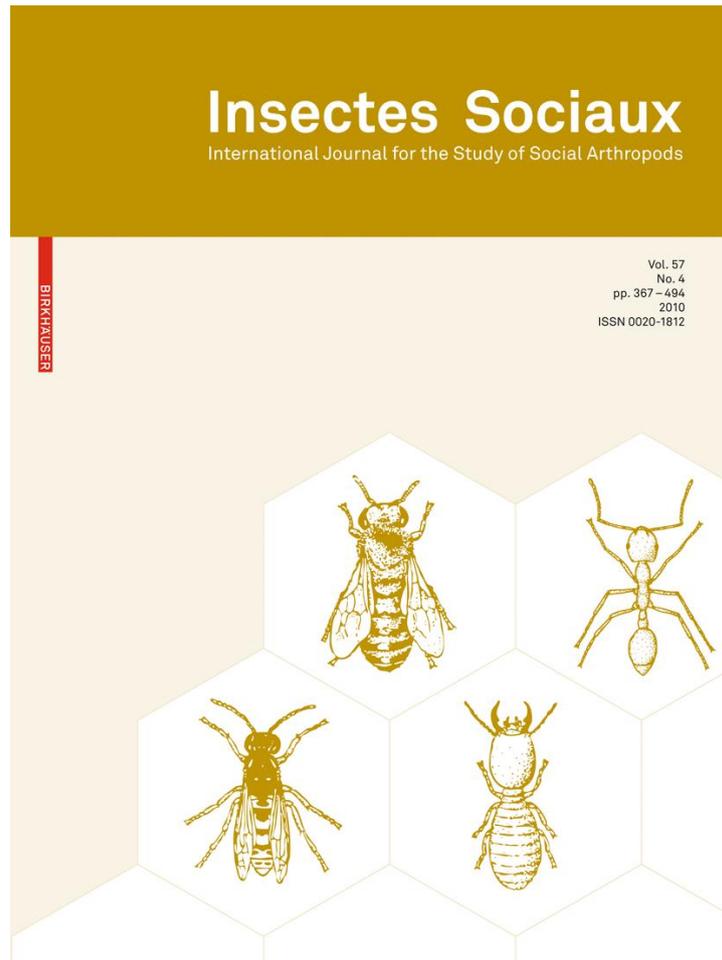


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## Impact of orphaning on field colonies of Southeast Asian *Macrotermes gilvus* (Hagen) and *M. carbonarius* (Hagen) (Termitidae, Macrotermitinae)

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**Abstract** Field colonies of *Macrotermes gilvus* (Hagen) and *M. carbonarius* (Hagen) were experimentally orphaned to examine their potential for producing replacement reproductives. Orphaned colonies were investigated only once for caste composition at selected time intervals at 3, 6, 9 or 12 months after orphaning. Of the 38 orphaned colonies of *M. gilvus*, 15 colonies (39.5%) re-established. In *M. carbonarius*, three colonies out of 20 (15%) re-established. Re-established colonies were headed by adultoids which were morphologically indistinguishable from primary reproductives. In naturally orphaned colonies of *M. gilvus*, we often found multiple adultoids with normal pigmentation but torn wings, i.e. the colonies retained alates as replacement reproductives. The number of reproductives probably declines over time. It may take alates of *M. gilvus* 6 months to develop into functional adultoids, and up to 12 months for alates of *M. carbonarius*. Our results also demonstrate that the presence of sexual castes (nymphs or alates) at the time of orphaning does not necessarily guarantee the success of colonies in re-establishing themselves as breeding colonies. We also found a high prevalence of occupation of the mounds by other termite species, after the death of *M. gilvus* (18.4%) or *M. carbonarius* (30.0%) colonies, probably using them as foraging sites.

