Polyethism in *Odontotermes formosanus* Shiraki

Ehsan Soleymaninejad1 Bao-Zhong Ji* Shu-Wen Liu Jin-Jin Yang Hong-Jiang Wang Fang Ding

1Nanjing Forestry University Southern Modern Forestry Collaborative Innovation Center, College of Forest Resources and Environment, Nanjing Forestry University, Nanjing 210037, China

*The Administration Bureau of Dr.Sun Yat-sen Mausoleum, Nanjing 210014, China

**Corresponding Author:** Ehsan Soleimani-Nezhadian

Nanjing Forestry University Southern Modern Forestry Collaborative Innovation Center, College of Forest Resources and Environment, Nanjing Forestry University, Nanjing 210037, China

jbz9885@njfu.edu.cn

1. Introduction

During the evolution of social insects polyethism is one of the key phenomena (Traniello and Rosengaus, 1997). Polyethism makes the colony works harmoniously (Soleymaninejad et al. 2014). To put it in a nutshell, polyethism means division of duties among the insect colony member. As Evans (2006) revealed in his paper, in Nasutitermes exitiosus, worker shifted their work from foraging to building behavior and vice versa very rarely. This research has shown that duties are somehow divided in different individuals in a fix manner. Recent studies have shown that age (Watson, McMahan 1978, Gerber et al. 1987, Lys and Leuthold 1991, Crosland et al. 1997, Hinze and Leuthold, 1999), size (McMahan and Watson, 1975, Campora and Grace, 2004, Crosland et al. 1998, Nalepa, 2011) and sex (Machida et al. 2001) or combination of them (Oster and Wilson, 1978) are the fundamental criteria in polyethism.

Size of the termites especially workers is one of the important morphological characters that confirm the activity of the workers. Jones (1980) studied the construction behavior of Nasutitermes costalis. He showed that workers with different sizes had different roles in construction behavior. He divided workers into two small workers (SW1, SW2) and three large workers (LW1, LW2, LW3) based on a research was done by McMahan (1970). Building behavior of all of them had been analyzed by him. He found that SW1 and LW3 were moderate in abundance at the gallery repair site, they were vigorous yet. In spite, the SW2 were the active builders. Also, LW3 were taking part in grooming. As well McMahan et al. 1984 attested that 96% of small workers were attending in queen chamber on the contrary 99% of large workers take part in foraging and 70% of them can be found around swarming sites exit holes. Studying the sternal gland size of higher instars in Trinervitermes bettonianus have shown that the higher instars have the bigger sternal glands. The result have cleared that only the workers in their highest instars take part in foraging activity (Oloo, 1981). Recently we have shown that workers with medium head sizes are the major foragers in *Odontotermes formosanus* Shiraki (Soleymaninejad et al. 2014). Campora and Grace (2004) mentioned that the size played a critical role in tunneling galleries. They analyzed their data using ANOVA and regression analysis. The research confirmed that large workers could make wide with less segmentation tunnels, while the small workers are good enough to make tunnels with more segmentation still less width. Size polyethism is not involved only among the workers, small and large soldiers of *N. exitiosus* act differently in facing with enemies. In this species small soldiers are more attracted to the intruders and they use their frontal gland to...
secret and repel the invaders. In contrast, large soldiers are non combative and flee at the time of danger. Non combative large soldiers may serve as messenger and make the nest ready by raising the danger signals (McMahan 1974, Kriston et al. 1977). Furthermore, one study on N. costalis has shown that soldiers have a key role in foraging by recruiting other soldiers and large workers to the source of food (Traniello, 1981). This phenomenon is called chemical regulation of polyethism during foraging (Traniello et al. 1985). It was clear that large instar workers have bigger sternal glands than soldiers, subsequently produce more trail pheromone to recruit more foragers (Oloo, 1981, Traniello and Busher, 1985).

In this paper O. formosanus Shiraki has been studied to see polyethism among foragers and inside the nest. To indicate this phenomenon, the head width of foragers and workers in the nest measured and put into different groups. Then, the major group of workers in and out of the nest found. Also, sizes of the sternal glands of the foragers and workers have been checked to see the differences among them. At the end tunneling behavior of the minor and major workers have been checked.

