STUDIES OF THE NESTS OF THE FUNGUS-GROWING TERMITE *MACROTERMES NATALENSIS* (ISOPTERA: MACROTERMITINAE)

by

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ABSTRACT

Monthly sampling of 71 laboratory nests, each with a pair of adults, revealed that eggs and first-instar larvae were observed in the third month, minor workers in the fourth month and minor soldiers in the seventh month. Mortality of the pairs was high, with only five pairs surviving over the 10 month period. Laboratory nests did not develop further than the copularium.

Excavation of 30 nests, of differing sizes, revealed that five were juvenile nests, consisting of only thin shelving with a few flattened fungus combs scattered throughout, and all, even the youngest nest (3 to 5 years), had a small mound. The queens from these young nests were small and had white pleural and intersegmental membranes. Twenty mature nests had a medium to large mound with large air passages and a medium to large hive with a well defined fungus garden containing large fungus combs. The queens from these nests were medium- to large sized, with white to brown pleural and intersegmental membranes. The remaining five nests had mounds often covered with grass, and a hive that contained less fungus comb than expected. The mounds of these nests were classified using their sandy pediment or crumbly texture. These were senescent or declining nests. The queens had pale brown pleural membranes and brown intersegmental membranes, and were often flaccid. The royal cell was commonly found in the middle to upper part of the nursery (20 nests), but sometimes was found at the edge of the nursery (five nests). The royal cells of five nests were not found or had been destroyed during excavation. The "youngest" mound was one to two years old and the "oldest" was more than 25 years old. The youngest queen was estimated to be three to five years old and the oldest queen more than 27 years. The nest seems to remain subterranean for two or less years before producing a mound. Thirteen nests were vigorous and five declining. The remaining 12 nests could not be classified as no fungus comb was collected from the nests.

KEY WORDS:
Fungus-growing termites, Macrotermitinae, *Macrotermes natalensis*, nest structure, nest age, queen age, nest vigour, KwaZulu-Natal.

PREFACE

The work described in this dissertation was carried out in the Department of Zoology and Entomology, University of Natal, Pietermaritzburg, from January 1992 to December 1994, under the supervision of Doctor R.M. Miller and Doctor P.R. Atkinson (Institute for Commercial Forestry Research, University of Natal).

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others it is duly acknowledged in the text.

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CHAPTER 1
INTRODUCTION

1.1 TERMITES

The Order Isoptera comprises five families: Kalotermitidae (dry-wood termites), Hodotermitidae (harvester termites), Termopsidae (damp-wood termites), Rhinotermitidae (subterranean termites), and Termitidae (common termites) (Ruelle, 1978, 1985). The Termitidae is the largest of these families, including about 80% of all species, and is divided into four sub-families: Apicotermitinae, Termitinae (common termites), Macrotermitinae (fungus-growing termites) and Nasutitermitinae (snouted termites) (Ruelle, 1978, 1985). The Macrotermitinae comprises 12 genera, 10 of which are found in the Afrotropical region: *Acanthotermes*, *Allodontermes*, *Ancistrotermes*, *Macrotermes**, *Microtermes**, *Odontotermes**, *Protermes*, *Pseudacanthotermes*, *Synacanthotermes* and *Sphaerotermes* (Krishna, 1970; Hickin, 1971). Five of these are of economic importance in southern Africa: *Ancistrotermes*, *Allodontermes*, *Microtermes*, *Macrotermes* and *Odontotermes* (Ruelle, 1985).

Many authors have reviewed the distribution of termites according to the biogeographical regions (Krishna & Weesner, 1970). Most termites are found predominantly in the tropical regions of the World (Krishna, 1969; Hickin, 1971) because of their need for a closed, warm, saturated environment (Krishna, 1970). Certain taxa (some Hodotermitidae, Nasutitermitinae and Macrotermitinae) have invaded temperate regions as far as 48°N and 45°S (Lee & Wood, 1971a; Nel, 1983). Where annual rainfall is low, the economic importance of grass- and wood-feeding termites increases dramatically (Cowie, 1988). Deshmukh (1989) reviewed current data and found that Macrotermitinae increased in percentage, of the total termite population, from 36% in high (1292 mm) rainfall regimes to 99% in low (230 mm) rainfall regimes.

Termites are polymorphic, social insects that live inside nests (termitaria) which they construct from various materials including soil, wood, and carton (organically rich material). Noirot (1970) and Lee & Wood (1971a) have reviewed the materials from which termitaria are constructed.

* also found in the Oriental region

Termites have also been grouped into four ecological categories: dry wood (Kalotermitidae), damp wood (Termopsidae), subterranean (Rhinotermitidae) and harvester (Hodotermitidae), usually depending on where the nests are built (Hickin, 1971; Nutting & Jones, 1990). However, there appears to be considerable variation, and species of the Termitidae have representatives in each ecological category. Termite nests are classified into five structural types (Noirot, 1970): nests in wood, nests in soil (subterranean), nests above the soil (epigeous), nests in trees (arboreal) and nests within nests (inquilinism). Nests may be diffuse (Kalotermitidae and Termopsidae), have interconnected, partitioned chambers (Hodotermitinae), or be discrete, concentrated systems (some Macrotermitinae) (Lee & Wood, 1971a).

The termite species living on plant material are phylogenetically primitive taxa (Kalotermitidae, Hodotermitidae, Termopsidae and Rhinotermitidae) which rely heavily on gut symbionts (protozoa and bacteria) for the breakdown of cellulose and lignin (Honigberg, 1970; Hickin, 1971, Romoser, 1981). However, the Macrotermitinae rely on external fungal decay of the food source to render it digestible (Adamson, 1943; Romoser, 1981). These termites, therefore, collect the food material which is then brought into the nest and processed before it is added to the fungus combs. The food material is collected from a variety of sources, depending on the species (Hickin, 1971).

1.2 ECONOMIC IMPACT

Harvester termites are recognised as pests in South Africa and Australia because they compete with livestock for grass (Harris, 1961). Dry-wood termites generally live in the dead branches of living trees, but the two species *Neotermes gestri* (Silvestri) and *N. aburiensis* (Sjöstedt) are pests in a number of crops such as teak, cocoa and tea (Harris, 1961; Hickin, 1971). Several *Coptotermes* spp. usually inhabit rotting logs in forests, but have become pests of *Havea, Eucalyptus*, and *Citrus* (Harris, 1961).

One group in particular, the fungus-growing termites, which is the predominant group of the sub-Saharan and Oriental biogeographic regions, is a serious soil pest affecting both agriculture (Hickin, 1971; Cowie & Wood, 1989; Dindal, 1990; Nutting & Jones, 1990) and forestry (Harris, 1961; Atkinson, 1986, 1989; Varma, 1990; Atkinson *et al.* 1992). In South Africa, indigenous trees are seldom attacked, whereas exotic trees, particularly *Eucalyptus* spp. and Black wattle (*Acacia* *mearnsii* De Wild), are often heavily damaged (Atkinson, 1986, 1989, Atkinson *et al.* 1992). The genus *Macrotermes* is the most prominent of the subfamily with respect to agriculture (Coaton, 1950) and forestry (Atkinson, 1986, 1989; Atkinson *et al.* 1992). The most common species of the genus in Kwazulu-Natal is *Macrotermes natalensis* Haviland (Ruelle, 1970; Mitchell, 1980). For this reason, this genus forms the basis of the present study.

1.3 GENERAL BIOLOGY AND ECOLOGY OF THE MACROTERMITINAE

1.3.1 Life cycle

Dispersing alates, once settled, shed their wings by a quick shrugging movement. The female remains stationary with her abdomen extended upwards, at an angle, releasing a pheromone to attract the male. When the male finds the female they adopt a tandem position, with the male following the female (Nutting, 1969; Stuart, 1969). Once the female selects a suitable site for the nest the insects dig into the soil. They dig to a depth ranging from 220-300 mm (Coaton, 1949a; Abe & Darlington, 1985). Once they have ceased digging the insects construct a cell (copularium) in which the female will lay her fertilised eggs. When the eggs hatch the nymphs are fed by the female until the workers appear and can forage on the surface. After a variable number of years, depending on the species, winged reproductives are produced (Collins, 1981; Darlington & Dransfield, 1987; Nutting, 1969; Pomeroy, 1976a) which remain inside the nest until environmental conditions are suitable for them to emerge and found a new nest.

Very little has been done on development, growth and especially aging of mounds and colonies, particularly from the foundation of the nest to the visible, epigeal phase (Pomeroy, 1976a; Noirot, 1970, 1977; Collins, 1981; Darlington & Dransfield, 1987). This is probably because fungus-growing termites are not easily cultured (Becker, 1969). As a result, exact information regarding the relationship between age and colony size is available for only one species (*Macrotermes bellicosus* (Smeathson)) from Uganda (Pomeroy, 1976a) and Nigeria (Collins, 1981). Although Abe & Darlington (1985) did studies on structure and populations of nests of *Macrotermes michaelseni* (Sjösted) from Kenya, they did not quantify growth. Furthermore, no studies have been done for the southern African species. Thus, the information and data collected in this study are of major importance, not only for comparison with equatorial species, but to better the control of termites, especially in the forestry industry. A general account of the development and growth of nests is given in Noirot (1969) and Abe & Darlington (1985). The latter authors refer to three stages in the life cycle of the nest: founding (the period until the first brood of workers reaches the adult stage), ergonomic (subdivided into three sub-groups depending on the presence or absence of a mound and colony size), and reproductive (when the first alates are produced). However, no actual ages were assigned to these stages in terms of years. Noirot (1969) gives a more accurate account by recognising three phases in the aging of nests: juvenile period (only neuters are differentiated), adult period (developing alates), and senescent period (decline in productivity). Collins (1981), who estimated maximum life span of *M. bellicosus* from mound growth measurements, was able to fit approximate ages to the three phases as follows: juvenile (zero to four or six years), adult (four or six to 10 or 12 years), and senescent (more than 10 or 12 years).

1.3.2 Nest shape and structure

Subterranean nests are frequent in the Macrotermitinae, as are epigeal nests, and possess many degrees of complexity. Some have diffuse chambers and galleries: *Microtermes*, *Ancistrotermes*, *Allodontermes* and certain *Odontotermes*, while others have chambers gathered together or concentrated: *Odontotermes badius* (Haviland), *O. formosanus* (Shiraki) and *Macrotermes* spp. (Noirot, 1970; Cowie & Wood, 1989). In concentrated nests there is a central part, variously referred to as the habitacle, hive or nursery. For the ~~purposes of clarity~~, in this study, the cavity beneath the mound, containing the fungus combs and the insects, will be referred to as the hive. The hive is divided into an outer area of fungus combs (the fungus garden) and an inner nursery zone which is filled with thin, horizontal shelving (Darlington, 1985b). It is in the nursery that the young brood is found. Characteristically, all Macrotermitinae have a royal cell, containing the king(s) and queen(s), which varies in position within the hive, depending on the species. In *Odontotermes badius* the cell is positioned next to the hive, and not in the hive itself (Coaton, 1949a); in *M. subhyalinus* the royal cell is positioned at the side of the hive (Darlington, 1984b); in *M. natalensis* the cell is in the middle of the hive (Coaton, 1949a,b), as it is in *M. michaelseni* (Darlington, 1985b). *Macrotermes* mounds are massive in parts of Kenya (Darlington, 1984a, 1985b) and sometimes contain ventilation structures and flues for the circulation of air (Lüscher, 1961; Ruelle, 1964; Darlington, 1984b). But *Macrotermes* spp. may build mounds without an obvious ventilation system; for example, in South Africa the mounds of *M. natalensis* (Haviland) nests do not have any holes leading down into the hive but often contain large air passages (Fuller, 1915; Coaton, 1949a) (see Fig. 4).

1.3.3 Fungi and fungus combs

Members of the sub-family Macrotermitinae share a mutualistic relationship with fungi of the two genera *Termitomyces* and *Xylaria* (Fuller, 1915; Sands, 1960; Coaton, 1961; Batra & Batra, 1979), although the growth of *Xylaria* may be suppressed (Batra & Batra, 1979; Crouch, *pers. comm.*). The fungus grows on a substratum (the fungus combs) which is situated in large chambers, surrounding the nursery. The nursery appears to be almost barren of fungus combs, although, a few flattened combs may be found here. There appears to be some controversy concerning the exact composition of the fungus comb. Sands (1960) observed the construction of fungus comb from faeces by *Ancisterotermes guineensis* (Silvestri). However, Batra & Batra (1979) claim the combs are built of finely divided plant tissues, a proportionately very small amount of insect remains, and insoluble particulate matter. The faecal-like particles are referred to as mylospheres (Grassé, 1978) to distinguish them from true faecal pellets, which they are not (Garnier-Sillam, 1989).

The comb material is moulded into intricate, spongy masses by the workers and inoculated with fungal spores. In the case of *Microtermes* sp. nr. *usambaricus* (Sjöstedt), *Macrotermes subhyalinus* (Rambur) and *Macrotermes bellicosus*, spores are introduced into the nest by the founder pair, which carry viable spores in the gut, from the old nest (Johnson, 1981). However, in the case of *Ancisterotermes guineensis* and some *Odontotermes* spp., spores have to be brought into the nest by the incipient foragers (Sands, 1960; Johnson *et al.* 1981). No information regarding the initiation of fungus combs in *Macrotermes natalensis* was available at the commencement of this study.

The fungal spores germinate on the combs and produce mycelia which spread over the surface and through the matrix of the comb. The germinating spores produce long, thin growths (hyphae), from which small, white, spherical bodies arise. These are what are variously termed conidia, conidiophores, mycotêtes spherules, and synemata (Coaton, 1961; Harris, 1961; Martin & Martin, 1978; Batra & Batra, 1979; Wood & Thomas, 1989). The conidia have periodically been seen to be eaten by nymphs and workers and it was thought that the conidia were acting as a nutrient or vitamin source (Coaton, 1961; Ruelle, 1985). It was later found that the conidia also provided the insects with an enzyme essential for the digestion of crystalline cellulose (Martin & Martin, 1978).

1.3.4 Ecology of the Macrotermitinae

Termites play a very important role in some ecosystems, particularly with regard to their contributions to energy flow and nutrient cycling (Ferrar, 1982a,b), as well as habitat modification (Lee & Foster, 1991; Pomeroy, 1989). The termites are important because they consume a wide variety of foods, including living vegetation, dead vegetation, dung, humus, fungi; even algae, lichens and dried tissues of vertebrate corpses may be eaten in certain cases (Nutting, 1990). Termites, therefore, return essential nutrients (such as nitrogen) and energy to the ecosystem by processing detritus. The two most important factors regarding the distribution of the fungus-growing termites are climate and soil type.

1.3.4.1 Climate

The limiting factors determining distribution appear to be mean annual rainfall and mean annual minimum temperature (Bouillon, 1970; Pomeroy, 1978). Ruelle *et al.* (1975b) found that *Ancistrotermes latinotus* (Holmgren) in southern Africa was not recorded where the annual rainfall was less than 250 mm. *Pseudacanthotermes militarus* (Hagen) and *P. spiniger* (Sjöstedt) were recorded in Zimbabwe only where the annual rainfall was 700 mm or more (Ruelle *et al.* 1975c). According to Pomeroy (1978) distribution of *Macrotermes subhyalinus* and *M. bellicosus* in Uganda is restricted to areas having a minimum annual rainfall of 300 mm and 700 mm, respectively, and a minimum temperature of 9°C and 12°C, respectively. In Namibia *Allodontermes* and *Microtermes* are restricted by an annual minimum rainfall of 100 mm and *Macrotermes* and *Odontotermes* by 200 mm (Coaton & Sheasby, 1972). Mitchell (1980) reported that in Zimbabwe, *Macrotermes subhyalinus* and *M. vitrialatus* (Sjöstedt) were not found where the minimum annual rainfall was less than 400 mm.

The concentrated subterranean nest system with large fungus combs may pre-adapt the Macrotermitinae to arid conditions, which, in part, explains why they have been able to invade some of the semi-arid to arid areas of the Oriental and Afrotropical regions. The fungus comb may assist the insects under arid conditions by 1) its role in temperature regulation (Geyer, 1951; Lüscher, 1961; Wood & Thomas, 1989), and 2) by increasing the efficiency of digestion of plant material (Wood & Thomas, 1989). It has also been suggested that the fungus comb may help to regulate the humidity within the nest, but it is probably the active transport of water by the workers which accounts for the majority of regulation of humidity (Wood & Thomas, 1989).

1.3.4.2 Soil

The majority of work concerning fungus-growing termites and soils has concentrated on how the termites affect the soil, mainly in terms of chemical constitution as well as physical properties of the soil (Nye, 1955; Lee & Wood 1971a; Pomeroy, 1976b, 1978, 1983; Malaka, 1977; Bagine, 1984). The most significant feature determining if a subterranean nest will survive on any particular soil type, is the proportions of sand, silt, and clay and their distribution throughout the soil profile (Hesse, 1955). A certain amount of clay is required to cement particles together, otherwise the intricate internal architecture would collapse. This accounts for the absence of mounds on pure sands (Lee & Wood, 1971a). However, too much clay results in the cracking of the soil as it dries. Mound builders are, therefore, seldom noticed on soils with a high proportion of the clay mineral montmorillonite (Lee & Wood, 1971a, Pomeroy, 1983). As *Macrotermes* mounds (in Africa) are constructed from soil derived from the subsoil (Hesse, 1955; Harris, 1961), it is in the sub-soil layer that the silt and clay:sand ratios are most important (Lee & Wood, 1971a). More *Macrotermes* mounds occur on soils with a clay content of 60% than on soils with a clay content of 50%, 40%, 30%, and 20% respectively (Lee & Wood, 1971a). However, soils with at least a 10% clay content are required to build the complex and intricate nests of Macrotermitinae (Boyer, 1982). Pomeroy (1977) proposed that the shape and size of mounds were probably determined by heat load, soil porosity, and the level of the water table, rather than by other soil characters. However, some mounds built by the same species, but on different soils are all similar which suggests a strong genetic component in the determination of the mound shape (Pomeroy, 1977).

Termites also affect the proportion of organic carbon (OC) of the soil, because the OC content of both the nest and the mound is higher than that of the surrounding soil (Lee & Wood, 1971b; Pomeroy 1976b). This is mainly due to the presence of organic materials (saliva and faeces) which are used to cement the soil particles together. It is only when the mound and hive erode away or collapse that the OC is released back into the system.

1.4 THE AFROTROPICAL SPECIES OF THE GENUS *MACROTERMES*

Of the 10 genera that occur in the Afrotropical region, *Macrotermes*, in particular, is responsible for most destruction of wood and other cellulosic materials (Fuller, 1915; Coaton, 1947; Coaton & Sheasby, 1972; Hickin, 1971; Varma, 1990). Of the 12 recognised species of *Macrotermes* (Ruelle, 1970) that occur in the region, six have been collected from southern Africa: *M. falciger*

(Gerstäcker), *M. mossambicus* (Hagen), *M. natalensis* (Haviland), *M. subhyalinus* (Rambur), *M. ukuzii* (Fuller), and *M. vitrialatus* (Sjöstedt) (Ruelle, 1970; Ruelle *et al.* 1975a). These species are all naturally open-woodland or savanna dwellers (Ruelle, 1978).

There has been some confusion concerning the distribution of *M. natalensis* as a result of misidentification and changes in species name. In 1970 Ruelle revised the genus *Macrotermes*, clarifying that *Bellicositermes natalensis* (Lüscher, 1955, 1961) was, in fact, *M. bellicosus*. *Macrotermes natalensis* occurs widely in southern Africa, with its distribution extending as far north as Mozambique and Zambia, according to the distribution maps of Ruelle (1970) and is the most common species of KwaZulu-Natal. Atkinson (1989) and Atkinson *et al.* (1992) clearly stated that *M. natalensis* is the cause of most forestry damage in KwaZulu-Natal.

1.5 THE PRESENT STUDY

Macrotermes natalensis is a particularly severe pest of forestry, especially in the initial establishment of *Eucalyptus* spp. (Gum) and *Acacia mearnsii* (Black Wattle) on grassveld. The termites attack young transplanted trees in their first six to nine months after planting (Coaton, 1957; Varma, 1990, Atkinson *et al.* 1992), attacks ceasing only after canopy closure. The termites are not a problem in the re-establishment of subsequent rotations, although there have been sporadic instances of serious mortality of seedlings (Atkinson, *pers. comm.*). Plants older than six months are still susceptible to attack for a further one and a half years (Atkinson, 1986, 1989; Coaton, 1957). If the trees survive the first two years, they appear to become resistant to attack. Although damage may still occur, it is often not serious enough to decrease the value of the timber (Atkinson, *pers. comm.*).

