Temporal Polyethism of Different Aged Individuals in the Worker Line of the Lower Termite *Reticulitermes fukienensis*

by

Wong Tai Choi Richard

Thesis submitted in partial fulfilment of the
requirement for the degree of Master of Philosophy

August, 1998
Division of Biology
Graduate School
The Chinese University of Hong Kong

Acknowledgements

I would like to express my thanks to my previous supervisor Dr. Michael W. J. Crosland, who not only offered me countless ideas over the duration of my studies but also trained me to solve problem independently.

I would like to express my sincerest thanks to my acting supervisor Prof. Vincent. E. C. Ooi, my internal examiners Prof. Wilkin W. K. Cheung, and Prof. P. O. Ang Jr, for their kind advice and useful suggestion. My gratitude is also extended to Prof. J. Kenneth Grace of Department of Entomology, the University of Hawaii at Manoa for his critical reading of my thesis and his valuable comments.

I also give thanks to Prof. Samuel S. M. Sun and Prof. H. S. Kwan for their help and support to me so that I can finish my graduate programme.

I am grateful to Prof. P. S. Chan of the Department of Statistics for data analysis, Prof. Johanna P. E. C. Darlington of the Cambridge University for morphological study, Prof. F. S. Huang of Institute of Zoology, Academia Sinica, Prof. D. R. Gao of Nanjing Institute of Termite Control, and Prof. G. X. Li of Guangdong Entomological Institute for termite identification.

I would like to thank all the staff members of the Department of Biology , the Chinese University of Hong Kong for their help during my study. In particular are Ms. Donna Lee, Mr. Edmond C. M. Lok and Mr. Patrick W. W. Leung.

I also highly appreciate to my parents and brother who have given me endless concern, endurance and support during my study.

Abstract

Temporal polyethism refers to the division of labor correlated with age in eusocial insect colonies. The division of labor amongst workers is very important to the success of eusocial insects. However, the topic is relatively less understood in termites. A local lower termite, *Reticulitermes fukienensis*,was studied in order to gain a better understanding of the division of labor of termites.

Individuals in the development line of workers in *Reticulitermes fukienensis* were separated into five age classes based on biometric measurements of their head width. The first and second age classes were identified as small and large larvae classes because of their inability to feed themselves and because of their dependence on the older individuals. The third, fourth, and fifth age classes were the workers having sclerotized mouthparts and body parts and named as small, medium and large worker classes respectively. Based on various morphological measurements, the large worker classes were further separated into six possible sub-age classes.

In order to study all the behavioral patterns of the five age classes of *Reticulitermes fukienensis*, ethograms were constructed. Most task-related behaviors were performed only by the workers. The repertoire sizes of task-related behaviors of the age classes increased with age. An accumulation of the number of behaviors was exhibited by the age classes so that no behaviors were lost with aging. In general, the older the individuals the more active they were. The frequencies of task-related behaviors exhibited by age classes increased with age. Repertoire behavioral size reached a maximum in the fourth instar, but there were significant differences in the frequencies of some task-related behaviors between the medium and large workers. Although there were no significant differences in the frequencies of task-related behaviors among the sub-age classes in large worker classes, frequencies of some task-related behaviors, such as longitudinal vibration behavior and entrance stationary behavior, were found correlated with age.

For the five age classes, most of the behaviors exhibited by the individuals followed a continuous temporal polyethism pattern (i.e. all age classes performed the behavior and the behavioral frequencies correlated with age). Discrete temporal polyethism patterns were only exhibited by large worker and medium worker in some behaviors such as larval carrying behavior and foraging behaviors. Large worker was recognized as the most active task performer in this study.

Unlike the discrete temporal polyethism exhibited by the workers of most eusocial hymenopterans (different age classes performed different sets of tasks as aging), the lower termite *Reticulitermes fukienensis* exhibited continuous temporal polyethism in various behaviors which were similar to those of other *Reticulitermes* species. The difference in the temporal polyethism patterns between termites and social hymenopterans may be due to their developmental difference. It is possible that temporal polyethism exhibited by the isopteran may be influenced by interacting factors such as body size, sclerotization, maturation of the physiological and morphological systems, exocrine changes, gene activities and by learning with aging. Further studies in the determining factors which control the exhibition of temporal polyethism are required to gain a better understanding of termites.

摘要

在完全社會性昆蟲的部落裏，勞動分工會因應各個體年齡而有所改變，這種情況被稱作時間性的行爲多元化。職蟲間的勞動分工對完全社會性昆蟲的成功構成了非常重要的一環。然而，在白蟻方面，這類的題目的認識則較其他社會性昆蟲相對地少。爲求在白蟻的勞動分工方面有深一層的認識，一種本地低等白蟻—福建花崗白蟻被用作爲研究的對象。

福建花崗白蟻的職蟲發育路徑上的不同個體白蟻乃因應其頭寬而被劃分爲五期齡蟲或年齡班別。由於第一及第二期齡蟲不能自飼，並要倚賴其他年長個體白蟻生存，故此，牠們分別被辨認爲小幼蟻和大幼蟻。第三、第四和第五期齡蟲各自擁有硬化及顏色變深後的口器和外骨骼，牠們依次分辨爲小職蟲，中職蟲和大職蟲。另外，基於不同的形態量度，大職蟲又被細分作六個有可能的亞年齡班別。

行爲統計圖表被利用來幫助瞭解福建花崗白蟻五期齡蟲的所有行爲模式。大多數與職務有關係的行爲只由職蟲表現出來。而與職務有關的行爲類別亦隨年紀增長而增加。行爲類別隨年齡增長而累積性地增加，沒有任何行爲會隨年齡增長而失去。通常白蟻的年齡越大，白蟻會更活躍。此外，職務性行爲的頻度亦隨年齡增長而增加。行爲類別在第四期齡蟲時便達至最大數目，但中職蟲與大職蟲彼此在一些與職務有關的行爲的頻度上仍存在著顯著的差別。雖然在大職蟲六個亞年齡班別相互之間已沒有職務性相關行爲的頻度顯著差別，但其中一些行爲的頻度仍隨年齡有著顯著的增減趨向。縱長擺動行爲和入口處靜止行爲便是箇中例子。

以五期年齡班別而言，大部分的行爲都是以連續的時間性行爲分工〔全部的年齡班別皆表現某行爲，而該行爲頻度與年齡相關〕被白蟻展示。行爲分工模式只發生在幼蟲提攜行爲及取食行爲，並由中職蟲和大職蟲擔任。在這研究裏，大職蟲被認定爲最活躍的職務表現者。

低等白蟻福建花崗白蟻展示了一種連續的時間性分工模式，這與其他鼻白蟻所展示的相似，但與其他屬於膜翅目的完全社會性昆蟲所展示的間斷的時間性分工模式有別。這樣的不一致可能由於牠們〔白蟻和社會性膜翅目昆蟲〕的發育不同所致。等翅目昆蟲所展示的時間性分工可能受著多種互爲因素隨年齡增長的影響所致。這些因素包括體型、硬化作用、生理系統和形態系統的成長、外分泌的轉變、基因活動性及學習等等。爲求對白蟻有進一步的瞭解，掌管時間性分工的決定性因素的研究是極爲需要的。

List of Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

TABLE OF CONTENTS

Chapter I
GENERAL INTRODUCTION

1.1 Introduction for the whole thesis

This thesis is an attempt to provide more information on the division of labor in termites. It is composed of six chapters in which the division of labor among different age classes in the worker line of the termite species *Reticulitermes fukienensis* Light was studied and discussed. In the first chapter, background knowledge of termites was introduced and the identification of the sample species *R. fukienensis* was discussed. Temporal polyethism and its importance was defined with a brief review of literature on polyethism of eusocial insects (termites, social wasps, ants and social bees). A comparison between eusocial hymenopterans and isopterans in development was given.

Chapter 2 describes the experiment for the separation of the individuals in the worker line of the sample species into five age classes. Another experiment has been performed for the identification of larvae (which cannot feed themselves) and workers (which can feed other individuals) among the age classes.

Chapter 3 is about the experiment to observe all the behaviors of the age classes. Results were displayed in the form of ethograms so that different behavioral patterns can be easily investigated.

In chapter 4, two experiments are descrobed. The aim of the first experiment was to determine the feeding and larval behaviors of medium worker and large worker.

The aim of the second experiment was to determine the feeding behaviors of the worker age classes.

In chapter 5, all the behaviors of marked large workers were observed in an experiment. Then the marked individuals were separated into six possible sub-age classes based on various biometric measurements. Next, the behaviors of these six sub-age classes were investigated. General discussion and conclusion for all the experimental results can be found in chapter 6.

1.2 Termites

1.2.1 Termites are eusocial insects

All termites are eusocial insects (Wilson, 1971). Eusocial insects can be regarded as a group of insects showing three distinctive biological features (Wilson, 1971; Oster and Wilson, 1978; Elzinga, 1997) as follows:

1. Members of the same species use the same composite nest.
2. Division of labors exists within the colony, and reproduction is not carried out by all individuals. Individuals may co-operate in caring for the offspring of the colony breeders.

3. The parental generation and one or more descendent generations co-exist at the same time as an integrated unit or colony (Wilson, 1971; Oster and Wilson, 1978; Elzinga, 1997).

Eusocial insects often demonstrate behaviors such as group foraging, group defense, pheromone diversity and associated communicative behavior, inquilinism (i.e. sharing a nest by symbionts and social insects) and other symbiotic associations, and caste differentiation (Hermann, 1979). Eusocial insects exist in the order Isoptera (termites) and some groups of Hymenoptera including the ants (e.g. Formicoidea), the social bees (e.g. *Apis*, *Bombus* and *Melipona*) and the social wasps (Vespidae) (review by Elzinga, 1997).

1.2.2 Families of termites

Termites made up the order Isoptera. There are more than 2000 species of termites (Pearce and Warte, 1994; Elzinga, 1997). Termites may be found in the regions between the 47° north and south latitudes of the world but there are abundant in tropical areas (Elzinga, 1997). Many authors such as Wilson (1971), Huang *et al.* (1989), and Bagine (1992) accept the classification by Snyder (1949), and Emerson (1965) that termites can be divided into six families: Mastotermitidae, Kalotermitidae, Hodotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae.

The first five families are referred to as the 'lower' termites and can be distinguished from the other one family in possessing symbiotic intestinal flagellate protozoans (Noirot, 1969a). Termitidae is the 'higher' termite. Termites of this family can digest cellulose with cellulase secreted by the termites themselves or with the use of anaerobic bacteria for internal digestion (Bignell *et al.*, 1979, 1980; Noirot, 1992).

1.2.3 Classification of sample termite: *Reticulitermes fukienensis*

The animal used for study in this thesis was the lower termite *Reticulitermes fukienensis* Light. *Reticulitermes fukienensis* Light (Gao and Lam, 1986) was classified as follows:

Order Isoptera
Family Rhinotermitidae
Genus *Reticulitermes*
Species *fukienensis* Light

1.2.4 Distribution of *Reticulitermes*

Reticulitermes is a temperate genus (Roonwal, 1970). They can be found in southern Europe, southern North America, and in southern Asia including China, Korea and Japan (Roonwal, 1970; Harris, 1971). In China they are widely distributed in Guangdong, Guangxi, Guizhou, Hunan, Fujian Provinces and Hong Kong (Huang *et al.*, 1989). At present over twenty species of the genus *Reticulitermes* have been recorded from the temperate region of the northern hemisphere (Huang *et al.*, 1989). Specimens of soldiers and workers of *R. fukienensis* were collected in buildings in Hong Kong by J. D. Romer in 1955. This species was indentified by Light from Foochow in Fujian Province in China in 1924 (cited in Harris, 1971).

1.2.5 Nest of *Reticulitermes*

Most *Reticulitermes* nests are found in separated pieces of damp wood associated with soil (Gao *et al.*, 1992). They are often buried. A colony may simultaneously occupy a large number of pieces of wood and can colonize new ones or abandon others depending on the situation. *Reticulitermes* can build tunnel through the soil, construct galleries within wood and build surface shelter tubes from soil. Thus the brood is scattered into several centers which are interconnected by a network of galleries. This dispersion is further accentuated by the presence of many replacement reproductives which are the rule in this genus (Gao *et al.*, 1992):

1.2.6 Economic Importance of *Reticulitermes*

Termites are important pests causing serious economic losses by damaging houses, trees, crops, river banks, dams and underground cables. It is estimated that the damage to electric cable by termites comprised 60-70% of the electrical faults in southern China (Gao *et al.*, 1992).

Reticulitermes together with *Coptotermes*, *Odontotermes* and *Macrotermes* are found to be the wood destructors in Hong Kong (Gao *et al.*, 1992). *R. speratus* is an important pest of Indian corn *Zea mays* L. and of the wheat *Triticum aestivum* L. (review by Gao *et al.*, 1992). Another species *R. flavipes* has been known to cause damage to Indian corn *Zea mays* L. as well (review by Gao *et al.*, 1992). *Reticulitermes* can infest London plane tree and Chinese fir (Gao *et al.*, 1985). It also cause damage to buildings in northern China and regions along Yangtze River and is responsible for the damage of underground cables in China. In 1984, it was estimated that a total area of 2.2m x 10^7 m^2 had been affected by termites, causing an estimated loss of over US \$ 0.4 billion in China (review by Gao *et al.*, 1992). The species *R. flavipes* and *R. hesperus* are also the two most common pest species in the United States (Elzinga, 1997).

1.3 Definition of temporal polyethism

Polyethism is a phenomenon of job allocation or the division of labor in social insect colonies (McMahan, 1979). It may be based on morphological character, sex or age of individuals, or a combination of these factors (Wilson, 1971; McMahan, 1977, 1979). Temporal polyethism is a phenomenon in which individuals in social insect colonies tend to change their tasks with age (Hölldobler and Wilson, 1990). The first record of temporal polyethism in eusocial insects (the honeybee workers) was documented by Charles Butler in 1609 (cited in Wilson, 1971). It is the frequently observed correlation between age and task in social insects that led to the view that aging provides the mechanism underlying the division of labor (Bourke and Franks, 1995).

Wilson (1976b) referred to the term temporal polyethism to include all behaviors among different age classes in the same colony (most in eusocial hymenopterans). He introduced two possible extreme alternatives of temporal polyethism: continuous temporal polyethism and discrete polyethism (Figure 1.1). In the continuous temporal polyethism, although behavior changes with age, there are no clear stages. All age classes overlap broadly in the behaviors they perform. In discrete temporal polyethism, different age classes perform different behaviors which do not overlap.

Some authors (e.g. McMahan, 1979) who study temporal polyethism considered only one or two behaviors of eusocial insects (most in termites). These authors use continuous temporal polyethism to describe all age classes performing a particular behavior. Discrete temporal polyethism is used to describe some age classes performing a particular behavior.

In this thesis, continuous temporal polyethism refers to a change of the division of labor in a particular behavior with age among the age classes so that the frequencies of this behavior exhibit by all of these age classes change in either increasing or decreasing order. Discrete temporal polyethism refers to a change of the division of labor in a particular behavior with age among the age classes, but not all age classes exhibit this behavior.

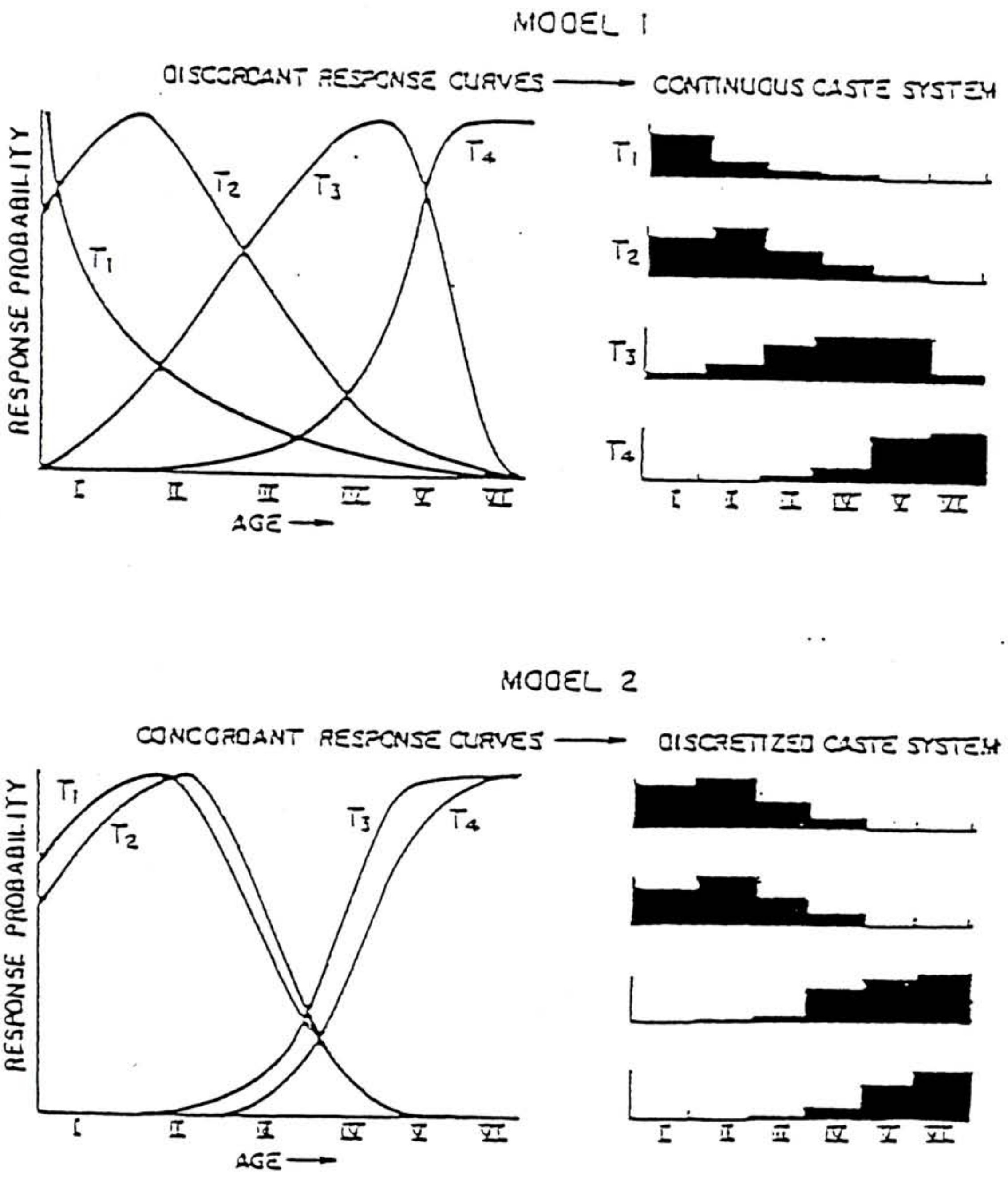


Figure 1.1 Two schemas represent the extreme alternativess open to an ant species in the evolution of temporal classes.
In the first model the four tasks are performed out of phase by different age classes. In the second model two pairs of tasks are performed in phase by different age classes. The first model defined continuous temporal polyethism. The second model defined discrete temporal polyethism. (Modified from Wilson, 1976b)

1.4 The general significance of polyethism

Polyethism or division of labor is fundamental to the organization of insect societies and is thought to be one of the principal factors in their ecological success (Wilson, 1985). Division of labor within the worker caste is found in nearly all social insects (Oster and Wilson, 1978; Robinson, 1992) and the tasks performed by eusocial hymenopteran workers are usually correlated with age (Beshers and Traniello, 1995). Oster and Wilson (1978) explain that it is more efficient for a colony to accomplish tasks concurrently with ensembles of specialized individuals than to perform tasks sequentially with undifferentiated members: castes marked by size or other physical differences work efficiently by doing tasks whose requirements match their physical abilities. Through the division of labor, behavioral repertoire of the individuals is simplified and mistakes are minimized, synergistic (i.e. working together) effects are promoted, and the ergonomic (i.e. biotechnological) efficiency of the colony is greatly increased and colony integrity is maintained (Oster and Wilson, 1978; Wilson 1985).

1.5 Developmental differences between isopteran and other social hymenopterans

The fundamental difference between termites and other social hymenopterans (ants, bees and wasps) is in their development (Howse, 1970). Termites are bisexual and hemimetabolous (i.e. designating development that produces gradual changes in form during growth from immature to adulthood). They have direct development: individuals hatch from eggs, molt at intervals and increase their body size gradually and lack a pupal stage. For the reproductives, wing-pads are acquired and become larger at each molt. The development paths for each caste (workers, soldiers, reproductives), and for each sex (male and female) in various species (e.g. *Macrotermes bellicosus, Anacanthotermes ahngerianus, Hodotermes mossambicus*, etc.) are quite complicated and divergent (review by Noirot, 1990).

The development of social hymenopteran is indirect. It undergoes complete metamorphosis (i.e. change in shape from one stage to another in the life cycle). The eggs hatch into larvae which are very unlike the adult. After a series of molts, the larvae transform into pupae from which the adults emerge. The developmental pathway for the basic castes (queen and workers) for different species are quite simple. In hymenoptera the males are haploid and the females are diploid. Elzinga (1997) mentions that hymenopteran societies have the following characteristics that termites lack:

1. Colonies are predominantly female with most tasks being carried out by the adults. Males are present for reproduction only in a brief reproductive period.

2. Castes consist of only the reproductives and the workers. Reproduction is performed by the queen while the task of giving food to the larvae is done by the workers.

3. Colony can be started by a single fertile female, by multiple queens, or by colony division. The males do not participate in the colony formation.

The society of termites is virtually composed of several immature stages of individuals whereas in social hymenopterans only one stage of adults makes up the functional elements of the society (Howse, 1970; Wilson, 1971).

1.6 Polyethism of termites and social hymenopterans

The areas in termite behavior that have been studied mainly focused on the dispersal, pairing, nest formation and construction, and regulation of castes. Most observations were mainly performed in the field (e.g. Sands, 1965; Howse, 1968; McMahan, 1970, 1977; Watson and McMahan, 1978; Badertscher *et al.*, 1983; Gerber *et al.*, 1988; Shellman-Reeve, 1990). As there are very few studies of polyethism in termites in the past (discussed in section **1.7**), only a few examples of these studies are introduced below. Most of these studies were specialized only in a few behaviors across different instars. Attemps were made to try to synthesize general principles applicable for each behavior of termites. For the social hymenoptera, polyethism for the individuals of different species is very similar. Behavioral changes of the adult in some typical species are also reviewed below. Therefore the difference in polyethism between isopterans (termites) and the social hymenopterans can be compared and discussed.

1.6.1 Caste-based polyethism

In eusocial insect colonies, castes can be identified by both their morphological characters and their specialized functions. Oster and Wilson (1978) define castes as a set of colony members, smaller than the total colony population, that specialize in particular tasks for prolonged periods of time. They distinguish two types of castes: physical castes with distinct anatomical traits and behavior, and the temporal castes with respect to behavior and age. For convenience, 'castes' referred to in this literature review are the physical castes. The physical castes of termites, ants, social wasps and social bees are introduced but those of termites are introduced in more detail to give more information for the study of temporal polyethism in *R. fukienensis*.

A. Termite

In termite colonies, the three principal castes are reproductives, workers and soldiers. Individuals in the reproductive caste are highly sclerotized. They are capable of flying and have fully developed reproductive systems. Together with the soldier caste, they are developmentally terminal (i.e. no further molting occurs) (Brian, 1979). They carry out reproduction, distribution of the species by swarming or colonizing flights, establishment of galleries, feeding and care of the first young in a new colony. After the dispersal flight, the female and male reproductives lose their wings and form pairs and dig a hole in the soil or in wood to establish a new colony (Wilson, 1971).

The male termite reproductives play a role which is quite different from that of the social hymenopteran. In hymenopteran, the male will die following the nuptial flight and the female is responsible for the establishment and construction of a new nest and for rearing its first brood. The male termite reproductives remain with the female, periodically inseminating her for the duration of his life (Nutting, 1970; Wilson, 1971).

Most termites have one pair of king and queen carrying out colony reproduction for 10 to 20 years or more (Elzinga, 1997). Reproductive replacement may occur if one or both of the reproductive pair die or if the sizes of the colony become large and dispersed. Such supplementary or replacement reproductives may be adultoids (i.e. they are derived from the adults remaining in the nest), or nymphoid (i.e. they are derived from the nymphs with wing pads), or ergatoids (i.e. they are from the workers) (Wilson, 1971; Elzinga, 1997).

Workers and pseudergates (i.e. the last, immature instar worker without wings in lower termites that are capable of molting into any caste), contribute to the majority of the population of the colony (Brian, 1979). Unlike the larvae which are pale and have soft mouthparts and are unable to feed themselves, workers have strong and large jaws and are responsible for taking care of the eggs and young, foraging for food, feeding and for cleaning the other individuals, nest construction and repairs. (Stuart, 1969).

In some termite species, there is no further molting after the molting of workers. But in other species, individuals in the worker line may go through a series of molts. The molting may be accompanied by an increase in pigmentation and a slight growth of appendages (McMahan, 1979).

In higher termites, sex is often associated with the difference in size. In some species there are two types of worker: major (large) and minor (small) although there is no general rule about which sex is the bigger (Brian, 1979). For example, *Macrotermes bellicosus* has large female workers and small male workers (Gerber *et al.*, 1988), while *Odontotermes distans* Holmgren and Holmgren has small female workers and large male workers (McMahan *et al.*, 1984).

Termite soldiers have powerful mandibles which are structurally modified for defense against predators. They depend on workers for food and are sterile (Brian, 1979; Stuart, 1969). In some subfamilies such as Nasutitermitinae, termite soldiers have enlarged frontal glands in the head that open into a rostrum through which toxic chemical and repellent can be ejected for defense (Noirot, 1969a).

In the families of lower termites, soldiers of both sexes are usually physically similar. The sex of soldiers differs in various termite genera of higher termites (Noirot, 1969b). In some species, all soldiers are of a single sex. For example, soldiers of *Microtermes*, *Cephalotermes* and *Globitermes* are almost always females. However, the soldiers of *Syntermes* and *Nasutitermes* are usually males. The young and immature individuals or larvae may develop into soldiers, workers or winged alates (Gerber *et al.*, 1988; McMahan, 1979).

B. Ant

Wilson (1971) emphasizes that three basic female castes are found in the ants: worker, soldier and reproductive. Typically, ant queens are larger than workers. The reproductive caste is a fully developed reproductive female with functional but deciduous wings which are shed after mating, a fully developed thoracic skeleton, and large ovaries with a receptacle for sperm storage. The reproductive male has a generalized hymenopterous thorax but fully developed, nondeciduous wings. The worker caste is composed of sterile females having reduced ovarioles and a greatly simplified thorax, the ovaries are smaller or absent in some species. They perform functions like foraging, building, defending the nest, attending the queen, or nursing the brood. Soldier castes are females possessing a disproportionately large head (this may be the main difference from the workers). They have adapted to one of the three basic defensive techniques (Wilson, 1971):

1. large mandible in massive heads for shearing;
2. pointed and sickle or hooked shaped mandibles for piercing in order to defend the colony;
3. shield shaped or plug shaped head for blocking nest entrances .

It must be mentioned that the classification of the physical castes of ants are complicated depending on the authors. Elzinga (1997) defined only two castes: the queen and worker of which the soldier is recognized as a major worker. *Pheidole dentata* was classified by Wilson (1976b) into five discrete female castes: the queen, one major worker (which has massive head filled mostly with the adductor muscles of the mandibles for colony defense, and it may resemble soldier castes). Three temporal subcastes of the minor worker (these may be grouped into the worker caste if the temporal behavioral change within this caste is ignored); and only one male caste based on their physical differences and their functions (Wilson, 1976b).

Calabi *et al.* (1983) found that except for trophallaxis, there were no overlaps in task performance between the major and minor workers in the ant *Pheidole hortensis.* The minor workers performed brood care, food acquisition, and allogrooming while the major workers performed defensive tasks.

C. Social bee and social wasp

The castes of honeybees consist of the fertile diploid female (queen), the stunted diploid females (workers), and haploid males (drones). The queens are larger than the workers and possess proportionately larger abdomens of which 300 or more elongate ovarioles are packed. They are highly specialized for reproduction and possess reduced mouthparts, smaller eyes, shorter antennae, and a smaller brain than the workers. Unlike the workers, the queens lack pollen-collecting hairs. Their hypopharyngeal and wax glands, which in the workers are the principal source of larval food and building material, are underdeveloped but they can use pheromones secreted from the mandibular gland to control the worker behavior. Compared with the queen, the drone is similar in size but thicker (not slender). It only mates with the queen for reproduction and does not perform nest works. The workers perform all the other functions, including foraging, defending the nest, attending the queen and brood care (Wilson, 1971).

In primitive wasps, it is quite difficult to distinguish the queen from the sister workers (Wilson, 1971). In the more advanced social wasps, castes become distinct whereas the queen is considerably larger than the workers (Wilson, 1971; Elzinga, 1997). The most extreme example of queen-worker dimorphism is found in the wasp *Steloppolybia flavipennis* from Brazil (Wilson, 1971). A large size difference is accompanied by an allometric (i.e. describing a relative proportional change) thickening of the body of this species.

1.6.2 Sex-based polyethism

One of the differences between termites and the social hymenopterans is that both sexes of termites are socialized (i.e. living and making fit for life in companionship with others in the same colony) (Wilson, 1971), while only the females of eusocial hymenopterans originate the colonies (mentioned in section **1.5**). Thus, the workers and soldiers in eusocial hymenopterans are females and nearly all the tasks are performed by the females (the males become present in a reproductive period, mentioned in section **1.5**). As mentioned before (section **1.6.1**), the sex of soldiers differs in different termite genera (Noirot, 1969b). In *Nasutitermes exitiosus*, the small (male) soldier is aggressive but the large (female) soldiers in *N. exitiosus* are not (McMahan, 1974).

A. Termite

i) Foraging behavior

Foraging behavior is normally exhibited in higher frequencies by the sex that is larger than that which is of smaller size.

Pasteels (1965) found that although the species *Nasutitermes lujae* has three stages of large (female) workers and two stages of small (male) workers, only the third stage large workers, having most highly developed sternal glands among the other worker stages, can lay a foraging trail and are the ones most frequently present outside the nest. In the fungus-growing *Macrotermes michaelseni, M. subhyalinus* and *M. bellicosus*, foraging behavior was observed only in major (male) workers (Sieber and Leuthold, 1981, Badertscher *et al.*, 1983). McMahan *et al.* (1984) found that over 99% of the foraging population of *Odontotermes distans* Holmgren and Holmgren are made up of large (male) workers.

In *M. bellicosus*, after the accidental discovery of a food source by a major (male) worker, the proportion of major workers that left the nest for the new food source reaches more than 75% of all workers, while there is no corresponding increase in the frequency of excursion observed in minor (female) workers (Lys and Leuthold, 1991).

ii) Nest-construction behavior

Lys and Leuthold (1991) found that in *Macrotermes bellicosus* during the exploration phase for food, construction of the network of subterranean galleries from the nest is almost exclusively participated by minor (female) workers. This is further confirmed by Gerber *et al.*, (1988) that minor (female) workers of *M. bellicosus* made up 97% of the construction population.

iii) Feeding behavior

Shellman-Reeve (1990) found that only in the pre-egg stage of the nest-founding period of *Zootermopsis nevadensis*, males give females significantly more proctodeally-produced pellets (food derived from the hindgut intestine) than vice versa, whereas females feed on pellets significantly more often than males do.

B. Social hymenopteran

In ants (all are eusocial insects) the three basic castes are females while males may be recognized only during the reproductive period (Wilson, 1971; Elzinga, 1997). The female castes basically perform all functions, i.e. reproduction (the queen caste), nest construction, brood care and foraging, etc., (the worker caste) and nest defense (the soldier caste). In social bees and social wasps, there are basically two castes: the queen caste for reproduction and the worker castes for brood care, queen attending, nest building, entrance guarding and foraging (Wilson, 1971). Sex-based polyethism cannot be applied to hymenopteran colonies because only the females originate the colonies, and division of labor exists only among the females (Wilson, 1971; Elzinga, 1997).

1.6.3 Age-based or temporal polyethism

Division of labor within the worker castes is found in nearly all social insects and is a central feature of colony organization (Beshers and Traniello, 1995). Age polyethism is nearly universal in the social Hymenoptera and appears to follow a consistent general pattern (Hölldobler and Wilson, 1990; Robinson, 1992). Eusocial hymenopteran workers progress from inside to outside tasks as they age, and there is a sharp discretization of the forager groups (Wilson, 1976b). For the reason that the termites are hemimetabolous, age-based polyethism can be studied among different instars respective to the difference in their body size. For the social hymenopterans, age determination is based on the body pigmentation (e.g. Wilson, 1976b) or directly observing the individual of the adult just after molting from the pupa.

The general principle for termites is that the older the age of the individuals of termites, the higher the frequencies exhibited in the behaviors or the larger the repertoire size of the behaviors (i.e. number of kinds) performed. For the social hymenopterans, the general principle is that task shift or behavioral discretization occurs with the change of the individuals' ages. Following is a review about the age-based polyethism in various behavior and evidence of these general principles. For the reason that this thesis is concerned with the study of temporal polyethism of the workers in termites, evidence for temporal polyethism of termites is displayed more than those of the social hymenopterans here. This enables us to gain a better understanding of termites.

A. Termite

i) Foraging behavior

In general, the frequencies of the foraging behavior are correlated with the age of the individuals. In some species, foraging behavior is only exhibited in the latest stage of the workers. The following are some examples of this general principle.

Age-related behaviors are found to be correlated with changes in glandular size and their activities (McMahan, 1979). Pasteels (1965) found that although the species *Nasutitermes lujae* has three stages of large (female) workers, only the oldest stage (third stage) have well developed sternal glands for laying a foraging trail outside the nest. Pasteels (1965) also found that the development of the sternal gland is correlated with age.

Sands (1965) found that workers in incipient (i.e. in the early stage) colonies of *Trinerviterm ebenerianus* tunnel out to forage after 3 weeks of nest tasks. Sand (1965) hypothesized that the foragers must build up a critical mass before their first time of foraging. The timing of the first foraging is not altered by any artificial reduction in the workers numbers of the colony.

Watson and McMahan (1978) discovered that over 75% of the Australian harvest termite, *Drepanotermes perniger,* found in the foraging parties were the fourth and fifth (the latest) stages of workers, and this percentage is much higher than those present in nest. The frequencies of younger instars decrease sharply with age, and virtually no first stage of workers join in the foraging group above the ground. The first, second and third stages of workers can be found in the galleries under the foraging exit holes, but the frequencies of them are much lower than those found in the central part of the underground nest.

The major worker and minor worker in *Macrotermes subhyalinus* are all of one stages. Badertscher *et al.* (1983) discovered that 51 % of these major and minor workers on foraging trails were 41-55 days old while only 0.5% were under 26 days. He explained the small proportion of the foragers over 55 days old was due to natural mortality.

McMahan *et al.* (1983) found that young major and minor workers of *Nasutitermes dunensis* are more common in establishing foraging areas and that older workers and soldiers are more common in risky, initial foraging sites. Gerber *et al.* (1988) confirmed the major workers in *Macrotermes bellicosus* made up 70% of the foraging populations all of which are old workers.

ii) Nest and gallery construction behaviors

Similar to foraging behavior, nest and gallery construction are mainly performed by the later instars, thus the older of the individuals, the higher the frequencies of these behavior performed by these individuals.

Howse *et al.* (1968) found that the primitive termite *Zootermopsis nevadensis* has no worker caste but the nymphs and larvae with six stages undertake the activities of the worker caste. The larvae, as functional workers progress from the young to the older stages, generally have a tendency to spend more time for building with increasing age. The first nymph and the sixth instar larvae take up highest amount of nest building.

Noirot (1969b) stated that there was only one stage of worker in *Bellicositermes natalensis*. The older workers can be identified by their whitish appearance because of the accumulation of urates in the fat bodies. He pointed out that most of the construction behavior are exclusively performed by the older workers with whitish appearance.

There are two stages of small workers and three stages of large workers in *Nasutitermes costalis* (McMahan, 1970; Jones, 1980). McMahan (1970) found that the first stage small workers in *Nasutitermes costalis* constituting about 24% of all workers in a nest, composed over 46% of total population collected at the construction site. It was considered that the larger the population size in the related site, the higher the contribution for the nest construction over there.

Jones (1980) discovered that the first stage small worker and the older (third) stage large worker of *Nasutitermes costalis* were vigorous builders and there was moderate abundance at a gallery repair site. They contributed overall to the construction than any other worker castes. The second stage of small worker were the most active builders.

iii) Nest repair behavior

The nest repair behavior is generally performed by the older instars or in some case it was exclusively performed by the oldest instars of the workers.

