Interspecific agonistic behaviour of *Odontotermes javanicus* and *Microcerotermes* sp. (Isoptera: Termitidae): Preliminary study

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Abstract. Termite interaction from the same or different colonies or species can display a variety of agonistic behaviours. The study aimed to observe the interspecific agonistic behaviour of *Odontotermes javanicus* and *Microcerotermes* sp. For this purpose, an assay was carried out using a test arena separated into two groups. Termite responses were tested against each other in the following pairwise test (worker versus worker, worker versus soldier, and soldier versus soldier) with a 1:1 ratio (ten individuals) from each colony. Specimens were collected at the area of Forestry Faculty, Universitas Hasanuddin, Indonesia. The observed agonistic behaviour of both species is dominated by examination (antennation) and aggression (attacking, grappling, and biting). There were three treatment combinations that classified as no aggression, except for the treatment (Microcerotermes sp. soldier versus *O. javanicus* worker) that classified in aggression level 3 (aggressive). Based on termite survival after five minutes of testing, *O. javanicus* is more competitive than *Microcerotermes* sp.; no more individual termites of both species survive after 24 hours of testing.

1. Introduction
One of the wood biodeterioration agents is termites. Most of these organisms are decomposing agents of dead organic matters, but also some species cause a large financial loss, even up to billions of rupiahs annually (LIPI, 2018). Currently, as many as 3,106 species have been identified worldwide, which are distributed in all parts of the earth with tropical and subtropical climates (Su and Scheffrahn, 2000), and are even known to have spread to temperate regions up to a limit of 50° N and 50° South [1,2]. The latest termite classification proposed by Beccaloni and Eggleton (2013) places the order Isoptera into the infraorder Isoptera, and together with cockroaches under one order, namely the order Blattoidea [3]. The termite family was also expanded into nine families, which are under the epifamily Termitidae. In Indonesia, termite diversity is estimated at almost 10% of the total termites in the world, which consists of three families them (Kalothermitidae, Rhinotermitidae, and Termitidae) [4]. In South Sulawesi, especially in the area of Universitas Hasanuddin Campus, several species have also been identified, namely the Rhinotermitidae family with two species, namely Coptotermes curvignathus and Schedorhinotermites sp. [5].

Termites are known as social insects that live in colonies with a caste-based work arrangement system in nests. Workers are responsible for almost all the work in the nest, such as foraging for food and helping soldiers secure nests. The proportion of workers reached >80-90% of the total members of the colony. Termites have different characteristics and behaviour from other social insects. In the nest, the behaviour of termites is controlled and regulated by a system of glands, well known as pheromones. One of the behaviours in termites is agonistic. This behaviour is a response shown when interacting with other or different species. Agonistic behavior is related to efforts to maintain survival. This study aims to examine/investigate the interspecific agonistic behaviour of *Odontotermes javanicus* with *Microcerotermes* sp.
2. Material and method

2.1. Study site
Determination of the location for the collection of specimens using the purposive sampling method. The location was chosen based on the presence of *O. javanicus* and *Microcerotermes* sp., which is located in the Hasanuddin University Campus area.

2.2. Termite caste segregation
The termites that will be used for behavioral testing are collected from nests found in the field, using direct sampling. Some of the nests containing termites are taken with a machete and put in plastic containers/boxes for easy transport to the laboratory. Individual termites are then slowly removed from the nest to facilitate the process of separating termites from each caste. The termites that have been removed from the nest are then placed on moistened filter paper in a plastic container to retain moisture and prevent dehydration of the termites. Separation of individual termites from the worker and soldier castes from the nest was carried out on the day of testing and observation of termite behavior.

2.3. Interspecific agonistic behavior test
Observation of termite agonistic behavior of termites colony *O. javanicus* and *Microcerotermes* sp. carried out by referring to the procedure of Wong and Lee (2010), with the following stages [6]:

2.3.1. Preparation of testing arena. The testing arena uses a 9 cm diameter Petri dish to see the response of the two species when interacting. Filter paper of the same diameter as the petri dish was moistened with distilled water and placed in the Petri dish. Then, the test arena was partitioned into two parts using a plastic sheet placed in the center of the Petri dish. This partition is maintained until just before recording for five minutes.

2.3.2. Observation of agonistic behaviour. Individual termites from the colonies of *O. javanicus* and *Microcerotermes* sp., which will be tested are made in pairs (pairwise tests), namely workers versus workers, workers versus soldiers, soldiers versus soldiers. All castes were paired with a 1:1 ratio, i.e., 10 individual soldiers or workers from each test pair. Termites introduced into the test area were allowed to acclimatize for 10 minutes before the partitions were removed and observations were made. Events due to termite activity were observed and videoed for 5 minutes. Each combination of test pairs was repeated five times, using different test arenas. After observing for 5 minutes, the test arena was closed using petri pairs and placed in a dark room at room temperature of 25±1.5 °C and humidity of 56.3±0.7% for 24 hours before the number of live termites was counted.