The problem is prevalent in deep, well drained soils (*i.e.* the better soils with high clay content, but low organic carbon), in drier (<*ca.* 950 mm annual rainfall) and warmer (<*ca.* 1400 m altitude) regions of KwaZulu-Natal and Mpumalanga Provinces. *Macrotermes natalensis* survives extensive cultivation, including deep ripping followed by harrowing, notwithstanding the complex structure of the nest which must presumably be destroyed in the process (Atkinson, *pers. comm.*). Pomeroy (1976a) noted this for *Macrotermes bellicosus*. The larger, epigeal mounds, which are prominent in the area, do tend to be destroyed, but, a considerable problem still remains. This could be emanating from hypogeal nests, because Abe & Darlington (1985) have indicated that there may be as many as 100-150 founding nests of *M. michaelseni* existing for every three mature mounds, in a Kenyan savanna. Furthermore, damage to forestry, crops and gardens also arises in situations where no epigeal nests are discerned, owing to previous cultivation, disturbance or development.

Epigeal nests are sometimes encountered which appear to be declining in vigour. These nests are characterised by their sandy, rounded external appearance, often covered with grass. Are such nests really declining, might they be recovering from disturbance, or does the nest appear to be recovering due to re-colonisation? Other colonies may occupy a mound after the death of the original builders (Darlington, 1984b, 1985b; Pomeroy, 1976a). The recolonisers may be of the same or different species as the original inhabitants (Pomeroy, 1976a). Darlington (1985b) reported that recolonisation of old nest sites of *M. michaelseni* in Kenya was common, the young nest having its hive partly or wholly above the external soil surface and often displaced to one side. Darlington (1984b) reported that recolonisation was widespread for *M. subhyalinus*, but did not give criteria for establishing whether a nest had been recolonised or not.

Invisible hypogeal nests, and the recovery of damaged, aged, or recolonised nests , are relevant to the effective chemical treatment and control of *M. natalensis*. Is it effective to treat mounds in general; or alternatively, is it better to protect the crop itself by using non-harmful controlled release granules which break down quickly, or the wooden structure of a building by the use of concrete foundations to raise the structure above ground level? Furthermore, fumigation of nests may be highly destructive to populations of an otherwise very useful insect (from the viewpoint of plant litter decomposition and nutrient recycling), whereas protection of the crop or wooden structure of a building would probably be the more desirable approach from an ecological and human point of view.

This project sets out to examine the structure, growth, aging and decline of nests of *M. natalensis* with the objectives to:

1) Determine and record the early growth phases and development of young, hypogeal nests from laboratory rearings, and to compare with data collected from nests excavated in the field.

2) Examine the structure, size and other variables of excavated nests on various soils and from various locations and to compare these nests with the laboratory nests.

3) Estimate the age of nests with different-sized mounds and estimate the age of the queens from these nests, and to compare the data with laboratory nests.

4) Determine whether a nest is vigorous and growing, or if it is declining and dying, so that appropriate control measures may be taken.

CHAPTER 2
METHODS AND MATERIALS

2.1 IDENTIFICATION OF SPECIMENS

To determine the genus of collected alates, 25 specimens were identified as *Macrotermes* alates using the keys in Hickin (1971) and Coaton & Sheasby (1972). From the distribution maps given in Ruelle (1970) and Mitchell (1980) it was obvious that the specimens were collected from localities within the range of *M. natalensis* only.

To accurately identify nests of *Macrotermes natalensis*, in the field, the mound was opened using a pick, and not less than 10 major and 10 minor soldiers collected. These samples were killed in 70% ethanol and the heads of 10 individuals, of each soldier caste, were removed. Three measurements were taken, viz. head length, head width and head depth (Fig. 1). Measurements were taken using a Wild dissecting microscope fitted with a graticule. Measurements thus obtained were compared with figures (means and ranges) given in Ruelle (1970). The observed measurements were found to fall within the data given for *M. natalensis*. In addition, specimens were keyed out as *M. natalensis* using the keys provided in Ruelle (1970) and Mitchell (1980). Distribution maps given in Ruelle (1970) and Coaton and Sheasby (1972) indicate that the collecting localities in this study fall within the distribution area of *M. natalensis* only.

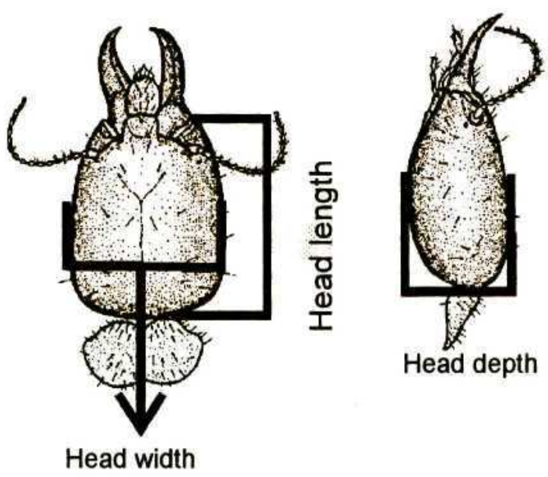

Fig. 1: The three measurements taken from the head capsules of randomly selected soldiers. Head length was measured from the posterior of the head to the base of the mandibles. Head width was measured as the widest part of the head, perpendicular to the length. Head depth was measured as the widest part of the head, including the gula, in lateral aspect.

2.2 GROWTH AND DEVELOPMENT OF HYPOGEAL NESTS: LABORATORY STUDIES

Hypogeal growth of the nests of fungus-growing termites is one of the least studied and understood phases of nest growth and development (Pomeroy, 1976a; Noirot, 1970, 1977; Collins, 1981; Darlington & Dransfield, 1987). For example, the duration of the hypogeal nest for *M. bellicosus* has been estimated at two years (Pomeroy, 1976a; Collins, 1981), but no data are available for *M. natalensis* or any other *Macrotermes* species. In this study, laboratory nests were initiated from collected alate pairs to examine the growth and development of young nests, and to try to determine the approximate duration of the subterranean or hypogeal phase, before the emergence of epigeal mounds.

2.2.1 Collection of alates and foundation of nests

In KwaZulu-Natal, the seasonal regularity of flights of *Macrotermes natalensis* is remarkable, the alates nearly always flying over a short period at the end of October and start of November, and only occasionally in December with late rains (Atkinson, *pers. comm.*). A total of 177 adult *M. natalensis* were collected during two nuptial flights, 10 and 23 November 1992. Alates were captured between 18h00 and 19h00, from the ground and off the walls of the University of Natal, Pietermaritzburg, buildings (John Bews building, Agricultural faculty) with a pair of forceps or from the air with a sweep net. Once caught, the termites were placed into a large plastic sorting dish (diam. = 300 mm) where they shed their wings and formed tandem pairs. Once a pair had formed, it was removed from the sorting dish and placed in a plastic petri dish (diam. = 120 mm) along with a few drops of water to prevent desiccation.

Pairs were transferred from the petri dish to one of a collection of plastic containers (Fig. 2) ranging in volume from one litre to 25 litres. The rearing containers had been filled with soil collected from areas where mounds were common, as well as from mounds themselves. The soil that was collected was not sterilised, as is usually the case (Johnson, 1981; Johnson *et al.* 1981; Okot-Kotber, 1981), so that the first brood would not starve. The tops of some containers were cut off (Fig. 2) so that the soil could be placed in them as well as to allow easy excavation of the nests. All containers were uncovered *i.e.* they did not have lids. The soil in the rearing containers was lightly moistened with tap water to allow easy penetration by the insects.

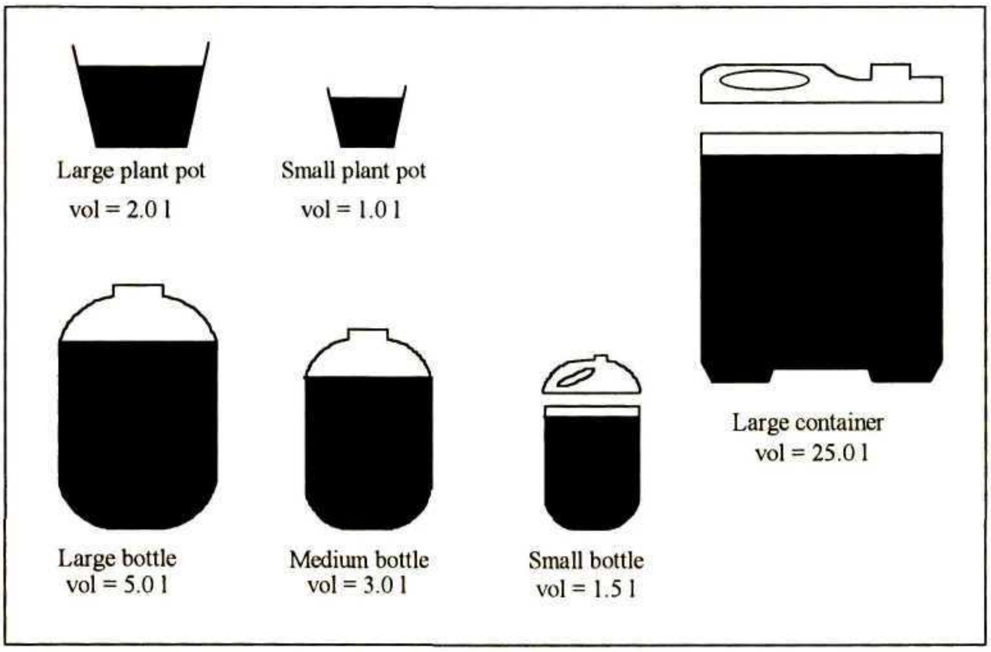

Fig. 2: The various containers used to initiate nests in the laboratory studies.

Once the pair had ceased digging a vertical shaft, and formed a copularium (a sealed off cavity in the soil), the container was placed on a shelf in the insectary which was kept at a constant temperature of 28°C (Sieber & Leuthold, 1981) and a relative humidity of 80-90%. Okot-Kotber (1981) watered colonies of *M. michaelseni* once to twice a week to maintain the high humidity required, but did not mention exactly how high this was. Tap water was added to each container (Table 1) to prevent the soil from drying out. All containers were watered twice a week for the first five months. However, once it became apparent that the insects were not surviving, the watering regime was changed to once a week. By this time additional adults could not be collected as the swarming period had finished.

The ability of incipient colonies to produce a viable fungus comb under sterile conditions in the laboratory has been demonstrated for five species of *Microtermes* (Johnson, 1981; Johnson *et al.* 1981), and three species of *Macrotermes*: *M. bellicosus* and *M. subhyalinus* (Johnson *et al.* 1981), and *M. michaelseni* (Sieber & Leuthold, 1981; Sieber, 1983). It is not known whether *M. natalensis* alates carry fungal spores within their guts or if they have to collect them. Rearing containers were therefore seeded with fragments of fungus comb from a mature nest when covered runways first appeared on the surface of the soil.

Table 1. Number and dimensions of the rearing containers and amount of water applied to these containers.

CONTAINER VOL. (ml)	NUMBER OF CONTAINERS	HEIGHT (mm)	DIAMETER (mm)	VOL. WATER ADDED (ml)
1000	15	120	150	50
1500	16	150	120	50
2000	7	150	180	75
3000	17	170	150	100
5000	11	230	180	150
25000	5	310	320	250

2.2.2 Sampling laboratory nests

Between seven and nine rearing containers, were selected for destructive sampling once a month to determine the size of the nest and the developmental phase of the colony. Selected containers were excavated to expose the nest and population, using a small spoon for the smaller containers, and a combination of small spoon and garden trowel for the larger containers. The queen, king, and brood (if any) were carefully removed with a pair of fine forceps and placed in a freezer (stand-up Kelvinator fast freeze) until dead (an hour was adequate). The king, queen and brood were then weighed on an analytical balance (Zeiss Metler H10) accurate to 0.01 g. The height, long diameter, short diameter and depth below soil surface, of the exposed copularia were recorded in the hope to use the data in comparison with nests sampled in the field. However, due to the high mortality of pairs no such comparison was possible, using the limited data. The entire population of each sampled nest was preserved in 70% ethanol as voucher specimens.

2.3 STUDIES OF FIELD NESTS

2.3.1 Sampling Sites

Because the study set out to examine the growth and decline of nests, sampling was carried out on as many sizes of nest as possible, rather than examining many outwardly similar nests on specific sites. In the course of selection nests were sampled from 10 localities (Figure 3), including road verges (sites 1, 3, 4, 6 and 10), wasteland (site 2), suburban garden (site 5), pasture (sites 7 and 9), and a *Eucalyptus* sp. stand (site 8). See Appendix 3 for map co-ordinates, altitude and mean annual rainfall of the 10 sampling sites from which nests were selected in this study.

Soil types

A short description is given below of the soil types found at each of the 10 sampling sites. Soil types were kindly identified by the soil science staff of the Institute of Commercial Forestry Research (ICFR), University of Natal, Pietermaritzburg using *Soil classification. A taxonomic system for South Africa* (MacVicar & De Villiers, 1991). Soils are described in terms of depth and colour of both the A- and B-horizons, structure, clay and sand content, drainage, and parent material(s). Table 2 lists each of the 30 nests sampled and which particular soil type the nest was found in.

A) Avalon

The A-horizon is a dark brown, orthic layer 0.3 m deep, with a yellow-brown, apedal, B-horizon from 0.3 m to 1.0+ m deep, grading into soft plinthite. This is a deep soil with a medium (40%) clay content that increases with depth. Parent material is Pietermaritzburg formation shale (Pfs). The presence of a soft plinthic layer indicates relatively poor drainage.

B) Hutton

The A-horizon is orthic, 0.4 m deep, and with weak structure. The B-horizon is from 0.4 m to 1.0 m deep, yellowish-red, apedal, friable with a 40% clay content. This is a deep, well drained soil and the parent materials are dolomite and Pfs.

C) Kranskop

The A-horizon is a greyish, humic layer 0.2 to 0.3 m deep, with a red, apedal, B-horizon from 0.3 m to 1.2+ m deep. This is a well drained soil with a medium clay (50%) content that increases with depth. The parent material is again Pfs. This deep soil is well drained.

D) Mispah

The A-horizon is greyish-brown, orthic, 0.1 m to 0.3 m deep with no B-horizon. This is a very shallow soil with a high clay (58%) and low sand (13%) content. Drainage is poor, and parent materials include Pfs and other black shale.

E) Shortlands

The A-horizon is red, weakly structured, orthic, 0.3 m deep with a > 1.0 m deep B-horizon.This is a deep soil with a high clay (69%) and a low sand (8%) content. The soil is welldrained, and the presence of concretions indicates a relatively dry climate. Parent materials include dolerite and Pfs.

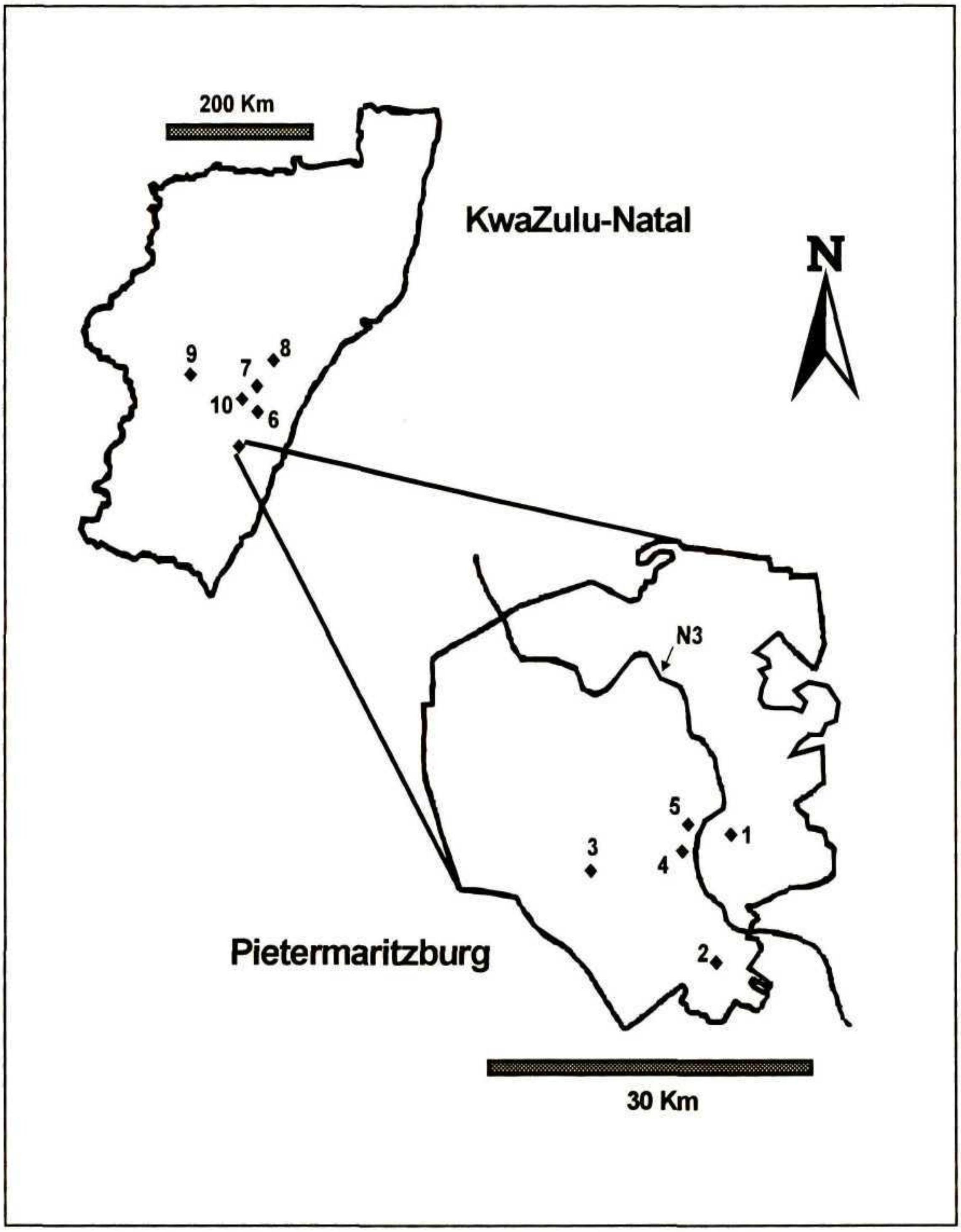

Fig. 3: The 10 sampling sites, from which nests were selected, in this study. See Appendix 3. N3 indicates a nationalroad.

F) Unnatural

These soils could not be classified according to natural soil profiles in MacVicar & DeVilliers (1991). In the case of sampling locality C (all nests), the road bank soil was made up largely of sand and gravel. The soil at sampling locality D (nest 50), the drainage ditch bank was artificially deepened and contained sand, topsoil and shale fragments. At sampling locality B (nest 14) the rubble dump contained mostly ash, sand and old crockery. At sampling locality E (nest 56) the flower-bed soil had been chemically altered by fertilisers and had been much deepened.

G) Westleigh

The A-horizon is greyish-brown, orthic, 0.3 m deep, with a B-horizon that is split into a soft plinthic layer (0.2 m deep) and a hard plinthic layer (> 0.5 m deep). This is a deep soil with a low clay (20%) and a medium sand (30%) content. The plinthic layers indicate yet again that drainage is relatively poor. The parent material is Pfs.

2.3.2 Sampling field nests

Nests were excavated according to Darlington (1984a), by digging a trench halfway around the mound and slowly deepening and widening it until the first fungus combs appeared. The height and both long and short diameters of the mound were measured before excavation. A total of 30 nests were excavated and processed between February 1993 and August 1994. Eleven nests (4, 24, 25, 26, 28, 32, 45, 46, 61, 62 and 63) were excavated to determine structure only. The queens from these nests were removed and treated the same as for the remaining nests. One side of the 11 nests was totally removed to reveal a section which was then photographed. The photographs were used to make diagrams of the nests. The height and long diameter of the hive and nursery were measured. The first appearance of fungus comb, during excavation, was marked in the soil. The distance from the centre of the slab of earth containing the royal cell, to the mark in the soil was taken as the radius. The short diameter of the hive could then be estimated by multiplying the radius by two. The short diameter of the nursery was also estimated in this way.

For 19 nests the contents of the hive (fungus combs and royal cell) were totally removed and taken back to the lab. The height, and long and short diameters of the four nest components: mound, hive, nursery and royal cell were measured. Each queen was immediately placed into a sealed container of known mass after removal from the royal cell, so as to reduce water loss. The

Table 2: Soil type found at each of the 10 sampling sites. See text for a short description of the soil types. For explanation of sampling sites see Appendix 3.

