).

The internal structure of the epigeous mound is very simple: it consists of a solid core in the lower part of the mound, where most of the inner cells have been back-filled with mound material. The upper and outer areas of the mound consist mainly of cells where food is stored and that are connected by small tunnels, which allow the movement of termites through the mound (Fig. S2.3, supplementary material). There are no specific ventilation structures, underground nest nor a well-defined nursery. Some slightly larger, flat cells which probably serve as nurseries (Hill 1942) are located in the core close to the ground (Fig. S2.3, supplementary material).

Figure 1.2
Food stores in the outermost cells of *A. meridionalis* mounds

Contrary to its simple internal structure is the unique meridional shape and orientation of these mounds: one axis of the mound is highly elongated and invariably aligned north-south. This elongation leads to a thin wedge mound shape which almost resembles a tombstone (Fig. 1.1).

A. meridionalis is endemic to monsoonal northern Australia and restricted to low lying habitats within 120 km of the city Darwin (Fig. 5.1 b; Gay & Calaby 1970; P. Jacklyn, pers. comm.). These habitats are water-logged from December to around May (Jacklyn 2010), because the monsoonal rains during the rainy season (December to March; Taylor & Tulloch 2006) raise the groundwater table above the ground line (Fig.1.1; P. Jacklyn, pers. comm.). Termite colonies inhabiting these seasonally-flooded depressions are viewed as subpopulations of variable sizes that range from only a few mounds to many hundreds (Fig.1.3).

In other seasonally-flooded habitats across northern Australia, at least two other *Amitermes* species (*A. laurensis* and one unnamed *Amitermes* species) also construct meridional mounds (Fig. 1.1; Jacklyn 2010). However, *A. meridionalis* is an obligate meridional mound builder while *A. laurensis* builds meridional mounds only in flooded habitats but small dome-shaped mounds in well-drained areas (Duelli & Duelli-Klein 1978).

Figure 1.3 © Donald Franklin

Field of ‘magnetic’ termite mounds in northern Australia

The spectacular appearance of a ‘magnetic’ termite field (Fig. 1.3) has ever since risen the interest of explorers and researchers. Nowadays, ‘magnetic’ termite mounds are an important tourist attraction of northern Australia and have reached a reputation as local ‘celebrities’ (Jacklyn 2010). Particularly the shape and its orientation have left researchers wondering about the biological significance of ‘magnetic’ termite mounds, because it distinguishes them from all other termite mounds (Fig.1.4; Korb 2003a; Jacklyn 2010; Korb 2011). In order to shed light on the evolution of these mounds, many hypotheses have been advanced and some experimental studies have been undertaken.

Figure 1.4

Mounds of *Nasutitermes triodiae* (left) and *Cubitermes spec.* (right)

The shape and orientation of ‘magnetic’ termite mounds

Hypotheses and ideas

Jack (1897) was one of the first explorers who published his ideas about the unique north-south orientation of ‘magnetic’ termite mounds. According to Jack (1897), the north-south orientation of thin flat mounds creates eastern and western faces which are exposed to high amounts of solar radiation during the day. This intense radiation could enhance the drying of new construction work despite the humid conditions during the rainy season (Jacklyn 2010). Mjöberg (cited in Hill 1942) remarked that the north-south orientation could serve as protection from prevailing winds in these open habitats. The drying of new building work and the protection from winds as explanation for the meridional orientation was doubted by Hill (1942) who was the first to propose a *thermoregulatory* advantage of the north-south orientation. According to Hill (1942), this orientation minimizes the effects of rapid temperature changes on the termite colony. Comparable to other tropical termites that seek refuge in the subterranean nest parts because of excessive day temperatures inside the mound, ‘magnetic’ termites would move to those parts of the mound with the most suitable temperatures at certain times of the day. This idea was elaborated by Gay and Calaby (1970) who stated that, because escaping to underground refuges is not an option for termites in seasonally flooded habitats, a nest shape evolved which resulted in large heated areas during the cooler times of the day (the eastern face in the morning and the western face in the afternoon) while in the hottest time of the day, the mound presents only a small aspect to the sun.

Serventy (1967) was the first to propose a *ventilatory* advantage of the north-south orientation. He suggested that ‘magnetic’ termite mounds were aligned to create thermal gradients within the mound, resulting from the differently heated mound faces. One face would be sunlit and heated up while the other face would be shaded and cool and these temperature gradients could drive convective heat flows across the mound, producing ventilation.

None of these ‘early observers’ (Jacklyn 2010) tested any of his ideas experimentally – they were all derived from either observation or speculation. The first one to study the shape and orientation of ‘magnetic’ termite mounds experimentally was Grigg (1973).

Experimental studies on ‘magnetic’ termite mounds

Grigg (1973) initially measured the temperature of one ‘magnetic’ termite mound, built by *A. laurensis*, in the natural north-south orientation. He showed that this orientation helps the colony to maintain a core temperature plateau around 34 °C for most of the day (10:30 - 17:30h), resulting from the interaction of mound's exposed surface area and solar radiation intensity. Then he experimentally rotated the mound by 90° so its long axis was aligned east-west instead of north-south. By doing so, the mound's core temperature quickly increased up to 40 °C and there was no evidence of a temperature plateau. Because a temperature of 40 °C is supposed to be rather uncomfortable for the termites, he concluded that the meridional orientation of ‘magnetic’ termite mounds reduces temperature variation during the day and aids the thermoregulation of the termite colony.

Grigg (1973) was also the first who focused not only on the meridional orientation of ‘magnetic’ termite mounds. He realized that while the orientation aided thermoregulation, the shape itself was unlikely to do so. A thin flat mound with a wedge shape is much more prone to external temperature fluctuations than a large spheroidal mound with a dome shape. This is due to the surface area: mass ratio and the thermal inertia. A large dome-shaped mound has a low surface area: mass ratio and thus a high thermal inertia. The dome shape leads to very stable core temperatures that are nearly independent of external temperature fluctuations and thermostability is generally seen as a main factor for functional adaptive mound architecture (Korb 2011). In contrast, a wedge-shaped mound has a very high surface area: mass ratio and thus a low thermal inertia (Korb 2003a; Jacklyn 2010). The core temperatures of wedge-shaped mounds are therefore much more dependent on external temperature conditions and generally fluctuate more than core temperatures in spheroidal mounds (Grigg 1973; Jacklyn 1992; Korb 2003b).

Thus, Grigg (1973) proposed a new explanation for the wedge shape of ‘magnetic’ termite mounds that refined Serventy’s (1967) suggestions: the shape could facilitate gas exchange by diffusion through the large mound walls, especially during the rainy season when dampness reduces the porosity of the mound walls and no chimneys or other openings are available to assist ventilation. An orientation that increases irradiance during mid-morning and mid-afternoon would further enhance drying and thus gas exchange.

Similar rotation experiments were conducted by Jacklyn (1992). By rotating mounds of *A. meridionalis* colonies by only 20° east or west, he found that the eastern face of unmanipulated *A. meridionalis* mounds reaches a daily temperature plateau of approximately

30 °C during the dry season that remains stable until the evening. The observed small variation of mound axes' orientation is therefore an adaptive response to local environmental variation (long-term wind speed and shading conditions) to maintain the eastern face plateau ('eastern face plateau hypothesis' hereafter). Concerning the biological significance of the wedge shape, Jacklyn (1992) concluded that meridional mounds might have evolved to enlarge the eastern face surface area and thus, an area with stable temperatures throughout the day. According to Jacklyn (1992), constructing a surface could be an alternative solution to make a simple mound thermo-stable. The thermostability of this surface would be determined by its orientation to the sun, wind and shading conditions. This idea, derived from the eastern face plateau-hypothesis, is the currently accepted hypothesis to explain the evolution of the 'magnetic' termite mound shape.

A more recent study by Korb (2003b) tested the eastern face plateau-hypothesis by determining the concentration of termites inside ten *A. meridionalis* mounds during the dry season with a nondestructive method. As predicted by this hypothesis, termites concentrate on the eastern face of the mounds – but only in the morning. During sunrise and noon, the termite activity does not differ between the eastern and western mound face. These results indicate that it is not the eastern face plateau that is beneficial for the termites, but the morning heating, at least during the dry season. This finding makes sense because the nights during the dry season can become very cold (minimum night temperature during the study: 8.9 °C). Korb (2003b) proposed alternative explanations for the evolution of the elongated mound shape which partly incorporate previous hypotheses by Jack (1897) and Grigg (1973): Elongated mounds with a high surface area : mass ratio and thin walls are better suited to (a) dry the food storage that is located in the outer cells of the mounds (Fig.1.2), (b) facilitate respiratory gas exchange through the porous mound walls, especially when dampness decreases the porosity of the mound walls and (c) increase the stability of wet mounds during the rainy season by enhanced drying. One aim of the current study was therefore to test the first two hypotheses in an experimental setting.

Amitermes meridionalis subpopulations: islands in an ocean of grass

The exclusive occurrence of *A. meridionalis* colonies on seasonally-flooded depressions results in an island-like distribution of the species. Termite colonies inhabiting one depression are viewed as a single subpopulation that is separated from other subpopulations by a seemingly unsuitable ‘habitat matrix’ of savanna woodland with well-drained red soil. The subpopulations can vary in size and degree of isolation and some are very small and/or isolated. The distribution of *A. meridionalis* is particularly interesting, because it differs from other island-like distributions such as metapopulations or patchy populations: while highly dynamic metapopulations are characterized by several local extinction and recolonization events, *A. meridionalis* subpopulations are very stable with low colony turnover (Bowman 2002; Peter Jacklyn unpublished data (but see Table S4.1)). Patchy populations, in contrast, have high rates of dispersal and gene flow. Regarding the poor flying ability of termite alates (Nutting 1969), dispersal between *A. meridionalis* subpopulations might well be limited. In this case, the question arises how such a system can persist over time. However, recent genetic studies of termite populations suggest that dispersal in general might be not that limited (Vargo & Husseneder 2011). Thus, the dispersal abilities and gene flow are yet unclear for *A. meridionalis.*

Negative effects of genetic drift in small or isolated *A. meridionalis* subpopulations?

A small effective population size and high degree of isolation makes subpopulations susceptible to the negative effects of genetic drift (Rousset 2004): (i) an increased probability to go extinct through environmental or demographic stochasticity; (ii) an increased risk of inbreeding, potentially associated with inbreeding depression; and/or (iii) genetic impoverishment (Prugh et al. 2008; Walker et al. 2008; Frankham et al. 2009; Amos et al. 2012). Genetic drift can also result in random fixation of different alleles in different subpopulations independent of natural selection. With restricted gene flow, this will lead to significant genetic differentiation between subpopulations. An influence of genetic drift on the orientation of ‘magnetic’ termite mounds has been postulated to explain the observed variation of mound orientation between termite subpopulations although the currently accepted explanation for the variation is the eastern face plateau hypothesis (see above) (Jacklyn 1992).

Thus, another aim of the current study was to determine possible negative effects of genetic drift in a species that lives under patchy conditions, is lacking metapopulation dynamics and for which dispersal abilities are unclear. Additionally, we wanted to gain first insights into general population genetics of this species, such as gene flow, colony composition and neutral genetic diversity.

Study aims

The aims of this study were twofold:

(1) What is the biological significance of the shape of ‘magnetic’ termite mounds?

    (a) Are the mounds functionally adapted to facilitate gas exchange and/or the preservation of food stores during the rainy season?

    (b) Is the shape of thermoregulatory significance?

(2) Is there a possibility for genetic drift and its (negative) effects on *A. meridionalis’* subpopulations?

(a) Is gene flow between subpopulations restricted and are subpopulations genetically differentiated?

(b) What are the possible consequences of genetic drift for a patchily distributed species lacking metapopulation dynamics?

To explore the first question, ‘magnetic’ termite mounds were manipulated by altering shading conditions or shape (chapter 2 and 3). We measured internal and external temperatures (chapter 3) as well as gas exchange and assessed food quality and quantity in these mounds over several years and seasons (chapter 2). The results were compared with those of unmanipulated control mounds which were matching in size and environmental conditions.

To answer the second question, species-specific neutral genetic markers (microsatellites) were developed as no genetic markers were yet available for *A. meridionalis* (chapter 4). Several microsatellites were employed for the genetic analyses of multiple subpopulations with multiple colonies and individuals. With the help of these markers, it was possible to investigate basic genetic features of colonies, subpopulations and the species itself and to determine the effects of genetic drift on *A. meridionalis* subpopulations (chapter 5).

The results of this study will help to elucidate several questions regarding the evolution of ‘magnetic’ termites in particular and contribute to the knowledge of mound building and patchily distributed species in general.

CHAPTER 2

‘Magnetic’ termite mounds: is their unique shape an adaptation to facilitate gas exchange and improve food storage?

Published in: *Insectes Sociaux* 61 (1), 41-49

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Abstract

Social insects can build impressive nest mounds but the functional significance of their architecture is rarely studied in experiments. The ‘magnetic’ termite mounds of monsoonal northern Australia built by *Amitermes meridionalis* are notable for their elongated wedge shape and north-south axial orientation. We tested whether the shape is an adaptation to facilitate gas exchange and the preservation of food stores by two experimental manipulations of mounds *in situ* covering all seasons. First, mounds were shaded to limit drying after rain and second, mound shape was amended from wedge to (approximate) sphere. Food storage, fungal contamination, and internal CO_2 concentration were unaffected by manipulation, but showed a distinct seasonal dynamic, with storage peaking towards the onset of rains and fungal load towards the end of the rainy season. Internal CO_2 concentrations were subject to a diurnal cycle, but also showed elevation during rains. We propose that one advantage of the wedge shape is the efficient use of building effort to achieve good passive ventilation for the food storage areas.

Introduction

Building behavior in animals is the outcome of natural selection and can result in structures ranging from simple earthen hillocks to the most delicate and complex formations. Building protective homes can increase survival probability, and thus the fitness of their builders: the builders are protected against climatic extremes and predators, they can optimize microclimatic conditions to suit their requirements and they can store food and thus, become less dependent on fluctuations in food availability (Theraulaz et al. 2003; Hansell 2005).

Among the most amazing structures built in nature are the nest mounds of social insects, but studies on the functional significance of termite mounds are rare (but see: Korb & Linsenmair 1998, 1999, 2000; Turner 2000b). The so-called ‘magnetic’ or ‘meridional’ termite mounds of monsoonal northern Australia, constructed by at least three endemic *Amitermes* (Isoptera: Termitidae) species (*Amitermes meridionalis*, *Amitermes laurensis* and one unnamed species), are characteristically meridional with an elongated north-south orientated axis (Jack 1897; Gay & Calaby 1970). This elongation leads to a thin, wedge-like shape with an elliptical cross-section and a large surface to volume ratio, as compared to mounds of other termite species which are generally more or less spheroidal (Grassé 1984). Meridional mounds are built only in low lying habitats that are water-logged during the rainy season.

One of the described ‘magnetic’ termite species is A. *meridionalis*. It is endemic to areas within120 km of the city Darwin and local populations can be comprised of hundreds of mounds (Gay & Calaby 1970; Jacklyn 1991). Despite their popularity and the various hypotheses that have been put forward to account for their distinctive shape, experimental studies on the mounds of ‘magnetic’ termites are rare. These studies concentrated on investigating the variation in north-south orientation, the significance of the north-south orientation per se and the magnetic cues used by the termites to align their mounds (Grigg 1973; Spain et al. 1983; Jacklyn 1991, 1992; Jacklyn & Munro 2002).

Jacklyn (1992) found that the eastern face of *A. meridionalis* mounds reaches a daily temperature plateau of approximately 30 °C during the dry season that remains stable until the evening. Mound axes vary slightly and this is assumed to reflect local irradiance patterns (e.g. differences in shading), optimizing morning face temperatures and producing subsequent stable conditions throughout the day. Concerning the biological significance of the wedge shape, Jacklyn (1992) concluded that meridional mounds might have evolved to enlarge the eastern face surface area and thus, an area with stable temperatures throughout the day. However, if thermostability is the key factor, this explanation leaves open the question as to why ‘magnetic’ termites build mounds in a way that renders them more susceptible to fluctuations of ambient temperature in the first place.

Mound architecture is generally thought to be an adaptation to maintain homeostatic conditions for the termite colony, including thermostability (Korb 2011). Studies of the large epigeal mounds of *Macrotermes bellicosus* and *Macrotermes natalensis* show that the most thermostable mounds are spheroidal with a small surface area to volume ratio which results in internal diurnal temperature fluctuations of only a few degrees (Lüscher 1961; Ruelle 1964; Korb & Linsenmair 1998; Turner 2000a). Grigg (1973) showed that the north-south orientation of the mound axis provides *A. laurensis* colonies with a core temperature plateau around 34 °C during most of the day (10:30-17:30 h), achieved by the interaction between exposed surface area and solar radiation intensity, considering this the main explanation of the wedge shape. In an experimentally east-west rotated mound, the core temperature rose quickly up to 40 °C and no temperature plateau was reached. However, Grigg (1973) argued that a meridional orientation could not explain the evolution of the wedge shape in the first place, as two separate attributes were involved: shape and orientation. Shape could facilitate gas exchange, especially during the rainy season when dampness reduces the porosity of the mound walls and no chimneys or other openings are available to assist ventilation. An orientation that increases irradiance during mid-morning and mid-afternoon would further enhance drying and thus gas exchange.

Korb (2003a) agreed with Grigg (1973) and added another possible explanation for the biological significance of the wedge shape: As the termites cannot leave their mounds and forage during the rainy season when their habitat is water-logged, they depend on plant material stored inside the mounds. Moist conditions, however, are supposed to enhance infection of the plant material by microorganisms such as bacteria and fungi (Holt 1998; Rosengaus et al. 2003). Interestingly, the stores are just beneath the mound surface; hence large surface areas might be selected to improve drying after rain, by irradiation or by wind. This idea is supported by the observation that *A. laurensis* builds meridional mounds only on poorly drained, seasonally flooded areas while small spheroidal mounds are constructed in well-drained areas (Ozeki et al. 2007).

Here, we tested whether the distinctive wedge shape of *A. meridionalis* mounds facilitates gas exchange and/or improves food storage conditions during the rainy season when mound walls become damp and foraging is impossible. If mounds are locally adapted to serve these functions, we expect that a change in the relevant local environmental conditions or an alteration of mound shape will lead to less efficient gas exchange and/or a decline in the quantity or quality of stored food: a more spheroidal mound will have a reduced surface area for gas exchange and/or ventilation of food stores as compared to the volume of living space capable of producing respiratory gases. This could lead to less efficient gas exchange and higher levels of CO_2 inside the mound. Reduced surface to volume ratios are also expected to result in less efficient drying and increased humidity in the food storage area that could lead to an increased microbial infection of the stored food. Food preferences for *A. meridionalis* are unknown, but in general, the presence of pathogens (often measured as ‘microbial load’, Rosengaus et al. 2003) has a negative impact on colony fitness and is actively controlled by the termites (Rosengaus et al. 2011). Since *A. meridionalis* forages and stores dead plant material that is used as the colony’s food supply during the rainy season, we expect a negative effect of high fungal load on the quality of the food stores. The microbial breakdown is also expected to decrease the quantity of available food during the 4 months of rainy season.

Our experiments involved first, manipulating the degree of shading of mounds, an environmental factor proposed to be important for thermoregulation in *A. meridionalis* mounds (Jacklyn 1992). Shading is expected to reduce the drying of mounds by changing irradiance patterns and hence affect gas exchange and food storage. Second, we manipulated mound shape by altering it to a more spheroidal structure. In both experiments, the efficiency of gas exchange was measured by recording CO_2 concentrations, while food quantity and quality were recorded by determining the amount of plant material and its fungal load in standardized samples taken from the mound’s food stores.

