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CASTE REGULATION IN THE TERMITE, NEOTERMES JOUTELI (BANKS)

A thesis submitted to the Faculty of The Rockefeller University  
in partial fulfillment of the requirements  
for the degree of Doctor of Philosophy

by

Richard Nagin  
iii

*approval for publication*

*Wm. T. T. T.*

*Professor*

22 April 1970

The Rockefeller University

New York

DEDICATION

This Thesis Submitted April 22, 1970,  
the Hundredth Anniversary  
of the Birth of Lenin,  
is Dedicated

to

All Rebellious Students, Teachers and Workers

## ACKNOWLEDGEMENTS

I would like to express my deep appreciation of my thesis advisor, William Trager. It has been very intellectually stimulating and a great pleasure for me to work in his laboratory. We have both shared a deep love of invertebrate biology and a belief that science is best pursued in an open and free atmosphere. Trager has been a constant source of ideas and encouragement.

Many others have helped me carry out this work. They have included laboratory helpers, library workers, secretaries, people who have obtained supplies and equipment, people who have maintained the laboratories in working condition, people who have carried out administrative work. To all of them I am very grateful.

There have also been some things that have made doing research very hard. There is a very oppressive system of careerism and a very backward social and political structure at this University. These things only serve to alienate everyone from everyone else. They tend to repress the creative abilities of the employees, to dehumanize the students and junior faculty, and to create in the senior faculty a kind of moral paralysis at a time when their prestige is desperately needed in a number of important social crises. I am very grateful to the hundreds of faculty members, employees and students who are fighting to abolish these things and to construct a fully human, democratic university.

## ABSTRACT

The southern Florida termite Neotermes jouteli (Kalotermitidae) was found to provide exceptionally suitable material for the study of caste regulation, with special reference to the regeneration of reproductive forms. It was possible to collect entire natural colonies of this termite, including the reproductive pair, and to maintain them for long periods in the laboratory. In this way the characteristics of individual colonies could be studied in a way not done in most earlier work on caste regulation.

Most interesting was the finding that the capacity to regenerate lost reproductive forms was a characteristic of individual colonies. By removing groups of pseudergates (advanced larval forms that serve as workers) to experimental nests where they could be observed daily, the rate and extent of appearance of supplementary reproductive forms could be measured. A quantitative measure of a colony's regenerative intensity,  $I_R$ , was developed. Low  $I_R$  was found to be associated with large-sized pseudergates and soldiers and the seasonal production of alates, while the opposite characteristics were found in colonies with high  $I_R$ . It was therefore concluded that  $I_R$  was a function of colony age and theoretical reasons were given why this should be the case. In addition it was found that a colony's  $I_R$  temporarily decreases if it is forced to regenerate reproductives. Also, in colonies with low  $I_R$  it is only the pseudergates that can transform to supplementaries. Earlier stage larvae with the same morphological characteristics as pseudergates of high  $I_R$  colonies lack this ability.

Previous work has indicated, but by no means proved, that the presence of a reproductive pair in a termite colony prevents transformation of pseudergates into supplementary reproductives by means of postulated inhibitory pheromones. Accordingly, many different kinds of experiments were devised centering on this hypothesis. In these studies, too, the index  $I_R$  was very useful. More clear-cut results than have been previously reported were obtained by focusing on high  $I_R$  colonies. The present findings can be summarized as follows:

1. All pseudergates in high  $I_R$  colonies are genetically capable of transformation to supplementaries. However, at any given time, only a certain proportion can immediately transform.

2. Male and female reproductives play different roles in the inhibitory process with the female totally inhibiting female pseudergates and partially inhibiting males and the male equally but partially inhibiting both sexes. In contrast to previous findings, the sum of the separate effects accounts for the total inhibition observed. The separate effects of the two sexes imply the existence of at least two inhibitory substances if the pheromone theory is accepted. In addition, a pair of reproductives of either sex is fully as inhibitory as a normal bisexual pair.

3. The extent to which a colony regenerates reproductives is proportional to the amount of time each day that reproductives are absent. The surplus inhibition experienced by female pseudergates can be seen under conditions of part-time orphaning.

4. Reproductives with sealed anuses are probably just as inhibitory as normal reproductives and therefore proctodeal feeding is not the means by which the postulated inhibitory pheromones are transmitted. Likewise, restricting pseudergates to a diet of filter paper impregnated with rectal fluid has no significant inhibitory effect. These results conflict with what has previously been reported.

The results of all these studies can be interpreted most easily in terms of the pheromone theory. However, the source and nature of the pheromones remain unknown.

The overall results fit into the general view that a termite colony is a "superorganism." The superorganism has differentiated parts; its members are integrated primarily through chemical interactions mediated by the behavioral systems of grooming and trophallaxis; it carries out coordinated behavioral patterns such as constructing and maintaining complex and finely regulated nests; and it has a life history of its own with physiologically different stages corresponding to the embryonic, juvenile and sexually mature stages of an individual organism.

Finally, a previously unnoticed and unusual protozoan, Staurojoenina sp. is present among the hindgut symbionts of N. jouteli pseudergates. It is occasionally lost in mature colonies of the termite.

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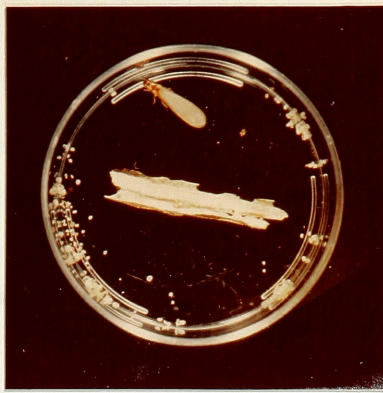
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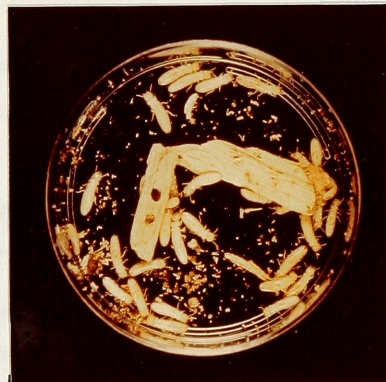
ALATE



WHITE SOLDIERS



SOLDIERS



WHITE and BROWN  
PSEUDERGATES



SUPPLEMENTARY REPRODUCTIVE  
PAIR with EGG



FIRST INSTAR LARVAE

POLYMORPHISM IN NEOTERMES JOUTELI (BANKS)



## I. INTRODUCTION

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Social organization is a common phenomenon in biology. The integration of organisms into well-defined colonies is found in diverse phyla including Protozoa (e.g., Volvox, Epistylis), Cnidaria (e.g., Hydractinia, Physalia - "The Portuguese man-of-war"), Ectoprocta (Bugula), Entoprocta (Pedicellina), Chordata (Botryllus) and Arthropoda (termites, bees, ants, wasps). The examples chosen all have highly integrated colonies, but loosely aggregated, sub-social or semi-social forms are also known in each phylum.

The highly integrated colonies share certain common characteristics. Generally speaking, they have a definite structure, they display organized behavior, they show division of labor usually associated with morphological differentiation and they have a life cycle. The individuals composing the colonies maintain these overriding social characteristics, although often the characteristics of the society are not the characteristics of the individuals. Thus, honeybees cannot regulate their body temperature, but hive temperature is maintained at  $32 \pm 3^{\circ}\text{C}$  throughout the year (see Simpson, 1961). The individuals specialized for defense in Hydractinia and termite colonies cannot reproduce, but their colonies do.

The maintenance of social characteristics implies the existence of self-regulatory mechanisms at a social level. One can speak of social homeostasis. The elucidation of the mechanisms of social homeostasis is the basic problem posed by each form of social organization. This defines the philosophy of the present study of the caste system in termites. The question asked is: How is the caste system, the peculiar form of internal differentiation in termite societies, regulated?

In order to understand this particular problem it is necessary to know something of the general biology of termites. In this introductory chapter, therefore, a discussion is presented of the phylogeny, classification, life cycle and castes of termites. In addition, the other insect societies (those of the bees, ants and wasps) are briefly described for comparison. Finally, the scope of the present experimental work is described.

### Phylogeny

Termites diverged from cockroaches in the Paleozoic, perhaps as early as the late Devonian period (Martynov, 1938), and became the first insects to evolve true social organization. Probably their ancestors were quite similar to Cryptocercus punctulatus which is considered to be the most primitive living cockroach (McKittrick, 1964). Cryptocercus, which inhabits the southern Appalachians and the northwest coast of the United States, lives in loosely organized multi-family colonies. It feeds on wood which it digests through symbiosis with intestinal flagellates of the same genera as are found in lower termites (Cleveland et al., 1934). Furthermore, in two independent conservative character systems (the proventriculus and the female genitalia) Cryptocercus is much more similar to the most primitive living termite, the North Australian Mastotermes darwiniensis, than to its nearest cockroach relative (McKittrick, 1965). In addition, Mastotermes has a number of roach-like characteristics which are not found in any other termite. Like the roaches it deposits its eggs cemented together in an ootheca whereas all other termites simply drop eggs one at a time. It also possesses in its fat body intracellular bacteriocytes like cockroaches but unlike any other termite (Jucci, 1952). The similarities between Mastotermes and Cryptocercus are so close that several authors have proposed (e.g., McKittrick, 1965) that the termites (Order Isoptera) be placed with the roaches (Suborder Blattaria) and the mantids (Suborder Mantodea) as a third suborder of the Dictyoptera.

### Classification

At the present time about 2100 living and fossil species of termites are known (Snyder, 1949; Emerson, 1955). These have been

classified into six families (Emerson, 1965) which are listed below from most primitive to most advanced together with the number of living species in each.

		Living Species
Family I	Mastotermitidae	1
Family II	Kalotermitidae	331
Family III	Hodotermitidae	29
Family IV	Rhinotermitidae	166
Family V	Serritermitidae	1
Family VI	Termitidae	1518

The first five families are known collectively as the lower termites. The termites in these families eat wood and possess cellulose-digesting symbiotic protozoa in their hind intestine. The Termitidae, referred to as the higher termites, is the largest and most diversified family, containing three-fourths of the known species. Termites of this family feed on a variety of things including leaves, grass, vegetable debris, wood and fungus. They do not possess symbiotic protozoa but digest cellulose with the aid of bacteria and fungi. Some species maintain elaborate gardens in which fungus is cultivated.

For reasons to be described, a primitive species was wanted for carrying out the present study. Experiments centered upon a species of the family Kalotermitidae. The family Mastotermitidae was not studied since it is represented by the single species of Mastotermes found only in Australia.

All species of termites referred to in the present work are listed under their family in Addendum B.

#### Life Cycle of the Lower Termites

Termite colonies are founded by a pair of winged adults. The winged forms or alates emerge under definite seasonal and meteorological conditions in huge swarms from mature colonies. The swarms contain equal numbers of males and females, which pair up, break off their wings and then excavate a cell in the earth or a dead log. Having enclosed themselves in the cell, the founding pair, which are now known as the

primary reproductives, mate for the first time and the female begins to lay eggs. From the eggs emerge larvae, which in the early stages are fed by the reproductives. The larvae grow rapidly by molting and are soon able to carry out the work of the colony. At this point they are known as pseudo-workers or pseudergates.

The work of the pseudergates involves enlarging the nest and, most importantly, helping to raise the maximum number of sibling larvae. The reproductives lose their ability to eat anything but predigested food fed to them by the pseudergates and become restricted to reproductive activities. The pseudergates feed and groom the young larvae, the reproductives and each other. The feeding is carried out proctodeally, that is, via the anus.

The pseudergates are functional workers but do not constitute a caste. Rather, they are larvae whose development has been temporarily arrested. At some point in the colony's history when its population has developed sufficiently, the pseudergates proceed to become sexually mature adult winged forms which in a short time swarm to establish new colonies. At the point that a colony is capable of producing alates it is said to be mature. In the lower termites, colony maturity is reached in two to six years and may last ten more years, after which the colonies die out. In the higher termites, colonies can remain mature and healthy for decades, in some cases for as long as a century (see Grassé, 1949). For more detailed information about the foundation and early development of colonies, see Nutting (1969).

#### The Castes

Not all pseudergates eventually develop into alates. Some differentiate into one of the castes. In the lower termites there are essentially two castes: the soldiers, which fulfill a purely defensive function, and the supplementary or replacement reproductives, which arise if the reproductives present in a colony are lost and which then take over the task of larva production. These castes are bisexual. They differ from the pseudergates in morphology, behavior and physiology. In particular, they lack the ability to molt. They also are unable to eat wood and must be fed proctodeally by the pseudergates.

In the higher termites, the situation is slightly more complicated. For one thing, some larvae become true workers which, though capable of soldier differentiation, can never develop towards the alate. For another, sexual dimorphism with respect to size is very common so that the larvae, workers, soldiers and alates of one sex may be distinctly larger than those of the other. In some species the sex ratio of the soldiers is highly skewed. In some cases soldiers of one sex are never produced. Finally, supplementaries are much rarer in the higher termites and very little information is available about them. Noirot (1969) has recently reviewed caste formation in the higher termites.

A color film, showing larval forms, pseudergates, castes and primary reproductives of the Kalotermitid, Neotermes jouteli, was made by the author with the help of Odi Kloesman of the Graphic Services Division of The Rockefeller University. A number of these forms are shown in the Frontispiece. The film also shows behavior patterns such as mating, eating wood, proctodeal feeding and grooming. A copy is filed with the Graphic Services Division.

For more detailed information on the biology of termites see Kofoid (1934), Grassé (1949), Weesner (1960), Harris and Sands (1965) and Krishna and Weesner (1969).

#### Other Social Insects

Discussions of the social organization of termites invariably lead to comparisons with the societies of bees, ants and wasps. The societies of these latter insects, members of the order Hymenoptera, share certain general features in common with termite societies. For example, their colonies are founded by winged sexuals whose offspring cooperate in the rearing of their siblings. The societies have polymorphic castes and show a high degree of integration.

On the other hand, the Hymenopteran societies differ sharply from termites in a number of respects. First of all it should be made clear that Isopteran and Hymenopteran societies are phylogenetically unrelated. Although the Hymenoptera emerged as an order in the upper Paleozoic, the fossil evidence (Wilson et al., 1967) indicates that social organization



did not arise until the late Mesozoic approximately 200 million years after the evolution of the social termites. The Hymenoptera were already a diverse group of non-social insects before true sociality evolved. In fact, social organization arose independently in at least 10 different branches of the order (Wilson, 1966). Thus, even within the Hymenoptera different examples of social organization are phylogenetically unrelated.

The Hymenoptera are also very advanced insects whereas the termites are primitive. One important illustration of this is in development. The Hymenopterans are holometabolous, that is, they undergo complete metamorphosis. This involves going through a number of highly distinct phases: egg, a number of grub-like larval instars, pupa, winged adult. The termites, on the other hand, undergo the gradual metamorphosis common to primitive insects; they are hemimetabolous. Development and differentiation occur without radical morphological changes at any molt.

This is not to say that termite societies are less complex than Hymenopteran ones. Probably they are more complex than those of the bees and wasps and as complex as those of most of the ants. It is simply that the individuals that make up termite societies are more primitive.

One major difference between the societies of the two orders lies in their caste systems. In the Hymenoptera the castes are restricted to the female sex whereas, generally speaking, termite castes are bisexual. The Hymenopteran societies have two basic castes: workers and reproductives (queens). The workers are sterile female offspring which help to raise the brood through nursing, foraging, nest-building, defense and queen-care activities. In the ants there may be two or more distinct size classes of workers with functional specialization loosely associated with each size. (For a theoretical discussion of the relation between functional and morphological specialization see Wilson, 1968). For example in the genus Pheidole the largest workers are primarily involved in defense activities and are called soldiers.

It appears that in the Hymenoptera a number of different mechanisms of caste determination are at work in different species, as might be expected, considering that social organization arose independently several times in that order. The mechanisms include genetic determination in Melipona bees, determination of larvae in the honeybee and many ants, and behavioral determination in Polistes wasps and Lasioglossum bees. These various mechanisms have been reviewed by Weaver (1966).

In one Hymenopteran species the problem of caste regulation and determination has been extensively studied. This is the honeybee, Apis mellifera. In this organism the queen secretes a number of substances which are highly attractive to the workers. The workers lick the queen and one of the substances so obtained, which has been identified as 9-ketodec-2-trans-enoic acid, has two inhibitory effects upon them. First, it represses the development of their ovaries and, second, it prevents them from constructing queen cells. In the absence of the queen the workers lay eggs which develop into males (drones) and rear new queens by feeding larvae in queen cells a special diet known as royal jelly. The active substance in royal jelly has not yet been identified but is known to have low molecular weight and to be very labile. For a general review of caste determination in the honeybee, see Pain (1968).

#### Scope of the Present Work

The present work was carried out to obtain information related to the question: What is it that determines caste in termites? How is it decided that some larvae will deviate from their normal development to winged adults and instead differentiate to become supplementary reproductives or soldiers?