2. Materials and Methods

Nanjing Forestry University (NFU), located in the east of Nanjing, at the foot of Zijin Mountains and east of Xuanwu Lake (32° 45′50.66″ N, 118° 48′41.06″ E), was the study site. All the termites captured in a sunny day (May 2014) with temperature of 15° C, relative humidity of 70% and a breeze blowing (0.5 Km/H). Hot damp summers and cold damp winters with short spring and fall are the main characteristic seasons in Nanjing. As the temperature is not high and changing dramatically during days and nights in spring of Nanjing (March-May), the foragers are on the ground and under rotten fallen logs. In summer and fall seasons (Jun-October and early November) it is easier to find the foragers by finding the moisture shelter tubes on trees.

To collect the foragers in the spring, logs had been moved to see if there is any sign of foraging activity. Samples captured by the means of painting brush into plastic boxes. In the case the foragers could not be captured by painting brush, they were collected by surrounding soils or pieces of logs into the containers. In summer finding shelter tubes on the trees considered as a sign of foragers of a nests. Making a breach in the tube gently (tweezers can be used) and then by the means of smooth painting brush samples were collected into a plastic containers.

To collect the samples from nest, foraging activity under rotten log means a high probability of finding a colony nearby. In summer and fall seasons, finding a shelter tube can be a sign of nest in proximity. The shelter tube or trail of foragers under the log must be followed to get to the ground. The foraging tunnel to the nest found and followed to get to bigger tunnel that was in vertical with ground, main tunnel, and ended with fungal gardens and then main fungal garden (Fig. 1).
In this case the workers could not come out of the hole and they forced to make the tunnels. Before starting the experiment the sand washed with distilled water and pour with 10 ml of water to keep the humidity.

In addition, sternal glands width of minor, major workers and soldiers (from out and inside the nest) had been studied. Each step had done with a great care to get the gland intact. Termite placed on the wax dish and entomological needles (zero size) had been used to fix them on the dish. Abdomen opened from dorsal part by the means of seizer or simply a shaving blade. Before start dissecting the abdomen it is better to get rid of legs. As far as tergites opened, insect needles were used to attach them to the wax dish for less interference. Fats and guts must be removed with gentle manner to get to the insect nerve system. The ganglions are stretched through the body on the anterior-posterior axis. The ganglions are like nodes with a thread of nerve system between them. The ganglions must be removed with high cautious as the sternal gland lies under the nerve system. It is better to wash the abdomen from time to time to remove the fats and other interfering particles that block the vision. After removing the majority of the fats and guts the insect removed from wax dish and transferred into a Petri dish. Instead of using top light of microscope, it is better to turn on the down light to have better contrast and better vision of sternal gland. The sternal glands found between fourth and fifth sternite, posterior part of fourth sternite and anterior part of fifth sternite (Fig 3).

To test the tunneling behavior of the termite, alive termites captured both minor and major workers. In first experiment a mixture of 10 workers, minor and major workers; have been studied to see the tunneling behavior of them. Ten workers have been put in a lead of Petri dish with diameter of 20 cm that filled in an equal manner with sea sands. The sand covered the bottom of the Petri dish up to 3mm. A circular hole made in center of Petri dish with diameter of 3mm and put all the ten workers inside and the Petri dish placed on the hole Fig 2. Then the system put in dark and every day checked to not the sand become dry.

Figure 2: As it is illustrated here the positions of Petri dish lead and the Petri dish itself has shown. The Petri dish lay on the sands to first not let the termites get out of the hole and second to prevent evaporation of water. In the center of Petri dish lead that is covered with sands, we made a hole with diameter of 3mm and put the workers inside and then the Petri dish itself placed on the hole and sands.

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To measure head width and sternal gland width of the samples, JSZ65 (20030568) microscope (manufactured by Nanjing Jiangnan Novel Optics Co., Ltd) with a camera (JIFEI) (manufactured by Nanjing JIFEI Technology Co., Ltd.) assembled on it has been used. The microscope was connected with a computer. JIFEI software helped us to measure and capture photos in the computer.