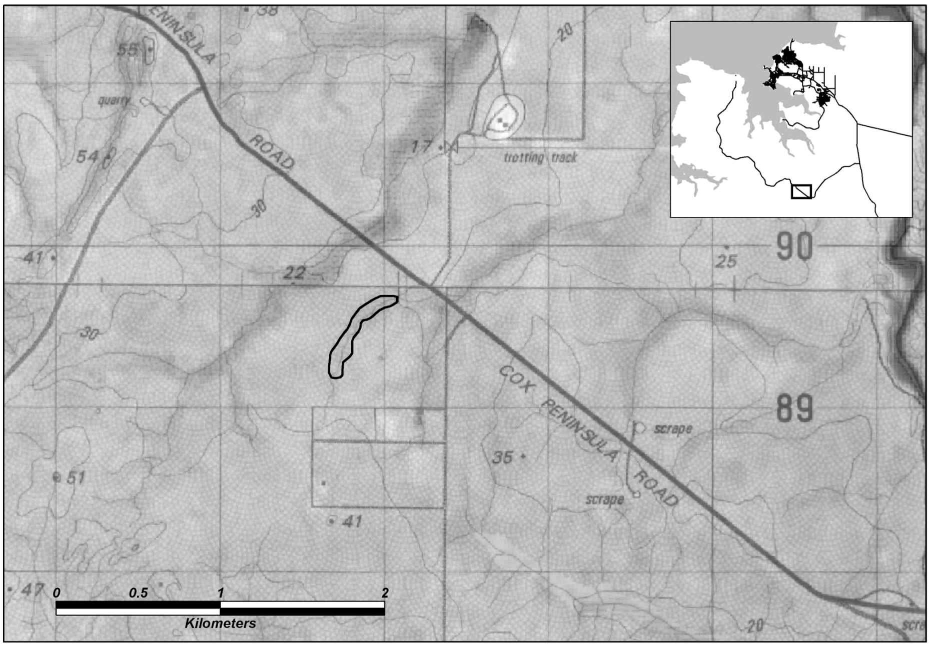

Figure 2.1

Study site in Northern Australia (black outline), west of the Blackmore River on the Cox Peninsula Road (map created by Craig Hempel).

Materials and methods

Study area

The study site was in a typical, seasonally inundated *A. meridionalis* habitat containing several hundred mounds, approximately 30 km southeast of Darwin (12.75° S,130.91° E, approximately 23 m above sea level), Northern Territory, Australia (Fig. 2.1). It was a typical grey soil plain with mainly grassy vegetation (spear grass; *Sarga intrans*) and few trees. The area was regularly burnt during the dry season and grasses reached up to three meters in height during the rainy season. The dry season typically ranges from June to August, while the rainy ('wet') season covers December, January, February and March. The transitional periods with less marked characteristics than the rainy and dry seasons include September, October, November ('dry-wet transition') and April, May ('wet-dry transition', all after Taylor & Tulloch 2006). However, rainfall patterns around Darwin are highly variable at a very small spatial scale (Table S2.1, supplementary material; Taylor & Tulloch 2006). With regard to the scope of this study, the exact amount of daily rainfall on the study site was considered less relevant as all studied mounds were exposed to the same rainfall conditions. The inundation period typically lasted from December until May (A.M.S., pers. obs.).

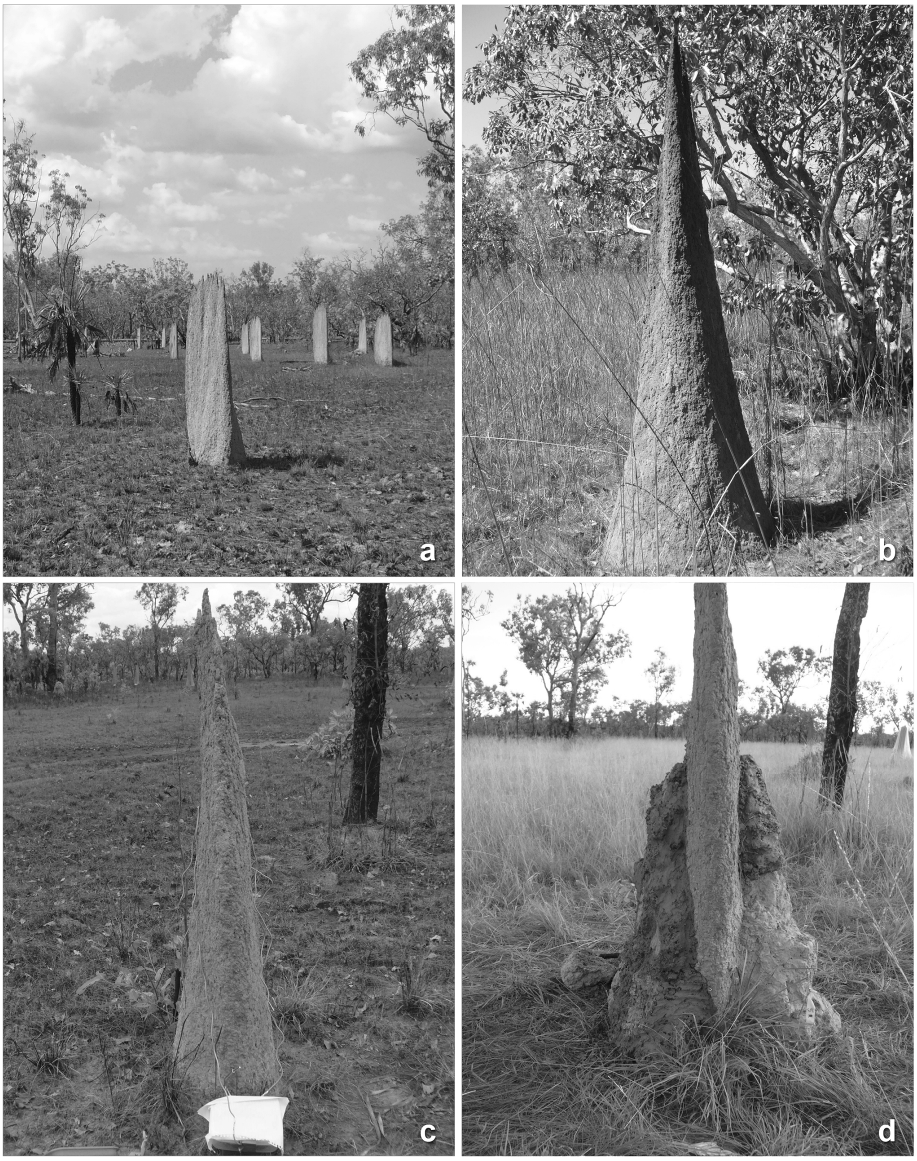

Figure 2.2
'Magnetic' termite mounds of the species *Amitermes meridionalis*. **a** naturally wedge-shaped mounds, **b** naturally spheroidal mound, **c** wedge-shaped mound before shape manipulation, and **d** the same mound as in **c** after manipulation. Note that the shape is now rather cone-shaped and resembles the naturally spheroidal mound.

Experimental manipulations

In September and October 2005, we selected ten sets of three inhabited mounds, each set comprising mounds of similar dimensions in close proximity. Within each set mounds were randomly allocated to one of the following treatments: (a) change in mound shape, (b) shading, and (c) control with no further manipulation:

(a) To change the mound’s shape, we attached pieces of recently abandoned *A. meridionalis* mounds to the eastern and western face of the ten experimental mounds. These pieces did not differ in any obvious way from structures of inhabited mounds, i.e. they had tunnels and cells as those found in inhabited mounds. We used a mixture of water and the soil surrounding the mounds to attach the pieces onto the mounds’ surface. Pieces were attached to the lower part of the mound until the cross-section reached an approximately circular shape and was overall cone- rather than wedge-shaped (Fig. 2.2c, d). The pieces were integrated into the mound structure by the termites (Fig. S2.1, supplementary material). Later, we discovered a naturally spheroidal mound of the same species which had a similar shape as our manipulated mounds (Fig. 2.2b). (b) Ten mounds were shaded with 1.44 m^2 shade cloths made of ultra mesh tarpaulin, commonly used in botanical shading experiments, to simulate natural shade produced by trees or tree branches (Fig. 2.3). We were able to show that the amount of shading was sufficient to reduce mean and maximum core temperatures of these mounds as compared to the control mounds during the rainy season when most measurements were taken [mean $\text{temperature}_{\text{shaded}}$ (±SE) = 29.89 °C ± 0.06; mean $\text{temperature}_{\text{control}}$ = 31.01 °C ± 0.07; maximum $\text{temperature}_{\text{shaded}}$ = 36.39 ± 0.15; maximum $\text{temperature}_{\text{control}}$ = 39.07 ± 0.16]. (c) Ten mounds served as control and were not manipulated but otherwise treated like the manipulated mounds.

Figure 2.3

Shade roofs were used to manipulate the amount of radiation that mounds were receiving from above. The shade roofs were designed to resemble the natural shade produced by tree branches from above.

Measuring food availability

Each mound was sampled once per month in July 2006 (‘dry season’) and January, February, March, September, October, November and December 2007 (‘wet season’ and ‘dry-wet transition’). The plant material is stored in many small cells in the upper part of the mound located beneath the surface. Since older storage cells are often re-filled with solid mound material, it was best to take plant material from areas of recent building activity which are easily recognized by their darker and more friable material and rough surface. A hole-saw adapter with a known volume of 42 ml was drilled in the mound to get a standardized sample size. The samples were taken to the laboratory in sealed plastic bags. In the laboratory, the samples were washed through a sieve with 300 µm mesh to separate the plant material, which was then dried to a constant weight and weighed to obtain a standardized relative estimate of the amount of stored plant material. Using a protocol developed by Rosengaus et al. (2003) to quantify the microbial load in termite nests, 100 mg of the dried plant material was placed in sterile 1.5 ml Eppendorf tubes with 1 ml of 0.1 % Tween 80 suspension medium, then vortexed and centrifuged at 15,000 rpm at 4 °C for 15 min. 10 µl of the supernatant was plated with 50 µl of Tween 80 solution onto a cultural media of Potato Dextrose Agar (PDA) plates with 3 replicates per sample. 50 µl of the Tween 80 solution served as a control (3 replicates). The plates were inverted and incubated at 37 °C for 2 days, when the numbers of colony-forming units (CFU) with at least 1 mm diameter were counted as a measure of fungal load. If the number of CFUs was too high to be counted we repeated the plating with only 2 µl of the supernatant and corrected the values accordingly.

Measuring gas exchange

Internal CO_2 concentrations of all termite mounds were measured once per month every 4 h over a 24 h period in July 2006 and January, February, March, September, October, November and December 2007. Readings were taken at a height of 50 cm and 1 m in mound cells in the centre of the western face. A screwdriver was used to probe the mound until a cell was found. A plastic tube attached to the CO_2 gas analyzer (BINOS 100.1 2M, Emerson Process Management GmbH, Germany) was inserted and the surrounding area sealed with clay and mud. To test the sealing, we exhaled next to the hole and checked for rises of CO_2 concentrations. Only completely sealed holes were measured and the readings were recorded when stable. At the time of measurement the dryness of the measuring point was assessed qualitatively as dry, damp from previous rain, or wet from current rain.

Data analyses

All data were tested for assumptions of parametric analyses and tests were chosen accordingly. All analyses were performed with SPSS 15.0; all tests were two-tailed. If not noted otherwise, mean ± standard errors are given. For the seasonal analyses, we had to pool the data from different years and sorted them by within-year data starting in the dry season and progressing to the rainy season.

Food availability

To describe the general change in food availability with season we used plant quantity and fungal load data from the control mounds and sorted the sampling dates from the middle of the dry season (July) to the end of the rainy season (March) and correlated the standardized amount of stored plant material and the number of CFUs with sampling date. These relationships were not linear; hence, we used the curve estimation procedure in SPSS 15.0 to find the best regression model.

To analyze the number of CFUs for each mound, we averaged the three replicates from each sampling date. Since mounds were analyzed repeatedly, we performed a repeated measure ANOVA with treatment as within- and sampling date as between-subject factor. A corresponding analysis was performed with the amount of stored plant material.

Gas exchange

To analyze the relationship between CO_2 concentrations and time of year we used a curve estimation procedure as implemented in SPSS 15.0. We did not find a significant correlation between CO_2 concentrations and time of year but season had a strong impact on CO_2 concentrations (see ‘Results’). Therefore, we analyzed our experimental data separately for each season (i.e. dry season, dry-wet transition, wet season, wet-dry transition; see above). We then used a linear mixed model analysis for each season to test whether treatment, measuring height and dryness (all fixed factors) and their interactions had an influence on CO_2 concentrations. Time of day (sorted from morning to nighttime) and a sampling ID (number of measurements) were included as covariate and random factor, respectively. To fulfill the requirements of normally distributed residuals, CO_2 concentration values were log-transformed.

Mound drying

To test the impact of shading on the mounds' dampness, we performed Mann-Whitney *U* tests for shaded and unshaded mounds, separately for each sampling date.

Results

Change in food availability with season

The plant material stored in *A. meridionalis* mounds decreased in quantity as well as in quality (measured as the inverse of the fungal load) with progression of the rainy season (Fig. 2.4a). The amount of plant material as estimated from monthly mound samples of control mounds was highest in November and decreased during the rainy season until March. This decrease was not linear due to a comparatively smaller decrease of plant material in March. This might be explained by the increased fungal load of the plant material (Fig. 2.4b) which could limit the ability of the termites to feed on the plant material. During the dry season and the dry-wet transition the amount of plant material increased again. The relationship was best explained by a quadratic regression (R^2 = 0.23, $F_{2,225}$ = 32.65, $P < 0.001$; Fig. 2.4a). Conversely, the platable fungal load of the stored plant material in control mounds increased during the rainy season and was best explained by an exponential regression (R^2 = 0.29, $F_{2,71}$ = 10.76, $P < 0.001$; Fig. 2.4b) and was lowest in September and highest in March.

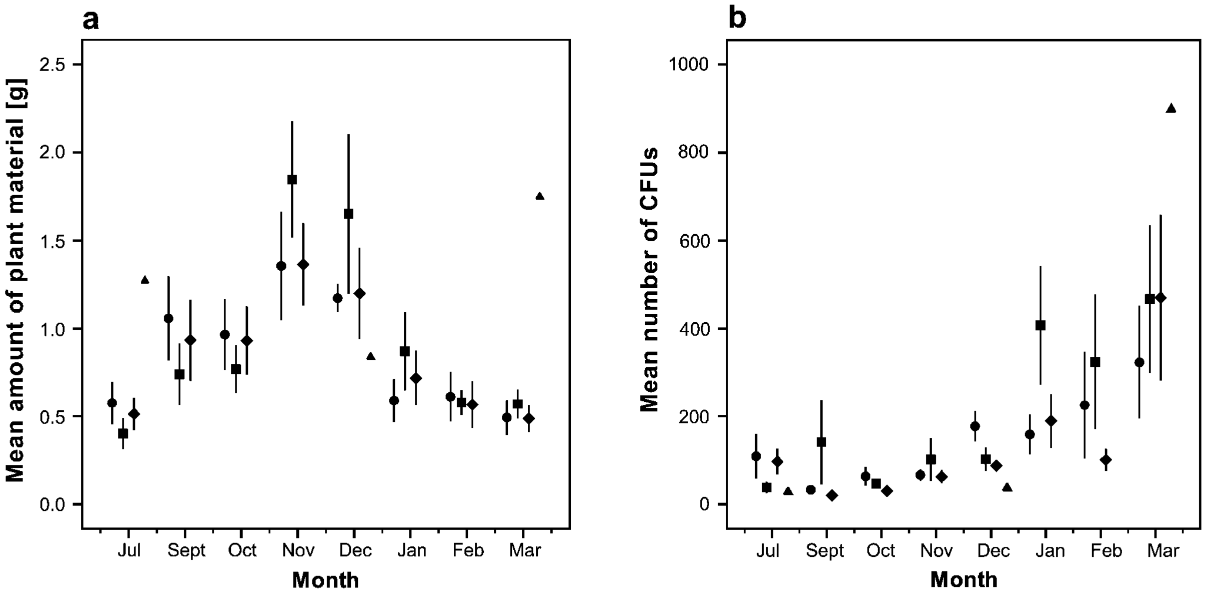

Figure 2.4

Mean amount of **a** plant material and **b** fungal load measured as colony-forming units (CFUs) from monthly samples of more spheroidal (*filled circle*), shaded (*filled square*) and control (*filled diamonds*) mounds and the one naturally spheroidal mound (*filled triangle*). The relationship between amount of plant material and month was significant (Quadratic regression: $R^2 = 0.178$, $P = 0.001$), but differences between treatments were not (Repeated measure ANOVA: mound shape: $F_{1,62} = 0.048$, $P = 0.828$; shading: $F_{1,67} = 0.406$, $P = 0.526$). The number of CFUs and the time of the year correlated significantly (Exponential regression: $R^2 = 0.290$, $P < 0.001$) but differences between treatments were not significant (Repeated-measure ANOVA: mound shape: $F_{1,60} = 0.451$, $P = 0.505$; shading: $F_{1,62} = 1.195$, $P = 0.279$). Note that samples of August are lacking because no sampling was done.

Change in gas exchange with season

CO_2 concentrations in control mounds were generally relatively low (mean of all data: 0.09 ± 0.004 %; Fig. 2.5). They differed significantly between seasons and were highest during the rainy season, lower during the dry-wet transitional period and lowest during the dry season (Linear mixed model: $F_{1,572} = 5.35$; $P = 0.021$; Fig. 2.5). Height had a significant effect on CO_2 concentrations during the rainy season (Linear mixed model: $F_{1,435} = 65.33$; $P < 0.001$; Fig. 2.5). This effect was due to significantly higher CO_2 concentrations at a height of 0.5 m than at 1 m height ($T_{1,377} = -4.50$; $P < 0.001$). During the rainy season, CO_2 concentrations significantly correlated with the time of the day, although the differences were small (Linear mixed model: $F_{1,156} = 8.59$, $P < 0.002$, Fig. S2.2, supplementary material). CO_2 levels were highest at night (0.08 %) and lowest in the morning (around 0.04 %). Interestingly, the effect of the wetness of mound walls on gas exchange was pronounced: during the rainy season, wet and damp control mounds had significantly higher CO_2 levels than dry control mounds (dry: 0.069 ± 0.003 %; damp: 0.082 ± 0.007 %; wet: 0.3 ± 0.03 %; Linear mixed model: $F_{2,365} = 61.37$; $P < 0.001$). After the rain, CO_2 built up very quickly and slowly decreased when mounds were drying.

Manipulation of shading

There was no significant effect of shading on the amount of plant material (Repeated-measure ANOVA: $F_{1,67} = 0.41$, $P = 0.526$; Fig. 2.4a) or fungal load (Repeated-measure ANOVA: $F_{1,62} = 1.20$, $P = 0.279$; Fig. 2.4b). However, in January and February, shaded mounds seemed to have higher fungal loads than control mounds but these differences were not significant due to the high variability between mounds (January: $\text{microbes}_{\text{shaded}}$: 407.4 ± 134.42; $\text{microbes}_{\text{control}}$: 189.6 ± 61.60; February: $\text{microbes}_{\text{shaded}}$: 324.5 ± 153.20; $\text{microbes}_{\text{control}}$: 101.1 ± 24.79; Fig. 2.4b). Although shaded mounds were wetter than control mounds in February (Mann-Whitney U test: $N = 231$; $U = 4021.50$, $P < 0.001$), we did not detect a significant effect of shading on the gas exchange in manipulated mounds (Linear mixed model: $T_{1,390} = -1.52$; $P = 0.130$).

Manipulation of mound shape

Manipulating mound shape did not significantly affect the amount of stored plant material (Repeated-measure ANOVA: $F_{1,62} = 0.05$, $P = 0.828$; Fig. 2.4a) or fungal load (Repeated-measure ANOVA: $F_{1,60} = 0.45$, $P = 0.505$; Fig. 2.4b). In addition, we did not detect significant effects of mound shape on gas exchange in mounds (Linear mixed model: $T_{1,300} = 0.82$; $P = 0.415$; Fig. 2.5).

