

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>

Contents lists available at [SciVerse ScienceDirect](http://www.sciencedirect.com)

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Physiological changes in major soldiers of *Macrotermes gilvus* (Isoptera: Termitidae) induced by the endoparasitoid *Misotermes mindeni* (Diptera: Phoridae)

Foong-Kuan Foo, Ahmad Sofiman Othman, Chow-Yang Lee*

Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

ARTICLE INFO

Article history:

Received 5 May 2011

Received in revised form 28 July 2011

Accepted 29 July 2011

Available online 5 August 2011

Keywords:

Termite

Parasitoid

Host physiology

Metabolic rate

Water balance

Desiccation tolerance

ABSTRACT

The majority of true parasitoids manipulate their host's physiology for their own benefit. In this study, we documented the physiological changes that occurred in major soldiers of the subterranean termite *Macrotermes gilvus* (Hagen) (Isoptera: Termitidae) parasitized by the koinobiont larval endoparasitoid *Misotermes mindeni* Disney and Neoh (Diptera: Phoridae). We compared the metabolic rate, body water content, body water loss rate, cuticular permeability, and desiccation tolerance between parasitized and unparasitized major soldiers. The metabolic rate of parasitized hosts was significantly higher than that of unparasitized termites. Mean total body water content of parasitized major soldiers ($64.73 \pm 3.26\%$) was significantly lower than that of unparasitized termites ($71.99 \pm 2.23\%$). Parasitized hosts also had significantly lower total body water loss rates ($5.72 \pm 0.06\%/h$) and higher cuticular permeability ($49.37 \pm 11.26 \mu\text{g}/\text{cm}/h/\text{mmHg}$) than unparasitized major soldiers ($6.75 \pm 0.16\%/h$ and $60.76 \pm 24.98 \mu\text{g}/\text{cm}/h/\text{mmHg}$, respectively). Parasitized major soldiers survived almost twice as long as unparasitized termites ($LT_{50} = 6.66 \text{ h}$ and $LT_{50} = 3.40 \text{ h}$, respectively) and they had significantly higher tolerance to water loss compared to unparasitized termites ($45.28 \pm 6.79\%$ and $32.84 \pm 7.69\%$, respectively). Body lipid content in parasitized hosts ($19.84 \pm 6.27\%$) was significantly higher than that of unparasitized termites ($6.17 \pm 7.87\%$). Finally, parasitized hosts had a significantly lower percentage of cuticular water content than unparasitized major soldiers ($10.97 \pm 1.84\%$ and $13.17 \pm 2.21\%$, respectively). Based on these data, we conclude that the parasitism-induced physiological changes in the host are beneficial to the parasitoids as the alterations can clearly increase the parasite's chances of survival when exposed to extreme environmental conditions and ensure that the parasitoids are able to complete their larval development successfully before the host dies.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

The process of successful parasitism requires a hierarchy of steps, including habitat location, host location, host acceptance, and host regulation (Brodeur and Boivin, 2004). Host regulation may involve intricate interactions between a parasitoid and its host including induction of numerous modifications in the physiology of the host insect (Poulin, 1998). Many of these interactions can be viewed as adaptations to ensure successful parasitism and to create a host environment suited to meet the demands of the parasitoids (Godfray, 1994). Changes in metabolic rate (Alleyne et al., 1997; Roces and Gnatzy, 1997; Haspel et al., 2005), lipid and carbohydrate contents (Thompson and Redak, 2008), growth and development (Alleyne and Beckage, 1996; Alleyne et al., 1997; Consoli and Vinson, 2004), and hemolymph solutes and

protein content (Baker and Fabrick, 2000) are some aspects of the physiological interactions related to host regulation.

Recently, Neoh and Lee (2010) found the koinobiont fly larval endoparasitoid *Misotermes mindeni* Disney and Neoh (Diptera: Phoridae) exclusively in the head of major soldiers of the fungus-growing termite *Macrotermes gilvus* (Hagen) (Isoptera: Termitidae). The fly larva consumed the entire contents of the major soldier's head capsule. Parasitization by this parasitoid causes morphological and behavioral changes in the major soldiers (Neoh and Lee, 2010). For example, parasitized major soldiers possess a rounded head capsule with a pair of short mandibles, and they show a lower level of interspecific aggressiveness compared to unparasitized individuals. However, little is known about the effect of parasitism on the termite host's physiology.

Parasitization by *M. mindeni* can occur in the early termite larval stage and remain through at least three termite development stages (Neoh and Lee, 2010; Foo et al., 2011a). Neoh and Lee (2010) found that parasitized termites were always aggregated in the dry

* Corresponding author. Tel.: +60 4 6533523; fax: +60 4 6565125.