Sample site	Nest Code No.	Soil Type
1	4	Shortlands
2	14	Unnatural (rubble dump)
	15	Westleigh
	16	Westleigh
3	24	Unnatural (road bank)
	25	Unnatural (road bank)
	26	Unnatural (road bank)
	28	Unnatural (road bank)
	30	Unnatural (road bank)
	32	Unnatural (road bank)
	45	Unnatural (road bank)
	46	Unnatural (road bank)
	55	Unnatural (road bank)
4	50	Unnatural (drainage ditch bank)
5	56	Unnatural (flower-bed soil)
6	61	Shortlands
	62	Shortlands
	63	Shortlands
7	65	Kranskop
	66	Unnatural (filled depression)
	67	Mispah *
8	70	Kranskop
	72	Kranskop
9	80	Avalon
	81	Avalon
	82	Avalon
	83	Avalon
	84	Hutton
	85	Hutton
10	90	Unnatural (filled depression)

* This mispah was 0.4 m deep.

sealed container was weighed, using a top pan balance (Mettler BasBal BB300), accurate to 0.01 g, on return to the laboratory. The queen's fresh mass was then determined from the difference of the container mass before and after the queen was placed inside. In the laboratory the larger fungus combs and fragments were separated from the rest of the rubble by hand. The combs and fragments were crushed with a five pound hammer in a plastic container to make drying time similar to the smaller pieces separated by flotation. The crushed fungus combs were laid out on a tray and dried in an oven (Memmert oven with removable trays) at 50°C for 24 hours. The smaller fungus comb fragments were separated from the rest of the nest contents by the process of flotation (Darlington, 1984a), as follows: A specially designed tank was half filled with water and compressed air was pumped into the tank through nozzles located along the length of a pipe mounted in the bottom of the tank. The nest contents were then sprinkled over the bubbling water surface with the result that stones and soil sank while the fungus comb fragments floated. The floating fungus comb fragments were scooped from the water surface using a round, metal sieve and placed in an oven at 50°C to dry for three days after which the fungus comb fragments were combined with the crushed combs and weighed with a spring balance, accurate to 100g.

2.3.3 Estimating the ages of nests and queens

Nest age can be considered to consist of three aspects: mound age, colony age, and queen age. Due to the long life span of *Macrotermes* colonies, age cannot be recorded directly. In this study the ages of mounds and queens were estimated. Conventional methods of determining growth (from which age and longevity may be calculated) involve repeated measurements over a certain period of time, for each individual (Ebert, 1973; Pomeroy, 1976a; Collins, 1981). Pomeroy (1976a) measured the volume of mounds of *Macrotermes bellicosus*, but did not relate volume to age. Collins (1981), on the other hand, was able to relate mound size to age, although the parameter he used was mound height. This was because height was well correlated to mound width. Collins (1981) estimated that the maximum longevity of a *M. bellicosus* nest was 15 to 20 years. The mounds of this species, incidentally, grow to a height of three to four metres. Due to lack of information relating mound size to age, the ages of *Macrotermes natalensis* mounds were estimated directly, from a series of aerial photographs or indirectly, from interviews with land owners or their labourers.

Queen age

Most insects have a life cycle lasting from a few days to a few weeks or months. However, some stages (*e.g.* cicada nymphs and termite reproductives) may live for considerable periods of time. Aging insects is never easy, except in cultures where the age of the subject is known. For field populations, physical variables such as number of daily growth rings in the tibial or other body sections (Zuk, 1986), amount of wear and tear on wing margins (Buxton, 1955), cuticular growth

(Bordereau, 1982) and chemical variables, such as mitochondrial-DNA analysis (Miquel, 1992) have been used. Yet these techniques were used for adult insects with a relatively short life span. Although Bordereau (1982) has accurately aged termite queens from sections of the cuticle, no queens older than one year were studied. Queens of the genus *Macrotermes* may live for very long periods of time and do not moult during their adult life. They appear to be unaffected by seasonal fluctuations of temperature and humidity (Lal, 1987), so that seasonal changes may not be reflected in physiological growth processes.

There is, therefore, no method at this time to determine the exact age of a termite queen. However, the colour of the cuticle and degree of physogastry have been used as relative indices of maturity. For example, Darlington (1985a) described the colour of the inter-segmental membranes of the abdomen of *M. michaelseni* queens as pale cream or white in young physogastric queens, whereas it darkened to buff, brown or putty-coloured in old queens. Darlington (1987) also used degree of physogastry, turgidity and sheen in combination with colour to estimate maturity of *M. michaelseni* queens. Younger queens were very turgid and had a glossy sheen, whereas older queens were wrinkled and flaccid and had a dull sheen. In all these cases maturity refers to the physiological condition of the queen, *i.e.* whether the queen is fully physogastric or not. No measures have been made relating these conditions to absolute age, for this or any other *Macrotermes* species.

Queen age may differ from mound age because old, dying queens may be replaced by younger queens recruited from alates left inside the nest after a swarming period. Coaton (1949b) recorded multiple queens in two *M. natalensis* nests; in one nest there was an old, flabby queen and a smaller younger queen in a single cell. In the other nest two royal cells were found, one uninhabited (old queen presumed died and removed) and the other cell contained two similar sized, young queens. In both these cases it was obvious to the author that the queens in both nests were supplementary (replacement) queens and were derived from adult, winged imagoes. Noirot (1969) states that queens of the Macrotermitinae can only be replaced by alates which become sexually mature instead of swarming. Sieber & Darlington (1982) showed this to be the case for *Macrotermes michaelseni*. Queen age may differ from colony age or mound age, because mounds of dead or dying colonies might be recolonised after the original colony has died. But recolonisation of dead or dying nests has not been recorded in *M. natalensis*, although it has in *M. michaelseni* and *M. subhyalinus* (Pomeroy, 1976a; Darlington, 1984b, 1985b, 1991b).

Because of the difficulty of estimating the ages of queens, the ages of the mounds were estimated and the possible ages of the queens inferred. For the purposes of estimating nest age, and therefore queen age, it was assumed that the nest remains hypogeal for two years, as suggested for *M. bellicosus* by Pomeroy (1976a).

In this study the colour of the inter-segmental membranes (ISM), joining tergites, was categorised as follows: creamy-white, creamy-white with dark streaks, pale-brown, pale-brown with dark streaks, medium-brown, medium-brown with dark streaks and dark-brown. The pleural membranes (PM), joining tergites to sternites, were categorised: creamy-white, creamy-brown and creamy-brown with dark streaks. Turgidity was categorised: very turgid, fairly turgid, not very turgid and flaccid. Queens so categorised are listed in Appendix 4.

2.3.4 Estimating the vigour and decline of nests

Young nests are vigorous because they grow rapidly, however, once the nest has attained maximum size (reached maturity) it is not always easy to determine if the nest is vigorous or declining. If the nest is vigorous it is not always possible to determine whether or not the nest has, at some stage, been recolonised or the king and queen replaced. Darlington (1982) used proportion of larvae in the total nest population to determine whether nests of *M. michaelseni* were young, mature or declining. The young, fast-growing nests had 60% or more larvae in the population. When the proportion of larvae was 40% to 50% the nests were considered mature, but when it was less than 40% and the colony was not occupying the whole of the nest chamber, nests were considered to be declining. However, separating the insects from the fungus comb fragments (to determine proportions of castes) prolongs the excavation and processing of the nest. Because a very strong correlation exists between the total sterile population and the queen and fungus comb masses of two closely related species (Table 3), it was decided to use queen mass and fungus comb mass as measures of total population and, therefore, as measures of vigour.

Table 3: Correlation coefficient (c) of total population vs. queen mass (QM) and total population vs. fungus comb mass (FCM) for two *Macrotermes* species.

Species	QM	FCM	Reference
M. michaelseni	0.919	0.928	Darlington & Dransfield, 1987.
M. subhyalinus	0.931	0.882	Darlington, 1990, 1991a.

Queen and fungus comb masses were related to measures of external size and appearance of the mound. Mound circumference was considered easier to work with than height or either diameters and better represented the size of the mound than any of the above variables separately. Mound circumference was calculated using the following formula used to calculate the circumference of an ellipse:

$$C = 2\pi\sqrt{\frac{(D1/2)^2 + (D2/2)^2}{2}}$$ (where D1 and D2 are perpendicular diameters, see Appendix 5)

Queen and fungus comb masses were also related to the internal size of the nest (hive volume), to reach conclusions about the vigour or decline of the nests. Hive volume was estimated by assuming that the hive is a three dimensional globular structure, *i.e.* an ellipsoid (squashed sphere) from observations. The volume of this ellipsoid may then be calculated using the formula:

$$v = 4/3\ \pi\ \{(H/2)(D1/2)(D2/2)\}$$

(where H, D1 and D2 are the height, and two diameters of the hive, see Appendix 5)

The fungus comb mass can then be divided by the hive volume to give a ratio. This ratio shows how many kilograms (dry mass) of fungus comb would exist in every cubic metre of hive space. The value of this ratio may then be used to determine if a nest is vigorous or declining.

Hesse (1955) observed that living mounds of *M. subhyalinus* in Tanzania were almost barren of vegetation. However, Pomeroy (1976b) reported that grass cover bore little relation to whether mounds of *M. bellicosus* in Uganda, were alive or not. Grass cover was recorded to see if it was related to vigour. Degree of grass cover over the mound was expressed as a percentage estimated by eye.

The amount of construction produced on the mound, during the last season, was also considered to be related to the vigour of a nest. If a nest grows rapidly the amount of construction should be high, on the other hand senescing nests would have little or no construction work. New construction (bubbly textured areas) was expressed as a percentage, estimated by eye.

The internal (core) temperature of a nest is related to metabolic heat, especially of the fungus combs (Batra & Batra, 1979; Darlington, 1984b; Wood & Thomas, 1989) and therefore can be expected to be related to vigour. Collins (1981) mentioned a reduction in the metabolic heat of the fungus during the senescent period of an *M. bellicosus* nest. It was for this reason that temperature was recorded (*i.e.* to determine if a nest was vigorous or declining). Temperature was recorded as the difference between the internal temperature of the nest and that of the adjacent soil, at equal depths. First a hole was drilled into the mound at an angle (with a petrol driven soil auger), in the hope that the hole would end up in the middle of the hive. A copper/constantan thermocouple, attached to a 1.5 m long dowel rod for support, was inserted into the drilled hole and the temperature of the nest recorded. The thermocouple was plugged into a digital thermometer (made by the Physics Department., University of Natal, Pietermaritzburg) accurate to 0.1° C. The thermocouple was removed and a hole drilled into the soil and the temperature of the soil recorded.

CHAPTER 3

RESULTS OF STRUCTURE AND DEVELOPMENT OF HYPOGEAL NESTS: LABORATORY STUDIES

Of the 177 alates initially collected, 25 failed to form pairs and were used for identification. Of the remaining 76 pairs, one or both partners of five pairs did not shed their wings and were unable to dig into the soil. Thus 71 pairs successfully dug into the soil to construct a copularium.

3.1 FOUNDATION OF NESTS

Once a pair of termites was placed into one of the soil containers they moved to the periphery in the tandem position. The pair walked around the periphery, occasionally stopping for a few seconds. This behaviour lasted for a varying amount of time usually lasting 5 to 10 minutes, but in some cases lasting for two or three hours. After the female had selected a site for the nest, she initiated digging. At this point the pair broke apart and both individuals contributed towards excavating a vertical tunnel. Soil particles were removed from the soil surface with the mandibles and placed in a ring around the developing excavation, or in a semi-circle if the excavation was against the container wall. As the vertical shaft was deepened the debris carried to the surface was added to the ring of particles to form a conical or domed pile covering the hole. As the hole was deepened, the excavated material was used to plug up the hole above the insects.

When the excavation reached a mean depth of 80 mm (n=37) an elliptical chamber was then constructed with a horizontal floor and an arched roof. This chamber, called the copularium, had a mean length of 127 mm (n=28), a mean width of 101 mm (n=28) and a mean height of 18 mm (n=25). The material removed from the developing copularium was used to plug up the vertical shaft and seal in the insects. The male and female remained stationary inside this sealed off copularium for one to two hours, probably resting. Some containers had clear plastic walls and a total of eight copularia were constructed against the walls of these containers; the occupants of these copularia could therefore be observed. These visible copularia were termed window nests and were put aside for long term observations on the development of the nest and were not sampled at monthly intervals.

3.2 DEVELOPMENT AND STRUCTURE OF HYPOGEAL NESTS

3.2.1 Survival of founding pairs

Monthly destructive sampling of rearing containers showed a high mortality of pairs (Table 4). All pairs from containers sampled in the second and third months had died, as had all pairs sampled in the fifth, eighth, ninth and tenth months. Only five live nests (in addition to the window nests) were sampled over the whole ten month period. Dead insects that were found inside copularia were covered in a blue or yellow fungus, or seemed to have had their tissues liquified. Some copularia did not contain any insect remains at all and it is thought that the insects either decomposed completely, or the dead insects were removed, during excavation. Some copularia were never found at all. These copularia may have caved in or the construction of copularia may not have been completed before the insects died.

3.2.2 Structure of hypogeal nests

For the first four months the nest consisted of only the oval-shaped copularium containing the king and queen. During the fifth month, covered runways were observed on the surface of 10 containers (which were placed with the window nests), and small tunnels (1-2 mm diameter) were observed in four of the window nest, with minor workers moving to and fro from the soil surface to the copularium. Containers that were sampled during the sixth, seventh and eighth months consisted of a copularium with two or three tunnels (3-5 mm diameter) leading away from the copularium. These tunnels were too small for the royal pair to move through. No fungus comb was ever observed in any of the nests.

3.2.3 Development of hypogeal nests

Two months after the pairs had dug into the soil, eggs were noticed in six of the window nests, but mating was never observed. No live nests were sampled at this time. At three months the first nymphs were observed moving around inside three of the window nests; again no live nests were sampled at this time. A total of at least 20 colonies were alive at this time, eight other containers had active window nests and two live nests were sampled in this month. One of the sampled nests only contained a king and queen, but the other nest not only contained a king and queen, but also had five minor workers and one larva present (Table 5). One live nest was sampled during the sixth month, but only the king and queen were present, no eggs, larvae, or workers were found (Table 5). Minor soldiers first appeared in the seventh month. All nests sampled during the eighth, ninth and tenth months were dead, but the last window nest, which was seen to be alive in the ninth month was dead when sampled in the tenth month.

Table 4: Data collected from the 71 rearing containers, sampled over a 10 month period, after foundation. MF= Months after foundation, NS= number of containers sampled per month, NL= number of live nests, ND= number of dead nests, NDI= number of dead nests with insect remains, NDW= number of dead nests without any insect remains, NN= number of copularia not recovered.

MF	NS	NL	ND	NDI	NDW[a]	NN[b]	Developmental phase of nest
2	7	0	7	5	1	1	Nests consisted of a copularium only. No eggs or larvae.
3	7	0	7	2	2	3	Nests consisted of a copularium only, eggs and larvae first observed.
4	8	2	6	2	1	3	Nests consisted of a copularium only, first minor workers.
5	8	0	8	5	1	2	Nests consisted of a copularium only, covered runways first observed.
6	9	1	8	5	1	2	Nests consisted of a copularium with tunnels, no new runways.
7	9	2	7	0	0	7	Nests consisted of a copularium only with tunnels, first minor soldiers.
8	9	0	9	3	1	5	Nests consisted of a copularium with tunnels, no new runways.
9	7	0	7	3	1	3	Nests consisted of a copularium only, no new runways.
10	7	0	7	1	1	5	Nests consisted of a copularium only, no new runways.

[a] The insects inside these copularia must have decomposed totally or have been removed when excavated.

[b] These copularia must have collapsed or have been removed during excavation.

Table 5. Population structure of the five nests which contained living insects when sampled. All these nests contained a king and queen. No major workers or major soldiers were observed. No fungus combs were observed. MF= months after foundation.

MF	Nest	Workers	Soldiers	Larvae
4	1	5 minor	0	1
	2	0	0	0
6	3	0	0	0
7	4	16 minor	1 minor	8
	5	5 minor	1 minor	2

3.3 DISCUSSION

Seventy-one pairs successfully burrowed into the soil and constructed copularia. All of these incipient colonies had died 10 months after founding. Due to the high mortality of pairs in rearing containers resulting in a low number of live nests sampled per month, no concrete conclusions can be drawn about the structure or development of the hypogeal nest during the first ten months after foundation. However, development may be summarised as: eggs first appeared in the second month, larvae first in the third month, minor workers in the fourth month and minor soldiers in the sixth month. What became apparent was that the nest appeared to grow very slowly. Nests sampled in the seventh month were little larger than nests sampled in the second month.

CHAPTER 4

STRUCTURE OF *Macrotermes natalensis* NESTS

The nests of *Macrotermes* spp. excavated in this study consisted of an epigeal component, the mound, and a hypogeal component, the hive. The hive contained the fungus garden, the nursery and the royal cell. The excavation of 29[*1] *Macrotermes natalensis* nests indicated that this species constructs nests (Fig. 4) similar to other members of the genus. Five nests (80, 81, 82, 83, 84) differed from the rest in that there was no fungus garden present, only the nursery and royal cell were present within the hive (Fig. 5). The hives of these nests had similar dimensions to the nurseries of mature nests. One small nest (85) with a small hive, did contain a fungus garden, as well as a very small nursery (Fig. 6). The size and structure of the nests excavated in this study are given in Table 6.

4.1 EPIGEAL COMPONENT (MOUND)

4.1.1 Mounds of mature nests

In this study mounds were either conical or dome-shaped, some being bare of vegetation (Figs. 7, 8, 9), but others were covered in grass (Figs. 10, 11, 12). Appendix 5 lists all nests and the variables recorded from each nest. In this study, mature mounds of *M. natalensis* had a mean height of 0.73 m ($\pm$ 0.07 m, n= 24[*2]) and a mean circumference of 5.75 m ($\pm$ 0.42 m, n=24). Mature mounds contained large air passages which appeared to originate from the top of the hive and branch out into the mound where they ran close beneath the surface of the mound (Fig. 4). No large air passages were observed that rejoined the hive around the edges, instead, the descending air passages appeared to lead directly into horizontal tunnels radiating away from the mound some 150 mm to 200 mm below the soil surface.

4.1.2 Mounds of juvenile nests

The mounds of the five juvenile nests (80 to 85) differed from the mounds of mature nests both in size and structure. The mounds of juvenile nests had a mean height of 0.16 m ($\pm$ 0.02 m, n= 5) and a mean circumference of 2.14 m ($\pm$ 0.71 m, n= 5), but lacked the large air passages characteristic of mature mounds. Instead, numerous small tunnels were found that ramified throughout the structure.

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*1 One nest (56) was not fully excavated as it was among shrub roots and awkward to get at.

*2 Five nests were juvenile and nest 56 did not have a mound.

Table 6: Estimated hive volume and structure. Nest 56 was not excavated and is excluded from this table.

Nest code number	Hive volume (m^3)	Subterranean nest structure
4	1.04	Fungus garden and nursery present, royal cell to side and above nursery.
14	1.36	Fungus garden and nursery present, royal cell in middle of nursery.
15	0.53	Fungus garden and nursery present, royal cell in middle of nursery.
16	1.69	Fungus garden and nursery present, royal cell in middle of nursery.
24	0.82	Fungus garden and nursery present, royal cell in middle of nursery.
25	1.61	Fungus garden and nursery present, royal cell not found.
26	0.44	Fungus garden and nursery present, royal cell not found.
28	1.04	Fungus garden and nursery present, royal cell not found.
30	0.49	Fungus garden and nursery present, royal cell in middle of nursery.
32	0.72	Fungus garden and nursery present, royal cell in middle of nursery.
45	0.71	Fungus garden and nursery present, royal cell in middle of nursery.
46	2.14 *	Fungus garden and nursery present, royal cell in middle of nursery.
50	0.65	Fungus garden and nursery present, royal cell not recovered.
55	0.42	Fungus garden and nursery present, royal cell in middle of nursery.
61	0.94	Fungus garden and nursery present, royal cell in middle of nursery.
62	0.49	Fungus garden and nursery present, royal cell above and in middle of nursery.
63	0.55	Fungus garden and nursery present, royal cell to side of nursery.
65	0.77	Fungus garden and nursery present, royal cell in middle of nursery.
66	0.26	Fungus garden and nursery present, royal cell in middle of nursery.
67	0.47	Fungus garden and nursery present, royal cell in middle of nursery.
70	0.60	Fungus garden and nursery present, royal cell to side of nursery.
72	0.93	Fungus garden and nursery present, royal cell to side of nursery.
80	0.07	No fungus garden present, only nursery, royal cell in middle of nursery.
81	0.12	No fungus garden present, only nursery, royal cell in middle of nursery.
82	0.17	No fungus garden present, only nursery, royal cell in middle of nursery.
83	0.10	No fungus garden present, only nursery, royal cell in middle of nursery.
84	0.09	No fungus garden present, only nursery, royal cell in middle of nursery.
85	0.19	Small fungus garden present with small nursery, royal cell in middle of nursery.
90	1.26	Fungus garden and nursery present, royal cell to side of nursery.