Although this problem was first clearly recognized by Grassi and Sandias in 1893-1894, it remains to this day unsolved. A large body of descriptive and experimental evidence has accumulated so that the theories of caste determination in termites have been considerably narrowed down. In this author's view only one of these theories fits all the known experimental facts. This is the substance or "pheromone"

theory which holds that the reproductives secrete a substance which is picked up by the pseudergates and which inhibits their development to supplementary reproductives. A major step toward proving this theory would be the isolation of a substance or extract with inhibitory activity. So far this has not been possible.

A more fruitful line of research, and the one which has been primarily pursued in the present work, has consisted of experiments aimed at defining the process of caste regulation more closely. Thus, in the present work, attempts have been made to describe the process of regeneration of lost reproductives, to define the factors determining the ability of pseudergates to transform to supplementary reproductives, to delineate the separate roles of the male and female reproductives in the inhibitory process and to determine the relationship between pseudergate transformation and extent of separation from inhibitory reproductives. In addition, some attempts were made to obtain direct evidence for pheromones.

## II. MATERIALS AND METHODS

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### Collecting Kalotermitid Termites

Location Southern Florida is the nearest place to New York where species of the primitive family, Kalotermitidae, can be found. In this region, the family is represented by five genera and nine species (Weesner, 1965). These are listed in Table I.

The purpose of my first collecting trip, which occurred in February, 1967, was to obtain a large quantity of as many species as possible. The aim was to establish stock colonies in the laboratory and to select the species which would be the best experimental subject. Dr. Margaret Collins of The Department of Zoology of Howard University, who had collected Florida termites on numerous occasions, accompanied me. We were able to find colonies of every species except Calcaritermes nearcticus, which is rare. Only one colony of another rare species, Incisitermes milleri, was found and only one very small colony of less than 100 individuals of I. schwarzi could be obtained. A medium-sized colony of Kalotermes approximatus was collected but was found, upon extraction, to be contaminated with a colony of Cryptotermes cavifrons inhabiting the same log. Large colonies containing several thousand individuals were obtained for the remaining five species. The actual collection locations of all the species are listed below.

TABLE I

Collection Locations of Kalotermitidae found in  
Southern Florida, February 10-14, 1967

Species	Location	Abundance
<u>Calcaritermes</u> <u>nearcticus</u> (Snyder)	not found	very rare
<u>Cryptotermes</u> <u>brevis</u> (Walker)	boards from condemned houses in Miami	very abundant
<u>Cryptotermes</u> <u>cavifrons</u> Banks	woods in Key Largo	very abundant
<u>Incisitermes</u> <u>milleri</u> (Emerson)	woods in North Key Largo	rare (one colony found)
<u>Incisitermes</u> <u>schwarzi</u> (Banks)	woods surrounding Gumbo Limbo Trail, Everglades Natl. Park	one small colony found
<u>Incisitermes</u> <u>snyderi</u> (Light)	woods in Key Largo	very abundant
<u>Kalotermes</u> <u>approximatus</u> Snyder	Highlands Hammock State Park	one colony found contaminated with <u>C. cavifrons</u>
<u>Neotermes</u> <u>castaneus</u> (Burmeister)	Highlands Hammock State Park	one very large colony found
<u>Neotermes</u> <u>jouteli</u> (Banks)	woods in Virginia Key and Key Largo	very abundant

In subsequent trips (January, 1968; January, 1969; July, 1969) collection was restricted to a single species, Neotermes jouteli (Banks), and a single location -- the woods across the highway from the Miami Seaquarium on Virginia Key. In these woods, N. jouteli is the dominant species although occasionally colonies of N. castaneus and I. schwarzi were found.

Collecting technique and equipment Termites are extremely abundant in the woods of Southern Florida, which contain a large quantity of dead wood. The soil is sandy and quite shallow, lying usually no more than a few feet over the bed limestone rock, Miami oolite, in the Everglades and Virginia Key. The same is true of the Florida Keys, such

as Key Largo, which are basically the remains of an old coral reef. The bedrock known as Key Largo Limestone is overlaid by little soil and in many places is completely exposed. Trees that grow in these areas must spread their roots in a shallow pancake. Hurricanes and other strong winds can blow the trees over or break off large branches or sections of trunk. In addition, a heavy insect fauna, including many that bore wood, further decreases the strength of the trees. Frequently one finds dead branches on living trees. Often these are infected with termites.

All of Kalotermitid species of Southern Florida with the exception of the house termite, Cryptotermes brevis, can be found in these woods. Searching for them involves slashing at logs lying on the ground or at the periphery of the trunks of dead trees with a short handled ax or machete. The termites or their galleries, if present, will be exposed. If the desired species is found, the log is then sawed into pieces three to four feet in length which can be taped together with masking tape. The tape is labeled with the date, location and tentative field identification of the species. The taped bundle can then be placed in the trunk or back seat of a car. A further precautionary measure, which is usually unnecessary, is to enclose the bundles in large plastic bags. This reduces drying and prevents invasions of ants from other logs. Colonies of ants, which are the chief predator of termites, are often found in the same logs as termites. In fact, when collecting, it is best to avoid logs containing obvious ant colonies, since they will only present a problem when the termites are extracted.

Excellent keys are available (Emerson and Miller, 1943; Weesner, 1965) for identifying the termites of Florida. Species identification is carried out by examining the soldiers or alates. To do this properly, one immerses the insects in 85% ethanol and examines them with a dissecting microscope. Generally, in the field one can pick up a soldier with tweezers and examine it with a high magnification hand lens, such as the 14X Hastings Triplet Magnifier made by Bausch and Lomb. In many cases the distinguishing characteristics can be seen at this level of magnification. For example, N. jouteli is the only species in which the

soldier has a pigmented eye spot. This is visible using a hand lens. If several different species are being collected, sample soldiers and, if possible, alates from each colony should be dumped in vials containing 85% alcohol which are then taped to the logs from which the termites came. This obviates having to break open the logs again simply to find out what species is inside after the entire collection has been brought to the laboratory.

The woods in Southern Florida are very thick, and one should be well covered. The author generally wore boots, khaki work pants, thick knee-length socks pulled up over the khakis, and long-sleeved shirts. Such clothing provides adequate protection from most scrapes and cuts and also from biting or stinging insects such as ants. Minor cuts seem to be inevitable so one should bring along a first aid kit. In some areas mosquito repellent and hats with face netting are necessary. Inasmuch as there is some danger of poisonous snakes, one should proceed through the woods carefully and noisily. It is advisable to have basic equipment -- razor blades and tourniquet -- to deal with snakebite.

To collect the house-dwelling Cryptotermes brevis we learned from the Miami slum clearance authorities the location of abandoned houses. In such houses the floor boards, window sills and door frames are usually infested. These can be broken open with an axe and taken if colonies are found.

The bundles of logs and boards can be transported to New York in a car, a two or three-day trip. It is better to keep the logs in the back seat than in the trunk since the temperature can be regulated while driving. A thermometer should be brought along for this purpose and the ambient temperature maintained at  $25 \pm 5^{\circ}\text{C}$ .

Kalotermitid species can readily be collected in Southern Florida at any season. The colonies vary seasonally inasmuch as there is an annual alate production cycle. In these species alates are present in the logs in large numbers in the spring and summer months. In the process of extracting such colonies from logs the alates are exposed and tend to fly out. Therefore, from this point of view, it is best to collect in the fall or winter.

### Extraction of Termites from Logs

The log bundles can be stored on a laboratory cart. They should be doused with water nearly every day so that they are kept moist.

Extraction is carried out on an operating table that has been covered with a long sheet of brown paper. A section of log is sawed off, 8-12" in length. The softer the wood the longer the section that can be handled at a time. The section is stood upright in a metal tray. It is then split lengthwise by hammering an ax into it. If galleries containing termites are exposed by this initial splitting, the half sections are tapped so as to knock the termites onto a second metal tray. Termites that fall into the first tray can be transferred with a cardboard spatula or a splinter of wood. Eggs can be transferred with a moist fine brush. If alates are present they should either immediately be picked up by the wings with tweezers and transferred to covered dishes or they should be killed. Otherwise they may become excited and start to fly.

One continues to split the sections and tap the termites into the tray until every gallery has been exposed for its entire length and the original section has been reduced to narrow strips of wood. Sometimes a screwdriver or an awl are useful for prying open galleries. There is always some mortality when the soft-bodied termites are crushed as the wood is deformed. This problem increases the harder the wood is. With care, mortality can be kept to a few percent. One continues to saw off sections, split them open and tap out the termites until one has gone through an entire natural colony.

It often happens that colonies of two species will inhabit the same natural log. Usually the two colonies are in different parts of the log and can be kept apart. Sometimes there will be two well-developed colonies whose galleries intermingle or connect. In this case contamination is necessarily associated with extraction.

One also finds many other arthropods inhabiting the same logs as the termite colonies. These include millipedes, centipedes, spiders, pseudoscorpions and bark insects such as silverfish, proturans and



beetles and their grubs. None of these presents any problems and they can even be kept in the laboratory stock colonies. The only serious problem arises with ants, which should be killed when they appear and scrupulously excluded from stock colonies of termites.

When the crude extraction of a termite colony has been completed, one has in the metal tray a single pair of reproductives, soldiers, eggs, larvae of many instars and possibly nymphs. These are mixed in with large quantities of wood particles, splinters, sawdust, fecal pellets and dead or dying termites. It would be very difficult to separate out the colony at this point. It is possible, however, to get the termites to separate themselves out by transferring them to a temporary nest consisting of a large petri dish containing a layer of thick ( $\sim 4\%$ ) agar (for moisture) on which lie several strips of wood from the remains of the colony's natural log. One pours the "crude extract" over the strips of wood and covers the dish. For larger colonies several such dishes are needed. By the next day the healthy termites will have completely reaggregated themselves under the strips together with their eggs and will have transferred all contaminating particles outside the strips. One can then scoop and brush away the particles. The termites can then be easily transferred with the strips of wood, to which most of them cling, to a permanent nest. Often, however, they are first transferred to a clean dish so that a census can be carried out on the entire colony.

The remains of the log from which the colony has been extracted are discarded, with the exception of a group of broad strips to be used for maintaining the stock colony. These are placed in a plastic bag together with a label indicating with which colony they are associated. The bag is closed with a rubberband and stored.

#### Culturing Termites

Becker (1969) has reviewed various methods of rearing termites in the laboratory. Stock colonies of Kalotermitidae are relatively easy to maintain since all they require is wood and moisture. They do not need soil, nor do they make elaborate constructions. Thus natural

colonies in logs can be maintained indefinitely in the laboratory so long as the wood is kept moist and the temperature is kept in the range 20-30°C.

Stock colony nests The termites are, of course, not accessible in the logs and so, for this study, a stock colony nest was developed that approximates natural conditions. The nest consists of a glass battery jar 15 cm in diameter and 20 cm high in which a layer of stiff (~4%) Difco Bacto agar containing ~0.5% Drosophila mold inhibitor has been poured. The agar gel, which the termites ingest and burrow into, is a simple way of providing a long-term source of moisture. Strips of wood from the colony's natural nest are placed on top of the agar. These include short strips that lie on the agar layer and long strips that stand on the agar and lean against the wall of the jar. The strips should be broad and thin so there is enough wood to cover and feed the termites but not enough that they will become inaccessible upon burrowing. If the termites do burrow into a thin strip they weaken it, and it is easy to open up their galleries. Usually, however, they remain on the underside of the wood strips which can be easily removed whenever termites are needed for experiments.

The jar is covered with the top of a 15 cm petri dish which is taped in place. A piece of masking tape is placed on the jar on which is written the colony code number, the species, the date and place of collection, the colony census and census date. In addition a vial containing several soldiers and alates in 85% alcohol is taped to the jar. The vial should contain a label with the same information as is on the tape. Thus, if there is ever any question about the identification of termites used in a given experiment, one can refer to the vial after the colony is gone.

For small colonies the same type of nest can be constructed in 150 x 20 mm glass petri dishes. The stock nests are stored in an incubator to maintain constant temperature and to keep out light which termites prefer to avoid. Trays of water are placed in the bottom of the incubator to maintain high humidity. Oven-incubators are prefer-

able to refrigerator-incubators since air circulates in the latter and tends to dry out the colonies. A standard temperature of  $26^{\circ}\text{C}$  was somewhat arbitrarily chosen. In the summer when the room temperature may rise above  $26^{\circ}\text{C}$  the oven-incubator was moved to an air-conditioned room.

The stock nests require minimum maintenance. Occasionally, when it is clear that the agar is drying out, fresh strips of agar can be added. Fresh wood must be added every few months also. About once a year when the nests have accumulated excessive fecal material the termites should be transferred to a new nest.

Experimental colony nests Most of the experiments carried out in this work required that all the individuals in an experimental colony be examined or counted each day. Thus, the first requirement in constructing an experimental nest is that the termites be accessible. Second, in order to facilitate the study of a large number of experimental colonies, experimental nests should be simple, easily assembled, and reasonably standardized. Finally, outside of these constraints the nests should be as close to "natural" as possible. Of course, logs, the natural habitation of termites, are neither simple nor standardized, nor are the termites inside accessible without irreversibly destroying the nest.

The experimental colony nests used in this work were designed according to the same principle as the stock colony nests. They consist of small 60 x 20 mm plastic petri dishes or larger 100 x 20 mm glass petri dishes in which a thin layer of stiff agar containing mold inhibitor is poured. Broad, thin strips of wood from the termites' natural log are placed on the agar gel.

The termites form a nest by digging out the agar under the wood. They then seal the wood to the surrounding agar with fecal cement. They also dig tunnels in the agar. Thus, under the wood the situation is similar to that in a gallery in a log. The wood pieces can be easily removed and the termites tapped out of the dish. Agar tunnels can easily be opened up to remove the termites. The wood strips must be thin,

otherwise burrowing will occur and getting at the termites will incur the risk of injuring or killing them. After carrying out whatever experimental manipulation is required on the termites, the wood strips are replaced in the position from which they were taken and the termites are returned to the nest. They rapidly reorganize themselves and repair any damage done.

Colonies of approximately 50 individuals fit comfortably in the small plastic dishes. The larger glass dishes can maintain colonies of up to 250 individuals. All normal colony activities including mating, egg-laying, hatching, molting, alate production and trophallaxis have been observed in such nests. Four plastic dishes can fit in a large 150 x 20 mm glass dish. This is necessary since termites are capable of eating their way through plastic, although they rarely do. The large glass dishes can then be stacked in an incubator. The supply of agar lasts about a month which is long enough for most experiments. For longer experiments the colony must be transferred to a new nest.

Observation nests The petri dish nests described above cannot be used for experiments involving observation of behavior patterns, since the termites are nearly always under the wood or in tunnels in the agar. For experiments involving long-term observation it is necessary to construct nests consisting of transparent plates which are separated by no more than the height of one termite. Such nests have been designed by Adamson (1941) and Lüscher (1949). The nest used in these studies was basically a simplified version of Lüscher's nest. It consists of two plexiglas rectangles separated by narrow plexiglas strips  $3/32$ " high, glued to the bottom rectangle. Plexiglass strips are also used to divide the nest into two connecting chambers -- one three times the size of the other. In it are placed long, broad and extremely thin (less than  $3/32$ ") strips of wood that have been shaved from the wood of the natural nest with a razorblade. This chamber also contains some sand. The smaller chamber and the connecting passage contain moist sand which the termites use for constructions.

The termite colony is placed in the large chamber and the upper plexiglas rectangle is clamped to the lower one with rubber tube clamps

or plastic clothespins. By removing the clamps over the smaller chamber and lifting a corner of the upper rectangle, water can be added to the sand without disassembling the nest. Water must be added approximately once every other week.

The nests lie horizontally and the termites can be observed by using a dissecting microscope mounted in a movable holder arm attached to a stand such that the microscope can move in any direction and be used to observe any part of the nest without disturbing it. A nest is placed on a dark background in diffuse light. Room lights are usually adequate and spotlights should be avoided since they obviously disturb the termites. The termites seem undisturbed by diffuse light, and behavior patterns such as mating, egg-laying, grooming, trophallaxis, eating and making constructions are routinely observed. If a nest is left constantly in diffuse light the termites will eventually smear the upper rectangle with fecal material. Therefore, except when a nest is being observed, it should be stored in a dark incubator at 26°C.

Colonies of up to 75 individuals can be maintained in nests with overall dimensions of 9 x 12 cm. Larger nests can be constructed for larger colonies.

## III. INSTAR ANALYSIS OF NEOTERMES JOUTELI

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Introduction

When one first examines a termite colony freshly extracted from its log, it is difficult to avoid a feeling of bewilderment at the great diversity of forms. The vast majority of individuals are unpigmented forms -- larvae, nymphs with wingbuds and white soldiers of many different sizes. In addition there are the pigmented castes: reproductives, alates and soldiers. The soldiers may also come in different sizes. A number of questions arise. Can all these forms be defined and categorized? Can each form be assigned to a different instar? Developmentally, what are the connections among the different forms? It is necessary to have a general answer to these questions before the problems of caste formation and determination can be approached.