E-mail address: chowyang@usm.my (C.-Y. Lee).

peripheral zone of the mound, which was isolated from areas where healthy termites usually were found. Based on the different micro-environments inhabited by parasitized and unparasitized major soldiers, we hypothesized that certain physiological changes must occur in the parasitized termites that allow them to survive in the dry habitat; these changes likely also allow the parasitoids to complete their larval development before their host dies.

Hadley (1994) reported that insects inhabiting warm and dry environments exhibit a variety of adaptive differences in water balance. Among them, cuticular permeability (CP) is the most important factor for regulating water loss. The permeability of the cuticle (which affects cutaneous water loss) is influenced by abiotic factors such as temperature and humidity (Edney, 1957; Hadley, 1994). Insects that live in a drier environment tend to have lower permeability or water loss rate (Appel, 1991; Shelton and Grace, 2003; Kaersgaard et al., 2004) compared to those living in a wetter environment, and thus they are more tolerant to desiccation (Sponsler and Appel, 1990). CP may be affected by the water content of the endo- and exocuticle and the epicuticular lipid layer (Appel and Tanley, 1999). In addition to CP, insect body water content and lipid reserves may also affect water loss rates (Mazer and Appel, 2001).

In this study, we compared the water balance, desiccation resistance and metabolic rate between parasitized and unparasitized *M. gilvus* major soldiers. Based on these data, we evaluated the implications of physiological changes in parasitized major soldiers on the ecological success of *M. mindeni*.

2. Materials and methods

2.1. Termite sampling

Parasitized and unparasitized major soldiers were freshly collected from previously surveyed colonies at Minden Campus of Universiti Sains Malaysia (5° 21' N, 100° 18' E), Bayan Lepas (5° 17' N, 100° 15' E), and Gelugor (5° 22' N, 100° 18' E) in Penang, Malaysia (Foo et al., 2011b). Parasitized major soldiers were identified based on Neoh and Lee (2010) (i.e., rounded head capsules with a pair of short mandibles). The mounds were opened partially by trenching around the base of the nest. The hive (the inner part of the nest where termite larvae and the royal pair reside) was revealed by applying sideways force to remove the outer casing of the mound. Parasitized major soldiers were commonly aggregated at the inner part of the outermost layer of the mound (Neoh and Lee, 2010; Foo et al., 2011b). The soldiers were placed in a container and then transported to the laboratory. Only intact termites (age unknown) were selected for further analysis.

2.2. Metabolic rates

To measure metabolic rate of the termites, the amounts of oxygen (O₂) uptake and carbon dioxide (CO₂) release were measured using a Wardburg constant-volume respirometer (model V 220, B. Braun Melsungen AG, Melsungen, Germany) based on the method described by Umbreit et al. (1972). Two sets of 10 parasitized and 10 unparasitized major soldiers were tested. For a given test, 10 termites were placed in a single sidearm reaction flask (10–15 ml internal volume) without potassium hydroxide (KOH). A second reaction flask, which was attached to the one containing the termites, contained 0.1 ml of 10% KOH in the center well to absorb carbon dioxide. The reaction flasks and manometers were paired and placed in the shaker with the flasks immersed in a water bath maintained at 28 °C. Readings were recorded every 15 min for 6 h and corrected based on the changes in a thermobarometer containing 1 ml of water and 0.1 ml 10% KOH in the center well. Metabolic

rates were calculated based on Umbreit et al. (1972). This experiment was conducted with termites from each of the three colonies.

2.3. Water balance

Total body water (% TBW) content, % TBW loss over desiccation time, and CP of 103 parasitized and 103 unparasitized major soldiers from three colonies were determined gravimetrically (Sponsler and Appel, 1990; Shelton and Grace, 2003). All soldiers were weighed to the nearest 0.01 mg with a digital analytical balance (Sartorius BP 190S, Goettingen, Germany). The specimens were placed individually in uncovered 15 ml glass vials and then put into an air-tight polyethylene container (370.0 cm × 300.0 cm × 150.0 cm) containing ~1.5 kg of silica gel at the base to obtain 5% relative humidity (RH) inside the container. The silica gel was pre-dried at 110 °C for 24 h prior to the test. The desiccator chamber that contained the specimens was placed in an Incucell incubator (MMM Medcenter Einrichtungen GmbH, München, Germany) kept at 28 °C. Temperature and RH in the desiccator chamber were measured at each weighing using a thermo-hygrometer (Model RH 520, Waltham, MA, USA). The specimens were weighed hourly for 10 h. Mortality was also recorded. Mortality was defined as failure of a test insect to show any perceptible movement. It was assumed that weight loss between weighing was the result of water loss. After 10 h, specimens were removed from the desiccator chamber and dried at 60 °C for 48 h in an oven, then reweighed to obtain the dry weight. % TBW and % TBW loss were calculated as follows:

% TBW content

$$= \frac{[\text{initial weight} - \text{dry weight}]/\text{initial weight}}{\times 100}$$

% TBW loss

$$= \frac{[\text{initial weight} - \text{weight at each hour}]/[\text{initial weight} - \text{dry weight}]}{\times 100}$$

The time by which 50% of the major soldiers died (LT₅₀) and % TBW that was lost before immobility occurred also were determined.