McMahan (1970) found that in *Nasutitermes costalis* nest repairs appear to be initiated almost exclusively by the oldest stage (third stage) workers, while the oldest stage (second stage) small workers are the next most active in making immediate repair.The younger workers of both worker lines are rare at disturbed surface breaches.

McMahan (1977) found that in *Nasutitermes exitiosus* most of the immediate repairs of the mound are done by the oldest instar (fifth stage) of the large worker line, whereas the forth stage large workers participated minimally, younger instars of both large and small workers lines take no obvious part.

Watson and McMahan (1978) found that in *Drepanotermes rubriceps* repair to the damaged mound is carried out primarily by the older instars, principally the fourth and fifth instars. They increase their numbers at the damaged mound very rapidly and stay there for more than 24 hours. It was found that the earlier the instar is, the greater the delay in joining nest repair.

iv) Oscillatory moving behavior

Howse *et al.* (1968) found that the larvae in *Zootermopsis nevadensis,* as functional workers progress from the young to the older stages, generally tend to spend more time to take on a greater share of activities with increasing age for oscillatory movements.

v) Trophallaxis behavior

Howse *et al.* (1968) also found that the older termites in *Zootermopsis nevadensis* spent less time in trophallaxis than the younger termites.

B. Social Hymenopteran

Temporal polyethism in honey bees (*Apis mellifera*) involves a correlation between age and task (e.g. Free 1965; Wilson, 1971; Robinson, 1992) and has also been shown to occur in a large number of ants (e.g. Otto, 1958). The young workers remain in the central part of the nest and are close to the queen and the brood and perform the nursing tasks. Slightly older workers also remain in the nest but live closer to the entrance and act in nest maintenance such as clearing debris, building and repairing the nest. The oldest workers are responsible for foraging outside the nest. The frequently observed correlation between age and task in social insects gives evidence that aging provides the mechanism underlying the division of labor (Bourke and Franks, 1995). This kind of polyethism can also be called centrifugal polyethism. Centrifugal polyethism describes the tendency of social insect workers to start their employment at the center of the nest, and later to perform each successive task closer to the periphery of the colony's activities (Wilson, 1985).

a. Ant

For example, Wilson (1976b) classified the minor workers of ant *Pheidole dentata* into six groups based on their body color and body pigmentation correlated to their age. He found that there are three age classes that can be defined based on their behavioral performance. The first age class of minor workers are below 9 days of age after eclosion. They mostly attend mother queen, groom, roll and carry eggs and microlarvae; assist eclosion of the adults from pupa and groom the pupa. The second age class below 16 days old mostly roll and carry pupae and mature larvae, groom and exchang oral liquid with mature larvae. The third age class, which are over 16 days old, mostly carry out guard nest entrance, guard food site, excavate nest, forage and defend nest behaviors (Figure 1.2).

Lenoir (1979) found out that 35% of young ant workers (less than one month old) in *Tapinoma erraticum* performed 20 to 80% of observations in foraging while 65% of them performed this behavior less than 20% of observation. 60% of the old workers (more than one month old) performed 20 to 80% of observations in foraging while 30% of the old workers (older than intermittent ones) performed more than 80% observations in foraging. He found that 65% of the young performed as nurses for giving food to the queen or the larvae. Only 10% of the old workers performed this behavior.

b. Social wasp

Jeanne *et al.* (1988) studied the social wasp, *Polybia occidentalis* (Figure 1.3). Workers switch from nest work (building, brood care, nest maintenance and defense) to foraging at a mean age of 19.6 days in one colony and 25.7 days in second colony. Simões (1977) studied worker age polyethism in both *Protopolybia exigua* and *Agelaia pallipes* and suggested that the workers become older, they move progressively to tasks less and less directly related to brood care. Workers aged 6-10 days spend most of their time in the comb, whereas workers aged 11-15 days move to the walls and floor of the nest, and workers which are older than 16 days start foraging. For the workers in social wasp *Metapolybia* sp., workers aged 6-10 days begin the nest construction and brood care task, but begin to forage after 10-15 days (Jeanne, 1991).

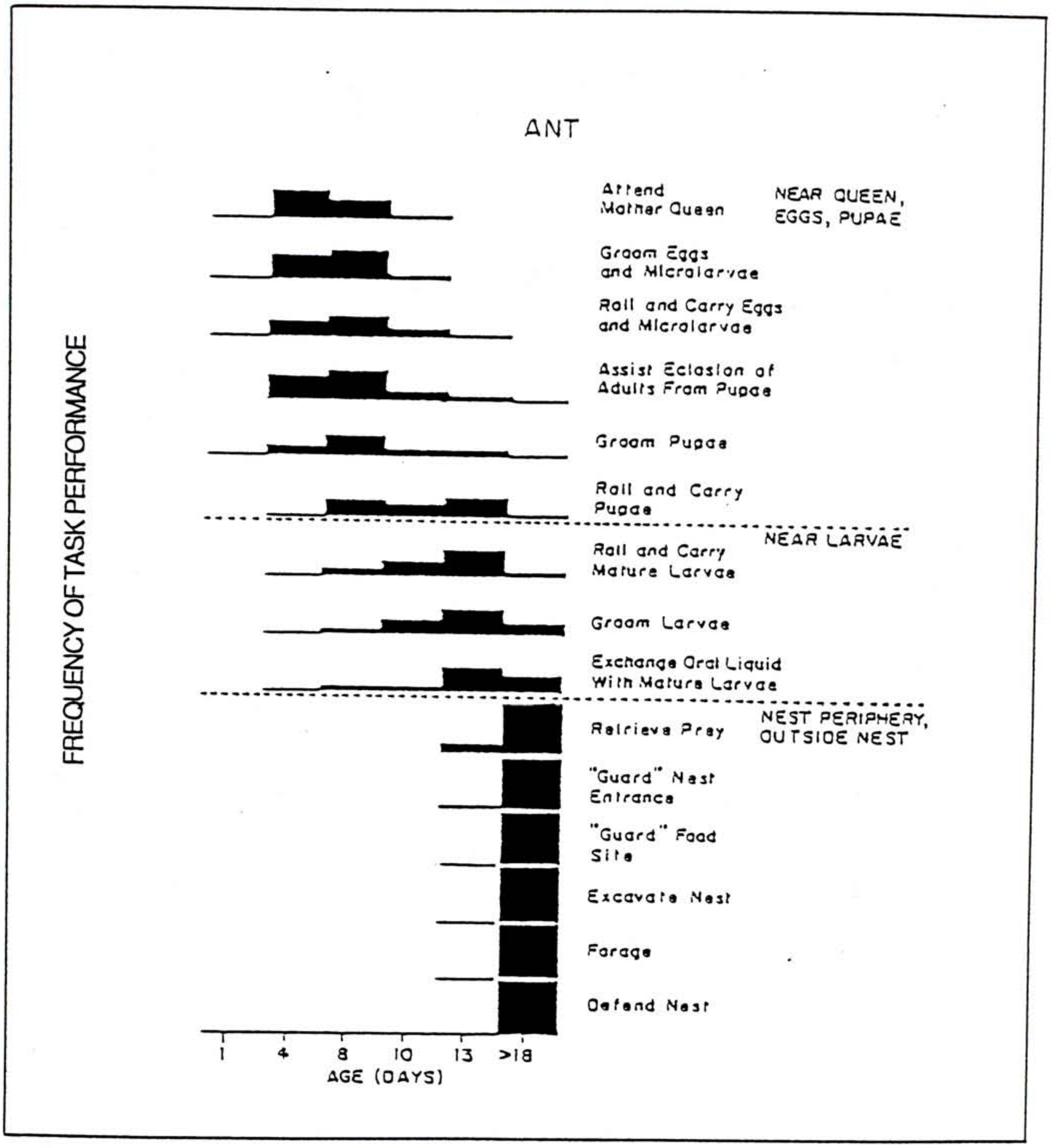


Figure 1.2 Temporal polyethism among workers of ant *Pheidole dentata*. The workers change their task and shift their activities from the queen, egg , to larvae , to nest periphery as they age. (From Wilson, 1976b)

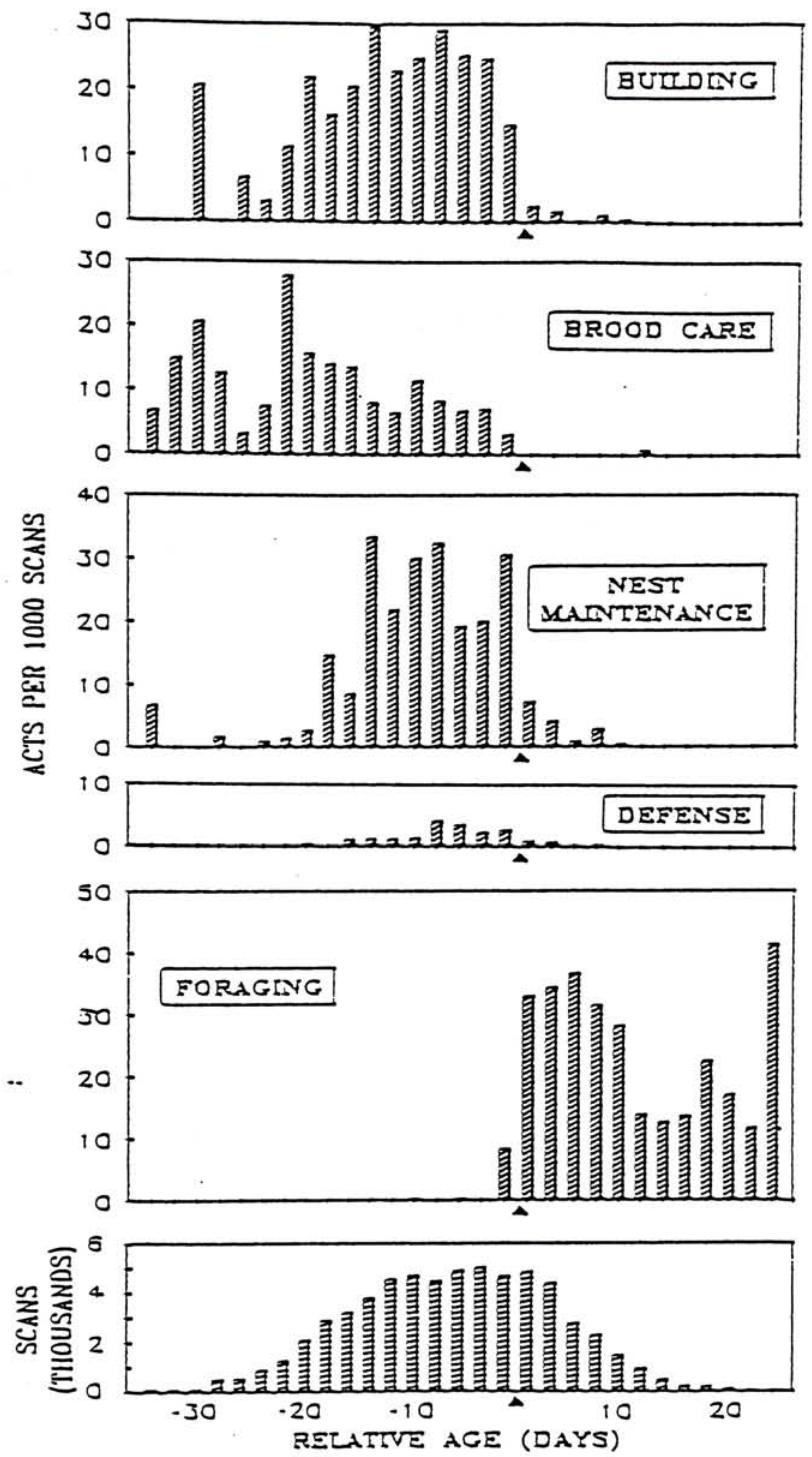


Figure 1.3 Temporal polyethism among workers of *Polybia occidentalis*. Frequency distribution of on-nest tasks (building, bood care, nest maintenance and defense) and foraging for each individuals in the sample is plotted so that its age of switching from on-nest tasks to foraging is centered on the same point (arrow) on the x-axis. The data are based on 38 individuals scanned at five-minute intervals. (From Jeanne *et al.*, 1988 and review by Jeanne, 1991.)

c. Social bee

Temporal polyethism of the workers in honeybee *Apis mellifera* (Figure 1.4) is described as a 'discretized' caste system, since the shifts in the various 'job-performance' probabilities are fairly sharp (review by Nowogrodzki, 1984). In respect to the spatial segregation of castes within the nest, honeybees resemble the ant species *Pheidole dentata* (Nowogrodzki, 1984).

1.6.4 Conclusion on the polyethism of termites and eusocial hymenopterans

Investigations of division of labor or job allocation according to termites, eusocial bees, eusocial wasps and ants based on sex, caste or age of the individuals are summarized in sections **1.6.1**, **1.6.2** and **1.6.3**. In castes based polyethism, three basic castes of both sexes can be found in termites while in the social hymenopterans, only two female castes (if the soldiers are classified as major workers) originate the colony.

Sex-based polyethism in various castes are mostly exhibited in higher termites such as *Nasutitermes exitiosus* (McMahan, 1974, 1977), *N. lujae* (Pasteel, 1965), *N. costalis* (Jones, 1980), *Macrotermes subhyalinus* (McMahan, 1970, 1977; Badertscher *et al.*, 1983), *M. bellicosus* (Sieber and Leuthold, 1981; Gerber *et al.*, 1988; Lys and Leuthold, 1991), *Odontermes distans* (McMahan *et al.*, 1984), *Trinerviterms ebenerianus* (Sand, 1965), *Drepanotermes perinigner* and *D. rubriceps* (Watson and

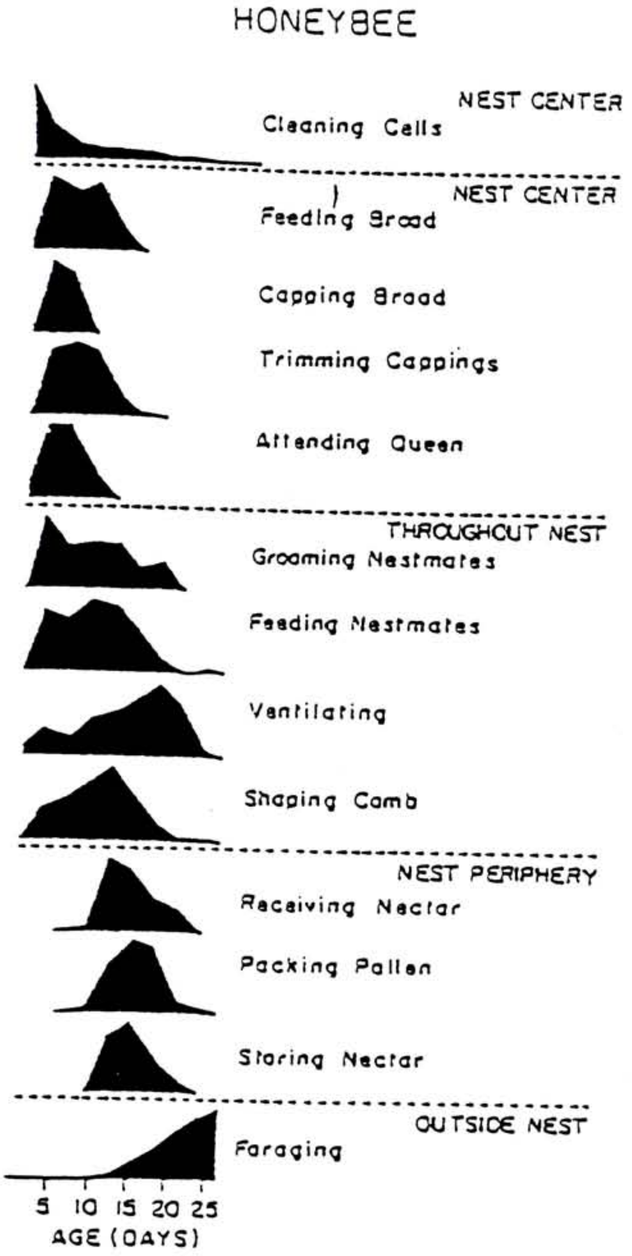


Figure 1.4 Temporal polyethism among workers of honey bee *Apis mellifera*.
The workers shift their tasks to another as aging and move their avtivities outward from the nest center. (From Seeley, 1982)

McMahan, 1978). For example, tasks are shared between the large and small workers in *M. bellicosus* during food acquisition (Lys and Leuthold, 1991). The last stage of the large workers in *N. lujae* are exclusive for foraging trail laying (Pasteels, 1965). In general, the major workers perform the foraging behavior while the minor workers perform nest construction behavior. In eusocial hymenopterans, only female castes perform all the nest tasks whereas the male are only present during the reproduction period.

Most of the age based polyethism studies concern about the initiation of foraging trails, the foraging behavior, nest repair and construction (Pasteels, 1965; Howse, 1968; Noirot, 1969b; McMahan, 1977; Watson and McMahan, 1978; Jones, 1980; Baderscher *et al.*, 1983; Gerber *et al.*, 1988 etc.). The older workers in termites mainly participate in extranidal activities such as exploration by foraging trail laying (Pasteels, 1965), foraging (Watson and McMahan, 1978), nest construction (Noirot, 1969b; Jones, 1980) and mold repair (Watson and McMahan, 1978) while the young workers mostly stay in the nest. The involvement of older individuals in activities outside the nest is a basic rule in social insects (Oster and Wilson, 1978). The involvement of young workers in nest tasks within the nest such as brood care, queen attending, nest construction etc., and they shift to the outside of the nest and perform foraging as they age is a basic rule in social hymenopterans (Oster and Wilson, 1978).

Although the development pathways of various termite species are widely different from those of the hymenopterans, some studies of age based polyethism of termites show similarities that are demonstrated by social hymenopterans:

1. workers tend to change the frequencies of their work or tasks correlated to their age;

2. the older individuals perform the outside nest task;

and differences:

1. the older workers of termites also perform the inside nest work which is only done by the young hymenopterans;

2. Most tasks are performed by the older individuals in termites while some tasks (such as brood care, queen attending) are performed by the younger workers and some task (e.g. foraging) are performed by the older workers in eusocial hymenopterans.

As a whole, polyethism or division of labor leads to task specialization and greater colony efficiency in insect eusociality (Wilson, 1985; Alexander *et al.*, 1991). In most studies of the social Hymenoptera, the temporal polyethism among the workers of honey bees mentioned in section **1.6.3** (the workers change their tasks and activities from the center of the nest to the periphery) is recognized as the common pattern of temporal polyethism in social insects (Winston, 1987; Hölldobler and Wilson, 1990; Ross and Matthews, 1991). A similar pattern is also suggested in the early studies of lower and higher termites (Pasteels, 1965; McMahan; 1979; Brian, 1979; Waller and La Fage, 1987).

1.7 Purpose of study

Among the four kinds of social insects (social bees, social wasps, ants and termites), only termites compose the order of Isoptera with phylogenetic traits which are quite different from the social hymenopterans (Wilson, 1971). For example the immature individuals of termites participate in colony labor while the larvae and pupa of hymenopterans are inactive (McMahan, 1979; Noirot and Pasteels, 1987).

Based on the fundamental differences between the Isoptera and the social Hymenoptera, comparative studies of division of labor should broaden our understanding of the ecological and evolutionary significance of polyethism (Rosengaus and Traniello, 1993). Most research on polyethism has been done on the social Hymenoptera while few study cases are concentrated on the relationship between polymorphism of workers of termites and the division of labor under natural conditions (Miura and Matsumoto, 1995). There are many studies regarding the division of labor among workers in social hymenopterans resulting in the establishment of many ecological, evolutionary and organizational principles (Oster and Wilson, 1978; Hölldobler and Wilson, 1990; Bourke and Franks, 1995; Gordon, 1996; Traniello and Rosengaus, 1997; Crosland *et al.*, 1996, 1997a, 1997b; Crosland and Traniello, 1997). However, there is little information about the division of labor among workers in the termites (McMahan, 1979; Robinson, 1992; Miura and Matsumoto, 1995). It is very surprising that most of the references related to termites are written more than twenty years ago (For example, Krishna and Weesner, 1969, 1970; Howse, 1970; Harris, 1971; Wilson, 1971; Oster and Wilson, 1978, etc.).

The poor background knowledge of termites can be reflected by the fact that there are very few references specializing in termites in Hong Kong. With the help of the computer network provided by the Chinese University of Hong Kong, there were no more than 10 copies to be found in all the university libraries in Hong Kong. In the papers found from the computer network databases published within the period from January 1991 to April 1997 with the following key words: Isoptera, Hymenoptera, termites, honeybees, wasps, ants, bees and social insects; only 7 papers are concerned with the behavioral studies but none of them are related to termites. Therefore, there are very few examples of temporal polyethism in termites.

This thesis is intended to investigate the temporal polyethism amongst age classes in the worker line of the lower termite *Reticulitermes fukienensis* in order to gain a greater depth of understanding of the temporal polyethism of termites. The thesis project is also designed to testify the hypothesis of division of labor pattern as honeybee workers that same-age individuals perform particular sets of tasks even to the extreme of evolving one class for each task (Oster and Wilson, 1978; Rosengaus and Traniello, 1993). Experiments were carried out in order to answer these questions:

1. Is there division of labor among age classes within worker castes of the sample termite *Reticulitermes fukienensis*?

2. If there is temporal polyethism in behaviors among the age classes, what kinds of the behaviors are present and are they continuous or discrete temporal polyethism for each behavior?

3. If there is temporal polyethism in some behaviors, what is the frequency distribution pattern of each behavior exhibited in the age classes of the worker line?

4. Are there any interesting or important findings in temporal polyethism?

5. Is there any discovery that initiate other studies in polyethism of termites?

1.8 Reason for the choice of sample species

Reticulitermes fukienensis was chosen for this study because of the following reasons:

1. A pattern of temporal polyethism of a species with only one single developmental line of workers (it is expected to be simpler than the study with more than one developmental line of workers) was expected to be found so that comparison can be made with the temporal polyethism of the workers in the social hymenopterans. The past studies of *Reticulitermes* (e.g. Buchli, 1958; Husby, 1980) have shown evidence that *R. fukienensis* fulfills the requirement.

2. *Reticulitermes fukienensis* and other species of the same genus are one of the most common pests in China and Hong Kong. It was expected that study of this species could give more understanding of the genus of *Reticulitermes* for termite control.

3. *Reticulitermes, Coptotermes* and *Nasutitermes* species are widely found in the Tai Po Kau Nature Reserve which is near to The Chinese University of Hong Kong. These species are quite common in Hong Kong and sampling is not a problem.

1.9 General Methodology

1.9.1 Identification of *Reticulitermes fukienensis*

Although there are many methods used for the taxonomy of termites, studying morphological characters is still widely used for the identification (e.g. Tsai *et al.*, 1977; Ping *et al.*, 1980, 1982; Li and Ping, 1983). There are few morphological differences among the workers of various species in the same genus or workers of various genera. The soldiers of various genera or species are quite morphologically different because their head specialized for different defensive techniques (Wilson, 1971). Therefore many authors identify the species mostly based on the morphology of soldiers (e.g. Yu and Ping, 1964; Hsia and Fan, 1965; and Huang *et al.*, 1989, etc.) *R. fukienensis* can be identified by using the keys constructed by Yu and Ping (1964), Hsia and Fan, (1965), Gao and Lam (1986) and Huang *et al.* (1989).

Among the 16 termites species found in Hong Kong, only two genera the *Coptotermes* (2 species) and the *Reticulitermes* (4 species: *R. fukienensis, R. affinis, R. labralis* and *R. speratus*) of the Family Rhinotermitidae are included (Gao *et al.*, 1992). The family Rhinotermitidae can be identified by the following characteristics:

1. head with fontanelle (a median dorsal pore);

2. pronotum narrower than the head; and

3. pronotum flat and slightly kidney shaped.

Reticulitermes can be identified by having a slightly rectangular head with parallel sides while *Coptotermes* can be identified by having a short head which is narrowed anteriorly. Soldiers of *R. fukienensis* have a body length ranging from 4.89 mm to 6.51 mm. Frontal area of the *R. fukienensis* soldier is distinctly raised. The head is rectangular, subparallel or slightly tapering anteriorly, rather more yellow than the pale body. Compared with others *Reticulitermes* species, the soldier head is relatively short and small. The head length is less than 3 mm and less than twice its width, the black-tipped mandibles are relatively short and stout, and they are slightly slender and slightly incurved with the posterior part of gulamentum broader. The labrum is lanceolate with a width 0.27-0.46 mm and mostly with paraterminal hairs (Hsia and Fan, 1965). The antennae have 16 to 18 segments but most have 16 segments.

R. fukienensis and *R. flavipes* are very close to each other while *R. affinis* is much larger (Morimoto, 1968). Soldiers of *R. fukienensis* have head widths ranging from 0.99 mm to 1.11 mm while *R. flavipes* are 0.89 mm to 1.14 mm. *R. fukienensis* have scatter pigmented spots on their yellow pronotum whereas *R. flavipes* have a yellow pronotum without any pigmented area (Morimoto, 1968).

1.9.2 Sample collection

Collection of moist and decaying logs of China fir (*Cunninghamia lanceolata* Hook.) containing mature colonies of *Reticulitermes fukienensis* from Tai Po Kau Nature Reserve in Hong Kong were carried out from September 1995 to December 1996.

The Tai Po Kau Nature Reserve (Figure 1.5) is situated above the sea level extending from 50 meters to 650 meters at the top of Grassy Hill. The area is heavily wooded with more than 100 different species of trees such as Litsea, Giant Bean, Sweet Gum, Camphor, China Fir, Acacia and Paper-bark Tree.

Every field trip for the collection of the colonies was taken on sunny days with the temperature above 18 °C. *R. fukienensis* termites migrate from the logs into the sub-nests in the soil when the weather is cool and dry or rainy (Esenther, 1961) The logs were identified as China fir based on the criteria that the tree bark was scale-like and brown in color similar in appearance to the tree bark of the living China fir found nearby. The logs were straight in shape and were normally over 30 cm and up to 60 cm in diameter. The branches of the logs (if present) were arranged in whorls at 70-90° to the main trunk. The remains of the leaves of China fir attached on the log gave further confirmation. The dead wood was damp, decaying and appeared deep brown in color accompanied with black soil inside. The logs containing mature colonies of *R. fukienensis* were usually found lying on the ground or slopes of the forest under the canopies.

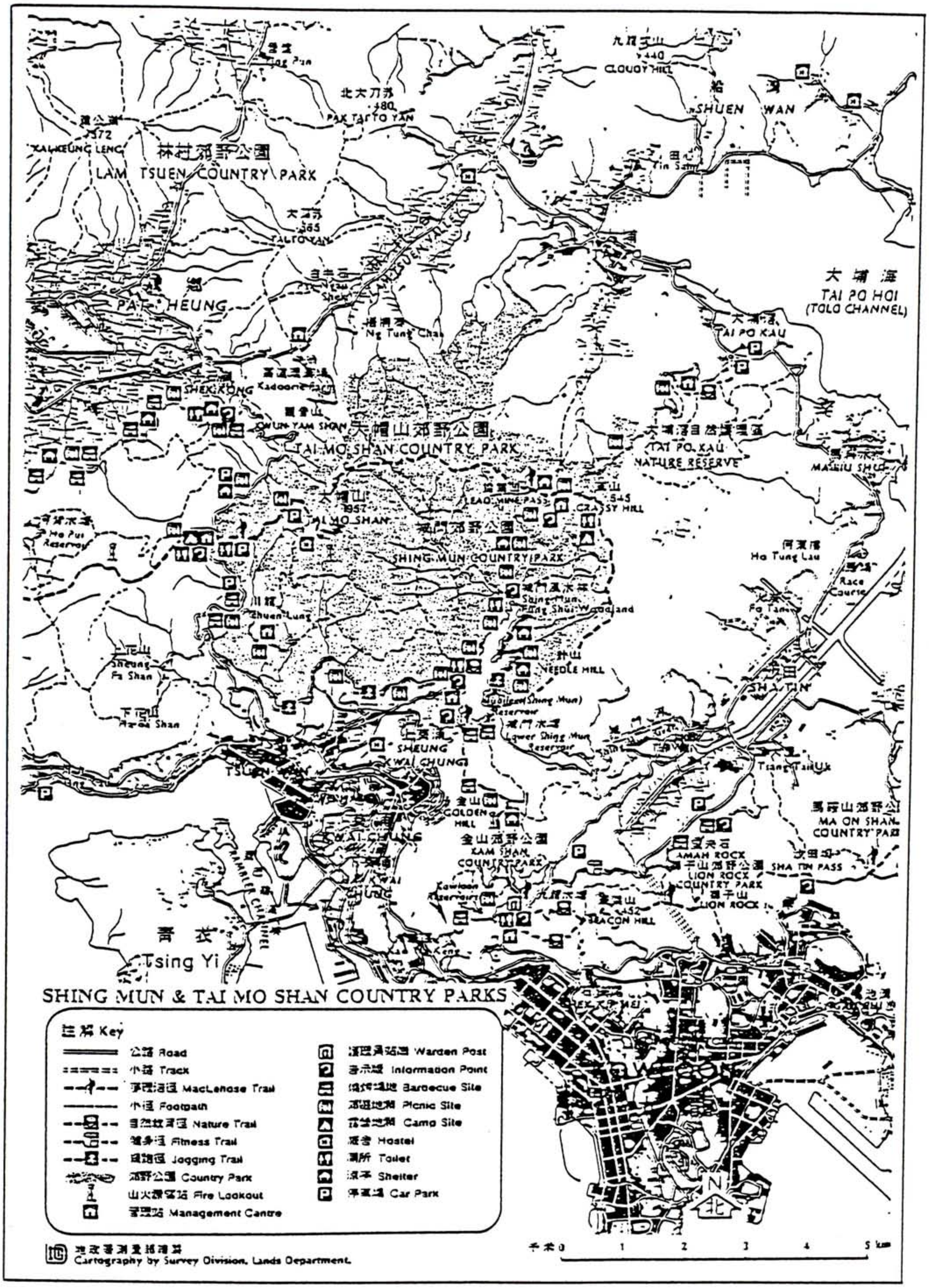


Figure 1.5 The map of the Tai Po Kau Nature Reserve in Hong Kong.

Once discovery of a colony occurred, the log was cut into sections not more than 1 meter in length. They were held in heavy plastic bags with label and transported to the laboratory.

1.9.3 Storage, preservation and further confirmation of sample in laboratory

In most instances, termites are difficult to rear. The individuals in a colony have delicate integration which is easily disturbed in handling and isolating them, resulting in a high mortality rate (Becker, 1969). In spite of this and other difficulties, many investigators have cultured termites in the laboratory with varying success, including Adamson (1941), Light and Weesner (1947), Lüscher (1949), Gay *et al.* (1955), Osmun (1957), and Becker (1961). In general, it is possible to rear termites belonging to the family Hodotermitidae, the subfamily Termopsinae of the family Kalotermitidae, and the genus *Reticulitermes* of the Rhinotermitidae family with success, but many of the Rhinotermitidae and especially the Termitidae are very difficult to culture (Becker, 1969). Because *Reticulitermes* can be cultivated in laboratory, the collected samples were stored in the laboratory with the following methods :

The collected logs containing *R. fukienensis* colonies were further cut into short lengths no longer than 50 cm and kept in covered big plastic boxes (55 cm in length) at 22°C±2°C in the laboratory. Water was sprayed on the logs once every week in order to keep the logs moist.

Between ten and fifty soldiers were taken from each colony (one piece of log) newly collected from the field and were preserved in 80% ethanol in glass vials. Ten preserved soldiers were randomly taken out and their morphological characters were observed such as head width before drying out under a calibrated microscope to further identify whether the species were *R. fukienensis* or not.

Because the soldiers of *R. fukienensis* are quite similar to those of *R. flavipes* and *R. affinis*, further confirmation is required. Specimens were confirmed to be *R. fukienensis* by using the following criteria: a head width in the range of 0.99 mm to 1.11 mm, a head length less than 3 mm, a body length ranging from 4.89 mm to 6.51 mm, a slightly rectangular head with parallel sides, a lanceolate labrum with a width 0.27-0.46 mm and mostly with paraterminal hairs, antennae with 16 to 18 segments, slightly slender and slightly incurved mandibles as compared with *R. flavipes*, and a yellow pronotum with scattered spots. Soldiers of *R. flavipes* were identified by the following morphological characters: subparallel-sided head with wider in median part, labrum generally without paraterminal hairs, and less incurved mandible as compared with *R. fukienensis*. Head width of *R. flavipes* soldiers fall in the range of 0.89-1.14 mm. Both the head length of *R. fukienensis* and *R. flavipes* soldiers are less than 3 mm whereas those of *R. affinis* are more than 3.07 mm. The head length is 2.5 times as long as wide. Preserved samples were also sent to the Institute of Zoology in Beijing for further taxonomic confirmation.

1.9.4 Methods for the removal of termites from nests

In order to avoid any possible deviation of the behavior of the sampled termites once they were taken from the field, they were taken out for experiment as soon as possible after the field trip collection. Removal of the termites from the log was performed within 12 weeks from the collection date. To ensure that the termites used for experiments were not damaged during removal, they were placed in Petri dishes with moist filter for 3 days after removal. After 3 days, living termites were taken out for experiments.

In order to take an efficient, time saving removal and minimize the degree of damage of the specimens, concentration of termites method' can be introduced. First, the short lengths of the same log except one were cut into small pieces and were let to air dry. The length without further cut was kept moist all the time. Then all the lengths were placed together. Moisture was very important to the termites to prevent their soft cuticle and bodies from rapid desiccation in a dry environment.

Then the moist lengths were taken out and peeled piece by piece. Most of the termites in the wood were easily tapped out into a collector box. Next, they were transferred into Petri dishes (90 mm in diameter) with a piece of moist filter paper (Whatman No. 1, 90 mm in diameter) on the floor of the dish. The filter paper provided food for the termites. Water was sprayed to the filter paper frequently in order to keep moisture inside the Petri dishes and to prevent desiccation of the termites. Then the Petri dishes were placed in covered plastic boxes (31.5 cm in length) lined at the bottom with moistened tissue paper to help maintain a moist environment.

1.9.5 Experimental conditions

All experiments were performed in the laboratory maintained at a temperature of 21-23°C with 24 hours of luminescence of fluorescent lamp of 60W. The benches for the placements were kept away from disturbances such as shaking on the benches.

Chapter 2

SEPARATION OF AGE CLASSES AND TEMPORAL POLYETHISM IN FEEDING BEHAVIOR OF AGE CLASSES

2.1 Introduction

This chapter composed of two part: separation of age classes and temporal polyethism in feeding behavior of the age classes. In the first part, experiment had been done for the separation of age classes in the development line of worker in *Reticulitermes fukienensis*. Review of the separation of age classes based on biometric or morphological characters used by different researchers was described. Then the methods designed for the separation were given. Next the number of age classes and morphological differences among the age classes were discussed.

In the second part, experiment had been done for the study of temporal polyethism in feeding behavior of the age classes. Experiment was carried out to determine the larvae and workers among the age classes. Individuals which can live independently and which can live dependently on the other individuals were identified as worker and larvae respectively. Thus other behavioral studies (such as brood care behavior) can be designed based on feeding ability of the age classes (i.e. or based on larvae and workers). The experiment was carried out by placing the instars on filter paper which were stained with indigestible green dye. If the termites feed on the filter paper, their gut will be stained green in color. Survivorship of termites was determined at different time interval. Feeding ability of the age classes was discussed last.

2.2 Separation of age classes

2.2.1 Review of separation of age classes

In past studies of temporal polyethism of termites, many researchers identified the number of instars or age classes respective to biometric measurements. Because the size and age of the instars are correlated: later instars are bigger in body size than the earlier instars through the increment of body size by molting. McMahan *et al.* (1984) concluded that studies of polyethism must be preceded by biometric analyses of polymorphic differences among the various kinds of termites whose different behaviors are to be compared. Noirot (1955) pointed out that the most important parameters for distinguishing different termites instars are head width, antenna length, and tibia length.

There were many examples of the separation of age classes based on head width. Howse (1968) separated a colony of about 100 individuals in *Zootermopsis nevadensis* into 6 larval instars, 2 nymphal instars, a soldier caste and a replacement reproductive caste using the criterion of head width. Rosengaus and Traniello (1993) found 7 instars in *Z. angusticolli* by measuring their head width using a stereo microscope. Miura (1995) separated 3 age classes of workers in *Hospitalitermes medioflavus* for the temporal polyethism in foraging behavior.