In the present study individuals from a freshly extracted colony of Neotermes jouteli were measured for head width and number of antennal segments. These were the measurements that Miller (1942) used to characterize the instars of the Rhinotermitid Prorhinotermes simplex and that Lüscher (1952a) used in studying Kalotermes flavicollis. The reason for

measuring these particular characteristics is as follows. Termites are soft-bodied insects. Obvious bodily characteristics such as weight and length vary considerably with the state of health, nutrition and hydration of the individual. Therefore it is necessary to restrict one's observations to the hard sclerotized portions of the body if one hopes to find characteristics that are instar specific. The most accessible of these are the head capsule and the antennae. In addition, in the present study the presence or absence of wingbuds was noted. Finally, larval molts involving caste formation were followed.

### Materials and Methods

Head measurements Termites were anaesthetized by blowing carbon dioxide over them in a dish. An individual's position was adjusted with metal probes so that its head was level. The head width at the widest point was measured with an ocular micrometer in a Zeiss dissecting microscope. All measurements were made at the same magnification (25x ocular, 1x objective). Under these conditions 1 ocular micrometer division =  $.444 \pm .004$  mm. A stage micrometer was used for this calibration. Readings were made to the nearest .05 ocular micrometer divisions.

Antennal measurements Termites possess beaded or moniliform antennae common to primitive insects. Such antennae consist of a string of discrete beads that are not morphologically very different. Since the antenna grows with the rest of the body at each molt, it was thought that the antennal segment number might be correlated with head width and that the two numbers might constitute a definite instar characteristic. The number of antennal segments was determined for each larva examined by direct count as observed in a Zeiss dissecting microscope at 25x.

Wingbuds Wingbuds, as defined in this study, are small outgrowths at the lateral posterior edges of the mesonotum and metanotum in larvae. They are not the same as the fully developed wingpads, clearly visible with the naked eye, which characterize the two nymphal stages in termites. The wingbuds observed here are precursors of nymphal wingpads which are precursors of wings in the imago.

### Results

Larvae The head widths of 247 larvae were measured and the larvae were categorized according to the number of antennal segments. The number in each group is given in Table II together with the number having wingbuds. It can be seen that wingbuds do not appear before larvae have 13 antennal segments and that individuals with 14 or more antennal segments nearly always have wingbuds. Wingbuds are never found in larvae with head widths of less than 3.00 micrometer divisions. Larvae with 13 antennal segments and wingbuds all had heads at least that wide. Larvae with 14 antennal segments and lacking wingbuds had small heads, usually less than 3.00 divisions.

TABLE II

Distribution of 247 Neotermes jouteli Larvae According  
to Number of Antennal Segments and Their Possession of Wingbuds

Antennal segments	Number of individuals measured	Number with wingbuds
10	10	0
11	3	0
12	10	0
13	34	11
14	50	45
15	84	81
16	41	41
17	15	14

For each antennal segment number a head width distribution curve was drawn. These curves are shown in Figure 1 for antennal segments 13, 14, 15, 16, and 17. Too few termites having 10, 11, or 12 antennal segments were found to draw meaningful distribution curves. In these cases the actual measurements are presented in Table III. Finally, the average head width associated with each antennal segment number was calculated and these results are presented graphically in Figure 2.



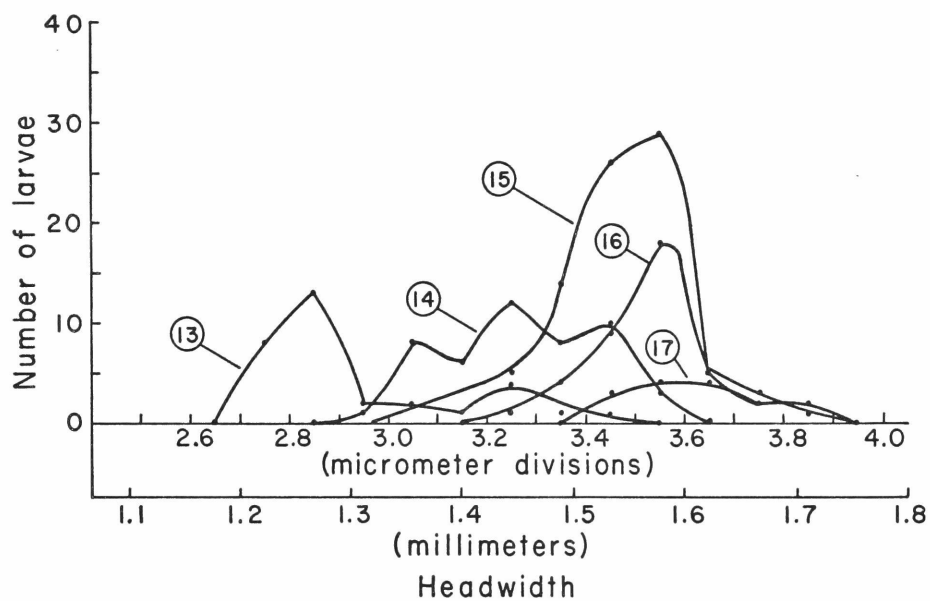


Figure 1. Head width distribution of 234 larvae of Neotermes jouteli. Circled numbers refer to antennal segments.

TABLE III

Head Widths of Larvae  
Having 10, 11 or 12 Antennal Segments

Micrometer divisions (1 div. = .444 mm)		Number of individuals	Number of antennal segments
1.0 - 1.1	.44 - .49 mm	2	10
1.1 - 1.2		7	
1.2 - 1.3		0	
1.3 - 1.4		1	
1.7 - 1.8	.89 - .93 mm	2	11
1.8 - 1.9		0	
1.9 - 2.0		0	
2.0 - 2.1		0	
2.1 - 2.2		0	
2.2 - 2.3		1	
2.5 - 2.6	1.33 - 1.37 mm	1	12
2.6 - 2.7		3	
2.7 - 2.8		1	
2.8 - 2.9		3	
2.9 - 3.0		1	
3.0 - 3.1		1	

Supplementary reproductives In the course of examining the larvae that had been removed from their stock colony nest, twenty supplementary reproductives -- ten males and ten females -- arose. The supplementaries arose in a single molt from larvae and were identifiable by their caramel pigmentation. Males and females were found with 14, 15, 16 and 17 antennal segments. One female arose with 18 antennal segments. The head widths were essentially the same as in larvae with the same number of antennal segments. Aside from pigmentation and the development, especially in the female, of external genital characteristics the only other change in bodily morphology from a larva is in the wingbuds, which are usually reduced or totally resorbed in this molt.

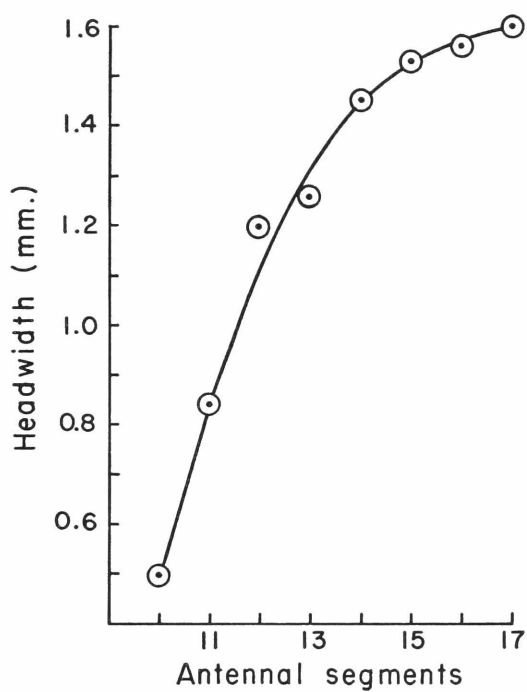


Figure 2. Average head widths of 247 *Neotermes jouteli* larvae as a function of number of antennoal segments.

The supplementary reproductives are therefore considered to be an example of neoteny -- sexual maturation within a juvenile morphology. This form lacks all the salient characteristics associated with adult termites such as heavy pigmentation, fully developed complex eyes and, of course, wings. On the other hand, it is an adult caste in the sense that it can never molt again.

The data regarding the supplementary reproductives is presented in Table IV.

TABLE IV  
Supplementary Reproductive Measurements

Antennal segments	Head width (micrometer divisions)	Wingbuds	Sex
14	3.35	+	F
14	3.45	(+)	F
14	3.50	(+)	M
14	3.55	-	F
15	3.21	+	M
15	3.35	(+)	F
15	3.35	+	F
15	3.40	+	M
15	3.45	-	M
15	3.80	-	M
16	3.40	-	F
16	3.40	(+)	M
16	3.50	-	F
16	3.50	+	M
16	3.55	+	M
16	3.85	-	M
17	3.60	(+)	M
17	3.65	-	F
17	3.80	(+)	F
18	3.80	+	F

Soldiers In Neotermes jouteli soldiers are large, darkly pigmented forms with large, sclerotized heads and long, toothed black mandibles. They arise in a single molt from an intermediate form, the white soldier, that is unpigmented and larva-like but has an enlarged head and mandibles.

The white soldier arises in a single molt from a larva. In the colony under study, 31 white soldiers were found with 13, 14, 15 and 16 antennal segments and 53 soldiers with 15, 16 and 17 segments. In four cases white soldier development occurred from larvae with known antennal segment numbers. One case involved a larva with 13 antennal segments,  $L^{13}$ , which molted to a white soldier with 13 antennal segments,  $WS^{13}$ . Another case can be represented as  $L^{14} \rightarrow WS^{14}$  and there were two cases of  $L^{14} \rightarrow WS^{15}$ .

Development along the soldier line involves wingbud resorption. Wingbuds are small or absent in white soldiers and nearly always absent in soldiers.

Alate formation No alates arose in the colony in this study. This by definition means that the colony was not fully mature. In other colonies alates were observed to arise via two nymphal stages from the largest larvae. Nymphs are characterized by the presence of wingpads. In the first nymphal stage the wingpads are thin and brownish. In the second nymphal stage the wingpads are thick, prominent and white. In this stage also the abdomen is very white, which suggests that the second stage nymph does not eat any wood and is fed by larvae.

### Discussion

Larval head width distributions A number of conclusions can be drawn from the larval head width distributions. First, although only a small number of individuals could be measured, the first two instars are clearly distinguishable from each other and all other instars. This corresponds to what Lüscher (1952a) found in Kaloterms flavicollis and is confirmed by gross observation of a termite colony. The early instars are clearly different in size from later instars by naked eye observations. This is evidently because growth is rapid in the early stages of termite development.

When one looks at the head width distribution of larvae with 12 or more antennal segments one finds considerable overlap. It is difficult to determine the importance of the overlap in the case, 12 antennal segments, since so few individuals were measured. In this case overlap occurs with the distributions of individuals with 13 and 14 antennal

segments. However, the curves of individuals with 13, 14, 15, 16 and 17 antennal segments all overlap with each other. In fact, the head width distributions for the cases 15, 16 and 17 antennal segments are very similar, if not identical. These forms presumably correspond to Lüscher's pseudergates.

One conclusion, then, is that growth is rapid in the early instars but thereafter slows down. Once a termite reaches a certain size it is impossible to say with certainty how many antennal segments it has or what instar it is in.

Thus it could well be that the question of the number of larval instars is unanswerable and somewhat meaningless. The situation is one in which discrete definable growth occurs in the early instars and thereafter individuals develop at different or varying rates so that an entire spectrum of individual types exists and one can find larvae with 14 antennal segments of the same size as larvae with 17.

One can think of reasons why this situation might exist. In most insects the larval instars are characterized by pronounced differences in size. The larval stage is the period of vegetative growth in which the organism accumulates the mass necessary for sexual maturity and reproduction. Speaking teleologically, it is in the interest of the species to get through the larval stage as rapidly as possible. However, termite larvae do not simply fulfill a vegetative function. Termites are social insects and probably starting with the third instar termite larvae fulfill social functions. From this stage on, they are functional workers that eat wood, clean other termites, feed individuals of the first two instars as well as soldiers and reproductives, and carry out all other worker activities. Light and Weesner (1951), for example, found that colonies of Zootermopsis angusticollis containing only third instar larvae could be maintained in the laboratory. Colonies containing only second instar larvae always died out. There are, in fact, no striking differences in behavior between small and large functional workers. Furthermore, at least in immature colonies, essentially all the larvae that are functional workers are capable of differentiation to soldier or supplementary reproductive.

It is therefore not surprising to find no great morphological differences between larvae having no great functional differences. Since larvae are used as workers, a rapid development to sexual maturity is not needed. What is needed and what does exist is a rapid development to the stage of being a functional worker. Thereafter, continuous growth can occur. Growth is still necessary since large larvae can probably do more work than small ones and since at some point in the colony's development the large larvae will be needed for alate production.

The overall growth and development of the larvae can be detected when one looks at the average head widths associated with a given number of antennal segments. This is shown in Figure 2. Average head width does progressively increase with antennal segment number, rapidly at first and gradually for 14 or more segments.

The result that a continuity of larval states exists, especially starting with the fifth instar, is very similar to what Miller (1942) found in the closely related Kalotermitid, K. flavicollis. In Miller's study, head width distributions were fairly discrete for the first four instars (defined according to antennal segment number) but overlapped considerably for the last four. This was also true if mesonotum widths were measured. In Lüscher's study it was found that larvae, after the fifth stage was reached, could undergo molts with only very gradual changes in size or form.

The larvae then reached a final stage known as the pseudoworker or "pseudergate," a term invented by Grassé and Noirot (1947). The pseudergate could undergo an indefinite number of stationary molts without significant change in size or form. From an earlier study (Lüscher, 1951a) it was clear that the pseudergate stage actually included forms with different numbers of antennal segments which presumably could change even in a "stationary" molt.

In the present work with Neotermes jouteli the term pseudergate will be taken to be synonymous with large functional workers. More specifically, pseudergates can be defined by three characteristics, which

describe their morphology, their developmental capabilities and their behavior.

1. They are the largest apterous larvae in any given natural colony.
2. They are capable of developing into supplementary reproductives, white soldiers or first stage nymphs, or they can undergo stationary or nearly stationary molts.
3. They carry out all non-soldier and non-reproductive work functions in the colony. They are to be distinguished from the first stage nymphs and juvenile larvae although these forms may share some of the behavioral and developmental characteristics of pseudergates.

Error analysis The overlap in the curves in Figure 1 is enhanced because of two sources of systematic error. The first is antennal breakage. Occasionally, individuals are found that have wide heads and are very large, but have very short antennae. These are obviously cases in which loss of part of the antenna has occurred during development. If both antennae are affected these cases can be excluded. However, if only a few antennal segments have been lost, this cannot be detected and such individuals will tend to skew the distribution curves to the right. In an effort to correct for this error, whenever there was a difference in antennal segment number between two antennae, the larger number was taken.

The other source of uncertainty is in the actual counting of antennal segments. The third segment seems to be the growing point for the whole antenna, and at this point one finds a continuity of states including a single discrete segment, various intermediate forms and two discrete segments. The presence of an intermediate state makes the decision as to the number of segments on a given antenna somewhat subjective. Since growth at the two antennae occurs at slightly different rates, one can often resolve the question for a given larva by comparing the third segment of each antenna. There is usually no difficulty in visualizing any of the other segments. The fact that intermediary states exist at the growth point is a further indication of the fact that growth is rather continuous in larval termites and that instars are ill defined.



Caste formation The observations made on soldiers, supplementary reproductives and alates in this study were aimed not so much at defining morphologically these castes as at attempting to determine their relationship to the undifferentiated larvae. Reproductives were found with up to 18 antennal segments and larvae were found with up to 17 antennal segments. This suggests that the supplementary reproductive molt can involve an increase in antennal segments. The smallest number of antennal segments found on a reproductive was 14, suggesting that perhaps as early as the fourth instar (13 antennal segments) but certainly as early as the fifth instar, larvae have the capability of differentiating into reproductives. Castle (1934) found that all immature forms starting with the fourth instar could become supplementaries in Zootermopsis angusticollis. Lüscher (1952a) found that it was not until the fifth instar in K. flavicollis that larvae could develop into supplementaries.

White soldiers were observed with as few as 13 antennal segments and in one case it was known that such a white soldier arose from a larva that also has 13 antennal segments. Thus, larvae can differentiate towards soldiers as early as the fourth instar. This was also found by Lüscher (1952a) in K. flavicollis.

The ability to differentiate into soldiers or reproductives is preserved in all larval stages after it first arises. Both differentiation processes involve loss of wingbuds. This is reminiscent of the resorption of wingpads that Lüscher (1952a) observed when nymphs of K. flavicollis developed into soldiers or supplementary reproductives.

The formation of alates via two nymphal stages is identical to what Lüscher found in K. flavicollis. Lüscher also found that the two nymphal stages could develop into soldiers or reproductives or could totally resorb their wingpads and dedifferentiate back to the pseudergate stage. No information on these questions was obtained in this study; however, it seems reasonable to assume that nymphs possess these capabilities in N. jouteli as well.