* This figure may be over estimated as the shape of the hive of this nest was more of a bell-shape than an ellipsoid-shape.

4.2 HYPOGEAL COMPONENT (HIVE)

4.2.1 Hive of mature nests

Beneath the epigeal mound lies the subterranean part of the nest (hive) which is made up of an outer, elliptical shell containing large, convoluted fungus combs, resting on thick shelves (Figs. 11, 12). This outer shell (fungus garden) had a mean height of 0.75 m ($\pm$ 0.03 m, n=24) and a circumference of 4.63 m ($\pm$ 0.21 m, n=24). The hive, which may extend into the mound, just above the external soil surface, was found, in this study to be largely subterranean. In the middle of the fungus garden there was a spherical structure made up of many horizontal, paper-thin shelves (Figs. 13, 14). A few flattened fungus combs were also found here. It is here that the eggs and larvae are found and is therefore termed the nursery. Eggs tended to be found in single concentrated masses (Fig. 14). The nursery had a mean height of 0.55 m ($\pm$ 0.2 m, n= 24) and a mean circumference of 1.80 m ($\pm$ 0.07 m, n=24). In the middle to upper part of the nursery, directly beneath the egg mass, there was a large, dense slab of earth with few, small tunnels (Figs. 15, 16). This slab contained the royal cell which was an elliptical chamber, with a mean height of 15 mm ($\pm$ 3.8 mm, n= 16) and a mean circumference of 385 mm ($\pm$ 45 mm, n= 16). The royal cell had a flat floor and an arched roof (Fig. 16) which was scattered with many small tunnels. Small tunnels were also scattered around the sides of the cell where the roof meets the floor. The queen and king were found inside the royal cell. However, the queen of nest 50 was found in a tunnel at the edge of the hive. It appeared as if workers were both pulling her and pushing her along the tunnel to escape. In two nests (46 and 65) two large physogastric queens of similar size and colour were found in a single royal cell. In yet another nest (45) three queens were found in a single cell. One of these queens was a large physogastric queen (destroyed), but the other two queens were small, similar in size to alates (Fig. 17).

4.2.2 Hive of juvenile nests

The hive of the five juvenile nests (80 to 84) did not have a defined fungus garden with large fungus combs resting in chambers separated by thick shelving. Rather, the hive consisted of paper-thin shelving with flattened fungus combs scattered throughout. The hive of these nests had a mean height of 0.57 m ($\pm$ 0.06 m, n= 5) and a mean circumference of 1.87 m ($\pm$ 0.24 m, n= 5). The royal cell was found in the middle of the hive and had a mean height of 11.0 mm ($\pm$ 0.2 mm, n=5) and a mean circumference of 240.4 mm ($\pm$ 40.1 mm, n= 5).

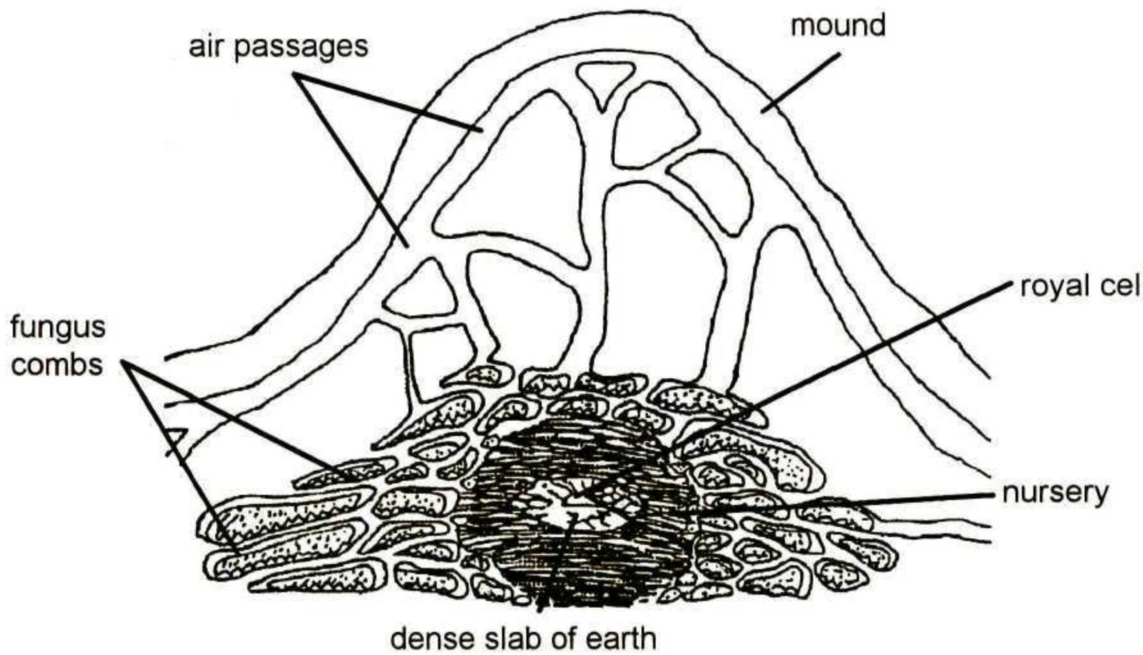

Fig. 4. Diagrammatic vertical section through a mature nest of *M. natalensis* (based on 24* observations), showing mound with air passages, fungus garden with fungus combs and nursery. The royal cell was found inside a dense slab of earth which was positioned in the middle of the nursery. Five nests had the royal cell positioned to one side of the nursery.

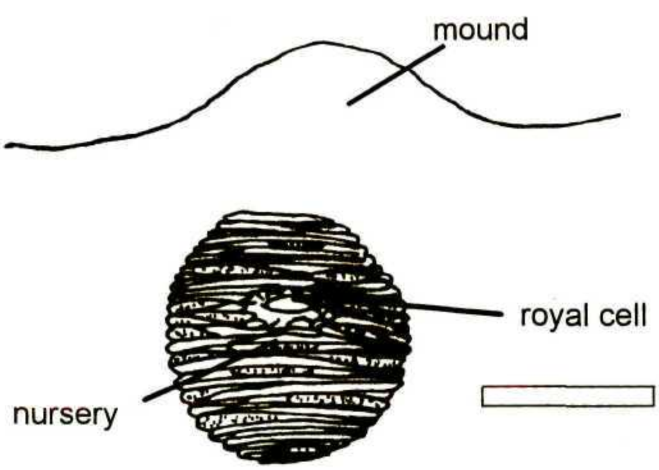

Fig. 5. Diagrammatic section through a juvenile nest (number 80). Only nursery galleries were present, no large fungus combs were present, but small flat combs (stippled areas) were scattered throughout the nursery. Scale bar = 0.3 m.

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* One nest (56) was not completely excavated and five nests (80 to 84) were juvenile.

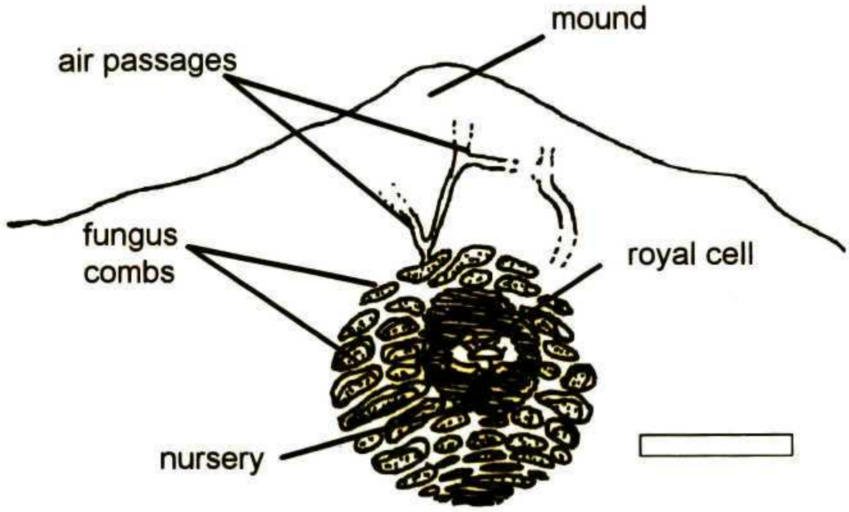

Fig. 6. Diagrammatic section through a transitional nest (number 85), showing the structure between juvenile and mature. Scale bar= 0.3 m.

Fig. 7. Mound of nest 66 showing sandy pediment (arrow). Scale bar = 0.3 m. Dashed line indicates boundary between sandy pediment and mound.

Fig. 8. Mound of nest 72 showing sandy pediment (arrow). Scale bar = 0.3 m. Dashed line indicates boundary between sandy pediment and mound.

Fig. 9. Mound of nest 55 showing bubbly texture (*i.e.* not smooth), due to the addition of new material, added on to the mound in the last season. Scale bar = 0.3 m.

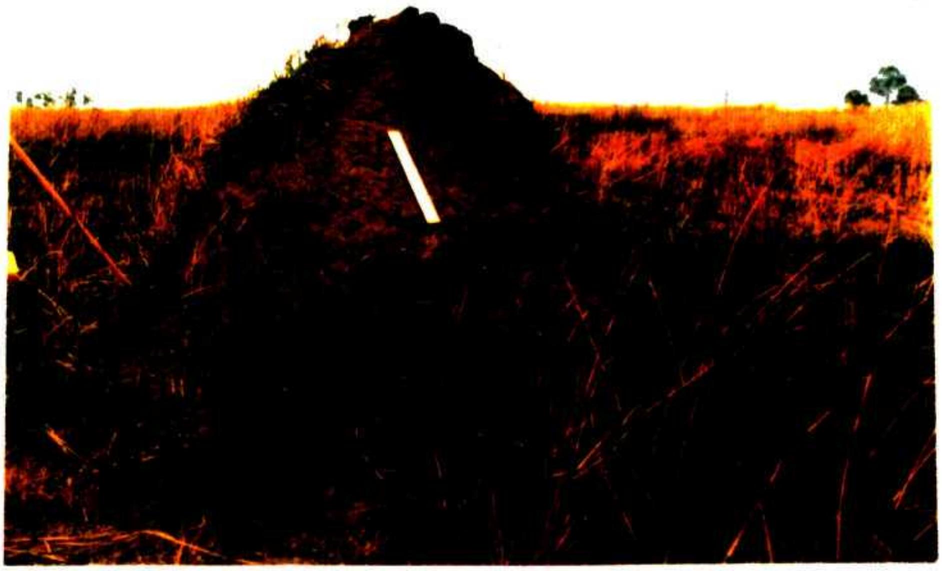

Fig. 10. Mound of nest 15 showing crumbly texture (weathered). Scale bar = 0.3 m.

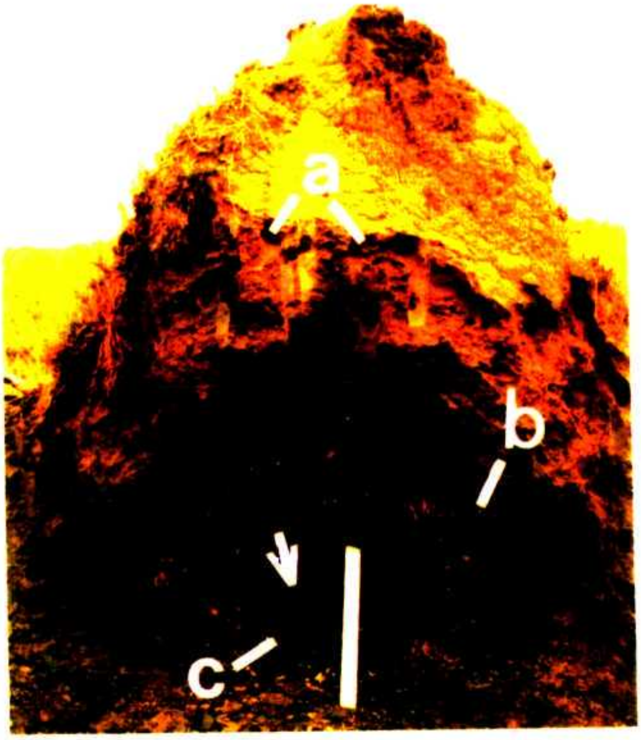

Fig. 11. Section through *M. natalensis* nest (number 15) showing the air passages in the mound (a), heavy clay shelving (b) of the fungus garden (fungus combs removed), and thin lamellae of nursery (c). The royal cell, inside a dense slab of earth, has been removed. Original position marked with arrow. Scale bar = 0.3 m.

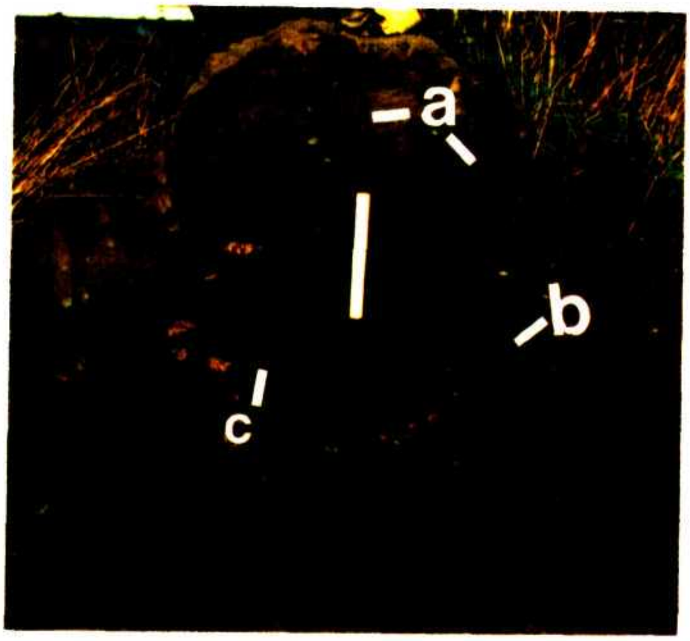

Fig. 12a. Section through *M. natalensis* nest (number 16) showing the air passages in the mound (a), the thick shelving (b) of the fungus garden (combs removed) and the thin lamellae of the nursery (c). Scale bar (0.3 m) is resting on the dense slab of earth, housing the royal cell.

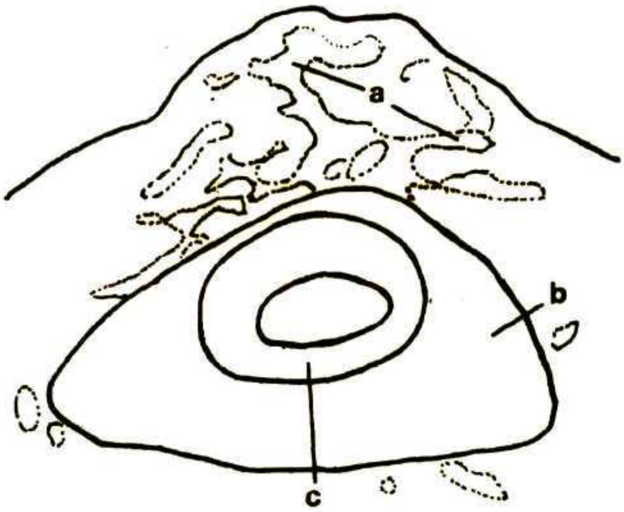

Fig. 12b. Diagram of above nest showing structure (labels as above).

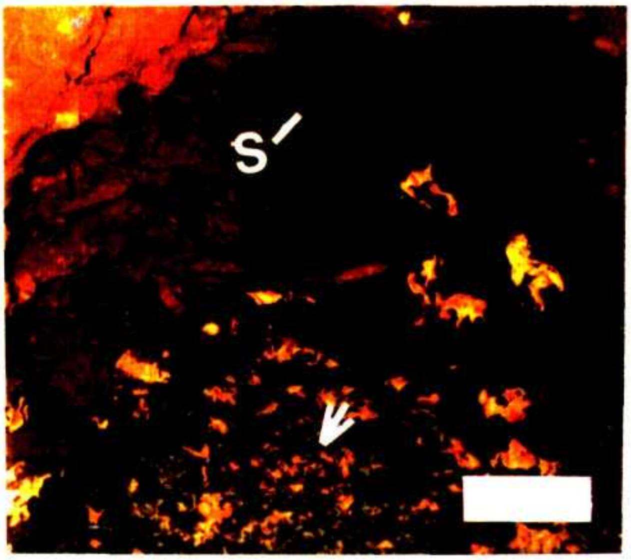

Fig. 13. Close-up of the upper half of the nursery of nest number 46, showing the paper thin shelves (s) and the top of the dense slab of earth (arrow) containing the royal cell, which is covered with eggs (small white specks). Scale bar = 100 mm.

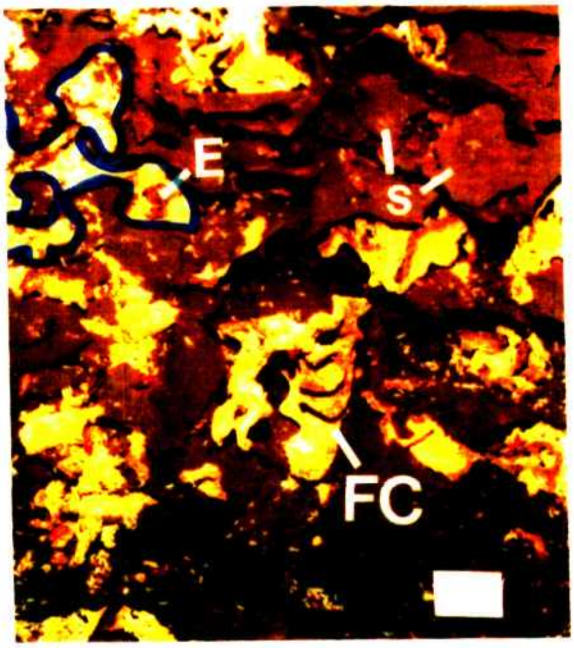

Fig. 14. Close-up of dense slab of earth housing royal cell showing mass of eggs (E) a flattened fungus comb (FC), many of which were found scattered throughout the nursery, and paper-thin shelves (s). Scale bar = 10 mm.

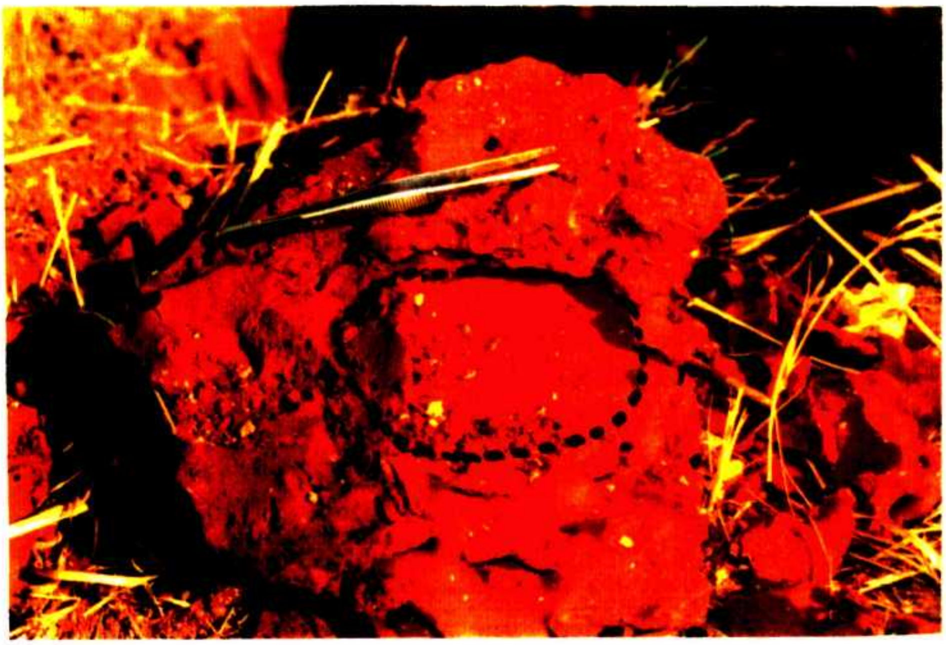

Fig. 15. The dense slab of earth removed from nest number 62 (dorsal aspect), showing the exposed floor (dotted area) of the elliptical royal cell (roof removed). Forceps = 150 mm.