Only data from the first 2 h of the experiment were used to generate CP values; this time interval represented the maximal water loss period due to the maximum water gradient between insects and the chamber (Sponsler and Appel, 1990; Shelton and Grace, 2003). Although desiccation can affect termite shape, this was not a problem during the first 2 h. No significant relationship was found between CP and initial weight in unparasitized major soldiers ($P > 0.05$). However, CP was positively correlated with the initial weight in parasitized major soldiers, but the relationship was relatively weak ($r^2 = 0.08$; $P > 0.01$). This shows that Meeh's formula is an accurate estimation of surface area in major soldiers, so it was used to calculate surface area as follows: $S = 12M^{2/3}$, where S = surface area (cm²) and M = initial weight (g) (Collins, 1969; Sponsler and Appel, 1990). CP then was calculated as water loss [initial weight – weight loss at 2 h] (μg)/surface area (cm²)/time (h)/saturation deficit (mmHg). Because only one temperature (28 °C) and RH (5%) were used, the saturation deficit of 26.95 mmHg remained constant throughout the experiment.

2.4. Body lipid content

Termites from the water balance study (101 parasitized and 99 unparasitized major soldiers) were used to determine the body lipid content. When determining the lipid content of parasitized termites, the termite head that contained larval parasitoid was excluded from the bioassay to eliminate the parasitoid's lipid content from the measurement. Thorax and abdomen were used for both types of soldiers. Individual specimens were preweighed and broken into two pieces, and the pieces were transferred to a 15 ml glass vials. The specimens were soaked in 2 ml of chloroform/methanol (2:1 v/v)

solution for 48 h to extract lipid from the body parts. After extraction, the solution was removed from the glass vial and the specimens were dried at 60 °C for 48 h in an oven to ensure that the remaining solution had evaporated. The specimens were then reweighed. Percentage of body lipid content was calculated as $([\text{initial weight} - \text{dry weight}]/\text{initial weight}) \times 100$.

2.5. Cuticular water content

Cuticular water content was determined following the methods of Appel and Tanley (1999) with some modifications. Abdominal tergites and sternites of the specimens were removed. The adhering tissues were scraped off gently from the cuticle. Cuticles were weighed and dried at 60 °C for 48 h in an oven and then reweighed. A total of 1–6 replicates from each of the five colonies were used and each replicate contained 4–5 randomly selected individuals. Percentage of cuticular water content was calculated as $([\text{initial weight} - \text{dry weight}]/\text{initial weight}) \times 100$ per individual.

2.6. Nest materials

To determine whether parasitized major soldiers inhabited in a drier region of the mound, samples of nest materials were randomly collected from six *M. gilvus* mounds infected by *M. mindeni* at Minden Campus of Universiti Sains Malaysia. The nest materials were taken from two regions of each mound: the peripheral and habitacle (nursery) zones (Noirot, 1970). Freshly collected nest materials were ground into smaller pieces and sieved using a 16-mesh sieve. Three subsamples (5 g each) of the materials from each region per mound were weighed in Petri dishes (9.0 cm diameter and 1.5 cm depth). The samples were dried at 60 °C for 7 d in an oven and then reweighed. The percentage of moisture content was calculated as $([\text{initial weight} - \text{dry weight}]/\text{dry weight}) \times 100$ (Fyfe and Gay, 1938).

2.7. Statistical analysis

Data in percentages were subjected to arcsine square transformation prior to analysis. One-way analysis of variance (ANOVA) was used to test whether the colonies varied physiologically for both parasitized and unparasitized major soldiers. Individual data from the three colonies were pooled for all of the bioassay studies except for cuticular water content, in which data from five colonies were pooled if no significant colony differences were detected. A Student's *t*-test was conducted to compare the O₂ uptake and CO₂ release rates, initial weight, % TBW content, CP, % TBW that was lost before immobility, body lipid content, and cuticular water content between parasitized and unparasitized major soldiers. Differences in moisture content of the nest materials between peripheral and habitacle zones were also compared using Student's *t*-test. LT₅₀ values at 5% RH and 28 °C were generated using probit analysis. The relationship between CP and initial weight of both

parasitized and unparasitized major soldiers was analyzed using linear regression. The same test was applied to determine how % TBW loss was related to desiccation time, how mean % TBW loss was related to mean % TBW content, and how percentage of body lipid content was related to % TBW content. Regression lines were considered to be significantly different if the slopes \pm SE did not overlap. All analyses were performed using SPSS 2002 version 11.0 for Windows (SPSS Inc., Chicago, IL, USA) and were performed at $\alpha = 0.05$.