A combination of measurements of different body parts for the separation of age classes was also used in the past. For example, Husby (1980) separated age classes in *Reticulitermes flavipes* based on head length, number of antenna segments, pronotum length, pronotum width, mesonotum width, metanotum width, tibia length and femur length and head. He found that there were two larval instars, five worker instars and eight nymphal instars. For the workers in *R. lucifugus santonensis,* Garnier-Sillam (1983a and 1983b) separated the 8 instars based on the measurements of antenna length, hind tibia length and body length and the number of antenna segments, McMahan *et al.* (1984) distinguished 5 instars of large and small workers, 1 soldier caste and 1 pre-soldier caste in *Odontotermes distans* based on measurements of head width, antenna length and metatibia length.

Besides biometric measurements, other researchers separated the age classes based on the shapes of various body parts. McMahan (1970) separated the age classes chiefly on the basis of size and head pigmentation. Jones (1980) distinguished the five worker classes in *Nasutitermes costalis* based on the body color (especially the color of the head) without any marking on the termites for the direct observation of their gallery construction behavior.

In conclusion, biometric measurements are widely used for the determination of the number of the instars or age classes. The appearance (i.e. color and shape of the body parts) of the individuals of some species can permit direct observation of the individual without any need to mark the bodies for recognition during the experiment.

In this thesis, the number of instars (or age classes) of the development line of workers in *R. fukienensis* was determined based on the head width measurement and their morphological appearance.

2.2.2 Materials and methods

The separation methods based on head width measurement was widely used by other investigators such as Heath (1927) and Miura and Matsumoto (1995). In those studies, head widths of individuals of the same colony were measured. Frequency of the individuals of various head widths was plotted on a graph. The number of the peaks on the graph represented the number of age classes or instars. Then morphological differences among the five age classes were determined so that each age class can be identified quickly with visual evidence in the followings experiments of this thesis.

2.2.2.1 Head width measurements

Only individuals of the same colony (only one piece of log was used) of about 200 individuals were chosen randomly and preserved in 80% ethanol. A microscope (KYOWA Model SZM) with a zoom range of 0.6x - 3.0x and scale was calibrated with a standard micrometer under the power of 2.7x.

The head width of the individuals of the worker line were measured at the widest point of the head. Head width was chosen because the head plates are highly united into socket-like form, and are harder than the other body parts of the termites. Thus the least degree of expansion or contraction in the head exhibited was compared with other body parts under various conditions such as dehydration or evaporation of termite body.

The preserved individuals were placed on the rough surface of a black card with the head horizontally orientated. Cool light was used for illumination to reduce the risk of evaporation of the specimen during measurement. The head width was measured in into the nearest 0.02 mm. Individuals with the same head width were stored together in 80% ethanol for further morphological study.

When measurements were finished, a graph of the complete range of head widths of workers was constructed. Expected peaks appeared on the graph. The number of the age classes and their head width ranges were determined by the number of the peaks and the peak ranges from the graph.

2.2.2.2 Morphological study

Appearances of the preserved individuals and living individuals of the five age classes were inspected. The colors and shapes of various body parts of these age classes were inspected and the approximate ratio of head size and body length among these age classes were expected to be found.

2.2.3 Results

2.2.3.1 Biometrics measurements

Head widths of two hundred individuals of the same colony of *Reticulitermes* *fukienensis* were measured on December 1995. Measurements were also performed by Crosland *et al.* using several hundreds of larvae and workers of the same species (Crosland *et al.* 1997a, 1997b).

Figure 2.1 illustrates that the head widths fall into five distinct peaks with five size categories of individuals. The most abundant size category appeared at 1.00 mm, and the other peaks at 0.40, 0.50, 0.64 and 0.90 mm.. The relative proportion of the individuals categorized into the five size categories increased from the first peak to the last peak. It should be noticed that there were gaps between some of the peaks such as 0.42 - 0.46 mm, 0.56 - 0.60 mm and 0.72 - 0.78 mm. The sizes of head width increased with the peaks of the different categories tend to merge together, and narrow ranges of head width were defined at the fourth and fifth peaks. Nevertheless, five size categories were temporary named as the 1st, 2nd 3rd 4th and 5th instars with the following range of head widths respectively: 0.40 to 0.42 mm, 0.48 to 0.56 mm, 0.60 to 0.72 mm, 0.78 to 0.88 mm and 0.94 to 1.10 mm.

2.2.3.2 Morphological study

There were no major detectable morphological and chromatic differences between the preserved specimens and the living specimens. Each age class has it appearance described below:

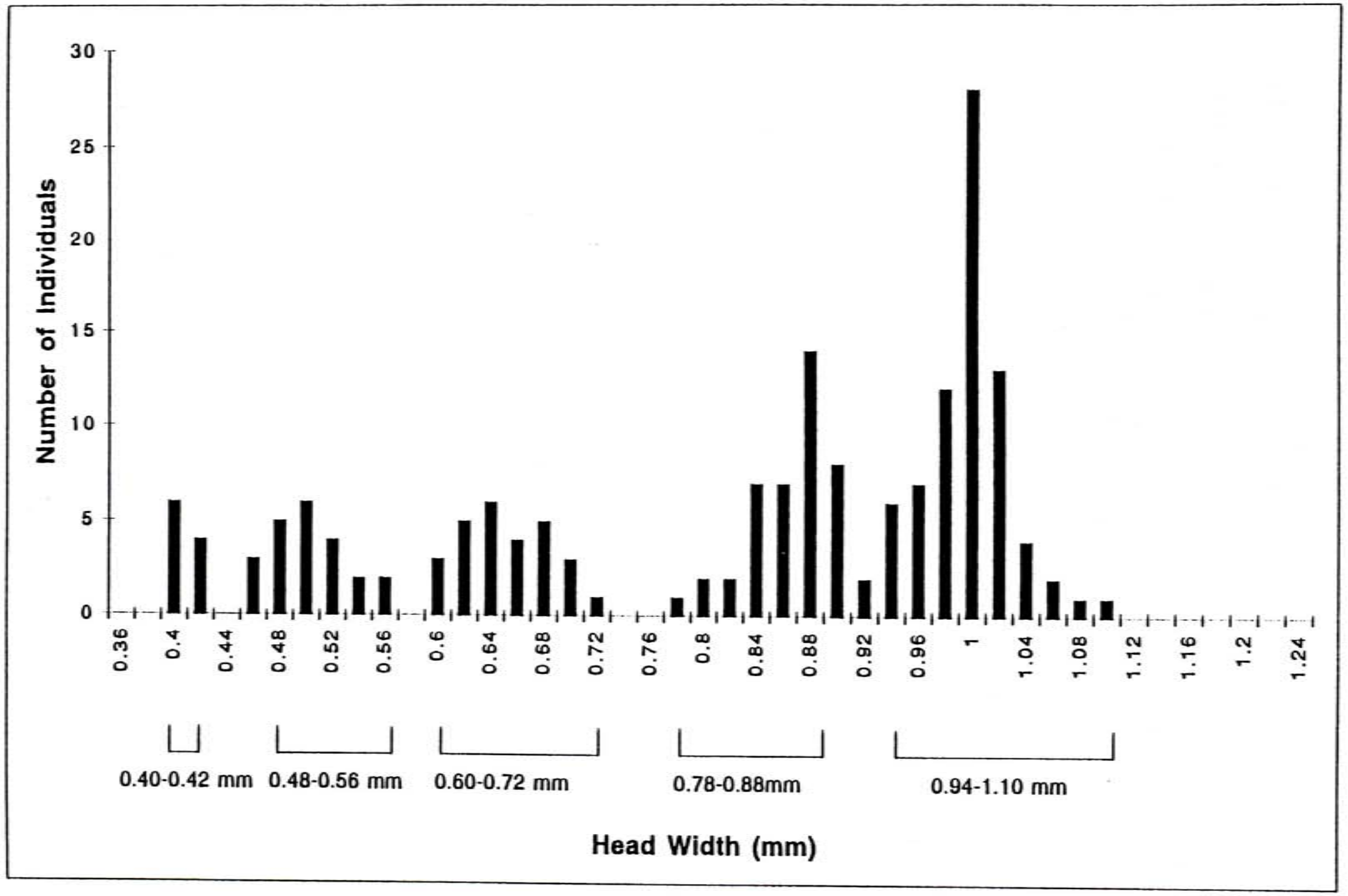


Figure 2.1 Head width distribution of the individuals in the worker line of ***Reticulitermes fukienensis.***

1. For the 1st age class:
    - white bodies;
    - white mandibles (not sclerotized);
    - transparent and white guts;
    - head was the smallest among the five head width size categories.

2. For the 2nd age class:
    - white bodies;
    - white mandibles (not sclerotized);
    - transparent and white guts;
    - head was approximate to 1.5 times the head of 1st age class.

3. For the 3rd age class:
    - tips of most white mandibles were brown;
    - some bodies were white (not sclerotized), some bodies were pale yellow (lightly sclerotized);
    - guts of some individuals were brown (wood fiber inside);
    - head was approximate to 2.5 and 1.5 times of those of the 1st and 2nd age classes respectively.

4. For the 4th age classes:
    - most bodies were pale yellow (pigmented and sclerotized), few bodies were white;
    - heads were deeper in color than abdomens;
    - brown mandibles (pigmented and sclerotized);

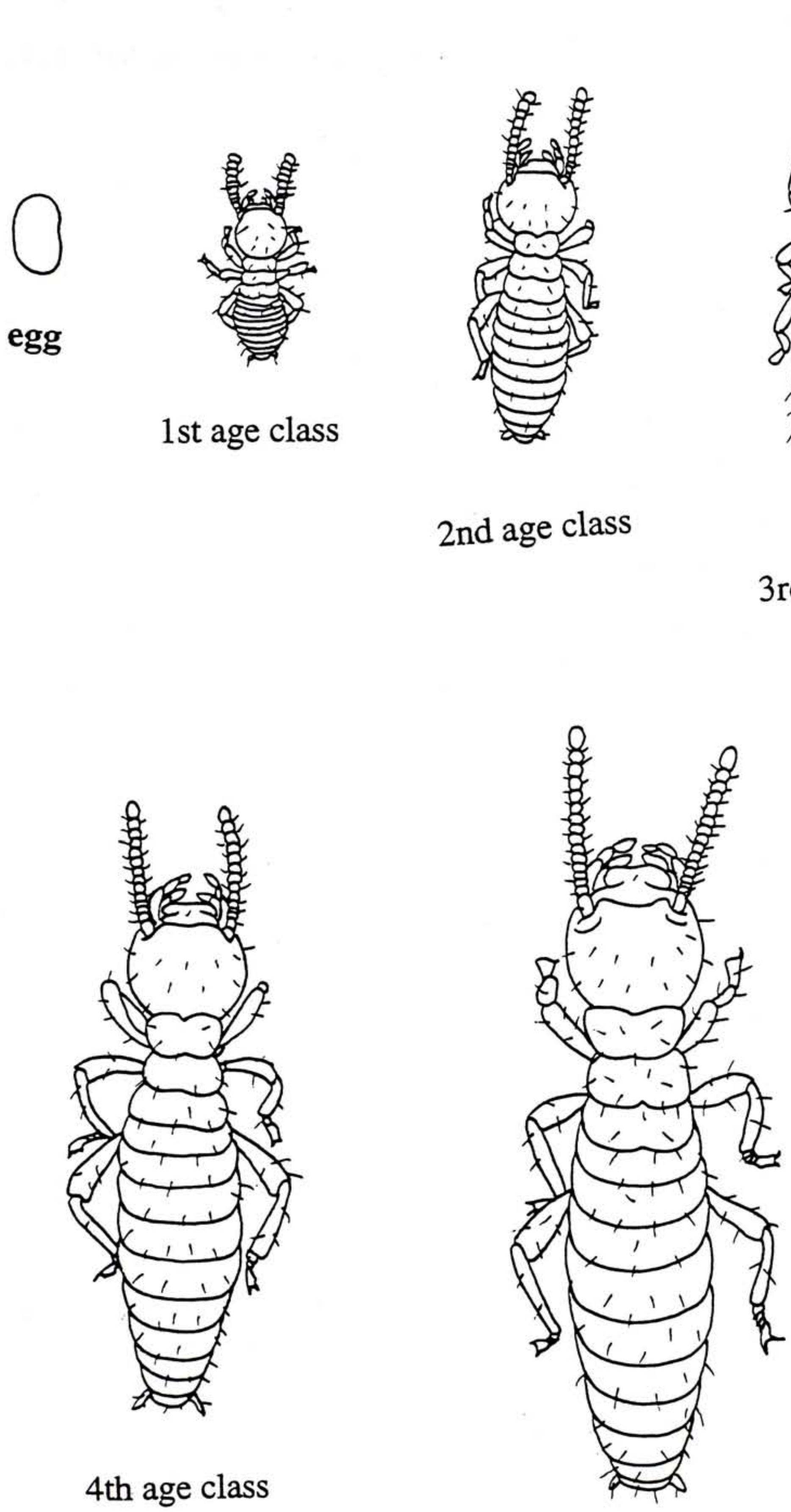
- guts of some individuals with pale yellow bodies were brown (wood fiber inside);
- pronotum, mesonotum and metanotum arranged as a trapezoid with deeper slope than those of the 5th age class;
- highly constriction between head and thorax;
- head was approximate to 4 and 1.5 times of those of the 1st and 3rd age classes respectively.

5. For the 5th age class:
    - most bodies were pale yellow, few bodies were white;
    - heads were deeper in color than abdomens;
    - brown mandibles (pigmented and sclerotized);
    - guts of some individuals with pale yellow bodies were brown;
    - pronotum, mesonotum and metanotum arranged as a trapezoid with more gentle slope than those of the 5th age class;
    - lightly constriction between head and thorax compared with the 4th age class;
    - head was the greatest among the five head width size categories;
    - head was approximate to 1.2 times of those of the 4th age class.

The relative size of the five age classes was shown on Figure 2.2 and the approximate ratio of head size, body length of the five age classes were summarized as:

age class head size body length

1st : 2nd : 3rd : 4th : 5th = 1 : 1.5 : 2.5 : 4 : 5 = 1 : 1.25 : 2 : 3.5 : 4



egg

1st age class

2nd age class

3rd age class

4th age class

5th age class

Figure 2.2 Relative sizes of the age classes in the worker line of *Reticulitermes fukienensis*

2.2.4 Discussion and conclusion

Five possible age classes in the development line of worker in *Reticulitermes fukienensis* were separated. Individuals in the development line of worker in *R. fukienensis* were separated into five size categories based on head width measurements. The five size categories were considered into five possible instars or age classes. The peaks of the frequency distribution of head widths graph were explained by the increment of body sizes through molting. If there are more than 5 instars, it is quite difficult to distinguish the real number of instar because of the decrease of increments of head widths and the tendency of merging of the peaks in the late instars. Possible event was supported by Buchli's (1958) seven years study in *R. lucifugus santonensis*. Buchli found that the *R. lucifugus santonensis* workers went through nine instars from egg to alate and were capable to molt without any conspicuous morphological changes in the late stages. Nevertheless, the 5th instar of *R. fukienensis* was regarded as the oldest stage of the development of workers.

Buchli (1958) made important observation of feeding relationship among various forms in *R. lucifugus santonensis*. He concluded that there were two larval instars which were very much alike, and seven worker instars which were very similar. He found that further molting of worker occurred after nine stage in the laboratory condition although no conspicuously morphological differences were detectable.

Husby (1980) found out seven stages in the development line of *R. flavipes* workers based on biometric measurements including number of antenna segments, head length, head width, pronotum length, pronotum width, mesonotum length, mesonotum width, hind tibia length and hind femur length. He separated them into two larval stages and five workers instars by using the Clustan 1C program. Most groups of *R. flavipes* formed 'tight' clusters, however, there was overlap in the clusters of the late-instar workers. It is similar to the merging of the peaks of different head width size categories of *R. fukienensis* in this chapter.

Together with the studies done by Buchli (1958), Husby (1980), Crosland *et al.* (1996, 1997a, 1997b), Crosland and Traniello (1997) and the result of this chapter, 5 to 11 instars was probably found in the development line of the genus *Reticulitermes* workers.

Compared with the head width measurements of *R. fukienensis* workers done by Zhang (Crosland *et al.*, 1996, 1997a, 1997b, Crosland and Traniello, 1997) (Figure 2.3) and Lok (1995) (Figure 2.4), the head width size ranges and peaks of *R. fukienensis* are various in different colonies but five age classes can be found in difference colonies. Therefore, one colony for one experiment was used for behavioral observation in this thesis.

The five instars or age classes can be easily separated and identified based on their morphological differences and their body size. Pigmented (pale yellow) bodies with brown mandibles were only found in very small portion of the 3rd age class, but in large portions of the 4th and 5th age classes. It was explained by pigmentation in the later stages of *R. fukienensis* termites. The white body color of a small portion of the individuals from the 3rd to 5th age classes was explained that they were just after molting, and need more time for pigmentation and exoskeleton thickening.

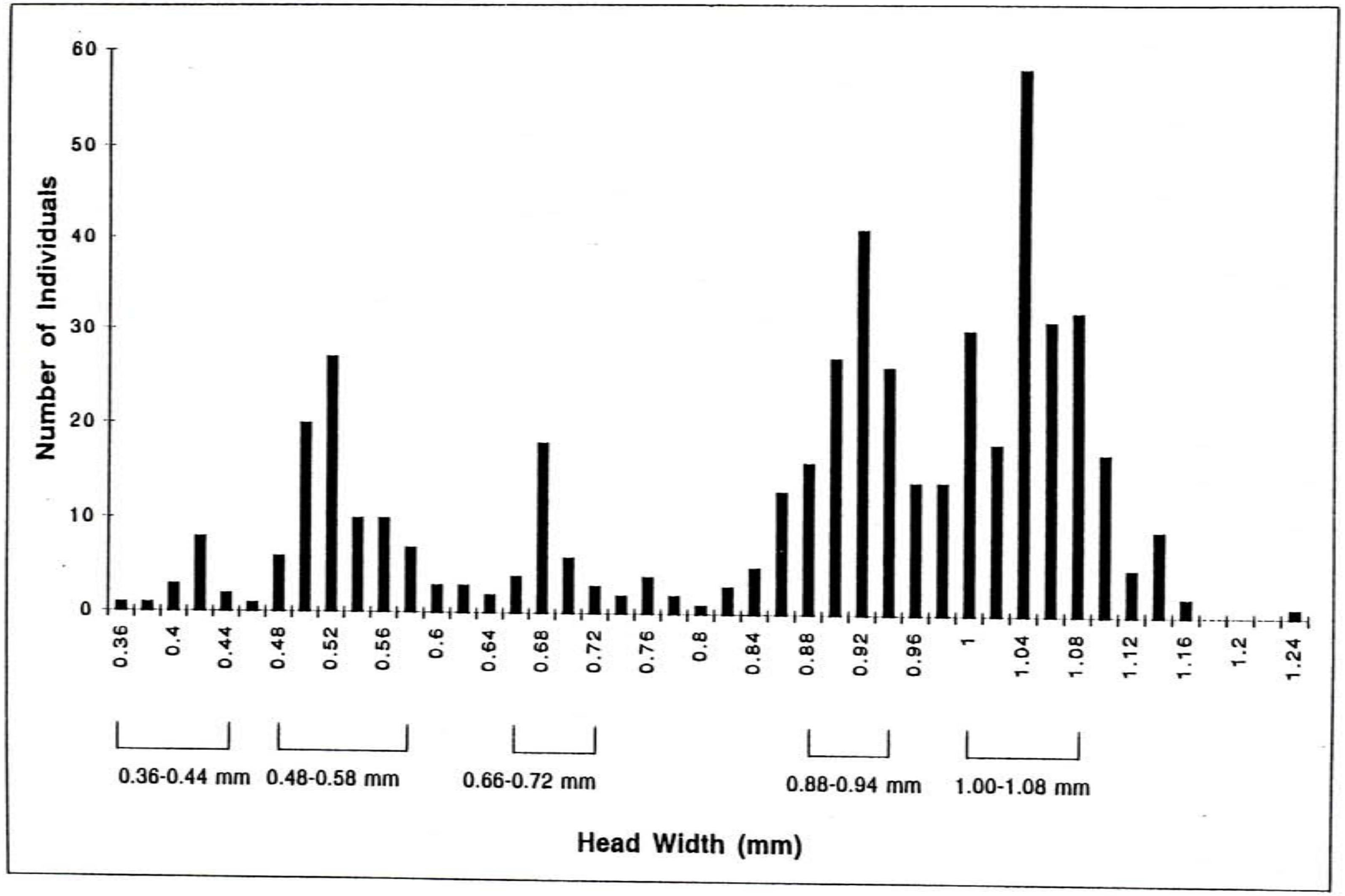


Figure 2.3 Head width distribution of the individuals in the worker line of *Reticulitermes fukienensis*.
(from Crosland *et al* ., 1997b)

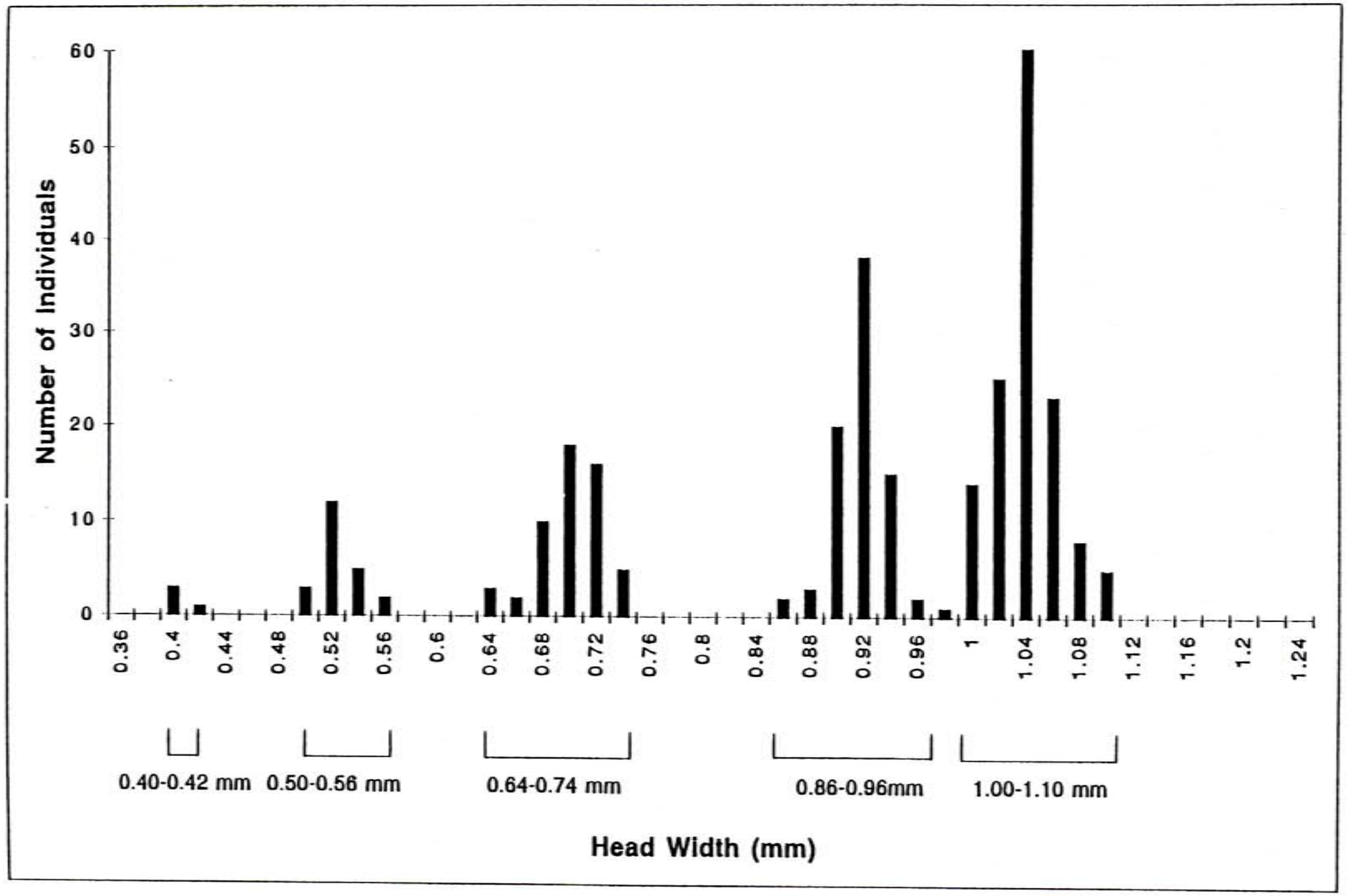


Figure 2.4 Head width distribution of the individuals in the worker line of *Reticulitermes fukienensis*.
(from Lok, 1995)

After the visual inspection of the five age classes, each age class presents a combination of several characters. The white bodies of a small portion of the 4th and 5th age classes are found changed into pale yellow in color. Nevertheless, the combination of the characters and body size (section 2.2.3.2) are quite enough for the separation of age classes without the use of microscope. It is not necessary to perform any biometric measurements before or after behavioral observation of *R. fukienensis* termites. Therefore, any influences caused by mark on the individuals are avoided and the separation of the size group is quite easy.

2.3 Temporal polyethism in feeding behavior of age classes

2.3.1 Definition of larva and worker

The term 'larva' (pl. larvae) of termites is quite different from the term 'larva' of hymenopterans or other homometabolous insects. Homometabolous larvae are quite morphologically and physiologically different from adults. Except social hymenoptera larvae, homometabolous larvae feed themselves and survive independently. Larvae and workers of termites are morphologically similar. It is quite difficult to find out a critical point to get a clear cut of larvae and workers in the development line of worker (Harris, 1971; Wilson, 1971; Watson *et al.*, 1985).

Different authors made different definition of larvae. Some authors (i.e. Noirot and Pasteels, 1987) distinguished larvae and workers based on their caste differentiation after molt. Noirot (1985) described larvae as early instars which can molt to become nymphs in the reproductive line or become workers in the worker line. Some authors referred individuals which do work in different stages, and can molt to become nymphs, soldiers or reproductives, to either larvae (Robson and Fourvaissié, 1995) or workers (Su, 1994; Grace *et al.*, 1995; Crosland *et al.*, 1996, 1997a, 1997b, Crosland and Traniello, 1997).

The traditionally accepted distinctions between larvae and workers (Watson *et al.*, 1985) are: workers in their earliest worker stages have sclerotized mandibles, are able to survive alone, and have a dark gut containing wood fragments. Many documents about *Reticulitermes* state that small larvae will grow and molt to become large larvae. Then large larvae will develop into small worker and then large worker through a series of moltings (Buchli, 1958; Noirot, 1985). Individuals which can feed themselves (can perform the task: feeding) and survive at the end of the experiment of this chapter are identified as workers, if not, they are identified as larvae.

2.3.2 Materials and methods

2.3.2.1 Experimental set-up

Fast Green dye is harmless to termites. It is not absorbable, but disposable in feces, and does not affect the osmoregulation of termites (Oi and Su, 1994). 0.05 ml of 1 % Fast Green dye solution was transferred to stain filter paper (Whatman No.1) on Petri dishes (5.5 cm in diameter). Excess droplets of dye solution on the filter paper were removed by tissue paper. Moisture of the filter paper was confirmed in suitable level if there was no shinning from the filter paper under light. If filter paper is too moist, termites may suck the dye solution from the filter paper.

2.3.2.2 Observation methods

Three individuals of the same age classes (selection based on the head peaks in section 2.2.3.1 and characters in section 2.2.3.2)were transferred into Petri dish. Eight replicates were done for each age classes. Therefore 24 individuals of each age class and a total of 120 termites were used in this experiment. All Petri dishes were placed in a covered plastic box with wet tissue inside. The replicates in plastic box were kept at 22±2°C under 24 hours fluorescent light (65 watts). Available evidence indicates that under constant light (not bright light), termites behave normally and perform all nest tasks (Howse, 1968; Stuart, 1969; Garnier-Sillam, 1983a; Rosengaus and Traniello, 1993). Number of survivors and number of individuals of each age class with their guts stained green were recorded at 0, 1st, 3rd, 6th, 9th, 12th, 15th, 18th and 21st days. (Most of the experiments in this thesis last more than 2 weeks but less than 3 weeks.)

2.3.3 Results

Only the 3rd, 4th and 5th age classes survived at the end of the experiment. The % of survivors of the five age classes were displayed on Table 2.1 and plotted on

Table 2.1 Survivorship of the five age classes in the worker line of *Reticulitermes fukienensis*.

Day	Age classes				
	1st	2nd	3rd	4th	5th
0-1	100	100	100	100	100
3	100	100	96	100	100
6	79	79	92	100	100
9	12.5	50	79	100	100
12-21	0	0	79	100	100

Table 2.2 Percentage of the survivors of the five age classes with their guts stained green.

Day	Age classes				
	1st	2nd	3rd	4th	5th
0-1	0	0	83	100	100
3	0	0	96	100	100
6-21	0	0	100	100	100

Figure 2.5 The trend of survivorship of the five age classes with time.

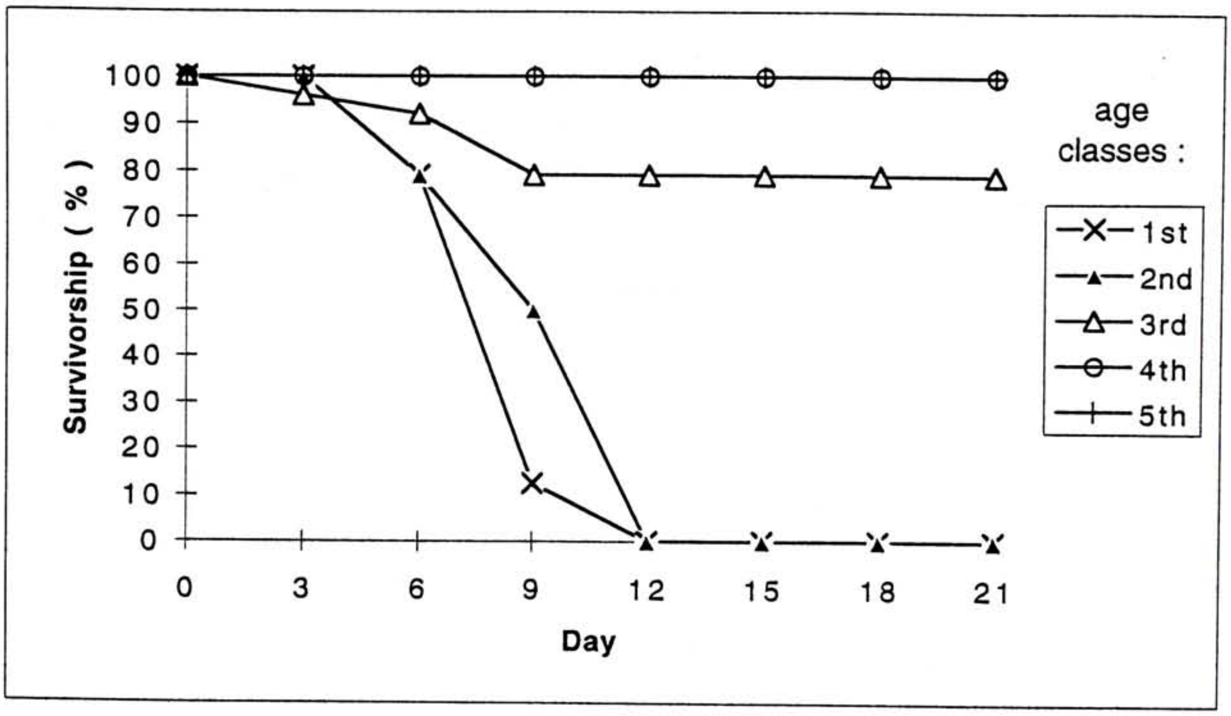


Figure 2.6 The trend of the percentage of the survivors of the five age classes with their guts stained green.

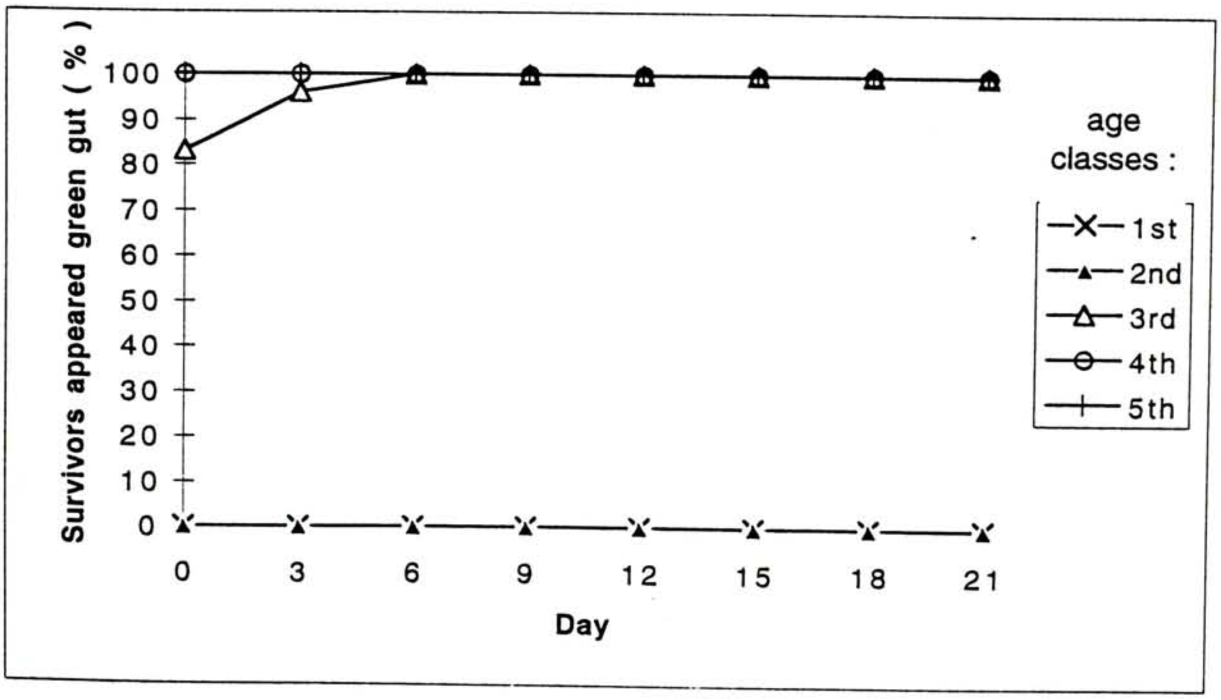


Figure 2.5. Both % of survivors of 1st and 2nd age classes declined rapidly from the 6th day and dropped to zero before the 12th day. The % of survivors of 3rd age class declined to 79% on the 9th day and then remained constant throughout the experiment. All the 4th and 5th age classes survived at the end of the experiment.

Only the 3rd, 4th and 5th age classes' gut were stained green. The % of the survivors with their gut stained green at different time intervals were displayed on Table 2.2 and Figure 2.6. None of the survivors of 1st and 2nd age classes had their guts stained green. The % of survivors of 3rd age class with their gut stained green increased from 83% to 100% from the 1st day to the 6th day and then remain constant in the following days. All guts of the 4th and 5th age classes were stained green throughout the experiment.

2.3.4 Discussion and conclusion

Temporal polyethism in feeding behavior among the five age classes is discrete (two age classes did not exhibit feeding behavior). Individuals older than 2nd age class perform feeding behavior (i.e. it can live and feed themselves independently on the individuals of other age classes).

Result of % of survivors was explained by feeding ability of the individuals. Workers which could feed themselves survived in the experiment. Larvae which could not feed themselves died within several days after using up their body stored food. 100% of survivors with their guts stained green indicated the 3rd, 4th and 5th age classes could intake food themselves. Zero % of survivors with their guts stained green indicated the 1st and 2nd age classes could not feed themselves.

Referred to the morphological characters study in section **2.2** and the anatomy study by Crosland *et al.* (1996, 1997a, 1997b), mandibles of the 1st and 2nd age classes are not well sclerotized for tearing and chewing the filter paper. White bodies and virtually transparent guts indicated no wood fragment present in their gut.

Buchli (1958) spent seven years to study the species *Reticulitermes lucifugus santonensis.* He concluded that the first two instars were larvae. Husby (1980) pointed out that seven stages in the development line of *R. flavipes* worker based on biometric measurements and gave a cut on the worker line that the first and the second instars were the larvae. Results of the experiment in section **2.3.3** and the results by Buchli (1958) showed that the first and second age classes of genus *Reticulitermes* were incapable to feed themselves.

After identification of larval stages and worker stages of the development of the worker line of *R. fukienensis*, various behaviors can be studied in two dimensions: a) behavioral differences among different instars; and b) behavioral differences between worker instars. For convenience, the 1st and 2nd age classes were described as small larvae (SW) and large larvae (LL) while the 3rd, 4th and 5th age classes were named as small worker (SW), medium worker (MW) and large worker (LW) respectively in the following chapters (Table 2.3).