Microsoft Excel has been used to calculate Average and Standard Error. After measuring the head width, the data has been loaded into Microsoft Excel and column charts made using head width and frequency of them as x and y axis respectively. Based on the peaks, workers and soldiers have been classified into different groups (Crosland et al. 1998, Rosin, 1988, Miura et al. 2000, Miura, 2006). For division of the workers groups we consider a gradual rise ended with a peak and a gradual down. So a sudden rises or falls did not consider as groups. Also, lowest peak is reflected as starting of one group.

3. Results
3.1 Polyethism of workers and soldiers in nest

After nest excavation, one peripheral fungal garden plus main fungal garden with queen and king chamber have been found. The fungal garden was cut into different parts based on the top, middle, and bottom of the fungal hives at the site of excavation (Fig. 1).

3.1.1 Peripheral Fungal Garden

Top: Twelve workers and 4 soldiers were wandering at the top of the fungal garden. Average head width of workers was 1.29mm with standard error of 0.01. Average head width of soldiers was 1.17mm.

Middle: Nine workers and 7 soldiers had been seen in the middle section of fungal garden. Average head width and standard error of the workers were 1.32mm and 0.01mm respectively. On the other hand soldiers average head width was 1.23mm with standard error of 0.02mm.

Bottom: In this section 13 workers and 3 soldiers were captured. Average head width of workers was 1.24mm with standard error of 0.05mm. Average head width of soldiers was 1.25mm and standard error was 0.003mm.

It should be mentioned that there was not seen any larvae or eggs in the peripheral fungal garden.

3.1.2 Main Fungal Garden

In the main fungal garden biomass was a little different in compare to the peripheral fungal garden. Main fungal garden was covered with larvae all over, from top to bottom. Thousands of larvae were moving and consuming from the fungus combs. In addition, the number of workers and soldiers were higher than peripheral fungal garden. As similar as peripheral fungal garden, at the site it was cut into three parts, top, middle and bottom plus queen and king chamber population.

Top: Only 1 soldier with head width of 1.21mm found in the top section of fungal garden. Number of workers was 29mm with average head width of 1.13mm and standard error of the data was 0.03mm.

Middle: In this part only workers were available. There was not any soldier in the middle part. However the number of workers increased dramatically. Fifty-two workers had captured in the middle section with average head width of 1.2mm and standard error of 0.01mm.

Bottom: Four soldiers and 114 workers serving at the bottom of the fungal garden. Average head width of the soldiers was 1.23mm with standard error of 0.003mm. On the other hand, workers average head width was 1.11mm and standard error was 0.01mm.

3.1.3 King and Queen Chamber

Fifty-seven workers and 19 soldiers were captured in queen and king chamber. Average head width of the soldiers was 1.27mm with standard error of 0.002mm. Number of workers around king and queen chamber was 57 with average head width of 1.24mm and standard error of 0.002mm. An overview of groups in different part of the nest brought in Table 1.

3.1.4 Total Workers and Soldiers in the Nest

Drawing a column chart based on head width of the workers inside the nest can be seen in Fig. 4a. Based on the diagram the workers can be divided into minor and major workers. The workers with head width of 0.83-0.9 were classified as minor workers and workers with head width of 0.2-0.31 categorized as major workers. Average head width of total workers was 1.17mm with standard error of 0.0005mm. On the other hand soldiers had the average head width of 1.24mm with standard error of 0.001mm (Fig. 4b).

3.2 Polyethism in foragers

One hundred and twenty workers plus 2 soldiers captured at the foraging site, under fallen log. Average head width of foragers was 1.37mm with standard error of 0.003mm, whilst, the average head width of soldiers were 1.29mm. Column chart of foragers can be seen in Fig. 4c.
Figure 4: Charts for the workers and soldiers inside the nest (a, b) and foraging workers (c) can be seen in this figure. a) Workers has been divided into 2 groups based on head width in the nest, major and minor workers. Workers with head width of 0.83-0.9mm classified as major workers and workers with head width of 0.2-0.3mm categorized as minor workers. b) Soldiers column chart. As it can be get from the chart, there is no special rule for dividing the soldiers into different groups. c) Foraging workers column charts, all of them are with head width of bigger than 1.28mm.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Workers</th>
<th>Soldiers</th>
<th>Average Head width of Workers(mm)</th>
<th>Average Head width of Soldiers(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral Fungal Garden</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>12</td>
<td>4</td>
<td>1.29</td>
<td>1.17</td>
</tr>
<tr>
<td>Middle</td>
<td>9</td>
<td>7</td>
<td>1.32</td>
<td>1.23</td>
</tr>
<tr>
<td>Bottom</td>
<td>13</td>
<td>3</td>
<td>1.24</td>
<td>1.25</td>
</tr>
<tr>
<td><strong>Main Fungal Garden</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>29</td>
<td>1</td>
<td>1.13</td>
<td>-</td>
</tr>
<tr>
<td>Middle</td>
<td>52</td>
<td>-</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td>Bottom</td>
<td>114</td>
<td>4</td>
<td>1.11</td>
<td>1.23</td>
</tr>
<tr>
<td><strong>King and queen chamber</strong></td>
<td>58</td>
<td>19</td>
<td>1.24</td>
<td>1.27</td>
</tr>
</tbody>
</table>