### Conclusion: Scheme of Developmental Pathways

The above results and discussion allow one to derive a general scheme of the developmental pathways in Neotermes jouteli. This is shown in Figure 3. Figure 3 summarizes the following findings. After hatching larvae go through a number of instars characterized by discrete size distributions and well-defined antennal segment number. They rapidly reach a point, perhaps as early as the fourth instar, after which molting continues but developmental changes become extremely gradual and ill defined. After this point larvae are known as pseudergates, forms which can be loosely defined by their morphological, developmental and behavioral characteristics. At least as early as the fourth instar ( $L_4^{13}$ ) larvae become capable of soldier development, and somewhere around the fifth instar ( $L_5^{14}$ ) larvae become capable of supplementary reproductive development. These capabilities are preserved in all further larval stages. Nymphs derive from the pseudergates and develop via two stages into alates. They are probably also capable of soldier and supplementary development and dedifferentiation back to the pseudergate. Wingbuds appear somewhere around the fourth instar ( $L_4^{13}$ ) and are kept in more advanced stages. They are resorbed during soldier or supplementary reproductive development.

The scheme shown in Figure 3 is essentially identical to that derived by Lüscher (1952a) for the closely related Kalotermes flavicollis. The only difference is that Lüscher found that the pseudergate stage was preceded by five rather than four larval stages. Since it is doubtful that pseudergates can be clearly demarcated from nearby larval stages, this difference may only be a matter of definition. It is reasonable that development should be similar if not the same in N. jouteli and K. flavicollis. Both species are members of the same family. Until Krishna's revision (1961) of the Kalotermitidae, both species were considered to be in the same genus, Kalotermes.

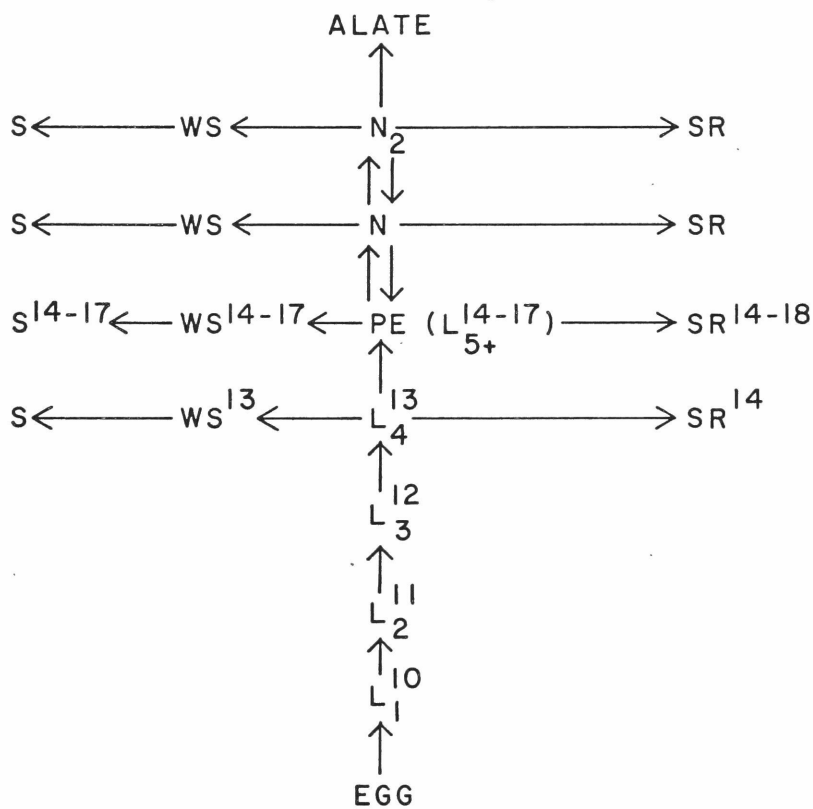


Figure 3. Developmental pathways in Neotermea jouteli.

Abbreviations: L = larva, PE = pseudergate, N = nymph, WS = white soldier, S = soldier, SR = supplementary reproductive. Superscript refers to number of antennal segments. Subscript refers to stage.

## IV. CASTE REGENERATION

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Introduction

The maturation of a termite colony to the point where its larvae can develop into swarming alates takes a long time. There must first be a period of vegetative growth in which the colony's population develops and its nest grows to full size. In the case of Neotermes tectonae this may last six or seven years (Kalshoven, 1930), in Zootermopsis, four years (Heath, 1931). The single pair of reproductives are therefore critical individuals in this growth period. After a new colony has been initiated and sufficient functional workers are present, the reproductives become totally specialized for colony growth. They lose their ability to eat wood and do work and they devote themselves entirely to mating and egg-laying (Grassé, 1949). This is associated with abdominal swelling, particularly in the female but to some extent in the male as the internal reproductive organs grow. This abdominal hypertrophy reaches an extreme in the highest family of termites, the Termitidae, where in some species the female's abdomen may increase in volume 300 times. These females, known as physogastric queens, are essentially giant egg-laying machines. In Macrotermes natalensis the female's abdomen may grow to 5" long and 1½" wide. Grassé (1949) reported observing a female of this species expel eggs at the rate of one every 2.4 seconds and stated that this probably was exceeded in other species that had even larger queens. He estimated that in some cases the queen might produce as much as two-fifths of its own weight in eggs each day.

A crucial question, then, is: What happens to a young growing colony if it is suddenly deprived of its reproductives? This could easily happen in all or part of a natural colony if a branch or tree

trunk were broken by the wind or if the reproductives were killed through predation, disease or various physical occurrences such as flooding. The answer to the question is that, at least in the lower termites, a process of regeneration immediately begins and within a short time a colony will have returned to normal.

Grassi and Sandias (1893-1894) were the first to study the production of supplementary reproductives in laboratory colonies of orphaned larval termites. Working with the Southern European dry-wood species, Kaloterme flavicollis, they observed that within 4-7 days following orphaning, larvae acquired pigmentation and became sexually mature in artificial laboratory colonies of 15-40 individuals. Furthermore, they found that if larvae were isolated from their reproductives for even 24-48 hours, then supplementaries would sometimes arise. They considered that the supplementaries were neotenic larvae that had acquired sexual maturity through being fed a special diet. They also noted that excess supplementaries that arose in response to orphaning gradually disappeared from the colony leaving a single pair and that thereafter no new supplementaries arose.

Despite this very promising beginning it was a long time before much more was known about the actual regenerative process. Supplementary reproductive production was studied in two species of Zootermopsis, a genus of the family Hodotermitidae, by a number of authors including Castle (1934), Light (1942-43), Light and Illg (1945) and Light and Weesner (1951). Their findings were similar to those of Grassi and Sandias for K. flavicollis, except that in Zootermopsis regeneration did not seem to be as sharply controlled. Castle (1934) found that groups of isolated Z. angusticollis larvae gave rise to pigmented individuals that produced eggs within 35-50 days. Light (1942-43) found that reproductives could be regenerated in as few as two weeks in Z. nevadensis but that there was great variability in laboratory colonies and that it might be as long as six weeks before pigmented individuals appeared. He also found that reproductives could arise in small colonies (of 20 individuals) that already had a pair of reproductives, although not as many arose as

in orphaned groups. Excess reproductives were tolerated and no mention was made of their disappearance. Light and Weesner (1951) found that reproductives could be removed from colonies of Z. angusticollis for at least a week before reproductive production became irreversible.

It was not until 1946 that Grassé and Noirot (1946a), working with K. flavicollis, showed that the transformation of a larva to a supplementary always involved a molt. Previous authors had assumed that larvae simply became pigmented and sexually mature without molting. As was the case for all molts this one was preceded by an emptying of the digestive tract, following which the larva appeared white (Grassé, 1949).

Lüscher (1952b) studied this molt in detail and found that it differed in a number of respects from normal larval molts. Using marked larvae, he found that the emptying of the gut occurred on the average 5.4 days before a supplementary molt but 7.0 days before a larval molt. Second, he found that the duration of the molting interval before a supplementary molt was less than half as long as that before a larval molt. That is, the supplementary molt is specially induced by the removal of reproductives. Finally, he found that supplementary reproductives never molt again and therefore constitute a final adult caste. Later work (Lüscher, 1960a) showed that the prothoracic glands, the source of ecdysone, the molt-inducing hormone, degenerate following a supplementary molt.

Both Grassé and Noirot (1946a) and Lüscher (1952b) observed that excess reproductives produced in response to orphaning were eaten by the larvae until only a single pair remained. Lüscher (1952b) found that this process of caste elimination began 1-3 days after the production of a second male or female.

In none of the studies that have been mentioned were daily censuses reported of colonies undergoing regeneration. This is perhaps due to the fact that the intensity of the regeneration process was never great enough to warrant daily observation. Judging from the data presented by Grassé and Noirot (1960a) approximately 5% of the larvae transform to supplementaries in laboratory colonies of K. flavicollis which have been

orphaned for ten days. In Z. nevadensis, Light's data (1942-43) indicate that an average of 10% of the larvae transform in 17 weeks after orphaning. As will be seen in the following experiments the regeneration intensity is much higher in Neotermes jouteli. In ten days after orphaning, 15% of colonies consisting of pseudergates will have transformed, and in three weeks the level can reach 40%. Because of the strength of its response to orphaning, N. jouteli is an excellent species for studying mechanisms of caste determination.

### Experimental Procedure

In the following experiments the details of the regeneration process in Neotermes jouteli were investigated. The procedure was as follows. On day 0, fifty larvae were removed from a stock colony nest and placed in an experimental colony nest.

The larvae were chosen from the most advanced stages present in the stock colony. As had been clear from the instar analysis carried out on N. jouteli it is not meaningful to make morphological distinctions between larvae after a certain stage. In early experiments larvae of a uniform number of antennal segments were used. Thus Figure 4A shows regeneration in a colony containing larvae that all had 16 antennal segments. It soon became clear that there was no detectable difference in regeneration behavior in larvae with 14, 15, 16 or 17 antennal segments, and thereafter experimental colonies were simply provided with "large" larvae or "pseudergates" that presumably represented a number of different advanced stages and antennal segment numbers.

In addition to the pseudergates, each experimental colony was provided with a few (2-5) soldiers to suppress the tendency towards soldier regeneration that appears in the absence of this caste. The number of soldiers was determined by the proportion of this caste in the original stock colony. Each day beginning on day 1, the experimental nest was opened and a census was performed. Except during the census the nests were incubated in the dark at 26°C.

Pseudergates normally have brownish bodies due to the fact that their guts are full of wood particles. The first sign that a pseudergate

is going to molt is that it stops eating, absorbs or excretes all wood particles and appears totally white. This may be related to the fact that molting involves shedding of the cuticle that besides covering the external surface of the larva's body also lines the foregut, the region consisting of the esophagus, crop and gizzard. The numbers of white and brown pseudergates were both noted in the daily census, which also included the number of reproductives, white soldiers and soldiers.

### Results

The censuses as a function of time are presented in Figure 4A for a single experimental colony established in 1967 and in Figure 5 for the average of eight experimental colonies in 1969. Figure 4B shows the behavior of a control colony that contained a pair of supplementary reproductives from the beginning.

It can be seen that the regeneration process begins very soon after orphaning. White pseudergates begin to appear in 1-3 days and the white pseudergate population builds at a very rapid rate. Starting on day 4, this rate begins to level off but the population of white pseudergates continues to grow until day 8 or 9 when the first white pseudergates molt to become supplementary reproductives. Thereafter the brown pseudergates stop becoming white and essentially all the white pseudergates are transformed into supplementaries. The number of supplementaries that accumulate in a colony is never as large as the number of white pseudergates that accumulate, because as excess supplementaries arise a process of caste elimination sets in. Starting on day 10 or 11 the remaining brown pseudergates begin to cannibalize supplementary reproductives. This is reflected in the fall of the total population, which has heretofore remained essentially constant. In some cases supplementaries are forced outside of the colony's galleries where they apparently starve to death. Finally the last white pseudergate becomes a reproductive and the reproductive population is reduced to a single male and female. By approximately day 24 the colony has been restabilized. Within the next four days the first eggs appear. Therefore, the regeneration process has cost the colony four weeks plus 40% of its pseudergate population.



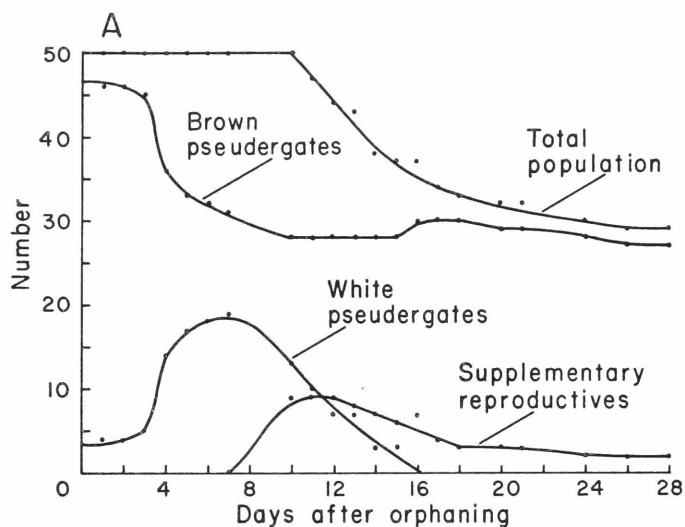


Figure 4A. Reproductive regeneration in a single colony of *Neotermes jouteli*, 7-8/67. Colony maintained at 26°C in the dark. All pseudergates with 16 antennal segments.

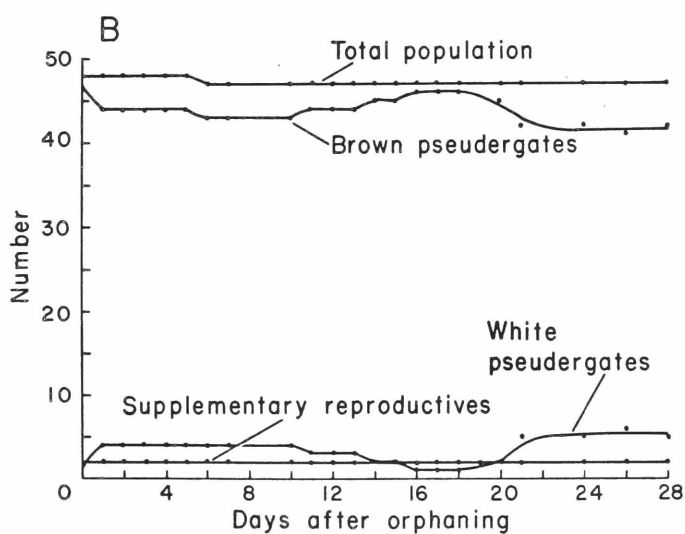


Figure 4B. Control colony from same stock run concurrently. All pseudergates with 15 antennal segments.

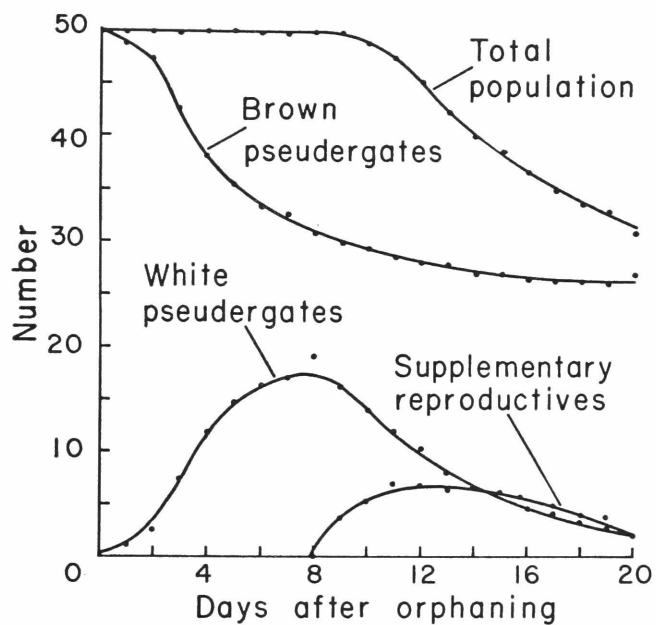


Figure 5. Reproductive regeneration in Neotermea jouteli. Average of eight colonies of pseudergates from Stock 69-2. Colonies maintained in dark at 26°C, January-April 1969.

It should be noted that the pseudergates from the colonies on which Figures 4 and 5 were based were taken from stock colonies that contained a single pair of supplementary reproductives. There would have been no difference in the results if the stock colonies had been headed by primary reproductives instead.

### Other Species

Reproductive regeneration was briefly surveyed in two other species of the southern Florida Kalotermitidae, Neotermes castaneus and Incisitermes snyderi. Neither seemed to be as promising experimental organisms as N. jouteli. In both species the regeneration response seemed considerably weaker. In N. castaneus an average of 8% of the pseudergates transformed in the same amount of time. In addition, these species present other difficulties. Very little pigmentation is acquired in the supplementary molt in N. castaneus and it is difficult to distinguish supplementaries from pseudergates. In I. snyderi the pseudergates are normally very pale and it is difficult to perceive the pre-molt white phase.