3. Results

3.1. Metabolic rates

O₂ uptake and CO₂ release rates of parasitized major soldiers were significantly higher than those of unparasitized major soldiers (Table 1). No significant colony differences in O₂ uptake and CO₂ release rates were detected (Table 2).

3.2. Water balance

3.2.1. Body weight and water composition

Parasitized major soldiers had significantly greater initial body weight and lower mean % TBW content than unparasitized major soldiers (Table 1). No significant colony differences in initial body weight and % TBW content were observed (Table 2).

3.2.2. Percentage of total body water lost

Cumulative % TBW loss for parasitized and unparasitized major soldiers increased linearly between 0 and 10 h. % TBW loss rates ranged from $5.72 \pm 0.06\%/h$ in parasitized major soldiers to $6.75 \pm 0.16\%/h$ in unparasitized major soldiers (Fig. 1). Generally, the slope of the linear regression of cumulative % TBW loss for parasitized major soldiers was significantly lower than that for unparasitized ones, indicating that parasitized termites had lower % TBW loss rates. There was a significant positive relationship between mean % TBW loss rates and mean % TBW content for parasitized ($r^2 = 0.70$; $P < 0.01$) and unparasitized major soldiers ($r^2 = 0.96$; $P < 0.01$) (Fig. 2).

3.2.3. Cuticular permeability

Parasitized major soldiers had a significantly lower CP value than unparasitized major soldiers (Table 1). There were significant colony differences in CP (Table 2).

3.2.4. Mortality and tolerance to water loss

Parasitized major soldiers survived almost twice (LT₅₀ = 6.66 h; range = 6.10–7.30 h; $n = 91$) as long as unparasitized major soldiers (LT₅₀ = 3.40 h; range = 2.79–4.01 h; $n = 102$) in the desiccation chamber, with 10.78% of parasitized major soldiers remaining alive after 10 h, whereas all unparasitized termites died. Unparasitized major soldiers had significantly lower tolerance to water loss than

Table 1
Metabolic rates, water relation, and lipid composition of parasitized and unparasitized *M. gilvus* major soldiers.

Parameters	Parasitized major soldiers			Unparasitized major soldiers			Test statistics
	<i>n</i>	Mean \pm SD	Range	<i>n</i>	Mean \pm SD	Range	
Oxygen uptake ($\mu\text{l O}_2/\text{h}/\text{individual}$)	72	0.89 \pm 0.25	0.52–1.85	72	0.40 \pm 0.21	0.13–1.48	$t = 12.855$, $df = 142$, $P < 0.01$
Carbon dioxide release ($\mu\text{l O}_2/\text{h}/\text{individual}$)	72	0.69 \pm 0.14	0.31–0.94	72	0.23 \pm 0.11	0.06–0.74	$t = 20.950$, $df = 142$, $P < 0.01$
Initial body weight (mg)	103	42.61 \pm 4.59	34.50–54.20	103	25.09 \pm 2.69	19.70–32.30	$t = 33.507$; $df = 163$; $P < 0.01$
TBW content (%)	102	64.73 \pm 3.26	54.26–77.21	102	71.99 \pm 2.23	68.07–84.59	$t = -18.316$; $df = 187$; $P < 0.01$
Cuticular permeability ($\mu\text{g}/\text{cm}/\text{h}/\text{mmHg}$)	102	49.37 \pm 11.26	23.75–81.70	102	60.76 \pm 24.98	18.85–150.67	$t = -4.196$; $df = 140$; $P < 0.01$
Total body water that can be lost (%)	102	45.28 \pm 6.79	28.00–62.12	102	32.84 \pm 7.69	15.20–72.90	$t = 11.761$; $df = 191$; $P < 0.01$
Body lipid content (%)	101	19.84 \pm 6.27	3.95–45.21	99	6.17 \pm 7.87	0.00–33.33	$t = 13.77$; $df = 133$; $P < 0.01$
Cuticular water content (%)	17	10.94 \pm 1.84	6.41–13.75	23	13.17 \pm 2.21	10.29–18.80	$t = -3.443$; $df = 38$; $P < 0.01$

Table 2
Results of one-way analysis of variance (ANOVA) testing for colony variations.