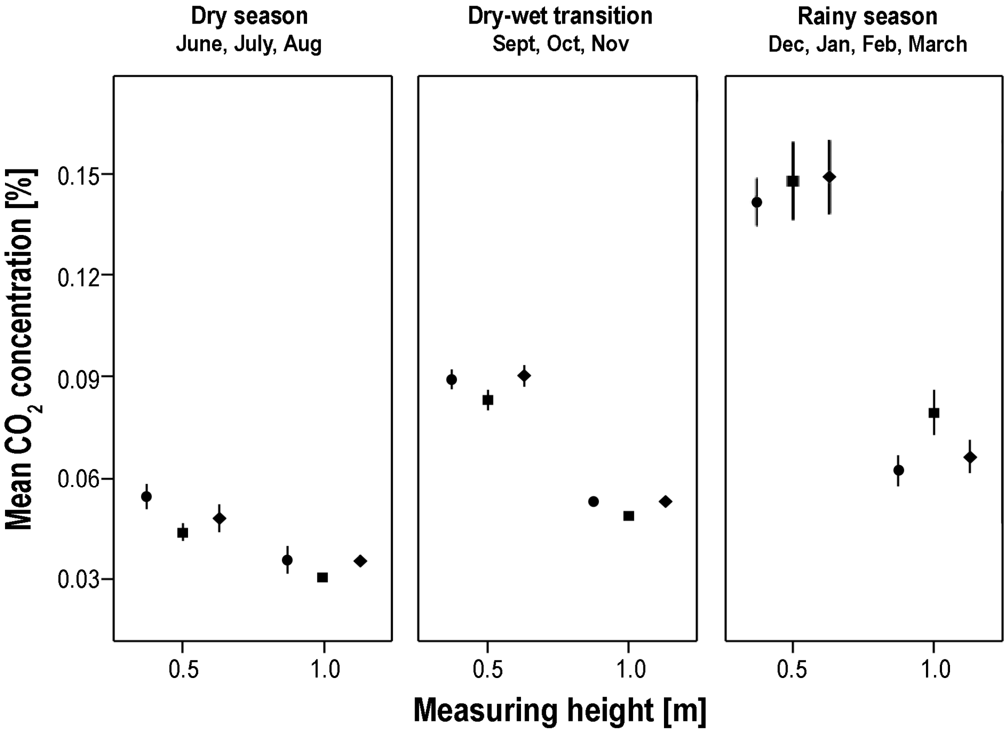

Figure 2.5

Mean concentrations of CO_2 in more spheroidal (*filled circle*), shaded (*filled square*) and control (*filled diamond*) mounds for the two different measuring heights and in the three different seasons. CO_2 concentrations differed significantly between seasons (Linear mixed model: $F_{1,572} = 5.35$, $P = 0.021$) but the two measuring heights differed only during the rainy season (Linear mixed model: $P < 0.001$). Differences between treatments were not significant (mound shape: Linear mixed model: $P_{\text{rainy season}} = 0.415$; $P_{\text{dry season}} = 0.721$; $P_{\text{dry–wet transition}} = 0.481$; shading: Linear mixed model: $P_{\text{rainy season}} = 0.130$; $P_{\text{dry season}} = 0.758$; $P_{\text{dry–wet transition}} = 0.119$).

Discussion

We tested whether *A. meridionalis* mounds are functionally adapted to facilitate gas exchange and/or improve food storage conditions during the rainy season when mound walls become damp and foraging is impossible. Neither changing shading conditions nor altering mound shape revealed a significant large-scale effect although rainy conditions affected both gas exchange and food availability: first, gas exchange was diminished during the rainy season, especially in wet mounds as the mound's pores became water-blocked. Second, food quantity and quality decreased during the rainy season, so food could become a limiting resource. Nevertheless, we did not detect a significant effect of our manipulations on these functions.

Are these findings due to a lack of a real effect of shading and mound shape on mound functioning or are they due to insufficient manipulation to reveal an effect? For shading the latter seems less likely as a similar shading experiment in the fungus-growing termite *M. bellicosus* resulted in the death of the mounds due to decreasing nest temperatures (Korb & Linsenmair 1998). So it seems more probable that shading did not have a large-scale effect on respiratory gas exchange and food storage. Nevertheless, in January and February, we found much higher fungal load on the stored plant samples of shaded mounds than of control mounds but the results were not significant probably due to the high variability in the data.

The finding that altering the mound shape did not affect CO_2 concentrations suggests that gas exchange in general does not seem to be a critical issue for *A. meridionalis* colonies: Compared to CO_2 concentrations in mounds of other termite species such as fungus-growing *Macrotermes* (Lüscher 1955; Darlington et al. 1997; Korb & Linsenmair 2000), the CO_2 concentrations in *A. meridionalis* mounds were very low and increased only temporarily. In addition, gas exchange in *A. meridionalis* seemed to be largely accomplished by passive diffusion through porous mound walls as there are no specific ventilation structures: the inner architecture consists of a solid core in the lower third of the mound, while the upper and outer areas of the mound consist mainly of cells, where food is stored, connected by small tunnels which allow the movement by the termites through the mound (Fig. S2.3, supplementary material). This contrasts strongly with fungus-growing termites where the metabolism of up to a few million termites together with the cultivated fungus requires efficient gas exchange mechanisms (Lüscher 1961; Darlington et al. 1992) and the mounds generally consist of a network of differently shaped tunnels that serve as an efficient means of ventilation (Darlington et al. 1997; Korb & Linsenmair 2000; Turner 2001). But compared to these species, *A. meridionalis* has rather small colonies of a few thousand individuals (Garnett et al., pers. comm.), producing low amounts of respiratory gases for exchange.

However, the necessity of an efficient gas exchange seems not to be dependent on the colony size but closely linked to fungus cultivation: *Nasutitermes exitiosus* and *Nasutitermes triodiae* have colony sizes with 1-2 million individuals but lack fungus cultivation and they do not have ventilation channels in their mounds but have a similar internal structure as *A. meridionalis mounds* (Gay & Calaby 1970). So, we suggest that the importance of gas exchange and the necessity for elaborate ventilation mechanisms are related to the presence of fungus cultivation and are hence less of a problem for *A. meridionalis.*

The finding that altering the mound shape did not affect CO_2 concentrations also argues against the cross-mound ventilation hypothesis proposed by Serventy (1967). He suggested that ‘magnetic’ termite mounds were aligned to create thermal gradients within the mound resulting from the differently heated mound faces. One face would be sunlit and heated up while the other face would be shaded and cool and these temperature gradients could drive convective heat flows across the mound, producing ventilation. Temperature data and analysis of the heating of the faces are needed to support our argument. However, we consider that significant convective heat flows across the mound are unlikely given the dense nature of the inner core (Fig. S2.3, supplementary material).

Our experimental results suggest that a change to a more spheroidal shape does not necessarily lead to impaired mound functioning and the death of an *A. meridionalis* colony. Four out of the ten colonies with a manipulated shape died or where close to death after the experiment, while the corresponding values for control colonies were two out of ten. This difference was not significant (Fisher’s exact test: $P = 0.628$). For shaded mounds like for the control mounds two out of the ten colonies were unhealthy (Fisher’s exact test: $P = 1.000$). Assuming that passive ventilation is used, a more spheroidal mound still has a large surface area to store plant material and to allow for sufficient gas exchange. However, a colony that constructs a large spheroidal mound might waste more effort on building areas that are less useful to store plant material and facilitate gas exchange, as the inner parts of the mound consist of solid re-packed soil material. *A. meridionalis* has only small colonies, therefore, building effort could be a limiting resource which, in the long run, selected elongated mounds over more spheroidal mounds.

Conclusion

We found no large-scale effect on gas exchange or food storage when mounds were manipulated by shading and the change of shape. However, moist conditions significantly affected CO_2 concentration and food quality and our results imply that passive diffusion is the main mode of gas exchange in these mounds. We propose that one advantage of the wedge shape in these mounds is the efficient use of building effort to achieve good passive ventilation for the food storage areas. This study is one of very few that tested the functional significance of mound architecture experimentally in the field. More such studies are needed to test ideas about the function of termite mound architecture.

Acknowledgments

We thank Christine Schauer, Phil Hickey, and Craig Hempel for assistance in the field, the members of the School for Environmental Research at the Charles Darwin University for their logistic support and Environment Australia for permission to do field work. We also thank colleagues and the reviewers for helpful comments on the manuscript. This study was supported by a research grant to J. K. provided by the German Science Foundation (KO 1895/7-1).

CHAPTER 3

The unique shape of ‘magnetic’ termite mounds: what role does temperature play?

Submitted to: Behavioral Ecology

Authorship: Anna Maria Schmidt, Peter Jacklyn, Judith Korb

Abstract

Social insects can build impressive nest mounds but the functional significance of their architecture is rarely studied experimentally. Although mounds display a wide variety of forms, the basic mound structure is always more or less spheroidal. Mounds built by ‘magnetic’ termites are striking as this spheroidal shape is altered to an elongated north-south oriented wedge-shaped architecture. We tested the functional significance of shape on temperature regimes in *A. meridionalis* mounds by comparing experimental, more spheroidal mounds to wedge-shaped control mounds that were matching in size and environmental conditions *in situ* covering all seasons. The core temperatures of more spheroidal mounds were more stable than those of natural elongated mounds implying that thermostability alone cannot explain the peculiar shape of ‘magnetic’ termite mounds. Yet, wedge-shaped mounds had higher mean core temperatures that might be advantageous for offspring development. Hence, our data clearly reject the thermostability hypothesis, but cannot rule out other thermoregulatory advantages. Yet, the fact that ‘magnetic’ termite mounds are only built on seasonally-flooded plains suggests that the reason for this shape and orientation is directly or indirectly linked to flooding.

Introduction

Termite mounds are one of the most impressive extended phenotypes of organisms (Turner 2000b). They are the result of the interactions among many individuals that act according to simple local rules and lack centralized control or blueprints (Korb 2011). They increasingly serve as models of swarm intelligence and are used to inspire new construction technologies (Werfel et al. 2014). Yet, the functional and adaptive significance of the architecture of these self-organized structures has rarely been studied experimentally. Mound architecture varies widely, ranging from simple earthen hillocks to elaborate cathedral-like structures that can reach up to 7 meters in height but the adaptive significance of this variability is largely unknown (reviewed in Korb 2011). Among this structural diversity, mounds built by ‘magnetic’ termites are striking as a spheroidal shape is altered to an elongated north-south oriented wedge-shaped architecture (Fig.1.1). Such otherwise simple mounds are only built by three termite species (*Amitermes meridionalis*, *Amitermes laurensis*, and an unnamed *Amitermes* species) and exclusively occur in low-lying, seasonally-flooded areas of northern Australia (Jack 1897; Gay & Calaby 1970). So what is the functional significance of this extraordinary architecture?

Mounds generally serve as a safe home for the colony that is well protected against predators, provides space for food storage and is less influenced by external environmental conditions. Where studied, the architecture seems to provide homeostatic conditions within the central nest and, depending on species and environmental conditions, ventilation, thermoregulation and humidity control are major influencing factors (reviewed in Korb 2011). Given that ventilation and gas exchange are sufficient (Schmidt et al. 2014), we expect more spheroidal mounds with a small surface area: mass ratio and a high thermal inertia to be selected as they are better insulators against external conditions and hazards than elongated wedge-shaped structures with a high surface area : mass ratio. And this is what we find in termite mounds with the striking exception of the ‘magnetic’ termite mounds. Regarding the biological significance of this elongated wedge-shape, several ventilatory and thermoregulatory hypotheses have been advanced (Korb 2003a; Jacklyn 2010). As one thermoregulatory hypothesis, Jacklyn (1992) proposed that constructing a mound as a flat surface could be an alternative solution that created a thermostable area in a non-massive (high surface area: mass ratio) mound with a simple structure. The thermostability of this surface would be determined by its orientation to the sun, wind and shading conditions. In this study, we aimed at testing the significance of shape on temperature regimes in *A. meridionalis* mounds by comparing experimental, more spheroidal mounds to wedge-shaped control mounds that were matching in size and environmental conditions.

Materials and methods

Study area

The study was done on a typical, seasonally inundated *A. meridionalis* habitat containing several hundred mounds, approximately 30 km southeast of Darwin (12.75° S, 130.91° E, approximately 23 m above sea level), Australia (Fig. 2.1). In this area, the dry season typically ranges from June to August, while the rainy (‘wet’) season covers December, January, February and March. The transitional periods with less marked characteristics than the rainy and dry season include September, October, November (‘dry-wet transition’) and April, May (‘wet-dry transition’) (after Taylor & Tulloch 2006).

Experimental manipulations

In September and October 2005, we selected ten pairs of inhabited mounds, each pair comprising mounds of similar dimensions in close proximity. Within each pair, mounds were allocated by chance to one of the following treatments: (a) change in mound shape (in the following ‘more spheroidal mounds’), and (b) control with no further manipulation: (a) to change the mound’s shape, we attached pieces of recently abandoned *A. meridionalis* mounds to the eastern and western face of the ten experimental mounds. These pieces did not differ in any obvious way from structures of inhabited mounds, i.e. they had tunnels and cells as those found in inhabited mounds. We used a mixture of water and the soil surrounding the mounds to attach the pieces onto the mounds’ surface. Pieces were attached to the lower part of the mound until the cross-section reached an approximately circular shape and the overall shape was cone- rather than wedge-shaped (Fig. 2.2b, d). The pieces were integrated into the mound structure by the termites (Fig. S2.1, supplementary material). Later, we discovered a naturally spheroidal mound of the same species which had a similar shape as our manipulated mounds (Fig. 2.2b). (b) Ten mounds served as control and were not manipulated but otherwise treated like the manipulated mounds.

Temperature monitoring

Temperatures of all mounds were measured at the surface of the northern, eastern and western face (HOBO 08 four-channel loggers; Synotech GmbH, Linnich, Germany (face temperatures) and inside the centre (core temperature) of the mound (HOBO Water Temp Pro loggers). The external sensors were attached in the middle of the surface at one-third of a mounds height as this was the area where termites are often found (Jacklyn 1992) and the internal sensors were installed inside the centre of the mound at the same height. The termites integrated these loggers into their mound structure. To control for microclimatic differences between control mounds and mounds that were to be manipulated, we monitored the temperature of all mounds for about one month before mounds were manipulated and compared the temperature data of manipulated and control mounds during this pre-manipulation time period (see *Data analysis).* The temperatures in the centre of the termite mounds were monitored from October 2005 until end of August 2007. The surface temperatures were measured over most of the study period but due to technical constraints (e.g. battery replacement, burning of the study site) gaps occurred. The most complete data set included all four seasons, starting from April 2006 (dry-wet transition) through to the end of March 2007 (rainy season), this data set was used in the current study.

Data analysis

For the main analyses, we used daily mean, minimum and maximum temperatures as well as the coefficient of variation (CV; standard deviation divided by mean) of daily temperatures (in the following ‘temperature summaries’). We tested the effect of season (as defined by Taylor & Tulloch 2006; see *Study area*) on these temperature summaries using ANOVAs as implemented in SPSS 15.0. As there was a strong seasonal effect on temperatures (P always < 0.001), we performed the following analyses separately for each season. Mounds were measured repeatedly and measuring points (‘direction’, i.e. northern, eastern, western face and core) were ‘nested’ in mounds. To account for this, we used linear mixed models with independent mounds as subject variables and measuring day as covariate so that separate regression models were fitted to each measuring point (Norusis 2005).

First, we analyzed the temperature summaries of unmanipulated wedge-shaped mounds. For this, we used only the control mounds and used ‘direction’ as fixed factor.

Second, we compared control mounds and mounds that were to be manipulated during the pre-manipulation time period to test for microclimatic differences unrelated to mound manipulation. The temperature summaries were used as dependent variables and ‘treatment’ (control, more spheroidal) and ‘direction’ as well as their interactions as fixed factors.

Third, we tested for effects of our treatment. To do this we did the same analysis as described under point 3 but using data of the manipulation period.

Fourth, we did the same ‘treatment’ analyses but separately for the directions ‘eastern face’ and ‘core’ to be able to compare the effects of treatments specifically for single directions.

Results

Temperature patterns of unmanipulated mounds

The core temperatures fluctuated in line with the face temperatures but generally with dampened amplitudes (Fig. S3.1, supplementary material). Core temperatures always had the lowest CVs of all directions, except during the rainy season (Table 3.1, Fig. 3.1).

Comparing temperature variability between faces, the eastern face had the lowest CV compared to all other faces during the dry season ($F_{1,24} = 4449.88$; $P = 0.014$; Fig. 3.1) and the highest compared to all other faces during the rainy season ($F_{1,23} = 6088.13$; $P_{\text{compared to western face}} < 0.001$; Fig. 3.1). During the transitional periods no significant differences were found between different directions (wet-dry transition: $F_{1,13} = 102.94$; $P_{\text{compared to western face}} = 0.245$; dry-wet transition: $F_{1,24} = 14826.02$; $P_{\text{compared to western face}} = 0.067$; Fig. 3.1).

Pre-manipulation comparison

During the pre-manipulation time period, control mounds and mounds that were to be manipulated differed in none of the temperature summaries with the exception of the mean core temperature. By chance, control mound had about 0.3 °C higher mean core temperatures than mounds that were to be changed in shape, respectively ($F_{1,28}=36108.02$; $P_{\text{shape-control}} = 0.004$).

Table 3.1

Influence of mound face and core on temperatures during different seasons. Shown are the results of linear mixed models comparing core temperatures to the different face temperatures (East, North, and West) in unmanipulated control mounds, separately for each season and each daily temperature summary.

Temperature summary	**Results**	**Comparison**	**Dry-wet trans.**	**Rainy season**	**Wet-dry trans.**	**Dry season**
CV of daily temperature	Df		3,3571	3,3579	3,639	3,3259
	F		326.64	79.13	25.83	118.44
		Core vs. East	< 0.001	< 0.001	< 0.001	0.045
	P	Core vs. North	< 0.001	0.058	< 0.001	0.001
		Core vs. West	< 0.001	< 0.001	< 0.001	0.001
Mean daily temperature	Df		3,3570	3,3632	3,651	3,3254
	F		167.40	157.29	4.68	56.43
		Core vs. East	< 0.001	< 0.001	0.874	< 0.001
	P	Core vs. North	< 0.001	< 0.001	0.069	< 0.001
		Core vs. West	< 0.001	< 0.001	0.003	< 0.001
Minimum temperature	Df		3,3569	3,3582	3,644	3,3261
	F		272.91	294.47	15.33	50.00
		Core vs. East	< 0.001	< 0.001	< 0.001	< 0.001
	P	Core vs. North	< 0.001	< 0.001	< 0.001	< 0.001
		Core vs. West	< 0.001	< 0.001	< 0.001	< 0.001
Maximum temperature	Df		3,3569	3,3578	3,655	3,3255
	F		266.08	140.05	43.33	206.27
		Core vs. East	< 0.001	< 0.001	< 0.001	< 0.001
	P	Core vs. North	0.321	< 0.001	< 0.001	0.219
		Core vs. West	< 0.001	< 0.001	< 0.001	< 0.001

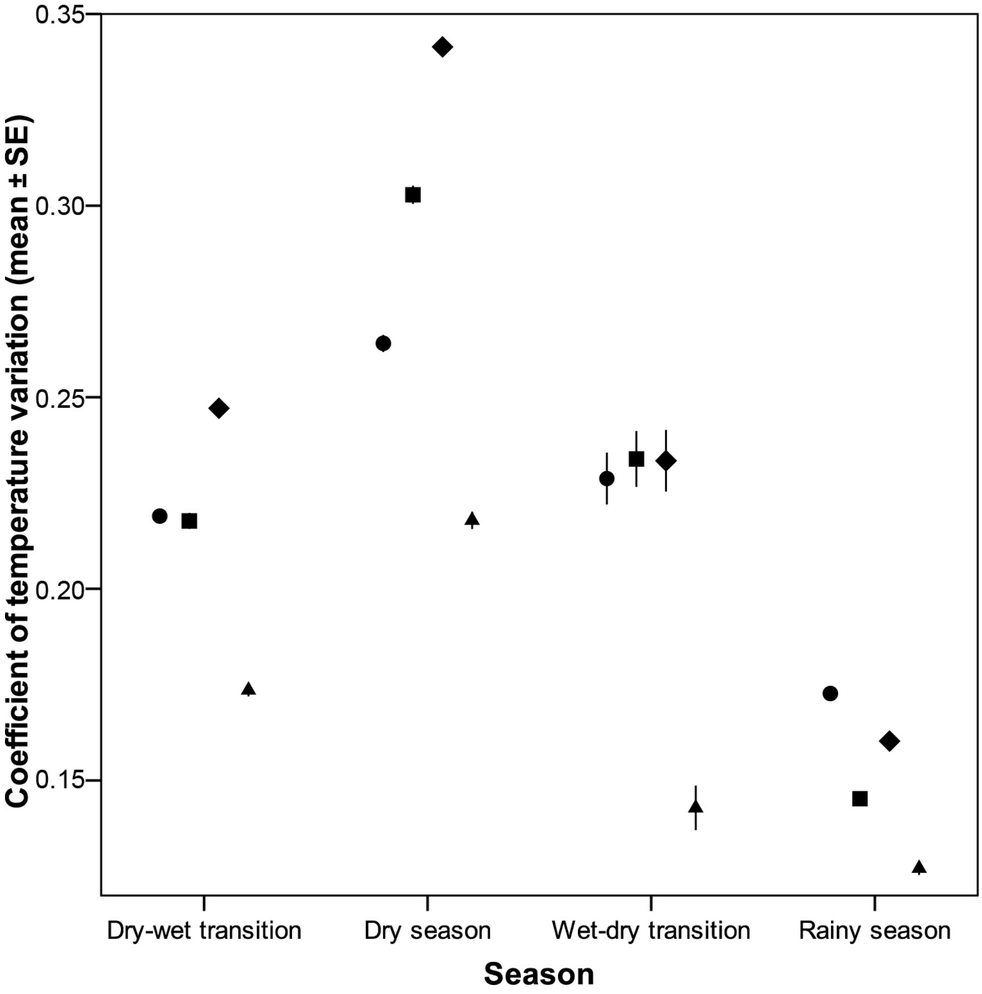

Figure 3.1

Mean (± 1 SE) of the daily temperature variation (measured as coefficient of variation (CV)) of the three directions (*filled circle* = eastern face, *filled square* = northern face, *filled diamond* = western face) and in the mounds' core (*filled triangle*) over the seasons in unmanipulated mounds. CVs of core temperatures were always significantly lower than face temperatures (except during the rainy season, Table 3.1).