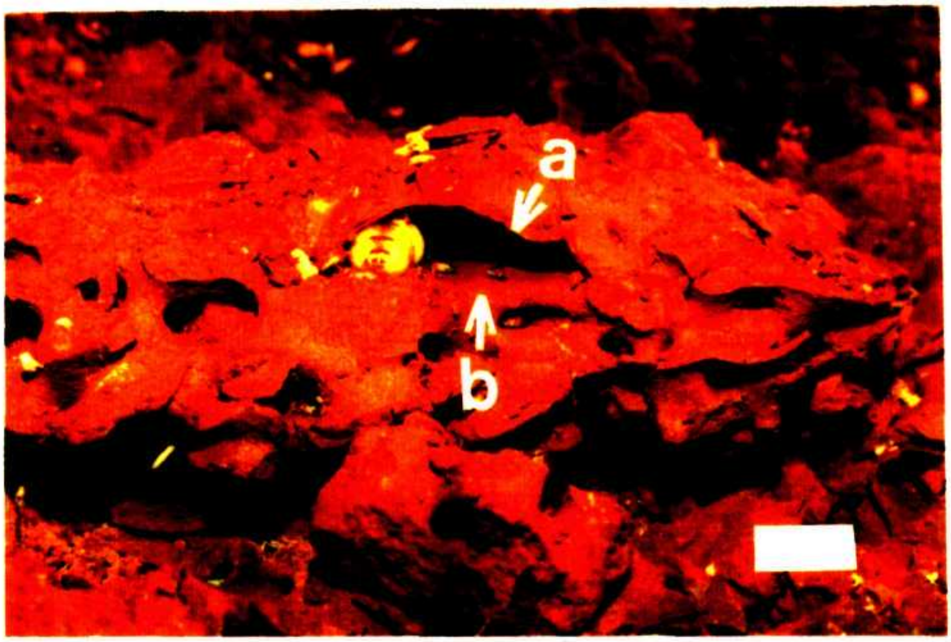

Fig. 16. The dense slab of earth removed from nest 61 (lateral aspect), showing the arched roof of the royal cell (a) and the flat horizontal floor (b). Queen visible at left of royal cell. Scale bar = 15 mm.

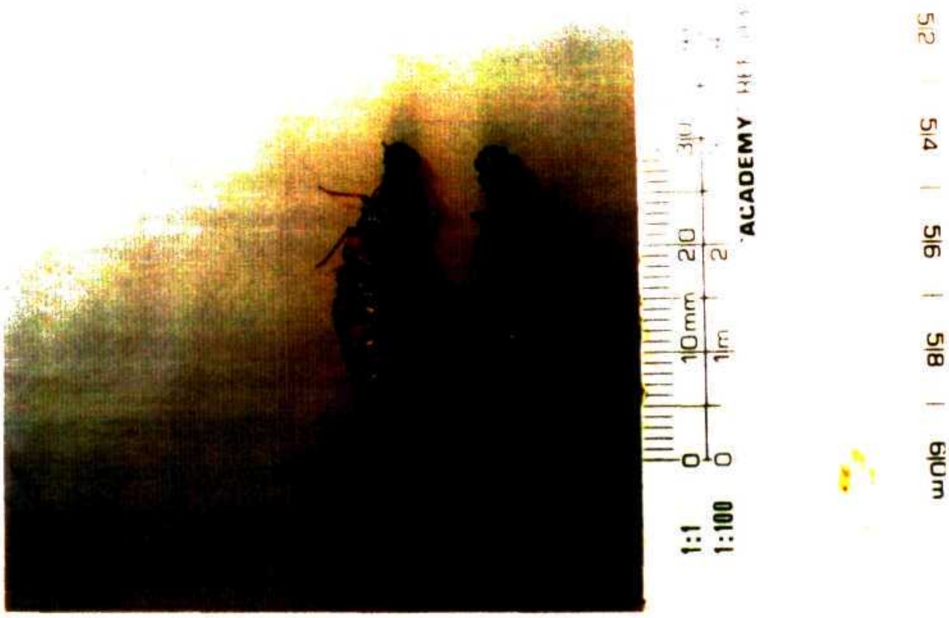

Fig. 17. The two smaller queens found in the royal cell of nest 45. Notice physogastry in early stages. The large, physogastric queen was destroyed.

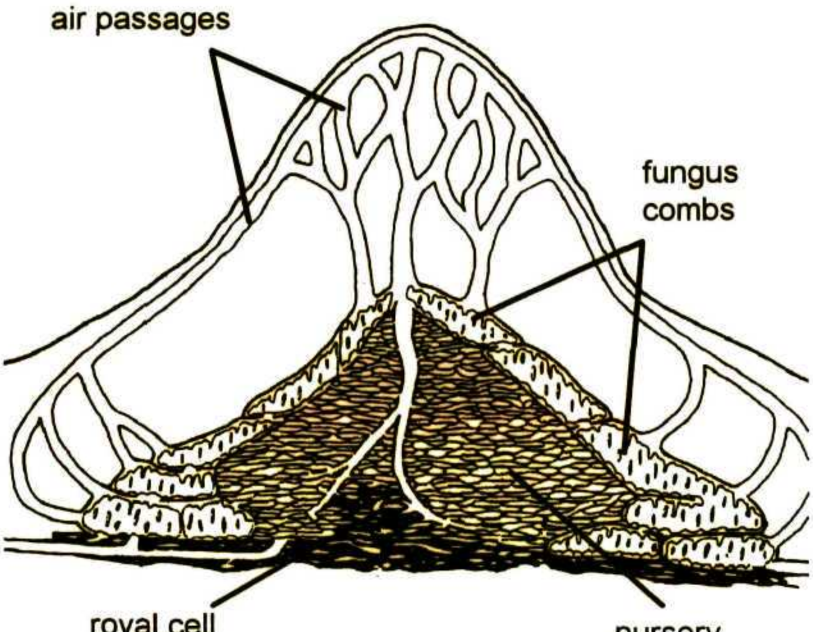

Fig. 18. Diagram of nest of *M. michaelseni*, in Kenya, showing closed ventilation system. The fungus combs enclose the nursery. The shelving at the bottom of the nursery is thicker than at the top. The hive is below the mound.Reproduced from Darlington (1985b).

4.3 STRUCTURE ON DIFFERENT SOIL TYPES

The type of soil that the nest was found in was recorded to gain some insight into the range of soil types (and characteristics) that this species is able to tolerate. Because nests were sampled on the basis of mound size and not soil type, not enough data were collected to statistically analyse for differences in structure and abundance on soils. However, it was observed that the hives of nests occurring on deeper soils tended to be spherical in shape, whereas hives in shallower soils tended to be more flattened. This is probably due to the hive of nests on shallow soils being restricted by the depth of the bedrock. As the hive can only grow downwards to the bedrock, and not through it, it must grow outwards to accommodate the increasing insect population. Table 7 shows the variation of physical and chemical properties of soils where *Macrotermes natalensis* nests were sampled.

Table 7: Physical and chemical properties of soils collected adjacent to each of the 30 nests sampled in this study. Soil types: A= avalon, H= hutton, K= kranskop, M= mispah, S= shortlands, W= westleigh. S-value represents the sum of exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) and is measured in milli-equivalents per hundred grams of soil (meq/100g) "-" represents no data.

PROPERTY OF SOIL	A	H	K	M	S	W
SILT %	32.00	22.00	24.00	29.00	23.00	50.00
pH (Kcl)	4.23	4.96	4.28	3.85	4.70	5.50
CALCIUM meq/100g	2.06	3.07	0.60	2.01	7.09	10.52
MAGNESIUM meq/100g	1.49	3.65	0.43	2.28	4.34	2.35
POTASSIUM meq/100g	0.11	0.93	0.09	0.72	0.16	1.71
SODIUM meq/100g	0.05	0.11	0.24	0.20	0.16	0.11
S-VALUE meq/100g	3.72	7.75	1.36	5.21	12.56	14.69
S-VALUE/100g CLAY meq/100g	-	-	4.50	8.98	18.21	73.47
ORGANIC CARBON	0.92	1.25	2.92	5.28	3.25	3.04
TEXTURE	CLAY/ LOAM	CLAY	CLAY	CLAY	CLAY	SILT/ LOAM

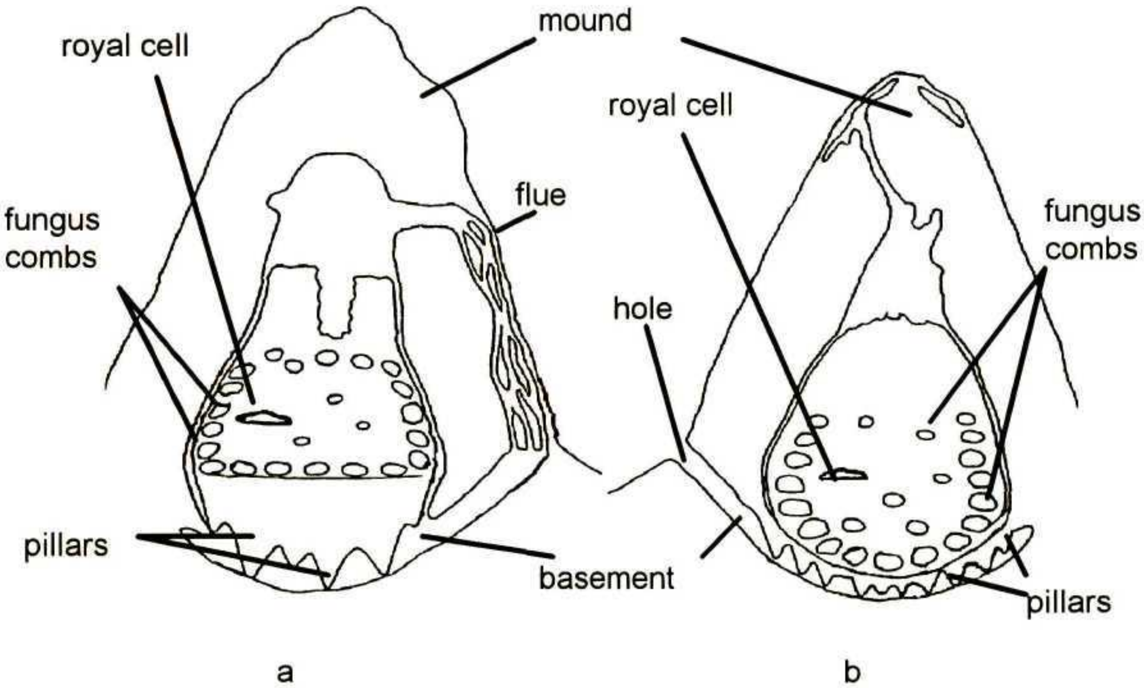

Fig. 19. Diagram of the nest of *M. bellicosus* in a) the Ivory Coast and b) Uganda. The Ivory Coast type mound has a closed ventilation system. Gaseous exchange occurs in the flue. The Uganda type mound has an open system with a large hole leading into the basement. In both cases cool air moves up through the nest, is heated, rises and diffuses out through the thin crust of the mound. In both nests the hive is contained within the mound. Reproduced from Lüscher (1955).

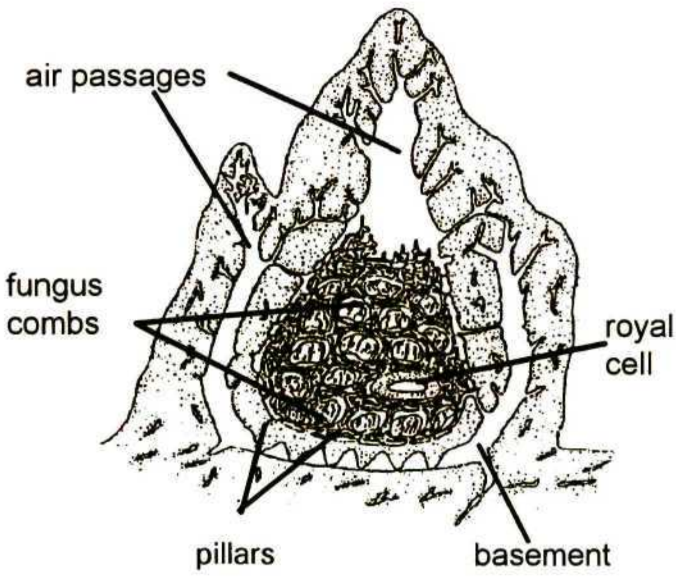

Fig. 20. Diagram of the nest of *M. bellicosus* from the Ivory Coast with closed ventilation system. Diagram shows large air passages in the mound and hive resting on pillars. The hive is contained within the mound. Reproduced from Noirot (1970).

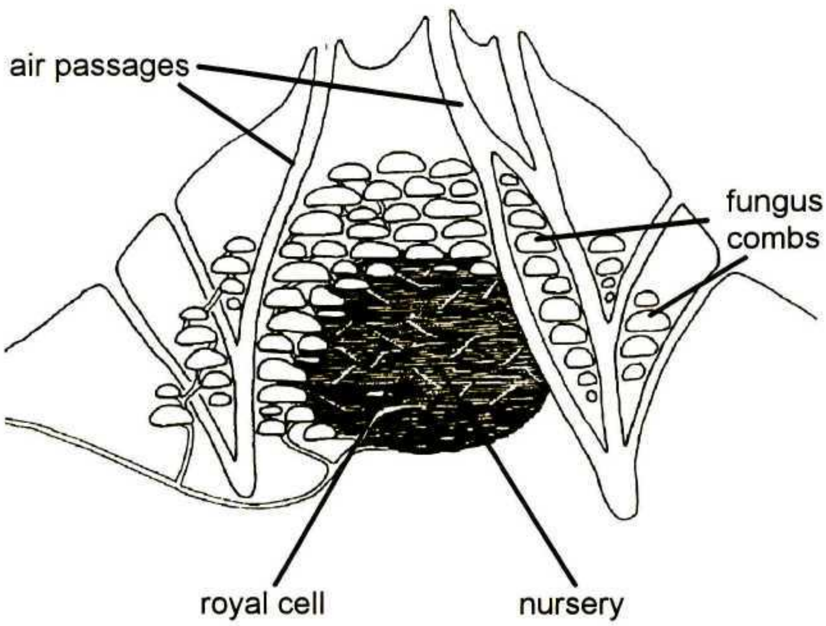

Fig. 21. Diagram of the nest of *M. subhyalinus* in Kenya (Bissel type) with open ventilation system. Air passages open to the exterior. The fungus combs surround the nursery. The royal cell is positioned in the lower half of the nursery, just off centre. The hive is contained within the mound. Reproduced from Darlington (1984b).

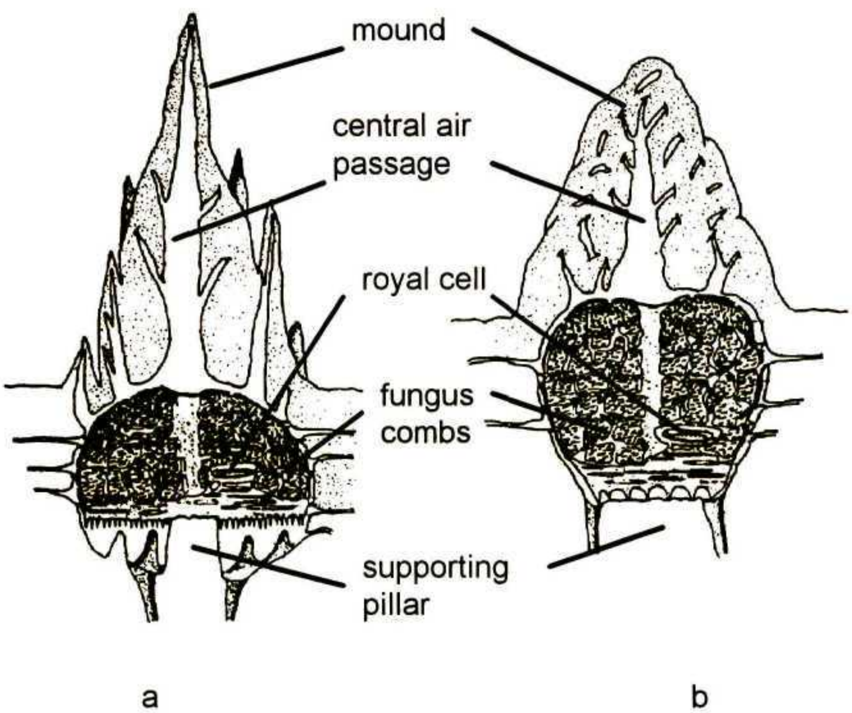

Fig. 22. Diagram of two types of nest built by *M. bellicosus* in Nigeria. Both nests appear to have a closed ventilation system with a large air passage in the middle of the hive and mound. The hives rest on a supporting pillar and are below the mound. Reproduced from Collins (1979).

4.4 DISCUSSION

4.4.1 The mound of mature nests

The system of air passages present in mature *M. natalensis* nests appeared to be similar to that of *M. michaelseni* in Kenya (Fig. 18), but unlike those of *M. bellicosus* (Figs. 19, 20) and *M. subhyalinus* (Fig. 21). Thus, in this study, the ventilation of *M. natalensis* nests appear to be closed, unlike *M. subhyalinus* or *M. bellicosus* which have an open ventilation system with large holes at the base of the mound to draw cool air into the bottom of the hive or large air spaces within the centre of the mound, respectively. The height of *M. natalensis* mounds was lower than that of other species; however, the circumference of *M. natalensis* mounds was greater than that of other species (Table 8).

Table 8: Height and circumference of mounds of *M. natalensis* compared with other species of *Macrotermes*. "-" represents no data.

Species	Mound height (m)	Mound circumference (m)	Reference
M. natalensis	0.73	5.75	present study
M. natalensis	0.60	-	Fuller, 1915
M. natalensis	0.80-1.20	-	Coaton, 1949a
M. natalensis	>2.00	-	Skaife, 1979
M. bellicosus	6.00	3.00-4.00	Collins, 1979
M. subhyalinus Bissel type	1.00-1.50	1.50-2.50	Darlington, 1984b
M. subhyalinus Marigat type	2.00-5.00	3.00-5.00	Darlington, 1984b
M. michaelseni	0.70-1.00	1.00-1.50	Darlington, 1985b

4.4.2 The mound of juvenile nests

Most studies concerning the structure of *Macrotermes* nests and mounds have been centred on the mature nests. Only one study of *Macrotermes michaelseni* nests gave a detailed account of the juvenile mound stages (Darlington & Dransfield, 1987). In this paper the mound of the juvenile nest was described as first appearing when the nest population reached 90 000 adults and was amorphous in structure. By the time the nest population had increased to 127 000 adults, the mound had begun to show evidence of a ventilation system. A mature nest had 700 000 adults, which suggests that the mound first appears above ground at a relatively early stage (1 -2 years?) in the life of the colony.

4.4.3 The hive of mature nests

In some equatorial *Macrotermes* species, the hive is contained entirely within the mound and is, therefore, above the external soil surface (Figs. 19, 20, 21). In this study the hives of 26 nests were completely below the external soil surface (Fig. 4), whereas four nests had the hive extending into the mound, above the external soil surface. The structure of the hive of sampled *M. natalensis* nests was similar to those of *M. bellicosus* nests in Nigeria (Fig. 22). There was no large air space (basement) below the hive of sampled *M. natalensis* nests as there is in some *M. bellicosus* nests (Figs. 19, 20, 22). The hive of *M.natalensis* nests was not supported by pillars, as in *M. bellicosus* in Uganda (Fig. 19). However, the galleries did become more compact towards the bottom of the hive, as they appear to do in *M. michaelseni* nests (Fig. 18).

These differences may be due to adaptations to environmental factors such as mean annual minimum temperature and rainfall, rather than simply due to genetic variation (*i.e.* in hot climates the hive is placed within the mound, the whole nest resting above the external soil surface. In cooler climates the hive is placed directly underneath the mound and is, therefore, partially or totally subterranean). However, as nests of closely related species were not sampled in this study, this would be difficult to substantiate.