**Keywords** Replacement reproductives · Adultoids · Re-colonization · Nymph production · Physogastry · Termitariophiles

### Introduction

A termite colony is usually headed by the pair of dealated primary reproductives which founded it following a nuptial flight. If the primary reproductives become weak or die, two outcomes may be observed: (1) the colony dies; (2) the termite society produces replacement reproductives from precursor stages which vary with termite species. The replacement reproductives can develop from several termite castes, e.g., alates retained in the parental colony (adultoids), nymphs (nymphoids), or workers (ergatoids). The types of replacement reproductives which develop in any one given colony are highly variable across species, especially in the genus *Nasutitermes* (Roisin and Pasteels, 1986a, b; Lefeuvre, 1987) and *Reticulitermes* (Buchli, 1956). However, reproductive replacement in *Macrotermes* is straightforward, as reproductives can only develop from the nymphal line (alates) to become normal adultoid replacement reproductives (Myles, 1999). Myles (1999) divided adultoids into three types: pseudoimagos which show poor pigmentation and irregular broken wings, microimagos (dwarf alates) which possess a pair of shortened wings, or normal adultoids.

Polygyny may happen in termite societies by: (1) several females cooperating during the colony foundation and reproducing actively (pleometrosis); (2) budding, especially in polycalic nesting termite species (Roisin and Pasteels, 1986b); (3) substituting dead primary reproductives with multiple replacement reproductives (Roisin and Pasteels, 1986b; Lenz and Runko, 1993).

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*Macrotermes gilvus* (Hagen) and *M. carbonarius* (Hagen) are common fungus-growing, mound-building termites in Southeast Asia. Reproductives are produced in a single annual brood. The species exhibit a forked developmental pathway, separating the reproductive line (nymphs/alates) from the sterile line (workers/soldiers) at the first moult. In the reproductive line, nymphs undergo four moults from the first (N1) to the fifth instar (N5) before moulting into winged adults (Neoh and Lee, 2009a). In northern peninsular Malaysia (Penang), nymph production in *M. gilvus* occurs between December and April, followed by flights. These have been recorded over a remarkably long 7-month period from March to September and small swarms have been observed between October and November (Neoh and Lee, 2009c). *M. carbonarius* produces nymphs between July and November. Swarming of *M. carbonarius* takes place from November to January (Neoh and Lee, 2009c). The functional reproductives of *Macrotermes* are confined to a single well-built royal cell. Holes in the cell walls allow soldier and worker access (Inoue et al., 1997).

Sieber and Darlington (1982) state that replacement reproductives in *Macrotermes michaelseni* (Sjöstedt) may only develop if alates or nymphs are present in the nest when orphaning takes place. We carried out a field orphaning experiment to test this hypothesis for two of the southeast Asian species of *Macrotermes*. In the present study, we also report on the replacement reproductives found in colonies orphaned by natural causes, the initial condition of replacement reproductives, and include some other observations; for example, the diversion of effort by workers in the colony and occupation of mounds by other termite species.

## Materials and methods

Study sites were located at the Minden Campus of Universiti Sains Malaysia (USM), Penang, Malaysia (5°21'N, 100°18'E). The campus encompasses an area of approximately 100 ha. The density of termite mounds on the campus can exceed 8 mounds/ha. *M. gilvus* and *M. carbonarius* are the two most common mound-building termite species found on the campus, accounting for 43.7 and 32.4%, respectively, of all termite mounds ( $n = 978$ ) (Wong, 2006).

In the orphaning experiments, 38 colonies of *M. gilvus* (height: 0.05–0.50 m; diameter: 0.60–1.80 m) and 20 colonies of *M. carbonarius* (height, 0.10–0.54 m; diameter, 0.65–1.90 m) were selected. The mounds were orphaned between January 2007 and April 2008 by removing the royal cell. The mounds were cut at the base and the outer wall was removed. Under the wall, there is a hive which

contains the nursery zone, fungus combs and food store. We gently removed fungus combs and broke up clay pillars and lamellae as necessary to reach the royal cell. In this study, we found that the royal cell was usually located from a depth of 0.25 m up to ground level. Fungus comb material and crevices were surveyed for the presence of nymphs or alates, since they tend to aggregate there. However, some parts of the mounds (e.g., nest galleries and nursery) were not too closely examined to ensure that the mound stayed as undamaged as possible to enhance the chances of colony survival. Nymphal instars present at the time of orphaning were subjected to morphological analysis to determine their stages based on the description by Neoh and Lee (2009a). After removal of the royal cell, fragments of mound material, fungus combs and outer casing were used to seal the breaches in the mounds to prevent predation or damage through extreme weather events.