### Soldier Regeneration

Much less attention has been paid to the problem of the regeneration of soldiers than to that of reproductives. The scattered observations that exist point to the view that the proportion of soldiers in a colony is rather stable at least for large and mature colonies. Soldier development is inhibited so long as this proportion is present, and furthermore the presence of soldiers in excess of this proportion leads to caste elimination. Thus Castle (1934) found that addition of soldiers to incipient colonies of Zootermopsis angusticollis prevented further soldiers from arising whereas removal of soldiers stimulated the production of more soldiers. Miller (1942), working with the Rhinotermitid, Prorhinotermes simplex, found that in groups of larvae from which the soldiers had been removed, about 7% of the individuals transformed to soldiers in about a month whereas in control colonies with soldiers only 0.7% became soldiers. Lüscher (1961a) found that colonies of K. flavicollis with more than 300 individuals maintained a relatively constant level of approximately 3% soldiers. The departure of alates in the

seasonal swarm momentarily increases the proportion of soldiers and the larvae proceed to cannibalize the soldiers back to their normal level.

One simple experiment was carried out in the present work to show that if soldiers were removed from a colony of N. jouteli, soldier regeneration occurs. The experiment was the same in principle as the one already described for reproductive regeneration. On day 0, 50 pseudergates were removed from a stock colony nest and placed in an experimental colony together with a pair of reproductives. In the initial stock colony the soldier:pseudergate ratio was approximately 1:10. Control colonies were also set up that contained 50 pseudergates plus five soldiers. The results for two experimental colonies are shown in Table V and graphically in Figure 6. It can be seen that it takes five to seven weeks for a white soldier to arise from a pseudergate, and then two to three weeks for the white soldier to molt to a soldier. (It should be remembered that the white soldier arises from a pseudergate via a molt and is not analogous to the white pseudergate, which is simply the phase of the intermolt cycle of a pseudergate that precedes its next molt). By fifteen weeks the colony has regenerated its normal complement of soldiers and is thereafter stable. Control colonies showed no change in soldier number.

Soldier elimination has frequently been observed though not studied systematically in the course of this work. As has been seen, the process of reproductive regeneration involves the destruction of a large part of the non-soldier population and therefore increases the proportion of soldiers in the colony. When the destruction of the excess reproductives is finished, the pseudergates nearly always then turn on the soldiers and reduce their numbers to the proportion originally present. For example, in the colony shown in Figure 4A that underwent reproductive regeneration, two soldiers were killed, one on day 20 and one on day 28. Thus both the soldier and pseudergate populations were reduced and the ratio was restored to 1:10.

TABLE V

Soldier Regeneration in N. jouteli

Day	Colony 1					Colony 2					Control 1					Control 2				
	WS	S	WL	L	SR	WS	S	WL	L	SR	WS	S	WL	L	SR	WS	S	WL	L	SR
0	0	0	5	45	2	0	0	1	49	2	0	5	4	44	2	0	5	1	47	2
29	0	0	3	47	2	0	0	8	42	2	0	5	7	40	2	0	5	5	42	2
32	1	0	4	45	2	0	0	8	42	2	0	5	2	45	2	0	5	8	39	2
35	1	0	5	44	2	0	0	14	36	2	0	5	3	44	2	0	5	6	41	2
39	1	0	3	46	2	0	0	13	37	2	0	5	7	40	2	0	5	9	38	2
46	1	0	5	44	2	2	0	7	41	2	0	5	3	44	2	0	5	12	35	2
49	1	1	5	43	2	2	0	7	41	2	0	5	7	40	2	0	5	10	37	2
53	1	1	6	42	2	2	0	3	44	2	0	5	9	38	2	0	5	8	39	2
62	3	1	5	38	2	1	2	2	44	2	0	5	5	42	2	0	5	7	38	2
103	0	6	2	38	2	0	5	2	42	2	0	5	1	45	2	0	5	2	43	2
151	0	6	0	40	2	0	5	4	39	2	0	5	1	45	2	0	5	2	43	2

Abbreviations: WS = white soldiers; S = soldiers; WL = white larvae (white pseudergates);

L = larvae (brown pseudergates); SR = supplementary reproductives.

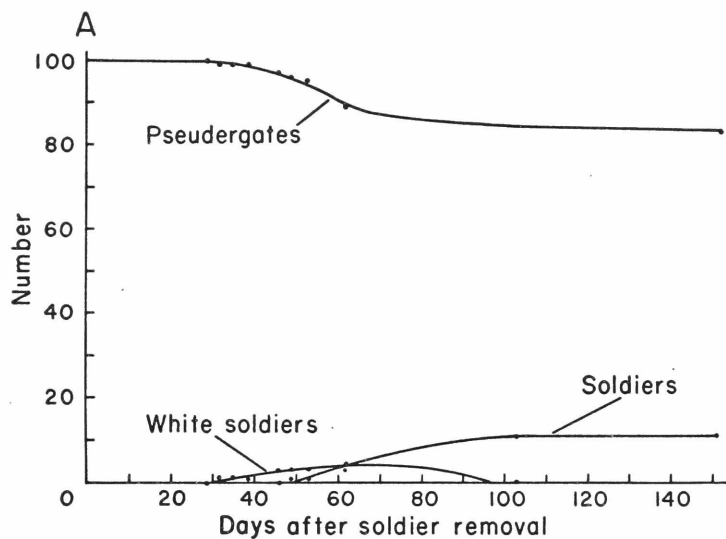


Figure 6A. Soldier regeneration in *N. jouteli*. Sum of two colonies kept at 26°C in the dark, June–November, 1967.

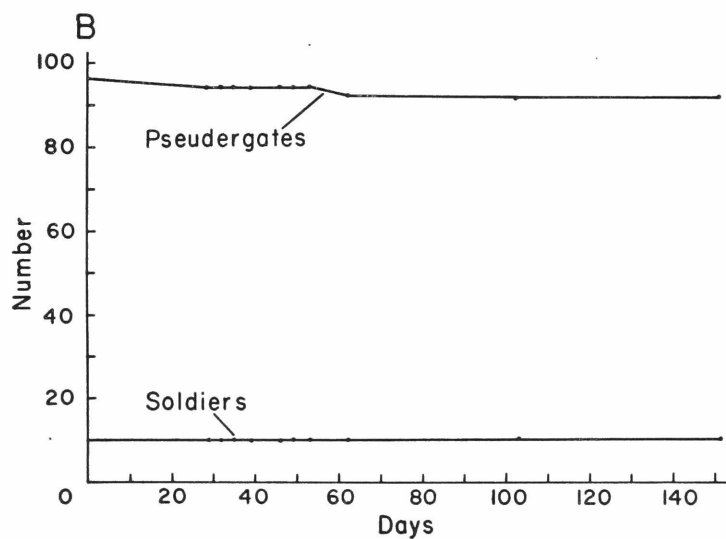


Figure 6B. Control colonies with full complement of soldiers, June–November, 1967.

### Discussion

The events leading to the restoration of a functioning pair of reproductives in an orphaned laboratory colony of N. jouteli can be summarized as follows. First, there is an activation period of 1-3 days in which the first pseudergates become "aware" physiologically of the absence of the reproductives. This period ends when these pseudergates empty their guts and turn white. The white premolt period lasts 6-8 days. There then follows a molting period in which pseudergates transform to supplementaries. This usually lasts 7-10 days since new pseudergates continue to be activated until there is at least one male and one female reproductive present. In 1-2 days after the start of the molting period, caste elimination begins. This continues for usually about two weeks until a single reproductive pair is left. It is not clear when the first matings occur, but eggs begin to appear almost as soon as the colony has been restabilized. The entire process lasts four weeks.

It is evident that reproductive regeneration in this species is qualitatively similar to that in K. flavicollis. Lüscher's data (1952b) indicate a slightly shorter white premolt period and possibly also a shorter time before the first pseudergates are activated. Thus, whereas the first supplementaries appear 7 days after orphaning in K. flavicollis, they usually do not appear until day 9 in N. jouteli. The general time course for caste elimination and molting seem to be the same in the two species. Egg-laying, on the other hand, does not occur until 2-4 weeks later in K. flavicollis (Grassé and Noirot, 1946a).

One significant difference appears to lie in the regenerative intensity. However, it must be pointed out that, as will be shown in the next chapter, this property varies with colonies within the same species. The important variable seems to be the age of the colony, and evidence will be presented that regenerative intensity in N. jouteli decreases as a colony grows older. Many colonies have been found in the course of this work with regenerative intensities as low as those reported for K. flavicollis.

The data presented here also support Lüscher's conclusion that the supplementary molt is specially induced by removal of reproductives. Thus the number of white pseudergates in control colonies fluctuates around a constant level of 6% (Figure 4B). The fact that a large bloom of white pseudergates occurs in experimental colonies shows that reproductives are not simply arising from pseudergates that are at the end of the normal intermolt cycle.

The vigorous response to orphaning of groups of N. jouteli pseudergates taken from young natural colonies makes such colonies an excellent subject for studying the mechanisms controlling and determining caste in termites. For example, orphaned colonies of N. jouteli pseudergates could serve as clear-cut assay systems to test the inhibitory power of chemical extracts.

Soldier regeneration, on the other hand, is much less vigorous, both in the time required and in the number of soldiers produced. There does not seem to be much, if any, overshoot in soldier production.



## V. COLONY VARIATIONS

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Introduction

In the course of this study it was soon observed that the vigorous regeneration displayed by some orphaned colonies was not obtained when other stock colonies were tested. The differences between colonies were often drastic.

This phenomenon was first noticed by Light (1942-43) in Zootermopsis nevadensis and was later investigated in some detail by Light and Illg (1945). Light and Illg concluded that "reproductivity," defined as the number of reproductives that arose in a given group of larvae in some given time after isolation from their parent colony, was the same in groups of larvae of the same instar composition taken from the same parent colony at the same time. However, great differences could be found if any of these three variables were changed. That is, groups of larvae having the same constitution and taken at the same time from different natural colonies, or groups taken from the same colony at the same time but having different instar compositions, or groups having the same composition and taken from the same colony but at different times, generally had different reproductivities.

Light (1942-43) proposed three possible explanations for the colony variations in regeneration intensity. They might result, he thought, either from genetic differences between the colonies or from individual historical differences in the development of each colony, or they might represent characteristic shifts during the life history of a colony with the possibility that changes in reproductivity were pro-

gressive, regressive or cyclic. Light doubted that the difference had a genetic basis but offered no evidence related to any of the three possibilities.

In the present work a number of correlations were found that suggest the colony variations can at least partly be accounted for as a regressive shift in regenerative ability with increased colony age. In addition, it was found that some of the data presented by Light and Illg (1945) could be interpreted as support for this hypothesis.

#### Experimental Procedure

On January 6 and 7, 1969, 18 natural colonies of Neotermes jouteli were collected in the same woods on Virginia Key, Florida. These were brought to New York on January 9 and extractions were carried out beginning January 10 and ending February 12. As soon as possible after extraction the following studies and procedures were carried out on each colony.

1. Nest description: Size, location and extent of galleries; wood samples were kept.
2. Population: Census of pseudergates, white soldiers, soldiers and primary and/or supplementary reproductives; presence of eggs and juveniles; ratio of pseudergates to soldiers plus white soldiers.
3. Antennal segments: The antennal segments of a number of typical pseudergates were counted.
4. Weight: 25 pseudergates and 10 soldiers from each colony were weighed.
5. Protozoa: The hindgut protozoa of larvae from each colony were qualitatively scanned with the aim of checking to see whether the major species characteristic of N. jouteli (Krishna, 1961 and Addendum) were universally present.
6. Response to orphaning: 25 pseudergates plus a proper number of soldiers were isolated from each stock colony and reproductive regeneration was monitored through daily censuses. The regenerating colonies were incubated at 26°C in the dark. In two colonies (69-14 and 69-15) reproductives were removed as they arose, but in the other groups the normal course of events were allowed to occur. The reproductives

present each day were sexed. A simple regenerative index,  $I_R$ , was invented and measured using the formula

$$I_R = \frac{\% \text{ of original pseudergates that transformed in 20 days}}{\text{days to the appearance of first reproductive after isolation (T-day)}}.$$

Thus  $I_R$  will be larger the more pseudergates transform and the sooner transformation begins.

7. Preservation: A group of pseudergates and soldiers and, if possible, alates from each colony were preserved in 85% ethanol.

8. Alates: If alates arose in the stock colonies in the following summer, the date on which they were first noticed was recorded.

### Results

Table VI lists the  $I_R$ 's, the average weights and numbers of antennal segments of the pseudergates, the average weights of soldiers, and the presence or absence of alates. The data is presented in order of decreasing  $I_R$ . In addition, regeneration graphs for sample colonies are presented in Figure 7. In the cases of colonies 14 and 15, from whose test colonies reproductives were removed as they arose,  $I_R$  was estimated on the basis of the number of white pseudergates present the day before the first supplementaries appeared. If the formula for  $I_R$  given above was used,  $I_R$  would of course be considerably higher for these colonies [ $I_R$  (colony 15) = 9.5,  $I_R$  (colony 14) = 8.0]. The true  $I_R$  for colonies 69-11 and 69-18 was probably somewhat higher than that found since the mortalities for these colonies were high. It can be seen that  $I_R$  varies continuously from 0 (colony 69-4) to 6.5 (colony 69-15).

When the data are presented as in Table VI, it is seen that the colonies fall into two groups: those in which the pseudergates and soldiers are large and those in which they are small. The large individuals weigh almost twice as much as the small ones and usually have more antennal segments. The colonies with large individuals tend to have low  $I_R$ 's and to produce alates. The colonies with small individuals tend to have high  $I_R$ 's and do not produce alates. By definition, colonies

TABLE VI

Some Characteristics of 18 Natural Colonies of Neotermes jouteli  
Collected in the Same Woods on Virginia Key, Florida 1/6-1/7/69.

Colony 69-	I <sub>R</sub>	T-day	Ave. wt. PEs (mg)	Ave. no. antennal segments	Ave. wt. soldiers (mg)	Population (PE)	Ratio PE: (S+WS)	Repro- ductives	Alates in summer 69
15	6.5(a)	8	9.7	14.6	18.7	150	15:1	2SR	-
9	6.2	9	11.0	13.3	11.4	280	7:1	2SR	-
14	5.5(a)	8	12.8	14.5	16.6	1040	15:1	2SR	+
2	5.5	8	13.3	15.0	18.8	760	38:1	?	-
1	3.8	10	13.5	16.4	29.3	1780	17:1	♀PR, ♂SR	-
8	3.6	9	10.7	14.2	13.0	500	9:1	2SR	-
12	3.6	9	11.3	14.1	25.4	670	8:1	2PR	-
16	2.7	9	28.5	17.2	48.3	700	8:1	♀PR, ♀SR	+
10	2.4	10	11.4	14.0	14.4	920	15:1	2SR	-
7	1.6	10	12.7	16.0	30.4	670	8:1	2PR	-
11	1.5(b)	11	9.9	13.9	15.5	310	8:1	2SR	-
6	1.1	11	22.9	16.5	49.3	910	5:1	2PR	+
5	1.0	12	21.0	16.1	39.7	550	8:1	2SR	+
13	0.86	14	21.1	17.0	41.8	1320	12:1	2PR	+
18	0.71(b)	14	24.9	16.6	37.9	210	10:1	2SR	+
17	0.40	10	28.2	17.3	49.1	1270	24:1	2SR	+
3	0.24	17	20.8	16.4	43.1	960	16:1	2SR	+
4	0.0	--	22.8	14.8	28.9	400	12:1	2PR	+

Abbreviations: (a) = based on peak of pretransformation

white pseudergate bloom

(b) = test colony pathological

T-day = transformation day - day following  
orphaning on which first repro-  
ductive arose

PE = pseudergates

SR = supplementary reproductive

PR = primary reproductive

S = soldiers

WS = white soldiers

I<sub>R</sub> = index of regeneration

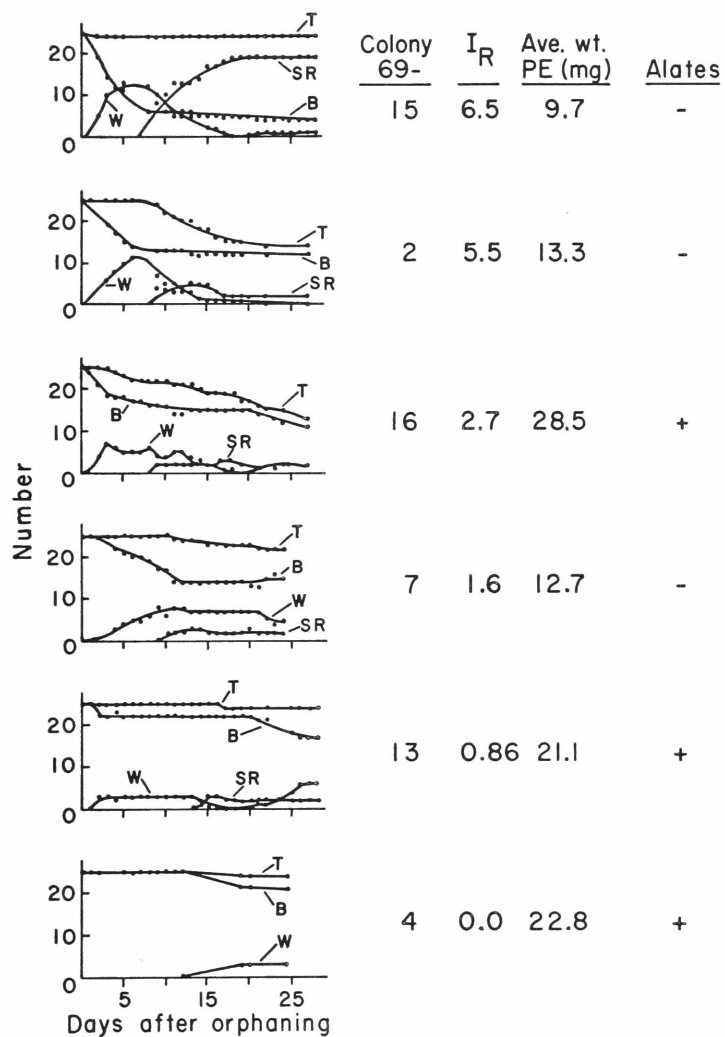


Figure 7. Reproductive regeneration in selected colonies of *N. jouteli* collected in the same woods 1/6/69. T = total population (sum of curves); B = brown pseudergates; W = white pseudergates, SR = supplementary reproductives.

that do not produce alates are immature. In addition, colonies with small individuals begin to regenerate sooner than colonies with large individuals.