The royal cell of 20 nests was centrally positioned. However, in five other nests it was found at the side of the nursery. In yet five other nests the royal cell was not found. These missing cells were most likely due to the royal cell being missed during excavation, as these nests were the first to be excavated when the author was, as yet, unfamiliar with the structure of the nest. The position of the royal cells of the majority of excavated nests in this study differed from the literature. Coaton (1949a) illustrated the royal cell in the lower half of the hive, but I only found it in the middle or upper half of the hive, never in the lower half. The royal cell did not appear to be separated from the dense slab of earth by an air space, as it is in *M. michaelseni* (Darlington, 1985a, 1985c) and *M. subhyalinus* (Darlington 1984b).

4.4.4 The hive of juvenile nests

Abe and Darlington (1985) described three juvenile nests of *M. michaelseni*. Two nests consisted of only a royal pair and their brood in a ellipsoidal cell, below the soil surface, however; one nest was found which consisted of a large round space (25cmx25cmx26 cm) containing a fungus ball. The royal cell was found to be inside the fungus ball. This must have been the beginning of a nursery area. The discovery of a nest (in the present study), with an apparently transitional structure between juvenile and mature (nest 85, Fig. 6), seems to indicate that the nest grows (increases in size) by a process of remodelling, as was suggested by Lüscher (1961) and Noirot (1977), rather than simply by chambers being excavated from the surrounding soil (Noirot, 1970), although it may well be that the two processes act simultaneously. The chambers, which are first excavated to enlarge the size of the hive, may then be remodelled as the insect population increases.

CHAPTER 5

ESTIMATES OF MOUND AND QUEEN AGES

5.1 ESTIMATES OF MOUND AGES

Estimated mound ages are given in Table 9 last column. The youngest mound recorded was that of nest number 80, which was estimated to be one to two years old, by the owner of the farm on which the nest was found. The small external dimensions of the mound, the small hive size (see Appendix 5) and structure (no fungus garden present) indicated that this mound was in the juvenile phase of development. Four other nests (81-84) on the same farm, also were estimated to have young mounds (less than five years old). However, one nest (85), was at least five years old (photographic evidence), but there was no way to determine an upper limit of age for this nest. Interestingly, a small fungus garden was differentiated from the nursery (Fig. 6), which was considerably smaller than the previous nests. This led me to believe that this nest was in a transitional phase between juvenile and mature phases of development. Seven mounds (24, 25, 26, 28, 30, 32 and 55) were all estimated to be less than eight years old. All these nests were sampled on an area that was drained and built up into a terrace, construction being completed in 1984. If the nest remains hypogeal for two years, none of these mounds could be older than eight years. Any nests that were founded in 1984 would not have produced a mound until 1986. All these seven nests were sampled during 1993 and were structurally mature. The oldest mound recorded was that of nest number 90, which was estimated to be 25 years old by the owner of the farm on which the nest was located. The large, external dimensions of the mound and hive support this viewpoint. The remaining 23 nests were all estimated to have mound ages between 8 and 20 years.

5.2 Discussion

Mounds appear to survive for considerably longer periods than the colony does. This is because complete erosion of large mounds may take as long as 20-25 years (Pomeroy, 1976a; Lepage, 1984). The nest may also be recolonised (Darlington, 1985b), although the recolonising species may not be the same as the original species (Pomeroy, 1976a). This may give the impression that the nest has an extended life. However, Pomeroy (1976a) has estimated that the average life of a *Macrotermes bellicosus* mound in Kampala, Uganda, was 10 years. Lepage (1984) calculated that a mound of *M. bellicosus*, in the Ivory Coast, reached 1m height 2 to 3 years after it appeared above ground and that a 3m mound was probably 8 to 10 years old. No other comparable mound ages could be found in the literature for *Macrotermes natalensis* or any other species, although Grassé (1949) seemed to think that the reproductive pair lived for 80 years.

Table 9: Mound dimensions, estimated mound age and source(s) of evidence for these estimates. "-" represents no data.

Nest code number	Mound dimensions (m)			Evidence in support of age	Estimated age (years)
	H	D1	D2		
4	0.80	2.80	1.70	Mound was identified on 1975 photograph.	> 19
14	0.90	1.70	1.30	Mound was identified on 1975, 1980 and 1993 photographs.	> 19
15	1.15	2.10	1.05	Mound was identified on 1975, 1980 and 1993 photographs.	> 19
16	0.95	3.10	2.90	Mound was under tree, therefore not visible on photographs.	-
24	0.70	2.35	1.45	Mound could not be older than 8 years as area drained and terraced in 1984.	< 8
25	0.35	1.60	1.40	Mound could not be older than 8 years as area drained and terraced in 1984.	< 8
26	0.40	0.80	0.70	Mound could not be older than 8 years as area drained and terraced in 1984.	< 8
28	0.40	2.20	1.75	Mound could not be older than 8 years as area drained and terraced in 1984.	< 8
30	0.25	2.00	1.15	Mound could not be older than 8 years as area drained and terraced in 1984.	< 8
32	0.20	1.25	0.85	Mound could not be older than 8 years as area drained and terraced in 1984.	< 8
45	0.65	1.30	0.90	Mound was not identified on 1981 photographs, but was sampled in 1992.	< 13
46	1.10	2.10	1.30	Mound was identified on 1981 photographs.	> 14
50	0.55	1.55	1.20	Mound was not identified on photographs. Anecdotal evidence from University worker gives mound age as 10-15 years.	10-17
55	0.60	1.20	1.10	Mound could not be older than 8 years as area drained and terraced in 1984.	< 8
56	-	-	-	No epigeal mound was present. Anecdotal evidence from house owner gives age of nest as 40 years.	40
61	0.60	1.50	1.45	Mound under tree, therefore not visible on photographs.	-
62	0.50	1.30	1.00	Mound was not identified on 1981 photographs.	< 14

Nest code number	Mound dimensions (m)			Evidence in support of age	Estimated age (years)
	H	D1	D2		
63	1.00	3.00	3.00	Mound was identified on 1981 photographs.	< 15
65	0.85	2.30	2.10	Mound was identified on 1981 photographs.	< 15
66	0.65	2.10	1.65	Mound was identified on 1981 photographs.	< 15
67	0.70	2.60	2.20	Mound was identified on 1981 photographs.	< 15
70	1.20	2.30	2.20	Mound was identified on 1981 photographs. Anecdotal evidence from forest labourer gave mound age as 10-15 years.	< 15
72	0.75	1.95	1.70	Mound was identified on 1981 photographs. Anecdotal evidence from forest labourer gave mound age as 10-15 years.	< 15
80	0.13	0.45	0.30	Mound not identified on photographs. Anecdotal evidence from farmer gives age as 1-2 years.	1 to 2
81	0.18	0.90	0.80	Mound not identified on photographs. Anecdotal evidence from farmer gives mound age as at least 1 year old.	< 5
82	0.14	0.70	0.50	Mound not identified on photographs. Anecdotal evidence from farmer gives mound age as at least 1 year old.	< 5
83	0.18	1.00	0.90	Mound not identified on photographs. Anecdotal evidence from farmer gives mound age as at least 1 year old.	< 5
84	0.16	0.75	0.45	Mound not identified on photographs. Anecdotal evidence from farmer gives mound age as at least 1 year old.	< 5
85	0.40	1.40	1.30	Mound was identified on 1989 photographs. Anecdotal evidence from farmer also indicated that mound was at least 5 years old.	> 5
90	1.00	3.30	2.80	Mound was identified on 1981 photographs. Anecdotal evidence from farmer gives age as 25 years.	> 25

5.3 ESTIMATES OF QUEEN AGES

Appendix 4 summarises the ages of queens derived from their mass, colour and from the age of the mound. The smallest and estimated youngest queen was from nest number 80 (Fig. 23). This was the smallest nest excavated and had a juvenile structure with only nursery galleries present and no separate fungus garden. The mound was estimated to be one to two years old; so the queen from this nest could have been three to four years old. The queen from this nest weighed 1.90 g, was 39.5 mm long and 9.7 mm wide, had creamy white pleural membranes (PM) and creamy white intersegmental membranes (ISM) (Fig. 23). The queens from three other, small nests (81, 82 and 84) were estimated to be between three and seven years old, because the ages of the mounds were estimated to be between 2 to 5 years. One nest (83), however, showed some peculiarities: it had a small mound but was covered with grass; there was only a nursery present; the queen was large (46.6 mm long, 14.4 mm wide), had pale membranes, and was heavier than expected, weighing 5.26 g. The grass-covered mound and large queen mass seem to suggest a mature nest, whereas the structure indicates a young nest.

The queen from the oldest nest (90) had a very wrinkled abdomen and dark intersegmental membranes (Fig. 24) although the pleural membranes were pale. The dimensions of this queen were: length 53.1 mm, width 14.0 mm, and she weighed 5.36 g. The queens from the other nests varied in colour, turgidity and mass (Appendix 4 and Figs 25-27).

5.4 Discussion

Assuming that a) a single queen found inside the royal cell is the original founding queen and b) the nest remains hypogeal for two years before producing an epigeal mound, the queens in this study could be expected to have a maximum life span of approximately 30 years. These figures are greater than those derived for *M. bellicosus* by Collins (1981). This may in part be due to the small sample size, or it may be that an entirely different system is operating in the study area. It appears that the nests of *M. natalensis* produce an epigeal mound when the subterranean nest (hive) is small in size (0.50 m to 0.60 m in diameter) and that both the hive and mound remain small for about 4 years. Mounds seemed to become mature at an age of about 5-8 years. *M. natalensis* mounds seemed to survive for a maximum of 25 years or more. It also seemed that queens could survive for a maximum of 27 years, except for the queen of nest 56 which appears to have survived for 40 years.

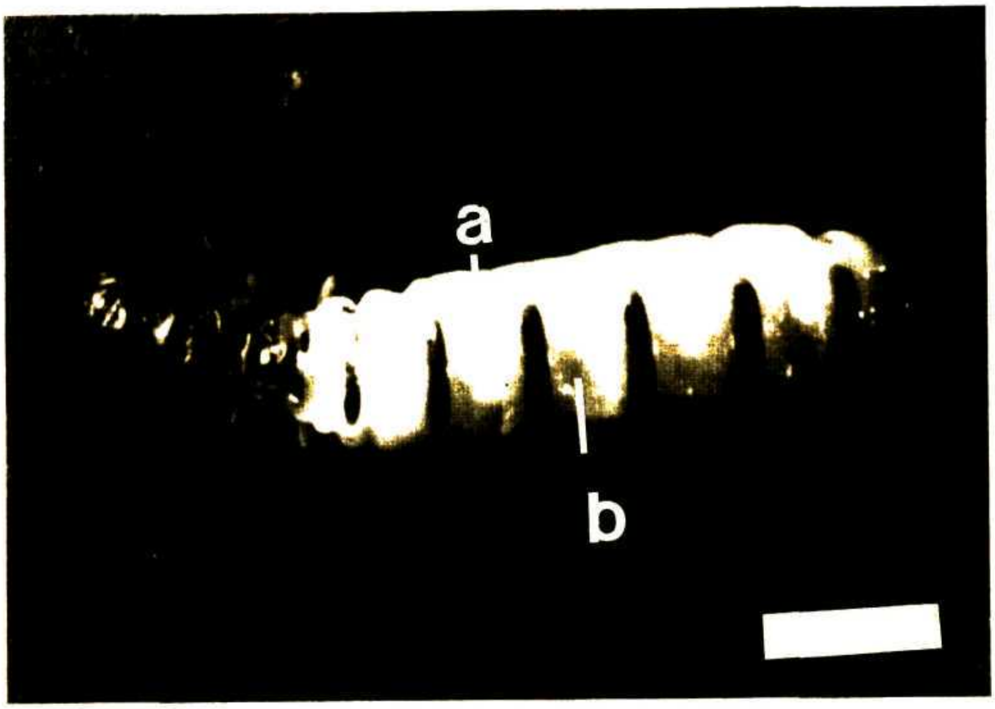

Fig. 23. The queen from nest 80 showing the white pleural membranes (a), white inter-segmental membranes (b) and turgid abdomen, indicating that this queen was a juvenile. Scale bar = 10 mm.

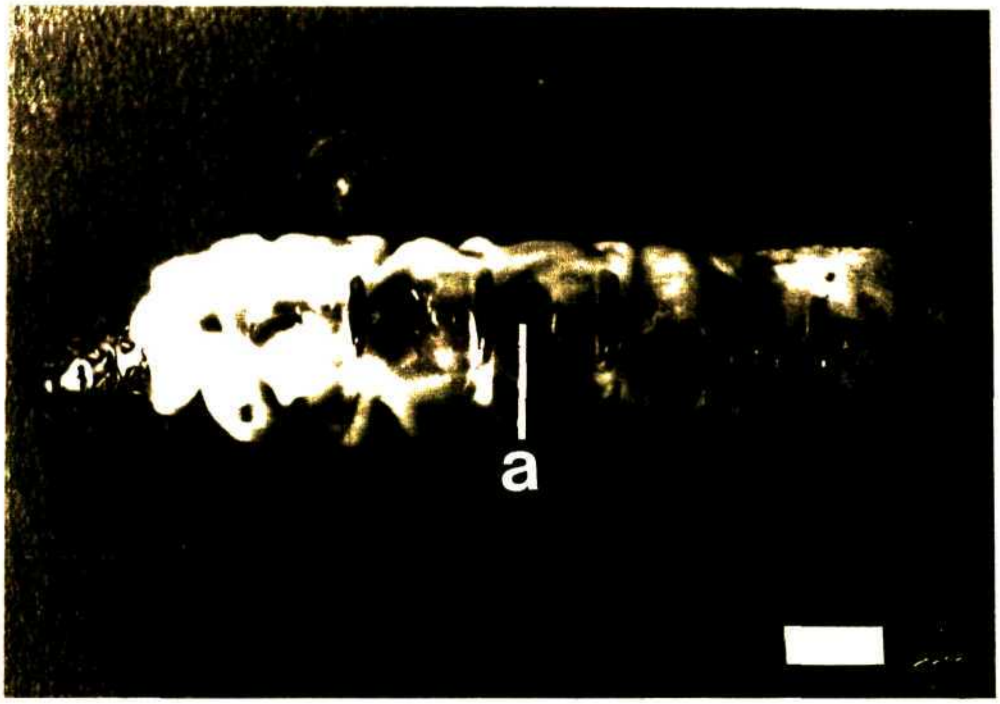

Fig. 24. The queen from nest 90 showing very wrinkled abdomen and dark intersegmental membranes (a), indicating that this queen was old and senescing. Scale bar = 10 mm.

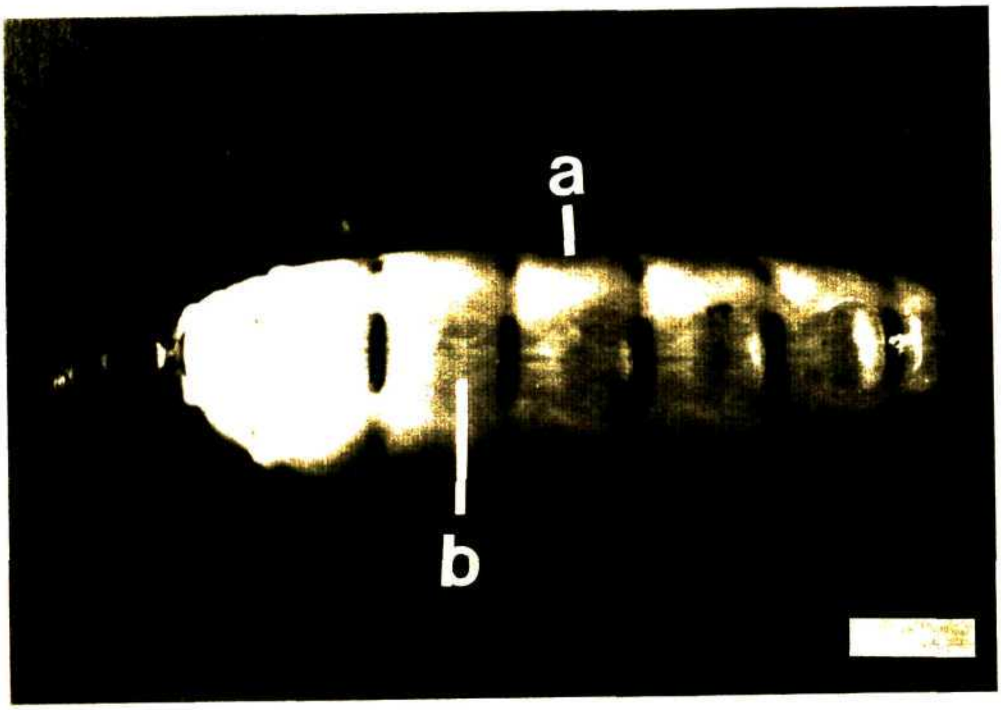

Fig. 25. Queen from nest 55 showing pale pleural membranes (a), very pale intersegmental membranes (b) and very turgid abdomen. Scale bar = 10 mm.

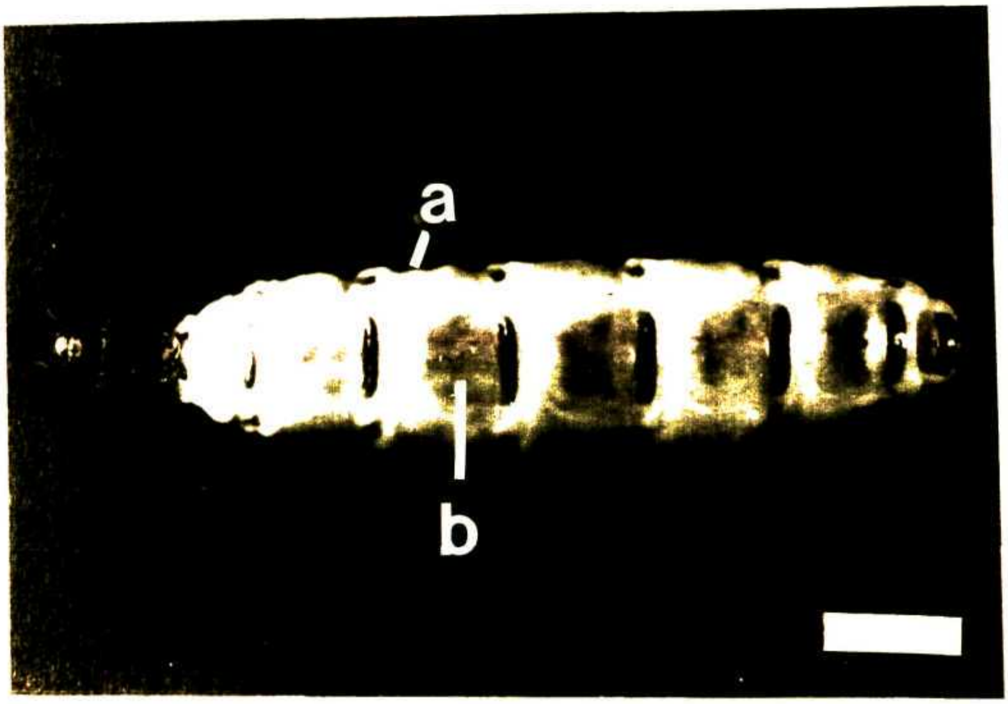

Fig. 26. Queen from nest 62 showing pale pleural membranes (a), pale intersegmental membranes (b) and fairly turgid abdomen. Scale bar = 10 mm.

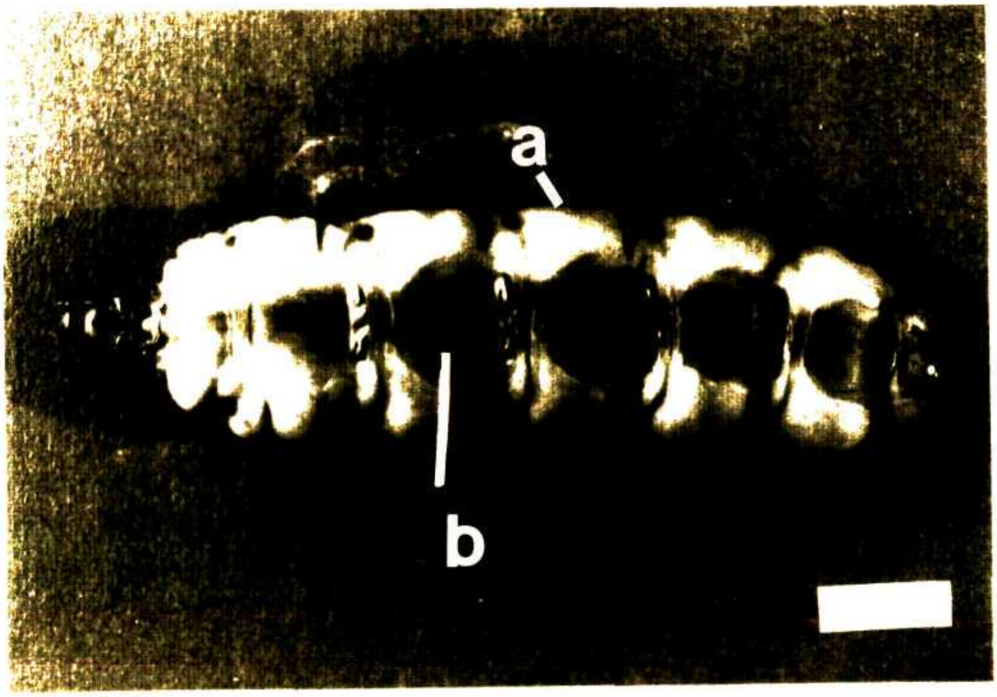

Fig. 27. The queen from nest 70 showing dark pleural membranes (a), very dark inter-segmental membranes (b), and wrinkled abdomen, indicating that this queen was old. Scale bar = 10 mm.