2.4. Data analysis
Data on the frequency and number of termites that survived were analyzed descriptively and classified based on the classification of Wong and Lee (2010) [6].

3. Results

3.1. Aggression level
The aggression level of the two types of termites when interacting was based on the agonistic behavior of the termite colonies *O. javanicus* and *Microcerotermes* sp., which are tested in pairs (pairwise tests), namely each caste of soldiers versus soldiers, workers versus workers, and soldiers versus workers. The frequency data of agonistic behavior is described as follows:

3.1.1. Aggression level of *Microcerotermes* sp. workers versus *O. javanicus* workers. Responses of *Microcerotermes* sp workers and *O. javanicus* workers when interacting showed different frequencies, with the average frequency as shown in Table 1.
Table 1. Aggression level of *Microcerotermes* sp. workers versus *O. javanicus* workers

| No | Classification   | Behavior         | Frequency | Mean |
|----|------------------|------------------|-----------|------|
|    |                  |                  | 1 | 2 | 3 | 4 | 5 |      |
| 1. | No aggression    | Antennation      | 5 | 2 | 6 | 12 | 6 | 6.2  |
|    |                  | Jerking          | 2 | 2 | 4 | 7  | 4 |      |
| 2. | Less aggression  | Avoidance        | 1 | 0 | 0 | 0  | 0 | 2.4  |
|    |                  | Chasing/escaping | 3 | 1 | 1 | 1  | 1 |      |
|    |                  | Attack           | 1 | 1 | 3 | 2  | 2 |      |
| 3. | Aggressive       | Grappling        | 4 | 4 | 7 | 7  | 7 | 4.3  |
|    |                  | Biting           | 3 | 6 | 5 | 8  | 5 |      |

In Table 1, the dominant behavior shown by the workers castes of the two different species when interacting in the testing arena is a determination by doing antennation with an average of 6.2; then followed by aggressive behavior with an average of 4.3, namely attacking, wrestling, biting; and less aggressive behavior with an average of 2.4, namely jerking, avoiding, chasing or escaping. Based on the classification of the aggression level, the worker termites of both species are no aggressive [6].

3.1.2. Aggression level of *Microcerotermes* sp. workers versus *O. javanicus* soldiers. Responses of *Microcerotermes* sp worker and *O. javanicus* soldier when interacting showed different frequencies, with the average frequency as shown in Table 2.

Table 2. Aggression level of *Microcerotermes* sp. workers versus *O. javanicus* soldiers

| No | Classification   | Behavior         | Frequency | Mean |
|----|------------------|------------------|-----------|------|
|    |                  |                  | 1 | 2 | 3 | 4 | 5 |      |
| 1. | No aggression    | Antennation      | 5 | 4 | 4 | 5  | 10 | 5.6  |
|    |                  | Jerking          | 1 | 2 | 4 | 2  | 2  |      |
| 2. | Less aggression  | Avoidance        | 1 | 5 | 2 | 4  | 4  | 2.9  |
|    |                  | Chasing/escaping | 2 | 3 | 3 | 3  | 5  |      |
|    |                  | Attack           | 1 | 1 | 2 | 4  | 1  |      |
| 3. | Aggressive       | Grappling        | 7 | 5 | 8 | 7  | 1  | 5.3  |
|    |                  | Biting           | 8 | 10| 7 | 9  | 9  |      |

In Table 2, the dominant behavior shown by the different castes of the two different species when interacting in the testing arena is a determination by doing antennation with an average of 5.6; then followed by aggressive behavior with an average of 5.3 such as attacking, grappling, biting; and less aggressive behavior with an average of 2.9, such as jerking, avoiding, chasing or escaping. Based on the classification of the aggression level, the worker and soldier of both species are not aggressive [6].

3.1.3. Aggression level of *Microcerotermes* sp. soldiers versus *O. javanicus* workers. Responses of *Microcerotermes* sp soldier and *O. javanicus* worker when interacting showed different frequencies, with the average frequency as shown in Table 3.
Table 3. Aggression level of *Microcerotermes* sp. soldiers versus *O. javanicus* workers

| No  | Classification   | Behavior       | Frequency | Mean |
|-----|------------------|----------------|-----------|------|
|     |                  |                | 1 2 3 4 5 |      |
| 1.  | No aggression    | Antennation    | 5 7 4 6 10| 6.4  |
|     |                  | Jerking        | 3 9 4 7 7 |      |
| 2.  | Less aggression  | Avoidance      | 3 6 3 6 4 | 4.4  |
|     |                  | Chasing/escaping| 1 0 1 3 4|      |
|     |                  | Attack         | 2 1 2 5 8 |      |
| 3.  | Aggressive       | Grappling      | 7 6 8 9 8 | 6.9  |
|     |                  | Biting         | 10 12 4 8 | 13   |

In Table 3, the dominant behavior shown by the different castes of the two different species when interacting in the testing arena is by aggressive behavior with an average of 6.9 such as attacking, grappling, biting; then followed determination by doing antennation with an average of 6.4; and less aggressive behavior with an average of 4.4, such as jerking, avoiding, chasing or escaping. Based on the classification of the aggression level, the workers and soldiers of both species are no aggressive [6].