3.3 Polyethism in tunneling behavior

3.3.1 Mixture of minor and major workers

The experiment had been done for 12 hours. Every one hour they went through one centimeter of the sands. After 5.0cm they start to make an oval or circular structure with diameter of 1.5 to 2.0 cm and after that they started to make branches with different angels between 25-85 degrees. Diameter of main tunnels reached up to 5.0mm while the branches diameters were only 3.0mm. Lengths of the branches vary between 1.0-3.0 cm.
3.3.2 Minor and Major workers in the case of tunneling

One major and one minor worker put into different Petri dishes with 10.0 cm in diameter to study their tunneling behavior. Petri dish lead has used as the surface to be covered by sands and the Petri dish itself was used to cover the sand to keep the termite in a hole that was made to put the termite inside it. A hole with diameter 5.0 mm made in the center of Petri dish inside the covering sands and slowly put the termite inside the hole. After three days the length of the tunnel and the diameter of the tunnel measured.

Major worker made a direct tunnel and without any branch. The length of the tunnel was 2.0 cm and the diameter of was 4 mm. On the other hand the minor worker made a main tunnel with length of 2.3 cm and diameter of 3.0 mm. In addition it made a branch vertical to the main tunnel with diameter of 3.0 mm and length of 8 mm.

Table: Tunneling Behavior among Major and Minor Workers.

<table>
<thead>
<tr>
<th>Workers</th>
<th>Tunnel length(cm)</th>
<th>Tunnel diameter(mm)</th>
<th>Branches</th>
<th>Angel to the main tunnel(degree)</th>
<th>Length(cm)</th>
<th>Diameter(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture of Workers</td>
<td>10.0</td>
<td>5.0</td>
<td>3</td>
<td>25-85</td>
<td>1.0-3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Major worker</td>
<td>2.0</td>
<td>4.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Minor worker</td>
<td>2.3</td>
<td>3.0</td>
<td>1</td>
<td>90</td>
<td>8.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

3.4 Sternal Gland Width

In total 20 workers (10 foragers and 10 nest keeping workers) had been examined for their sternal glands’ size. Average sternal gland width of nest keeping workers was 0.28 mm. In contrast, average width of foraging workers sternal glands was 0.33 mm. Moreover, soldiers from out and inside the nest checked for their sternal gland width; average sternal gland width inside the nest was 0.24 mm, while the measurement for the foraging soldiers’ sternal gland width was 0.29 mm.