Parameters	F	df	P
Oxygen uptake	2.788	2	P > 0.05
Carbon dioxide release	1.340	2	P > 0.05
Initial body weight	2.196	2	P > 0.05
% TBW content	3.102	2	P > 0.05
Cuticular permeability	31.386	2	P < 0.01
% Total body water that can be lost	1.798	2	P > 0.05
% Body lipid content	0.206	2	P > 0.05
% Cuticular water content	1.431	4	P > 0.05

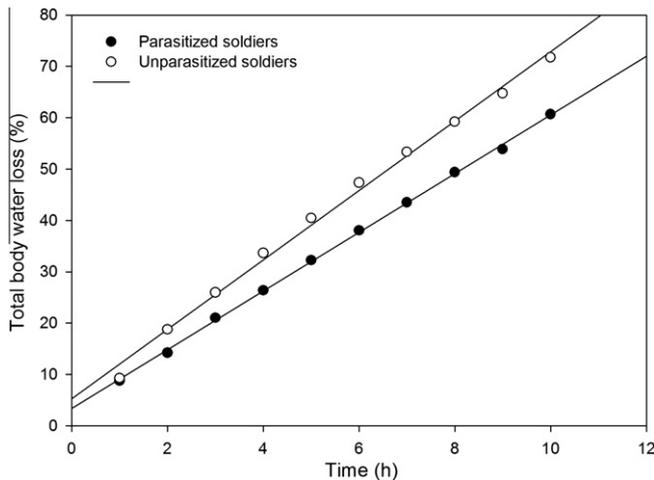


Fig. 1. Relationship between mean cumulative percentage of total body water loss and desiccation time (h). $Y = (5.715 \pm 0.055) X + (3.388 \pm 0.341)$; $r^2 = 0.9993$; $P < 0.01$ for parasitized major soldiers and $Y = (6.754 + 0.163) X + (5.251 + 1.009)$; $r^2 = 0.9954$; $P < 0.01$ for unparasitized major soldiers.

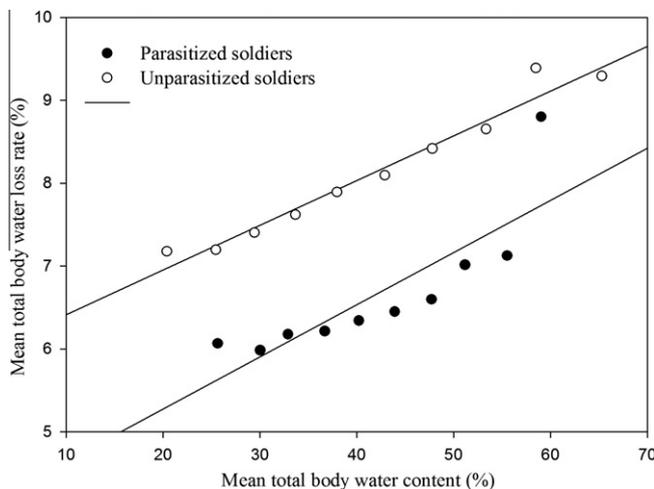


Fig. 2. Relationship between mean percentage total body water loss rate and mean percentage total body water content. $Y = (0.063 \pm 0.015) X + (4.016 \pm 0.633)$; $r^2 = 0.7010$; $P < 0.01$ for parasitized major soldiers and $Y = (0.054 + 0.004) X + (5.872 \pm 0.161)$; $r^2 = 0.9641$; $P < 0.01$ for unparasitized major soldiers.

parasitized major soldiers (Table 1). No significant differences were observed among colonies (Table 2).

3.2.5. Body lipid content

Body lipid content in parasitized major soldiers was significantly higher than that of unparasitized major soldiers (Table 1). There was a significant negative relationship between % body lipid

content and % TBW content ($Y = [97.197 \pm 8.651] - [1.231 \pm 0.126] X$, where $X = \% \text{ body lipid content}$ and $Y = \% \text{ TBW content}$; $r^2 = 0.32$; $P < 0.01$) in both types of major soldiers. Major soldiers from different colonies showed no difference in body lipid content (Table 2).

3.2.6. Cuticular water content

Cuticular water content of parasitized major soldiers was significantly lower than that of unparasitized ones (Table 1). No significant colony variation in cuticular water content was found (Table 2).

3.2.7. Moisture content of the nest materials

Moisture content of the nest materials from the habitacle zone (mean = $22.64 \pm 4.38\%$; range = 15.17–27.65%; $n = 6$) was significantly higher than that of the peripheral zone (mean = $16.24 \pm 2.73\%$; range = 11.13–18.85%; $n = 6$) ($t = -2.971$; $df = 10$; $P < 0.01$).