The royal cells were transported to the laboratory and opened to obtain the royal pair(s). In the study, we found that four colonies contained replacement reproductives (adultoids with broken wings) implying that the colonies had previously been orphaned by natural causes. The replacement reproductives were sexed according to the shape of abdominal segments. The fresh weight of each reproductive was recorded using an analytical balance (Sartorius AG, Göttingen, Germany). For each naturally orphaned colony the fresh weights of the females were subjected to one-way Anova (SPSS analysis version 11.0) (SPSS, 2002) to compare their degree of physiogastry.

Pearson correlation and Spearman's rho test (SPSS analysis version 11.0) (SPSS, 2002) were used to correlate the individual fresh weights and the number of replacement reproductives with the time elapsed after orphaning, respectively.

The orphaned colonies were investigated only once at selected time intervals: 3 (only *M. gilvus*), 6, 9 or 12 months after orphaning. We categorized the examined colonies as: (1) re-established colony (colony that contained reproductives with eggs or larvae or both); (2) survived colony (colony that contained only workers or soldiers but no reproductives, eggs or larvae); (3) dead colony (colony that contained no individuals of the original *Macrotermes* species, although the mound may have been invaded by other termite or ant species).

## Results

### *Macrotermes gilvus*

Table 1 provides details of the reproductives in orphaned colonies at different time intervals. Of the 38 orphaned

**Table 1** Number and fresh weight of reproductives before and after orphaning in colonies of *Macrotermes gilvus*

Colony no.	Date of orphaning	Date of re-sampled	Colony prior to orphaning		Orphaned colony		
			Fresh weight (g)	King	Status	Fresh weight (g)	
			Queen			♀ adultoid	♂ adultoid
<i>Inspected after 3 months</i>							
1	08.01.07	08.04.07	5.4240	0.0653	Alates present		
2	26.01.07	26.04.07	NA	0.0564	Alates present		
<i>Inspected after 6 months</i>							
21	22.05.07	22.11.07	1.4584	0.0720	Survived		
22	22.05.07	22.11.07	1.0263	0.0599	Dead		
23	24.05.07	24.11.07	2.2069	0.0725	Torn-winged adultoids present	0.0638	0.0519
						0.0575	0.0630
						0.0678	0.0659
						0.0703	0.0655
						0.0582	0.0612
						0.0645	0.0625
						0.0605	(n = 11) <sup>a</sup>
						0.0598	(0.0617 ± 0.0051)
						0.0636	
						(0.0629 ± 0.0043)	
26	25.05.07	25.11.07	R	R	Dead		
28	25.05.07	25.11.07	R	R	Re-established	1.7202	0.0819
29	05.06.07	05.12.07	0.7802	0.0630	Dead		
			0.9647	0.0710			
			(0.8725 ± 0.1305)	(0.0670 ± 0.0057)			
30	05.06.07	05.12.07	1.7645	0.0698	Torn-winged adultoids present	0.0653	
						0.0735	
						NA (0.0694 ± 0.0058)	
36	08.01.08	08.07.08	2.9029	NA	Re-established	2.7873	0.0670
37	24.01.08	24.07.08	2.6309	0.0678	Survived		
38	25.01.08	25.07.08	3.3559	0.0770	Re-established	2.0021	0.0668
39	18.03.08	18.09.08	3.8302	0.0686	Dead		
40	21.03.08	21.09.08	2.4758	0.0751	Survived		
<i>Inspected after 9 months</i>							
31	06.09.07	06.06.08	4.1035	0.0819	Re-established	4.1266	0.0696
32	11.09.07	11.06.08	1.7191	0.0715	Re-established	1.6079	0.0596
33	31.10.07	31.07.08	1.8487	0.0611	Re-established	2.1101	0.0829
34	09.11.07	09.08.08	1.3947	0.0623	Survived		
			1.7442 (1.5695 ± 0.2471)	0.0659 (0.0623 ± 0.0025)			