There seem to be a few exceptions to this generalization. Colony 69-16, with a medium  $I_R$  of 2.7, consisted of large individuals and did produce alates. On the other hand, colony 69-14, with a high  $I_R$  of 5.5 and small individuals, produced alates. Colony 11, which had very small individuals, began regenerating rather late (day 11) and produced few reproductives ( $I_R = 1.5$ ). However, the test colony used in this case was pathological. Five pseudergates died before any reproductives arose, by day 20 ten pseudergates had died, and by day 35 only six pseudergates remained and all the reproductives had died.

Table VI also shows the average weights and numbers of antennal segments for colonies with  $I_R \geq 1.5$  and for colonies with  $I_R < 1.5$ . In the former case the pseudergates on the average weighed 13.2 mg and had 14.8 antennal segments and the soldiers weighed an average of 22.0 mg. In the latter case the pseudergate weight was 23.1 mg with 16.4 antennal segments and the soldier weight was 42.8 mg. If one averages the data on a totally different basis -- namely, the presence or absence of alates -- then the results are essentially the same and are presented in Table VII.

TABLE VII

Data Averaged on the Basis  
of Presence or Absence of Alates

	Ave. wt.(mg) pseuder- gates	Ave. no. antennal segments	Ave. wt.(mg) soldiers
Colonies not pro- ducing alates	11.5	14.6	19.7
Colonies producing alates	22.6	16.3	40.5

In addition, it was found that colonies with high  $I_R$  often had less well-developed gallery systems in their natural nests than colonies with low  $I_R$ . For example, in colony 69-15 ( $I_R = 6.5$ ) the galleries were of irregular shape and were restricted to the peripheral layer of the log. The heart of the log was untouched. On the other hand, colony 69-3 ( $I_R = 0.24$ ) had a fully developed nest with galleries throughout the log. There were, however, many exceptions to this generalization. Very little, if anything, is known about nest development in the Kalo-termitidae.

The populations of the natural colonies, as determined by censuses immediately upon extraction, do not seem to be correlated with  $I_R$ . The same negative result obtains for the pseudergate to soldier plus white soldier ratio and for the type of reproductives that were present in the natural colony. The protozoa were the same in all colonies. The pseudergates tested in each colony all contained the oxymonad described by Zeliff (1930) and the three devescovichids described by Kirby (1942, 1945) in Neotermes jouteli. In addition, they all contained the large unusual hypermastigote Staurojoenina that does not seem to have been previously noticed (see Addendum). The reason the protozoa were surveyed was that some stock colonies that had been maintained for over a year lacked the Staurojoenina. In addition, these colonies had low  $I_R$ . However, in the present study this correlation was not found.

### Discussion

When the striking differences between colonies in pseudergate size and regenerative power were noticed it was thought at first that perhaps there had been a mistake in identification and one was dealing with two different species. Samples of soldiers, alates and larvae from the different colonies were sent to Alfred Emerson, whose life-long interest in termites has included many contributions in the field of their taxonomy. Emerson confirmed that the colonies were all of the same species, Neotermes jouteli (Banks), and suggested that the differences observed might be due to differences in colony age.

This suggestion seems extremely plausible for a number of reasons. First, it is supported by the correlation between alate production and

lack of regenerative power. Colonies that produce alates are considered to be mature and are normally older than colonies that do not. Second, there is the much weaker correlation between degree of nest development and lack of regenerative power. Older colonies would be expected to have more developed galleries. Third, it makes biological sense for regenerative power to fall off as the colony ages. The immature phase of a colony's development is devoted to creating a large larval population. Therefore, the restoration of lost reproductives is a much more pressing need than in the case of fully developed colonies that are devoted primarily to transforming larvae into alates. In fact, low  $I_R$  in mature colonies tends to preserve pseudergates for alate production. There are, of course, many examples of loss of regenerative power with aging in other biological systems.

The difference in function between immature and mature colonies would also explain the difference in size of the individual pseudergates. A young colony diverts the matter and energy it obtains from wood into population production. An old colony diverts these things into fattening up pseudergates so that they will be successful sexuals. This could explain the phenomenon of stationary molts encountered previously (Chapter III). From the fourth or fifth instar on, termite larvae have the possibility of molting without changing size or shape. It would be this author's suspicion that these molts occur primarily in immature or growing colonies. That is, the pseudergates would essentially "mark time" while the pseudergate population built up. The colony that supplied the larvae studied in Chapter III was, in fact, immature. The exceptional case of colony 69-14, which had small pseudergates but produced alates, suggests that sexual maturation precedes the process of enlargement of the pseudergates in a colony's development. From observations of the East Asian species Neotermes tectonae, Kalshoven (1930) also concluded that "the individuals which first reach a further stage in a developing colony show a smaller size than those which reach the same stage later on." Heath (1931) observed the same phenomenon in Zootermopsis.



Light and Illg (1945) carried out censuses of natural colonies of Z. nevadensis with different regenerative powers in an effort to see whether the differences could be related to differences in colony composition. Their data is presented in Table VIII. Each colony listed represents a different natural colony whose census is given in the table. The colonies are arranged in groups corresponding to Light and Illg's experiments. Within each group the colonies are listed in decreasing order of regenerative power, which was based on the percent of test colonies that transformed to supplementaries in some given length of time (17-28 weeks, depending on the experiment). In this species natural colonies may contain many pairs of supplementaries. Light and Illg concluded that there was no relation between colony composition and regenerative power. Although this conclusion is supported by the data for reproductives, larvae and soldiers, there does seem to be some correlation between small numbers of nymphs and high regenerative power. The nymphs are precursors of alates and generally only arise in mature colonies. Thus, this data supports the idea that younger colonies have higher regenerative powers in Zootermopsis as well as Neotermes.

The loss of regenerative powers together with decline of egg production with colony age would explain why it is that termite colonies die. If vigorous regeneration were a constant possibility, then termite colonies should be immortal. In fact, Kalshoven (1930) found that the longevity of Neotermes tectonae colonies was never more than 15 years and that a natural death might occur in as few as six years. In the highest family of termites, the Termitidae, longevity may be considerably greater. One case was reported by Hill (1942) of a colony of the Australian species, Nasutitermes triodiae, which was at least 75 years old when last observed and possibly considerably older. Grassé (1949) has estimated the ages of some nests of Macrotermes at approximately 80 years, but in these cases it was the original primary pair that survived so long, not a series of supplementaries.

The fact that in a given species of termite the regenerative, morphological and developmental characteristics are colony-specific leads to one final conclusion regarding methodology. In order to standardize

TABLE VIII

Data from Light and Illg (1945) on Composition of Colonies  
with Different Regenerative Powers\*

Experiment	Colony	Reproductives	Soldiers	Larvae	Nymphs
D2	8	5SR	40	300	0
	4	2PR	38	505	0
	1	2PR	53	650	3
	5	4SR	42	500	0
	6	2PR	7	2200	2000
	10	35SR	200	425	500
	9	2PR	45	1500	300
	2	2PR	102	800	200
	3	2PR	232	3000	2000
2DS	C8	19SR	21	545	222
	C4	2PR	82	912	288
	C1	4SR	106	1020	686
	C10	21SR	27	2247	527
	C2	16SR	60	462	248
	C3	17SR	81	1368	912
	C5-7	2PR	28	1392	1008
4DS	C2	126SR	132	2562	3767
	C5	12SR	160	1518	931
	C1	2PR	128	3080	1436
	C4	18SR	160	3705	492
	C3	2PR	117	2424	660
1DS	C1	4SR	87	779	161
	C2	5SR	20	527	1
	C3	2PR	85	1537	359
	C6	2PR	22	594	601
	C4	9SR	93	1231	308
	C5	2PR	235	3280	112

\* The colonies within each experiment represent different natural colonies and are listed in order of decreasing regenerative power.

results and compare findings obtained in different laboratories, the following procedure should be followed. First, in any given study the termites used in experimental and control colonies should all come from the same natural colony. Second, the characteristics of that colony should be given in detail, including the location of collection and the dates of collection, extraction and removal of the termites from the stock colony for the experiment. In addition, the characteristics listed in Table VI should be given.

#### Some Factors Affecting $I_R$

Light and Illg (1945) reported that there could be significant differences in regenerative power between groups of larvae of the same composition taken from the same colony at different times. They did not study the problem systematically and could only speculate about the causes. In the present study quantitative changes in regenerative power were observed but there were no striking qualitative changes. Strongly regenerating colonies never became weak unless they were obviously unhealthy and weak colonies never became strong. It must be admitted, however, that variations in  $I_R$  with time were never systematically studied in this work either.

Regeneration It is clear that there is one factor that would affect  $I_R$ , namely regeneration itself. If a colony is forced to regenerate reproductives, then the supply of transformable pseudergates will be temporarily depleted and a sample of pseudergates isolated from such a colony at this time would show a lowered  $I_R$ . Eventually  $I_R$  should return to its normal level. This, in fact, was found.

On day 0 the reproductives were removed from stock colony 69-2. On the same day and at various time intervals thereafter, 50 pseudergates were removed from the colony and allowed to regenerate reproductives. Daily censuses were carried out and  $I_R$  was measured at each time point. All colonies were maintained at 26°C in the dark. The results are presented in Figure 8.  $I_R$  falls from 5.5 on day 0 to a low of 2.5 by day 20. At this time the regeneration process in the stock colony is over and the number of transformable larvae is at a minimum. Thereafter  $I_R$  steadily increases and returns to its initial level on day 85.

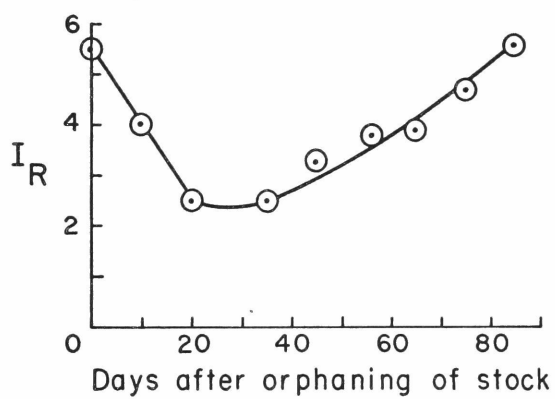


Figure 8. Loss and regeneration of regenerative ability following orphaning.  $I_R$  measured in samples of 50 pseudergates from stock 69-2.

Larval stage The fact that a low  $I_R$  was associated with large pseudergates raised the question: What is  $I_R$  for small and medium-sized larvae in a colony where the pseudergates have low  $I_R$ ? Is  $I_R$  a size/instar characteristic or is it a colony characteristic? To answer this question, groups of 50 uniformly small, medium and large larvae were isolated together with an appropriate number of soldiers from two colonies that had produced alates and whose pseudergates showed low  $I_R$ 's. The larvae in each group were weighed and the number of antennal segments determined. The groups were maintained at 26°C in the dark. The first day on which any larva transformed to a reproductive (T-day) was noted as was the total number of reproductives produced by each group. The results are presented in Table IX.

TABLE IX

Reproductive Regeneration by Larvae  
of Different Sizes from the Same Colony.

Colony	Ave. wt. (mg)	Ave. no. antennal segments	T-day	Total reproductives produced by end of experiment	Last day of experiment
69-17	9.9	14.0	--	0	150
	14.4	14.4	--	0	140
	24.2	16.8	41	1	140
69-26	9.5	14.0	--	0	63
	14.6	15.1	--	0	53
	23.4	16.5	18	4	53

These results were more extreme than anything expected. The groups of small and medium-sized larvae had morphological characteristics in the range of pseudergates with high  $I_R$  from other colonies. Yet these larvae produced no reproductives. Thus not only  $I_R$  but the ability of larvae to transform to reproductives is a characteristic of a colony, not an individual. From this experiment one concludes that in mature colonies with low  $I_R$  it is only the pseudergates that can transform and they transform at a very low rate.

The loss of differentiation abilities of early instars with increasing colony development has been observed in other species. Grasse (1949) has expressed the belief that it is a general rule that castes are formed from older larvae as a colony ages since cases are known in the Kalotermitidae, Hodotermitidae and Rhinotermitidae. For example, Kalshoven (1930) reported that only the two most advanced larval stages in Neotermes tectonae could differentiate to soldiers or supplementary reproductives. In a two-year-old colony these two stages were the third and fourth instars. In a mature colony, six or seven years old, they were the fifth and sixth instars. Grasse and Noirot (1946b) noted a similar phenomenon in the formation of soldiers in colonies of Kaloterms flavicollis. On the other hand, Light and Illg (1945) found that whereas the regenerative power of younger Zootermopsis larvae was less than that of older larvae in the same colony, this power was not totally lost.

The loss of differentiation abilities of early instars with increased colony development is extremely puzzling. It is clear that a simple mechanism by which old larvae inhibit young larvae cannot explain the phenomenon since the young larvae were unable to differentiate in isolation. How did the groups of small and medium-sized larvae in this experiment know that they came from an old colony? Perhaps the appearance of alates in a colony causes long-range physiological changes in the larvae.

The pseudergates seem to possess the special characteristic, at least in mature colonies, of being the only larvae capable of transformation. This unique capability could possibly be conferred upon the pseudergates at the time in the colony's history when the pseudergate population is building up. Thereafter, during the period when the pseudergates increase in size they would be the only ones to retain the ability to transform. Younger larvae, even when they became pseudergates, would lack this ability. The decrease in  $I_R$  would then be explained as a reduction in the colony concentration of transformable pseudergates as more and more became alates and as more untransformable larvae became pseudergates. At a certain point, however, a large portion of the pseudergates, even though they were of large size, would still be transformable. Thus exceptional colonies such as 69-16 would occasionally be found.

Using Lüscher's idea (1953) that transformability depends upon the possession of a special competence substance, one could say that the substance ceases to be produced after a certain point in colony history and thereafter either begins to break down or is diluted out as the transformable pseudergates become alates. Alternatively, the physiological basis for the reduction in  $I_R$  could be a gradual reduction in the transformable portion of the intermolt interval.

## VI. PSEUDERGATE TRANSFORMABILITY

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### Introduction

In the rest of the work to be presented, studies were restricted to immature or strongly regenerating colonies, since it is in such colonies that the mechanisms which inhibit pseudergate transformation must be most pronounced. First, can all pseudergates from such colonies transform to reproductives? If so, why don't they? What is it that stops the process? On the other hand, if they cannot all transform, what are the special characteristics of those that can?

The evidence that has accumulated so far for Zootermopsis (Castle, 1934) and for Kalotermes (Grassé and Noirot, 1946a; Lüscher, 1952a) indicates that all nymphal and advanced larval stages in lower termites are capable of molting to supplementary reproductives and that, barring some infrequent mutation, there are no larvae genetically incapable of developing into this caste. The technique used to demonstrate this totipotency has been to isolate individual larvae or nymphs. Those that survived eventually all became supplementaries (Castle, 1934; Grassé, 1956).

The technique used in this work was continual orphaning. Orphaned colonies were set up and maintained at 26°C in the dark as in the previous experiments. The pseudergates and soldiers came from stock colony 68-5, whose characteristics are presented in Table X. A census was taken each day. Whenever reproductives arose they were removed.