Table 2.3 Names, abbreviations and ranges of headwidths for 5 age classes in the worker line of *Reticulitermes fukienensis*

Name	Abbreviations	Range of head width
Small Larva	SL	0.40-0.42 mm
Large Larva	LL	0.48-0.56 mm
Small Worker	SW	0.60-0.72 mm
Medium Worker	MW	0.78-0.88 mm
Large Worker	LW	0.94-1.10 mm

Chapter 3

ETHOGRAMS OF THE AGE CLASSES

3.1 Introduction

Ethogram is a graph of a frequency distribution of an animal's behavior in which rectangles with bases on the horizontal axis are given widths equal to the class intervals and heights equal to the corresponding frequencies. It is a presentation technique through which the trends and changes of behavioral frequencies distribution of various classes of an animal can be easily understood by readers. Through ethogram, the similarities, differences, and evolutionary relationship among species can be predicted (Wilson, 1976a).

Wilson (1976a) introduced ethogram in the study of social insects. Wilson (1976a) mentioned that through ethogram, organization can be made in a way to permit a closely comparison among 12000 species and even 270 genera of ants. In the study of the ant *Zacryptocerus varians,* specialization of tasks can be easily seen: major workers only performed defense colony; the queen was responsible for egg laying while the other tasks such as brood care, grooming and feeding behaviors were exhibited by minor workers (review by Wilson, 1976a).

Ethogram exhibits the patterns of temporal polyethism in various behaviors among age classes of eusocial insects. The most typical examples are the studies of discretization of labor occurring in workers of honeybee *Apis mellifera* and ant *Pheidole dentata* with age (Wilson, 1976b, 1985; Seeley, 1982). Ethograms in

Figure 1.3 and Figure 1.4 illustrated the discretization of division of labor and the similarities between these two eusocial insects: the insects shift from one linked set of tasks to another with age and move their activities outward from the nest center. The similarities between these two species are evolutionarily convergent (Wilson,1976b). Ethogram is also applied in the studies of other species of eusocial hymenopterans such as ants *Formica sanguinea, Camponotus sericeus* (Möglich and Hölldobler, 1974), *Diacamma* sp. (Nakata, 1995), *Pheidole hortensis* (Calabi *et al.*, 1983*), Orectognathus versicolor* (Carlin, 1981), *Atta sexdens* (Wilson, 1980), and honeybee *Apis mellifera* (Calderone and Page, 1991; Robinson, 1987).

In the past studies of temporal polyethism of termites, frequencies of various behaviors scored for ethogram construction were based on one of the two methods: a) determination of portions of population of each age class in an area where particular behavior concerned; b) direct observation of behaviors of individuals. For the first method, populations of different castes and age classes of termites in separate places related to their task performance were trapped and collected (Sand, 1965; McMahan, 1970; McMahan *et al.*, 1984; Watson, 1973; Watson and McMahan, 1978; Gerber *et al.*, 1988).

The methods in this chapter were quite similar to the second method mentioned above: all expected behaviors of termites were observed at the same time (e.g. Wilson, 1976b, 1980; Calderone and Page, 1991; Sendova-Franks and Franks, 1995). However, many studies on termites mainly focused at foraging, nest construction and repair behaviors (e.g. Pasteels, 1965; Howse, 1968; McMahan, 1970, 1977; Watson and McMahan, 1978; Badertscher *et al.*, 1983; Shellman-Reeve *et al.*, 1990; Lys and Leuthold, 1991).

Unlike the above studies, Rosengaus and Traniello (1993) investigated division of labor of incipient colonies of the damped wood termite *Zootermopsis angusticollis* by recording repertoire size, and behavioral frequencies of the seven larval instars of this species. Rosengaus and Traniello (1993) found that behavioral repertoire size increased with age from the 1st instar to the 2nd instar but stopped at the 3rd instar. There was no correlation between age and task, temporal polyethism was suggested to be absent in the larvae (3rd to 7th instars) of *Zootermopsis angusticollis.*

Experiment in this chapter was designed to determine the temporal polyethism in various behaviors of the five age classes in the worker line of *Reticulitermes fukienensis* within two dimensional 'nest' or cage. Repertoire size, behavior frequencies, and time budgets spent on various behaviors of age classes were studied.

3.2 Definition of behaviors

The term "task" in this study was used to denote behavior achieving purpose in the colony (Oster and Wilson, 1978). Sixteen behaviors were defined for observation in the experiment (Table 3.1). These behaviors were classified into six behavioral categories: 1) individual maintenance, 2) larval carrying, 3) social interactions, 4) feeding, 5) nest construction, and 6) foraging (Table 3.2).

Table 3.1 Definition of behaviors exhibited by the five age classes in the worker line of *Reticulitermes fukienensis*

	Behavior	Behavior code	Description of behavior and its related functions
	Individual maintenance		
1	**General stationary**	GS	standing without any body motion (except the movement of antennae) in the nest.
2 @	**Entrance stationary**	ES	standing in the opening of the tunnel without any body motion (except the movement of movement of antennae) in the nest. It is related to guarding the nest (Wilson, 1971).
3	**Open stationary**	OS	standing without any body motion (except the movement of antennae) in the open area.
4	**Moving**	MV	body (except the antennae) moving without any displacement in the nest.
5 @	**Searching**	SR	head move to left and right while walking and standing. It is supposed to be related to search for food or nestmates.
6	**Walking in tunnel**	WT	walking inside the nest with its mandibles holding nothing.
7 @	**Longitudinal vibration**	LV	body vibrates to and fro rapidly along the longitudinal axis within 1 second in the nest or in the the nest or in the open area. It is related to giving signals or alarms (Stuart, 1969).
	Larvae transportation		
8 @	**Larval grasping**	GR	lifting up another individual clear from ground by mandibles. It is related to transportation of larvae (Wilson, 1971).
9 @	**Larval carrying**	CA	holding the larva (touching or clear from the groud) with its mandibles whether walking or standing in the nest or in the open area. It is related to transportation of larvae (Wilson, 1971).
	Social interactions		
10	**Antennae touching**	AT	using its antennae to touch another individual's antennae.
11	**Antenna-body touching**	AB	using its antennae to touch another individuals' body parts (excluding mouth) such as thorax, abdomen or legs.
12 @	**Mouth-body touching**	MB	using its mouth or mandibles to touch another individual's body part such as head, antennae, thorax, abdomen or legs. It is related to oral grooming or body cleaning (Wilson, 1971).
	Feeding		
13 @	**Mouth touching**	MT	using its mandibles or other mouthparts to touch the individual's mouthpart in the nest or in the open area. It is related to stomodal feeding larvae (Stuart, 1969; Wilson, 1971).
14 @	**Tunnel wall touching**	MW	touching the tunnel wall with mandibles. It is related to feeding on wood of the tunnel or for nest construction (Stuart, 1969; Wilson, 1971).
	Nest construction		
15 @	**Wood Carrying**	CW	holding wood fragments with mandibles when walking. It is related to nest construction (Stuart, 1969; Wilson, 1971).
	Foraging		
16 @	**Walking in open**	WO	walking in the open area. It is related to foraging (Stuart, 1969; Wilson, 1971).

@ = task-related behavior.

Table 3.2 Definition of behavioral categories exhibited by the five age classes in the worker line of *Reticulitermes fukienensis*

Behavioral categories	Description of behavioral acts
Individual maintenance	The behaviors involved no interaction with other individuals. The behaviors may involve no purpose such as general stationary, open stationary, moving, walking in tunnel; or task-related such as entrance stationary (guarding), searching and longitudinal vibration (giving alarm).
Larvae transportation	The behaviors involved the transportation of larvae. Behaviors includes larval grasping and larval carrying (trasportation of the larvae to target place).
Social interactions	The behaviors involved interaction with another individual. Behaviors include antennae touching, antennae-body touching, and mouth body-touching (may be related to body cleaning).
Feeding	The behaviors related to the individual feeding itself or feeding the other individual. Behaviors include mouth touching, touching tunnel wall.
Nest construction	The behavior involved carrying the wood fragments by any individuals.
Foraging	The behavior involved the activities outside the nest (except open stationary).

3.3 Methods and materials

3.3.1 Preparation of embedding materials

Rotten wood of the China fir, *Cunninghamia lanceolata* Hook in which the animals originally inhabited was chosen as embedding material. Wood was cut and ground into fine chips (<1 mm in any dimension).

3.3.2 Experimental set-up

Experimental set-up (cage) was shown in Figure 3.1. A circular loop of electric wire (AWG, 1.45 mm), 8.5 cm in diameter, was placed on a piece of transparent acrylic plate (0.3 x 13 x 18 cm) which surface was carefully evenly roughened with fine sand-paper. The thickness of the wire allowed termites to move freely in two-dimensional space so that their activities would not be obscured.

Five to six grams of wet wood chips was packed evenly on the left half of the confined area (shaded part of the set-up). In the middle of the occupied area, wood pieces were picked out to form a clear circular zone (1 cm in diameter). Then it was covered by a transparent acrylic plate with square grids of 1 cm^2.

A small hole 3 mm in diameter just above the 1cm^2 circular zone was drilled on the cover plate and was covered with a transparent plastic film (0.25 x 0.4 x 1 cm). The

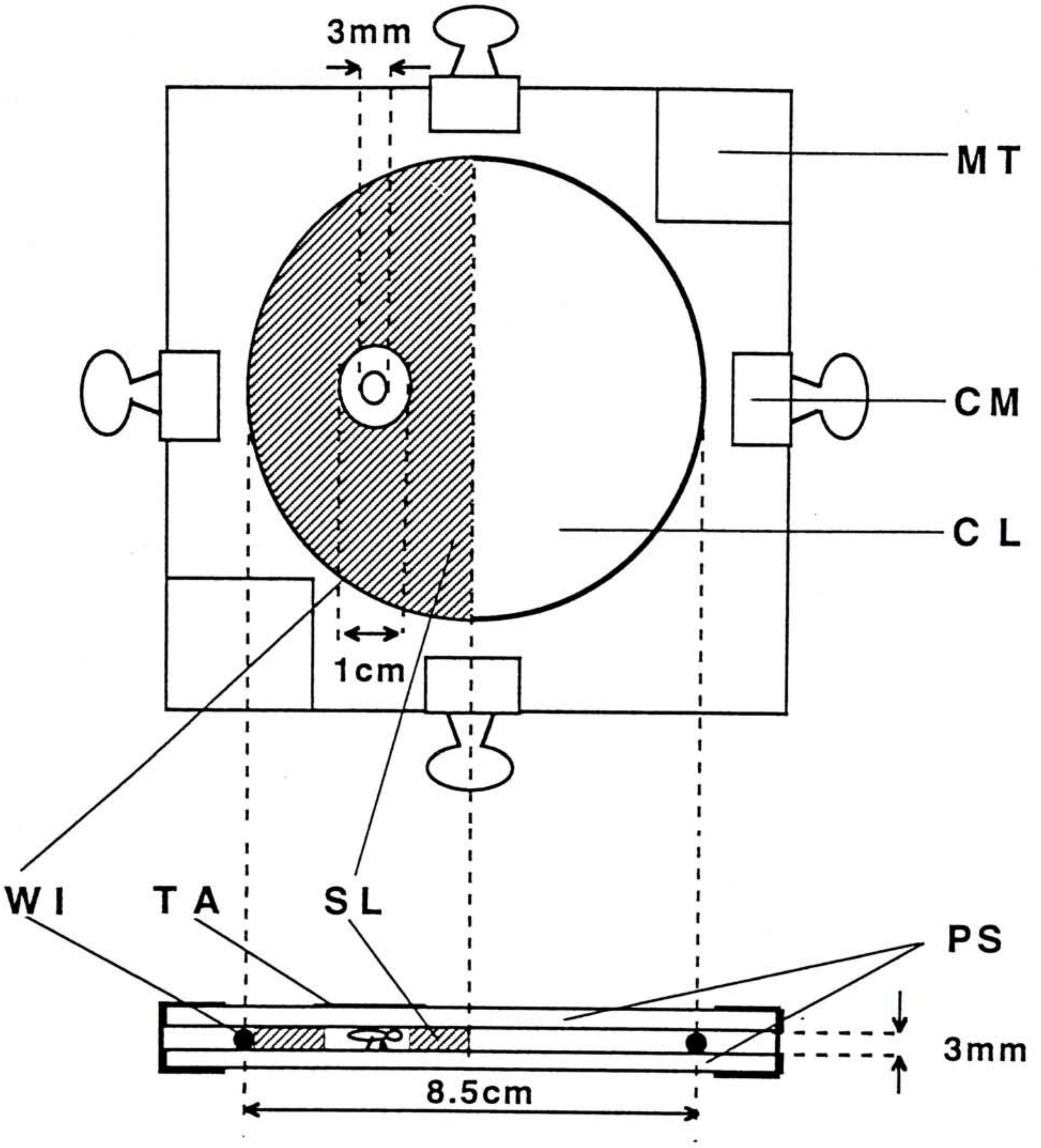


Figure 3.1 Experimental set-up

CL , Clear half of cage (no soil); *CM* , Clamp clip; *MT* , Moist tissue paper; *PS* , Perspex plates; *SL* , Soil; *TA* , Tape covering hole; *WI* , Wire, plastic coated acting as a spacer.

plates were then clipped tightly with two pieces of moist tissue paper (3 x 2 x 1.5 cm) sandwiched at diagonal corners. Tissue paper was kept moist in order to provide moisture to the colony.

3.3.3 Combination of termites in set-up

Ten individuals of each age class and 4 soldiers were put into the 1cm^2 circular zone through the 3 mm hole of the cover plate. Combination of termites in each cage was kept constant (more than 4 individuals of each age class and more than 1 soldier in the cage) throughout the experiment. Daily observation of the cage was performed if the population of termites in cage was constant. At the end of each daily observation, 10 individuals of each age class and 4 soldiers in each cage were restored by adding termites into the cage.

3.3.4 Observation and scoring methods

The cages were arranged in a row at constant temperature of 22 ± 2 °C under 24 hours fluorescent light (65 watts). Cassette recorder for clear voice recording, speed control, microphone sensitivity switch, counter and counter reset, was used to record the data. Termite was randomly chosen for observation by lot. Small sticks labeled with corresponding areas of the grids on the cover plate of the replicate were prepared. Small stick was randomly picked up with closed eyes. Identification of age classes by naked eyes was based on body sizes in section 2.3.2.1 and morphological characters in section 2.3.2.2. The termite in randomly chosen square was observed for 15 seconds. If there was no termite in the randomly chosen square, another stick was randomly picked up again. If there were more than one termite found in the area, termite which is near to the front bench side is chosen for observation.

Once the chosen termite was found, code number of the replicate, and the age class it belonged to were recorded by tape. One-second behavior within 15 seconds were spoken out just after speaking out the age class. Fifteen 1-second behavior was scored continuously in 15 seconds. If randomly chosen termites was found hidden within 15-second observation period, another termites of the same replicate was randomly chosen (as the same way mentioned above) for observation again. Data was written down on score sheet.

Scoring was started when all the 1st, 2nd and 3rd instars settled down inside the wood pieces and tunnels (none of them was found in the open area). Daily observation was performed in 20 cycles. The whole time for 20-cycle observation was about 16 hours. This experiment lasted for 10 week days and a total of 160 hours was spent on observation.

3.3.5 Data analysis

Percentage of total time budget spent on various behaviors of each age class of each cage was sorted out. Correlation of the behavioral frequencies (mean of the % of total time budget spent on a particular behavior of twenty replicates) of the five age classes with their age was tested by the Spearman's correlation coefficient test, in 95% confident level (Heath, 1995). If there is a correlation between particular behavioral frequencies of the age classes and age, continuous temporal polyethism (all age classes perform a particular behavior and the behavioral frequencies correlate with age) existed in this behavior.

Comparison between two successive age classes was held if there was correlation between frequencies of a particular behavior with age. Behavioral frequencies of a pair of successive age classes were compared by using the Wilcoxon signed rank test (two tailed, $p<0.05$). Wilcoxon signed rank test is used to test whether two random samples (with equal sample size) with the same frequencies (or means). If the ranked sum is less than the critical value the null hypothesis of same frequencies (mean) would be rejected (summary from Kanji, 1993; Heath, 1995; Zar, 1996). Multiple comparisons of frequencies of a particular behavior among all age classes are very complicated and relatively meaningless. Crosland *et al.* (1996) introduced Bonferroni procedure: when two pairs of comparisons are made, only probabilities less than 0.025 can be regarded to be significant; probabilities must be less than 0.0167 and 0.0125 for three and four pairs of comparisons to be significant. In order to simplify the statistical methods, one pair comparison was applied for all the experiments of this thesis.

Behavioral repertoire sizes (number of types of behaviors), and behavioral frequencies (mean of % of total time budget spent on a particular behavior of twenty replicates) were presented in pie charts. Behavioral frequencies of the same behavior among the five age classes were also studied in ethograms.

3.4 Results

Behavioral observation of five age classes (small larva, large larva, small worker, medium worker and large worker) was composed of 4000 counts of individuals and 60,000 1-second behaviors of twenty replicates in 20 working days. Sixteen behaviors were identified (Table 3.3).

3.4.1 Correlation between the frequencies of various behaviors and age of different age classes

Frequencies of all task related behaviors except mouth-body touching behavior (MB) were positively correlated with age (Spearman's correlation coefficient test, $p<0.05$) (Table 3.4). Frequencies of general stationary behavior (GS) was negatively correlated with age (Spearman's correlation coefficient test, $p<0.05$).

3.4.2 Repertoire size

Repertoire size of behaviors increased from the SL to LW in the order from 7, 8, 12, 16 and 16 behaviors (Figure 3.2). Considering the behaviors which exceeded 3% in frequency, number of types of behaviors also increased from the earlier instar to the oldest: 2, 3, 6, 8 and 14 (contributed over 90% of total time budget) from SL to LW respectively.

Table 3.3 Comparison of the percentage of total time budget of various behavioral acts of larval and worker age classes.

		AGE CLASSES								
Behavior act	Behavior code	**SL** (N=519)		**LL** (N=555)		**SW** (N=552)		**MW** (N=655)		**LW** (N=1719)
Individual manintenance										
General stationary	GS									
Mean ± SD		77.7±9.85		58.4±10.6		46.8±7.9		16.5±6.1		9.5±3.4
Probability			$p<0.05$		$p<0.05$		$p<0.05$		$p<0.05$	
@ Entrance stationary	ES									
Mean ± SD		0		0		0.5±0.5		0.8±1.7		6.8±3.7
Probability			NS		NS		NS		$p<0.05$	
Open stationary	OS									
Mean ± SD		0		0		0		0.2±0.5		0.3±0.3
Probability			NS		NS		NS		NS	
Moving	MV									
Mean ± SD		15.2±7		21.9±10.1		19.7±13.1		11.3±4.8		5.9±1.9
Probability			$p<0.05$		NS		$p<0.05$		$p<0.05$	
@ Searching	SR									
Mean ± SD		0		0		0.5±0.63		1.5±1.3		2.9±1.7
Probability			NS		$p<0.05$		$p<0.05$		$p<0.05$	
Walking in tunnel	WT									
Mean ± SD		2.1±2.9		10.9±10.7		12±5.7		13.6±6.4		13.2±5.6
Probability			$p<0.05$		$p<0.05$		$p<0.05$		NS	
@ Longitudinal vibration	LV									
Mean ± SD		0		0		1.4±0.7		1.9±1.9		3±1.1
Probability			NS		$p<0.05$		$p<0.05$		$p<0.05$	
Larvae transportation										
@ Larval grasping	GR									
Mean ± SD		0		0		0		0.5±0.3		1±0.7
Probability			NS		NS		$p<0.05$		$p<0.05$	
@ Larval carrying	CA									
Mean ± SD		0		0		0		0.7±0.5		4.7±1.2
Probability			NS		NS		$p<0.05$		$p<0.05$	

N = total number of counts for each instar.
@ : task related behavior
NS: not significantly different (Wilcoxon signed rank test, two paired, $p>0.05$).
p<005 : significantly different (Wilcoxon signed rank test, two paired, $p<0.05$)

Table 3.3 (continued)

		AGE CLASSES								
Behavior act	Behavior code	**SL** (N=519)		**LL** (N=555)		**SW** (N=552)		**MW** (N=655)		**LW** (N=1719)
Social interactions										
Antennae touching	AT									
Mean ± SD		1.5±1.9		2.5±1.7		6.1±2.5		9.4±5.5		4.7±4.1
Probability			NS		p<0.05		p<0.05		p<0.05	
Antenna-body touching	AB									
Mean ± SD		0.2±0.5		1.2±0.5		4.1±3.1		5.6±3.3		5.2±4.1
Probability			NS		p<0.05		p<0.05		NS	
@ Mouth-body touching	MB									
Mean ± SD		1.8±2.3		2.3±1.7		1.7±1.2		17.6±7.5		11.7±8.3
Probability			NS		NS		p<0.05		p<0.05	
Feeding										
@ Mouth touching	MT									
Mean ± SD		1.5±1.8		2.5±1.2		1.7±1.7		3.7±2.2		4.7±2.7
Probability			NS		NS		p<0.05		p<0.05	
@ Mouth-tunnel touching	MW									
Mean ± SD		0		0.3±0.6		5±5		14.6±5.3		10.8±3.6
Probability			p<0.05		p<0.05		p<0.05		p<0.05	
Nest construction										
@ Carrying wood	CW									
Mean ± SD		0		0		0		0.49±1		6.6±2.5
Probability			NS		NS		p<0.05		p<0.05	
Foraging										
@ Walking in open	WO									
Mean ± SD		0		0		0.5±0.3		1.5±2.7		9±5.3
Probability			NS		NS		p<0.05		p<0.05	

N = total number of counts for each instar.

@ : task related behavior

NS: not significantly different (Wilcoxon signed rank test, two paired, p>0.05).

p<005 : significantly different (Wilcoxon signed rank test, two paired, p<0.05)

Table 3.4 **Correlation between the frequencies of various behaviors and the age of different age classes in the worker line of *Reticulitermes fukienensis*.**

	Behavior	behavior code	Spearman's rank correlation test	
			Correlation	r value
	General stationary	GS	p<0.05	**-0.986**
@	Entrance stationary	ES	p<005	**0.975**
@	Open stationary	OS	p<0.05	**0.900**
	Moving	MV	NS	-0.70
	Searching	SR	p<0.05	**0.975**
	Walking in tunnel	WT	p<005	**0.900**
@	Longitudinal vibration	LV	p<0.05	**0.315**
@	Grasping	GR	p<0.05	**0.900**
@	Carrying	CA	p<0.05	**0.900**
	Antennae touching	AT	NS	0.700
	Antenna-body touching	AB	p<0.05	**0.900**
@	Mouth-body touching	MB	NS	0.600
@	Mouth touching	MT	p<0.05	**0.900**
@	Mouth-tunnel touching	MW	p<0.05	**0.900**
@	Carrying wood	CW	p<0.05	**0.900**
@	Walking in open	WO	p<0.05	**0.975**

@ : task related behavior

p<0.05 : significantly different (Spearman's rank correlation test, p<0.05)

NS : not significantly different (Spearman's rank correlation test, p>005)

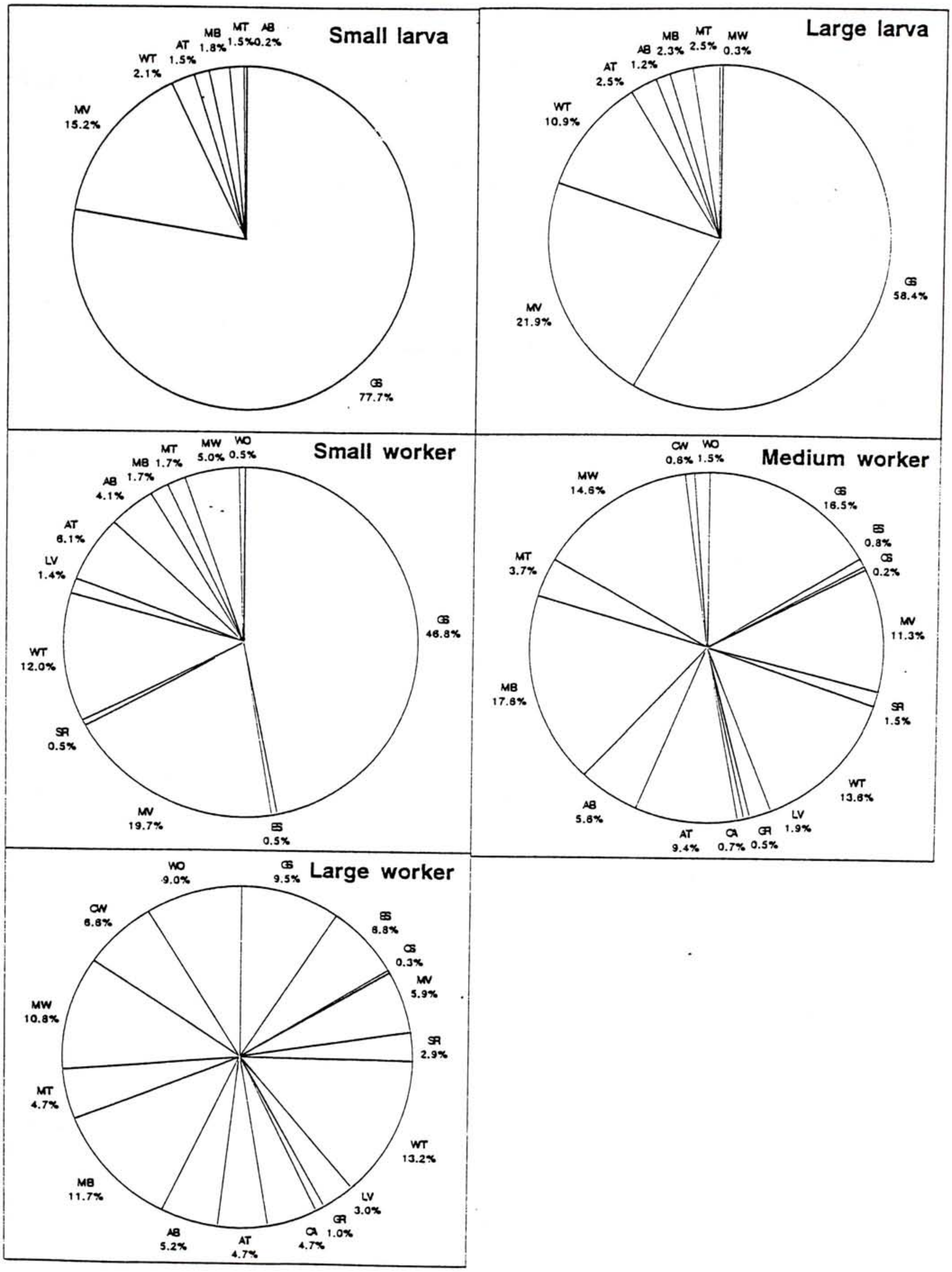


Figure 3.2 Distribution of the % of the total time budget spent on various behaviors by various age classes in the worker line of *Reticulitermes fukienensis*.

3.4.3 Task related behaviors

Number of task related behaviors and the frequencies (mean % of total time budget spent on a particular behavior of twenty replicates) of these behaviors increased from the 1st to 5th instars. Although general stationary behavior, moving behavior, and walking in tunnel behavior contributed over 90% of the total time budgets of SL and LL, none of them was task related. Number of task related behaviors increased from 7 to 9 from SW to LW. % of total time budget spent on tasks increased from 8.6%, 43.15% and 61.35 % is SW, MW to LW respectively.

3.4.4 Number of behavioral categories and % of time budget spent on various behavior categories within age

Number of behavioral categories increased from SL to LW. percentage of total time budgets spent on individual maintenance behavior decreased from SL to LW while the other behavioral categories increased with age. Individual maintenance, social interactions and feeding behaviors were found in all age classes (except 1st and 2nd age classes in feeding behavior). Individual maintenance behavior contributed over 90% of total time budget in all age classes, decreased from SL to LL (SL: 95%; LL: 91.2%; SW: 80.7%; MW: 45.8%; and LW: 41.6%). Task unrelated individual maintenance behavior decreased in the same way from SL (95%) to LW (28.9%). Social interactions behavior and feeding behavior increased from SL to LW but MW exhibited higher % than LW. Foraging behavior switched on from the 3rd instar in relatively low frequency whereas the nest construction behavior and larval carrying behavior started from the 4th instar. The nest construction behavior contributed the lowest % of time budget spent for MW (0.6%) and LW (6.6%). Percentage of total time budget spent on larval carrying behavior increased 4 times from MW (1.2%) to LW (4.8%). Percentage of total time budget spent on nest construction behavior increased 11 times from MW (0.6%) to LW (6.6%).

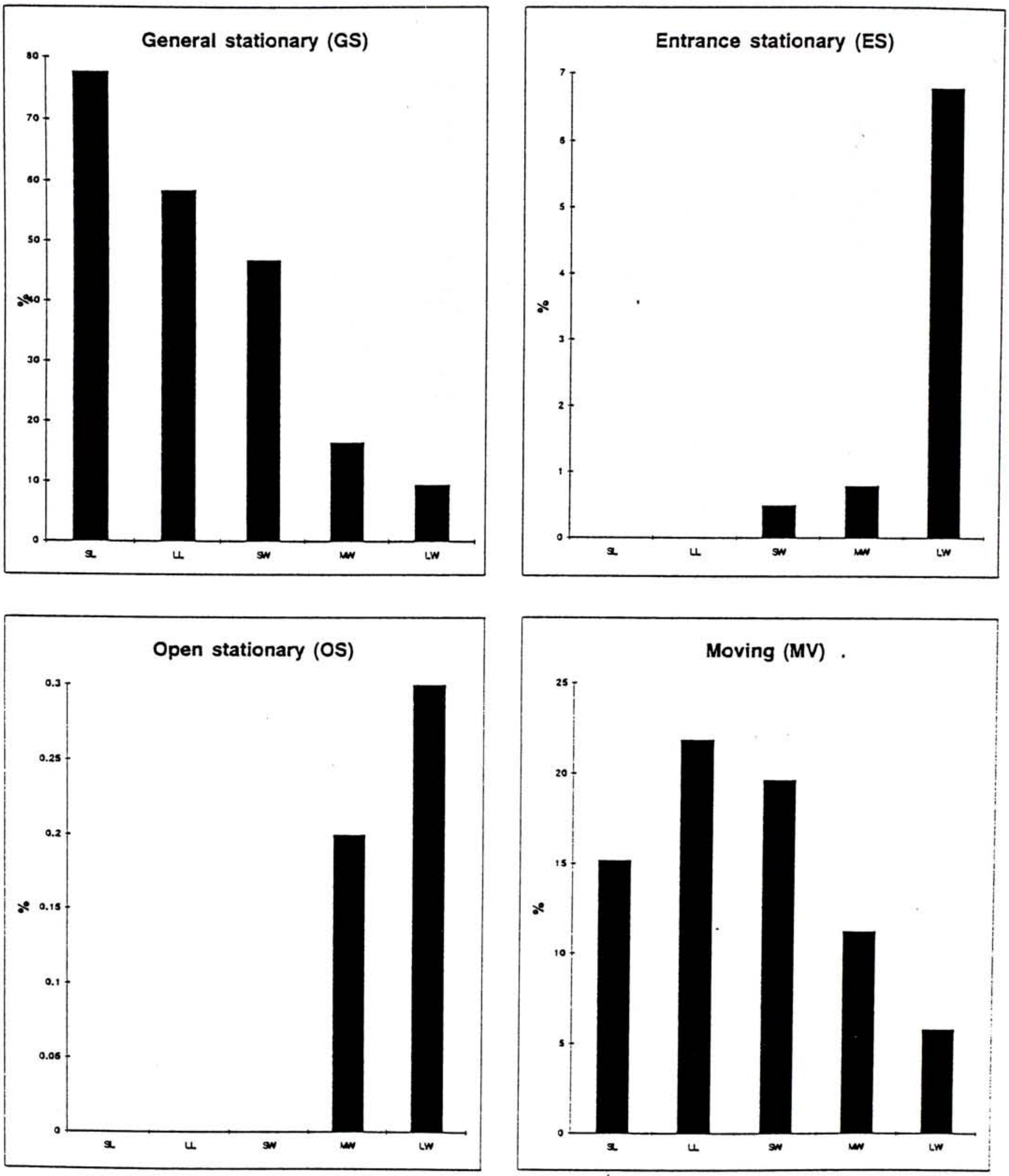
3.4.5 Patterns of behavioral frequencies

Except moving behavior, walking in tunnel behavior, all behaviors were performed significantly among successive stages of the age classes (Table 3.3 and Figure 3.3). Frequencies (mean of % of total time budget spent on a particular behavior of twenty replicates) of behaviors correlated with age were classified into four patterns :

1. Frequencies of non-task related behavior decreased with age. Older individuals inactively participated in this behavior.

Example 1. Frequencies of general stationary behavior decreased from SL to LW. LW was the most active whereas SL was the most inactive. Frequency of SL was 8 times of that of LW.

2. Frequencies of task related behaviors increased with age. Older individuals actively participate in these behaviors.


General stationary (GS)

Entrance stationary (ES)

Open stationary (OS)

Moving (MV)

Figure 3.3 Ethograms of the frequencies (%) of various behaviors of the five age classes in the worker line of *Reticulitermes fukienensis*. SL, Small larva; LL, Large larva; SW, Small worker; MW, Medium worker; LW, large worker.