Multiple queens occurred in three out of the 30 nests excavated (10%) compared with 23.3% for *M. michaelseni* and 42% for *M. herus* (Sjöstedt) (Darlington 1988). Because of the lack of evidence supporting the founding of a nest by more than one pair of alates, the multiple queens in this study were thought to have been replacement queens. In one nest (45) this was most likely the case as two small queens were found in a royal cell (Fig. 17), together with a large physogastric queen, which was damaged (by the author). One other nest (65) also with two queens, had the royal cell at the side of the nursery. Yet another nest with two queens (nest 46) had the royal cell in the middle of the nursery. Recolonisation of nests did not seem to have occurred, because no hives were found that were partly or wholly above the external soil surface, as was suggested by Darlington (1985b).

CHAPTER 6

VIGOUR AND DECLINE OF NESTS

Nests were sometimes found which seemed to be growing or declining on the basis of the following three criteria: a) mound texture and grass cover, b) queen mass and colour and c) fungus comb mass. For example, in canopied forests one frequently encounters nests which seem to have declined in vigour and the question arises as to whether or not they might constitute a problem in the re-establishment of trees at a subsequent rotation. In this chapter, Chapters 4 and 5 are brought together in an attempt to categorise the nests into vigorous or declining.

In the course of this study, nests sometimes appeared to be dead or dying (mound with a large sandy pediment and frequently with a high percent grass cover), but contained more fungus comb than expected and also often contained young-looking (pale) queens. Yet other nests were found which had a particularly low fungus comb mass and a large bare mound, but an old queen. These conditions might happen for two reasons. Firstly, the original, founding queen may have died from disease or old age (Coaton, 1949b; Darlington 1982; Darlington, 1988). If this is the case, an alate inside the nest, that has not yet escaped to swarm or was left from an earlier swarm, becomes a secondary queen. Secondly, the original founding colony, and queen, may die, but the nest might be recolonised by a new pair of reproductives (Pomeroy, 1976a; Darlington, 1985b).

Vigorous nests had mounds that varied in grass cover from 0%-70% and did not have large sandy pediments or a green tinge in colour. The texture varied from sandy to smooth, but was never crumbly. Queens varied from small to large in size and weighed from 1.90g to 7.84 g. They also had pale (creamy-white) pleural membranes and creamy-white to pale-brown intersegmental membranes, however, turgidity varied from very turgid (Figs 23, 25) to turgid (Fig. 26). The ratio of fungus comb mass to hive volume was higher than that of declining nests (Table 10).

Nests were regarded as senescent, or declining, when the mound was medium to large in size and showed signs of extensive erosion, indicating that the mound was no longer being maintained. These included a bare mound with a large sandy pediment (Figs. 7 and 8), a bare mound with a crumbly texture (Fig. 10) or a bare mound with an overall greenish appearance due to the growth of algae (nests 70 an 72 in an eight year old *Eucalyptus* stand). The queens of these nests tended to be dark in colour with pale-brown pleural membranes and medium- to dark-brown intersegmental membranes. The queens also tended to be flaccid as opposed to the turgid queens of vigorous nests. The ratio of fungus comb mass to hive volume of these nests was low (Table 10).

6.1 VIGOROUS NESTS

Based primarily on the ratio of fungus comb mass to hive volume, 12 nests (Table 10) could not be classified as either vigorous or declining, due to these nests not having the fungus combs removed. Five nests (14, 16, 65, 70 and 72) had a particularly low fungus comb mass to hive volume ratio (fcm:hv), in all cases being between 4 and 11. In contrast all other nests (classified as vigorous) had fcm:hv between 19 and 37.

Six nests (80, 82, 83, 84, 85, 90) had the highest fungus comb mass to hive volume ratio (Table 10), indicating that the subterranean nest was packed full of fungus combs (indicating a high insect population). Nests 82 and 84 had heavier queens than nests 80 and 81, but similar fungus comb masses. The sizes of the mounds, hive volumes, and queen and fungus comb masses seem to indicate young nests, the estimated ages of the mounds also supports this. Nest 83 had the same hive volume as nest 82, but almost twice the fungus comb mass. The queen in this nest was much heavier than the other nests, similar in mass to some queens from vigorous nests. The mound of nest number 85 had a larger circumference and subterranean hive volume than any of the previous nests, but the queen mass was similar to that of nest 83. The structure of nest 85 indicated that it was in a transitional period between the juvenile and mature phases. It appeared that the outer nursery had been remodelled to accommodate the large fungus combs. The mound of nest number 55 had a smaller circumference and hive volume than the previous nest, but a heavy queen and fungus comb mass. The hive volume, fungus comb mass and queen mass seem to indicate a vigorous nest, however the size and texture of the mound seem to suggest that the mound was young. This nest may have been entering the mature phase, which would explain the absence of alates when excavated. However, the amount of fungus comb was too high to assume the nest was young.

Seven other nests (15, 30, 50, 55, 66, 67 and 90) were also considered vigorous, because of the large mound size, large hive volume, heavy queen, high fungus comb mass and the presence of alates at the time of excavation. These nests had a lower fungus comb mass to hive volume ratio than the four young nests (80, 81, 82, 84), but a higher ratio than the declining nests (14, 16, 65, 70, 72). Although the mounds of nests 15, 30, 66 and 67 were of similar size, the hive volume, fungus comb mass, queen mass and membrane colour seem to indicate a vigorous nest. However, these mounds had a high percentage grass cover but varied in texture. Nest 90 also had a very large mound circumference and hive volume, fungus comb mass exceedingly high, but queen mass comparatively low. The mound size, the fungus comb mass and the hive volume seem to indicate a mature nest. However, the structure of the nest seems to indicate that the primary queen may have been replaced, because the slab of earth containing the royal cell spanned the width of the nursery, and the royal cell was found right at the edge of the nursery. The queen found in this royal cell was dark and flaccid and appeared to be old. The hive had also been extended outwards into two alcoves, one to the side and one to the back of the hive. The estimated mound age was 13 to 25 years, so it seems likely that the queen might have been the original, founding queen.

6.2 DECLINING NESTS

Five nests (14, 16, 65, 70 and 72) seemed to be declining because they had the lowest fungus comb mass to hive volume ratio (possibly suggesting that the amount of fungus comb might have decreased), a small, dark queen and a grassy eroded mound. The mound of nest 14 had a medium circumference (4.76 m), crumbly texture with 30% grass cover; the hive volume was very large (1.45 m^3), but the fungus comb mass (7.8 kg) was lower than that expected. The queen was large, had a pale colour, and was of medium mass (5.33 g). The mound size and texture, the hive volume, and the fungus comb mass seem to suggest a declining nest, however, the medium mass queen seems to indicate a mature nest. Because the royal cell was in the middle of the nursery the nest is unlikely to have been recolonised, but since the queen had a pale colour she may have been a secondary queen. The estimated mound age was over 20 years so it seems likely that the original queen had been replaced. The mound of nest 16 had a very large circumference and a sandy texture. The hive volume was also large, but the fungus comb mass was much lower than expected. The queen was brown and of medium mass. The mound, hive volume and the low fungus comb mass seem to suggest a declining nest, however, the queen seemed to be vigorous. The estimated mound age was 20+ years, so it seems likely that the original queen might have been replaced. The mound of nest 65 had a smaller circumference and hive volume. The fungus comb mass was again lower than expected. Two queens were found in the same royal cell in the middle of the nursery. Both queens were brown but one was heavier than the other. It might be possible that the smaller queen was the original queen and that the heavier queen was a secondary queen. The mound of

Table 10: Parameters used to determine vigorous, declining and unclassified nests. Alates = present (p) or absent (a), c = mound circumference, cl = colour of membranes, eqa = estimated queen age, ema = estimated mound age, fcm = fungus comb mass, fcm:hv = ratio of fungus comb mass to hive volume, g = grass cover, hv = hive volume, qm = queen mass, t = texture, tg = degree of turgidity. "-" represents no data. Ages of nest 25 and 26 were estimated from date of completion of road construction

	Nest number	Mound c	g (%)	t	Queen qm (g)	cl	tg	fcm (Kg)	hv (m^3)	Age eqa	ema	Date excavated	Alates	fcm:hv
U	4 •	7.28	5	sandy	5.10	white	fairly turgid	-	1.04	> 21	> 19	June 1993	p	-
N	24 •	6.14	20	crumbly	6.25	white	fairly turgid	-	0.82	< 10	< 8	July 1993	p	-
C	28 •	6.25	35	sandy	-	-	-	-	1.04	<10	< 8	April 1993	p	-
L	32 •	3.36	5	sandy	8.02	pale	fairly turgid	-	0.72	< 10	< 8	May 1993	a	-
A S	45 •	3.51	0	crumbly	0.76	pale	not turgid	-	0.71		< 13	June 1993	a	-
S					0.51	pale	not turgid							
I F	46 •	5.49	25	sandy	4.02	pale	fairly turgid	-	2.14		< 13	July 1993	a	-
I					4.24	pale	fairly turgid							
E	56 •	-	-	-	5.67	pale	very turgid	-	-	40?	40	July 1993	a	-
D	61 •	4.64	10	sandy	8.16	pale	turgid	-	0.94	< 17	< 15	July 1993	a	-
	62 •	3.64	50	crumbly	6.96	pale	fairly turgid	-	0.49	< 17	< 15	July 1993	a	-
	63 •	9.43	10	crumbly	5.79	brown	flaccid	-	0.55	< 17	< 15	Aug. 1993	a	-

• These nests were used primarily to determine the structure of nests, as a result the fungus comb was not completely removed.

	Nest number	Mound			Queen			fcm (Kg)	hv (m^3)	Age		Date excavated	Alates	fcm:hv
		c	g (%)	t	qm (g)	cl	t			eqa	ema			
VIGOROUS	15	5.22	70	crumbly	7.84	dark	flaccid	13.15	0.53	> 21	>19	Sept. 1993	p	24.8
	30	5.13	60	sandy	6.57	dark	flaccid	11.20	0.49	< 10	< 8	Sept. 1993	p	22.8
	50	4.36	55	sandy	4.83	pale	fairly turgid	15.25	0.65	12-19	10-17	June 1993	p	23.5
	55	3.62	0	bubbly	7.51	pale	turgid	10.03	0.42	< 10	< 8	July 1993	a	23.9
	66	5.94	30	sandy	6.12	pale	fairly turgid	5.06	0.26	< 17	< 15	Nov. 1993	p	19.5
	67	7.57	50	sandy	7.22	dark	fairly turgid	10.80	0.47	< 17	< 15	April 1994	a	21.4
	80	1.20	40	bubbly	1.90	pale	turgid	2.00	0.07	2-5	1-2	June 1994	a	28.6
	81	2.68	60	bubbly	2.90	pale	turgid	3.10	0.12	< 7	< 5	June 1994	a	34.4
	82	1.91	5	bubbly	3.75	pale	turgid	2.60	0.17	< 7	< 5	June 1994	a	20.0
	83	2.99	5	bubbly	5.26	pale	turgid	4.85	0.10	< 7	< 5	June 1994	a	37.3
	84	1.94	5	bubbly	3.05	pale	turgid	2.90	0.09	< 7	< 5	June 1994	a	36.3
	85	4.25	30	sandy	4.49	pale	turgid	6.10	0.19	> 7	> 5	June 1994	a	30.5
	90	9.62	0	sandy	5.36	dark	flaccid	27.45	1.26	> 27	> 25	July 1994	a	21.8
DECLINING	14	4.76	30	crumbly	5.33	brown	turgid	7.80	1.45	> 21	> 19	Sept. 1993	a	5.4
	16	9.43	75	sandy	6.26	brown	turgid	7.58	1.69	-	-	Oct. 1993	a	4.5
	25	4.72	5	sandy	-	-	-	-	1.61	< 10	< 8	May 1993	a	-
	26	2.36	10	sandy	-	-	-	-	0.44	< 10	< 8	May 1993	a	-
	65	6.92	60	sandy	3.96	brown	flaccid	8.25	0.77	< 17	< 15	Nov. 1993	a	10.7
					5.01	brown	turgid							
	70	7.07	5	crumbly	9.44	dark	fairly turgid	5.90	0.60	< 17	< 15	Mar. 1994	a	9.8
	72	5.75	5	crumbly	6.67	dark	fairly turgid	8.50	0.93	< 17	< 15	Mar. 1994	a	9.1

nest 70 had a large circumference, green colour and a crumbly texture. The hive volume was almost one third of the first two nests, but similar to nest 65. The fungus comb mass was less than nest 65. The queen was dark and heavy. The mound size, texture, hive volume, low fungus comb mass and dark queen seem to indicate a declining nest. The royal cell was positioned to one side of the nursery. The mound was estimated to be 10 to 15 years old, suggesting the lifespan of the queen was in the order of 12 to 17 years. The mound of nest 72 had a smaller circumference, a green colour and a crumbly texture. The hive volume was larger than the previous nest (70), but the fungus comb mass was almost similar to nest 65. The queen was dark and of medium mass. The mound size and texture, the hive volume, low fungus comb mass and dark queen seem to indicate a declining nest. The mound was estimated to be 10-15 years old.

For the remaining 12 nests, fungus comb mass was not measured as these nests were used to determine structure only. In three of these nests (25, 26 and 28) no queens were found. This may have been due to the royal cells being overlooked and discarded or the queens may have been moved out of the royal cells. This has been observed for *M. michaelseni*, when doryline ants attacked the nest (Darlington, 1985c).

6.3 UNCLASSIFIED NESTS

Nests 45 and 46 all had multiple queens in a single royal cell. Nest 45 had one large queen (destroyed while excavating nest) and two very small queens (Fig. 17). Nest 46 had two similar sized queens. As there is no evidence of multiple founding queens for *Macrotermes*, the original queen from this nest probably died, resulting in two alates becoming replacement queens.

Table 11 shows the correlation coefficients of the seven nest variables measured in this study. Temperature difference and grass cover were of little use in determining whether a nest was vigorous or declining. Both correlation coefficients for temperature vs. fungus comb mass and temperature vs. queen mass were very low (0.248 and 0.395 respectively). This result can be explained in part by it being impossible to know the exact position of the temperature probe within the nest, before the nest was excavated. When the nest had been exposed the hole drilled for the temperature probe could be seen. Sometimes the probe ended up in an air passage just below the mound, sometimes in a fungus comb chamber and sometimes in the nursery. The correlation coefficients for grass cover vs. queen mass and grass cover vs. fungus comb mass were also low (0.383 and -0.106 respectively). This may also be explained in part by the fact that a fire had passed through the site several months before, and only a fraction of the original vegetation cover was present on the mounds at the time of excavation. Interestingly, there appeared to be a weak, positive relationship between the fungus comb mass and the height of the mound. Thus, as height increased the fungus comb mass also increased. Another interesting outcome is that there appeared to be a negative relationship between the size of the mound (both circumference and height) and the amount of new construction added to the mound. Thus, when the mound is small, it is being added to (i.e. growing), but when it is big the mound is no longer added to (i.e. not growing). Also the expected strong, positive relationship (0.818) between the height and the circumference of the mound was clearly evident. Fungus comb mass was strongly, but negatively, correlated (-0.678) with the amount of construction produced on the mound. Thus when fungus comb mass was low the mound was being added to (i.e. growing). When fungus comb mass was high the mound was no longer being added to, (i.e. not growing) The correlation coefficient of queen mass vs. fungus comb mass was high (0.766) indicating that the amount of fungus comb was positive and exponentially related to the condition of the queen.

Table 11: Correlation coefficients (r) of the seven nest variables measured in this study. An exponential model ($y = ax^b$) gave the highest values of r.

	Fungus comb mass	Queen mass	Temperature difference	Grass cover	Mound additions	Mound circumf.	Mound height
Fungus comb mass	1						
Queen mass	0.766	1					
Temperature difference	0.248	0.395	1				
Grass cover	-0.106	0.383	-0.115	1			
Mound additions	-0.678	-0.512	0.027	-0.203	1		
Mound circumference	0.730	0.708	0.302	0.091	-0.617	1	
Mound height	0.618	0.689	0.232	0.011	-0.689	0.818	1

6.4 DISCUSSION

In this study an exponential relationship was found to exist among several of the studied variables (the model $y=ax^b$ gave higher correlation coefficients than a linear model). This may have been due to the small sample size (< 30 for most variables), or it may have been that this species does in fact display such a relationship. As no similar studies have been done on *Macrotermes natalensis*, in this or any other country at this time, there are currently no figures to compare the results of this study with. However, the results of this study can be compared with studies dealing with closely related *Macrotermes* species, such as *M. bellicosus*, *M. michaelseni*, and *M. subhyalinus* (Table 12).

Table 12: Queen mass (QM) and fungus comb mass (FCM) of two species of *Macrotermes*. (Compare with Appendix 5).

Abe & Darlington (1985) Darlington (1984b)		Darlington (1990)	
Macrotermes michaelseni		*Macrotermes subhyalinus*	
QM (g)	FCM (Kg)	QM (g)	FCM (Kg)
0.12	negligible	1.38	0.41
0.16	0	3.87	1.27
0.32	0.23	3.39	2.17
3.23	0.78	7.37	5.09
3.60	1.23	8.13	4.74
20.16	10.31	14.88	9.05
20.24	10.87	19.95	13.98
		18.42	21.10
		18.42	13.77
		21.93	19.78
		22.85	12.87

In Table 12 it can be seen that high fungus comb masses correspond to queen masses of more than 10 g. However, in the present study, similarly high fungus comb masses corresponded to queen masses of less than 10 g. Also the highest queen mass recorded in this study was 9.44 g. It is unclear what these differences could mean, in terms of productivity and maturation of the queens. The indications were that vigorous nests generally had a high fungus comb mass to hive volume ratio, a pale and heavy queen, and a high proportion of additions on the mound. However, the mounds of these nests varied in percentage grass cover. Declining nests had a low fungus comb mass to hive volume ratio (possibly reflecting a decrease in the nest population), a dark, wrinkled, flaccid queen and a low proportion of additions to the mound. Grass cover and temperature difference were of little use in determining if a nest was vigorous or declining.

CHAPTER 7
GENERAL DISCUSSION

This study has shown that 1) the nest remains hypogeal (in the form of a copularium) for as long as seven months after foundation, 2) the nest produces a mound in the third to fifth year after foundation, 3) the primary queen reaches an age of 17 to 21 years old, at which time she probably dies and is replaced by a secondary queen(s), 4) the nest may survive for more than 27 years, 5) the vigour of a nest cannot be determined from its outward appearance, 6) the structure of mature mounds, and hives, differs from early descriptions and depictions in the literature and 7) that juvenile nests do not have a distinctly separate fungus garden.

7.1 REARING STUDIES

Macrotermes spp. are not easy to rear in containers under laboratory conditions (Okot-Kotber, 1981). In this study, mortality of *Macrotermes natalensis* was high in all rearing containers used. A more efficient technique needs to be developed for rearing *Macrotermes* species, so that founding pairs have a higher chance of surviving. One of the two longest surviving colonies was in a 1 l container and the other in a 2 l container. Therefore containers could be restricted to a maximum of 2 l capacity. No major castes or fungus combs were ever observed in these hypogeal nests. For future rearing it is suggested that the containers, the soil and the surface of the termites (if possible) be sterilised so as to reduce pathogenic contamination. Initially, several alates should also be dissected to determine whether they carry viable fungal spores in the gut or not. A piece of fresh fungus comb may have to be placed inside the copularium, rather than on the surface of the soil, for successful establishment of a comb. It might also be useful to cover the rearing container with a dark piece of card or cloth, which should be removed only for short periods of time for observations. This would help to reduce the effect of light on the development of the colony, as the copularium was often positioned up against the side of the plastic container. Drainage holes in the base of the rearing containers would lessen the chance of the copularium becoming water-logged and the termites drowning.