Table 1 continued

Colony no.	Date of orphaning	Date of re-sampled	Colony prior to orphaning		Orphaned colony		
			Fresh weight (g)		Status	Fresh weight (g)	
			Queen	King		♀ adultoid	♂ adultoid
35	21.11.07	21.08.08	3.3230	0.0728	Re-established	1.9782 1.8915 (1.9349 ± 0.0613)	0.0704 0.0712 0.0706 (0.0707 ± 0.0004)
41	29.11.07	29.08.07	1.7202	0.0819	Re-established	2.1839	0.0697
<i>Inspected after 12 months</i>							
3	22.02.07	22.02.08	1.2900	0.0620	Dead		
4	10.03.07	10.03.08	1.0880	0.0602	Dead		
5	18.03.08	18.03.09	3.8302	0.0686	Dead		
6	21.03.08	21.03.09	2.4758	0.0751	Dead		
7	17.04.07	17.04.08	1.3433	0.0505	Dead		
8	17.04.07	17.04.08	1.5019	0.0709	Dead		
9	18.04.07	18.04.08	1.2000	0.0763	Re-established	1.9730	0.0718
10	18.04.07	18.04.08	1.5530	0.0621	Eggs and larvae present	NA	NA
11	04.05.07	04.05.08	1.2040	0.0703	Dead		
			1.2660 (1.2350 ± 0.0438)	0.0605 (0.0654 ± 0.0069)			
12	04.05.07	04.05.08	R	R	Dead		
13	08.05.07	08.05.08	1.2993	0.0743	Dead		
14	08.05.07	08.05.08	3.3955	0.0783	Re-established	1.5000	0.0787
15	14.05.07	14.05.08	2.5740	0.0748	Dead		
16	14.05.07	14.05.08	4.2427	0.0613	Re-established	1.1535	0.0581 0.0625 (0.0603 ± 0.0031)
17	15.05.07	15.05.08	2.2706	0.0800	Dead		
18	18.05.07	18.05.08	1.1363	0.0748	Re-established	0.1746 2.1138 1.3246 1.2128 (1.2065 ± 0.7962)	0.0581 0.0746 0.0667 0.0686 0.0760
19	18.05.07	18.05.08	(1.0553 ± 0.1199)				0.0669 (0.0685 ± 0.0064)
20	18.05.07	18.05.08	1.7160	0.0677	Eggs and larvae present	NA	NA
			R	R	Re-established	1.0017	0.0692

Mean ± SD are given in parentheses

R naturally formed replacement reproductives (see also Fig. 1), NA not available due to injuries during excavation or failure to locate

<sup>a</sup> The weights of five males with worn wings were not available, they were injured during excavation (total = 11)

colonies of *M. gilvus*, we found 15 colonies (39.5%) re-established. Two colonies contained eggs and newly formed larvae but we could not locate the reproductives.

Four colonies contained only workers and soldiers. In a further two colonies (Nos. 1 and 2), inspected 3 months after orphaning, nymphs and alates had been present initially: N2, N3, N4 in Colony 1 and N4 and N5 in Colony 2. Three months later in Colony 1, N5 and alates were found; while in Colony 2, only a small number of alates were still present, i.e., possibly retained in the nest (the nuptial flight had just happened as we noticed flight holes). These colonies might also have re-established if we had not sampled them.

Fifteen colonies had died out. In seven of those cases, other termite species were present in the mounds at the time of inspection, e.g., *M. carbonarius*, *Globitermes sulphureus* (Haviland), *Amitermes* sp., *Odontotermes* sp., *Pericapritermes* sp. and *Microtermes* sp. Only workers and soldiers of the invading species were found. Eight colonies were invaded by ants. Usually, dead termite bodies and fungus combs overgrown by a fast-growing fungus were observed in the dead mounds.

Six months after orphaning, three out of 12 colonies [Colonies 36 and 38 which contained nymphs (N1, N2, N3) at the time of orphaning; Colony 28 which contained alates] contained replacement reproductives consisting in each case of one physogastric queen and one king in a well-built royal cell. Eggs and a large number of larvae were also present. In Colony 38, the pair was significantly mutilated. The queen had lost the second left tibia and antennal segments; while the first and third right tibiae of the king were missing.