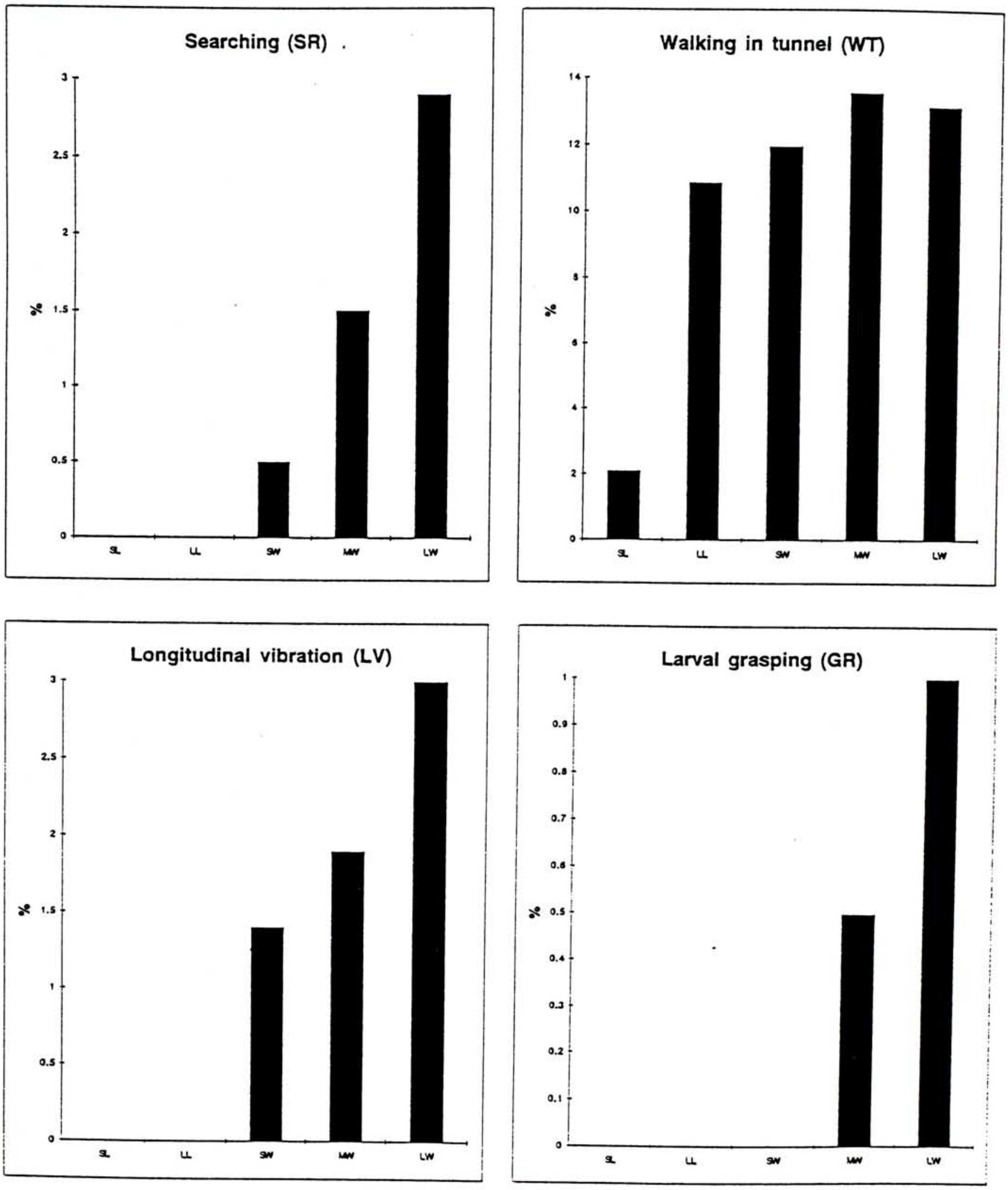


Figure 3.3 (Continued)

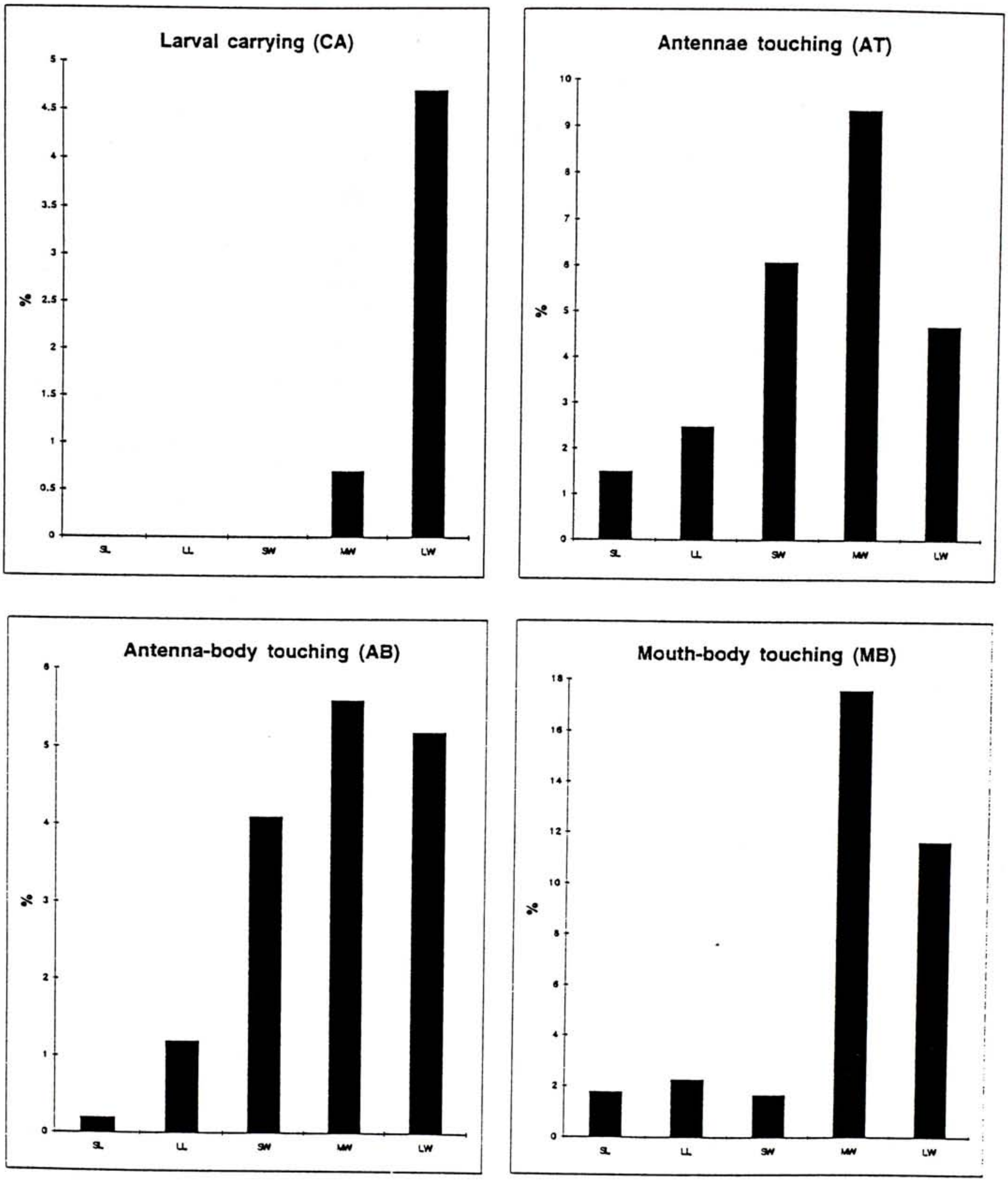


Figure 3.3 (Continued)

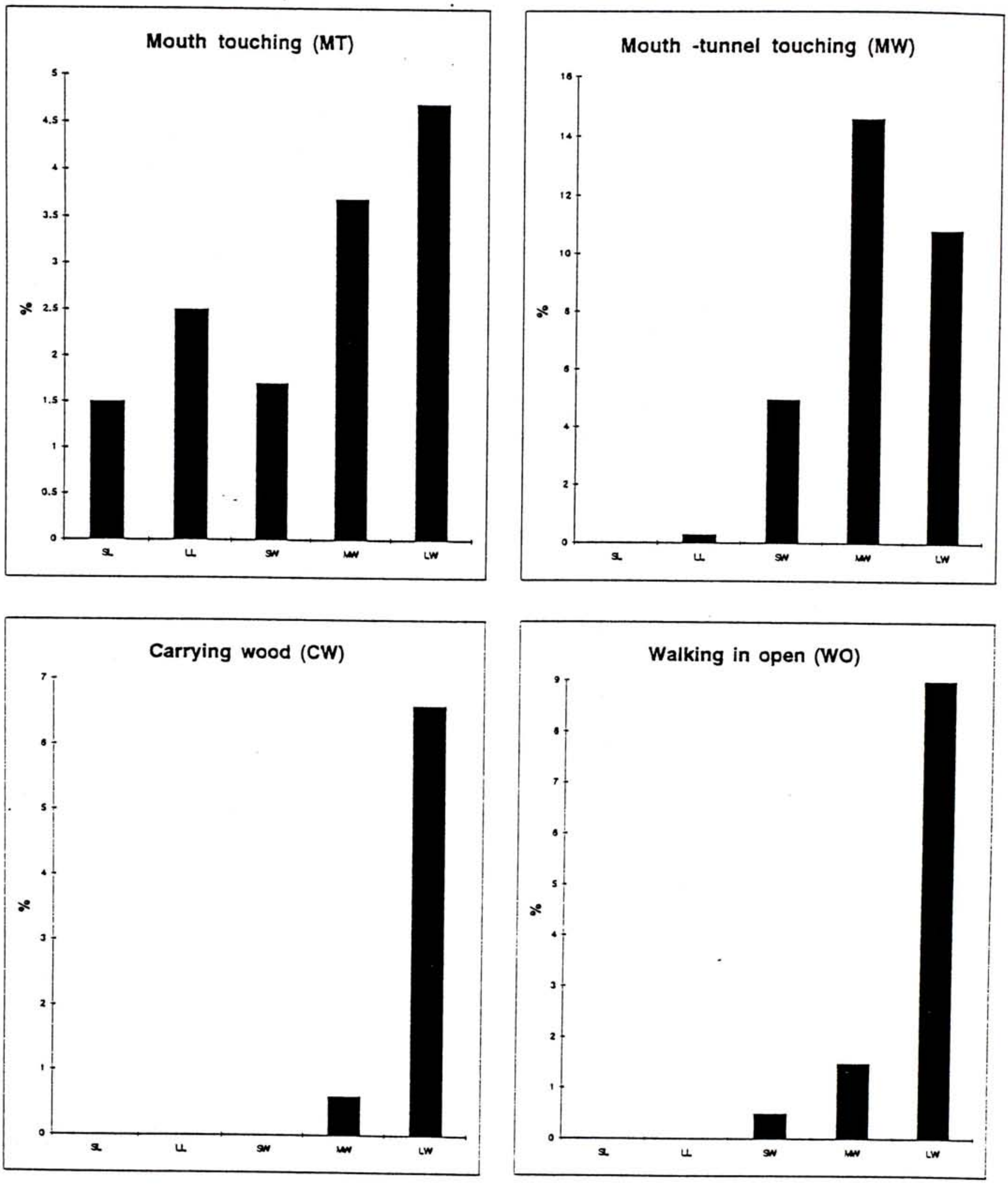


Figure 3.3 (Continued)

Example 1. Entrance stationary behavior was only performed by worker. Frequency increased from SW to LW. Frequency of LW was 8.5 times of MW, 13.6 times of SW. LW was main guard.

Example 2. Searching behavior only existed among workers. Frequency increased from SW (0.3%) to LW (3%). LW were main searchers.

Example 3. Longitudinal vibration behavior only existed among workers. Frequency increased from SW (1.2 %) to LW (3 %). LW was main signal giver.

Example 4. Only MW and LW performed grasping behavior and carrying (larvae) behavior. LW was main larvae carrier.

Example 5. Only MW and LW performed wood carrying behavior. LW was main wood carrier or nest builder.

Example 6. Only worker exhibited walking in open behavior. LW was the most active foragers.

3. Frequencies of task related or non-task related behaviors increased to a maximum and then decreased with age.

Example 1. LL exhibited the highest frequency in moving behavior.

Example 2. Frequency of walking in the tunnel behavior increased from the SL to SW and then decreased to LW. SW presented the highest frequency (13.6 %).

Example 3. Social interactions included antennae touch behavior, antenna-body touch behavior, mouth body touch behavior and mouth touch behavior. All frequencies of these behaviors increased from SL to LW (except that MW was the highest in mouth touch behavior). Sum of the % total time budget spent on these four behaviors: SL<LL<SW<LW<MW. MW and LW were main body cleaners (MW: 17 %; LW: 12 % in mouth-body touch behavior) and main ......larvae feeder (MW: 3.65 %; LW: 4.7 % in mouth touch behavior).

Example 4. Workers exhibited mouth tunnel touching behavior: SW (5.05%) < LW (10.8%) < MW (14.55%). Medium workers was the most active participator in this behavior.

3.5 Discussion

3.5.1 Discrete and continuous temporal polyethism

Temporal polyethism in various behaviors of five age classes in the worker line of *Reticulitermes fukienensis* was exhibited in several patterns. With ethogram (Figure

3.3) it is quite easy to identify discrete temporal polyethism (not all age classes exhibited the behavior) or continuous temporal polyethism (all age classes exhibited the behavior with significant correlation between behavioral frequency of age classes and age; Spearman's rank correlation coefficient test, p<0.05).

If only larval age class and worker age class were considered, discrete temporal polyethism (i.e. not all age classes exhibited the behavior) was exhibited in the behaviors: entrance stationary, open stationary, searching, longitudinal vibration, grasping, carrying (larvae), carrying wood and walking in open behaviors. Continuous temporal polyethism (all age classes exhibited the behavior with significant correlation between behavioral frequency with age, Spearman's rank correlation test, p<0.05) with significant difference in frequencies between LL and SW (Wilcoxon signed rank test, two tailed, p<0.05) was exhibited in the behaviors: general stationary, moving, walking in tunnel, antennae touching, antenna-body touching behaviors.

If all age classes (SL, LL, SW, MW and LW) were considered, continuous temporal polyethism (all age classes exhibited the behaviors with significant correlation between behavioral frequency with age; Spearman's correlation test, p,0.05) with significant difference in frequencies between successive age classes (Wilcoxon signed rank test, two tailed, p<0.05) was exhibited in behaviors: general stationary and mouth tunnel touching behaviors. Discrete temporal polyethism (not all age classes exhibited the behavior) was exhibited in behaviors: entrance stationary, open stationary, searching, longitudinal vibration, grasping, carrying, mouth-tunnel touching, nest construction, and walking in open behaviors.

3.5.2 Inactivity of larvae

Larvae, SL and LL, were the most inactive age classes, they performed no task related behaviors and their behavioral categories were only half of those of large worker. Task related behaviors were switched on from SL or ML and normally increased from the earlier instar to the oldest instar. Large worker was the most active among the age classes. Large worker was the most active guards, signal givers, larvae carrier, searchers, body cleaners, larvae carriers, wood carriers and nest builders, and the most active foragers, showing the highest frequencies among the others. Similar repertoire sizes were found within larvae (8 types) and within older worker, MW and LW (16 types). SW was intermediate between these two size groups.

Rosengus and Traniello (1993) mentioned that the first two instars of the lower termite *Zootermopsis angusticollis* spent approximate 90% of total time budget on task unrelated behaviors. In older instar, amount of time spent on individual maintenance was approximate to 40-50%. Results in this chapter showed that the 1st and 2nd instars of lower termites were always inactive, spending over 90% of their time budget in task unrelated behaviors. The inactivity of larvae might be due to their small body size, soft cuticle, lack of symbionts in their gut, and weak mouth parts which is unsuitable for wood mastication and digestion (Rosengus and Traniello, 1993).

3.5.3 Starting point for the task related behaviors

In the lower termite *Z. angusticollos,* individuals, when they reached the 3rd stage, suddenly exhibited 84 % of the oldest instar's repertoire size and performed 64% of all tasks similar to the older instar's performance (Rosengaus and Traniello, 1993). In this chapter, the 1st and 2nd instars of *R. fukienensis* exhibited 50% of the repertoire size of the large worker but the third instar, small workers, performed 75% of the oldest workers' repertoire size. If the task related behavior is considered only in the repertoire size, then small workers suddenly increased from 37.5% to 62.5% of the large worker's task related repertoire size.

3.5.4 Relationship between morphological characters and behaviors

The age classes can be divided into three groups based on their morphological characters: a) small larva and large larva with white bodies and white mandibles; b) small worker with mandibles partially brown in color and partially sclerotized; c) medium worker and large worker with brown mandibles and pigmented bodies and heads (highly sclerotized). Repertoire sizes of larvae and workers may also be classified into three groups as the same combination based on morphological characters. Highly seclortization of body part with aging may be one of the factors to control the performances of termites.

3.5.5 Task performance amongst worker age classes

SW, MW and LW performed tasks (behaviors achieving purpose). The task related behavioral repertoire sizes and frequencies increased from SW to LW. Larval carrying behavior and nest construction behavior performances were found to be discrete between SW and MW or LW (both MW and LW performed these tasks). The other task related behaviors (such as feeding and foraging) were performed continuously from SW to LW .

Nearly all task related behaviors were carried out at a higher frequency by large worker than by any other worker age classes (e.g. entrance stationary, searching, longitudinal vibration, grasping, carrying (larvae), mouth touching, carrying wood, and walking in open behaviors). Medium workers performed higher frequency in some behaviors (such as mouth-body touch and mouth-tunnel touch behaviors) than the larger worker.

Although frequencies of mouth-body touch and mouth-tunnel touch behaviors exhibited by MW are higher than those of LW, the efficiency and rate of task performance of LW may be higher than MW. It means LW may do task more quickly than MW and so the time spent on the task was relatively shorter.

3.5.6 Mouth-body touching and mouth tunnel touching behaviors

Frequencies of mouth body touching increased from small worker to larger workers and medium worker exhibited the highest frequency. It can be explained that poorly sclerotized teeth of small workers are too weak to handle the cleaning task (mouth body touch behavior) while the powerful and highly sclerotized teeth of large workers are too strong to clean the body, and may easily hurt the body. Teeth of medium worker are just suitable to handle cleaning job.

Frequencies of mouth-tunnel touching behavior increased with age, the medium worker exhibited the highest frequencies. Teeth of small worker are too weak for mastication of wood (food). Teeth of large workers are powerful to bite the wood and break it down very quickly and efficiently. Therefore large worker need not spend so much time for malaxation. Teeth of medium workers are less powerful than those of large workers, so they spend more time for mastication.

3.5.7 Division of task related behaviors

Among the ethograms (Figure 3.3), two sets of behaviors were performed out of phase by five age classes. General stationary and moving behaviors (non-task related) were performed in phase by different age classes. The other behaviors (most of them are task related) except general stationary and moving behaviors were performed in phase by different age classes. If task related behaviors were considered, the older stage of the age classes performed almost all the task related behaviors, small worker performed the least, and the two larval age classes took up none task related behaviors. Number of task related behaviors increased with age. No task related behaviors will be disappeared in the later stages of the age classes. The older the individuals were, the more tasks they performed. In eusocial hymenopterans, some tasks would be lost with age while some task would be gained with age. *In R. fukienensis*, Number of task performed by workers increased with age and no tasks would be lost.

In this chapter, the general patterns of temporal polyethism in various behaviors are studied. Important behaviors such as trophallaxis (related to feeding the dependent individuals) and foraging (related to finding food), which are mostly studied by other researchers in the temporal polyethism of termites, exhibited a small portion of the total time budget of individuals. Because of the importance of these behaviors, further studies are continued in chapters 4.

Chapter 4

TEMPORAL POLYETHISM IN TROPHALLAXIS, LARVAL CARRYING AND FORAGING BEHAVIORS OF WORKER AGE CLASSES

4.1 Introduction

This chapter discribes two experiments: the first experiment had been done to study the temporal polyethism in trophallaxis and larval carrying behaviors of medium and large worker classes, the second experiment had been done to determine the temporal polyethism in foraging behavior among the worker age classes. In chapter 3, it was found that temporal polyethism in various behaviors were exhibited in different patterns. In general larvae were the most inactive age classes. Medium and large workers were the most active task related behavior performers whereas small worker was intermediate between them. Larvae care , feeding and foraging behaviors are quite important (section 4.2, section 4.3) to the colony but they were allocated only small portion of time budgets by age classes. The following experiment was designed to gain a better understanding of temporal polyethism in the larvae care and foraging behaviors among the worker classes.

Between the small and large larvae, only large larvae were used in the first experiment because of their similar behaviors and morphological characters. Medium and large workers have fully sclerotized mandibles and high frequencies in the performances of the task related behaviors. They were recognized as the true workers (see chapter 3). These larvae and workers were put together, frequencies of vicinity of larvae, head touch, anus touch, front grasp and back grasp behaviors (they are defined in section **4.5.1.1**) were scored.

In the second experiment, worker age classes were placed together in a 'no food nest', and frequencies of their foraging behaviors (defined in section **4.5.2.3**) were scored at instance during observation period. The higher the frequencies of these behaviors, the greater the activity for foraging of termites is. The higher the frequencies of staying on filter paper and feeding on filter paper, the greater the ability of food exploration the termites have.

4.2 Trophallaxis of eusocial insects

Trophallaxis permits efficient use of nutrients, recognition of colony mates, inter-individual communication, distribution of pheromones involved in caste differentiation and in caste elimination, and transfer of cellulose-digesting protozoans in termites families (Wilson, 1971; Brian, 1983). It plays a 'group effect', which leads to greater working efficiency, greater longevity, and greater vigor of social insects in groups as opposed to individuals in isolation (Brian, 1983).

The aspect of trophallaxis concerned in this chapter is food exchange. Workers are responsible for distributing nutrients to all the other castes which are unable to feed themselves. Termites exchanged food by one or both of the two methods: stomodeal feeding and proctodeal feeding (Wilson, 1971). Stomodeal feeding is that donor offers food to recipient from its mouth. Stomodeal food may be a clear fluid, or a pastelike consistency containing fragments of wood in suspension. Proctodeal feeding is that donor offers food to recipient from its anus. All lower termites within the same colony participate in food exchange by mean of proctodeal and stomodeal feeding (Wilson, 1971).

Workers represent an engine to drive the society (Brian, 1983). Food malaxation is done by workers with well sclerotized teeth. Worker are principal donors of both stomodeal and proctodeal food (Brian, 1983). Head touch behavior and anus touch behavior (defined in section **4.5.1.1**) were supposed to be related to trophallaxis. The higher the frequencies of these behaviors, the higher the frequency of trophallaxis of termites performed.

4.3 Social carrying behavior of eusocial insects

In social ants societies, social carrying behavior is one of the most remarkable social activities (Möglich and Hölldobler, 1974). The worker ants frequently carry eggs, larvae, pupa, adult workers, queens and males to various target areas. Social carrying behavior serves as a "social timer" in *Camponotus herculeanus* during the nuptial flight activities. During the daily flight periods, the males that tend to take off too early or too late were carried back into the nest by the worker nestmates (Hölldobler and Maschwitz, 1964).

However, in many ants, carrying behavior is frequently employed for emigration from one nest site to another site if a nest becomes too small and cannot be extended, or the microclimatic conditions change (Möglich and Hölldobler, 1974). Moreover, adult transportation is the basic recruitment technique of most ant species (Möglich and Hölldobler, 1974). Carrying behavior patterns in ants are very specific. *Myrmecia* grasps another one at the mandibles or any other part of the body and drags it over the ground. Reteenmeyer (1963) noticed that workers of the New World army ants (tribe *Ecitonini*) carry larvae, pupa and adult slung beneath the body and between the legs of transporting worker. *Odotomachus* ant simply grasps the leg, the petiole or other body part of a nestmate, and lifts it up and carries it away (review by Möglich and Hölldobler, 1974).

In termites, abdomen of termite queen is swollen with eggs so that she cannot easily move her body. Normally, queen is surrounded and fed by attendant workers, eggs are transported by workers to suitable location for hatching (Wilson, 1971).

It is found that once termite nest is cut open, large and medium workers carries larvae (both small and large larvae) and eggs away from exposed area and enter into tunnels. Workers carry larvae in the following patterns: workers use their mandibles to grip larva's abdomen in front of the larvae or back of the larvae while the longitudinal axis of the worker and the longitudinal axis of the larvae are parallel or combine to form an axis. This pattern is suggested to be very efficient for transportation inside tunnels because termites meet the least hindrance in moving in tunnels. The purpose of larval carrying may be related to emigration of termites from nest site to another, and escape of the young from dangerous environment such as exposure to predators.

4.4 Foraging behaviors of eusocial insects

All individuals in an insect society share their food in a nest space but only some go out and get in; these foragers are usually older and larger than average (Brian, 1983). Involvement of older individuals in activities outside the nest is a basic rule in social insects (Oster and Wilson, 1978). Above statement is also true for termites, where older workers are recognized participating in the activities outside the nest such as exploration, foraging and mound repairing (Pasteels, 1965; McMahan, 1970, 1977, 1979; Garnier-Sillam, 1983a, 1983b, 1983c).

In the past, an important aspect of studies of temporal polyethism in foraging studies was the determination of the relative proportions of the foraging population of various castes and instars of each castes within typical colonies. For example, the caste composition of parties in *Drepanotermes perniger* engaged in foraging was determined by collecting groups from single foraging holes (Watson and McMahan, 1978). Foraging workers of *Nasutitermes exitiosus* were collected from dead wood lying within about 2m of the just-sample mould (McMahan, 1977). Gerber *et al.*, (1988) studied polyethism related to foraging in *Macrotermes bellicosus* by fast extraction of the forager population of a buried bucket which was filled with food, 10 m away from the mound. Many studies (Watson and McMahan, 1978; McMahan, 1977; Badertscher *et al.*, 1983.) also give evidences that workers in the later stages take foraging task of bringing food to the underground colony. The main principle is that foragers are usually older and larger than average.

4.5 Materials and methods

4.5.1 Trophallaxis and social carrying behaviors experiment

4.5.1.1 Definition of behaviors

Head touch, anus touch, front grasp, back grasp behaviors and larval vicinity were defined in Table 4.1. Frequencies (number of count) of head touch and anus touch behaviors are suggested to be proportional to the frequencies of stomodeal and proctodeal trophallaxis respectively. Frequencies of front grasp and back grasp behaviors are supposed to be proportional to the frequencies of larval carrying. Larval vicinity is supposed to be proportional to the frequency of larval care related behaviors.

4.5.1.2 Experimental set-up

Three LL, 3 MW and 3 LW were transferred to the surface of moist filter paper (Whatman, No. 1) in Petri dish (5.5 cm in diameter) with cover. Twenty replicates were prepared and arranged into rows and kept at $22\pm2^{\circ}C$ under 24 hours fluorescent light (65 watts). The arrangement was covered with a plastic box with moist tissue water if daily observation was finished. Petri dishes were not covered during observation because moisture condensed on the covers will hinder observation. The filter paper was moisten with water at the end of daily observation.

Table 4.1 Definition of behaviors exhibited by worker age classes in the worker line of *Reticulitermes fukienensis*

Behavior	Behavior code	Description of behavior
Head touch	HT	Both individuals are stationary. The anterior end of large larva's head or mouth touches the anterior end of worker's head or mouth.* It is related to stomodeal trophallaxis (Wilson, 1971).
Anus touch	AT	Both individuals are stationary. The anterior end of large larva's head or mouth touches worker's anus.* It is related to protodeal trophallaxis (Wilson, 1971).
Front grasp	FG	worker uses its mandibles to grasp the large larva's abdomen from the front of large larva, pick it off from the ground whilst walking.*
Back grasp	BG	worker uses its mandibles to grasp the large larva's abdomen from the back of large larva, picks it off from the ground whilst walking.*
Vicinity		
Larval vicinity		The total number of workers within 1 cm of large larva in 8 days.

* The behavior is scored one count whether the behavior is last 2 or up to 120 seconds. If the behavior last more than 120 seconds, one more count will be scored.

4.5.1.3 Observation methods

This experiment lasted 4 days. Larval vicinity was recorded twice daily. Larval vicinity of LL with MW and LW in each Petri dish was recorded at instance 15 minutes after removal of covers of Petri dishes, and after the observation of grasping and touching behaviors.

Observation of head touch, anus touch, front grasp and back grasp behaviors between LL and MW or LW were started in cycles for one hour just after the first score of larval vicinity. If a behavior was exhibited by an individual for more than 2 minutes, then the behavior was scored as second count. Air current was avoided in order to reduce evaporation of the moist filter paper, and to reduce disturbance of termites.

4.5.1.4 Data analysis

Wilcoxon signed rank test (two tailed, $p<0.05$) was used to compare the means (mean of the total number of counts of twenty replicates) of medïum worker and large worker. Definition of terms for statistic was stated below :

Larval vicinity = mean of total number of worker of the same age class within 1 cm of a larva of twenty replicates throughout the experiment (in 4 days).

Frequency of behavior = mean of total number of counts of a particular behavior of twenty replicates scored throughout the experiment (for 4 days).

4.5.2 Foraging behaviors experiment

4.5.2.1 Experimental set-up

Experiment was performed in 20 replicates. 10 SW, 10 MW and 10 LW of the same colony were placed in each replicate. In chapter 5, SL and LL seldom go out from the 'nest', so they were not introduced in this experiment.

Experimental set-up was shown in Figure 4.1. Four holes of 0.5 mm in diameter were made (two adjacent holes form 90^o with the center of the dish) on the edge (near the bottom) of Petri dish as entrances. A plastic box with cover (32 x 20.5 x 11 cm) was used as incubation chamber. The floor of the plastic box was scratched to be rough by sand-paper so that termites can walk steadily on the floor.

Sand (0.6 to 2.0 mm in length) was purchased from gardening shop. It was placed in tap water overnight. Then it was washed by continuous flowing tap water for 10 minutes in order to remove salt. Next it was dried in oven at 105°C overnight. 2.5g of sand was used to fill the Petri dish. 6.5 ml distilled water was pipetted to the sand. Thus sand provided water but no food to termites. Sand which covered the entrance was swept inward so that termite can pass through the holes. The Petri dish holding sand without cover was placed on an single layer of sand(1.5 g) in the center of the box. Food was provided by placing four regular pieces of unwet filter paper (2 cm x 2 cm) at the near corners (16 cm from the center of the box).

Termites were placed on the surface of the sand in the Petri dish. One day was used for termites settlement. Termites would move out from the Petri dish through the

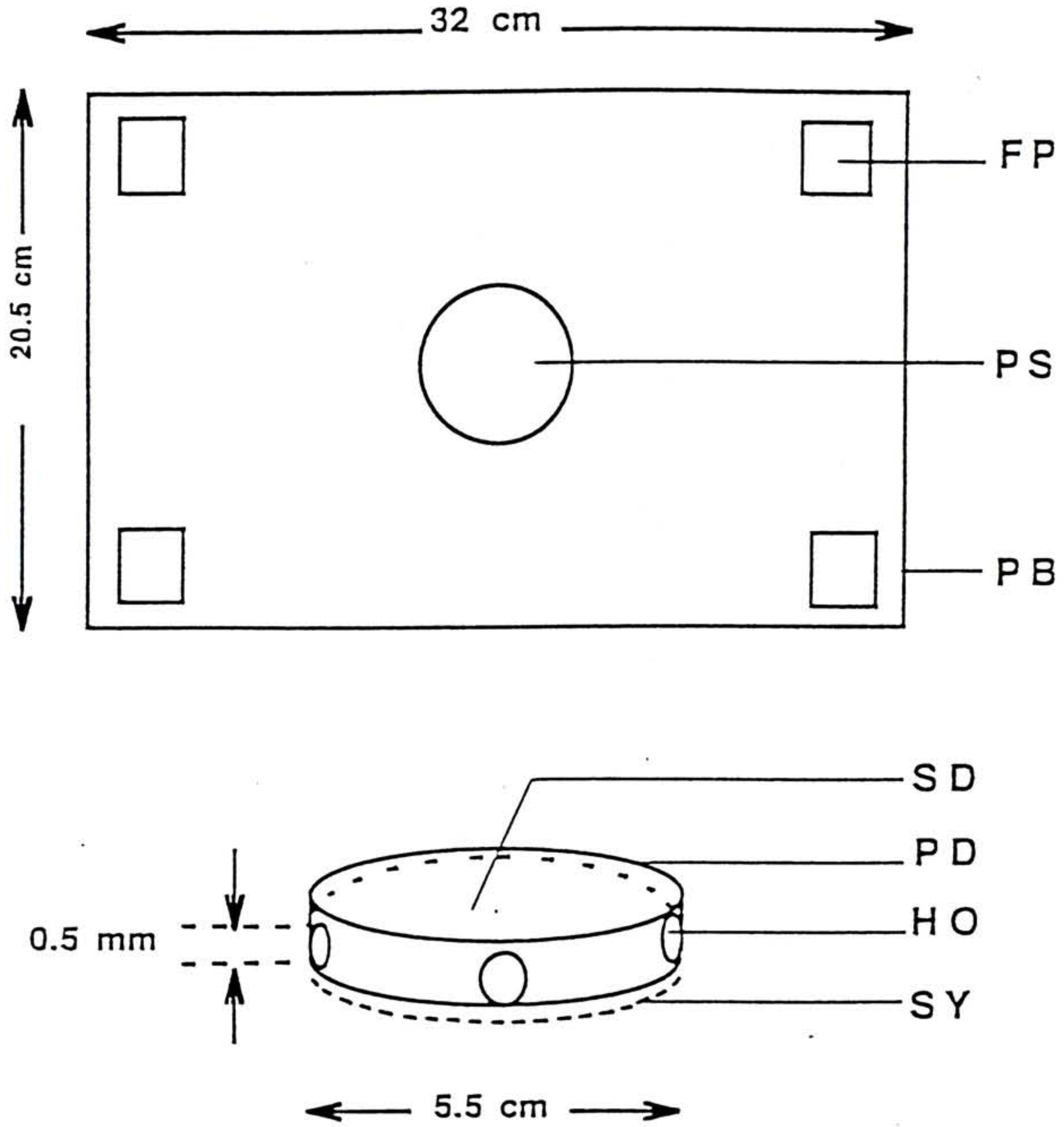


Figure 4.1 Experimental set-up

FP, Filter paper 2 x 2 cm; *HO* , Hole acting as entrance; *PB* , Plastic box 32 x 20.5x 11 cm, covered with transparent film; *PD* , Petri dish; *PS* , Petri dish containing moist sand; *SD* , Sand; *SY* , A layer of sand.

entrances and settle down in the sand layer. Plastic film (Saran Wrap Brand Plastic Film) was used to cover the box and sealed with rubber bands. A piece of circular transparent tape (1 cm in diameter) was stick on the center of the plastic film through which water could be injected by syringe to the sand bed. All replicates were kept at 22±2°C under 24 hours fluorescent light (65 watts).

4.5.2.2 Observation methods

Behaviors were recorded instantly (13 times per day) at intervals (at least half an hour). The experiment lasted 5 days. Observation lasted 13 hours or more from 9:00 to 22:00 daily. Three types of behaviors were recorded: a) foraging in the arena; b) staying on the filter paper; and c) feeding on the filter paper (see definition in section **4.5.2.3**). The replicate number, kind of workers, and their behavior were recorded on worksheet. At the end of the experiment, the number of survivors of each replicates were counted. The data is acceptable in the condition that at least 6 SW, 7 MW and 8 LW survived.

4.5.2.3 Definition of behaviors

Three types of behaviors were defined:

1. Foraging in arena (A) :	individual appears on the open area (arena), but is not walking or standing on filter paper.

2. Staying on : filter paper (B)	individual appears on the open area (arena), walking or standing on filter paper, but is not feeding on the filter paper.
3. Feeding on : filter paper (C)	individual appears on the open area (arena), using its mandibles to bite or tear the filter paper whether it is on the filter paper or not. While standing on the filter paper, its head is bending downward with mandibles perpendicular to the filter paper for feeding.

4.5.2.4 Data analysis

Five categories of data were analyzed :

1. frequencies* of foraging in arena behavior (A);

2. frequencies* of staying on filter paper behavior (B);

3. frequencies* of feeding on filter paper behavior (C);

* Frequency (or mean of total number of counts) was defined as follow:

$$\text{Frequency} = \frac{Yi \text{ x } yi + Yii \text{ x } yii + \ldots\ldots + Yxx \text{ x } yx}{N}$$

Y = Total number of a particular behavior exhibited by the same kind of workers in a replicate in the observation period.

y = correction factor = the number of survivor of the same kind of workers in a replicate at in the end of the experiment/ the number of these workers in the same replicates used for the experiment.

N = number of the replicates.

where *i, ii . . . . . . xx* are respective to the first, second to twentieth replicates.

Spearman's rank correlation coefficient test (refer to section **3.3.5**) was used to test the correlation between behavioral frequencies with age. Wilcoxon signed rank test (refer to section **3.3.5**) was used to compare the frequencies of various behavior of the workers classes of acceptable replicates. Probabilities presented for the test are two-tailed in 95 % (or 0.05).

4.6 Results

4.6.1 Trophallaxis and larval carrying behaviors

Among the four kinds of behaviors, only front grasp behavior was found significantly different (Wilcoxon signed rank test, two tailed, $p<0.05$) between medium worker and large worker. Frequency of head touch behavior (HT) of medium worker was higher than those of large worker (Table 4.2), while large worker exhibited higher frequency in anus touch behavior. Head touch behavior was exhibited in higher frequencies than anus touch behavior by worker age classes (15 to 50 times). Therefore

Table 4.2 Comparison of trophallaxis related behaviors in medium and large worker age classes

	Worker age classes	
	MW	LW
Head touching (HT)		
Mean ± SD [a]	9.8±11.0	6.3±5.0
Probability	NS	
Anus touching (AT)		
Mean ± SD	0.2±0.4	0.4±1.0
Probability	NS	

a : Mean of the total number of counts (frequency) during the observation period of 20 replicates.

NS : not significantly different (Wilcoxon signed rank test, 2 tailed, $p>0.05$)

head touches behavior was more popular than anus touch behavior. In other words, stomodeal trophallaxis is more popular than proctodeal trophallaxis among workers (trophallaxis was supposed to be related to head touch and anus touch behaviors).

Large worker exhibited higher frequency both in front grasp and back grasp behaviors than medium worker (significant difference in front grasp behavior; Wilcoxon signed rank test, two tailed, $p<0.05$) (Table 4.3). The frequency of front grasp behavior exhibited by large worker was 8.5 times of that exhibited by medium worker.

There is no significant difference in the frequencies of larval vicinity between medium and large workers (Wilcoxon signed rank test, two tailed, $p<0.05$). Larval vicinity for medium and large workers were 39.5 and 34.8 respectively. Larval vicinity of medium worker (39.5) was greater than large worker's (34.8) (Table 4.4).

4.6.2 Foraging behaviors

Seventeen replicates were acceptable at the end of the experiment. The means of survived small, medium and large workers in replicate were 10, 9 and 6 respectively. All the behavioral frequencies were found correlated with age (Spearman's rank correlation coefficient test, $p<0.05$).

Large workers exhibited the highest frequencies, medium workers exhibited very low frequencies while small worker exhibited zero frequencies of the three types

Table 4.3 Comparison of grasping behaviors in medium and large worker age classes

	Worker age classes	
	MW	**LW**
Front grasp (FG)		
Mean ± SD [a]	0.2±0.4	1.7±2.0
Probability	$p<0.05$	
Back grasp (BG)		
Mean ± SD	1.1±2.0	2.2±3.0
Probability	NS	

[a] Mean of the total number of counts (frequency) during the observation period of 20 replicates.

NS : not significantly different (Wilcoxon signed rank test, 2 tailed, $p>0.05$)

$p<0.05$: significantly different (Wilcoxon signed rank test, 2 tailed, $p<0.05$)

Table 4.4 Comparison of the vicinity of larvae with medium and large worker age classes

	Worker age classes	
	MW	**LW**
Vicinity of larvae		
Mean ± SD [a]	39.5±13.1	34.8±10.0
Probability	NS	

a Mean of the total number of counts (frequency) during the observation period of 20 replicates.