3.1.4. Aggression level of *Microcerotermes* sp. soldier versus *O. javanicus* soldier. Responses of *Microcerotermes* sp soldier and *O. javanicus* worker when interacting showed different frequencies, with the average frequency as shown in Table 4.

Table 4. Aggression level of *Microcerotermes* sp. soldier versus *O. javanicus* soldier

| No  | Classification   | Behavior       | Frequency | mean |
|-----|------------------|----------------|-----------|------|
|     |                  |                | 1 2 3 4 5 |      |
| 1.  | No aggression    | Antennation    | 9 10 5 10 5| 7.8  |
|     |                  | Jerking        | 3 3 2 5 5 |      |
| 2.  | Less aggression  | Avoidance      | 1 2 3 6 6 | 3.1  |
|     |                  | Chasing/escaping| 2 2 4 1 1|      |
|     |                  | Attack         | 6 5 7 3 4 |      |
| 3.  | Aggressive       | Grappling      | 6 5 5 7 9 | 5.9  |
|     |                  | Biting         | 3 4 7 9 8 |      |

In Table 4, the dominant behavior shown by the soldiers castes of the two different species when interacting in the testing arena is determination by doing antennation with an average of 7.8; then followed by aggressive behavior with an average of 5.9, namely attacking, wrestling, biting; and less aggressive behavior with an average of 3.1, namely jerking, avoiding, chasing or escaping. Based on the classification of the aggression level, the soldiers of both species are no aggressive [6].

3.2. Competitiveness
The competitiveness of both termites species when interacting was based on observing the number of survival termites in the testing arena which are tested in pairs (pairwise tests), namely each caste of soldiers versus soldiers, workers versus workers, and soldiers versus workers. The number and percentage of individual termites that survive are shown in Figure 3.
Figure 1. The average number and percentage of surviving *Microcerotermes* sp. versus *O. javanicus*: (a) MWo versus OWo, (b) MWo versus Oso, (c) MSo versus OWo, and (d) MSo versus OSo

Figure 1 shows that *O. javanicus* workers had a higher survival rate in the test arena than *Microcerotermes* sp. workers, which was indicated by a higher number and percentage of surviving termites. Based on the classification of Wong and Lee (2010), *O. javanicus* are competitive until very competitive with a survival percentage range of 66-92%, while *Microcerotermes* sp. are moderate until competitive with a survival percentage range of 26-54% [6].

4. Discussions

Termite responses that can be observed in the test arena are antennation, avoidance, attacking, chasing or escaping, jerking, and grappling. The agonistic response differs depending on the individuals, colonies, and species involved. In addition, genetic relationships, colony size, colony health and density, food availability, and other environmental barriers can influence the expression of agonists [7]. However, several other studies found several factors that did not affect agonistic behavior, including differences in cuticular hydrocarbon composition, geographic distance, and genetic similarity between different colonies [7–9].

Among the eight agonistic behaviors based on Wong and Lee (2010), the dominant behaviors shown by the two species are antennation, attacking, grappling, and biting [6]. The number of colonies of *O. javanicus* that can survive in the test arena is higher than that of *Microcerotermes* sp. However, mortality in both species reached 100% before 24 hours after the test was carried out, even though *O. javanicus* termites could survive longer than the termites. termites *Microcerothermes* sp. This is in line with research Delphia et al., (2003) study which states that agonistic behavior in Reticulitermes (Rhinotermitidae) worker termites lasts for five minutes and causes death in 24 hours [10].

Intercolony agonistic behavior of subterranean termites can vary widely. Differences in duration may influence the prevalence of agonistic behavior. There are some individuals who do not directly show
aggressive behavior towards individuals from different colonies, but show agonistic behavior after a long time. If this happens, it may be caused by differences in the components of the digestive system. This is related to the bacteria present in the digestive system, where the bacteria are spread from one individual to another in a colony through the process of trophallaxis. The bacteria that are excreted through the feces are used to identify individuals from one colony or individuals from different colonies.

5. Conclusions
Referring to the results of research and discussion, it can be concluded that no aggression was shown by three treatment combinations, except for the treatment (Microcerotermes sp. soldier versus O. javanicus worker) that classified in aggression level 3 (aggressive); In competitiveness, termites O. javanicus be classified as competitive to very competitive (66-92%), while Microcerotermes sp. termites are classified as moderate to competitive (26-54%).

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