7.2 NEST STRUCTURE

The structure of *M. natalensis* nests excavated in this study, was found to differ from descriptions and diagrams found in the literature. The only two comparable studies found concerning the nest structure of *Macrotermes natalensis*, in South Africa, are by Fuller (1915) and Coaton (1949a). The diagrams in Fuller (1915) depict a dome- or conical-shaped mound with a chamber below it. This chamber consists of many clay shelves, however, no differentiation was evident in the thickness of shelves within the chamber and no fungus comb garden was depicted. In the description, though, the fungus combs are mentioned as resting on the thicker shelves of the top half of the chamber, with the lower half containing thinner shelving, barren of combs. The royal cell is described as being found inside a heavy, rounded mass of clay, suspended in the middle of the lower half of the hive. Coaton (1949a), on the other hand, contains detailed diagrams of the nest in which the shelves are depicted as being thinnest in the lower half of the hive, with the fungus garden positioned on either side, and above, the thin shelves. The royal cell is positioned within a heavy slab, placed inside the thin shelved area. These early workers described the nests similarly, but during the current study it was found not to be the case with the nests excavated.

In this study, the mounds of mature *M. natalensis* nests contained many large air passages (with no apparent fixed arrangement), while mounds of juvenile nests contained only small tunnels ramifying throughout the structure. Mature nests contained a distinct fungus garden surrounding the nursery. The nursery consisted of paper-thin clay shelves placed closely together. The nursery was also positioned directly beneath the mound. Juvenile nests did not have a distinct fungus garden, but instead contained small, flattened fungus combs scattered throughout the nursery galleries. The royal cell was usually found in the centre (20 nests), but in five nests the royal cell was found to one side of the nursery.

These differences may be as a result of adaptation to micro-climatic conditions. Coaton (1949a) conducted his studies in the Northern Province where both the mean annual minimum temperature and the mean annual rainfall are less than in the KwaZulu-Natal midlands. It may be that the royal cell is under the fungus garden, in Coaton's diagrams, for insulation purposes. Fuller (1915) conducted his investigations throughout the Northern Province (Transvaal) and KwaZulu-Natal. Although he distinguished different mounds between these provinces, he did not do this for the hives. Thus, the picture he described did not account for any differences noted in the present study. It may also be possible that the differences are simply due to genetics, i.e. two or more populations of *Macrotermes natalensis* may exist within the southern African region. One population may have evolved a nest with the nursery restricted to the lower half of the hive in the colder areas of the region and the other one, in the warmer areas, constructing a nest with a globular nursery.

7.3 MOUND AND QUEEN AGES

The queens from three nests were not found, even though two of the nests were totally excavated. Possibly, the royal cells were not located where they were expected and may therefore have been overlooked. Another possibility is that the queen may have escaped from the royal cell. This has been observed in *M. michaelseni* (Darlington, 1985c). A hole was opened in the side of the royal cell and the queen moved above or below the original royal cell via a specially built ramp to safety. An escaping queen was found in one other nest (50) in which the royal cell was not found. She was located at the edge of the hive, 1 metre from the centre. The queen was moving peristaltically and workers behind and in front of her appeared to be dragging and pushing her along the tunnel.

7.4 NEST VIGOUR

Because of the apparent contradiction of young queens in nests with old mounds, and old queens in nests with young mounds, the vigour of nests was also studied. For the forestry industry it is important to know if a nest is vigorous and growing or declining and dying, so that the potential threat of a nest in an area can be estimated before the trees are planted. Then appropriate chemical treatment can be taken so as to reduce the likelihood that the seedlings will be attacked.

Mound size and appearance (texture, colour, grass cover), queen mass and colour, fungus comb mass and ratio of fungus comb mass to hive volume, were all used to determine if nests were vigorous and growing, or declining and dying. The most useful variables were (a) the ratio of fungus comb mass to hive volume, (b) the queen mass and colour and (c) the percentage grass cover. It was also hoped that temperature difference would be a useful variable, however, there was too much variation (probably due to the small sample size, n=17, and readings were also taken at different times of the year). It was determined that 13 nests were vigorous and that five nests were declining. However, due to the reliance on fungus comb mass to determine vigour, and the fact that the fungus combs were not collected from 12 nests, these 12 nests could not be classified as either vigorous or declining.

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APPENDIX 1:

Raw data collected from 37 of the 71 rearing containers (34 copularia were not found). All measurements are in millimetres."-" represents no data. H = height, D1 = diameter 1, D2 perpendicular diamenter.

MONTH SAMPLED	NEST No	COPULARIUM DIMENSIONS			DEPTH BELOW SOIL SURFACE	COMMENTS
		H	D1	D2		
Feb	1	20	50	25	120	
	2	10	20	15	70	
	3	15	25	15	75	
	4	10	30	20	50	
	5	10	35	25	120	
	6	-	-	-	100	Only part of floor of copularium found.
March	1	10	20	15	110	
	2	15	25	15	105	
	3	-	-	-	120	Only part of floor of copularium found.
	4	-	-	-	80	Only part of floor of copularium found.
April	1	10	25	20	50	
	2	10	25	20	90	
	3	10	27	15	60	
	4	10	20	15	85	
	5	-	-	-	60	Only part of floor of copularium found.
May	1	15	28	25	40	
	2	10	25	20	65	
	3	15	20	15	70	
	4	-	30	20	85	Roof removed during excavation.

MONTH SAMPLED	NEST No.	COPULARIUM DIMENTIONS			DEPTH BELOW SOIL SURFACE	COMMENTS
		H	D1	D2		
June	1	15	40	20	60	
	2	10	50	15	80	
	3	-	30	30	40	Roof removed during excavation.
	4	15	35	20	50	
	5	15	40	15	90	
	6	10	25	10	65	
	7	-	-	-	80	Only part of floor of copularium found.
July	1	10	50	15	70	
	2	-	-	-	100	Only part of floor of copularium found.
Aug	1	10	20	15	70	
	2	10	35	25	90	
	3	10	30	25	75	
	4	-	-	-	130	Only part of floor of copularium found.
Sept	1	-	30	25	60	Roof removed during excavation.
	2	5	30	25	100	
	3	-	-	-	70	Only part of floor of copularium found.
Oct.	1	10	40	25	75	
	2	-	-	-	60	Only part of floor of copularium found.

APPENDIX 2:

Head capsule measurements of both soldier castes used for identification purposes. Figures represent mean of 10 randomly selected individuals from both castes. HL= head length, HW= head width and HD= head depth. All measurements in mm.

NEST	MAJOR SOLDIERS			MINOR SOLDIERS		
	HL	HW	HD	HL	HW	HD
4	5.14	4.23	2.75	3.32	2.86	1.89
14	5.28	4.38	2.78	3.30	2.79	1.83
15	5.16	4.27	2.67	2.91	2.51	1.68
16	5.19	4.38	2.80	3.09	2.65	1.82
24	5.31	4.42	2.80	3.20	2.75	1.77
25	4.75	3.98	2.50	2.68	2.83	1.89
26	5.26	4.41	2.84	3.16	2.72	1.85
28	5.64	4.72	2.85	3.38	2.91	1.94
30	5.33	4.34	2.83	3.22	2.77	1.83
32	5.08	4.60	2.85	3.07	2.66	1.81
45	5.67	4.75	3.05	2.78	2.48	1.62
46	4.98	4.05	2.49	2.95	2.53	1.83
50	5.17	4.31	2.78	3.18	2.63	1.72
55	4.79	3.99	2.52	3.43	2.93	1.91
56	4.94	4.23	2.71	2.90	2.62	1.79
61	5.26	4.37	2.89	2.89	2.45	1.69
62	4.93	4.04	2.56	2.87	2.47	1.66
63	4.98	4.07	2.64	3.07	2.59	1.73
65	5.21	4.38	2.89	2.65	2.34	1.81
66	5.54	4.56	3.08	2.88	2.62	1.67
67	4.73	3.95	2.56	2.85	2.43	1.66
70	5.05	4.14	2.65	3.07	2.61	1.76
72	5.19	4.52	2.79	2.62	2.31	1.84
80	4.75	4.06	2.53	2.77	2.48	1.64
81	5.04	4.21	2.67	3.12	2.57	1.76
82	4.93	4.08	2.61	2.90	2.46	1.62
83	5.16	4.36	2.50	2.92	2.60	1.72
84	5.20	4.25	2.68	2.84	2.40	1.63
85	4.97	3.96	2.06	2.71	2.36	1.64
90	5.68	4.75	3.08	3.58	2.96	1.93

APPENDIX 3:

Map coordinates, altitude (Alt) and mean annual rainfall (Mar) of the various sampling localities where nests were sampled.

Sampling site	Nest code Numbers	Map Co-ordinates and site name	Alt (m)	Mar (mm)
1	4	29°37'S, 30°25'E PMB- Road side, Golf Course	630	750
2	14,15,16	29°37'S, 30°25'E PMB- Open wasteland, opposite Oribi Airport	740	775
3	24,25,26,28,30 32,45,46,55	29°37'S, 30°23'E PMB- Camps Drift	760	747
4	50	29°37'S 30°24'E PMB- Road side, UNP Zoology student car park	670	750
5	56	29°37'S, 30°24'E PMB- Suburban garden	660	750
6	61,62,63	29°24'S, 30°28'E Road side, opposite sub-station	740	820
7	65,66,67	29°20'S, 30°27'E York commonage	785	1046
8	70,72 *	29°16'S, 30°28'E *Eucalyptus* sp. stand, Blinkwater farm	965	1241
9	80,81,82, 83,84,85	28°50'S, 29°36'E Winterton - Welgekozen farm	1100	671
10	90	29°22'S, 30°28'E Road side, opposite Beaulieu farm	780	1046

* One additional nest was partly excavated at this locality, but was found to be dead.

APPENDIX 4:

Raw data collected from the queens and the dimensions of the royal cells from each of the 27 nests excavated in this study. In nests 25, 26 and 27 no queens or royal cells were found. PM= Pleural membrane, ISM= intersegmental membrane. Dimensions of the royal cells are given in mm. H= height, D1= long diameter, D2= short diameter.
"-" represents no data.

Nest Code	Mass (g)	Length (mm)	Width (mm)	Colour		Turgidity	Royal cell dimensions			Estimated age
				PM	ISM		H	D1	D2	Years
4 [*1]	5.10	61.6	18.0	Creamy white	Creamy white with dark streaks	Fairly turgid	-	-	-	21+
14	5.33	53.3	15.6	Creamy white	Pale brown with dark streaks	Fairly turgid	10	135	125	21+
15	7.84	58.0	14.8	Creamy brown	Medium brown with dark streaks	Not very turgid	10	150	115	21+
16	6.26	66.7	17.3	Creamy brown	Pale brown with dark streaks	Very turgid	10	150	110	[*4]
24	6.25	58.1	14.1	Creamy white	Pale brown with streaks	Fairly turgid	20	175	125	< 10
30	6.57	55.3	19.6	Creamy white	Medium brown	Flaccid	15	140	120	< 10
32	8.02	53.6	17.4	Creamy white	Pale brown with dark streaks	Fairly turgid	15	125	105	< 10
45 [*2]	0.76	21.2	9.6	Creamy white	-	Not very turgid	-	-	-	[*3]
	0.51	18.8	10.1	Creamy white	-	Not very turgid				
46	4.02	51.6	17.0	Creamy brown	Medium brown with dark streaks	Fairly turgid	25	150	140	[*3]
	4.24	54.5	16.9	Creamy brown	Medium brown with dark streaks	Fairly turgid				
50 [*1]	4.83	56.4	17.7	Creamy brown	Medium brown with dark streaks	Fairly turgid	-	-	-	12-19
55	7.51	61.5	17.4	Creamy white	Medium brown with dark streaks	Very turgid	15	115	105	< 10
56 [*1]	5.67	52.9	18.1	Creamy white	Creamy white with dark streaks	Very turgid	-	-	-	40?
61	8.16	55.3	18.9	Creamy white	Pale brown with dark streaks	Very turgid	15	130	120	[*4]

Nest Code	Mass (g)	Length (mm)	Width (mm)	Colour		Turgidity	Royal cell dimensions			Estimated Age
				PM	ISM		H	D1	D2	Years
62	6.96	51.1	16.3	Creamy white	Pale brown	Fairly turgid	15	110	105	< 16
63	5.79	50.6	18.9	Creamy brown	Pale brown	Flaccid	15	120	90	< 17
65	3.96	44.5	15.9	Creamy brown	Pale brown with dark streaks	Fairly turgid	15	125	120	[*3]
	5.01	48.9	16.7	Creamy brown	Pale brown with dark streaks	Flaccid				
66	6.12	57.9	16.3	Creamy brown	Pale brown with dark streaks	Fairly turgid	20	125	120	< 17
67	7.22	45.9	17.5	Creamy brown with dark streaks	Pale brown with dark streaks	Fairly turgid	15	120	90	< 17
70	9.44	61.9	17.3	Creamy brown with dark streaks	Medium brown with dark streaks	Fairly turgid	15	105	100	< 17
72	6.67	62.0	16.6	Creamy brown with dark streaks	Dark brown	Fairly turgid	15	135	100	< 17
80	1.90	34.8	11.1	Creamy white	Creamy white	Fairly turgid	10	65	45	3-5
81	2.90	44.4	13.2	Creamy white	Pale brown with dark streaks	Fairly turgid	10	80	75	< 7
82	3.75	46.6	14.0	Creamy white	Pale brown	Fairly turgid	15	105	60	< 7
83	5.26	47.4	15.1	Creamy white	Creamy white with dark streaks	Fairly turgid	10	100	75	< 7
84	3.05	41.2	12.2	Creamy white	Creamy white with dark streaks	Fairly turgid	10	80	70	< 7
85 [*1]	4.49	48.1	15.4	Creamy white with dark streaks	Medium brown	Fairly turgid	-	-	-	7+
90 [*1]	5.36	53.1	14.0	Creamy brown	Pale brown with dark streaks	Not very turgid	-	-	-	27+

[*1] The royal cells of these nests were broken.
[*2] The large, physogastric queen from this nest was destroyed.
[*3] Queens in these nests were secondary queens or recolonising queens.
[*4] These nests under trees, mound age not determined, therefore queen age not determined.

APPENDIX 5:

Raw data collected from the 30 *Macrotermes natalensis* nests excavated in this study. QM= queen mass, FCM= fungus comb mass, TD= temperature difference between the nest and surrounding soil at equal depth. Dimensions of the mound, hive and nursery are given in metres. H = height, D1 = long diameter, D2 = short diameter. "-" represents no data.

Nest Code	QM	FCM	TD	Mound			Hive			Nursery		
	(g)	(g)	(°C)	H	D1	D2	H	D1	D2	H	D1	D2
4[*1]	5.1	-	-	0.80	2.80	1.70	1.0	1.65	1.2	0.50	0.55	0.50
14	5.33	7800	4.6	0.90	1.70	1.30	0.70	2.00	1.85	0.55	0.60	0.60
15	7.84	13150	7.9	1.15	2.10	1.05	0.65	1.30	1.20	0.45	0.55	0.45
16	6.26	7580	6.7	0.95	3.10	2.90	0.85	2.00	1.90	0.70	0.80	0.70
24[*1]	6.25	-	-	0.70	2.35	1.45	0.65	2.40	1.00	0.65	0.60	0.55
25[*1]	-	16680	-	0.35	1.60	1.40	0.70	2.20	2.00	0.55	0.55	0.60
26[*1]	-	3450	-	0.40	0.80	0.70	0.60	1.40	1.00	0.40	0.50	0.50
28[*1]	-	17350	-	0.40	2.20	1.75	0.80	1.90	1.30	-	-	-
30	6.57	11200	3.1	0.25	2.00	1.15	0.75	1.20	1.05	0.65	0.50	0.45
32[*1]	8.02	-	-	0.20	1.25	0.85	0.70	1.45	1.35	0.60	0.70	0.65
45[*1]	0.76, 0.51	-	-	0.65	1.30	0.90	0.60	1.55	1.45	0.60	0.70	0.65
46[*1]	4.24, 4.02	-	-	1.10	2.10	1.30	0.95	2.15	2.00	0.70	0.95	0.80
50	4.83	15250	-	0.55	1.55	1.20	0.55	1.50	1.50	0.50	0.60	0.55
55	7.51	10030	7.9	0.60	1.20	1.10	0.85	1.00	0.95	0.50	0.45	0.40
56[*2]	5.67	-	5.3	-	-	-	-	-	-	-	-	-
61[*1]	8.16	-	-	0.60	1.50	1.45	0.90	1.90	1.05	0.55	0.65	0.55

Nest Code	QM (g)	FCM (g)	TD (°C)	Mound			Hive			Nursery		
				H	D1	D2	H	D1	D2	H	D1	D2
62[*1]	6.96	-	-	0.50	1.30	1.00	0.50	1.80	1.05	0.55	0.50	0.50
63[*1]	5.79	-	-	1.00	3.00	3.00	0.70	1.50	1.00	0.45	0.50	0.45
65	3.96, 5.01	8250	6.8	0.85	2.30	2.10	0.75	1.50	1.30	0.65	0.65	0.60
66	6.12	5060	-	0.65	2.10	1.65	0.55	1.30	0.70	0.40	0.40	0.35
67	7.22	10800	4.6	0.70	2.60	2.20	0.75	1.50	0.80	0.55	0.55	0.45
70	9.44	5900	6.1	1.20	2.30	2.20	0.80	1.30	1.10	0.60	0.70	0.70
72	6.67	8500	5.2	0.75	1.95	1.70	0.85	1.50	1.40	0.65	0.60	0.60
80[*3]	1.9	2000	2.1	0.13	0.45	0.30	-	-	-	0.50	0.50	0.55
81[*3]	2.9	3100	9.3	0.18	0.90	0.80	-	-	-	0.60	0.55	0.70
82[*3]	3.75	2600	7.2	0.14	0.70	0.50	-	-	-	0.65	0.65	0.75
83[*3]	5.26	4850	10.3	0.18	1.00	0.90	-	-	-	0.55	0.60	0.55
84[*3]	3.05	2900	2.7	0.16	0.75	0.45	-	-	-	0.55	0.55	0.55
85	4.49	6100	6.8	0.40	1.40	1.30	0.75	0.75	0.65	0.25	0.25	0.30
90	5.36	27450	5.3	1.50	3.30	2.80	1.00	1.60	1.50	0.60	0.65	0.70

[*1] These nests were excavated only to determine the structure of the nest.

[*2] This nest directly under shrub, amongst roots, fungus combs not collected or structure determined.

[*3] These nests lacked a fungus garden, hive dimensions equal nursery dimensions.

APPENDIX 6 :

Sources of aerial photographs used in this study to identify mounds and age nests.

Site	Date of Photography	Photograph Numbers	Scale of Photograph(s)	Source(s) of Photographs
1	1976	2930 CB 14	1:10 000	Map Sales, Regional and Land Affairs, rm 214, 300 Pietermaritz Street, Pietermarizburg.
2	1993 1980	243 55188	1:5 000 1:12 000	Map Section, The Air Survey Company of Africa (Ltd), 225 Umbilo Road, Durban.
	1976	2930 CB 19	1:10 000	Map Sales, Regional and Land Affairs, rm 214, 300 Pietermaritz Street, Pietermaritzburg.
3	1976	2930 CB 13	1:10 000	Map Sales, Regional and Land Affairs, rm 214, 300 Pietermaritz Street, Pietermaritzburg.
4	1976	2930 CB 14	1:10 000	Map Sales, Regional and Land Affairs, rm 214, 300 Pietermaritz Street, Pietermaritzburg.
5	1976	2930 CB 14	1:10 000	Map Sales, Regional and Land Affairs, rm 214, 300 Pietermaritz Street, Pietermaritzburg.
6	1981	2930 AD 15	1:10 000	Map Sales, Regional and Land Affairs, rm 214, 300 Pietermaritz Street, Pietermaritzburg.
7	1981	2930 AD 10	1:10 000	Map Sales, Regional and Land Affairs, rm 214, 300 Pietermaritz Street, Pietermaritzburg.
8	1981	2930 AD 5	1:10 000	Map Sales, Regional and Land Affairs, rm 214, 300 Pietermaritz Street, Pietermaritzburg.
9	1989	2829 DC 7	1:10 000	Map Sales, Regional and Land Affairs, rm 214, 300 Pietermaritz Street, Pietermaritzburg.
10	1981	2930 AD 15	1:10 000	Map Sales, Regional and Land Affairs, rm 214, 300 Pietermaritz Street, Pietermaritzburg.