NS : not significantly different (Wilcoxon signed rank test, 2 tailed, $p>0.05$)

of foraging behaviors. Differences in frequencies (or mean of total number of count) of various behaviors among these workers were significant (Wilcoxon signed rank test, two tailed, $p<0.05$) (Table 4.5).Large worker was the most active forager and explorer among worker age classes.

Considering frequencies of activities in food source (staying on filter paper and feeding on filter paper behaviors), large worker was the main explorer of food (i.e. their success in finding food source), they contributed 90% while medium worker was 10% of total frequencies (sum of the frequencies exhibited by small, medium and large workers) of activities in food source (filter paper).

High frequency in feeding on filter paper behavior exhibited by large worker and the highest survivorship (10 large workers were found in each replicates at the end of the experiment) among worker age classes suggest that LW has the highest ability to find and feed on food.

4.7 Discussion

There is no significant differences in larval vicinity, head touch and anus touch behaviors between medium and large workers, it reflects that medium and large workers are feeding the larvae in similar level. The frequencies of head touch behavior are much higher than anus touch behavior (15 to 50 times) reflect individuals of *R. fukienensis* mostly obtain food from their nestmates through stomodeal trophallaxis. Moreover large worker is the most active larva carrier.

Table 4.5 Comparison of foraging behaviors in different worker age classes.

	Worker age classes				
	SW		MW		LW
Foraging in arena (A)					
Mean ± SD [a] [b]	2.7±2.9		21.7±16.5		143.3±67.9
Probability [c]		p<0.05		p<0.05	
Staying in filter paper (B)					
Mean ± SD	0		1.8±4.0		8.5±5.3
Probability		p<0.05		p<0.05	
Feeding on filter paper (C)					
Mean ± SD	0		0.5±0.7		12.4±9.0
Probability		p<0.05		p<0.05	

---

[a] Mean of the total number of counts (frequency) during the observation period of 20 replicates and standard deviation.

[b] The frequency is already corrected by factor (the number of surviors over the initial number of individuals).

[c] significantly different (Wilcoxon signed rank test, 2 tailed, p<0.05).

Frequencies of front grasp behavior are significantly different between medium and large worker. Back grasp is more popular than front grasp behavior (frequency of back grasp is higher than frequency of front grasp). Large workers performed higher frequencies of front grasp and back grasp than medium worker. It is probably because large worker had bigger mandibles, bigger body parts than small and medium worker, and large worker can carry heavy weights from one site to another site

Large worker and medium worker are found as main feeder for the young (small worker performed the lowest frequencies in feeding related behaviors in chapter 3). This finding does not agree with the principle widely applied in honey bee and other social Hymenoptera that young workers perform the task of distributing food, feeding their nest mates in the center of the nest while the older workers are outside the nest foraging and do no larval care related tasks (Wilson, 1976b, 1985; Jeanne, 1991; Seeley, 1982).

Discrete temporal polyethism (not all worker age classes exhibited behaviors) in foraging behaviors exist in the worker age classes. Frequencies of foraging behaviors are correlated with age. All the behavioral frequencies in foraging between successive worker age classes are significantly different. Large worker is main active forager and explorer. In chapter 3, large worker is the main foragers (% time budget spent on walking in open area behavior: Small worker is 0.5 %, medium worker is 1.5 %, large worker is 9 %). Watson (1978) mentioned that in termite *Drepanotermes*, more than 75 % of above ground foraging parties are performed by the oldest instars (fourth and fifth instars), and frequencies of foraging behaviors increase with age. All these findings show that the latest instars are the main foragers. Large worker exhibits the highest frequency in the behavior of feeding on filter paper. It is partly because large worker is the most active workers to perform various tasks and partly because large worker may feed small worker, the inactive forager and inactive explorer in the 'nest'.

Past researches about temporal polyethism in foraging behaviors of termites are based on the proportions of foraging population in the field (McMahan, 1977; Watson and McMahan, 1978; Badertscher *et al.*, 1983, etc.). Although evidence is scant, foraging is mostly performed by larger and older workers. For example, 97% of the foragers of *Macrotermes subhyalinus* are of age over 30 days after molting while only 5% of the foragers are less than 26 days of age (Badertscher *et al.*, 1983). The fourth and fifth (the last) stages of workers of *Drepanotermes perniger* contribute over 75% of the foraging parties (Watson and McMahan, 1978). In *Hodotermes mossambicus*, workers which cut grass are older and stronger and darker than those which work in the nest (Watson , 1973).

Unlike social hymenoptera which have only one worker stage, there are two larval stages and at least three worker stages in the worker line of termite *R. fukienensis*. Both the oldest workers of termite *R. fukienensis* and eusocial hymenopteran perform foraging behaviors. Lenoir (1979) explained that old workers are bigger than the average of age classes and have highly developed systems or organs. They have well developed tough pigmented skin to meet the out door work which is heavy and dangerous and required sensitivity to environmental cues and intelligent flexibility.The big body size of large workers may give them more strength to collect food. Compared with the small worker and larvae, large worker can store more reserved body food. Large workers can perform long lasting work with consuming their reserved body food (such as foraging) especially when they meet the risk of food shortage.

Sudd and Frank (1987) pointed out foragers are almost always the oldest workers. Foraging is the most dangerous task. Workers run outside the nest will take the risk of meeting predators, falling into deadly territorial battles or simply getting lost (Lenoir, 1979). Foraging is best performed by the oldest workers because they are close to the end of their physiological lives. The young have useful life span to make contribution to the colonies without taking the risk of facing enemies during foraging (Sudd and Frank, 1987).

In conclusion, large worker is the active worker performing larval carrying behavior and foraging behaviors among the worker age classes because it possesses the most biggest body, strong and largest mandibles and well sclerotization among the worker age classes.

Chapter 5

TEMPORAL POLYETHISM OF VARIOUS BEHAVIORS AMONG SUB-AGE CLASSES OF LARGE WORKER

5.1 Introduction

In chapter 2, five age classes were separated based on head width measurements and morphological characters. Buchli (1958) pointed out that further molting of *Reticulitermes lucifugus santonensis* worker occurs after the 9th stage although no conspicuous difference can be detectable. Five to 11 instars may be found in the worker line of *Reticulitermes species* (Buchli, 1958; Husby, 1980) Therefore, the last (fifth) age class of *R. fukienensis* possibly consists of sub-age classes (section **2.3.4**). In chapter 3, it was found that large worker performed all task related behaviors in higher frequencies than medium worker and small worker. Because large workers were the most active age class performing all tasks, it is worth to determine whether temporal polyethism exist in various behaviors among the possible sub-age classes or not.

The experiment in this chapter was done to determinate the temporal polyethism in all behaviors among the possible sub-age classes of large worker. First, large larvae and paint marked individuals of large workers were put together in cages for direction at time interval. Frequencies of various behavior performed by individuals inside and outside the 'nest' were scored. Sub-age class separation of the marked large workers based on various biometrics measurements of these workers was followed. Then frequencies of various behaviors performed by sub-age classes were presented in ethograms.

Principal components analysis and discriminant analysis were used for the separation of the sub-age classes. Head width was found as the main factor for the separation of the sub-age classes by principle component analysis. Then Spearman's rank correlation coefficient test and Mann-Whitney U test were used for data analysis of the behavioral frequencies of the sub-age classes. Spearman's rank correlation coefficient test was used to test the correlation between a particular behavior with age, and the correlation between two behaviors. If frequency of a behavior was correlated with age, Mann-Whitney U test was used to test difference in frequencies of a particular behavior between successive sub-age classes (Mann-Whitney U test can be applied for pairs of data with unequal sizes).

Principal components analysis is an advance multivariate mathematical method for restating information in an original set of variables in terms of a new set of variables in a linear combination of the variables with successively smaller variances. It is difficult to deal with variables in more than two and three dimensions. Principal component analysis reduces the number of dimensions and allows the investigators to capture most of the information in the original data set as much as possible. Supposed that there are five variables, principal component analysis is used to restate them into five principal components with linear combination of the variables with the first component expressing most information whereas the fifth component expresses the least. Eigenvectors in principal component analysis are constant factors in a component. The higher the value of the constant, the more important the factor contributing to the component is. Eigenvalues are variances of the principal components. If the variables are highly correlated, the first few principal components can account for a large proportion of the total variance. If some of the eigenvalues are small, we can neglect them and represent the points with fewer dimensions (Rencher, 1995).

One of the applications of principal component analysis is group separation. Jeffers (1967) applied principal component analysis to a sample of 40 alate adelges (winged aphids) on which 19 variables (such as the body length, body width, forewing length, etc.) had been measured. The objective for the study was to determine the number of distinct taxa present in the habitat where the samples were collected. Since the first two components account for 85% of the total variance, four major groups can be found by plotting the first two components (Figure 5.1) (Rencher, 1995).

Discriminant analysis is a multivariate technique used for the description of group separation and for prediction or allocation. In describing of group separation, group membership is identified. In prediction or allocation, the measured values for an individual or object can be evaluated by the classification functions to see to which group it most likely belongs.

5.2 Materials and methods

5.2.1 Combination of individuals for experiment

Six replicates were used in this experiment. Each replicate had 30 large workers and 100 large larvae.

5.2.2 Labeling of individuals for identification

Heads of large workers were labeled with color paint (Brand: Gunze Sangyo)

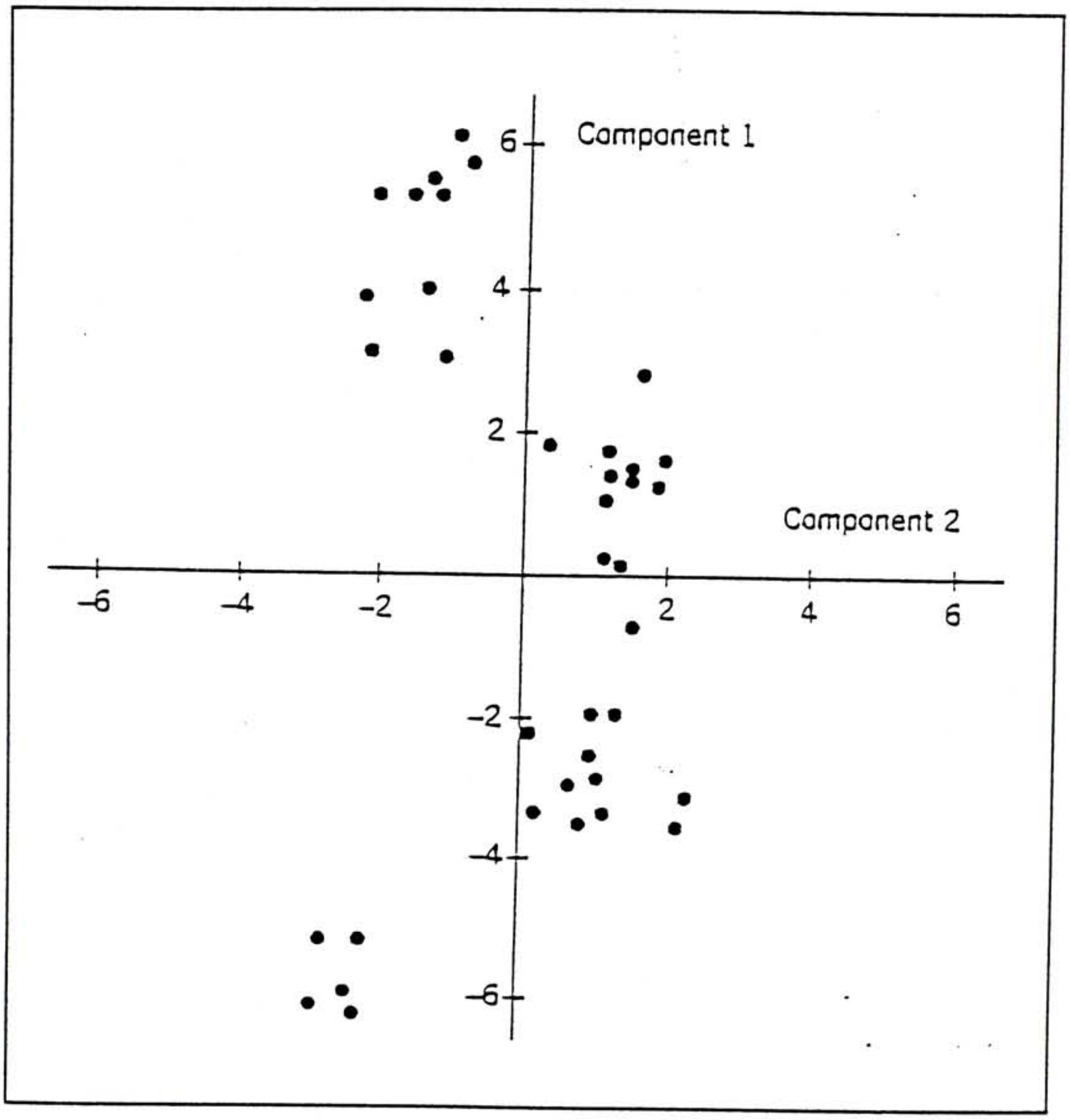


Figure 5.1 Plotted values of the first two principal components for winged aphid data (40 individuals). There are four major groups. The groupings form an interesting S shaped. (Modified from Jeffers, 1967 by Rencher, 1995).

dot. Dorsal side of head was chosen as the site for marking because it was highly sclerotized with thicker cuticle than the other body parts and of which no spiracles could be found. The marked paint would not cover spiracles and hinder the respiration of large workers.

A transparent plastic sheet 2 cm wide and 4 cm long was placed on the dorsal side of thoracic and abdominal part of a large worker. This kept termite in a position that it could not move its body, with the dorsal side of its head just facing the ceiling.

The tip of a thin plastic stripe (0.25 mm thick, 1 mm wide and 15 cm long) was dipped in the paint at a depth about 3 mm. The stripe was held horizontally with the face (not the ridge) facing the ceiling and the tip with the paint was managed very carefully to touch the dorsal side of head without touching antennae. Paint was marked on the head of termite after touching. Paints of different color were marked on the head of termites separately.

5.2.3 Experimental set-up

The experimental set-up is shown in Figure 5.2. A circular loop (8.5 cm in diameter) was placed on a piece of transparent acrylic plate (13 x 18 cm) whose surface was carefully roughened with fine sand-paper. Two grams of sand (desalted) were placed evenly into 8 squares (1 cm^2) on the left half of the confined area (named as area A). Each small square was 3 mm apart from the others. Tunnel width of the nest ranged from 1.5 mm to 3 mm as the tunnel width of their nest in the field. Two wires

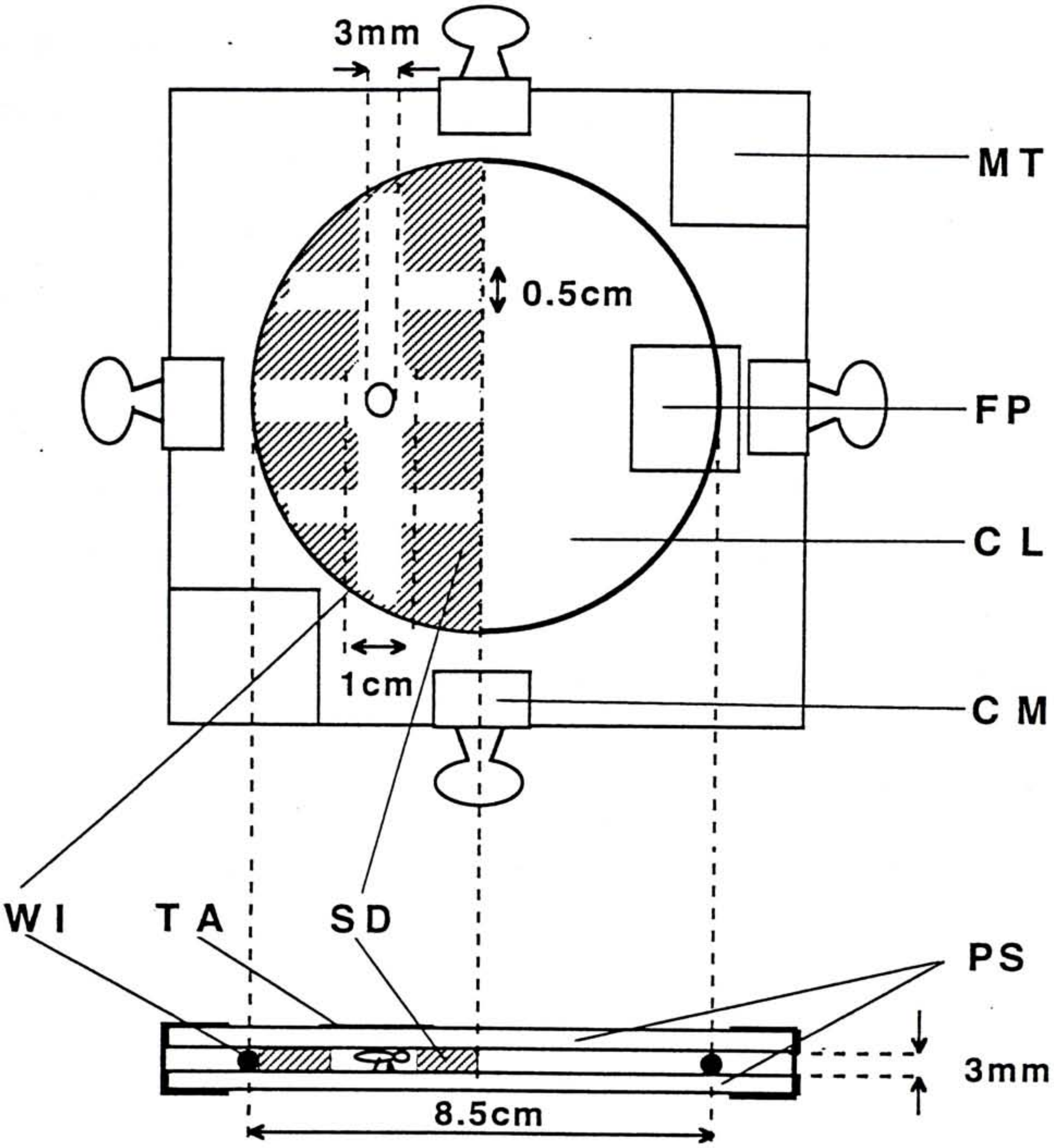


Figure 5.2 Experimental set-up

CL , Clear half of cage (no soil); *CM* , Clamp clip; *MT* , Moist tissue paper; *PS* , Perspex plates; *SD* , Sand; *TA* , Tape covering hole; *WI* , Wire, plastic coated acting as a spacer; *FP* , Filter paper.

4.5 mm long were placed on the diameter of the confined area with 3 mm apart (tunnel width) from the other and from the circular loop (this makes three entrances to the 'nest'(sandy area).

Twenty grams of nest fragments including wood and soil were ground with 100 ml of distilled water. The mixture was filtered, and the filtrate, named as soil water, was used to moisten the sand (soil water and its smell made termites settle down in the sandy area as their nest). The amount of soil water in the sand was controlled at less than 0.5 cm^2 in area of soil water that leaked from the sand. Sand and soil water provided no food to the termites.

An unwet filter paper (the only food source) with size 1 x 3 cm was placed on the furthermost point from area A on the right half of the confined area. Two pieces of tissue paper moistened with distilled water were placed at opposite corners in order to maintain the moisture of the colony (Tissue paper was kept wet all the time by spaying distilled water on it occasionally). A second transparent acrylic plate with a small hole 3 mm in diameter (it was drilled in a position just above the middle of area A of the first acrylic plate and covered with a small plastic film) covered the first acrylic plate. The plates were then clipped tightly. Paint marked termites were put into area A through the small hole of cover plate. Replicates were arranged into one row and were covered with plastic boxes (drying was prevented). The replicates were kept at constant temperature of 21-22°C under florescent light (60 watts). Termites were left for settlement at least 24 hours before observations.

5.2.4 400 observation cycles score

A color chart was constructed with the colors used for marking arranged in order: red, orange, yellow, . . . black. It was used to recognize the color marks of the large workers and gave sequencing order for all the large workers in the same replicates for observation. Behavior scoring started with the large worker having color mark corresponded to the first color of the color chart, and ended with to the large worker having color mark corresponded to the last color of color chart. Behaviors of all marked large workers in the same replicate were scored, followed by those in another replicate, and so on.

Once target large worker was found, the first behavior observed just after 2 seconds was scored. One cycle was completed when all the marked large workers in the six replicates were observed. Twenty cycles were performed per day for 20 days. Considering 12 seconds were used for one large worker behavior scoring, then to 20 cycles took a total of approximately 12 hours of observation per day.

Observation for one replicate was performed under the condition that at least 25 living large workers were present. If the number of living large worker is less than 25, then large workers without color mark were added into the replicates until there were 30 living large workers. Observations for the replicate with new immigrants were performed in the following day. At the end of twenty cycles, the number of living large workers was restored to 30 by adding large workers with no color mark. The number of large larvae was restored to 30 by adding large larvae. If all replicates fulfilled the acceptance criteria (section **5.2.5**), 36000 behavioral acts will be scored.

5.2.5 Acceptance criteria of behavior data

Data of each replicate is considered useful and acceptable if: at the end of the experiment, at least 15 color marked large workers survived. The whole set of data for the experiment is useful and acceptable if: at least 3 acceptable replicates were left at the end of the experiment. Data of dead individuals or of those lost their marks (paint) must be rejected. Only data of color marked survivors were considered.

5.2.6 Definition of behaviors and biometric parameters

Definition of behaviors was listed in Table 5.1. It is lightly different from those listed in table 3.1 because of the slightly difference in experimental set-up between chapter 3 and 5. Eight biometric parameters were defined in Table 5.2 and Figure 5.3.

One hundred and eighty large workers were used for behavioral observation. Only 115 of these large workers survived at the end of the experiment and were preserved in 80% ethanol separately. A microscope (KYOWA, Model SZW) with zoom 1.6x was calibrated with scale. The number of antenna segments, antenna length, head width, pronotum width, mesonotum width, metanotum width, pronotum length, hind tibia length, hind femur length, and body length of preserved large workers of various groups were measured by using the calibrated microscope.

Table 5.1 Definition of behaviors exhibited by the six sub-age classes in large worker.

	Behavior	Behavior code	Description of behavior and its related funtions
1	**General stationary**	GS	standing without any body motion (except the movement of antennae) and displacement in the nest.
2 @	**Entrance stationary**	ES	standing in the opening of the tunnel without any body motion (except the movement of the antennae) and displacemant in the nest. It is related to guarding the nest (Wilson, 1971).
3	**General moving**	MV	body moving without any displacement in the nest.
4	**General walking**	WT	walking inside the nest with its mandibles holding nothing.
5 @	**Longitudinal vibration**	LV	body vibrates to and fro rapidly along the longitudinal axis within 1 second in the nest or in the open area. It is related to giving signals or alarms (Stuart, 1969; Wilson, 1971).
6 @	**Larvae carrying**	CA	holding the larva (touching or clear from the groud) with its mandibles whether walking or standing in the nest or in the open area. It is related to transportation of the larvae (Wilson, 1971).
7	**General touching**	GT	using its antenna to touch another individual's body parts (except the mouth) such as thorax, abdomen or legs.
8 @	**Grooming with larva**	MB2	using its mandibles or other mouthparts to touch or bite the larva's body except mouthparts. It is related to body cleaning of the larvae (Wilson, 1971).
9 @	**Grooming with worker**	MB5	using its mandibles or other mouthparts to touch or bite the worker's body except mouthparts. It is related to body cleaning of the workers (Wilson, 1971).
10 @	**Feeding the larvae**	FL	using its mandibles or other mouthparts to touch the larva's mouthpart in the nest or in the open area. It is related to stomodal feeding the larvae (Stuart, 1969; Wilson, 1971).
11 @	**Oral trophallaxis with worker**	MT5	using its mandibles or mother mouthparts to touch the worker's mouthparts. It is related to stomodal feeding or exchanging body fluid with another worker (Wilson, 1971).
12 @	**Anal trophallaxis**	MA	using its mouthparts to touch the larva's or worker's anus. It is related to proctodeal feeding by sucking the anal fluid from another individual (Wilson, 1971).
13 @	**Self feeding**	SF	chewing a fragment of filter paper whith using its mandibles and other mouthparts whether it is walking or standing in the open area; or biting the filter paper in a posture that the head is bending downward with the mandibles perpendicular to the surface of the filter paper or tearing a small fragments from the filter paper whether th termite is on the filter paper or not. It is related to feed itself (Wilson, 1971).
14 @	**Sand handling**	SH	handling the sand particles by carrying, holding, pushing or pulling with using mandibles or other body part inside or outside the nest. It is related to nest construction (Stuart, 1969; Wilson, 1971).
15	**Open stationary**	OS	standing in the open area or in the filter paper without any motion
16 @	**Open walking**	OW	walking in the open area with its mandibles holding nothing. It is related to foraging (Stuart, 1969; Wilson, 1971).
17 @	**Open moving**	OMV	body moving without any displacement in the open area. It is related to foraging (Stuart, 1969; Wilson, 1971).
18 @	**Paper carrying**	PC	carrying a fragment of filter paper with its mandibles while walking or standing in the nest or in the open area. It is related to foraging (Wilson, 1971).

@ = task related behavior.

Table 5.2 Definition of biometric parameters of large worker.

1. Number of antenna segments (**NANT**)	counted from the first segment of antenna connected to head plate.
2. Head width (**HEAD**):	maximum width from left to right margin of head.
3. Pronotum width (**PRO**):	maximum width from left to right margin of pronotum.
4. Mesonotum width (**MESO**):	maximum width from left to right margin of mesonotum
5. Metanotum width (**META**):	maximum width from left to right margin of metanotum
6. Pronotum length (**PROL**):	maximum length from the anterior end to the posterior end of pronotum.
7. Hind tibia length (**TIBI**):	measured from the distal end to the proximal end of hind tibia.
8. Hind femur length (**FEM**):	measured from the distal end to the proximal end of hind femur.

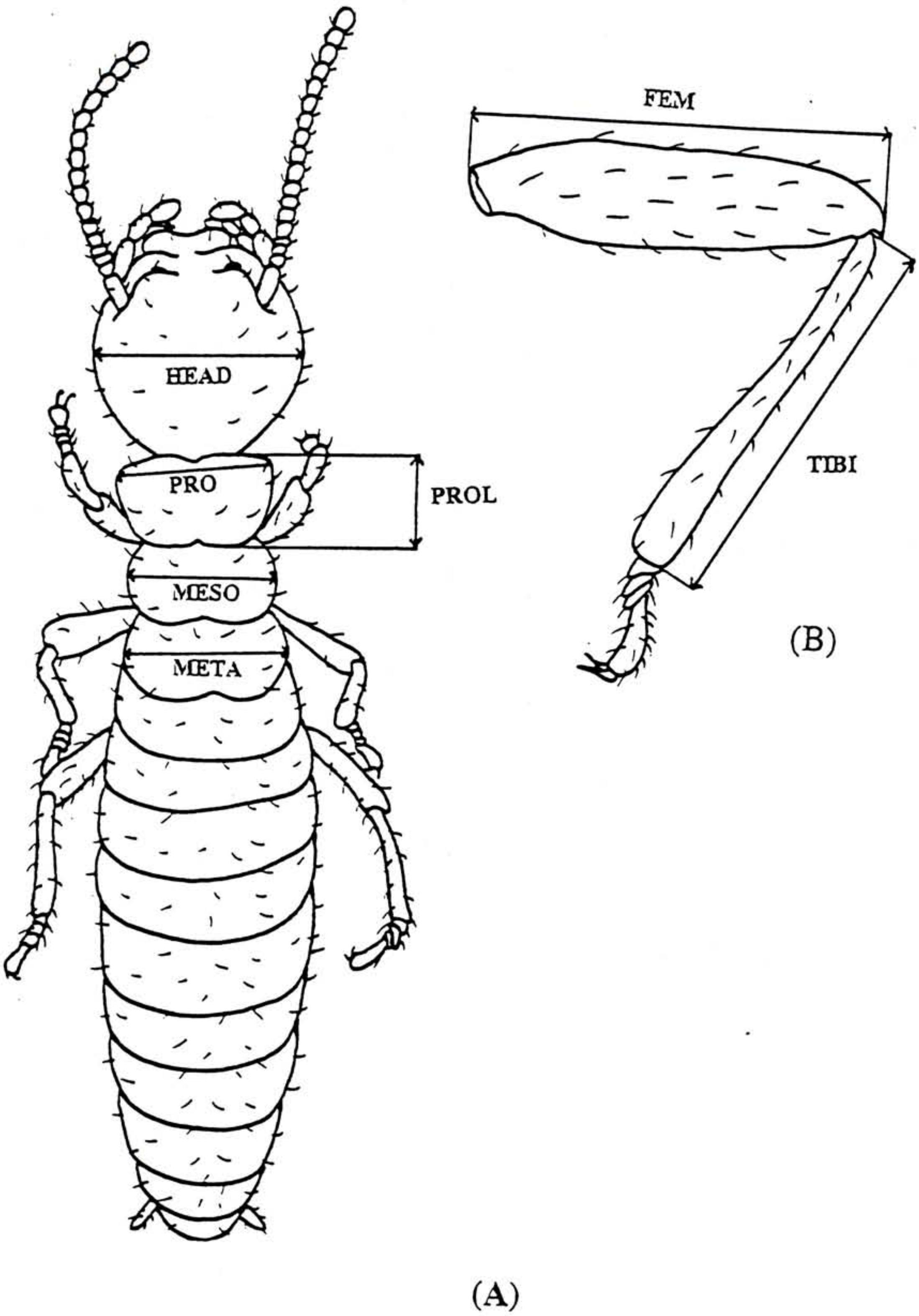


Figure 5.3 (A) Dorsal view of a large worker of *Reticulitermes fukienensis* ; (B) lateral view of right hind leg of a large worker of *Reticulitermes fukienensis.*

FEM, Hind femur length; HEAD, Head width; MESO, Mesonotum width; META, Metanotum width; PRO, Pronotum width; PROL, Pronotum length; TIBI, Hind tibia length.

5.2.7 Principal component analysis (PCA) for sub-age classes separation

Principal component analysis was used for sub-age classes separation in large workers. The 8 variables used were head width, pronotum width, mesonotum width, metanotum width, pronotum length, hind tibia length, hind femur length, and number of antenna segments. These parameters were used because their dimensional size were constant (exoskeleton is too hard to expand in length and width due to osmotic condition of individual bodies). Both the body and the antenna have many junctions between segments. Because body length and antenna length varied due to influence of the osmotic condition of individual bodies, these two parameters were not chosen for sub-age classes separation. PRINCOMP of SAS program was used for PCA analysis. Data of 8 variable was input into the PRINCOMP program. Correlation matrix, eigenvalues of the correlation matrix, eigenvectors for the first four components of large worker of *Reticulitermes fukienensis* were generated. A scatter diagram of the PCA scores was plotted using the first two principal components as axes. Groups of data were separated based on the point distribution of the scatter plot.

5.2.8 Discriminant analysis for sub-age classes classification

Groups separated by PCA were classified by discriminant analysis of the SPSS program. All cases (one case consists a set of 8 biometric parameters of a large worker) belonging to each group were input into the discriminant analysis program. Memberships of the cases within group and among groups were identified in percentage. Number of unfit cases was identified and the best fit groups for these cases were recommended. Allocation of the unfit cases into other groups proceeded until 100% (or maximum %) membership of group cases correctly classified was reached.

5.3 Statistical analysis of the behaviors

Spearman's rank correlation coefficient test was used to assess the positive or negative correlation of behavioral frequencies with age. Behaviors can be separated into different groups based on the correlation between behavioral frequency and age.

Mann-Whitney test was used to assess the difference in frequencies of behavior between two successive sub-age classes (if there is a correlation between behavior and age).

5.4 Results

5.4.1 Sub-age classes separation

All replicates fulfilled the acceptance criteria stated in section **5.2.5**. One hundred and eighty individuals of large workers were used for the experiment but only 115 individuals survived at the end of the experiment. Eight parameters of these 115 marked individuals were analyzed by principal component analysis and discriminant analysis. The mean and standard deviation, the correlation matrix of the principal component analysis, the eigenvalues and first four eigenvectors of the biometric parameters data are shown in Tables 5.3, 5.4, 5.5 and 5.6 respectively. Principal component analysis converted the eight biometric parameters into eight principal components with linear relation. The first principal component is composed of eight parameters with nearly equal eigenvector values. It means that all the parameters contributed equal importance to the first component, which accounts for 71% of the

Table 5.3 Mean and standard deviation of the biometric measurement of 115 individuals of large workers.

Variables	Mean	Standard deviation
Number of antenna segments	15	1
Head width (mm)	1.04	0.07
Pronotum width (mm)	0.65	0.07
Mesonotum width (mm)	0.66	0.07
Metanotum width (mm)	0.78	0.07
Pronotum length (mm)	0.38	0.04
Hid tibia length (mm)	0.73	0.07
Hind femur length (mm)	0.68	0.06

Table 5.4 Correlation matrix for 8 variables of 115 large workers by principal component analysis.

Variables	NANT	HEAD	PRO	MESO	META	PROL	TIBI	FEM
NANT*	1.000							
HEAD	0.333	1.000						
PRO	0.621	0.441	1.000					
MESO	0.596	0.357	0.880	1.000				
META	0.607	0.420	0.769	0.855	1.000			
PROL	0.623	0.454	0.866	0.820	0.768	1.000		
TIBI	0.601	0.465	0.815	0.757	0.761	0.783	1.000	
FEM	0.611	0.438	0.752	0.701	0.669	0.713	0.829	1.000

*NANT, Number of antenna segments; HEAD, Head width; PRO, Pronotum width; MESO, Mesonotum width; META, Metanotum width; PROL; Pronotum length; TIBI, Tibia length; FEM; Femur length.
Confidence level : 95 %

Table 5.5 Eigenvalues of the correlation matrix of 8 variables of 115 large workers by principal component analysis.

Component	Eigenvalue	% of variance	Cummulative %
1	5.68	71.0	71.0
2	0.76	9.5	80.5
3	0.51	6.3	86.8
4	0.40	5.0	91.9
5	0.26	3.2	95.1
6	0.17	2.1	97.2
7	0.14	1.8	99.0
8	0.08	0.9	100.0

Table 5.6 Eigenvectors for the first four components of 8 variables of 115 large workers by principal component analysis.

Component	1	2	3	4
NANT*	0.31	-0.12	0.90	0.24
HEAD	0.38	0.95	0.00	0.17
PRO	0.39	-0.10	-0.19	0.04
MESO	0.38	-0.23	-0.27	0.27
META	0.37	-0.11	-0.18	0.36
PROL	0.38	-0.06	-0.14	0.15
TIBI	0.38	0.01	-0.07	-0.44
FEM	0.36	0.02	0.11	-0.71

*NANT, Number of antenna segments; HEAD, Head width; PRO, Pronotum width; MESO, Mesonotum width; META, Metanotum width; PROL; Pronotum length; TIBI, Tibia length; FEM; Femur length.

total variance. In the second principal component, head width is the most important factor (eigenvector of head width is 0.95, which is very large compared with the second important factor, femur length with eigenvector of 0.02) (Table 5.6). The second principle component accounts for only contributed 9.5% of the total variance. The first two principal components account for 80.5% of the total variance. Six major groups, apparently corresponding to the range of head width sizes of large workers could roughly be separated based on the scatter plot of the PCA scores against the first two PCA axes (Figure 5.4).

These six groups were then classified by discriminant analysis. Unfit cases were rearranged into another groups until 100% (or the maximum %) of group membership was found. The number of cases in each group was displayed in Table 5.7 with 100 % of group cases correctly classified. In Table 5.8 and Table 5.9 (standard canonical discriminant function coefficients, and structure matrix) head width was the most important factor for the first function for discriminant classification and the largest absolute correlation between each variable and any discriminant function. It was found that all members that belonged to the same group had the same head width sizes. Head width represents the other parameters for sub-age classes separation. With the assumption that head width is correlated with age, these six groups were named as sub-age classes **A**, **B**, **C**, **D**, **E** and **F** with their age in increasing order.