4. Discussion

The physiological changes induced by parasitism have never been reported for termites, but they have been studied previously in moths, cockroaches, and crickets (Alleyn et al., 1997; Roces and Gnatzy, 1997; Haspel et al., 2005). These authors reported that the presence of parasitoids interfered with the normal metabolism of the hosts. For example, metabolic rates were markedly reduced in tobacco hornworms (*Manduca sexta* (Linnaeus)) after they were parasitized by the braconid wasp *Cotesia congregata* (Say) (Alleyn et al., 1997). An analogous phenomenon was noted in the American cockroach *Periplaneta americana* Linnaeus parasitized by the wasp *Ampulex compressa* (Fabricius) (Haspel et al., 2005) and in the cricket *Acheta domestica* (Linnaeus) parasitized by the digger wasp *Liris niger* (Fabricius) (Roces and Gnatzy, 1997). However, contrary results were reported in the current study. Our data showed that parasitized major soldiers had greater respiratory metabolism than unparasitized ones. There are several possible explanations for these higher rates of metabolism: (1) to provide an adequate amount of energy for growth and development of the larval parasitoids (Smith-Trail, 1980, cited in Varo et al., 2000); (2) more energy is needed to recover from the damage caused by the parasitoids (Day and Oster, 1963); and (3) greater size of the parasitoid hosts (Berrigan and Partridge, 1997).

Korb and Linsenmair (1999) and Korb (2003) reported that gas exchange was restricted to the surface at the top of the dome-shaped *Macrotermes bellicosus* (Smeathman) mound. If the gas exchange pattern in *M. gilvus* mounds follows that found in *M. bellicosus* mounds, we propose that the parasitized hosts, with their higher metabolic rate, tend to be restricted to the peripheral zone of the mound for more efficient gas exchange.

The moisture content of the nest materials in the peripheral zone was lower than that of the habitacle zone in our study, and it was in the peripheral zone where we found aggregations of parasitized *M. gilvus* hosts. This result suggests that parasitized individuals were more tolerant of the drier environment than unparasitized ones, as is also indicated by the higher survivorship of the former when exposed to desiccation stress. Physiological characteristics of parasitized termites identified in the present study, such as larger size, lower water loss rates, higher water loss tolerance, and a greater amount of metabolic water stores compared to unparasitized termites correlate with this distribution pattern and suggest potential physiological adaptations that help parasitized soldiers (and parasitoids) to survive.

Smaller sized terrestrial arthropods have a large surface area to volume ratio, which makes them particularly vulnerable in dry surroundings (Gibbs et al., 1997; Danks, 2000; Kaersgaard et al., 2004). The fact that parasitized major soldiers were larger in size,

this suggests that they were less susceptible to desiccation compared to unparasitized major soldiers. As an apparent result of reduced CP, parasitized termites also had lower rates of water loss than unparasitized ones when exposed to desiccation stress. This may indicate that the presence of the larval parasitoid decreases CP, thus enabling the host to better withstand lower humidity. Moreover, CP of most insects that live in dry environments is usually lower than those living in wetter environments (Appel, 1991; Shelton and Grace, 2003; Kaersgaard et al., 2004). Two factors that may affect CP in an insect are cuticular water content and chemical and physical properties of the epicuticular lipid layer (Gibbs, 1998, 2002; Appel and Tanley, 1999). We found that parasitized major soldiers had lower cuticular water content than unparasitized termites. Riddle (1981) reported that in drier environments, cuticular water content was reduced in less permeable cuticles because water movement into the cuticle was restricted by the epidermis. Appel and Tanley (1999) found that the water content of the procuticle contributed significantly to permeability of the German cockroach *Blattella germanica* (Linnaeus). Chemical and physical properties of the epicuticular lipid layer were not evaluated in the present study, and they merit further investigation.

In the current study, significant colony differences were observed in CP (Table 2). However, the data were pooled across all colonies because water balance in insects is generally influenced by environmental factors (Edney, 1957). Because the three colonies used in this study were obtained from different locations, the colony differences in CP likely were due to the variation in environmental conditions. However, the general trend of parasitized major soldiers having lower CP values remained (Table 1).

Generally, lower body water content in insects contributes to water conservation and lower water loss rates (Danks, 2000; Mazer and Appel, 2001). Body fat and body water content are inversely proportional in many arthropods (Hadley, 1994), as was found in this study. Mean % TBW content increased with an increasing mean % TBW loss rate in parasitized and unparasitized major soldiers (Fig. 2). Concurrently, body lipid content of major soldiers was inversely proportional to the total % TBW content, indicating that lipid reserves may play a role in reducing water loss rates in parasitized major soldiers, as they contained significantly higher body lipid content than unparasitized major soldiers. Metabolic water gained from catabolism of fat bodies is an important process that allows insects to withstand long periods of desiccation (Danks, 2000; Mazer and Appel, 2001).

Hadley (1994) proposed various ways to measure tolerance to water loss, such as % TBW content at death and % TBW. In our study, parasitized major soldiers appeared to tolerate greater losses of body water than unparasitized ones, as indicated by % TBW that could be lost before immobility occurred. Tolerance of insects to water loss varies substantially depending on the environment (Hadley, 1994).