4. Discussion

As it can be seen from the data, only 34 workers and 14 soldiers were found in peripheral fungal garden. It means only 14.8% of total population of the nest were working on peripheral fungal garden and rest of the population were in the main nest and taking care of larvae and queen and king chamber. It was interesting that there was not seen any larvae in peripheral fungal garden. If average head width of the individuals coming from different part of fungal garden is compared to each other it can be seen that in top and middle sections of peripheral fungal garden the average head widths are more than that in bottom section. Also as it can be seen in the chart of foragers it is obvious that foragers head width are usually more than 1.28 mm. It can be interpreted that in top and middle part of the main nest with queen and king chamber at the bottom, majority of population of both workers, soldiers and larvae were serving the colony. Ninety-five percent of workers and 63% of soldiers were in the nest. Comparison among different parts of the main fungal garden can be informative. The average head width of the worker at bottom part of the main nest was 1.11 mm and it shows most of the workers in this section are minor workers. It can confirm the result by McMahan et al. (1984) that 96% of small workers were serving the queen and king chamber. To add more, the average head width of the workers at top and middle sections of the main nest is very low. It can clarify that the minor workers are the main player of main fungal garden. It can prove the results by Badertscher et al. (1983) that young workers had a key role in processing the cellulose rich material and distributing it among new born larvae. As the numbers of larvae were thousands in the main nest, it can be explained that minor workers usually taking care of the larvae and eggs. It was very amazing that 114 (58.4%) of the workers in the main nest are at the bottom section. It can be interpreted that in the main nest taking care of the larvae and eggs is the main task and mostly is doing by minor workers (as the average head width is very low). Different cellulase enzymes activities in the guts of different workers, soldiers and larvae have been studied by Veivers et al. (1991) in Macrotermes subhyalinus and M. michaelseni Sjøstedt to find the importance of polyethism in digestion and distribution of food in the colony. They found that fungal cellulase enzyme, endo-β-1,4 glucanase, took a part in 9% of digestion of cellulose in major worker. Also, young fungal nodules have more enzyme activity and they usually...
consume by young minor and especially young major workers. In contrast, old major worker and old minor worker feed on old nodules with less cellulase enzymes. Interestingly, larvae and soldiers only fed by minor workers’ saliva glands. However the numbers of total soldiers in the garden are a few, but the soldiers mostly found near the queen and king chamber. Only 5 soldiers had seen on the fungal garden of the main nest and rest of them protecting king and queen chamber. It can be elucidated that main task of the soldiers in O. formosanus Shiraki is to protect the queen and king. It was amazing that king was very active in the nest while we were collecting the main nest. The king was moving very fast inside the nest. It can illustrate that king has more duty than only periodical insemination of the queen as Maistrello and Sbrenna (1999) said. They mentioned in their paper that king played a key role in social structure and dynamic development of the colony as a mediator in social interaction between queen and other nest members. Furthermore, the average head width of soldiers around and in the queen and king chamber is higher than other sections of main fungal garden, 1.27mmVs 1.23mm.

Data have shown that workers with bigger head width usually taking part in foraging activity. In previous research also it was validated that 99% of large workers taking part in foraging activity (McMahan et al. 1984).However some the major workers in the nest taking part of foraging site, but the percentage of them is very low (4.1% of foragers). As we have shown in previous research (Soleymaninejad et al. 2014) soldiers with head width of bigger than 1.24mm are taking part in foraging activities. We have studied foraging soldiers of 30 nests at the feet of Zijin Mountains and found soldiers with head width of less than 1.24mm cannot be found in foraging activities. Here also only soldiers with biggest head widths taking part in foraging activity. It was thought provoking that soldiers with smaller head width were more frequently used their frontal glands to attack our fingers during the nest excavation. This phenomenon had been seen before in N. exitiosus by McMahan (1974) and Kriston et al. (1977).

Subject to tunneling behavior, however the mixture of workers can work together, but studying the major and minor workers showed that minor workers can make longer tunnels with more galleries. As Campora and Grace at 2004 mentioned that the size played a critical role in tunneling galleries in Coptotermes formosanus Shiraki. They said in this subterranean termites that large workers can make wide with less segmentation tunnels, while the small worker are good enough to make tunnels with more segmentation still less width. In usual in mixture of minor and major workers, they finish one tunnel first and then they start another one. In fact they follow the trail pheromone of the first workers that started the tunneling. Workers with smaller head sizes usually play as a role of header and pave the way for bigger ones.

In the course of sternal glands, the study has shown that the foragers of both casts, soldiers and workers, have bigger sternal glands in compare to those who are grooming the nest and queen and king chamber. Different instars of workers checked for their glands’ size in Trinervitermes bettonianus by Oloo in 1981. The sizes were not significantly different among 1, 2, 3 instar workers, whilst dramatically different from 4-7 instars. The author found that trail laying activity of workers increased from zero in lower instars to 50 in higher instar workers. This was important because 5-7 instars are highly available at the foraging site. This research can prove our data that foragers have bigger sternal glands. If the foragers with bigger head widths classified into higher instars it can be concluded that in addition to head width size, age also can play a key role in polyethism in O. formosanus Shiraki.

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