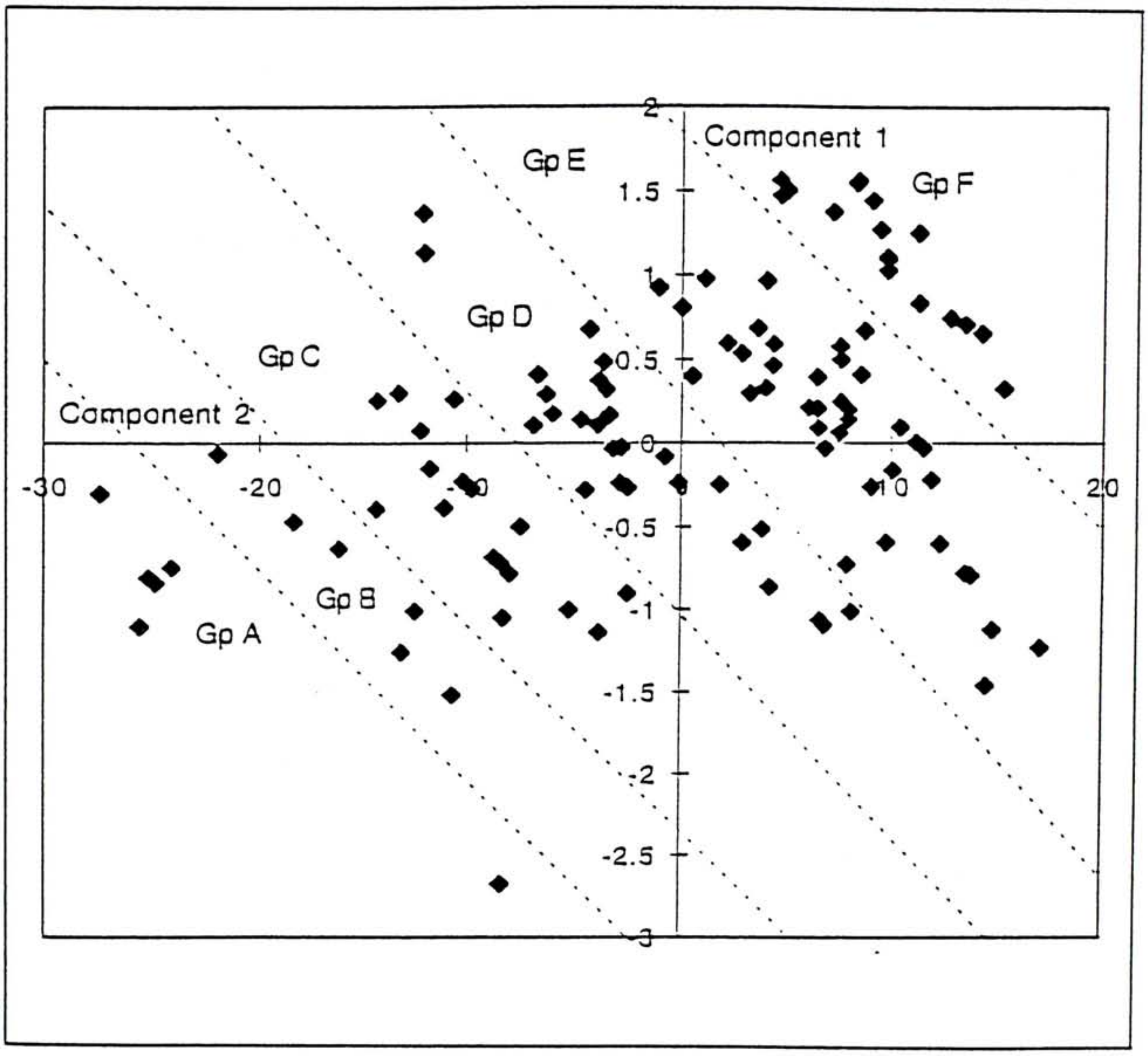


Figure 5.4 Plotted values of the first two principal components for large worker data (115 individuals) by Principal component analysis. There are six major groups: Gp A, Gp B, Gp C, Gp D, Gp E and Gp F.

Table 5.7 Predicted group membership of the six major groups: A-F of large workers by discriminant analysis.

Actual group	No. of cases*	Predicted group membership (%)					
A	5	100	0	0	0	0	0
B	7	0	100	0	0	0	0
C	15	0	0	100	0	0	0
D	30	0	0	0	100	0	0
E	40	0	0	0	0	100	0
F	17	0	0	0	0	0	100

Percent of 'group' cases correctly classified : 100%

---

*No. of cases: number of large workers classified into the group.

Table 5.8 Standardized canonical discriminant function coefficients of the 115 large workers data by discriminant analysis.

Function	1	2	3	4	5
NANT*	-0.05	0.10	0.10	0.46	0.40
HEAD	1.16	-0.11	-0.01	0.01	0.03
PRO	0.78	0.33	-0.05	-0.63	-0.36
MESO	-0.35	0.31	0.20	0.44	0.88
META	-0.25	0.23	-0.32	-0.01	0.11
PROL	-0.15	0.02	0.70	-0.55	-0.21
TIBI	0.25	0.20	-1.12	-0.09	-0.20
FEM	-0.01	0.14	0.70	0.73	-0.62

*NANT, Number of antenna segments; HEAD, Head width; PRO, Pronotum width; MESO, Mesonotum width; META, Metanotum width; PROL; Pronotum length; TIBI, Tibia length; FEM; Femur length.

Table 5.9 Pooled within-groups correlations between discriminating variables of the 115 large workers data by discriminant analysis.

Function	1	2	3	4	5
NANT*	0.05	0.33	0.17	0.43**	0.30
HEAD	0.83**	-0.21	0.09	0.19	0.31
PRO	0.09	0.84**	0.13	-0.36	-0.07
MESO	0.07	0.83**	0.11	-0.04	0.41
META	0.05	0.75**	-0.12	0.06	0.26
PROL	0.08	0.64**	0.42	-0.35	-0.07
TIBI	0.08	0.70**	-0.45	0.14	-0.38
FEM	0.07	0.58	0.18	0.53	-0.58

*NANT, Number of antenna segments; HEAD, Head width; PRO, Pronotum width; MESO, Mesonotum width; META, Metanotum width; PROL; Pronotum length; TIBI, Tibia length; FEM; Femur length.

** denotes largest absolute correlation between each variable and any discriminant function, $p<0.05$.

5.4.2 Temporal polyethism of the sub-age classes

The behavioral observation composed of a total of 46,400 behavioral acts exhibited in 20 working days. Eighteen behaviors were identified in the experiment. All sub-age classes exhibited similar repertoire size of 18 behaviors. There was no further increase in repertoire size with age (Table 5.10, Figures 5.5 and 5.6).

The older the age of the large workers, the higher the frequencies of their task related behaviors. The means of frequencies of task related behaviors among sub-age classes were significantly correlated with age (Spearman's rank correlation coefficient test, $p< 0.05$) (Table 5.11). Frequencies of task related behaviors increased from sub-age class **A** to sub-age class **F**. No significant difference in frequencies of any behaviors between two successive sub-age classes was found (Mann-Whitney U test, two tailed, $p>0.05$).

Six behaviors were found to be correlated with age (Table 5.11). These are entrance stationary, general walking, longitudinal vibration, general touching and open moving behavior whereas the general stationary behavior was negative correlated with age (Spearman's rank correlation coefficient test, $p<0.05$). Positive correlation between these behaviors and age means that the frequencies of these behaviors increased with age. Large workers of the last sub-age classes were the most active as entrance guard (highest frequency in entrance stationary) and active alarm giver (highest longitudinal vibration frequency).

Based on Spearman's rank correlation coefficient test, it was found that general walking, longitudinal vibrations and grooming with larvae behaviors were correlated

Table 5.10 Percentage of total time budget spent on various behaviors by the six sub-age classes of large worker.

Behavior act	Behavior code	Sub-age class					
		A (N=6)	B (N=6)	C (N=16)	D (N=30)	E (N=40)	F (N=17)
		a					
General stationary	GS	26.5 ± 6.7	27.1 ± 7.7	19.3 ± 8.5	19.0 ± 7.9	17.4 ± 6.0	17.9 ± 5.8
@ Entrance stationary	ES	1.0 ± 1.0	2.0 ± 2.2	2.1 ± 2.0	2.0 ± 2.0	2.5 ± 2.1	2.8 ± 2.3
General moving	MV	24.1 ± 2.6	20.0 ± 4.3	21.3 ± 3.5	21.1 ± 4.8	20.1 ± 4.1	20.6 ± 4.8
General walking	WT	13.7 ± 5.0	16.1 ± 4.8	18.1 ± 5.4	17.8 ± 5.6	18.8 ± 4.1	18.7 ± 4.0
@ longitudinal vibration	LV	1.5 ± 1.2	2.2 ± 2.3	2.3 ± 1.9	2.8 ± 3.4	3.4 ± 3.0	5.3 ± 4.4
@ Larvae carrying	CA	2.0 ± 1.8	1.8 ± 2.0	1.6 ± 1.4	1.6 ± 1.7	1.4 ± 1.5	1.6 ± 1.5
General touching	GT	0.2 ± 0.5	0.4 ± 0.5	0.4 ± 0.7	0.3 ± 0.5	0.5 ± 0.6	0.7 ± 1.9
@ Grooming with larvae	MB2	4.4 ± 2.4	3.3 ± 1.4	4.7 ± 2.5	4.9 ± 2.7	5.4 ± 3.2	5.1 ± 1.9
@ Grooming with worker	MB5	3.8 ± 1.8	4.9 ± 3.1	6.3 ± 1.7	6.3 ± 2.1	6.5 ± 3.1	5.4 ± 2.9
@ Feeding the larvae	FL	1.5 ± 1.3	1.3 ± 1.2	1.1 ± 0.5	1.5 ± 1.0	1.4 ± 1.0	1.8 ± 1.4
@ Oral trophallaxis with worker	MT5	2.9 ± 0.7	2.8 ± 1.0	4.0 ± 0.8	4.1 ± 1.7	4.3 ± 2.1	3.7 ± 1.9
@ Anal trophallaxis	MA	1.1 ± 0.8	1.1 ± 1.3	1.4 ± 1.3	1.2 ± 1.3	1.0 ± 1.1	1.2 ± 1.0
@ Self feeding	SF	2.0 ± 1.7	1.4 ± 0.6	1.5 ± 1.5	1.2 ± 1.6	1.4 ± 1.5	0.7 ± 0.9
@ Sand handling	SH	6.4 ± 3.4	6.3 ± 2.3	8.8 ± 3.9	8.3 ± 4.6	7.9 ± 5.2	7.8 ± 4.0
@ Open stationary	OS	2.7 ± 1.9	1.7 ± 1.8	1.8 ± 2.2	1.7 ± 2.2	1.8 ± 2.2	1.3 ± 1.5
@ Open walking	OW	4.7 ± 2.2	5.8 ± 1.2	4.6 ± 3.2	4.4 ± 2.9	5.4 ± 3.7	4.3 ± 2.5
@ Open moving	OMV	0.2 ± 0.2	0.2 ± 0.4	0.1 ± 0.3	0.2 ± 0.4	0.2 ± 0.3	0.2 ± 0.3
@ Paper carrying	PC	1.0 ± 1.1	0.8 ± 0.9	0.7 ± 0.8	0.7 ± 0.9	0.4 ± 0.7	0.7 ± 0.6

N = Number of individuals belonged to the sub-age classes.

@ task related behavior.

a = Mean ± standard deviation

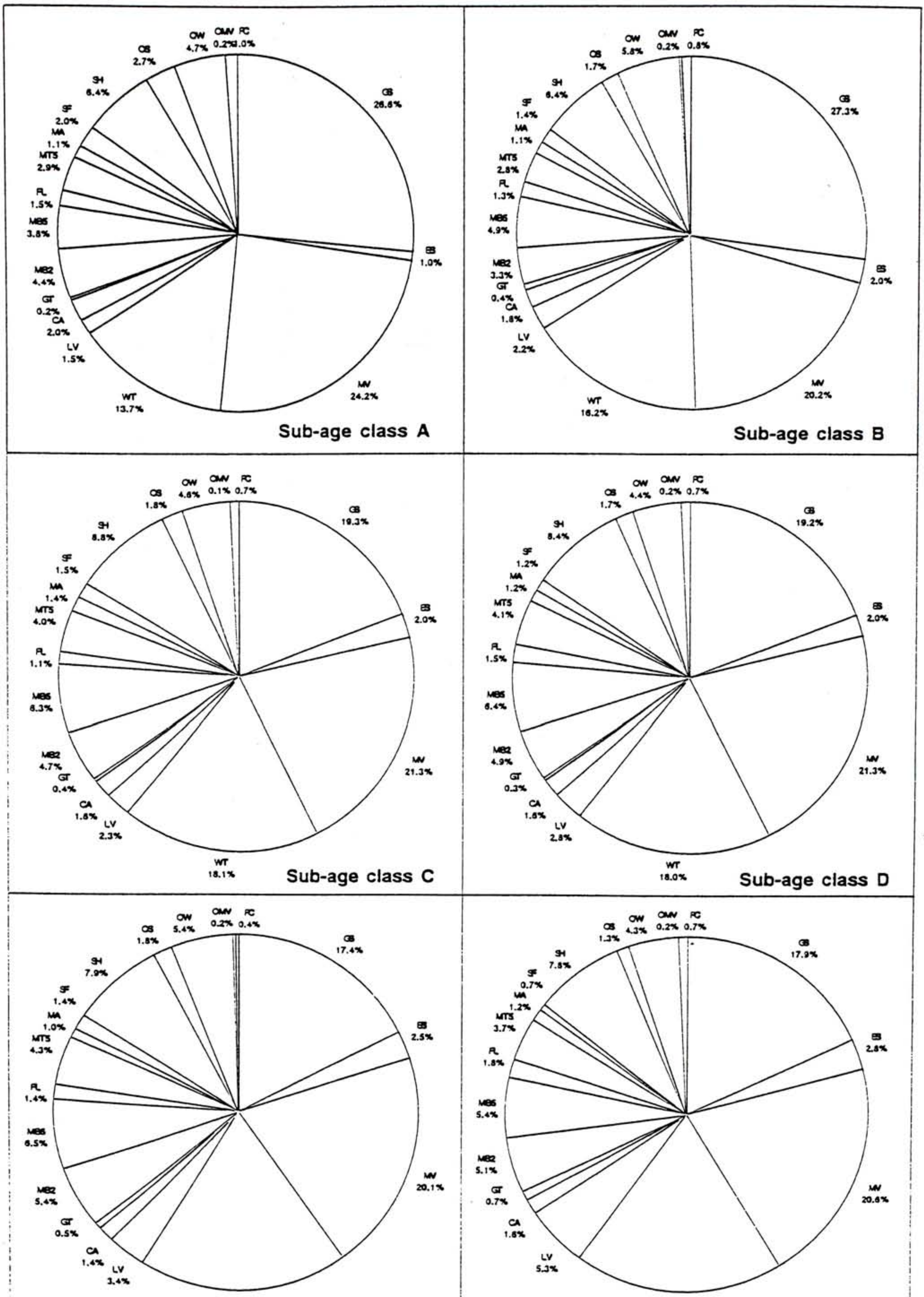


Figure 5.5 Distribution of the % of the total time budget spent on various behaviors by the six sub-age classes within the large workers of *Reticulitermes fukienensis*.

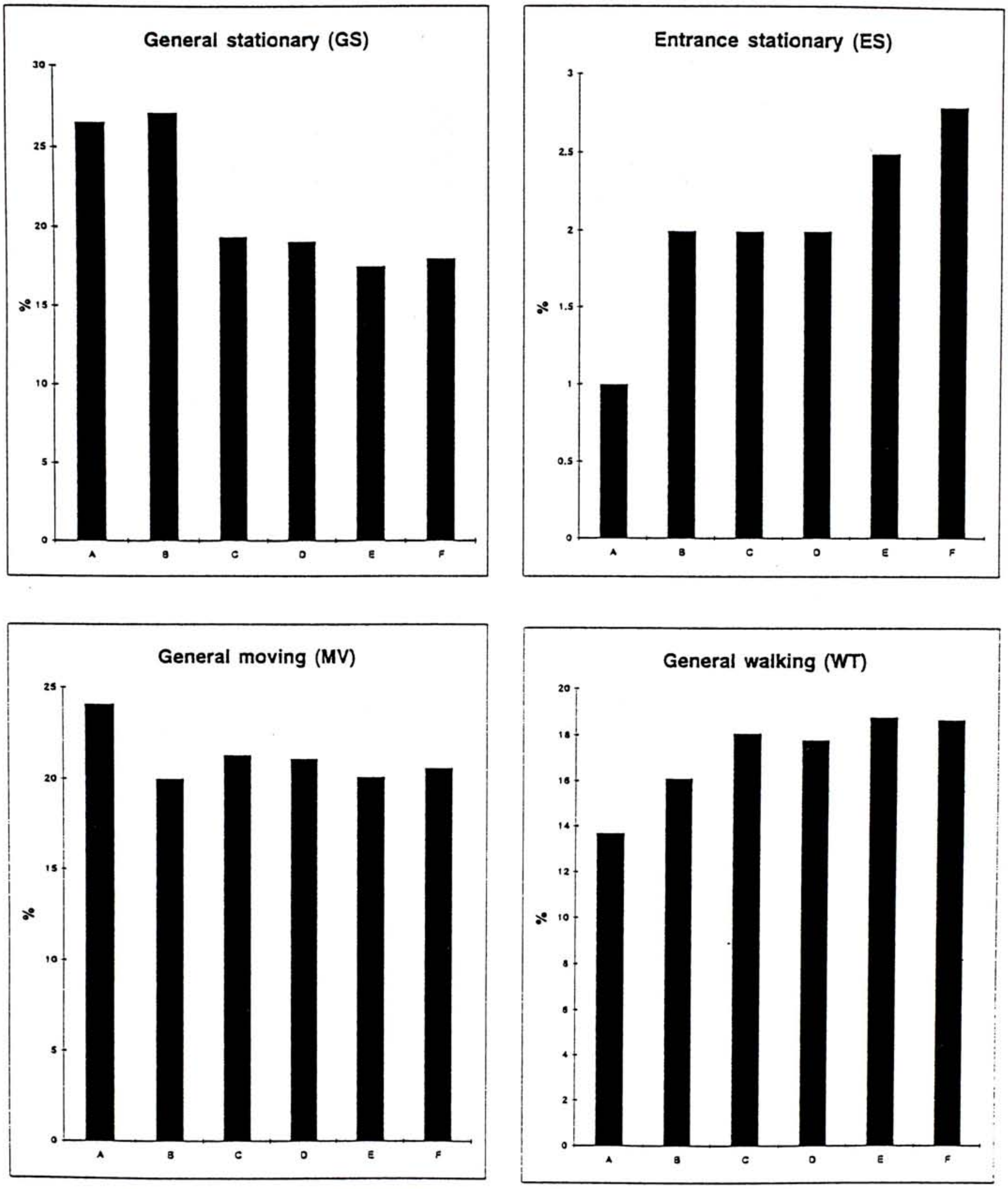


Figure 5.6 Ethograms of the frequencies (%) of various behaviors of the six sub-age classes within the large worker class in the worker line of *Reticulitermes fukienensis*.
Alphabets A-F correspond to the six sub-age classes within the large worker age class.

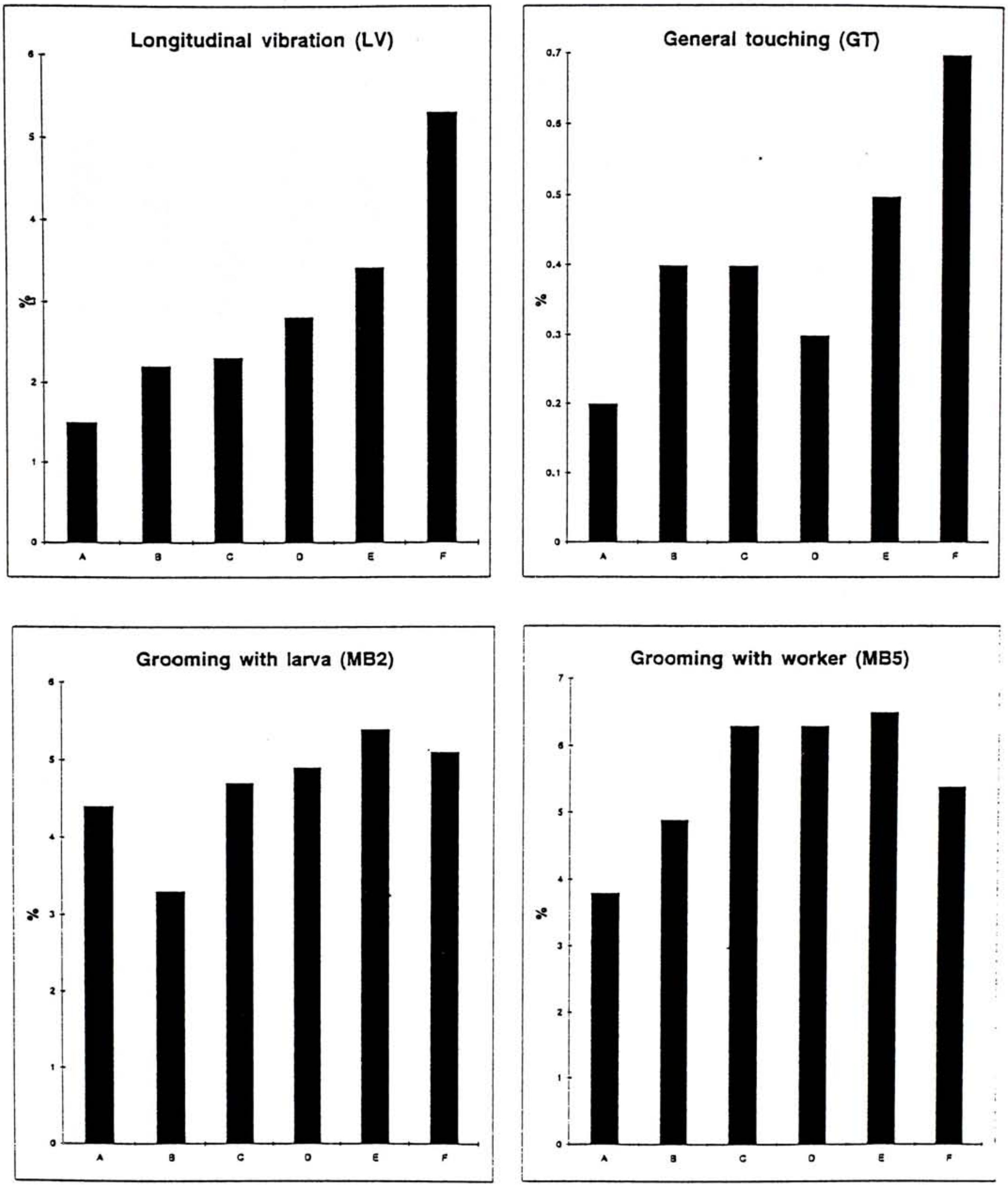


Figure 5.6 (Continued)

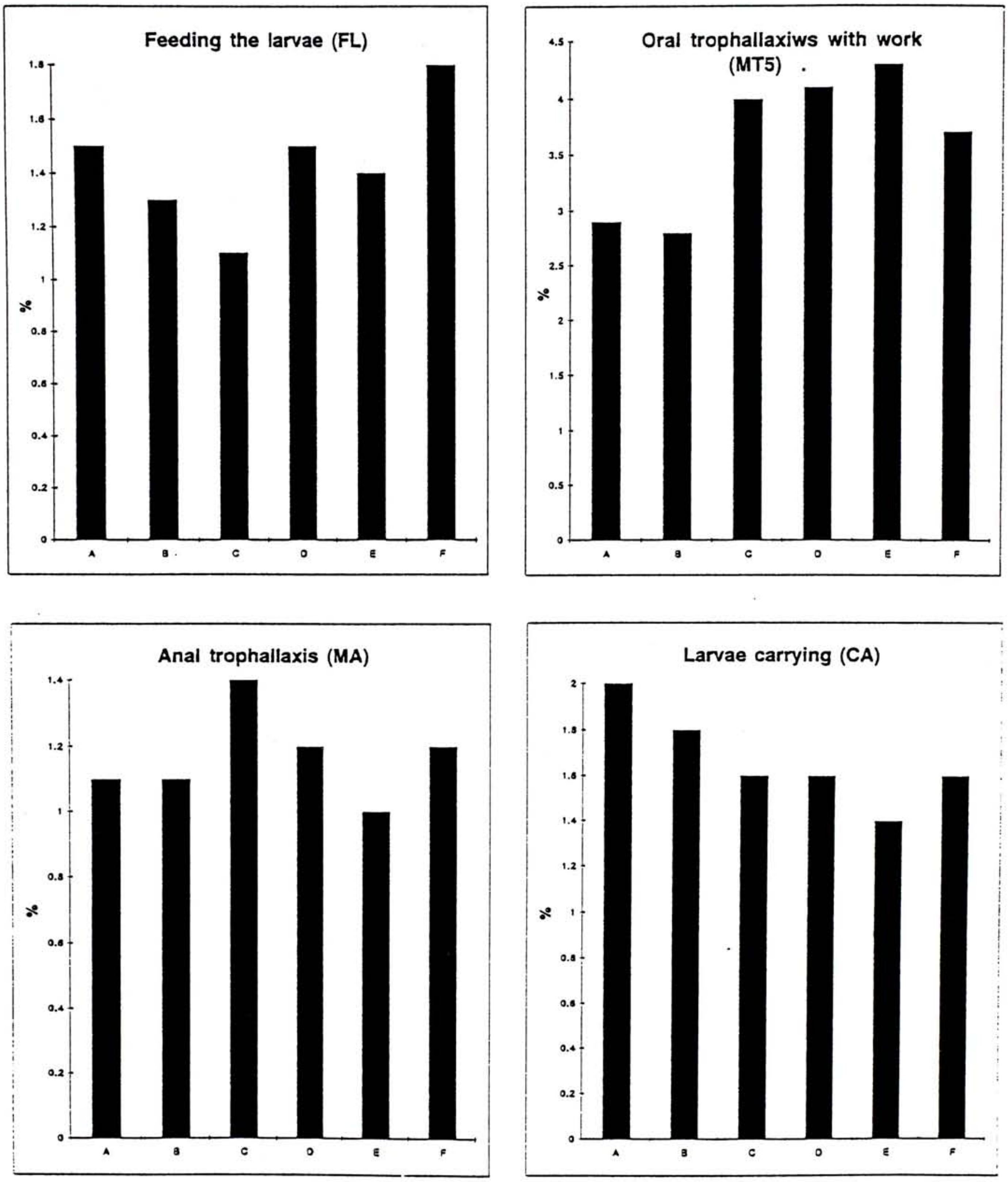
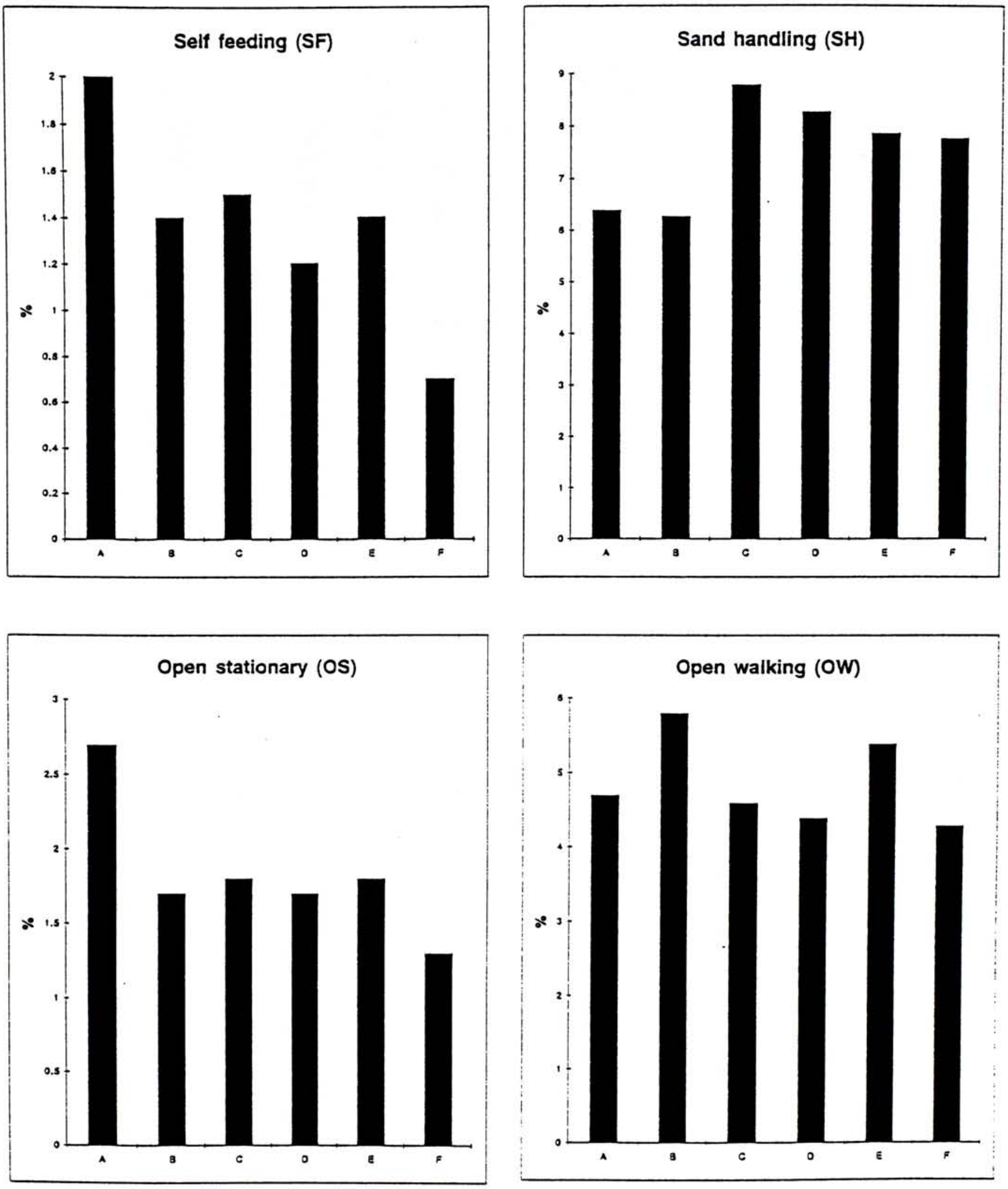


Figure 5.6 (Continued)


Self feeding (SF)

Sand handling (SH)

Open stationary (OS)

Open walking (OW)

Figure 5.6 (Continued)

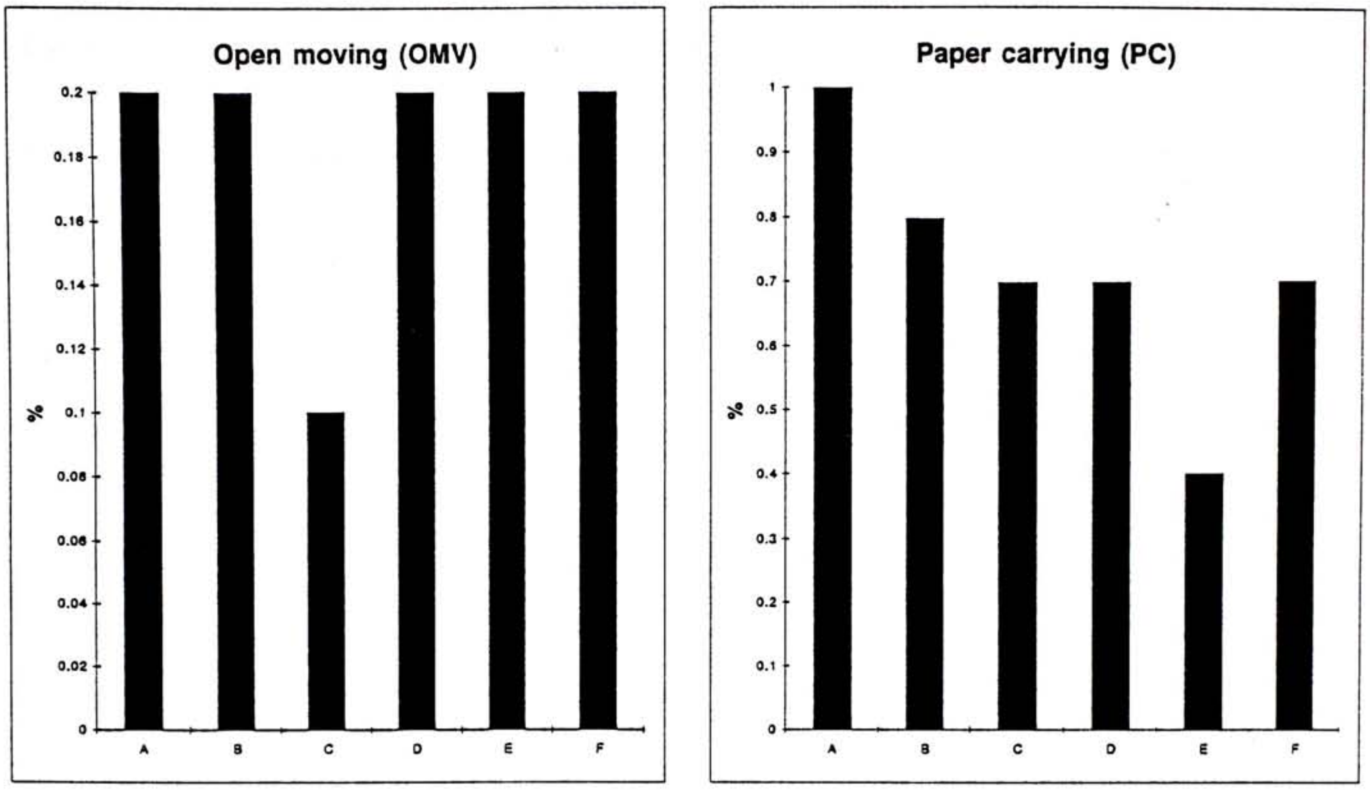


Figure 9.4b (Continued)

Table 5.11 Correlation between the frequencies of various behaviors and the age of different sub-age classes A-F in large worker class.

	Behavior	**beahvior code**	**Spearman's rank correlation test**	
			Correlation	**r value**
	General stationary	GS	**p<005**	**-0.186**
@	Entrance stationary	ES	**p<0.05**	**0.196**
	General moving	MV	**NS**	-0.106
	General walking	WT	**p<0.05**	**0.200**
@	Longitudinal vibration	LV	**p<0.05**	**0.315**
@	Larvae carrying	CA	**NS**	-0.048
	General touching	GT	**p<0.05**	**0.214**
@	Grooming with Larva	MB2	**NS**	-0.150
@	Grooming with worker	MB5	**NS**	0.030
@	Feeding the larvae	FL	**NS**	0.150
@	Oral trophallaxis with worker	MT5	**NS**	0.115
@	Anal trophallaxis	MA	**NS**	-0.007
@	Self feeding	SF	**NS**	-0.138
@	Sand handling	SH	**NS**	0.038
@	Open stationary	OS	**NS**	-0.113
@	Open walking	OW	**NS**	0.013
@	Open moving	OMV	**p<0.05**	**0.239**
@	Paper carrying	PC	**NS**	-0.029
	Task related behaviors		**p<0.05**	**0.222**

@ : task related behavior

p<0.05 : significantly different (Spearman's rank correlation test, p<0.05)

NS : not significantly different (Spearman's rank correlation test, p>0.05) with each other in frequencies(Table 5.12). Entrance stationary behavior was correlated with general touching behavior and its frequency increased with age. General stationary behavior was positively correlated with paper carrying behavior but was negatively correlated with general walking, longitudinal vibrations and grooming with larvae behaviors.

Larvae carrying, grooming with larvae and feeding the larvae behaviors can be grouped into larvae care behavior. Open stationary, open walking and open moving behaviors can be grouped into foraging behavior (excluded feeding in open area). Both frequency of larvae care and foraging behaviors among sub-age classes were not correlated with age (Spearman's correlation coefficient test, $p>0.05$).

5.5 Discussion

By using principal component analysis and discriminant analysis, six groups of large worker were separated. All members belonged to the same group had the same head width sizes. Head width represented the other parameters for sub-age classes separation (section **5.4.1**). It is very difficult to find out the real number of instars in the development line of the worker caste of *Reticulitermes* species because of the small 212increment intheir body size and the nearly absence of no morphological change in the later stages of their development (Buchli, 1958). Considering the relationship between the increment in body size of the termites of successive instars, head width measurement is the most reliable criterion to indicate the age of the termites.

Continuous temporal polyethism (all age classes exhibited the behavior and the

Table 5.12 Spearman's rank correlation (r) between frequencies of various behaviors associated with sub-age classes A-F in large worker class.