Further, in Colonies 23 and 30, which contained alates at the time of orphaning, torn-winged adults were found. These observations suggest that either alates were present at the time of orphaning or developed later on from existing nymphs and were retained in the parental colonies. The alates found at the inspection 6 months after treatment were non-physogastric and usually hidden in crevices in the inner nest carton. Both colonies appeared weak. Only a low number of mite-infested workers and dipteran-parasitized soldiers (see also Disney et al., 2009; Neoh and Lee, 2009b) were found. The workers were larger than in a normal colony and their abdomen had an abnormal whitish brown colour. Fungus combs were largely consumed. Clearly these two colonies were moribund.

At 9 months after orphaning, five nests out of six were found to contain replacement reproductives. Colony 35 included two physogastric queens and three kings.

At 12 months after orphaning, of the 18 colonies, three (Colonies 9, 14 and 20) contained a pair of reproductives. Colony 16 was headed by a queen and two kings. Multiple reproductives (four queens and six kings) were present

in Colony 18. The least physogastric queen (weight, 0.1746 g) was heavily mutilated. It had lost four tibiae and a pair of antenna. In two cases (Colonies 10 and 19), we found empty royal cells with a big hole. The pair could have moved out to a new location within the nest and the presence of eggs, larvae and newly formed fungus comb, indicated that breeding was taking place.

Only monogynous colonies were subjected to correlation analysis and we found there was no correlation between fresh weight of the replacement queens and the period elapsed after orphaning ( $P = 0.123$ ,  $r = -0.404$ ,  $n = 10$ ). There was also no significant correlation between the number of adultoids and the time elapsed ( $P = 0.822$ ,  $r = -0.059$ ,  $n = 17$ ).

#### *Macrotermes carbonarius*

Six months after orphaning, five out of the six colonies had survived (Table 2). However, neither reproductives nor any sign of breeding (eggs and larvae) were found. Usually, fungus combs were largely consumed. The abdomen of workers was speckled and whitish in appearance, often an indication of poor condition. In Colonies 18 and 19, we found dipteran-parasitized soldiers (see Neoh and Lee, 2009b).

Of the 20 orphaned colonies, only three colonies (Colonies 6, 8, 9) (15%) were alive and contained a pair of replacement reproductives 12 months after orphaning. In Colonies 6 and 9, the fresh weight of the replacement queens was about a third of that of the founding queens. Six colonies, which account for 30.0%, were re-colonized by *M. gilvus* and *Odontotermes* sp., in the latter case with a pair of physogastric imagoes.

#### Colony composition and the success rate of colony re-establishment

##### *Macrotermes gilvus*

In the present study, nymphs and alates were found in 13 colonies at the time of orphaning (excluding the colonies re-collected after 3 months), but only seven colonies re-established (53.8%). Nine colonies with no nymphs and alates recorded at the time of orphaning experienced reproductive replacement by primary pairs. In such cases, early nymphal stages could have been present but unnoticed since not all parts of the mound were thoroughly inspected at orphaning time (Fig. 1).

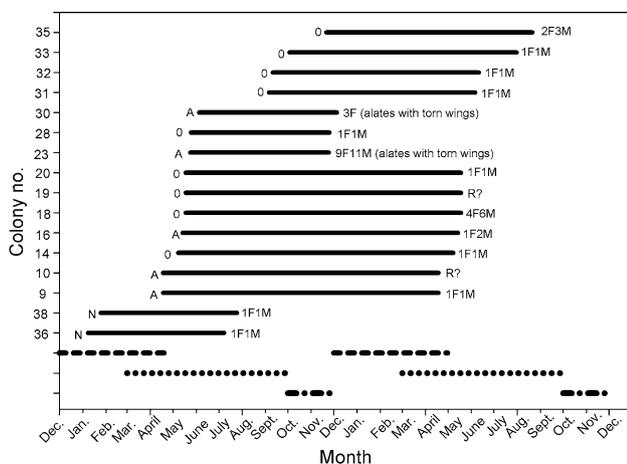
##### *Macrotermes carbonarius*

Three out of seven colonies (42.9%) orphaned at the time when nymphs were found to be in the colonies, contained