Effect of treatments

Effect of a manipulated shape on core temperatures

More spheroidal mounds had always significantly lower CVs of core temperatures than control mounds (except during the wet-dry transition; Table 3.2; Fig. 3.2). The minimum core temperatures of more spheroidal mounds were with 1 to 2 °C significantly higher than those of control mounds during all seasons (except the wet-dry transition; Table 3.2, Fig. 3.3a) and the maximum core temperatures were up to 5 °C lower during all seasons (except the dry season; Table 3.2, Fig. 3.3b). Overall, the mean core temperatures of more spheroidal mounds were lower than those of control mounds during the dry-wet transition and rainy season (Table 3.2) with no significant effect during the other seasons. Although there was a small but significant difference in the mean core temperatures before these mounds were manipulated (see *Pre-manipulation comparison*), the difference was more pronounced after the manipulation (about 0.3 °C before manipulation vs. about 0.7 °C after manipulation).

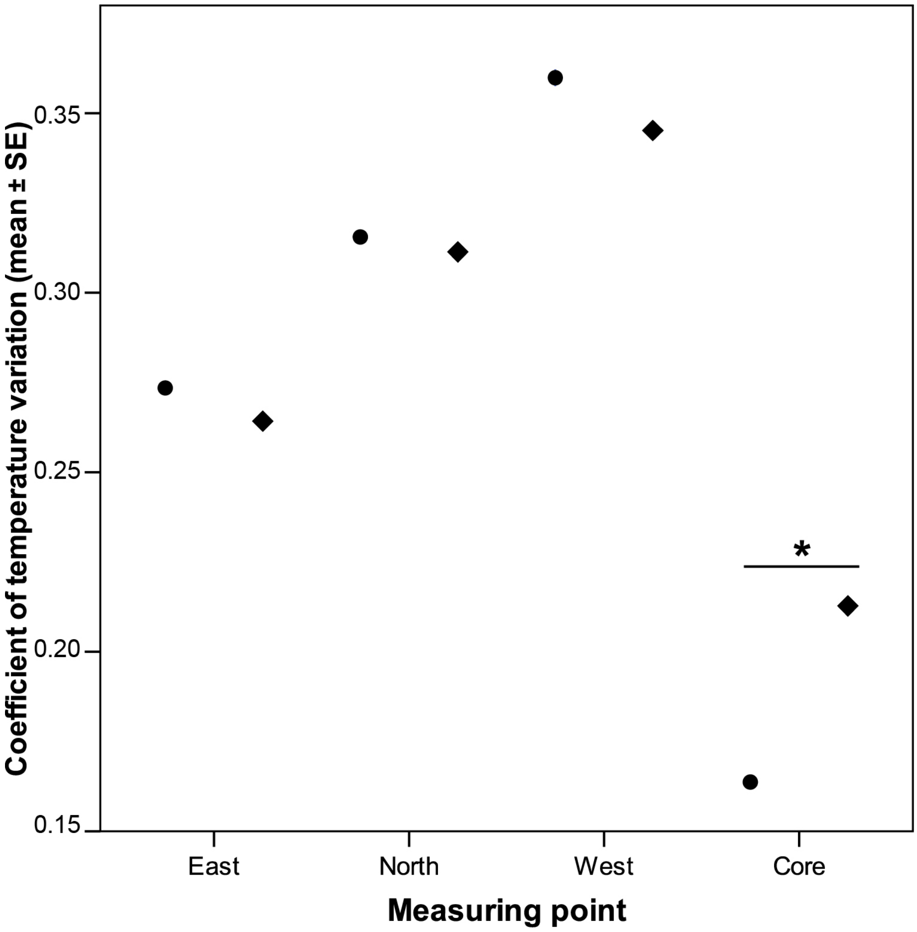

Figure 3.2
Mean (± 1 SE) of the daily coefficient of variation (CV) of the face and core temperatures of experimental mounds during the dry season (*filled circle* = more spheroidal, *filled diamond* = control, *asterisk* = significant difference.)

Table 3.2

Influence of a more spheroidal shape on mound core temperatures. Shown are the results of linear mixed models comparing core temperatures of unmanipulated mounds to core temperatures of shape-manipulated mounds, separately for each season and each daily temperature summary.

Temperature summary	**Results**	**Dry-wet trans.**	**Rainy season**	**Wet-dry trans.**	**Dry season**
CV of daily temperature	Df	2,23	2,27	2,17	2,26
	F	7.76	10.04	0.03	6.09
	P	0.001	< 0.001	0.824	0.017
Mean daily temperature	Df	2,23	2,28	2,18	2,20
	F	2.34	25.34	1.51	2.10
	P	0.046	0.005	0.104	0.118
Minimum temperature	Df	2,37	2,26	2,20	2,22
	F	6.27	6.46	0.54	5.60
	P	0.001	0.015	0.321	0.004
Maximum temperature	Df	2,23	2,25	2,16	2,26
	F	5.82	14.19	4.89	4.92
	P	0.008	< 0.001	0.017	0.101

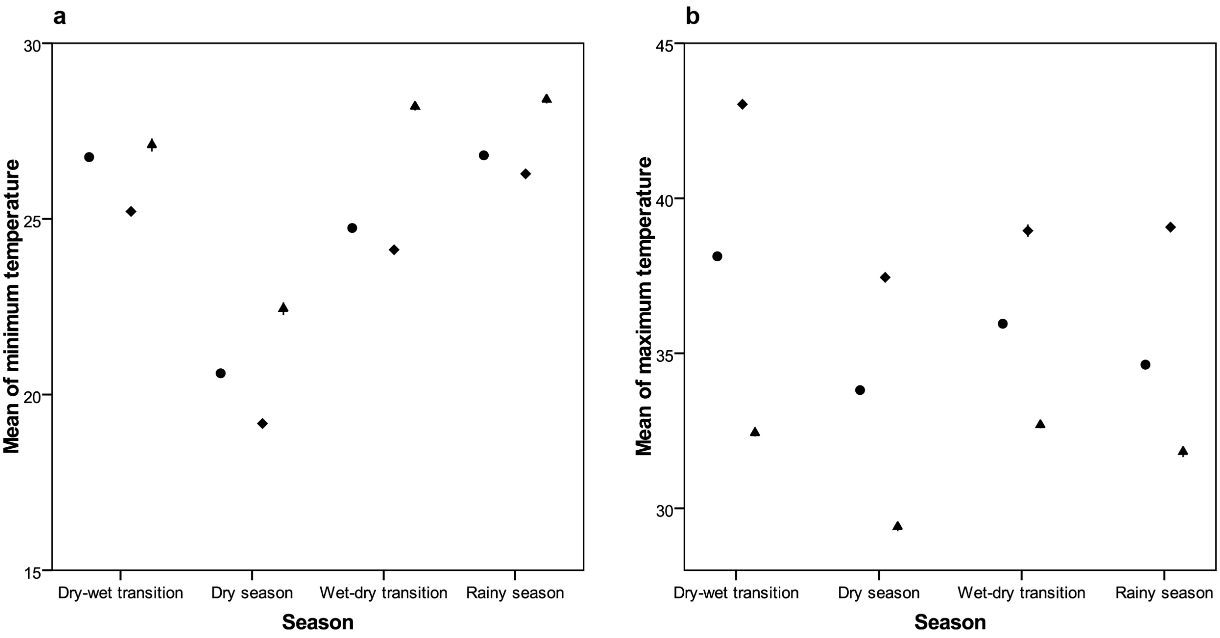

Figure 3.3

Mean (± 1 SE) of **a** daily minimum and **b** daily maximum core temperatures in [°C] of experimental mounds (*filled circle* = more spheroidal, *filled diamond* = control) and a naturally spheroidal mound (*filled triangle*) over the seasons. Only differences between core temperatures of experimental and control mounds were statistically analysed. These differences were always significant except between minimum temperatures during the wet-dry transition and maximum temperatures during the dry season (Table 3.2)

Effect of a manipulated shape on eastern face temperatures

The face temperatures also differed between treatments: The mean eastern face temperatures of more spheroidal mounds were significantly lower than those of control mounds during the rainy season but significantly higher during the dry season (Table 3.3). Additionally, more spheroidal mounds had lower maximum temperatures and lower CV in eastern face temperature than control mounds during the rainy season (Table 3.3).

The daily temperature curves of a representative manipulated and a naturally spheroidal mound during the dry season showed no eastern face plateau as opposed to the daily temperature curve of an unmanipulated mound (Fig.3.4).

Table 3.3

Influence of a more spheroidal shape on eastern face temperatures. Shown are the results of linear mixed models comparing eastern face temperatures of unmanipulated mounds to eastern face temperatures of shape-manipulated mounds, separately for each season and each daily temperature summary.

Temperature summary	Results	Rainy season	Wet-dry trans.	Dry season
CV of daily temperature	Df	2,24		
	F	3.97		
	P	0.027	ns	ns
Mean daily temperature	Df	2,24		2,27
	F	4.90		2.83
	P	0.017	ns	0.034
Maximum temperature	Df	2,26	2,20	
	F	5.26	6.02	
	P	0.003	ns	ns

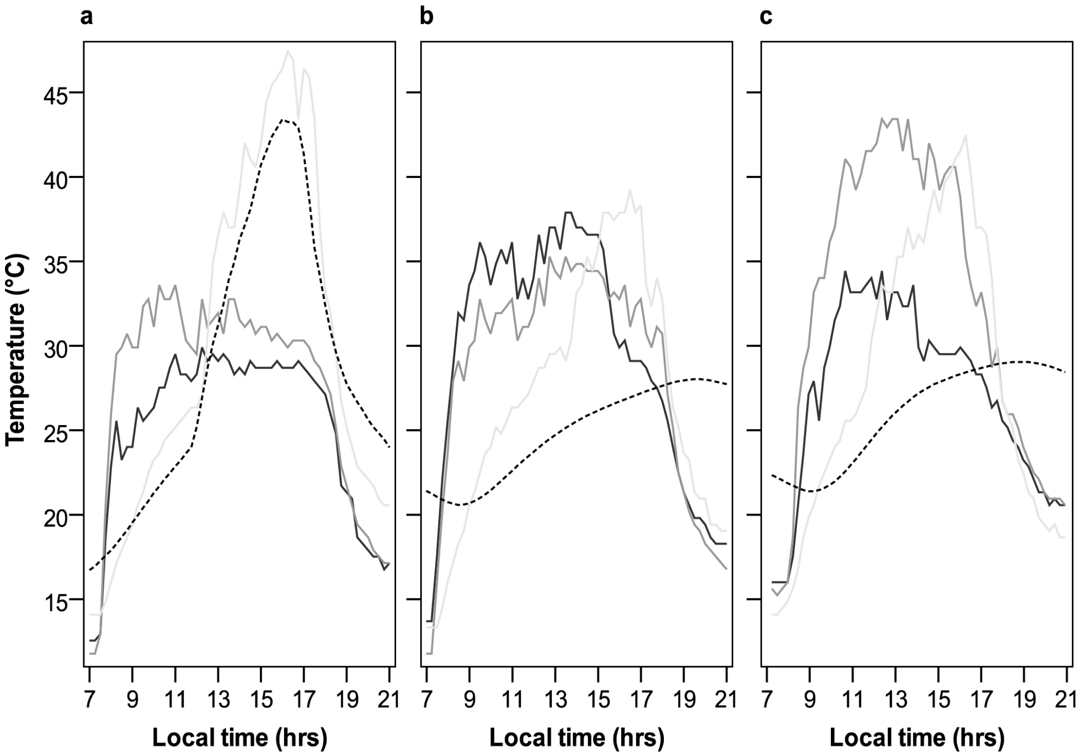

Figure 3.4

Daily temperature curve of *A. meridionalis* mounds on a representative dry season day: **a** typically large unmanipulated mound; **b** artificially more spheroidal mound and **c** the naturally spheroidal mound (*light grey* = western face; *medium grey* = northern face; *dark grey* = eastern face; *dotted line* = core).

Discussion

The objective of this study was to test the significance of shape on temperature regimes in *A. meridionalis* mounds. The core temperatures of more spheroidal mounds were more stable than that of natural elongated mounds implying that thermostability alone cannot explain the peculiar shape of ‘magnetic’ termite mounds. Yet, wedge-shaped mounds had higher mean core temperatures that might be advantageous for the termite colonies.

It was hypothesized that one solution to achieve thermostability in non-massive, simple mounds could be the construction of elongated mounds that have an adjusted orientation to provide stable temperature conditions at the eastern face during the dry season (Jacklyn 1992). During this season, ambient temperatures drop considerably and termites indeed concentrate on the eastern face in the cool morning hours (Korb 2003a). This implies that morning heating is beneficial for the termites. Our data confirm that the ‘eastern face plateau’ is connected to the elongated wedge shape as it disappeared in more spheroidal mounds (Fig. 3.4). Our findings suggest that even if there are benefits in having a thermostable eastern face at a certain time, overall mound thermostability alone cannot explain this peculiar shape as more spheroidal mounds were more thermostable than wedge-shaped mounds (Fig. 3.2): if the mound core area is constantly providing more thermostable conditions in more spheroidal mounds, then a wedge shape should not be selected due to thermostability reasons because it provides less stable conditions. According to our data, a thermoregulatory advantage of having a wedge-shaped mound could be their higher core temperatures compared to more spheroidal mounds during the dry-wet transition and the rainy season: During this time winged sexuals (alates) develop which will leave the nest attempting to found new colonies (unpubl. data). If the sexual development proves to be enhanced by increased temperature this would be a strong selective force favouring an elongated wedge-shaped architecture. Higher core temperatures could be an explanation for the wedge shape;

however, data on the optimal temperatures for alate development are needed to support this hypothesis. Also, given the largely solid nature of the mound bases, we would expect the thermal inertia of these mound bases to increase with size as seen in the walls of buildings (Arumi-Noe & Hamilton 1998) and a range of animals (Peters 1986). This should tend to reduce the diurnal range of core temperatures, and hence maximum core temperatures, in larger mounds and this trend has been confirmed by one of the authors (P.J.). So we would expect the adaptive benefits of a high core temperature to be reduced in larger mounds. Nevertheless, even for larger mounds, the higher surface area: mass ratio of the wedge shape should allow the inner parts of the mound to heat up more rapidly and reach higher temperatures than equivalent areas inside a similar sized more spheroidal mound.

Besides its distinctive mound shape in inundated areas, *A. meridionalis* does not differ in any obvious trait from other termites existing close by. For instance, *Nasutitermes triodiae* builds cathedral shaped mounds with internal mound architecture very similar to *A. meridionalis* mounds (Gay & Calaby 1970), next to them on slightly elevated positions (A. Schmidt, pers. obs). Both species forage on the same kind of dead plant material which they both store within their mounds. One difference is that *A. meridionalis* is restricted in its foraging activity and has to rely on stored food material within the mound because of the seasonal flooding. This might explain its small colony sizes. Wedge-shaped, meridionally oriented termite mounds are built by three *Amitermes* species but only under such distinct ecological conditions (Ozeki et al. 2007). This strongly suggests that the reason for this shape and orientation is directly or indirectly linked to flooding.

Our data suggest that thermostability alone cannot explain this unique shape and while there are potential thermal benefits of high core temperatures more research is needed to clarify these benefits. However the north-south orientation of the elongated mounds may be an adaptation to reduce temperature variability after building a wedge-shaped mound as this study observed that a daytime eastern face plateau was a feature of the control wedge mounds that was not seen in the spheroidal treatments. Grigg (1973) suggested that the wedge shape may be an adaption to facilitate gas exchange and a recent study suggests that passive diffusion between the surface layers of the mound, where the food material is stored, and the air is the main mode of gas exchange and a thin, wedge-shaped mound may minimise the building effort to achieve such gas exchange (Schmidt et al. 2014).

Acknowledgments

We thank Christine Schauer, Phil Hickey, and Craig Hempel for assistance in the field, the members of the School for Environmental Research at the Charles Darwin University for their logistic support and Environment Australia for permission to do field work. This study was supported by a research grant to J. K. provided by the German Science Foundation (KO 1895/7-1)

CHAPTER 4

Isolation and characterization of 10 microsatellite loci in the magnetic termite, *Amitermes meridionalis* (Isoptera: Termitidae)

Published in: *Molecular Ecology Notes*, 7: 1045-1047.

Authorship: Anna Maria Schmidt, Andreas Trindl, Judith Korb

Abstract

The elongated mounds of the ‘magnetic termite’, *Amitermes meridionalis* are a prominent feature of the Northern Territory in Australia. They are restricted to habitat patches of seasonally flooded plains which are largely isolated from each other. To investigate the population structure of *A. meridionalis* we developed 10 polymorphic microsatellite loci. We tested the variability of the markers on at least 20 individuals from two populations. We found three to 12 alleles per locus with a level of heterozygosity at each locus ranging from 0.05 to 0.74.

The mounds built by *Amitermes meridionalis* in the tropical savannahs of northern Australia are unique in shape and orientation (Grigg & Underwood 1977; Korb 2003a). The thin, wall-like shape and the astonishing alignment according to the north-south axis of the earth’s magnetic field has ever since risen the interest of explorers and scientists (Jack 1897). The distribution of *A. meridionalis* is limited to seasonally flooded plains, which can be separated from each other by several kilometres (Jacklyn 1991). As the dispersal abilities of the winged termites are poor (Nutting 1969) gene-flow is likely to be restricted.

To investigate the population structure of such island populations, neutral markers such as microsatellites are ideal means. As there are no genetic markers available for *A. meridionalis*, we developed 10 microsatellite markers.

Specimens were collected from mounds and stored immediately in 100% ethanol. For 60 workers or soldiers, the head and thorax were ground individually in liquid nitrogen. DNA was extracted following a modified cetyltrimethyl ammonium bromide (C-TAB) protocol (Sambrock & Russell 2001) and pooled. Approximately 50 μg DNA was digested with TSP 509 I and two adaptors (Tsp AD short and Tsp AD long; Tenzer et al. 1999) were ligated to the DNA, resulting in blunt-ended fragments. After purification with Ultrafree-4 spinning columns (Millipore), the ligation product was amplified using the adaptor Tsp AD short as a primer. Forty polymerase chain reactions (PCR) were carried out in a final volume of 25 μL each, containing 0.5 μL ligation product, 0.25 μL *Taq* DNA polymerase (5 U/μL; MBI Fermentas) and a final concentration of 1 μM Tsp AD short, 1 x buffer, 2.5 mM und 250 μM of each dNTP. We used a Biometra T1 thermocycler (Whatman) and the following temperature profile: initially 72 °C for 5 min to synthesize the nick between the linker and the genomic DNA followed by 20 cycles of initial denaturation at 93 °C for 1 min, primer annealing at 55 °C for 1 min and extension at 72 °C for 1 min. The PCR products were pooled, purified and concentrated using Centrifuge Filter Columns (Genomics). To enrich repeat motifs, the purified amplification product was hybridized with biotinylated oligonucleotides of either $(AC)_{13}$ or $(AG)_{13}$ linked to streptavidin-coated magnetic beads (Dynabeads M-280m Streptavidin; Dynal). Hybridization was carried out following Tenzer et al. (1999). Enriched DNA was recovered from the beads and amplified again using the same conditions as before without the initial extension step. The PCR was performed directly with 1 μL of bead solution. The enriched and amplified fragments were cloned in the plasmid pCR 2.1 and xL1 blue cells (TA Cloning Kit; Invitrogen) were transformed with these plasmids.

Clones with an insert were dot-blotted on nylon membranes (Hybond- N^+ Amersham) and probed with the oligonucleotides $(AC)_{13}$ and $(AG)_{13}$ labelled with fluorescein-11-dUTP (MWG Biotech).