### Results

The results for the average of two colonies, each initially containing 100 pseudergates (brown) and 6 soldiers, are shown in Figure 9. The curves for white and brown pseudergates represent daily censuses. The reproductive curve is cumulative. The total population curve is the sum of the other three. The fall in the total population reflects normal



mortality, not caste elimination. It can be seen that 241 days after the initial orphaning all pseudergates that survived (i.e., 66%) had transformed to reproductives.

TABLE X

## Characteristics of Stock Colony 68-5

---

Location collected:	Virginia Key, Florida
Date collected:	1/30/68
Date extracted:	2/68
Date pseudergates and soldiers removed from experiment:	5/2/68
Average weight of pseudergates used in experiment:	9.5 mg
Average weight of soldiers used in experiment:	12.4
$I_R$ :	6.1

---

Many of the features of normal regeneration are repeated here. By day 2 the bloom of white pseudergates had begun in full force; by day 4 it had begun to level off; and by day 7 it had reached its peak. By seven days later, essentially all the white pseudergates in this initial bloom had transformed to reproductives. There were about five more white pseudergates in the day 7 peak than cumulative reproductives on day 14. It is possible that these white pseudergates did actually molt into reproductives but were killed in the process. Like many other activities in termites, molting is a social phenomenon. The molting fluid is extremely attractive to nearby larvae, which help pull the old cuticle off and actively lick the newly emerging individual. Sometimes this termite is injured in the process and is then rapidly cannibalized.

What is most interesting is that after the initial bloom of reproductives the cumulative reproductive curve enters a new phase in which new reproductives appear at a much slower rate until the colonies have been totally depleted of pseudergates.

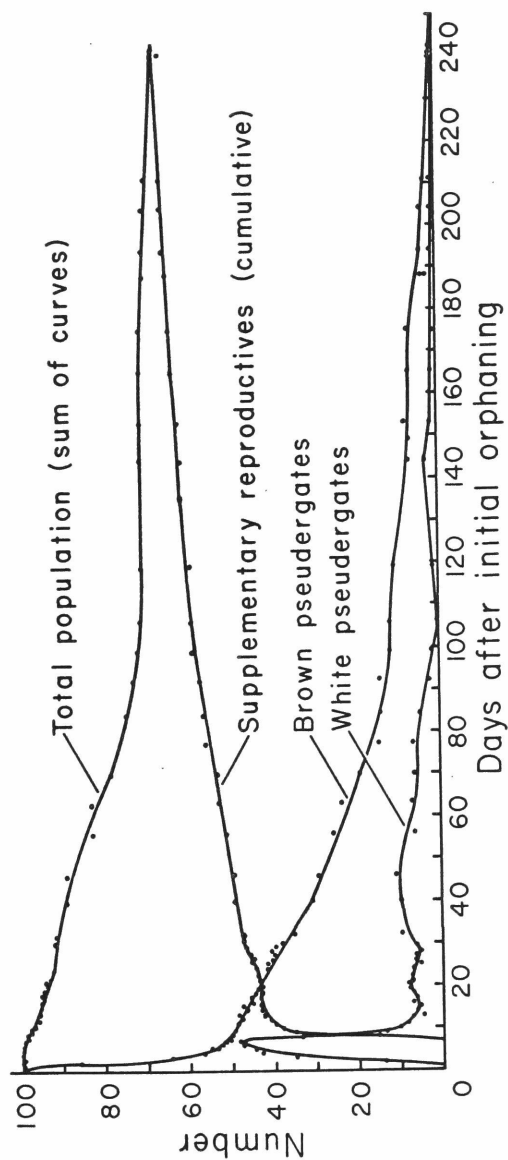


Figure 9. Transformability of pseudergates upon continual orphaning. Average of two colonies maintained in dark at 26°C, 5/68 - 1/69.

### Discussion

One can conclude from this experiment that all pseudergates in colonies with high  $I_R$  are genetically capable of supplementary reproductive development. The only escape from this conclusion would be if one could show that all pseudergates that were genetically incapable of this development died in the course of the experiment. This, of course, seems unlikely. The experiment lasted over 34 weeks and there was a 34% total mortality. It would probably be difficult to make the mortality rate less than 1% per week.

Two possible results might have been expected in this experiment. If all pseudergates are equally capable of becoming reproductives and it is only the arising of new reproductives that stops this process via negative feedback inhibition, then upon continual orphaning all pseudergates will rapidly be transformed and the colony will be depleted. On the other hand, if only a certain proportion of the pseudergates are transformable for genetic or other reasons, then only these will transform and the process will rapidly halt. This could also explain why only a fixed percentage of pseudergates in colonies transforms under conditions of normal regeneration.

The two-phase nature of the cumulative reproductive curve shows that the situation is more complex and that a mixture of the two mechanisms is probably at work.

One could say that at any given time only a certain proportion of pseudergates is transformable. This protects the colony from total depletion if the reproductives are lost. When the initial pair of reproductives is removed this proportion immediately transforms. The appearance of new reproductives prevents the transformation of any pseudergates that in the meantime have become transformable. With continual orphaning those pseudergates that gradually become transformable eventually do molt and become supplementaries.

What determines transformability? It could well simply correspond to a period in the molt cycle. The two colonies represented in Figure 9 each initially contained 100 pseudergates. If these 200 pseudergates were

randomly distributed as to position in their intermolt intervals then the transformable period must constitute about 45% of the intermolt interval since approximately this percent of the pseudergates immediately transformed.

Lüscher (1952b, 1953, 1956a) has presented a much more complicated theory of transformability, or what he has called "competence." He found that in the case of 30 pseudergates of K. flavicollis that transformed to supplementaries, competence decreased with the length of time since the last molt. That is, of 7 pseudergates that had molted within ten days prior to orphaning, 6 (86%) transformed. However, out of 13 pseudergates that had molted 10-20 days prior to orphaning, only 7 (54%) transformed. And, out of 25 pseudergates that had molted 70-100 days before orphaning, only 2 (4%) transformed. In addition, he found that for these pseudergates there was a rather loose correlation between length of time since the last molt and length of time required for transformation. That is, if one continually orphans a colony, those pseudergates that molt rapidly are those that have recently molted, and those that molt later on have molted a longer time ago.

Lüscher (1953) plotted the percent molting versus time since molting and concluded that the loss of competence corresponded to exponential decay and that it represented the loss of a substance that conferred upon a pseudergate the ability to transform when orphaned. As in radioactive decay the loss of this substance could occur at any time to any individual but the probability (or "half-life") was fixed.

A number of comments about Luscher's theory should be made since it has been rather uncritically accepted in the literature. In Luscher's experiment 30 out of 104 pseudergates transformed. The 30 were distributed into six time intervals since molting and thus there were six experimental points. It seems, first of all, unjustified to conclude that one has a curve of exponential decay on the basis of six points, each of which has a high degree of uncertainty. The actual curve might be linear or some other function. Similarly, more data is needed to substantiate a correlation between time required for transformation and time since the last

molt. Second, it seems intuitively more justified to measure competence as a function of fraction of the intermolt interval rather than days. Lüscher (1952b) found after studying 343 larvae and nymphs that the normal intermolt interval for non-supplementary molts varied widely, namely from 30 to 230 days. Thus a point 15 days after molting could be anywhere from 6.5 to 50% of the interval. Third, if competence corresponds to the all-or-none possession of a special substance it is difficult to explain the variation in length of time required for transformation that Lüscher believed to be related to time since the last molt. A simpler theory (for which the present author offers no evidence) is that competence corresponds to a certain fraction of the intermolt interval and the variation in transformation time reflects either the great variation in time required for any molt or the fact that it would take certain larvae longer to reach their competent periods because of normally longer intermolt intervals.

If this theory is correct, then Lüscher's evidence would suggest that this discrete period lies early in the intermolt interval. This would explain Lüscher's observation that the average time since the last molt is less when larvae transform to supplementaries than when they have a larval molt. And, as stated above, the period would last about 45% of the intermolt interval in Neotermes jouteli.

## VII. SEX SPECIFICITY

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Introduction

As long as a pair of reproductives is present in a colony of Neo-termes the transformation of larvae to supplementary reproductives is inhibited. To attempt to understand the mechanism of inhibition it is first necessary to analyze the role played by each sex. What happens in half-orphaned colonies? What effect does each reproductive have upon the larvae of its own and the opposite sex?

The evidence that has been obtained in other species is incomplete and somewhat confusing, but supports the idea that the two reproductives have separate and unequal effects in the overall inhibition. The data obtained in studies of Zootermopsis (Castle, 1934; Light, 1942-43; Light and Weesner, 1951) supported the conclusion that strong but incomplete sex specific inhibition did exist. For example, in Light and Weesner's study (1951) of Z. angusticollis, out of 32 colonies headed by a primary female reproductive, 27 produced only male supplementaries, 1 produced males and females and 4 produced only females. On the other hand, out of 13 colonies headed by a primary male reproductive 10 produced only female supplementaries, 2 produced both males and females and 1 produced only males. This data is somewhat unsatisfying for a number of reasons: the actual number of supplementaries produced was not given, the termites used came from different stock colonies, totally orphaned control colonies from the same stocks were not run for comparison and Zootermopsis colonies normally tolerate more than a single pair of supplementaries.

Some of these difficulties plagued the early experiments with Kalotermes flavicollis. The first detailed attempt to sort out the specific effects of the male and female reproductives was the study of Grassé and Noirot (1960a). In one part of this study one of the reproductives was removed from colonies that had been raised in the laboratory from mated primary pairs. The number of supplementaries present in each colony was noted 10, 20 and 40 days later. Their data for 10 colonies headed by a primary female and 9 colonies headed by a primary male is presented in summary in Table XI.

TABLE XI

Data from Grassé and Noirot (1960a)  
 Supplementaries Arising in Half-orphaned Colonies  
 of Kalotermes flavicollis

Primary	Number of colonies	Supplementaries present					
		day 10		day 20		day 40	
		M	F	M	F	M	F
Female	10	20	0	16	1	10	0
Male	9	11	43	6	14	5	10

Grassé and Noirot concluded from this data that inhibition was sex-specific but more complete in the case of the female than the male. This was supported by other experiments in which groups of orphaned pseudergates and nymphs of the same sex were allowed to regenerate reproductives. It was found that the female colonies were stabilized by the presence of a single female supplementary whereas the male colonies were not stabilized unless two or three male supplementaries were present.

Lüscher (1964) criticized Grassé and Noirot's experiments as well as his own previous experiments (1956a) with half-orphaned colonies on the grounds that totally orphaned control colonies were missing and

that therefore it was impossible to say whether the arising of fewer males than females in colonies headed by males is due to inhibition of male larvae or stimulation of female larvae. In addition, one can criticize Grassé and Noirot's experiment for providing too little data since few supplementaries arose despite a large number of half-orphaned colonies.

In Lüscher's study (1964) these problems were specifically avoided. In addition to providing each half-orphaned experimental colony with a totally orphaned control from the same stock, new supplementaries were removed as they arose so that the larvae were continually subjected to the effect of one reproductive and maximal reproductive production was obtained. Finally, he determined the sex of the larvae of each colony so that the number of reproductives of a given sex that arose could be used to calculate the percent of the larvae of that sex which transformed. This data, as of day 20 when his experiments were terminated, is summarized in Table XIII for four colonies.

TABLE XII

Data Calculated from Lüscher (1964)  
Supplementaries Arising in Continually Half-orphaned Colonies  
of Kalotermes flavicollis as of Day 20

Reproductive continually present	Number of colonies	Total number of larvae	Reproductives arising			
			number		as % of larvae of that sex	
			M	F	M	F
Female	4	107	23	5	48	8
Male	4	112	24	54	52	82
None (control)	4	111	22	36	51	53

Lüscher concluded from this data that neither reproductive by itself affected the development of male larvae and that whereas the female



reproductive inhibited the transformation of female larvae, the male reproductive stimulated it. This conclusion, while supported by Lüscher's data, is extremely difficult to accept since it suggests that the male and the female reproductives acting together should have no net inhibitory effect on larval transformation. Lüscher was aware of this difficulty and could only conclude that the reproductives must act differently when they are together from when they are alone. This would have to be especially true of the male reproductive, which would in the presence of a female, he felt, actively inhibit male larvae and cease to stimulate female larvae.

In support of this latter conclusion Lüscher cited evidence obtained using colonies headed by two male reproductives. Eight of these colonies were established and new reproductives were removed as they arose until day 20. The data obtained is summarized in Table XIII.

TABLE XIII

Data from Lüscher (1964)

Supplementaries Arising in Colonies of K. flavicollis

with Two Male Reproductives Continually Present,

as of Day 20

Reproductives continually present	Number of colonies	Total number of larvae	Reproductives arising			
			number		as % of larvae of that sex	
			M	F	M	F
2M	8	239	37	70	32	57
none	8	239	51	54	41	47

Lüscher believed that this data showed that a male in the presence of another male reproductive was partially stimulated to act the way it did in the presence of a female reproductive. That is, it showed more inhibition of male larvae and less stimulation of female larval transformation.

In these experiments of Lüscher's there was considerable variation in the strength of the response obtained from colony to colony. This reflected the fact that the experimental colonies in each experiment were derived from different natural colonies. Summing the results from such widely varying colonies is possibly misleading. In addition, Lüscher's colonies were actually mixtures of larvae and first-stage nymphs. It is not clear that the two forms respond in the same way to half-orphaning and this could have affected the results.

In the present study all individuals used were pseudergates that came from the same stock colony. This colony was chosen because it had a high  $I_R$  so that the response of pseudergates to half-orphaning would be maximized and any sex-specificity would become clear. In addition, new reproductives were continually removed for a very long time.

#### Experimental Procedure

Sixteen soldiers and 480 pseudergates were removed from stock colony 69-14, whose characteristics are listed in Table XIV. These termites were divided into three groups -- two containing 180 pseudergates plus 6 soldiers and one containing 120 pseudergates plus 4 soldiers. Each group was provided with a pair of foster supplementary reproductives with which it was incubated at  $26^{\circ}\text{C}$  for 49 days. This was done to insure that the reproductives were fully inhibitory. At the end of this time the pseudergates and soldiers of each group, whose numbers had changed because of death and soldier production, were divided into three equal colonies, one of which kept the male reproductive, one the female and one was totally orphaned. The day 0 census for each colony is presented in Table XV. Thus, the results from the colonies in each group can be compared as can the results from the colonies of the same experimental type since colonies from different groups came initially from the same stock. Censuses were made each day in each colony and all new supplementaries were removed and sexed. The colonies were maintained in the dark at  $26^{\circ}\text{C}$ .

#### Results

The results at a number of time points are given in Table XVI. The sum of the daily results for all three groups are presented graphically

in Figure 10. The results can be summarized as follows: In the presence of a single male reproductive, equal numbers of males and females arise. In the presence of a single female reproductive as many males arise as with a single male reproductive but essentially no females arise. In the absence of both reproductives, equal numbers of males and females arise but the numbers are approximately twice as high as with a single male.

TABLE XIV  
Characteristics of Stock Colony 69-14

---

Location collected:	Virginia Key, Florida
Date collected:	1/7/69
Date extracted:	1/12-13/69
Population upon extraction:	1043 pseudergates + 58 soldiers + 12 white soldiers + 2 supplementary reproductives + small larvae + eggs
Date pseudergates and soldiers removed for experiment:	3/2/69
Average weight of pseudergates used in experiments:	12.8 mg
Average weight of soldiers used in experiments:	16.6 mg
I <sub>R</sub> :	5.5

---

### Discussion

From these results it can be readily seen why a pair of reproductives should totally inhibit the production of supplementary reproductives. The male and female reproductives in isolation each partially inhibit pseudergate transformation. The sum of the separate partial effects would in fact produce total inhibition. The male reproductive indiscriminately half-inhibits both male and female pseudergates but additionally inhibits female pseudergates totally. Thus, female pseudergates experience surplus inhibition.