**Table 2** Number and fresh weight of reproductives before and after orphaning in colonies of *Macrotermes carbonarius*

Colony no.	Date of orphaning	Date of re-sampled	Colony prior to orphaning		Orphaned colony		
			Queen	King	Status	Fresh weight (g)	
						♀ adultoid	♂ adultoid
<i>Inspected after 6 months</i>							
1	28.03.07	28.09.07	1.6948	0.1207	Survived		
2	08.05.07	08.11.07	2.8184	0.1475	Dead		
3	15.05.07	15.11.07	2.0255	0.1390	Survived		
18	18.03.08	18.09.08	0.1620	2.9569	Survived		
19	21.03.08	21.09.08	1.5974	0.1412	Survived		
20	21.03.08	21.09.08	3.1973	0.1612	Survived		
<i>Inspected after 9 months</i>							
14	09.01.08	09.10.08	2.7930	0.1355	Dead		
15	09.01.08	09.10.08	2.1361	0.1286	Dead		
16	11.01.08	11.10.08	2.3430	0.1504	Dead		
17	11.01.08	11.10.08	3.3711	0.1471	Dead		
<i>Inspected after 12 months</i>							
4	16.05.07	16.05.08	2.3960	0.1307	Dead		
5	14.08.07	14.08.08	2.7334	0.1290	Dead		
6	03.09.07	03.09.08	2.2125	0.1534	Re-established	0.5922	0.1404
7	28.09.07	28.09.08	0.9734	0.1158	Dead		
8	30.10.07	30.10.08	NA	NA	Re-established	1.2180	0.1292
9	30.10.07	30.10.08	2.1015	0.1329	Re-established	0.7250	0.1457
10	31.10.07	31.10.08	2.7923	0.1273	Dead		
11	01.11.07	01.11.08	3.0750	0.1600	Dead		
12	01.11.07	01.11.08	1.9862	0.1428	Dead		
13	21.11.07	21.11.08	3.3782	0.1496	Dead		

NA not available due to injuries during excavation



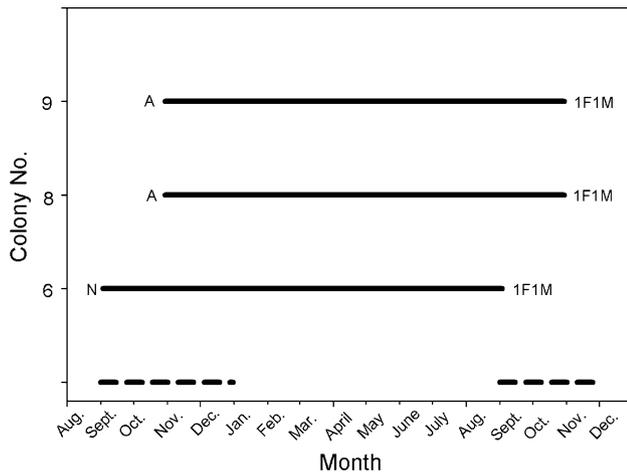
**Fig. 1** *Macrotermes gilvus*: occurrence of replacement reproductives in colonies orphaned between January 2007 and April 2008 in relation to the presence of nymphs (dashed line) and flight activity (dotted line peak flight; dash and dot line small flight). N nymphs, A alates, O: no sexual castes were found at the time of orphaning; F/M: mature female and male reproductives, R?: replacement reproductives escaped during final inspection

replacement reproductives. No successful cases were recorded in colonies which had been orphaned outside of the nymph production period (Fig. 2).

#### Naturally orphaned field colonies

In May 2007 in the course of orphaning, we discovered that four *M. gilvus* colonies (Colonies 12, 20, 26, 28) already contained replacement reproductives. The primary pairs had been replaced naturally. The new sets of female and male reproductives showed the same characteristics as the adult winged termites but possessed pairs of irregularly torn wings (Fig. 3). The female and male reproductives lived in modified royal cells with multiple chambers (at least two instead of one chamber as in normal colonies (K.B. Neoh and C.Y. Lee, unpubl.).