Positive clones (140) were identified, of which 99 were successfully sequenced with the Big Dye Cycle Sequencing version 1.1 Ready Reaction kit (PE Biosystems) and T7 and M13 reverse primers (MWG Biotech) in an ABI Prism 310 Genetic Analyser (PE Biosystems). Clones (61) contained repeat motifs and primer pairs were designed for 37 of these. Of the primer pairs, 20 yielded a single fragment and therefore the forward primer was labelled with FAM, HEX or TET at the 5'-end.

To assess the variability of the microsatellites, DNA from one to five individuals from 20 colonies from two sites, that is populations, was extracted and the microsatellites were amplified using the following standard PCR protocol: A final volume of 20 µL contained 1-50 ng DNA template, 0.025 U/µL *Taq* polymerase, 0.5 µM labelled forward and reverse primer, 1x *Taq* buffer, 1x Enhancer, 1.6-1.8 µL from a 25 mM $MgCl_2$ solution and 200 µM per dNTP (MBI Fermentas). The initial denaturation step was set at 95 °C for 2 min, followed by 34 cycles of denaturation at 95 °C for 45 s, locus-specific annealing temperature for 45 s and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 10-30 min. Because some loci showed an + A-effect that made interpretation ambiguous, $MgCl_2$ concentration was increased as well as the final extension time of up to 30 min. This increased the + A-effect strongly and simplified interpretation. The labelled products were diluted with water, mixed with Genescan-500 (Tamra) size standard and scored on the ABI.

Ten of the 20 primers were polymorphic, with three to 12 alleles (Table 4.1). Heterozygosities were calculated using HW-QUICKCHECK (Kalinowski 2006). The observed heterozygosity was lower than expected for all loci and significant deviation from Hardy-Weinberg equilibrium was detected for loci AG1-B10, AC2-H5, AG2-F4, AC2-F4 and AG1-

H9. This indicates population substructuring or inbreeding. There was no evidence for null alleles (exception: AC2-F4 which had only three alleles), large allele dropouts or stuttering (MICRO-CHECKER, Van Oosterhout et al. 2004). No linkage disequilibrium (GENEPOP, Raymond & Rousset 1995) was found, except for AG1-B10 and AC2-H10 loci pair that showed a significant deviation from random association (*P* = 0.03; Bonferroni corrected). Therefore, at least eight loci had sufficient variability to analyse population genetics of *Amitermes meridionalis.*

Acknowledgements

This study was supported by the German Science Foundation (Ko 1895 / 7-1). Environment Australia gave permission to export the termites (export permit No. PWS P513139).

Table 4.1 Primer sequences and characterization of 10 microsatellite loci in the termite *Amitermes meridionalis*

Locus	Primer (5'-3')	Repeat motif	Label forward primer	Insert size (bp)	T_a (°C)	*n*	+ A	N_A	Size of mca (bp)(range)	Freq. of mca	H_O	H_E	*HWE P* value	Accession no. (EMBL)
AG1-B10	F: TTAGAAAAACATGTGATTAG R:GACCAGATTTGGGGCTCTTAC	$(AG)_{32}AA(AG)_3$	FAM	382	55	27	-	9	119 (101-123)	0.37	0.48	0.80	0.0002	AM 420321
AC2-G1	F: TAAACCTGATGTCACTAGTC R: ACGTAGTCACTTTCATTGCC	$(AC)_{27}TCCG(TC)_5$	TET	372	55	24	-	8	135 (124 -141)	0.33	0.67	0.81	0.1	AM 420322
AC2-H5	F: GCAGTCTGCTCACCATTAGC R: GTGAGTTAAAATGCACATAC	$(AG)_{11}(TG)_{40}$	TET	321	59	20	-	10	181 (162-197)	0.28	0.45	0.85	0.0002	AM 420323
AC2-H10	F: GAAGTAGCAGTTTATCACTT R: GAACTGCTGTCTTAACCAAC	$(AC)_{38}$	TET	384	59	26	-	11	141 (125-158)	0.44	0.69	0.78	0.3	AM 420324
AG1-B11	F: TTAGGGTGGACATATGGA R: CCTACAGAGCAACGACCT	$(AG)_{23}TG(AG)_2$	FAM	603	55	26	-	5	133 (127-135)	0.56	0.54	0.65	0.2	AM 420325
AG2-C12	F: TACAGAAGGACTCACGACGC R: TGTATCTGTGACTAGGTCGC	$(AG)_{31}$	HEX	370	59-65	21	+	8	173 (169-183)	0.31	0.67	0.82	0.12	AM 420326
AC2-F4	F: ATAGGCATATATAGCCAT R: ACTGTGAACTTCCCGGTGAC	$(AC)_5AT(AC)_{21}$	FAM	307	55-58	20	-	3	128 (109-128)	0.55	0.05	0.57	0	AM 420327
AG1-A2	F: GGAAGGTAGCATGCTAAAGC R: CAACGAATATGACTAGCGTC	$(AG)_{18}AT(AG)_5GG(AG)_{15}$	TET	343	55	20	+	12	202/237 (202-239)	0.18	0.75	0.90	0.0814	AM 420328
AG1-H9	F: ATCTCCATGTTCTGAACTGC R: GGACAACTTAGAGACACCTA	$(AG)_{33}$	HEX	387	55	26	-	13	108 (109-162)	0.19	0.65	0.91	0.0004	AM 420329
AG2-F4	F: AACATTCACCTTAAGTTCGC R: CAGTCAACATTAAAATAATG	$(AG)_{29}$	HEX	431	50-55	20	+	11	126 (120-157)	0.25	0.65	0.88	0.0072	AM 420330

T_a, annealing temperature; *n*, sample size; +A-effect, PCR was carried out with 1.8-2.0 µL $MgCl_2$ (25mM) and with 30-min. final extension; N_A, number of alleles; mca, most common allele; range, observed range of alleles; Freq. of mca, frequency of the most common allele; H_O, observed heterozygosity; H_E, expected heterozygosity; *HWE*, *P* values of the test on Hardy-Weinberg Equilibrium.

CHAPTER 5

Isolated in an ocean of grass: low levels of gene flow between termite subpopulations

Published in: *Molecular Ecology* 22, 2096-2105

Authorship: Anna Maria Schmidt, Peter Jacklyn, Judith Korb

Abstract

Habitat fragmentation is one of the most important causes of biodiversity loss, but many species are distributed in naturally patchy habitats. Such species are often organized in highly dynamic metapopulations or in patchy populations with high gene flow between subpopulations. Yet, there are also species that exist in stable patchy habitats with small subpopulations and presumably low dispersal rates. Here, we present population genetic data for the ‘magnetic’ termite *Amitermes meridionalis*, which show that short distances between subpopulations do not hinder exceptionally strong genetic differentiation (F_{ST}: 0.339; R_{ST}: 0.636). Despite the strong genetic differentiation between subpopulations, we did not find evidence for genetic impoverishment. We propose that loss of genetic diversity might be counteracted by a long colony life with low colony turnover. Indeed, we found evidence for the inheritance of colonies by so-called ‘replacement reproductives’. Inhabiting a mound for several generations might result in loss of gene diversity within a colony but maintenance of gene diversity at the subpopulation level.

Introduction

Habitat fragmentation is one of the most important causes of biodiversity loss (Debinski & Holt 2000; Fahring 2003). It transforms formerly continuous populations into subdivided units, embedded in a matrix of unsuitable habitat with varying degrees of connectivity and decreased subpopulation sizes. Along with habitat fragmentation comes an increased influence of genetic drift relative to natural selection, which is predicted to lead to a decline of genetic diversity within fragments (Prugh et al. 2008; Frankham et al. 2009; Amos et al. 2012). With restricted gene flow, genetic drift results in higher genetic differentiation between fragments, which is not linked to adaptation. Thus, the small subpopulations can suffer from (i) an increased probability to go extinct through environmental or demographic stochasticity; (ii) an increased risk of inbreeding, potentially associated with inbreeding depression; and/or (iii) genetic impoverishment (Prugh et al. 2008; Walker et al. 2008; Frankham et al. 2009; Amos et al. 2012). These negative effects are most pronounced when the subpopulations are small and strongly isolated, and are dependent on the quality of the matrix habitat and the species' dispersal abilities (Rousset 2004). Yet, there are species that appear stable in a metapopulation or in naturally patchy habitats. In a highly dynamic metapopulation system, the subpopulations are connected by gene flow that decreases with distance (isolation by distance), and patches with local extinction events are recolonized by founder individuals from other subpopulations (Hanski 1999). For instance, water voles (*Arvicola terrestris*) sustain their genetic diversity by highly efficient and frequent long-distance dispersal, despite significant subpopulation differentiation (Aars et al. 2006). Stable patchy populations are characterized by high levels of gene flow, which connect all subpopulations and prevent subpopulation differentiation, so that the patchy population forms one genetic cluster. As a consequence, genetic diversity remains high and subpopulations have a low risk of extinction (Mayer et al. 2009).

Yet, there are also species that exist in stable patchy habitats with small subpopulations and presumably low dispersal rates. Our study species, the mound building ‘magnetic’ termite, *Amitermes meridionalis*, seems to be an example: *A. meridionalis* is endemic to the ‘Top End’ of northern Australia, where it occurs in islands of low-lying grasslands that are embedded in a matrix of savannah woodland, and that are characterized by alluvial flooding during the rainy season (Fitzpatrick & Nix 1970; Jacklyn 1992). This species builds elongated wedge-shaped mounds of up to four metres in height that are oriented along the north-south axis (Fig. 1.1). Termite colonies inhabiting these seasonally flooded islands are considered subpopulations of varying sizes and degrees of isolation that are expected to exchange migrants. These migrants are winged sexuals (‘alates’) that have a nuptial flight once a year after the first strong rain at the beginning of the rainy season. In contrast to other species such as water voles (Aars et al. 2006), saxicolous mice (*Phyllotis xanthopygus*, Kim et al. 1998) or Glanville fritillary butterflies (*Melitaea cinxia*, Saccheri et al. 1998) that survive in naturally patchy habitats in a highly dynamic metapopulation system with local extinctions and recolonizations, there is evidence that *A. meridionalis* subpopulations and colonies are very stable with little turnover (Bowman 2002; Peter Jacklyn unpublished data (but see Table S5.1)). The dispersal abilities are unclear: on the one hand, termite alates are generally regarded to be poor flyers (Nutting 1969), while more recent population genetic studies suggest that dispersal might be less limiting (Vargo & Husseneder 2011). Hence, the aim of this project was to study a species that lives under patchy conditions, is lacking metapopulation dynamics and for which dispersal abilities are unclear. Based on population genetic theory and existing data, we expected to find high dispersal among patches, which leads to gene flow and prevents a decline of the genetic diversity within subpopulations. Alternatively, if gene flow between patches is restricted, we expect genetic patterns typical for habitat fragmentation: high subpopulation differentiation, low genetic diversity within fragments and extensive inbreeding. In the latter case, the question arises how this system maintains stability without suffering from the negative effects of habitat fragmentation.

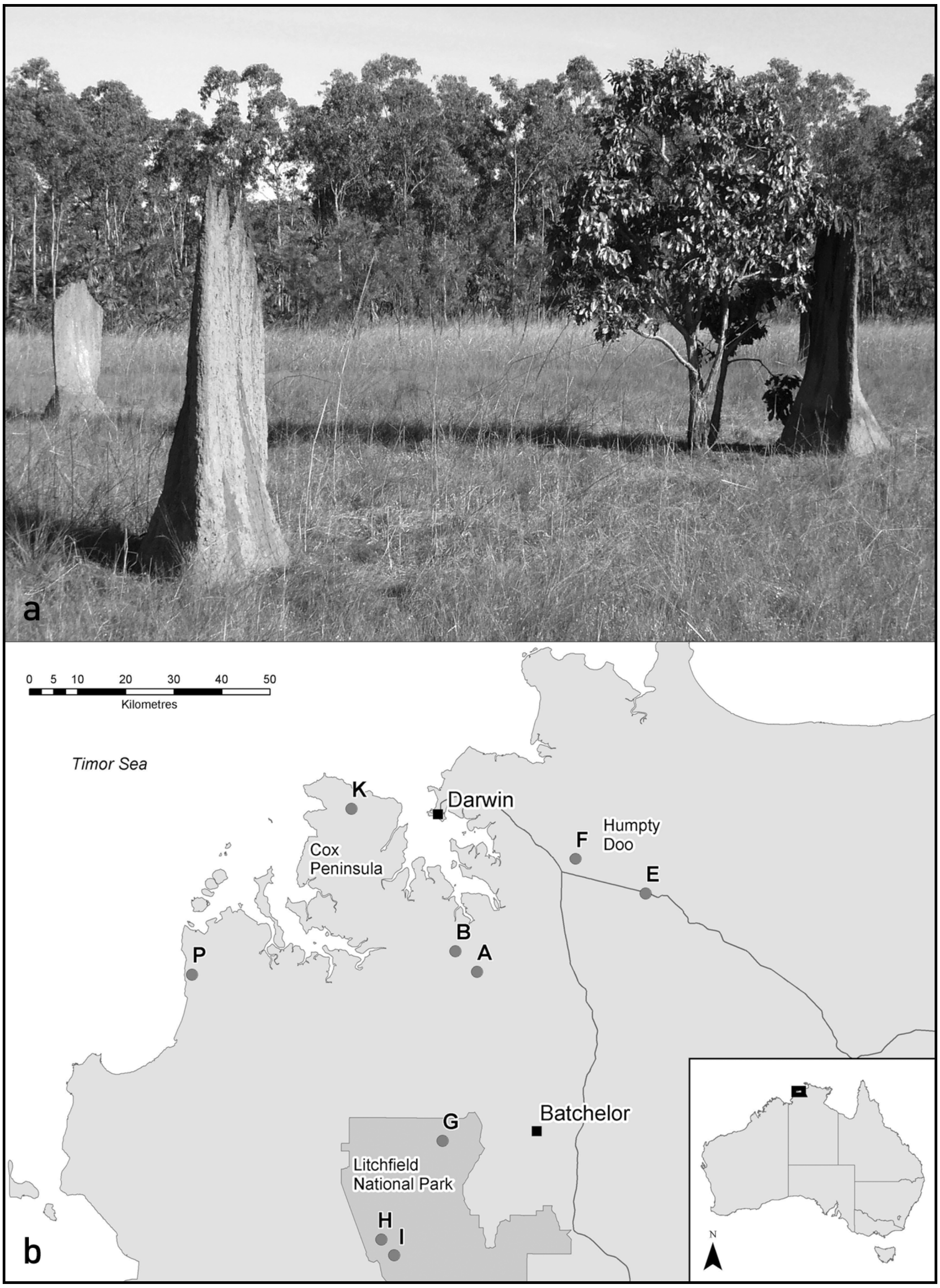

Figure 5.1

a Mounds of *Amitermes meridionalis*, photograph taken in the morning with view on the southern face. The broad eastern face is sunlit, while the western face is in shade. **b** Locations of the nine sampled subpopulations in northern Australia.

Material and methods

Sampling

Specimens of *Amitermes meridionalis* were sampled from nine subpopulations throughout the species' range between 2005 and 2007 (Fig 5.1b). The subpopulations differed in size from nine to a few hundreds of mounds and in degree of isolation from one km to about 45 km distance to the next subpopulation (Table 5.1). To determine the size of each subpopulation, GPS locations were recorded along the border of each subpopulation and the size was extrapolated using Hawth's Analysis Tools in ARCMAP (Beyer 2004). A nearest neighbour analysis of mounds within subpopulation A showed that mounds were randomly distributed (aggregation index = 0.91, $Z = -0.531$, $P > 0.05$). Hence, we assumed that the area size is approximately proportional to the number of mounds and thus the subpopulation size. The approximate isolation of subpopulations was assessed using GOOGLE EARTH (available at http://earth.google.de) where termite mounds can be visually recognized and distances can be determined between sites with visible termite mounds (Table 5.1). Distances between sampled subpopulations were calculated from GPS locations, also using Hawth's Analysis Tools in ARCMAP. All distances between subpopulations were measured from the borders of the subpopulations to infer the shortest possible dispersal distance.

We sampled at least ten neighbouring mounds in each of the nine subpopulations, except for subpopulation E where only nine mounds were present and subpopulation G where one mound was partly inhabited by an inquiline termite species that was accidentally sampled. Because the latter was noticed only in the laboratory, it was not possible to resample this mound for *A. meridionalis* (Table 5.1). According to Thompson et al. (2007), this is an appropriate sample size for population genetics in termites. All sampled mounds were built and inhabited by *A. meridionalis*. Approximately 20 termites were taken from each mound and stored in 100% ethanol until genetic analyses.

Genotyping

We screened 524 individuals at the following eight microsatellite loci that were specifically developed for *A. meridionalis*: AG1-B11, AC2-G1, AG1-H9, AC2-H10, AG2-C12, AG2-F4, AG1-A2 and AG1-B10 (Schmidt et al. 2007; Schmidt et al. 2013). However, because the locus AG2-F4 showed evidence for null alleles, we excluded this locus from the final population genetic analyses; the results from the reduced data set did not differ from those obtained when including AG2-F4 (see Results section).

The genetic analyses with microsatellite markers were carried out according to Schmidt et al. (2007). To get an idea of the colony composition and breeding system of *A. meridionalis*, we first genotyped ten specimens per colony in the arbitrarily chosen subpopulation B. We found a maximum of four alleles per locus and colony, which is consistent with colony foundation by two (unrelated) individuals (see Results section). Because the emphasis of this study was to analyse the population structure rather than the breeding system, we reduced the sample size of genotypes per colony and instead sampled more subpopulations (see also Garcia et al. 2002; Garcia et al. 2004; and Brandl et al. 2005 who used one and two samples per colony, respectively). To test whether a sample size of five individuals per colony is sufficient to estimate genetic diversity, we performed the analyses for subpopulation B with a sample size of ten and an arbitrarily selected subsample of five individuals per colony. The gene diversity and the number of alleles did not differ when using five or ten individuals (mean ± SE of gene diversity, five individuals: 0.65 ± 0.055, ten individuals: 0.65 ± 0.057, *t*-test for paired samples: $t_{1,6} = - 0.75$, $P = 0.482$; mean ± SE number of alleles, five individuals: 5.6 ± 0.75; ten individuals: 5.9 ± 0.91; *t*-test for paired samples: $t_{1,6} = 1.55$, $P = 0.172$). So, we genotyped at least five individuals per colony in all other subpopulations, that is, about 50 individuals per subpopulation (Table 5.1).

A constant sample size that does not correlate with the size of the subpopulation raises the issue of an underestimation of genetic diversity in large subpopulations. We chose a standard sample size (around 50 individuals, see Results section) for each subpopulation to exclude largely differing sample sizes as a confounding factor that could influence the results of the genetic diversity analyses. Thus, we followed the approach used in other population genetic studies that analyse correlations of genetic diversity with subpopulation size (England et al. 2002; Lienert et al. 2002; Tero et al. 2003).

Measures of genetic diversity

Workers within a colony are not genetically independent because they are related. To test for deviation from Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium, we thus used an approach suggested for social insects/termites (Vargo 2003) and generated five data sets with one arbitrarily chosen worker from each colony. Genotypic linkage disequilibrium was tested by means of Fisher's exact test using GENEPOP version 3.4 (Raymond & Rousset 1995), and the occurrence of null alleles was tested with MICRO-CHECKER (Van Oosterhout et al. 2004). Allelic richness, gene diversity according to Nei (1987) and deviation from HWE were calculated by means of exact tests using ARLEQUIN version 3.1 (Excoffier et al. 2005). We also determined the proportion of private alleles, that is, the number of alleles that are unique in a subpopulation relative to the overall number of alleles in that subpopulation. To test whether the genetic diversity of subpopulations correlates with their size or degree of isolation (see *Sampling* section), we performed Spearman rank analyses in SPSS 15.0.

Colony genetic structure and relatedness

To infer general features of the breeding system in *A. meridionalis* (e.g. number and relatedness of founding individuals, existence of inbreeding through secondary replacement reproductives), we estimated the number of alleles per locus and colony and determined whether the genotype composition within a colony was consistent with being the offspring of a single pair of founding individuals (e.g. a maximum of four different alleles and genotypes). We calculated the average relatedness coefficient of workers from the same nest in each subpopulation and the average relatedness within each subpopulation with RELATEDNESS 5.0.8 (Queller & Goodnight 1989). Relatedness estimates were bias corrected, and all individuals were weighted equally. 95% confidence intervals for relatedness estimates were calculated by jackknifing over loci.