Behavior	code	GS	ES	MV	WT	LV	CA	GT	MB2	MB5	FL	MT5	MA	SF	SH	OS	OW	OMV
General stationary	GS																	
@ Entrance stationary	ES	-0.26																
General moving	MV	0.14	-0.77															
General walking	WT	**-0.89**	0.54	-0.37														
@ Longitudinal vibration	LV	**-0.89**	0.60	-0.37	**0.89**													
@ Larvae carrying	CA	0.80	-0.09	0.29	-0.69	-0.69												
General touching	GT	-0.59	**0.90**	-0.59	0.84	0.81	-0.31											
@ Grooming with Larva	MB2	**-1.00**	0.26	-0.14	**0.89**	**0.89**	-0.74	0.61										
@ Grooming with worker	MB5	-0.77	0.09	-0.26	0.77	0.66	**-0.91**	0.38	0.77									
@ Feeding the larvae	FL	-0.36	0.07	0.16	0.04	0.41	0.00	0.11	0.39	-0.13								
@ Oral trophallaxis with worker	MT5	-0.83	-0.14	-0.03	0.71	0.60	-0.86	0.24	0.84	**0.94**	0.01							
@ Anal trophallaxis	MA	0.11	0.00	0.46	0.03	0.14	0.00	0.03	-0.06	0.03	-0.01	0.03						
@ Self feeding	SF	0.53	-0.61	0.53	-0.47	-0.79	0.57	-0.56	-0.50	-0.36	-0.49	-0.21	-0.11					
@ Sand handling	SH	-0.49	-0.26	0.37	0.49	0.37	-0.63	0.07	0.49	0.71	-0.21	0.77	0.60	-0.04				
@ Open stationary	OS	0.23	-0.69	0.57	-0.26	-0.57	0.33	-0.50	-0.17	-0.09	-0.31	0.11	-0.23	**0.93**	0.11			
@ Open walking	OW	0.43	-0.03	-0.43	-0.26	-0.54	0.31	-0.16	-0.43	-0.14	-0.59	-0.26	-0.66	0.53	-0.49	0.46		
@ Open moving	OMV	0.09	0.31	-0.77	-0.09	0.09	-0.20	0.07	-0.09	0.14	0.07	-0.09	-0.40	-0.47	-0.43	-0.46	0.37	
@ Paper carrying	PC	**0.89**	-0.26	0.37	-0.83	0.71	**0.91**	-0.53	-0.77	-0.83	0.13	-0.77	0.09	0.50	-0.54	0.64	0.26	0.03

@ = task related behavior.

Bold r value means there is significant correlation between the behaviors at $p<0.05$.

behavioral frequencies correlated with age; Spearman's rank correlation coefficient test, $p<0.05$) was exhibited by sub-age classes (Figure 5.6) in six behaviors: general stationary, general walking, entrance stationary, longitudinal vibration, general touching and open moving behaviors, but there was no significant difference in the frequencies of these behaviors between successive sub-age classes (Mann-Whitney U test, $p>0.05$). No continuous temporal polyethism existed in the other behaviors and no significant difference in the behavioral frequencies among the six sub-age classes was found.

All sub-age classes exhibited 18 behaviors , indicating that no behavior was lost or gained with aging. In other words, once a behavior was switched on at a particular stage (for example, longitudinal vibration behavior was switched on at small worker stage, in chapter 3), it was performed continuously with aging. In chapter 3, both medium and large workers exhibited same repertoire size (i.e. number of types of behaviors) but significant difference in behavioral frequencies between medium and large workers was found (e.g. general stationary, entrance stationary, moving, searching, larvae grasping, larvae carrying, longitudinal vibration, mouth body touching, mouth touching, mouth-tunnel touching, wood carrying, and walking in open behaviors). Thus, although behavioral repertoire size reached a maximum at medium worker stage, but difference in behavioral frequencies existed between medium and large workers.

There was decreasing trend in the frequency of general stationary, but increased frequencies of general moving, longitudinal vibration and task related behaviors with age (chapter 3). These general principles were also true among the sub-age classes: the higher the frequencies of general stationary behavior, the more inactive the individuals are, the higher the frequencies of task related behaviors, longitudinal vibration behavior or moving behavior, the more active the individuals are. Individuals becoming more active with aging is a general principle for all the age classes (including the sub-age classes in large worker) in the worker line of *R. fukienensis*.

As a whole, results found among sub-age classes of large worker was consistent with the three principles found among the other age classes (chapter 3):

1. frequencies of task related behaviors increase with age;
2. the older the individuals in the worker line, the more active the individuals are; large worker class is the most active;
3. the older the individuals in the worker line, the higher the frequencies of longitudinal vibration behavior and entrance stationary behavior.

Two more principles were set in this chapter:

1. behavioral repertoire size of possible age classes (including the sub-age classes in large worker stage) after medium worker stage remains constant;
2. behavioral frequencies performed by possible sub-age classes in large worker stage are similar (i.e. no significantly difference by Wilcoxon signed rank test and Mann-Whitney U test, two tailed, $p>0.05$).

In Chapter 3, behavioral differences among age classes were due to the difference in their body forms (section 3.5). Sub-age classes of large workers developed in similar form and so all sub-age classes performed the same kinds of behaviors. The activity of the individuals increased with age (frequency of general stationary behavior decreased with age). However, entrance stationary (guard) and longitudinal vibration (signal giving) become more important with age (frequencies of these behaviors increased with age). It reflected that morphological differences among different age classes are enough to explain behavioral change with age.

Chapter 6

GENERAL DISCUSSION AND CONCLUSION

6.1 Summary of chapters 1-5

Temporal polyethism is a phenomenon observed in social insect colonies in which there is a division of labor among individuals whose tasks changed as they age (Wilson, 1971, McMahan, 1977, 1979). Polyethism is one of the most important traits that contribute to the ecological success of social insects. Specialization of tasks and simplification of behavioral repertoire occur so that efficiency of a colony is greatly increased (Oster and Wilson, 1978, Wilson, 1985, Gerber *et al.*, 1988). Polyethism based on morphological character, sex and age can be seen in various examples of eusocial hymenoptera and termites. In general, temporal polyethism of eusocial hymenopterans is discrete (not all worker age classes perform a particular task; workers of different ages perform different sets of tasks), workers tend to change their work or tasks as they age and older individuals perform outside nest task more often than younger individuals. If temporal polyethism exists in termites, it is continuous in most task related behaviors (all worker age classes perform a particular behavior and behavioral frequencies are correlated with age). Old individuals (the workers) perform more tasks, including inside nest and outside nest work, than young individuals.

Individuals in the development line of *R. fukienensis* worker could be separated into five age classes (instars) based on head width measurements (chapter 2). Discrete temporal polyethism (not all age classes exhibit the same behavior) in feeding (one of the task related behaviors) was exhibited by these five age classes. The first two age classes were classified as larvae because they could not feed themselves. The three latter age classes were classified as workers because of their abilities to feed.

Repertoire size among individuals of *R. fukienensis* increased with age (chapter 3). All task related behaviors were performed by workers and most of these task related behaviors were switched on at the third age class (small worker stage). Repertoire sizes of medium and large workers were the same but some behavioral frequencies were significantly different among age classes. In general, frequencies (or % of total time budget spent on a particular behavior) in various task related behaviors increase with age. The older the individuals, the more active they participated in task related behaviors. Most task related behaviors were performed with the highest frequencies by the large worker while the larvae (first and second age classes) were the most inactive as they performed no task. Small worker (the third age classes) was found to be intermediate between larvae and the medium or large workers in behavior performances. Behavioral change can be explained by changes in morphological characters and sclerotization of termite body parts, especially mandibles and mouthparts. The first two age classes (small and large larvae) can be classified into another group: small worker into one group; medium and large workers into one group. Continuous temporal polyethism in some behaviors (such as general stationary, entrance stationary, searching, longitudinal vibration behaviors; section **3.4**) was found among five age classes and was exhbited with significant difference in frequency between successive age class. Discrete temporal polyethism (not all age classes perform behavior) in some behaviors (such as carrying wood, open stationary, grasping behaviors; section **3.4**) was found among the three worker age classes (i.e. small, medium and large workers).

Stomodeal trophallaxis was more common than proctodeal trophallaxis among workers (chapter 4). Large worker performed higher frequencies in larval carrying behaviors than medium worker. Large worker was the main forager and the differences in frequencies of foraging behaviors between successive classes were statistically significant. Discrete temporal polyethism in foraging behaviors existed among worker age classes.

Six possible sub-age classes in large worker were separated based on morphological measurements in head width, pronotum width, pronotum length, mesonotum width, metanotum width, hind tibia length, hind femur length, and number of antenna segments using principal component analysis and discriminant analysis,with head width found to be the main factor that was correlated with age (chapter 5). All the six sub-age classes have the same repertoire size (18 behaviors). There were no significant differences in the frequencies of 18 behavioral acts between the successive sub-age classes in large workers. The sub-age classes of large worker exhibited continuous temporal polyethism in six behaviors: general stationary, general walking, entrance stationary, longitudinal vibration, general touching and open moving behaviors with the frequencies increased or decreased with age.

Frequencies of some behaviors such as entrance stationary, longitudinal vibration behaviors increased with age both in the three worker age classes (chapter 3) and in the six sub-age classes of large worker (chapter 5). It is not required to have highly sclerotized mandibles, mouthparts, and exoskeleton for the exhibition of these behaviors. Some factors, which are correlated with age, may play a role in the performance of various behaviors among different age classes and sub-age classes.

6.2 General discussion

Results of the temporal polyethism studies in various behaviors among the age classes in the worker line of *Reticulitermes fukienensis* do not support the hypothesis of Oster and Wilson (1978) that different age classes in worker line of eusocial insects are expected to carry out discrete tasks, to the extreme that one class performed one task. This hypothesis is true for eusocial hymenopteran. The young workers perform the inside nest work such as feeding the larvae, and nest construction while old workers perform outside nest work such as foraging for food and water. In *Reticulitermes fukienensis*, the young and immature individuals on the worker line perform no task but the older workers perform more tasks both inside and outside the nest. No one age class performs one task in *R. fukienensis*. Nevertheless, both the oldest workers in eusocial hymenopterans and *R. fukienensis* perform the outside nest task.

Rosengaus and Traniello (1993) mentioned that the inactivity of the larvae of *Zootermopsis angusticollis* may be constrained by their small size, soft cuticle, lack of symbionts, and their soft masticatory apparatus, which is unsuitable for wood consumption. Because of their (the young individuals on worker line such as larvae and small worker) fragile bodies, and their need to go through further molts, the dependence of larvae on other workers allow them to reserve more energy and nutrients for growth and molts, and for differentiation to other castes. This may be true for cases involving tasks that must be performed with a certain degree of sclerotization of the body skeleton. However, tasks such as longitudinal vibration (alarm giving) and entrance stationary (nest guard) behaviors performed by worker age classes are not required to have highly sclerotization mandibles, mouthparts and exoskeleton for performance. There are several interacting factors which may play the role of controlling temporal polyethism of termites (Rosengus and Traniello, 1993). These factors may be temporal changes in exocrine and endocrine gland activity; temporal changes in the maturation of various systems such as the muscular, nervous and sensory systems; and the sequential activation and expression of genes (McMahan, 1979).

Degrees of maturation of various physiological and morphological systems of different age classes of termites are involved in temporal polyethism (McMahan, 1979). Pasteels (1965) pointed out that there is a correlation between the sternal gland size and the initiation of trail laying in *Nasutitermes lujae* workers. Although McMahan (1970, 1977) evaluated the sternal gland activity with the trail laying behavior in her studies of polyethism in nest repair behaviors of *Nasutitermes costalis* and *N. exitiosus*, she found that the oldest large workers of these species process highly developed sternal glands giving greater trail laying tendencies in foraging (McMahan, 1979). Richard (1969) suggested that the capacity to detect and to react to stimuli such as pheromones, air movements, humidity gradients, substrate, etc. changes with age with the accompanying maturation of the nervous and sensory systems (review by McMahan, 1979). The pattern of temporal polyethism in longitudinal vibration behavior (alarm giving) of *Reticulitermes fukienensis* workers can be explained by maturation of the sensory, nervous and muscular systems for stimuli reception, response to giving signals and for rapid motion.

Endocrine activity is also involved in temporal polyethism (McMahan, 1979, Robinson, 1987). McMahan (1979) pointed out that the titers of juvenile hormone (JH) influences insect behavior. In honeybees, the young bees (about 1-week-old queen attendant nurses) have low levels of JH, the guard bees (about 2-week-old) have higher levels, and the older bees (about 3-4 weeks-old pollen foragers, non-pollen foragers) have the highest level of JH (McMahan, 1979; review by Nowogrodski, 1984). If the honeybees are treated with JH, age polyethism is accelerated (review by Nowogrodski, 1984). Lenoir (1987) suggested that JH is involved in the regulation of age-related division of labor but it does not imply that polyethism is determined strictly by hormone regulation. The behavioral patterns shown by age classes in the worker line of *Reticulitermes fukienensis* may also be influenced by the level of JH, although there are few studies on the JH level in termites that are related to temporal polyethism. Studies in JH level in termites may be a new direction for the study of factors controlling temite temporal polyethism.

Gordon (1996) discussed gene expression as one of the internal factors for the organization of work in social insect colonies. He pointed out the idea of 'one gene, one protein', and the idea that each neuron performs a single function. Workers in social insect are thought to be intrinsically suited to a particular task and perform this task more or less exclusively (Gordon, 1996). Nowogrodzki (1984) reviewed two recessive genes for behavioral control in honeybee: one recessive gene controls the behavior of chewing open the capping of a cell containing a dead larva or pupa, and the another one gene controls the behavior of disposing of corpse. Lenoir (1987) mentioned the hypothesis of Stort (1975) that aggressive behavior of Africanized bees involved the control by two pairs of genes. Lenoir (1987) concluded that genes could influence behavior in unknown ways, and may explain part of the inter-individual variability, but is insufficient to understand it completely. Nevertheless, different genetic systems are presumably activated or inhibited by stage-characteristic concentrations of hormonal and other chemical substances, resulting in the altered behavioral capacities of different instars (McMahan, 1979). The pattern of the temporal polyethism in the entrance stationary behavior of age classes in the worker line of *Reticulitermes fukienensis* may be partly explained by the influenced by genes activities which is correlated with age.

Nowogrodzki (1984) suggested learning influences the division of labour in an insect society. For example bumble bees gain experience with a particular type of plant, gain skill in entering and manipulating its peculiar maze of floral structures so that their foraging efficiency is increased (Nowogrodzki, 1984). It may be possible that the young individuals (such as medium worker) learn to perform new task from the older individuals (large worker). The difference in frequencies of some task related behaviors between the young and old individuals in worker line of termites may be partly a result of learning. This may need more experiment to test this hypothesis.

6.3 Conclusion

There are two age classes of larvae and possibly more than three worker age classes found in the worker line of *Reticulitermes fukienensis*. In general continuous temporal polyethism (all age classes performed behavior with behavioral frequencies correlated with age) exists in most behaviors among the possible age. Activities, repertoire size (number of kinds of behaviors), and frequencies of task related behavior increase with age. No behaviors will be lost with aging. This pattern of temporal polyethism in termite *R. fukienensis* clearly shows the behavioral difference between termite (isopterans) and eusocial hymenopterans. Although discrete temporal polyethism of some behaviors such as foraging behavior, and larval carrying behavior of the possible age classes in *R. fukienensis* have been detected, it is quite different from the patterns of temporal polyethism exhibited by eusocial hymenopterans of which behaviors may be lost in the oldest stages. This difference may be based on the developmental difference in the order Isoptera and Hymenoptera. It is possible that continuous temporal polyethism exhibited by isopteran may be influenced by interacting factors such as body size, sclerotization, maturation of physiological and morphological systems, exocrine change, genes activities and by learning. Further studies in the determining factors which control the exhibition of temporal polyethism are needed to gain a better understanding of the biology of behavior of termites.

References

Adamson, A. M. 1941. Laboratory technique for the study of living termites. *Ecology*, 22: 411-414.

Alexander, R. D., Noonan, K. M. and Crespi, B. J. 1991. The evolution of eusociality. In: Sherman, P. W., Jarvis, J. U. M. and Alexander, R. D. eds., *The Biology of the Naked Mole-Rat.* Princeton University Press, Princeton, New Jersey, pp. 3-44.

Badertscher, S., Gerber, C. and Leuthold, R. H. 1983. Polyethism in food supply and processing in termite colonies of *Macrotermes subhyalinus* (Isoptera). *Behavioral Ecology and Sociobiology,* 12: 115-119.

Bagine, R. K. 1992. Problems in identification and control of termites. In:*Proceedings of the 1st Regional Workshop on Termite Research and Control,* pp. 44-49.

Becker, G. 1961. Beiträge zur Prüfung und beurteilung der natürlichen dauerhaftigkeit von Holz gegen Termiten. *Holz Roh-werkstoff,* 19: 278-290.

Becker, G. 1969. Rearing of termites and testing methods used in the laboratory. In: Krishna, K. and Weesner, F. M. eds., *Biology of Termites. Vol. I.* Academic Press, New York, pp. 315-385.

Beshers, S. N. and Traniello, J. F. A. 1995. Polyethism and the adaptiveness of worker size variation in the Attine ant *trachymyrmex septentrionalis. Journal of Insect Behaviour*, 9(1): 61-83.

Bignell, D. E., Oskarsson, H. and Anderson, J. M. 1979. Association of actinomycete-like bacteria with soil-feeding termites (Termitidae, Termitinae). *Applied Environmental Microbiology*, 37: 339-342.

Bignell, D. E., Oskarsson, H. and Anderson, J. M. 1980. Specialization of the hindgut wall for the attachment of symbiotic micro-organisms in a termites *Procubitermes aburiensis* (Isoptera, Ternitidae, Tetmitinae).*Zoomorphology*, 96: 103-112.

Bourke, A. F. G. and Franks, N. R. 1995. *Social Evolution in Ants.* Princeton University Press, Princeton, New Jersey.

Brian, M. V. 1979. Caste differentiation and division of labor. In: Hermann, H. R. ed., *Social Insects.* Vol. 1. Academic Press, New York, pp.121-222.

Brian, M. V. 1983. *Social Insects. Ecology and Behavioural Biology.* Chapman and Hall, London, New York.

Buchli, H. H. R. 1958. L'origine des castes et les potentialites ontogéniques des termites européens du genre *Reticulitermes* Holmgren. *Annales des Sciences Naturelles*, 20: 263-429.

Calabi, P., Traniello, J. F. A. and Werner, M. H. 1983. Age polyethism: its occurrence in the ant *Pheidole hortensis*, and some general considerations. *Psyche*, 90: 395-412.

Calderone, N. W. and Page, Jr. R. E. 1991. Evolutionary genetics of division of labor in colonies of the honey bee (*Apis mellifera*).*The American Naturalist*, 138: 69-92.

Carlin, N. F. 1981. Polymorphism and division of labor in the dacetine ant *orectognathus versicolor* (Hymenoptera: Formicidae). *Psyche*, 88: 31-244.

Crosland, M. W. J., Lok, C. M., Shakarad, M., Zhang, J. H., Wong, T. C. and Traniello, J. F. A. 1996. Temporal polyethism in the lower termite, *Reticulitermes fukienensis* (Isoptera: Rhinotermitidae). Proceedings of International Congress of Entomology, XX, Firenze, Italy. p.408.

Crosland, M. W. J., Lok, C. M., Wong, T. C., Shakarad, M. and Traniello, J. F. A. 1997a. Division of labour in a lower termite: the majority of tasks are performed by older workers. *Animal behaviour*, 54: 999-1012.

Crosland, M. W. J., Ren, S. X. and Traniello, J. F. A. 1997b. Division of labour among workers in the termites, *Reticulitermes fukienensis* (Isoptera: Rhinotermitidae). *Ethology* (in press).

Crosland, M. W. J. and Traniello, J. F. A. 1997. Behavioral plasticity in division of labor in the lower termite *Reticulitermes fukienensis*. *Naturwissenschaften*, 84: 208-211.

Elzinga R. J. 1997. *Fundamentals of Entomology*. Fourth Edition. Prentice-Hall, New Jersey, pp. 212-237.

Emerson, A. E. 1965. A review of the mastotermitidae (Isoptera), including a new fossil genus from Brazil. *American Museum Novitates,* 2236: 1-46.

Esenther, G. R. 1961. The biology of the eastern subterranean termites *Reticulitermes flaviceps* (Kollar) in Wisconsin, and related experiments with other species. Ph.D. Thesis, University of Wisconsin.

Free, J. B. 1965. The allocation of duties among worker honeybees. *Symposium of the Zoological Society of London*, 14: 39-59.

Gao, D. R. and Lam, P. K. S. 1986. Notes on the termites (Isoptera) of Hong Kong, including description of a new species and a checklist of Chinese species. *Memoirs of the Hong Kong Natural History Society,* 17: 67-83.

Gao, D. R., Lam, P. K. S. and Owen, P. T. 1992. The taxonomy, ecology and management of economically important termites in China. *Memoirs of the Hong Kong Natural History Society*, 19: 15-48.

Gao, D. R., Zhu, B. Z., Gan, B. R., He, S. S. and Yuan, S. X. 1985. A new toxic bait for the control of forest-infested termites. *Journal of Nanjing Institute of Forestry,* 3: 128-131. [In Chinese]

Garnier-Sillam, E. 1983a. Le polyéthisme *chez Reticulitermes lucifugus santonensis* (Isoptera-Rhinotermitidae). A. Différences dáctivités des ouvriers en fonction des stades. *Insectes Sociaux,* 30: 9-26.

Garnier-Sillam, E. 1983b. Le polyéthisme *chez Reticulitermes lucifugus santonensis* (Isoptera-Rhinotermitidae). B. Contribution a l'étude du comportement individuel de prospection et de recrutement lors de la recherche de nourriture chez les ouvriers. *Insectes Sociaux,* 30: 27-44.

Gay, F. J., Greaves, T., Holdaway, F. G. and Wetherly, A. H. 1955. Standard laboratory colonies of termites for evaluatng the resistance of timber, timber preservatives, and other materials to termite attrak. *Australia Commonwealth Science Industrial Research Organization Bulletin.*, 277: 1-60.

Gerber, C., Badertscher, S. and Leuthold, R. H. 1988. Polyethism in *Macrotermes bellicosus* (Isoptera). *Insectes Sociaux,* 35: 226-240.

Gordon, D. M. 1996. The organization of work in social insect colonies. *Nature*, 380: 121-124.

Grace, J. K., Wood, D. L., Kubo, I. and Kim, M. 1995. Behavioural and chemical investigation of trail pheromone from the termite *Reticulitermes hesperus* Banks (Isoptera, Rhinotermitidae). *Journal of Applied Entomology*, 119: 501-505.

Harris, W. V. 1971. *Termites: Their Recognition and Control.* Longman, London.

Heath, D. 1995. *An introduction to Experimental Design and Statistics for Biology.* University College London Press, London.

Heath, H. 1927. Caste formation in the termite genus *Termopsis. Journal of Morphology and Physiology*, 43: 387-425.

Hermann, H. R. 1979. *Social insects.* Academic Press, London.

Hölldobler, B. and Wilson, E. O. 1990. *The Ants.* Harvard Belknap. Cambridge, Massachusetts.

Howse, P. E. 1968. On the division of labour in the primitive termite *Zootermopsis nevadensis* (Hagen). *Insectes Sociaux*, 15: 45-50.

Howse, P. E. 1970. *Termites: a Study in Social Behavior.* Hutchinson University Library, London.

Hsia, K. L. and Fan, S. T. 1965. Notes of the genus *Reticulitermes* Holmgren of China (Isoptera. Rhinotermitidae). *Journal of Entomology,* 14: 360-382.

Huang, F. S., Li, G. X. and Zhu, S. M. 1989. *The Taxonomy and Biology of Chinese Termites*. Tianze Press, Beijing. [In Chinese]

Husby, W. D. 1980. Biological studies on *Reticulitermes flavipes* (Kollar) (Dictuoptera, Rhinotermitidae) in Southern Ontario. M.Sc. Thesis, University of Guelph, Canada.

Jeanne, R. L. , Downing, H. A. and Post, D. C. 1988. Age polyethism and individual variation in *Polybia occidentalis*, an advanced eusocial wasp. In: Jeanne, R. L. ed., *Interindividual Behavioral Variability in Social Insects*. Westview, Boulder, Colorado, pp. 323-357.

Jeanne, R. L. 1991. Polyethism. In: Ross, K. G. and Matthews, R. W. eds., *The Social Biology of Wasps*. Cornwell University Press, Ithaca, London, pp. 389-425.

Jeffers, J. N. R. 1967. Two case studies in the application of principal component analysis. *Applied Statistics*, 16: 225-236

Jones, R. J. 1980. Gallery construction by *Nasutitermes costalis*: Polyethism and the behavior of individuals. *Insectes Sociaux*, 27: 5-28.

Kanji, G. K. 1993. *100 statisticall tests*. Cromwell Press, London.

Krishna, K. and Weesner, F. M. 1969. *Biology of termites. Vol. I* .Academic Press, New York.

Krishna, K. and Weesner, F. M. 1970. *Biology of Termites. Vol. II* . Academic Press, New York.

Lenoir, A. 1979. Feeding behavior in young societies of the ant *Tapinoma erraticum* L.: trophallaxis and polyethism. *Insectes Sociaux,* 26: 19-37.

Lenoir, A. 1987. Factors determining polyethism in social insects. *Experientia Supplementum Vol. 54., Behavior in Social Insects*. pp. 219-240.

Li, G. and Ping, Z. 1983. Description of a new genus *tsaitermes* and its three new species from China (Isoptera: Rhinotermitidae; Heterotermitinae). *Journal of Entomological Taxonomy*, 5: 239-245.

Light, S. F. and Weesner, F. M. 1947. Methods for culturing termites. *Science,* 106: 131.

Lok, C. M. 1995. Behavioural changes correlated with age of the termite *Reticulitermes fukienensis.* Senior seminar report. The Chinese University of Hong Kong, Hong Kong.

Lüscher, M. 1949. Continuous observation of termites in laboratory cultures. *Acta Tropica*, 6: 161-165.

Lys, J. A. and Leuthold, R. H. 1991. Task-specific distribution of the two worker castes in extranidal activities in *Macrotermes bellicosus* (Smeathman): observation of behaviour during food acquisition. *Insectes Sociaux*, 38: 161-170.

McMahan, E. A. 1970. Polyethism in workers of *Nasutitermes costalis* (Holmgren). *Insectes Sociaux*, 17: 113-120.

McMahan, E. A. 1974. Nonaggressive behavior in the large soldier of *Nasutitermes exitiosus* (Hill) (Isoptera, termidae). *Insectes Sociaux*, 24: 225-232.

McMahan, E. A. 1977. Mound repair and foraging polyethism in workers of *Nasutitermes exitiosus* (Hill):)Isoptera: Termitidae). *Insectes Sociaux*, 24: 225-232.

McMahan, E. A. 1979. Temporal polyethism in termites. *Sociobiology*, 4: 153-168.

McMahan, E. A., Kumar, S. and Sen-Sarma, P. K. 1984. Male/female (size) polyethism in workers of *Odontotermes distans* Holmgren and Holmgren (Isoptera: Termitidae : Macrotermitinae). *Annuals of the Entomological Society of America*, 77: 429-434.

McMahan, E. A., Sen-Sarma, P. K. and Kumar, S. 1983. Biometic, polyethism, and sex ratio studies of *Nasutitermes dunensis* Chatterjee and Thakur (Isoptera: Termitidae). *Annuals of Entomology*, 1: 15-25.

Miura, T. and Matsumoto, T. 1995. Worker polyethism and division of labor in the foraging behavior of the black marching termite *Hospitalitermes medioflavus*, on Borneo Island. *Naturwissenschaften,* 82: 564-567.

Möglich, M. and Hölldobler, B. 1974. Social carrying behavior and division of labor during nest moving in ants. *Psyche*, 81: 219-236.

Morimoto, K. 1968. Termites of the genus Reticulitermes of Japan and Taiwan. *Report of Forest Experiment Station*, 217: 42-73.

Nakata, K. 1995. Age polyethism, idiosyncrasy and behavioural flexibility in the queenless ponerine ant, *Diacamma* sp. *Ethological Society*, 13: 113-123.

Noirot, C. 1955. Researches sur le polymorphisme des termites supérieurs (Termitidae). *Annales des Sciences Naturelles,*17: 399-595.

Noirot, C. 1969a. Glands and secretions. In: Krishna, K. and Weesner, F. M. eds., *Biology of Termites. Vol. I.* Academic Press, New York, pp. 89-123.

Noirot, C. 1969b. Formation of castes in the higher termites. In: Krishna, K. and Weesner, F. M. eds., *Biology of Termites. Vol. I.* Academic Press, New York, pp. 311-350.

Noirot, C. 1985. Pathways of caste development in the lower termites. In: Watson, J.A.L., Okot-Kotber, B. M. and Noirot, C. eds., *Caste Differentiation in Social Insects*. Pergamon Press, Oxford, pp. 41-57.

Noirot, C. 1990. Sexual castes and reproductive strategies in Termites. In: Engel, W., ed., *Social Insects: An Evolutionary Approach to Castes and Reproduction.* Springer-verlaf, Germany, pp. 5-36.

Noirot, C. 1992. From wood- to humus-feeding : an important trend in termite evolution. In: Billen, J. ed., *Biology and Evolution of Social Insects.* Leuven University Press, Leuven, Belgium, pp. 107-119.

Noirot, C. and Pasteels, J. M. 1987. Ontogenetic development and evolution of the worker caste in termites. *Experientia*, 43: 851-952.

Nowogrodzki, R. 1984. Division of labor in the honeybee colony: a review. *Bee World*, 65: 109-116.

Nutting, W. L. 1970. Flight and Colony foundation. In: Krishna, K. and Weesner, F. M. eds., *Biology of Termites. Vol. II.* Academic Press, New York, pp. 233-282.

Oi, F. M. and Su, N. Y. 1994. Stains tested for marking *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae). *Sociobiology*, 24: 241-268.

Osmun, J. V. 1957. Three entomological laboratory techniques. I. Rearing methods for subterranean termites. *Proceedings of the Indiana Academy of Sciences*, 66: 141-143.

Oster, G. F. and Wilson, E. O. 1978. *Caste and ecology in the Social Insects.* Princeton University Press, Princeton, New Jersey.

Otto, D. 1958. über die Arbeitsteilung im Staate *von Formica rufa rufo-pratensis minor* Gössw. und ihre verhaltens-physiologischen Grundlagen: Ein Beitrag zur Biologie der Roten Waldameise. *Wissenschaftliche Abhandlungen der Deutschen Akademie der Landwirtschaftswissenschaften zu Berlin,* 30: 1-169.

Pasteels, J. M. 1965. Polyéthisme chez les ouvriers *de Nasutitermes lujae* (Termitidae Isoptères). *Biologia Gabonica,* 1: 191-205.

Pearce, M. J. and Warte, B. S. 1994. A list of termite genera (Isoptera) with comments on taxonomic changes and regional distribution. *Sociobiology,* 23: 247-263.

Ping, Z. M., Xu, Y. L. and Li, S. 1982. Two new species of the genus *Reticulitermes* (Isoptera: Rhinotermitidae*). Journal of Entomological Taxonomy*, 4: 419-424. [In Chinese]

Ping, Z. M., Zhu, J. and Li, G. 1980. A new species of *Reticulitermes* from Dinghushan (Isoptera: Rhinoternitidae). *Journal of Entomological Taxonomy,* 2: 65-68. [In Chinese]

Rencher, A. C. 1995. *Methods of Multivariate Analysis.* Wiley-Interscience Publication, New York.

Rettenmeyer, C. 1963. Behavioral studies of army ants. *Kansas University Science Bulletin,* 44(9): 281-465.

Richard, G. 1969. Nervous and sense organs. In: Krishna, K. and Weesner, F. M. eds., *Biology of Termites. Vol. I.* Academic Press, New York, pp. 161-192.

Robinson, G. E. 1987. Hormonal regulation of age polyethism in the honey bee, *Apis mellifera. Neurobiology and Behavior of Honey Bees,* pp. 266-280.

Robinson, G. E. 1992. Regulation of division of labor in insect societies. *Annual Review of Entomology*, 37: 637-665.

Robson, S. K. and Fourcaissié, V. 1995. Nonrandom search geometry in subterranean termites. *Naturwissenschaften*, 82: 526-528.

Roonwal, M. L. 1970. Termites of the Oriental Region. In: Krishna, K. and Weesner, F. M. eds., *Biology of Termites. Vol. I.* Academic Press, New York, pp. 315-392.

Rosengaus, R. B. and Traniello, J. F. A. 1993. Temporal polyethism in incipient colonies of the primitive termite *Zootermopsis angusticollis:* a single multiage caste. *Journal of Insect Behavior*, 6: 237-252.

Ross, K. G. and Matthews, R. W. 1991. *The Social Biology of Wasps.* Cornell University Press, Ithaca, New York.

Sands, W. A. 1965. Mound population movements and fluctuations in *Trinervitermes ebenerianus guineensis* Sjöstedt (Isoptera, Termitidae, nasutitermitinae). *Insectes Sociaux*, 12: 49-58.

Seeley, T. D. 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology and Sociobiology*, 11: 287-293.

Seeley, T. D. 1995. *The Wisdom of the Hive: the Social Physiology of Honey bee Colonies*. Harvard university Press, London.

Sendova-Franks, A. B. and Franks, N. R. 1995. Division of labor in a crisis: task allocation during colony emigration in the ant *Leptothorax unifasciatus* (Latr.). *Behavioural Ecology and Sociobiology*, 36: 269-282.

Shellman-Reeve, J. S. 1990. Dynamics of biparental care in the dampwood termite, *Zootermopsis nevadensis*: response to nitrogen availability. *Behavioural Ecology and Sociobiology*, 16: 389-397.

Sieber, R. and Leuthod, R. H. 1981. Behavioral elements and their meaning in incipient laboratory colonies of the fungus-growing termites *Macrotermes michaelsen. Insect Sociaux*, 28: 371-382

Simões, D. 1977. Etologia e differenciação de casta em algumas vespas sociais (Hymenoptera, Vespidae). Ph.D dissertation, Universidade de São Paulo, Ribeirão Prêto.

Stort, A. C. 1995. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. IV. The number of stings in the gloves of the observer. *Behavioural Genetics*, 5: 269-274.

Stuart, A. M. 1969. Social behavior and communication. In: Krishna, K. and Weesner, F. M. eds., *Biology of Termites. Vol. I.* Academic Press, New York, pp. 193-232.

Su, N. Y. 1994. Field evaluation of a hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). *Journal of Economic Entomology,* 87: 389-397.

Sudd, J. H. and Franks, N. R. 1987.*The Behavioural Ecology of Ants*. Blackie, Glasgow.

Synder, T. E. 1949. Catalog of the termites (Isoptera) of the world. *Smithsonian Miscellaneous Collections*, 112: 1-490.

Traniello, J. F. A. and Rosengus, R. B. 1997. Ecology evolution and division of labour in social insects. *Animal Behaviour*, 53: 209-213.

Tsai, P. H., Hwang, F. S. and Li, K. S. 1977. Notes on the genus *Reticulitermes* (Isoptera) from China, with descriptions of new subgenera and new species. *Journal of Entomology*, 20: 465-475. [In Chinese]

Waller, D. A. and La Fage, J. P. 1987. Nutritional ecology of termites. In: Slansky Jr., F. and Rodriguez, J. G. eds., *Nutritional Ecology of Insects, Mites, and Spiders*. John Wiley, New York, pp. 487-532.

Watson, J. A. L. 1973. The worker caste of the hodotermitid harvester termites. *Insectes Sociaux*, 20: 1-20.

Watson, J. A. L. and McMahan, E. A. 1978. Polyethism in the Australian termite *Drepanotermes* (Isoptera, Termitinae). *Insectes Sociaux*, 25: 53-62.

Watson, J. A. L., Okot-Kotber, B. M. and Noirot, C. 1985. *Caste Differentiation in Social Insects*. Pergamon Press, New York.

Wilson, E. O. 1971. *The Insect Societies*.. Harvard Belknap, Cambridge, Massachusetts.

Wilson, E. O. 1976a. A social ethogram of the neotropical arboreal ant *Zacryptocerus varians* (Fr. smith). *Animal Behaviour*, 24: 354-363.

Wilson, E. O. 1976b. Behavioral discretization and the number of castes in an ant species. *Behavioral Ecology and Sociology,* 1: 141-154.

Wilson, E. O. 1980. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). *Behavioral Ecology and Sociobiology*, 7: 157-165.

Wilson, E. O. 1985. The sociogenesis of insect societies. *Science*, 228: 1489-1495.

Winston, M. L. 1987. *The Biology of the Honey Bee*. Harvard Belknap, Cambridge, Massachusetts.

Yu, S. and Ping, C. M. 1964. Systematics of Isoptera from China. Description of a new genus, *Operculitermes* (Rhinotermitidae, heterotermitinae), with reference to its two new species and three new subspecies. *Journal of Entomology*, 13: 344-361. [In Chinese]

Zar, J. H. 1996. Biostatistical Analysis. Prentice-Hall, New Jersey.