The number of replacement reproductives and the degree of physogastry in the queens (One-way Anova:  $F = 25.304$ ,  $df = 3$ ,  $P < 0.05$ ) were highly variable across the colonies (Fig. 4). Colony 20 was headed by 13 physogastric females (mean weight  $\pm$  SD,  $0.1978 \pm 0.0747$  g) and 13 males

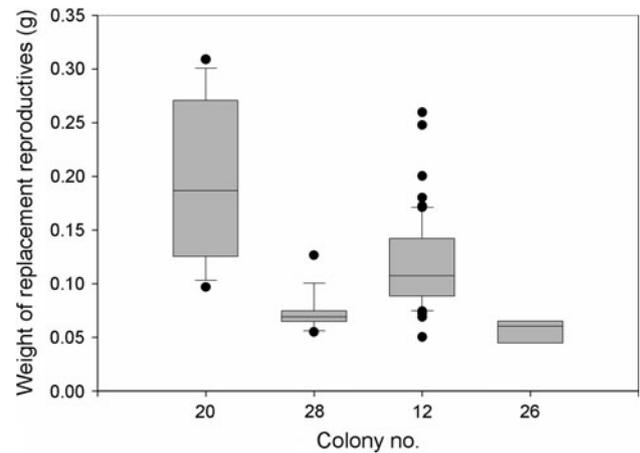


**Fig. 2** *Macrotermes carbonarius*: occurrence of replacement reproductives in colonies orphaned between January 2007 and April 2008 in relation to the presence of nymphs/alates (dashed line). N nymphs, A alates were found at the time of orphaning, F/M mature female and male reproductives present at the time of the final inspection



**Fig. 3** *Macrotermes gilvus*: partially physogastric female adultoids with torn and broken wings found in Colony 28, 25 May 2007

( $0.0621 \pm 0.0031$  g) ( $\text{♀}:\text{♂} = 1:1$ ). In Colony 28, 18 females ( $0.0735 \pm 0.0177$  g) and 28 males ( $0.0627 \pm 0.0056$  g) were present ( $\text{♀}:\text{♂} = 1:1.6$ ). Colony 12 contained 61 females ( $0.1179 \pm 0.0411$  g) and 107 males ( $0.0666 \pm 0.0074$  g) ( $\text{♀}:\text{♂} = 1:1.8$ ). The distribution of female fresh weight in Colony 12 appeared to be skewed to the right and the outliers indicate that some females were highly physogastric. In Colony 26, six females ( $0.0570 \pm 0.0115$  g) and 82 males ( $0.0528 \pm 0.0043$  g) ( $\text{♀}:\text{♂} = 1:14$ ) were found. In all likelihood, this colony had just replaced the founding reproductives.



**Fig. 4** *Macrotermes gilvus*: fresh weight of female replacement reproductives present in field colonies orphaned by natural causes. The box plot with error bars presents the median and the 10th, 25th, 75th and 90th percentiles. Solid circles outliers

In contrast, no reproductives with torn wings were found in *M. carbonarius* in the course of the investigation.

## Discussion

In the present study, we found that alates of *M. gilvus* were retained in the parental colony after the removal of the founding pair of primary reproductives. Reproductive replacement is likely to occur within 6 months after orphaning. In contrast, *M. carbonarius* took up to 12 months to develop functional reproductives.

During this time, workers might take intensive care of nymphs and any developing alates, and mutilate the wings of at least some alates to retain them as future kings and queens. The high consumption rate of fungus comb and lack of food stores imply that colony members, especially workers, directed more colony energy investment from foraging and mound fortification towards caring for the brood and reproductives (retained alates), hence in that period they may appear to largely rely on fungus combs for food. Conversely, Darlington (1991) postulated that the decrease of larval and worker population in one orphaning colony caused the degeneration of fungus combs.

The alates with torn wings found in experimental nests of *M. gilvus* (Colony 23 and 30) match in appearance exactly those found in the naturally orphaned nests. In cases where multiple torn wings reproductives were found, it would be of benefit to a colony. The work of reproduction may be shared by several females. This may produce a larger worker force or accelerate colony recovery, which, in turn, may increase the survival rate of a colony especially during the early stages of colony development (Darlington, 1988a; Brandl et al., 2001; Kaib et al. 2001).