Hierarchical analysis of genetic variance

At the total population level, we determined the partitioning of genetic variance between different hierarchical levels of the population structure with a four-level hierarchical AMOVA as implemented in ARLEQUIN version 3.1 (see Table 5.2 for levels and notations). We repeated the analysis with a data set consisting of only one individual per colony to ensure genetic independence of samples (Garcia et al. 2002): the overall results changed only marginally (Table 5.2). ARLEQUIN version 3.1 allows the employment of *F*- or *R*-statistics for the AMOVA. However, both statistics have their drawbacks and assume strict mutation models for microsatellite evolution (infinite alleles model (IAM) or stepwise-mutation model (SMM), respectively; reviewed in Balloux & Lugon-Moulin 2002). F_{ST} underestimates differentiation in highly structured populations, but R_{ST} is only independent of mutation under a strict SMM model. Therefore, we report both *F*- and *R*-statistics

Genetic differentiation

We calculated pairwise F_{ST} and R_{ST} between subpopulations and between colonies within subpopulations with ARLEQUIN version 3.1. We also calculated subpopulation-specific inbreeding coefficients with ARLEQUIN version 3.1. For the latter analysis, we used a reduced data set with only one individual per colony to exclude the influence of inbreeding within colonies.

Estimation of gene flow

We used a Bayesian approach with BAYESASS 1.3 (Wilson & Rannala 2003) to estimate migration rates between subpopulations. This approach estimates more recent migration rates and does not assume that the populations are in drift-mutation equilibrium. The burn-in length was set after log-likelihood values peaked and we accepted proposed changes in parameters that were between 40% and 60% of the total chain length, except for the migration rate. The delta value was set to 0.32 (Wilson & Rannala 2003), and the number of proposed changes was 87% of the total chain length. The results were consistent with different delta values and initial seeds (see Results section). We performed 3×10^6 iterations of which 60 000 were burn-in and the sampling frequency was 1000.

Isolation by distance

We investigated patterns of isolation by distance separately for each subpopulation, by calculating pairwise F_{ST} (R_{ST}) for colonies and testing the significance of the correlation between genetic distance F_{ST} / (1- F_{ST}) and the logarithm of the geographical distance (Slatkin 1993) using Mantel tests (10 000 permutations; Mantel 1967) as implemented in ARLEQUIN version 3.1. We also performed the same analysis for all sampled subpopulations and their pairwise distances to investigate isolation by distance on a large scale and infer information about the direction of gene flow between these subpopulations.

Table 5.1

Number of alleles (*A*) and gene diversity (*h*) according to Nei (1987) of microsatellite loci and subpopulations; number of individuals (*N*) and colonies (*col*) analysed in the study subpopulations; degree of isolation and area size of subpopulations. The number of private alleles is given as percentage values of all alleles in a subpopulation.

Sub pop	AG2-F4		AG1-B11		AG1-H9		AC2-G1		AG1-B10		AC2-H10		AG2-C12		AG1-A2		Gene div.	Mean *A*	Priv. allel. (%)	*N (col)*	Isolation (km)	Size (m^2)
	A	*h*	*A*	*h*	*A*	*h*	*A*	*h*	*A*	*h*	*A*	*h*	*A*	*h*	*A*	*h*						
A	9	0.64	4	0.52	9	0.82	5	0.77	8	0.76	7	0.69	4	0.67	7	0.82	0.71	6.6	17.0	87 (13)	1.47	84 834
B	6	0.60	4	0.61	10	0.86	5	0.59	3	0.42	6	0.78	8	0.57	5	0.69	0.64	5.9	17.0	100 (10)	1.11	29 212
E	6	0.55	5	0.46	3	0.45	3	0.46	2	0.50	2	0.15	3	0.22	3	0.55	0.42	3.4	3.7	45 (9)	1.38	1 438
F	8	0.81	3	0.40	6	0.76	5	0.58	7	0.63	2	0.41	1	0.00	5	0.68	0.53	4.6	10.8	50 (10)	9.50	49 618
G	6	0.75	5	0.63	2	0.11	5	0.67	3	0.60	5	0.68	7	0.76	5	0.57	0.60	4.8	10.5	45 (9)	1.70	48 603
H	4	0.60	6	0.59	6	0.72	4	0.52	6	0.77	6	0.66	2	0.02	2	0.12	0.50	4.5	8.3	48 (10)	2.20	52 242
I	7	0.72	10	0.83	10	0.86	7	0.76	8	0.76	10	0.84	6	0.57	3	0.41	0.72	7.6	14.8	49 (10)	1.77	15 284
K	3	0.48	6	0.71	4	0.56	4	0.47	5	0.40	4	0.61	6	0.70	3	0.53	0.56	4.4	25.7	50 (10)	2.60	41 924
P	8	0.75	8	0.80	4	0.60	4	0.66	3	0.52	4	0.62	4	0.59	3	0.20	0.60	4.8	18.4	50 (10)	~ 45	93 347
All	27	0.66	15	0.62	25	0.64	11	0.61	21	0.60	18	0.60	19	0.46	19	0.51	0.59	5.18	14	524		

Results

Descriptive diversity statistics

We genotyped 524 specimens at eight microsatellite loci (data deposited in the Dryad repository: doi: 10.5061/dryad.5q35k). The microsatellites showed high overall variability (11-27 alleles per locus, Table 5.1). There was no linkage disequilibrium between loci. The generated data sets did not show a consistent pattern of null alleles or deviation from HWE across loci, with one exception: the locus AG2-F4 showed evidence for null alleles and significant deviations from HWE (Fisher's exact test: $P < 0.05$) in 26 of 45 tests and in 24 of 45 tests, respectively. As suggested by Vargo (2003), this locus was excluded from further analyses. All other loci showed significant deviations in < 35% of the tests that is below the threshold of 50% suggested by Vargo (2003). There was also no consistent pattern of significant deviation from HWE across subpopulations with one exception: subpopulation G showed significant deviation from HWE in locus AG1–B10. However, there was no evidence for null alleles at this locus in any of the generated data sets. All other loci analyzed in subpopulation G showed significant deviation in < 5% of the tests. Thus, we employed seven loci for the genetic population analyses.

Due to our sampling scheme, we sometimes had slightly different sample sizes for different colonies and different subpopulations (Table 5.1). To keep the sample size constant and control for this parameter, we standardized the sample size to five individuals per colony and nine colonies per subpopulation and repeated these analyses. Some loci showed one allele less than showed in the analyses of the whole data set, but the overall results did not change. All reported results are therefore derived from the whole data set.

Correlations of genetic diversity

We performed Spearman rank analyses with all diversity measures and *F*-statistics to discover potential relationships with subpopulation size (measured as the size of the populated habitat) and degree of isolation (measured as the shortest possible distance to the next subpopulation as visually assessed by GOOGLE EARTH), but no significant correlations were found (Fig. 5.2). The gene diversity, mean and maximum number of alleles, the inbreeding coefficients and the proportion of private alleles did not correlate with either the distance to the next subpopulation or the subpopulation size. The proportion of private alleles in a subpopulation ranged from 3.7% in subpopulation E to 25.7% in subpopulation K (Table 5.1).

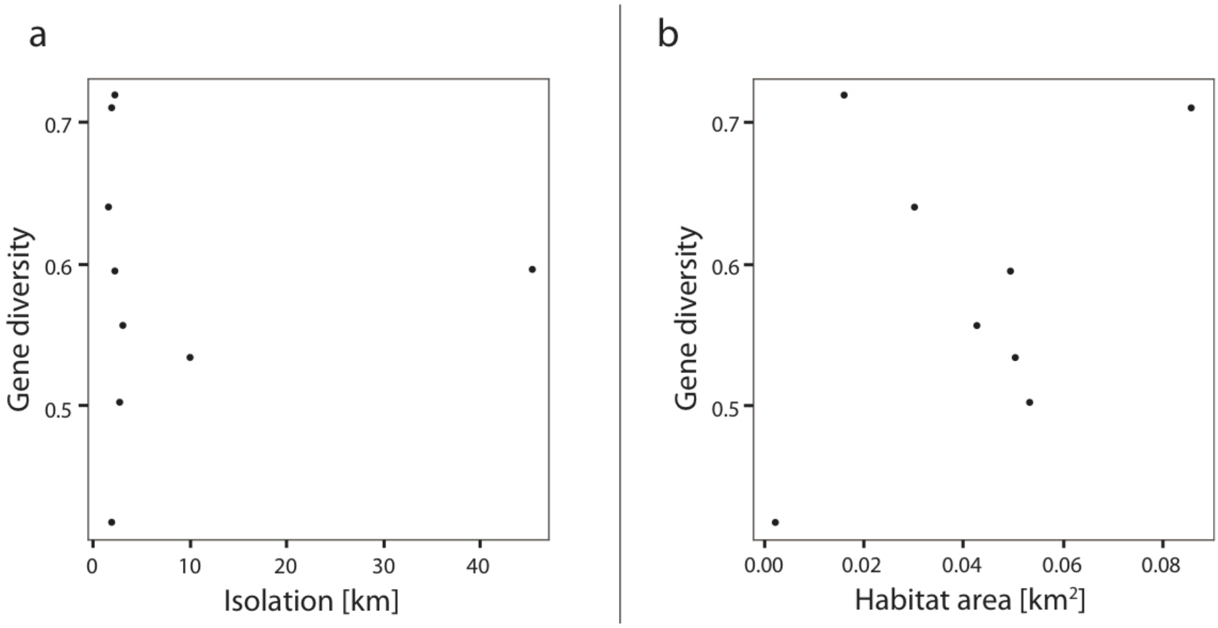

Figure 5.2
a Plot of gene diversity with subpopulation size (estimated by size of the habitat patch). No significant correlation was found (Spearman rank correlation: $r = 0.1$, $P = 0.798$). **b** Plot of gene diversity with degree of isolation to the next subpopulation. No significant correlation was found (Spearman rank correlation: $r = - 0.15$, $P = 0.700$).

Genetic structure of colonies

Visual inspection of the colony genotypes revealed a maximum of four alleles in a colony. The existing genotypes were often not consistent with being the offspring of only two reproductives; in many cases, at least three genetically different parents were necessary to explain the existing genotypes. We found on average 33% colonies with four alleles, 49% with three and 18% with two alleles.

The relatedness between workers of the same colony (‘nestmates’) ranged from r = 0.48 in a colony in subpopulation A to r = 0.98 in a colony in subpopulation H. Pairwise F_{ST} between colonies within subpopulations was significantly different from zero for most combinations, except for four pairs in subpopulation E, two in subpopulation G and one in subpopulation K. The values for colony differentiation were very high (F_{SG}: 0.394, R_{SG}: 0.369; Table 5.2b).

Table 5.2

Hierarchical analysis of variance and *F*-statistics. AMOVA with **a** region, subpopulation and colony level included; **b** subpopulation, colony and individual level included, below the black line is the partition of within-colony variance into within-colony and within-individual variance.

Source of variation	**% of total variation (F_{ST})**	**% of total variation (R_{ST})**	***F*-statistics**	***R*-statistics**
a				
Among regions	8.77	9.62	$F_{GT} = 0.088$	$R_{GT} = 0.096$
Among subpopulations within regions	28.15	63.14	$F_{SG} = 0.308$	$R_{SG} = 0.699$
Among individuals within populations	16.38	10.06	$F_{IS} = 0.260$	$R_{IS} = 0.369$
Within individuals	46.71	17.18	$F_{IT} = 0.533$	$R_{IT} = 0.828$
b				
Among subpopulations	33.9	63.6	$F_{GT} = 0.339$	$R_{GT} = 0.636$
Among colonies within subpopulations	26.0	13.4	$F_{SG} = 0.394$	$R_{SG} = 0.369$
Within colonies	40.0	23.0	$F_{ST} = 0.592$	$R_{ST} = 0.767$
Among individuals within colonies	-7.28	-3.9	$F_{IS} = -0.181$	$R_{IS} = -0.169$
Within individuals	47.3	26.9	$F_{IT} = 0.527$	$R_{IT} = 0.732$

Hierarchical analysis of molecular variance

The analysis of molecular variance (AMOVA) (Table 5.2a) showed that a substantial amount of the variation stems from variation between subpopulations (33.9% as calculated from *F*-statistics and 63.6% as calculated from *R*-statistics; $P < 0.001$) and some from differences between colonies within a subpopulation (*F*-statistics: 26.0%, *R*-statistics: 13.4%; $P < 0.001$). The remaining variation (*F*-statistics: 40%, *R*-statistics: 23%; $P < 0.001$) was due to differences between individuals within colonies. To explain this unexpected result, the individual level was included in the analyses. These analyses showed that the remaining variation was due to differences between microsatellite loci within each individual, while individuals within colonies were not significantly differentiated.

To test whether the strong differentiation could be due to historical vicariant effects that completely prevent gene flow, we additionally ran an AMOVA in which we *a priori* defined vicariance groups that incorporated certain subpopulations based on their relative distance to each other (Kelly et al. 2006). We defined five regions, in each of which subpopulations were reasonably close to each other (Table S5.3). Very little variation was due to this division, and only the F_{GT} value differed significantly from zero (*F*-statistics: 8.77%, $P = 0.017$; *R*-statistics: 9.62%, $P = 0.309$; Table 5.2b).

Table 5.3

Pairwise geographic (in km, lower diagonal) and genetic distance, measured as F_{ST} (upper diagonal) between subpopulations. All F_{ST} values were significantly different from zero ($P < 0.001$).

Subpopulation	**A**	**B**	**E**	**F**	**G**	**H**	**I**	**K**	**P**
A	*	0.24	0.38	0.26	0.30	0.36	0.26	0.32	0.29
B	5.94	*	0.40	0.31	0.27	0.42	0.28	0.39	0.34
E	38.23	41.13	*	0.42	0.47	0.53	0.41	0.50	0.46
F	30.50	31.13	16.12	*	0.38	0.45	0.34	0.44	0.41
G	35.19	39.12	66.20	64.30	*	0.43	0.31	0.41	0.38
H	58.25	61.30	90.10	88.17	23.62	*	0.20	0.43	0.42
I	60.80	64.17	91.32	90.19	25.50	3.80	*	0.32	0.31
K	42.59	36.45	63.53	47.43	71.26	89.18	92.92	*	0.39
P	58.88	54.60	95.61	82.86	62.11	67.05	71.43	47.50	*

Subpopulation differentiation, gene flow and isolation by distance

The values for subpopulation differentiation were very high (F_{GT}: 0.339; R_{GT}: 0.636; Table 5.2a). Values higher than 0.25 are considered to reflect very large differentiation (Hartl & Clark 1989). Even subpopulations in close proximity (e.g. H - I: 3.8 km; A - B: 5.9 km) were significantly differentiated, and pairwise F_{ST} ranged from 0.20 (H - I: 3.8 km) to 0.52 (H - E: 90 km; Table 5.3). Pairwise R_{ST} had generally higher values and ranged from 0.08 (H - I) to 0.90 (H - E).

The subpopulation-specific inbreeding coefficients differed slightly between *R*- and *F*-statistics, but most subpopulations were significantly inbred. With *F*-statistics, all but subpopulation G were significantly inbred, ranging from 0.174 (P, $P = 0.026$) to 0.661 (K, $P < 0.001$). With *R*-statistics, all but subpopulations A, B and P were significantly inbred, ranging from 0.379 (G, $P = 0.008$) to 0.846 (F, $P < 0.001$).

The average relatedness among individuals within subpopulations ranged from $r = 0.06$ in subpopulation G to $r = 0.23$ in subpopulation A.

The Bayesian approach for the estimation of migration rates assigned all individuals to their source subpopulation. More than 99% of the individuals in the sampled subpopulations were classified as nonmigrants (95% confidence interval (CI): 0.97-1.0) with very low migration rates from other subpopulations (always below 0.001). Although the 95% CI for the migration rates from other subpopulations was large at the lower limit, the means differed considerably from simulations that assumed no information in the data (mean of non-migration rate (95% CI): 0.83 (0.68-0.99) and mean of migration rate (95% CI): 0.02 (4.53 $\times 10^{-10}$ - 0.126). These results were consistent with other delta values or initial seed settings.

We detected significant isolation by distance (Mantel test: $r = 0.49$, $P < 0.001$) between subpopulations (Fig. 5.3), but there was no correlation between genetic and geographic distance between colonies within a subpopulation (always $P > 0.344$; Fig. S5.1).

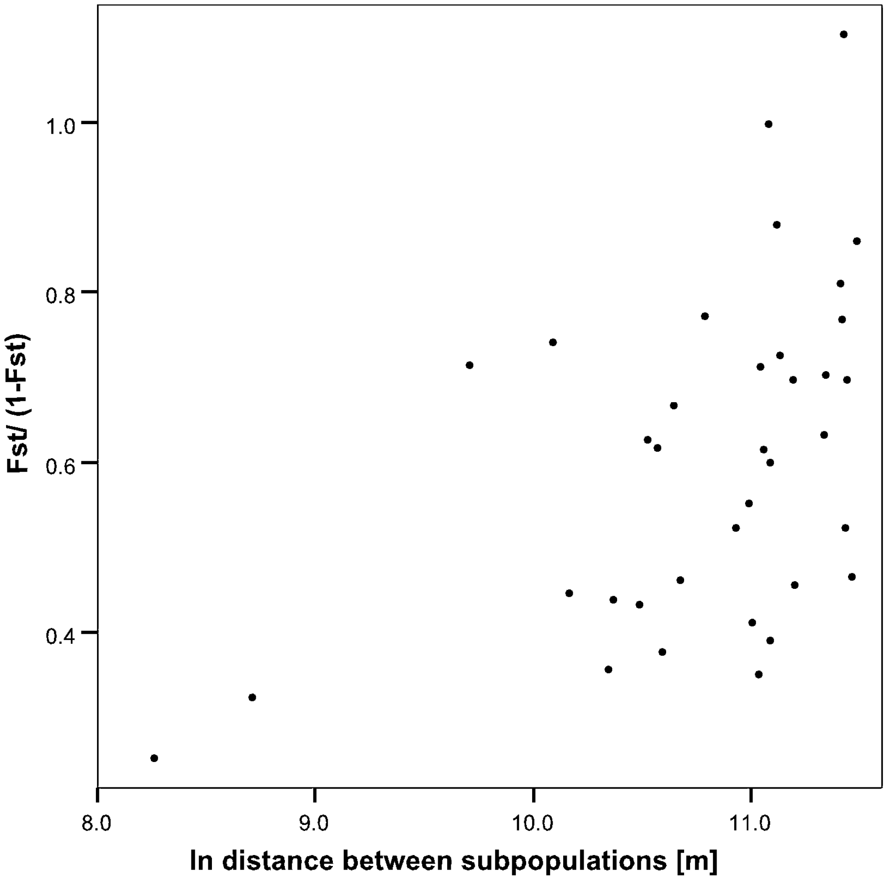

Figure 5.3

Isolation by distance analysis of the nine sampled subpopulations. The correlation between pairwise estimates of genetic and geographical distance was significant (Mantel test: $r = 0.49$, $P < 0.001$).

Discussion

The objective of this study was to evaluate the population genetics of a species with a naturally patchy distribution that seems to lack a dynamic metapopulation structure and strong dispersal ability and thus differs from other patchily distributed species. We found that *A. meridionalis* shows some typical characteristics of species whose habitat has been fragmented for a long time: low gene flow between subpopulations leading to significant differentiation of subpopulations and the occurrence of private alleles (Table 5.1). On the other hand, we find characteristics of species with patchy populations that are unusual in fragmented populations: the subpopulations did not show low genetic diversity (Table 5.1).

Compared to other termite species, *A. meridionalis* subpopulations were exceptionally strongly genetically differentiated (F_{GT}: 0.339; R_{GT}: 0.636; Table 5.2a), even over short distances (pairwise F_{ST} between H and I: 0.20; distance: 3.8 km, Table 5.3). Most termite studies report F_{ST} values below 0.2 over distances of tens of kilometers and values of 0.25 or higher are found only over distances of hundreds of kilometers (e.g. Thompson & Hebert 1998; Brandl et al. 2005; DeHeer et al. 2005; Dronnet et al. 2005; reviewed in Vargo & Husseneder 2011). Hence, gene flow in termites seems to be relatively unfettered over distances of tens of kilometers, and it was proposed that the assumed poor dispersal abilities of termites may not be true (Vargo & Husseneder 2011). In only a few species is considerable genetic substructuring found over distances less than hundreds of kilometers (e.g. *Mastotermes darwiniensis*: pairwise F_{ST} up to 0.4 over distances between 50 and 100 km, Goodisman & Crozier 2002; *Labiotermes labralis*: pairwise F_{ST} 0.16 - 0.25 over distances of 150 - 300 km, Dupont et al. 2009). Yet in *A. meridionalis*, pairwise F_{ST} estimates between subpopulations were up to 0.43 over distances between 16 and 40 km (e.g. between subpopulations E and F, Table 5.3). Such high values of population differentiation are generally interpreted as lack of gene flow due to isolation by distance (e. g. Palumbi et al.