Although higher respiration rates mainly lead to greater losses of water rates (Gibbs et al., 1997; Davis et al., 2000) the data in the present study contradict this premise. The respiration rates of parasitized major soldiers were greater but their water loss rates were lower than those of unparasitized termites. These conflicting results could be due to the integument that forms the main barrier to evaporative water loss, as 90% of total water loss occurs largely from the integument in several insects (Hadley, 1994; Gibbs, 2002) and loss through the respiratory surfaces is minimal (William et al., 1998; Rourke, 2000; Bazinet et al., 2010).

In conclusion, the physiological changes that occur in parasitized *M. gilvus* are apparently beneficial to the parasitoids, as the alterations can clearly increase the parasite's chances of survival when exposed to extreme environmental conditions and ensure that the parasitoids are able to complete their larval development successfully before the host dies. The physiological changes in

parasitized hosts may also explain why the fly parasitoids are so successful in *M. gilvus* nests, particularly in Penang, where 19.7% (137 of 697 surveyed colonies) of *M. gilvus* colonies were found to be infected by *M. mindeni* (Foo et al., 2011b).

Acknowledgments

We thank Kok-Boon Neoh for helpful comments on the manuscript. F.-K.F. was supported under Ph.D. fellowship schemes by Universiti Sains Malaysia. The research was funded by Universiti Sains Malaysia Postgraduate Research Scheme USM-RU-PRGS and Bayer CropScience (Singapore).

References

- Alleyne, M., Beckage, N.E., 1996. Parasitism-induced effects on host growth and metabolic efficiency in tobacco hornworm larvae parasitized by *Cotesia congregata*. *Journal of Insect Physiology* 43, 407–424.
- Alleyne, M., Chappell, M.A., Gelman, D.B., Beckage, N.E., 1997. Effects of parasitism by the braconid wasp *Cotesia congregata* on metabolic rate in host larvae of the tobacco hornworm, *Manduca sexta*. *Journal of Insect Physiology* 43, 143–154.
- Appel, A.G., 1991. Water relations and thermal sensitivity of several cockroach species (Dictyoptera: Blattellidae and Blaberidae). *Comparative Biochemistry and Physiology* 100A, 353–356.
- Appel, A.G., Tanley, M.J., 1999. Water composition and loss by body color and form mutants of the German cockroach (Dictyoptera: Blattellidae). *Comparative Biochemistry and Physiology* 122A, 415–420.
- Baker, J.E., Fabrick, J.A., 2000. Host hemolymph proteins and protein digestion in larval *Habrobracon hebetor* (Hymenoptera: Braconidae). *Insect Biochemistry and Molecular Biology* 30, 937–946.
- Bazinet, A.L., Marshall, K.E., MacMillan, H.A., Williams, C.M., Sinclair, B.J., 2010. Rapid changes in desiccation resistance in *Drosophila melanogaster* are facilitated by changes in cuticular permeability. *Journal of Insect Physiology* 56, 2006–2012.
- Berrigan, D., Partridge, L., 1997. Influence of temperature and activity on the metabolic rate of adult *Drosophila melanogaster*. *Comparative Biochemistry and Physiology* 118A, 1301–1307.
- Brodeur, J., Boivin, G., 2004. Functional ecology of immature parasitoids. *Annual Review of Entomology* 49, 27–49.
- Collins, M.S., 1969. Water relations in termites. In: Krishna, K., Weesner, F.M. (Eds.), *Biology of Termites*, vol. 1. Academic Press, New York, pp. 433–458.
- Consoli, F.L., Vinson, S.B., 2004. Host regulation and the embryonic development of the endoparasitoid *Toxoneuron nigriceps* (Hymenoptera: Braconidae). *Comparative Biochemistry and Physiology* 137B, 463–473.
- Danks, H.V., 2000. Dehydration in dormant insects. *Journal of Insect Physiology* 46, 837–852.
- Davis, A.L.V., Chown, S.L., McGeoch, M.A., Scholtz, C.H., 2000. A comparative analysis of metabolic rate in six *Scarabaeus* species (Coleoptera: Scarabaeidae) from southern Africa: further caveats when inferring adaptation. *Journal of Insect Physiology* 46, 553–562.
- Day, M.F., Oster, I.I., 1963. Physical injuries. In: Steinhaus, E.A. (Ed.), *Insect Pathology: An Advanced Treatise*, vol. 1. Academic Press, New York, pp. 29–63.
- Edney, E.B., 1957. *The Water Relations of Terrestrial Arthropods*. Cambridge University Press, New York, pp. 6–93.
- Foo, F.K., Othman, A.S., Lee, C.Y., 2011a. Morphology and development of a termite endoparasitoid, *Misotermes mindeni* (Diptera: Phoridae). *Annals of the Entomological Society of America* 104, 233–240.
- Foo, F.K., Veera Singham, G., Othman, A.S., Lee, C.Y., 2011b. Prevalence of a koinobiont endoparasitoid *Misotermes mindeni* (Diptera: Phoridae) in colonies of the fungus-growing termite *Macrotermes gilvus* (Blattodea: Termitidae) in Malaysia. *Journal of Economic Entomology* 105, in press.
- Fyfe, R.V., Gay, F.J., 1938. The Humidity of the Atmosphere and the Moisture Conditions within Mounds of *Eutermes exitiosus* Hill. Council for Scientific and Industrial Research, Melbourne, pp. 1–22.
- Gibbs, A.G., 1998. Water-proofing properties of cuticular lipids. *American Zoologist* 38, 471–482.
- Gibbs, A.G., 2002. Lipid melting and cuticular permeability: new insights into an old problem. *Journal of Insect Physiology* 48, 391–400.
- Gibbs, A.G., Chippindale, A.K., Rose, M.R., 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *Journal of Experimental Biology* 200, 1821–1832.
- Godfray, H.C.J., 1994. *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, Princeton, pp. 1–488.
- Hadley, N.F., 1994. *Water Relations of Terrestrial Arthropods*. Academic Press, New York, pp. 1–356.
- Haspel, G., Gefen, E., Gustavo Glusman, A.Ar.J., Libersat, F., 2005. Parasitoid wasp affects metabolism of cockroach host to favor food preservation for its offspring. *Journal of Comparative Physiology* 191A, 529–534.
- Kaersgaard, C.W., Holmstrup, M., Malte, H., Bayley, M., 2004. The importance of cuticular permeability, osmolyte production and body size for the desiccation resistance of nine species of collembola. *Journal of Insect Physiology* 50, 5–15.