However, conflicts between queens may happen over time as the colonies mature for gaining a dominant position, the attendance of workers (e.g., food, grooming) and the greatest share in the reproductive output of the colony (Darlington, 1988b; Thorne, 1984; Brandl et al., 2004; Adams and Atkinson, 2007). Only highly fertile and fit individuals succeed to take on breeding (Thorne, 1984; Myles, 1999). Evidence for the possible intensity of such conflicts was provided by the presence of greatly mutilated female reproductives in Colony 18 and 38 (Table 1) and other *Macrotermes* species (Darlington, 1988b). A similar situation may also occur in colonies orphaned by natural causes, as the colonies contained variable numbers of reproductives with varying degrees of physogastry.

No significant correlation was found between the degree of physogastric development (fresh weight) of the replacement queens and the elapsed time since the removal of the founding primaries. The results parallel the study done by Sieber and Darlington (1982) on *M. michaelseni*. This is to be expected as the time when replacement reproductives first start to develop may vary across colonies. However, we can extrapolate that a female's fresh weight can increase from the average female alate weight of 0.061 to 2.79 g (Colony 36) within 6 months.

Figure 1 shows that nine colonies of *M. gilvus* with no nymphs and alates present at the time of orphaning were re-established. Our result apparently contradicts the hypothesis of Sieber and Darlington (1982) who stated that replacement reproductives of *M. michaelseni* were only produced if nymphs or alates were present at the time of orphaning.

In our study, there are several possible origins of the replacement reproductives in *M. gilvus*: (1) with nymphs or alates present in the colonies at the time of orphaning, the reproductive caste is retained in the parental nests to become replacement reproductives, as evidenced by the presence of torn-wing alates in experimental and naturally orphaned colonies; (2) with nymphs or alates absent in the colonies at the time of orphaning, the eggs that are still present from the founding queen at the time of orphaning could be raised by the orphaned colonies through all the nymphal stages to the adult; although in some instances nymphs could have been overlooked at the time of orphaning (see "Materials and methods"; Sieber and Darlington, 1982). In African *Macrotermes*, Darlington (1991) observed colony collapse in less than 3 months after the removal of the reproductives, whereas it takes 7 months to rear nymphs to the alate. However, several of our *M. gilvus* colonies survived up to 9 months (Table 1) while queenless, long enough for the development of nymphs to alates. In fact, in *M. gilvus* only 3 months are required to produce alates from first nymphal instars (Neoh and Lee, 2009a); (3) the orphaned colony dies, but the mound is subsequently re-colonized by another conspecific termite

colony. While in 30% of the orphaned *M. carbonarius* colonies the mounds left empty after colony death were colonized by other Macrotermitinae, such as *M. gilvus* and *Odontotermes* sp. with reproductives found inside the orphaned nests, to date we have no case of the mound of a perished colony being re-colonized by the same species. However, this possibility cannot be excluded and molecular techniques may be required in some cases to ascertain the origin of replacement reproductives.

In contrast, replacement reproductives in *M. carbonarius* were found only if nymphs or alates were present in the colony at the time of orphaning, although to date our sample size is small.

In instances in which an empty royal cell was found with big holes in the walls, the reproductives could have moved out to a new location within the mound where workers would construct a new royal cell. This phenomenon has been documented in *M. michaelseni* (Darlington, 1987) and in the course of royal cell exchange experiment (K.B. Neoh and C.Y. Lee, unpubl.). Another interesting and new observation of this study is that numerous replacement reproductives housed in multi-chambered royal cells were found in naturally orphaned colonies. The varying shapes of royal cells in *M. michaelseni* reported by Darlington (1987), do not match the ones observed in the present study.

Colonization of dead *Macrotermes* mounds is very common in Africa (Pomeroy, 1976; Darlington, 1982; Inoue et al., 1997). In our study such mounds were taken over by Macrotermitinae from an adjacent healthy nest (see Darlington, 1982) as secondary sites for the cultivation of fungus comb (as evidenced by the absence of reproductive castes in the nests); or by termitariophiles taking advantage of congenial soil conditions: protection and valuable resources from the host nest [termite carrion, fungus combs (Batra and Batra, 1979), live and dead plant material (*Coptotermes lacteus*, Lenz et al. 1986)].

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