1997; Garcia et al. 2004; You et al. 2008), a history of invasion (e.g. the wasp *Vespula germanica*, Goodisman et al. 2001; the ant *Linepithema humile*, Jaquiery et al. 2005) or geographic or biological barriers (e.g. the giant springtail *Acanthanura sp. n.*, Garrick et al. 2007; and the fish *Sprattus sprattus*, Debes et al. 2008). We estimated a small but nonzero gene flow between *A. meridionalis* subpopulations that decreased with distance and showed no evidence of vicariant effects: the estimated migration rates were lower than 0.001. Hence, gene flow between subpopulations of *A. meridionalis* is insufficient to prevent strong differentiation and the occurrence of private alleles but sufficient to prevent complete genetic isolation, that is, speciation.

Despite the very low gene flow and strong genetic differentiation between *A. meridionalis* subpopulations, we did not find evidence for genetic impoverishment that would be expected after long-term fragmentation: although most subpopulations were significantly inbred, we found moderate to high genetic diversity within subpopulations (Table 5.1) and genetic diversity did not correlate with subpopulation size or degree of isolation (Fig. 5.2). This indicates that the subpopulations do not face the negative consequences of isolation and this despite high levels of inbreeding within subpopulations. One factor that could support high levels of genetic diversity is a large effective population size (Frankham et al. 2009). We cannot make direct inferences about the effective population sizes of the studied subpopulations. However, this factor appears less likely in this case as genetic diversity did not correlate with subpopulation size, and we can only assume that the effective population size correlates with subpopulation size if all other factors that could influence the effective population size are equal between subpopulations (e.g. number of reproducing colonies).

Genetic diversity could also be maintained by having long-lived colonies with low colony turnover. In termites, nest inheritance by colony members is common: when a founder of a colony dies, a nestmate, generally the offspring of the founder, can take over the breeding position as replacement reproductive (Korb & Hartfelder 2008). Through this inheritance, mounds can be inhabited for several generations by the same termite colony (reproductives and their offspring). This leads to inbreeding within colonies as is indeed indicated in *A. meridionalis* by the high relatedness among nestmates and by the inspection of genotypes within colonies, which showed that not all existent genotypes could have been produced by one single pair of unrelated reproductives (e.g. more than four genotypes in one colony). Replacement reproductives have been described in *A. meridionalis* (Gay & Calaby 1970). Inbreeding within colonies results in a decline of gene (allelic) diversity within a colony, but because colonies are inbred and do not regularly mix with each other, different alleles are lost by chance in different colonies. Thus, each colony may have its own set of alleles or allele frequencies (indicated by the significant differentiation of colonies within subpopulations). The significant differentiation and longevity of the termite colonies therefore might result in loss of gene diversity within a colony but maintenance of gene diversity at the subpopulation level.

Conclusion

Here, we give a striking example of a species that is able to survive under naturally patchy conditions despite being an extremely poor disperser. The small *A. meridionalis* subpopulations did not show any evidence of low genetic diversity despite strong genetic differentiation and very low gene flow (Prugh et al. 2008; Frankham et al. 2009). Our results might indicate that high colony longevity could be one factor that has pre-adapted *A. meridionalis* to live stably in a naturally patchy habitat. Other possible explanations for these unusual findings will need to be addressed in further studies. Studying the mechanisms that allow poorly dispersing species to survive under patchy conditions can contribute to improve predictions on species extinction risks in conservation biology.

Acknowledgements

We thank Christine Schauer for assistance with sampling and microsatellite analyses and Tobias Lenz and Sebastian Klaus for comments on an earlier draft of the manuscript. Additionally, we thank the editor and reviewers for helpful comments. This work was supported by a German Science Foundation (DFG) grant to J.K. Environment Australia gave permission to export the termites (export permit No. PWS P513139).

CHAPTER 6

General discussion

What is the biological significance of the unique shape of ‘magnetic’ termite mounds?

Although ‘magnetic’ termite mounds are a prominent feature of the tropical savannas in northern Australia and many researchers have speculated about their unique shape, all these hypotheses advanced so far remained speculative and have not been tested experimentally. The few studies performed by Grigg (1973; 1977; 1988), Jacklyn (1991, 1992; 2002) and others (Spain et al. 1983) that employed various rotation or magnetic field experiments focused on the meridional orientation or means to achieve the meridional orientation of these mounds while the shape itself was again just hypothetically explored.

Therefore, the present work is the first study that experimentally tested several hypotheses for the biological significance of the wedge shape in the field. The second and third chapter explored the following questions: (a) are the mounds functionally adapted to facilitate gas exchange and/or the preservation of food stores during the rainy season or (b) is the shape of thermoregulatory significance?

Is the shape an adaptation to facilitate gas exchange and/or the preservation of food stores during the rainy season?

We initially quantified the influence of rainy conditions on gas exchange and food availability in general and identified the predominant mechanisms of mound ventilation: first, gas exchange was diminished during the rainy season, especially in wet mounds as the mound’s pores became water-blocked. Accordingly, quick drying of mound walls is important and could be enhanced by a large surface area (chapter 2). Second, food quantity and quality decreased during the rainy season, so food could become a limiting resource (chapter 2).

The first results confirm Grigg's (1973) hypothesis, that respiratory gas exchange of *A. meridionalis* colonies is accomplished by passive diffusion through the large mound walls. Although shaded mounds were wetter than control mounds during the measurement in February 2007 and the mean mound temperature was decreased, CO_2 concentrations were not significantly different between shaded and control mounds (chapter 2). Thus, irradiance and temperature are obviously not the most important factors for gas exchange. Instead, the main driver of gas exchange in *A. meridionalis* seems to be wind that was not influenced by shade roofs. Therefore, the distribution of 'magnetic' termite mounds in open habitats with prevailing winds could aid respiratory gas exchange. Because we did not detect significant differences in gas exchange between faces (unpublished data) and the internal structure of the mound (dense core and no specific ventilation structures) makes convective heat flows across the mound rather unlikely, the cross-mound ventilation hypothesis proposed by Serventy (1967) was not supported by the data.

Wind as the driver of gas exchange in termite mounds is common and has been shown in several species of the genus *Macrotermes*, e.g. *M. michaelseni* (closed mounds as in *A. meridionalis*; Turner 2001), *M. subhyalinus* (Darlington 1989) and *M. jeanneli* (Darlington et al. 1997; both open mounds). Similarly, mound-building ants like the leaf-cutting ant *Atta vollenweideri* use wind to drive the passive gas exchange of their open nests (Kleineidam & Roces 2000). The structural adaptations to employ wind for passive gas exchange vary considerably between species and are much more sophisticated than the simple construction of a large surface area as in *A. meridionalis* mounds (mechanisms of gas exchange reviewed in Korb 2011).

If ventilation is passive and accomplished by wind, is the shape with its enlarged surface area an adaptation to facilitate this gas exchange? Because CO_2 concentrations did not differ significantly between control and more spheroidal mounds, the surface area of a more spheroidal mound is obviously still large enough for efficient gas exchange. Although CO_2 concentrations increased temporarily in wet mounds, they were generally very low (chapter 2). Also, the lack of specific ventilation structures inside the mounds argues against an adaptation of ‘magnetic’ termite mounds to facilitate gas exchange as there is simply no need for particular efficient ventilation: *A. meridionalis* colonies are very small and do not harbour a fungus that may produce comparatively high levels of CO_2 as has been shown in fungus-growing termites (Korb & Linsenmair 2000; Turner 2001) and ants (Kleineidam & Roces 2000). Similarly in the grass-harvesting termite *Trinervitermes trinervoides,* CO_2 levels were much lower than has been described in fungus-growing termites (Seiler et al. 1984). Therefore, the importance of effective gas exchange seems to be related to fungus cultivation and hence, not a problem for *A. meridionalis*.

If ‘magnetic’ termite mounds are not primarily adapted to facilitate gas exchange, what role does food availability play? While CO_2 is obviously not an issue for *A. meridionalis* colonies, food availability could well become one: both quantity and quality (measured as the inverse of fungal load) of the food stores decreased significantly with progression of the rainy season (chapter 2). For a colony that is restricted in its foraging activity to probably only a few months (because of habitat flooding and availability of dead plant material) and therefore completely dependent on stored food for approximately six months, preservation of food stores could become an important issue. However, we did not detect significant differences between more spheroidal and control mounds, probably because the surface area of a more spheroidal mound was also still large enough to store and ventilate the food, at least during the course of this study. But although the drying of mound walls by sun had no influence on gas exchange (see above), it seemed to have an influence on the quality of food stores: samples of shaded mounds had numerically higher fungal loads than those of control mounds at two out of three sampling dates during the rainy season. Due to high variability of the data and a relatively small sample size, this difference was statistically not significant. However, the particularly strong dependence of colonies from their food stores makes the importance of a large, well ventilated surface area for food storage reasonable. Of course there are other termite species that are also strongly dependent on their food stores due to a period of restricted foraging activity but do not built meridional mounds. Adam (2008) showed that it is crucial for *Trinervitermes trinervoides* workers to store as much grass as possible for the winter when foraging activity is restricted. But here, the cool winter is only three months long in contrast to nearly six months of flooding of *A. meridionalis* colonies. Thus, the pressure to maintain the food stores might be even higher for *A. meridionalis*, which could have selected a high surface area for well ventilated food stores.

Although the surface area of a large spheroidal mound is obviously still large enough to store and ventilate food, a lot of the dense internal mound space will be useless for this function. For a small colony as in *A. meridionalis*, building effort could be a limiting resource which, in the long run selected elongated mounds over more spheroidal mounds.

Is the shape of thermoregulatory significance for the colony?

A thermoregulatory explanation of the ‘meridional type of construction’ has long been hypothesized (Hill 1942; Gay & Calaby 1970) and rotation experiments have emphasized the importance of the meridional orientation for areas with relative thermostability (the core, Grigg 1973; the eastern face, Jacklyn 1992). But if the termite colony prefers warm and stable temperatures, especially during the colder and thermally more unstable dry season – is the ‘meridional type’, i.e. the north-south elongated wedge shape really the best option? Because we manipulated living *A. meridionalis* colonies to a more spheroidal and therefore in theory more thermostable shape, we were able to compare the temperature regimes of wedge-shaped control mounds and more spheroidal experimental mounds directly for the first time. The results clearly showed that i) compared to the mound faces, the core was the most thermostable area and ii) the core temperatures of more spheroidal mounds were even more stable than those of wedge-shaped control mounds. Thus, thermostability alone cannot explain the unique shape in *A. meridionalis*.

Although the meridional shape is apparently not an adaptation to enhance thermostability, it could offer other thermoregulatory advantages for the termite colony. According to the data of this study, one advantage could be the higher core temperatures of wedge-shaped mounds compared to more spheroidal mounds during the dry-wet transition and the rainy season. During the dry-wet transition, winged sexuals (alates) develop which will leave the nest attempting to found new colonies at the beginning of the rainy season (unpubl. data). Increased core temperatures could be important for their development. A similar explanation has been proposed for increasing core temperatures in *T. trinervoides* (Field & Duncan 2013), and *in vitro* studies of *Odontotermes wallonensis* also showed that higher temperatures resulted in higher development rates from nymphs to alates although this was not tested statistically (Prahlad & Chimkod 2012).

In contrast, higher core temperatures during the rainy season could be important for embryonic development. According to Garcia & Becker (1975) the optimum temperature for embryonic development in *Nasutitermes nigriceps* is 30 °C. For *O. wallonensis*, the optimum temperature for nymphs hatching from eggs was up to 40 °C (not tested statistically, Prahlad & Chimkod 2012). If embryonic and/or sexual development proves to be enhanced by increased temperature this would be a strong selective force favouring an elongated wedge-shaped architecture. Unfortunately, such data are lacking for *A. meridionalis*.

Conclusion on mound shape

There is no single answer regarding the biological significance of the meridional shape of *A. meridionalis* mounds. According to the results of this study, the facilitation of gas exchange through the large surface area seems to be less important. For food storage, on which the colony is highly dependent, a large surface area would be advantageous. Also for a quick and thorough heating of the core that might be favorable for the development of termites, a large surface area offers advantages. All these advantages could compensate for the decreased thermostability of a wedge shape that can be partly overcome by its meridional orientation. *Amitermes meridionalis* has small colonies where all colony members are mobile (queen and larvae) and can escape extreme temperatures (pers. obs). A colony that puts all its building effort in building a flat surface that offers the mentioned advantages might thus be selected over a colony that wastes building effort to build a dome-shaped mound that is slightly more thermostable but does not offer any of the other advantages.

The ultimate advantage of a meridional shape seems to be the opportunity to colonize an inhospitable habitat that suffers flooding during the rainy season: only termite species with the ability to build meridional mounds have so far managed to overcome the difficulties associated with flooding over a period of nearly six months. The upside of this habitat is its rich grass resource that is also exploited by other termite species during the dry season (e.g. *Nasutitermes triodiae*, pers. obs.). However, *A. meridionalis* is much more abundant on such flood plains, and they presumably get the biggest share.

The functional significance of termite mounds will rarely be the result of only a single function. Instead, most likely several factors have to be acknowledged for the explanation of the functional significance of mound shape and sometimes, mound architecture might be the result of a trade-off between different functions (Korb 2011). Evolution does not imply a purposeful design and consequently, the current state may not always be the optimum – it might be just the compromise between different suboptimal options.

The influence of irradiance and shading conditions on 'magnetic' termite mounds

Shade has been proposed as an important environmental factor for the functional adaptation of 'magnetic' termite mounds and long-term shading conditions predict the geographic variation in mean mound orientation (Jacklyn 1992). Additionally, there have been recent observations that *A. meridionalis* mounds seem to thrive less well in habitats subject to overgrowth by bushes and trees (P. Jacklyn, pers. comm.). Regarding their endemism and very limited distribution in northern Australia, this fact could be of significance for conservation. The results of this study show that shade from above only reduced face and therefore core temperatures significantly during the rainy season because of the high solar altitude during the tropical summer (Fig. S6, Table S6). However, this could be a crucial time for embryonic development, and the reduced temperatures might thus have a negative impact on the colony. Shading also significantly reduced the drying of the mound walls and wet mounds had significantly higher levels of CO_2 (chapter 2). Differences between shaded and control mounds were, however, not significant which might be due to the large variability of the data. More obvious was the influence of shade on the food stores: the samples of shaded mounds had numerically much higher fungal load than those of control mounds in January and February (chapter 2). Thus, a negative influence of shade on the functioning of *A. meridionalis* colonies is supported by the data of this study.

Genetic drift and its effects on *A. meridionalis* subpopulations: gene flow, subpopulation differentiation and genetic diversity

Although *A. meridionalis* has a unique island-like distribution and there are many questions regarding the founding of new colonies and the breeding system, no genetic studies tackling these questions have yet been undertaken.

To answer population and colony genetic questions, ten species-specific microsatellite markers were developed for *A. meridionalis* that proved to be variable and reliable (chapter 4). Of these ten markers, seven were employed for genetic analyses of nine subpopulations throughout the species' range. The genetic analyses revealed astonishing results: the genetic differentiation between subpopulations was one of the highest ever recorded in social insects and animals in general (F_{GT}: 0.339; R_{GT}: 0.636; Table 5.2a). Values higher than 0.25 are considered to reflect very large differentiation (Hartl & Clark 1989). Even subpopulations in close proximity (e.g. H - I: 3.8 km; A - B: 5.9 km) were significantly differentiated. Such high values of population differentiation are usually interpreted as a lack of gene flow due to large distances or geographic/biological barriers. Consequently, gene flow between subpopulations was very low and resulted in significant isolation by distance. Most subpopulations were significantly inbred and showed high values of overall relatedness. These results imply that the dispersal abilities of *A. meridionalis* are very low and the inbred subpopulations are likely to be influenced by negative effects of genetic drift. This in turn raises the question how stable these small and isolated subpopulations are over time.

But maybe even more surprising than the exceptionally strong subpopulation differentiation over short distances was its effect on the genetic diversity of small and isolated subpopulations: none of the subpopulations showed low genetic diversity. Consequently, different measures of genetic diversity did not correlate with subpopulation size or degree of isolation. Contrary to expectations from conservation genetics, very low levels of gene flow and high levels of inbreeding had no negative effects on the genetic diversity of *A. meridionalis* subpopulations.

The explanation of this unexpected finding lies in another result of the population genetic analyses: not only the subpopulations were significantly differentiated but also the colonies within subpopulations. The value for colony differentiation was of similar magnitude as the subpopulation differentiation (F_{SG}: 0.394; R_{SG}: 0.369; Table 5.2b). And while subpopulations had private alleles, also colonies within subpopulations had their own sets of alleles or allele frequencies, respectively. The genetic analyses of colonies showed that relatedness within colonies was very high and presumably caused by inbreeding. As indicated by the number of alleles and genotypes, colonies were founded by a monogamous pair of unrelated reproductives (maximum of four alleles at a locus) but not all genotypes could have been produced by this primary reproductive pair since five or more genotypes in a colony were common. This indicates that colonies are inherited by the offspring of the colony founders and indeed, so-called 'replacement reproductives' have been described in *A. meridionalis* (Hill 1942; Gay & Calaby 1970). Inheriting colonies makes also sense in light of the 'founding paradox' and other observations: as most other termite species (Eggleton 2011), *A. meridionalis* produces winged sexuals (alates) once a year that have a nuptial flight on one or few days after the first strong rain at the beginning of the rainy season (pers. obs.). After the nuptial flight and the subsequent mating, the founding pairs burrow into the soil to be protected against desiccation and predators. Paradoxically, the nuptial flight occurs shortly before the onset of the monsoon that causes water-logging of the habitat. So the termites seem to have little time to establish their colonies and to build up stocks for the rainy season when they are confined to their nests. In line with this, young colonies are rarely found and subpopulations seem to consist mainly of established colonies of a certain size range. According to Garnett et al. (in preparation), *A. meridionalis* mounds seem to be very old and new foundation events are very rare. Thus, established mounds could be a valuable resource that is continuously passed on to the next generation.

The longevity and significant genetic differentiation of colonies might therefore result in loss of genetic diversity through inbreeding within a colony but maintenance of genetic diversity at the subpopulation level. On the total population level, the strong differentiation between subpopulations and their specific sets of private alleles contribute to increased overall genetic diversity (Frankham et al. 2009).

Future perspectives

Investigations of mound shape

To reach further conclusions about the shape of ‘magnetic’ termite mounds, similar experiments with the widely studied ‘magnetic’ termite *A. laurensis* could offer interesting insights. *A. laurensis* is a facultative meridional mound builder which constructs small dome-shaped mounds on well-drained areas but meridional mounds on seasonally-flooded plains.

To test the effect of colony size on CO_2 levels in large spheroidal mounds, a comparison with the cathedral termite *Nasutitermes triodiae* seems promising: this species has a similar internal structure as *A. meridionalis*, is a grass-harvester and lives right on the non-flooded edges of the *A. meridionalis* habitats. Thus, it experiences similar temperature and rainfall regimes but its population sizes are probably several orders of magnitudes higher.

Genetic analyses

Now that variable and reliable microsatellites have been established as genetic markers for *A. meridionalis*, it would be possible to investigate its breeding system and take a closer look at the genetic structure of colonies (reviewed in Vargo & Husseneder 2011). The present study focused on population genetics and therefore we reduced the sample size of genotypes per colony and instead sampled more subpopulations. To analyze the breeding system reliably, a sample size of 20 workers per colony is appropriate (Vargo 2003; DeHeer et al. 2005; DeHeer & Vargo 2008). In contrast, it is not necessary to study many different populations (Vargo & Husseneder 2011).

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Acknowledgements

Firstly, I want to thank my advisor Prof. Judith Korb for the opportunity to work on this project and for her support during all of its phases - particularly the statistical analyses and the writing of the manuscripts.

Many thanks go to Peter Jacklyn for his assistance in the field and his support with data interpretation and development of ideas.

The support of Prof. Jürgen Heinze is also very much appreciated, particularly regarding the possibility to work in his lab and his willingness to write the “Zweitgutachten”.

Thanks also to the German Science Foundation (DFG) for their financial support of this project.