- Korb, J., 2003. Thermoregulation and ventilation of termite mounds. *Naturwissenschaften* 90, 212–219.
- Korb, J., Linsenmair, K.E., 1999. Ventilation of termite mounds: new results require a new model. *Behavioral Ecology* 11, 486–494.
- Mazer, C.L., Appel, A.G., 2001. Water loss and desiccation tolerances of longwing butterflies (Lepidoptera: Nymphalidae). *Environmental Entomology* 30, 631–636.
- Neoh, K.B., Lee, C.Y., 2010. Morphological and behavioural changes in soldiers of *Macrotermes gilvus* (Hagen) (Termitidae: Macrotermitinae) parasitized by the fly *Misotermes mindeni* Disney & Neoh (Diptera: Phoridae). *Environmental Entomology* 39, 835–840.
- Noirot, C.H., 1970. The nests of termites. In: Krishna, K., Weesner, F.M. (Eds.), *Biology of Termites*, vol. 2. Academic Press, New York, pp. 73–125.
- Poulin, R., 1998. “Adaptive” changes in the behaviour of parasitized animals: a critical review. *International Journal for Parasitology* 25, 1371–1383.
- Riddle, W.W., 1981. Cuticle water activity and water content of beetles and scorpions from xeric and mesic habitats. *Comparative Biochemistry and Physiology* 68A, 231–235.
- Roces, F., Gnatzy, W., 1997. Reduced metabolic rate in crickets paralysed by a digger wasp. *Naturwissenschaften* 84, 362–366.
- Rourke, B.C., 2000. Geographic and altitudinal variation in water balance and metabolic rate in a California grasshopper, *Melanoplus sanguinipes*. *The Journal of Experimental Biology* 203, 2699–2712.
- Shelton, T.G., Grace, J.K., 2003. Cuticular permeability of two species of *Coptotermes* Wasmann (Isoptera: Rhinotermitidae). *Comparative Biochemistry and Physiology* 134A, 205–211.
- Smith-Trail, D.R., 1980. Behavioral interactions between parasites and hosts. Host suicide and the evolution of complex life cycles. *American Naturalist* 116, 77–91.
- Sponsler, R.C., Appel, A.G., 1990. Aspects of the water relations of the Formosan and Eastern subterranean termites (Isoptera: Rhinotermitidae). *Environmental Entomology* 19, 15–20.
- Thompson, S.N., Redak, R.A., 2008. Parasitism of an insect *Manduca sexta* L. alters feeding behaviour and nutrient utilization to influence developmental success of a parasitoid. *Journal of Comparative Physiology* 178B, 515–527.
- Umbreit, W.W., Burris, R.H., Stauffer, J.H., 1972. *Manometric and Biochemical Techniques*, fifth ed. Burgess Publishing Company, Minneapolis, Minnesota, pp. 1–99.
- Varo, I., Taylor, A.C., Navarro, J.C., Amat, F., 2000. Effect of parasitism on respiration rates of adults of different *Artemia* strains from Spain. *Parasitology Research* 86, 772–774.
- William, A.E., Rose, M.R., Bradley, T.J., 1998. Using laboratory selection for desiccation resistance to examine the relationship between respiratory pattern and water loss in insects. *The Journal of Experimental Biology* 201, 2945–2952.