I sincerely thank the whole teams at the School for Environmental Research (Charles Darwin University) and at the Universities of Regensburg and Osnabrück for their support with field and lab work and for making all my different work places a friendly space.

Craig Hempel, Phil Hickey and many more helped me constantly in the field although the monsoon often hit us hard - thanks to all you guys!

Many thanks go to Julio Canales Delgadillo, Sebastian Klaus, Tobias Lenz and Barbara Hausberger for much valued input into this thesis and to Christoph-Lennart Wagener, Nina Schmidt-Jahre and Gesa Braun for their professional help with the artwork.

Lastly, I want to thank all my friends and family, all above Christoph Gawlik, for their ongoing moral support during the years of hard work - thanks for your understanding and encouragement to “pull through” … I would not have made it without you!

Appendix

Chapter 2 Supplementary

Description of Seasonal Rainfall Characteristics

The nearest weather station from the study site, Southport NT (014206) was opened in 2006 and recorded 2452.6 mm of rainfall in 2007 (when most measurements were done, see below), slightly more than in subsequent years. Particularly in March and November 2007 the amount of rainfall was higher than in subsequent years. The rainfall preceding the gas measurements is listed in Table S2.1 (Commonwealth of Australia 2012, Bureau of Meteorology; available from http://www.bom.gov.au/climate/data/).

Table S2.1

Amount of rainfall on days shortly before and during the CO_2 measurements, taken at two different weather stations. Rainfall readings are taken at 9 am local time.

Date	CO_2-Measurement	Rain at field site?	Rainfall Darwin Airport (mm)	Rainfall Southport (mm)
26.01.2007	no		0.6	2
27.01.2007	yes	yes	0	59.4
28.01.2007	yes	yes	18.4	125
29.01.2007	no		2.4	32.4
30.01.2007	no		1.6	6.6
31.01.2007	yes	no	0.2	16.4
01.02.2007	yes	no	1.6	0.8
23.02.2007	no		10	21.2
24.02.2007	yes	little rain	16.4	17
25.02.2007	yes	little rain	40	9.2
28.03.2007	no		0.2	14.6
29.03.2007	yes	little rain	4.8	0
30.03.2007	yes	little rain	4.2	13.4
27.09.2007	no		0	0
28.09.2007	yes	little rain	2.4	4.2
29.09.2007	yes	little rain	41.2	13
21.10.2007	no		0	0
22.10.2007	yes	no	0.6	3.4
23.10.2007	yes	no	0.4	0
19.11.2007	no		6.2	56.4
20.11.2007	yes	no	0	24.6
21.11.2007	yes	no	1.2	0.2
09.12.2007	no		0.2	0.2
10.12.2007	yes	yes	3.8	0.6
11.12.2007	yes	yes	10.8	4.8
Distance to field site			ca. 37 km	4.8 km

Figure S2.1

This photograph shows pieces of recently abandoned *A. meridionalis* mounds that were attached to the eastern and western face of one of the ten experimental mounds to alter the mounds' shape. Note the darker, more friable material between the pieces which is new mound material built by termites. The photograph shows that the added pieces were integrated into the mound structure by the termites.

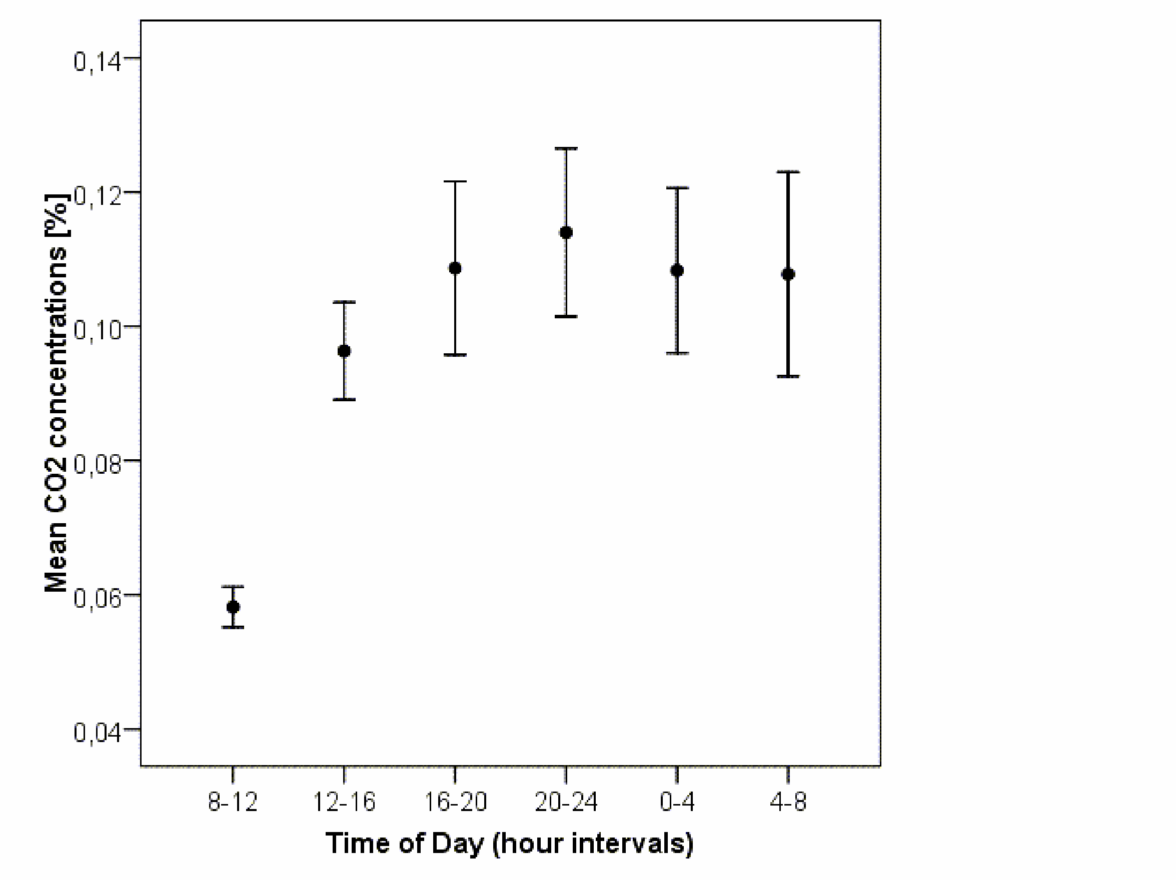

Figure S2.2

Mean CO_2 concentrations in 0.5 m height of dry control mounds during the rainy season. The correlation between the CO_2 concentrations and the time of the day was significant (Linear mixed model: $F_{1,156} = 8.59$, $P < 0.002$).

Figure S2.3

These photographs show the inner architecture of *A. meridionalis* mounds. The mounds consists of a solid core in the lower third of the mound while the upper and outer areas of the mound consist mainly of cells in which food is stored connected by small tunnels which allow movement by the termites through the mound.

Chapter 3 Supplementary

Seasonal effects on daily temperature summaries

During the dry season, daily face temperatures of unmanipulated mounds fluctuated greatly, ranging from about 5 °C to 58 °C (Fig. S3.1a). The mean and minimum temperatures of the dry season were significantly lower than during other seasons (always $P < 0.001$; Table S3.1). By contrast, during the dry-wet transition, significantly higher mean and maximum temperatures were recorded than during other seasons, while the CV was significantly lower during the rainy season (always $P < 0.001$; Table S3.1). During the rainy season, the daily temperature pattern was not as regular as during the dry season (Fig. S3.1b).

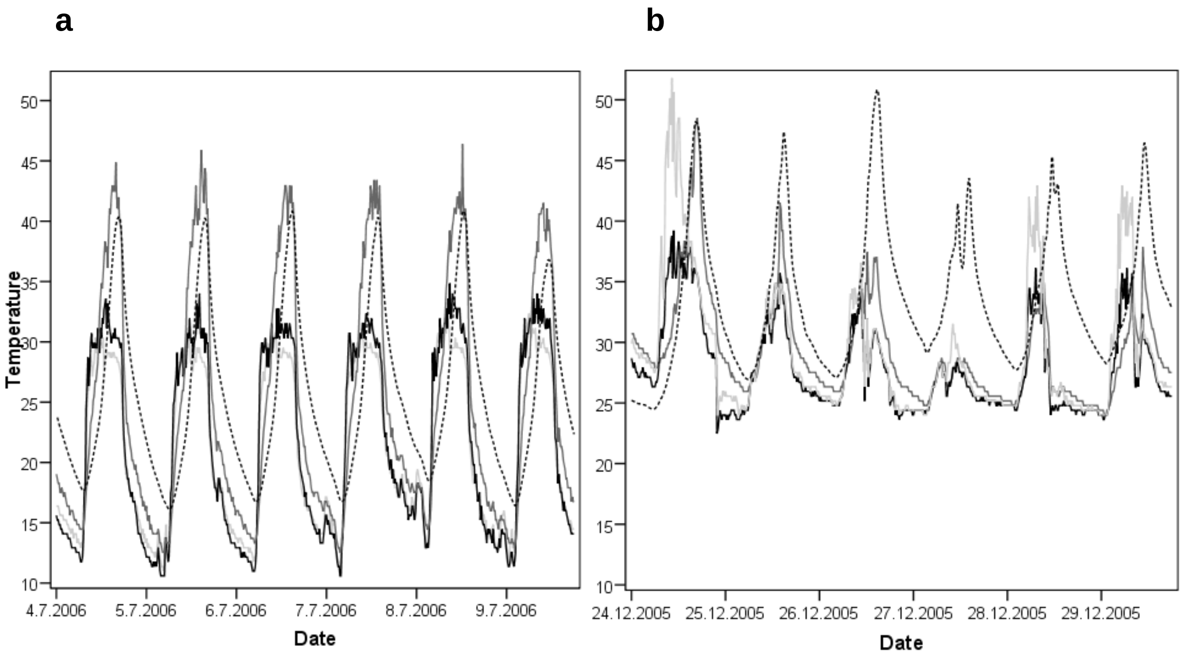

Figure S3.1

Face and core temperatures [°C] of a representative mound during six representative days during the **a** dry season and **b** rainy season, (*light grey* = eastern face; *medium grey* = western face; *dark grey* = northern face; *dotted line* = core).

Table S3.1

Result of ANOVAs that tested the effect of season on temperature summaries, separately for each face.

Direction	**Eastern Face**	**Northern Face**	**Western Face**
Df	5,8242	5,8128	5,7771
F	193129.300	257421.811	368972.275
P	< 0.001	< 0.001	< 0.001

Chapter 5 Supplementary

Table S5.1

Summary of a survey of mound height and survival on a field of *Amitermes meridionalis* mounds in 1987, 1996 and 2012. 20 out of 34 initial mounds were still alive in 2012 – 25 years after the initial survey.

Mound	October 1987 height (m)	May 1996 height (m)	April 2012 height (m)
1	1.95	2.3	2.35
2	2.1	2.5	2.4
3	2.0	2.5	2.6
4	3.5	3.5	3.4
5	2.5	fallen	not seen
6	3.0	1.29	0.75
7	2.4	2.3	not seen
8	2.0	2.1	not seen
9	2.35	Dead fallen (2.35m)	not seen
10	2.0	2.5	2.8
11	2.8	3.0	3.1
12	2.3	2.6	2.7
13	2.2	fallen	not seen
14	1.8	2.3	2.4
15	1.8	2.2	2.1
16	2.9	2.9	not seen
17	2.4	shallow dome	not seen
18	2.4	2.6	not seen
19	1.65	1.9	not seen
20	1.7	2.4	not seen
21	1.75	2.0	not seen
22	1.5	1.75	not seen
23	2.05	shallow dome	not seen
24	2.5	2.6	2.8
25	2.9	3.2	0.9
26	2.3	2.6	0.5
27	3.2	3.6	3.3
28	2.3	2.7	2.9
29	2.2	2.5	2.6
30	2.4	2.8	3.0
31	1.5	1.1	0.9
32	2.0	2.2	2.5
33	1.95	2.3	2.8
34	2.05	2.3	not seen
35	not seen	1.8	2.3
36	not seen	1.9	2.4

37	not seen	1.9	2.2
38	not seen	1.25	2.6
39	not seen	1.1	2.2
40	not seen	1.0	1.8
41	not seen	1.45	2.45
42	not seen	1.3	not seen
43	not seen	1.2	not seen
44	not seen	not seen	1.5
45	not seen	not seen	2.0
46	not seen	not seen	1.8
47	not seen	not seen	0.4
48	not seen	not seen	1.25
49	not seen	not seen	1.1
50	not seen	not seen	1.5
51	not seen	not seen	0.95
52	not seen	not seen	1.1
53	not seen	not seen	0.85
54	not seen	not seen	0.4
55	not seen	not seen	0.2
56	not seen	not seen	0.5

Only mounds large enough to be clearly meridionally elongated and of the distinctive *A. meridionalis* type were included in the survey. Mound locations were determined manually on a grid system for the first two surveys in 1987 and 1996 and with GPS for the 2012 survey. Two mounds were tagged, but the repeated surveys showed that nearly all mounds were easily identifiable from their location, size, orientation etc. Mound height was measured in metres from the mound base to highest point above the ground.

This field is close to a rural area and the western edge of it was cleared in the late 1990s. This seems to have resulted in the deaths of several mounds (16-23 in the Table S5.1).

Table S5.2

AMOVA of a dataset consisting of only one individual per colony to ensure genetic independence of samples. The results are qualitatively the same as those for the whole data set.

Source of variation	**% of total Variation (F_{ST})**	**% of total Variation (R_{ST})**	***F*-statistics**	***R*-statistics**
Among subpopulations	34.23	70.03	$F_{CT} = 0.342$	$R_{CT} = 0.700$
Among colonies within subpopulations	21.33	14.73	$F_{SC} = 0.324$	$R_{SC} = 0.491$
Within colonies	44.44	15.24	$F_{ST} = 0.555$	$R_{ST} = 0.848$

Table S5.3

A priori defined vicariance groups to test for vicariant effects. The geographic distances are given between the subpopulations within one group (e.g. between subpopulation A and B in Group 1) or between the single subpopulation and the nearest subpopulation (e.g. subpopulation K). For all other geographic distances between subpopulations, please see Table 5.3 in the main publication. For the location of subpopulations, please see Fig. 5.1b.

Vicariance group	**Included subpopulations**	**Geographic distance between subpopulations [km]**
1	A, B	5.94
2	E, F	16.12
3	G, H, I	3.80 (H-I)
		23.62 (G-H)
		25.50 (G-I)
4	K	36.45 (K-B)
5	P	47.50 (P-K)

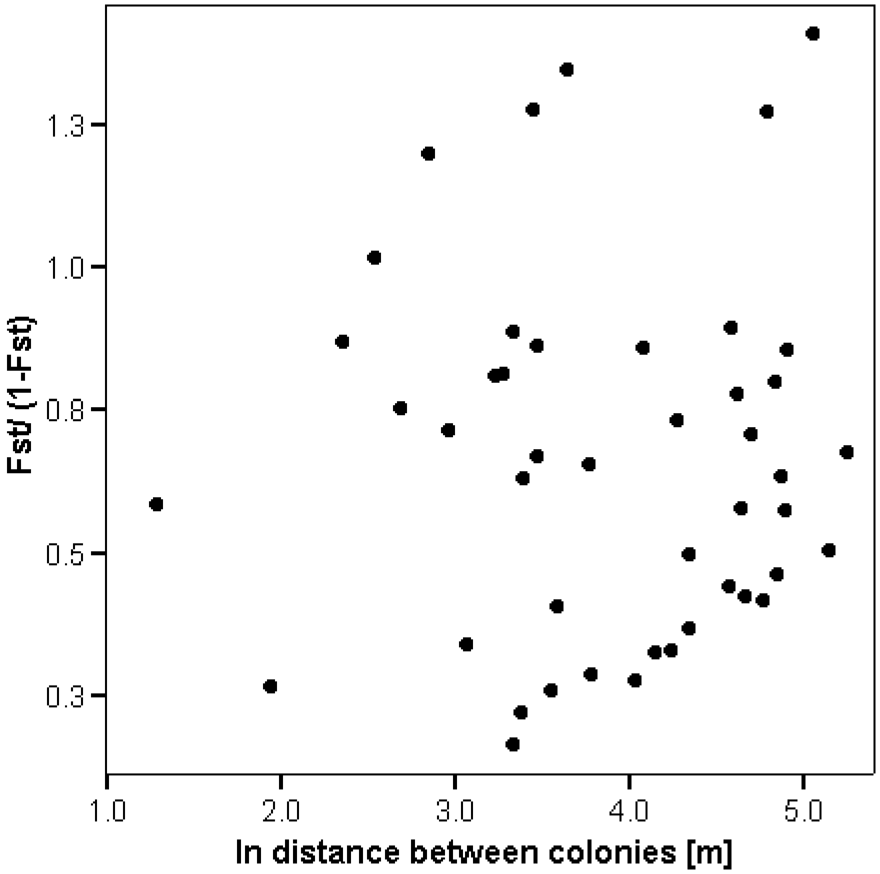

Figure S5.1

Example of an Isolation-by-distance analysis of the colonies in a study subpopulation, here subpopulation B. The correlation between pairwise estimates of genetic and geographical distance of colonies in all subpopulations was not significant (Mantel test: $r = 0.09$, $P = 0.348$).

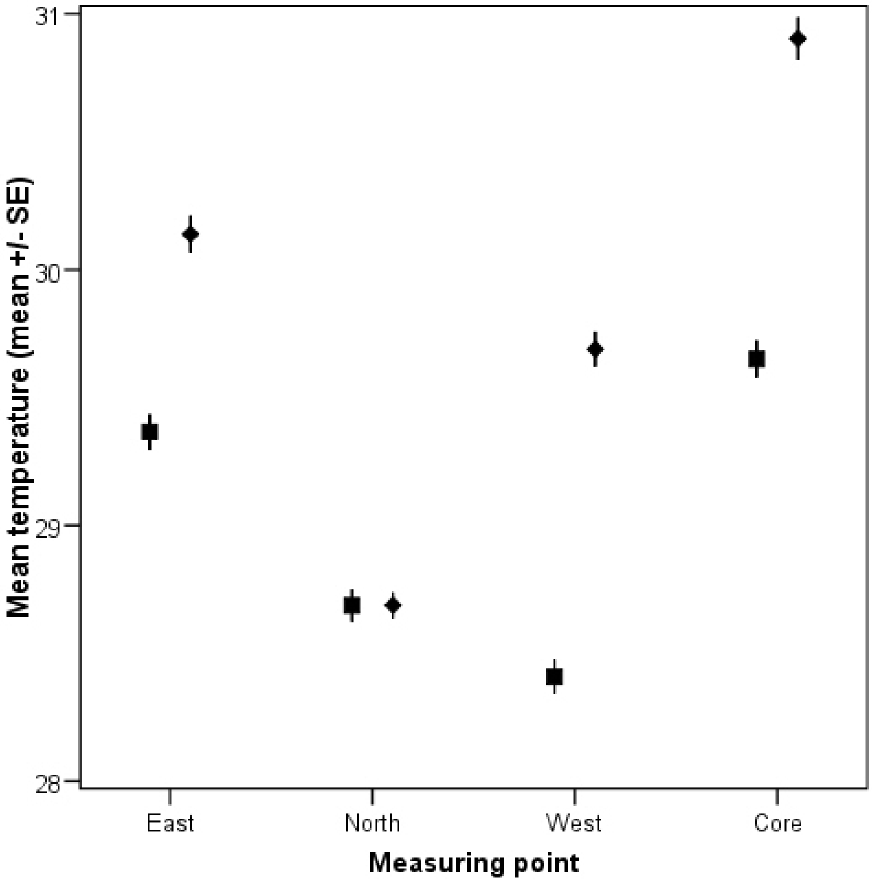

Figure S6

Mean (± 1 SE) of the mean face and core temperatures of experimental mounds during the rainy season (*filled diamond* = control mounds, *filled squares* = shaded mounds). Mean face and core temperatures of shaded mounds were always significantly lower than mean face and core temperatures of control mounds (except the northern face, Table S6).

Table S6

Influence of shade on mound core and face temperatures. Shown are the results of linear mixed models comparing core and face temperatures of unmanipulated mounds to core and face temperatures of shaded mounds during the rainy season.

Temperature summary	Results	Core	East	West
Mean daily temperature	Df	2,28	2,24	2,24
	F	25.34	4.90	3.77
	P (Control vs. shade)	< 0.001	0.011	0.015