TABLE XV

Day 0 Censuses for Colonies of Neotermes jouteli  
in Half-orphaning Experiment

Reproductive present	Female		Male		None	
	pseudergates white brown	soldiers	pseudergates white brown	soldiers	pseudergates white brown	soldiers
I	8	40	8	40	8	40
II	11	39	11	39	11	39
III	5	32	5	32	5	32
Total	24	111	24	111	24	111
		15		15		15

TABLE XVI

Supplementary Reproductives of Each Sex  
 Arising in Continually Half-orphaned and Totally Orphaned Colonies  
 of Neotermes jouteli

(Cumulative production as of various dates)

		<u>Reproductive Present</u>					
		Female		Male		None	
	Group	males	females	males	females	males	females
<u>Day 10</u>	I	6	0	3	1	7	9
	II	4	0	3	0	12	9
	III	2	0	2	0	4	4
	Total	12	0	8	1	23	22
<u>Day 20</u>	I	11	0	7	8	10	15
	II	4	0	8	5	17	13
	III	4	0	4	5	10	9
	Total	19	0	19	18	37	37
<u>Day 30</u>	I	13	0	10	8	10	17
	II	5	0	9	7	21	14
	III	5	0	5	8	11	10
	Total	23	0	24	23	42	41
<u>Day 70</u>	I	13	0	11	8	10	24
	II	8	1	9	10	21	19
	III	7	0	5	9	11	14
	Total	28	1	25	27	42	57

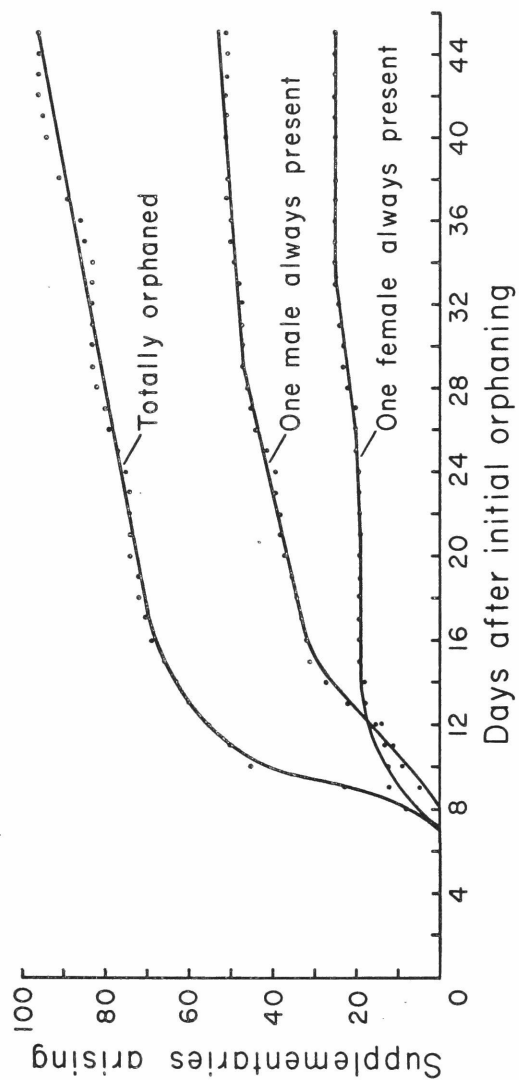


Figure 10. Supplementaries arising in continually orphaned and half-orphaned colonies of *N. jouteli*. Each curve represents the sum of three colonies, April - June, 1969.

If it is assumed that inhibition results from an inhibitory substance then the present results imply that at least two substances are at work in the overall inhibition. If there was only one substance, which inhibited females at one-half the dose necessary to inhibit males, then the effect of a single female reproductive would be explained. However, this idea is inconsistent with the fact that male and female pseudergates respond equally to the presence of a single male reproductive.

There are two ways in which there could be two substances. For example, there could be a female-produced substance and a different male-produced substance and the female-produced substance affects female pseudergates more strongly than males. Alternatively, there could be a sex-specific inhibitory substance produced only by female reproductives and acting only on female pseudergates and a sex non-specific substance produced by both reproductives. One can also imagine more complicated explanations involving more than two substances, which would also fit the data.

It is interesting to compare these results with what has been found previously. The two points of universal agreement are that the transformation of female larvae is strongly inhibited by female reproductives and that the male does not show a strong sex-specific inhibitory effect. However, Lüscher's results (1964) suggested that neither reproductive, at least in isolation, had any inhibitory effect on male larvae. This is contradicted by the present finding that both reproductives equally inhibit male pseudergates. In addition, the present results give no indication that male reproductives stimulate the transformation of pseudergates as claimed by Lüscher. Finally, the complicated interactions between male and female reproductives whose existence Lüscher was forced to postulate from his data are not suggested by the present results.

How can the differences between Lüscher's and the present results be accounted for? There are five major differences between the experiments. First, Lüscher determined the sex of the larvae in each colony and

could therefore calculate his results on the basis of the percent of larvae of a given sex which transformed. This is essentially a quantitative refinement and there are no qualitative differences in the results whether one looks at the absolute number of reproductives that arise or at the percent of a given sex of larvae. Thus, given the large number of pseudergates used in the present experiment, it is unlikely that the results would be substantially different if their sex were known. The second difference is that Lüscher's results consisted of sums of results of colonies with widely different regenerative intensities, whereas in the present experiment all pseudergates were initially derived from the same stock and were treated the same until the beginning of the experiment. Thus, anomalous results might not cancel each other out as well in Lüscher's experiment as in the present study. Third, Lüscher's colonies were rather small, consisting of only 20-30 individuals, whereas in the present study the colonies were twice as large. Thus, after an initial bloom of reproductives, Lüscher's colonies became quite small and it is probable that the tendency of larvae to transform would be abnormally low in such small colonies (Lüscher, 1956a). Fourth, different species were used in the two studies. It is possible that different inhibitory mechanisms are at work even though both species are members of the same family. Fifth, Lüscher's colonies consisted of both larvae and nymphs whereas only pseudergates were used in the present study. It is possible that the different forms used by Lüscher respond differently to half-orphaning.

It is interesting to speculate on the biological significance of the different inhibitory effects of the male and female. First, the phenomenon is such that if a termite colony loses a reproductive of either sex then a situation is produced in which a female reproductive will have many males as possible mates. Thus, if it is the female which is lost then the colony not only regenerates females but also produces more males. This is reminiscent of the fact that in the other social insects the queen is provided with excess males during the nuptial flight. Second, the fact that a termite colony's response is considerably less vigorous when one reproductive is lost rather than two seems extremely sensible from the point of view of colony efficiency. The loss of a single reproductive is probably the usual situation in nature.



### Sex Intensity

If the present interpretation is correct, namely that male reproductives half-inhibit both sexes and female reproductives half-inhibit male pseudergates while totally inhibiting female pseudergates, then the presence of either two male reproductives or two females should also be totally inhibitory. This can be tested since caste elimination will not necessarily occur when there are only two males or two females present.

Experimental procedure Three hundred pseudergates and 30 soldiers were removed from stock colony 69-21, whose characteristics are listed in Table XVII. These termites were divided into three groups -- two containing 120 pseudergates and 12 soldiers and one containing 60 pseudergates and 6 soldiers. Each group was provided with a foster pair of supplementary reproductives with which it was incubated at 26°C for 15 days. At the end of that time one of the large groups was divided in half with each half getting one of the two reproductives. The other large group was similarly divided in half, but the half which received the male reproductive was also given the male reproductive that had been present in the small group and the half receiving the female also was given the female from the small group. Thus there were five colonies of about the same size with different numbers of reproductives. The initial (day 0) censuses of these colonies are presented in Table XVIII. Censuses were carried out each day in each colony and all new supplementaries were removed as they arose and were sexed. Also, if any of the initial reproductives was killed a replacement was added to the colony. Thus it was necessary to add a male reproductive to colony "M" on day 4 and another one to colony "2M" on day 8. The other reproductives survived the course of the experiment.

Results The cumulative total of reproductives that had arisen in each colony as of day 31 is presented in Table XIX and Figure 11. The results for the first three colonies are similar to what was found in the half-orphaning experiment. More reproductives arose in totally orphaned colonies than in half-orphaned colonies. Only males arose in the presence of a female whereas both sexes arose in the presence of a male.

TABLE XVII

## Characteristics of Stock Colony 69-21

---

Location collected:	Virginia Key, Florida
Date collected:	7/30/69
Date extracted:	8/7/69
Population upon extraction:	313 pseudergates + 107 soldiers + 12 white soldiers + 2 primary reproductives + small larvae + eggs
Date pseudergates and soldiers removed for experiment:	9/3/69
Average weight of pseudergates used in experiment:	8.6 mg
Average weight of soldiers used in experiment:	11.8 mg
I <sub>R</sub> :	2.2

---

TABLE XVIII

## Day 0 Censuses of Colonies in Sex-intensity Experiment

---

Colony	Pseudergates white    brown		Soldiers	White soldiers	Supplementary reproductives
0	7	50	6	1	0
M	6	51	6	1	1M
F	6	51	6	0	1F
2M	5	51	6	0	2M
2F	5	51	5	1	2F

---

In this experiment the sex ratio of the reproductives that arose in the "M" and the "O" colonies is not even as previously found. This could reflect an uneven pseudergate sex ratio. The important new result is that regeneration is strongly suppressed in the presence of either two males or two females. Only a single male reproductive arose in the "2F" and "2M" colonies.

TABLE XIX

Reproductives Arising in Sex-intensity Experiment as of Day 31

Colony	O	F	M	2F	2M
# arising	24	14	15	1	1
Males	18	14	12	1	1
Females	6	0	3	0	0

Discussion The strong suppression of regeneration by two reproductives of the same sex is in accord with the finding that a single reproductive of either sex will partially suppress regeneration. It also supports the idea that the total inhibition produced by a mated pair of reproductives can be accounted for simply as the addition of the separate effects of each sex and that complex interactions between the sexes are unnecessary.

The fact that two male reproductives strongly inhibited regeneration in this experiment is in complete disagreement with Lüscher's result (1964) that two males had no effect on the number of supplementaries that arose but only shifted the sex ratio in favor of more females and fewer males. A number of differences between Lüscher's experiments and the present study have already been discussed. In this case the results are so strikingly different as to suggest that the cause is the difference in species rather than procedure. On the other hand, Grassé and Noirot's finding (1960a) that in colonies of Kalotermes flavicollis consisting only of male larvae total inhibition could only be achieved when two or three male supplementaries were present is in line with the present results.

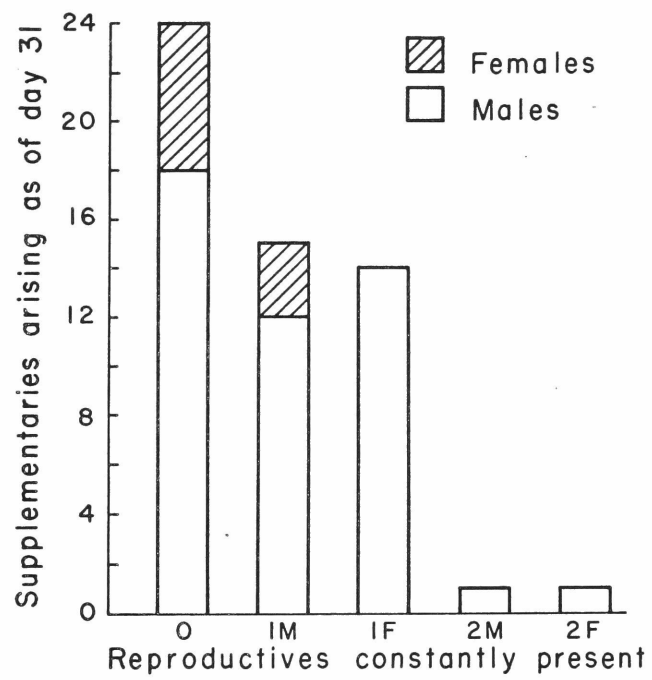


Figure 11. Sex-intensity experiment.

The one puzzling thing about the present results is that total inhibition by a pair of reproductives of the same sex would seem to be non-adaptive. A colony's growth could be cut off if it found itself headed by two male reproductives. In the case of two females the problem may be less severe since female reproductives can lay viable unfertilized eggs (Light, 1944a; Grassé and Noirot, 1960b). Colonies have been occasionally found in nature headed by a single pair of female reproductives (e.g., Colony 69-16 in Table VI). It would appear that the accurate ability of a colony's caste elimination process to reduce the reproductives that arise in response to orphaning or half-orphaning to a single bisexual pair is critical.

## VIII. PART-TIME ORPHANING

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Introduction

It is now clear how laboratory colonies of Neotermes jouteli respond if a pair of reproductives is either always or never absent. In the following experiments we ask how colonies respond if the reproductive pair is absent only a certain number of hours per day.

Experimental Procedure

Groups of 120-130 pseudergates together with some soldiers were removed from two stock colonies (69-1 and 69-20) whose characteristics are given in Table XX. The groups were each provided with a pair of foster supplementary reproductives with which they were incubated at 26°C in the dark for approximately six weeks. At the end of that time the pseudergates and soldiers in each group were divided into two equal colonies. For each group one colony then kept its reproductive pair for a fixed period each day and the other colony kept the reproductives for the rest of the day. The day the groups were divided and the reproductive transfers were begun was considered day 0. A census was taken each day thereafter and all new reproductives were removed as they arose and were sexed. The colonies were otherwise maintained in a dark incubator at 26°C.

Results

The number and sex of reproductives that arose as of day 26 for the colonies and time points studied are presented in Table XXI. In addition the overall percent of pseudergates that transformed is plotted in Figure 12 as a function of the number of hours per day the reproductives were absent. It can be seen from this data that the degree to which a colony produces supplementaries varies directly with the amount of time per day a functional reproductive pair is absent. While this variation is more or less continuous for the production of male reproductives, female

pseudergates can be orphaned for at least eight hours a day without being stimulated to transform. Even when reproductives are absent twenty hours each day many fewer females transform than males. The sex ratios of the supplementaries whose sex could be determined are shown for each time point in Figure 13.

TABLE XX

## Characteristics of Stock Colony 69-1 and 69-20

Stock colony	69-1	69-20
Location collected:	Virginia Key, Fla.	Virginia Key, Fla.
Date collected:	1/6/69	7/30/69
Date extracted:	2/5/69	8/5-6/69
Population upon extraction:		
pseudergates	1780	421
soldiers	99	48
white soldiers	7	6
reproductives	primary female supplementary pair	supplementary pair
small larvae	+	+
eggs	+	+
Date pseudergates and soldiers removed for experiment:	3/13/69	9/2/69
Average weight of pseuder- gates used in experiment:	13.5 mg	11.3 mg
Average weight of soldiers used in experiment:	29.3 mg	14.5 mg
$I_R$ :	3.8	2.3

Discussion

The fact that the extent of regeneration varies directly with the extent of orphaning suggests that the inhibitory process is a continuous and cumulative one. That is to say, we do not have an all-or-none situation in which the absence of reproductives for a certain threshold period suddenly unleashes massive regeneration. Certain theories about

TABLE XXI

## Reproductives Arising in Part-time Orphaning Experiments

Hours/day SRs absent	Actual time schedule	Source of PEs (stock colony)	Number of PEs (incl. white PEs) at day 0	Total no. of SRs arising as of day 26	Sex		Percent of day 0 PEs that trans- formed
					F	M	
24	always absent	69-1	50	11	7	4	0
		69-20	71	17	8	9	0
		total	121	28	15	13	0
20	2 PM - 10 AM	69-1	55	17	4	13	0
		69-1	52	13	8	4	1
		69-20	53	5	1	4	0
		total	160	35	13	21	1
16	6 PM - 10 AM	69-1	49	13	3	9	1
		69-20	50	3	1	2	0
		total	99	16	4	11	1
8	10 AM - 6 PM	69-1	49	8	0	7	1
		69-20	51	2	0	2	0
		total	100	10	0	9	1
4	10 AM - 2 PM	69-1	56	3	0	3	0
		69-1	52	6	0	6	0
		69-20	52	2	0	2	0
		total	160	11	0	11	0
0	never absent	69-1	49	1	0	1	0
							2.0

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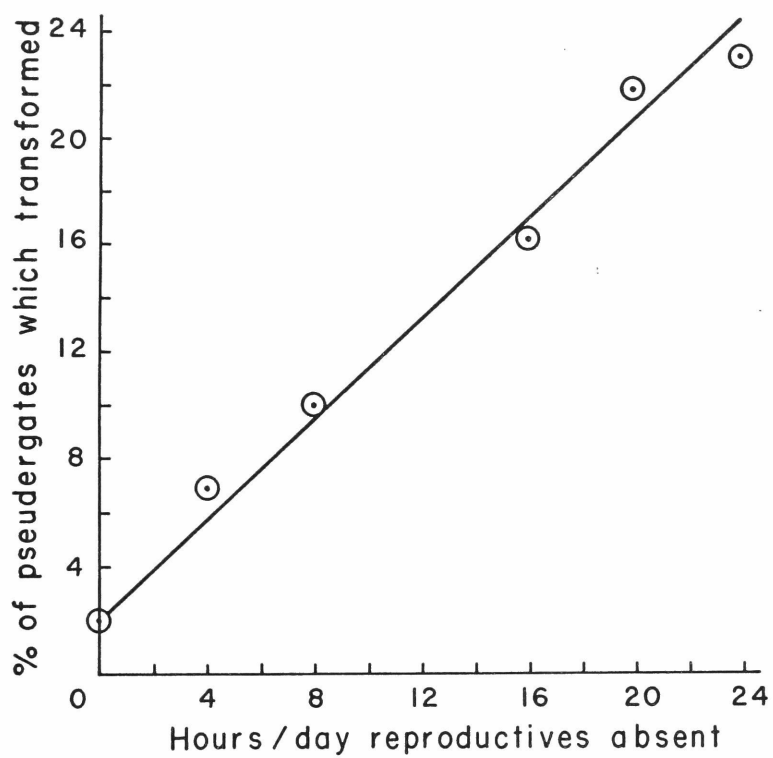


Figure 12. Reproductives arising in partial orphaning experiment as a percent of pseudergates present on day 0. Sum of two experiments. Results as of day 26.

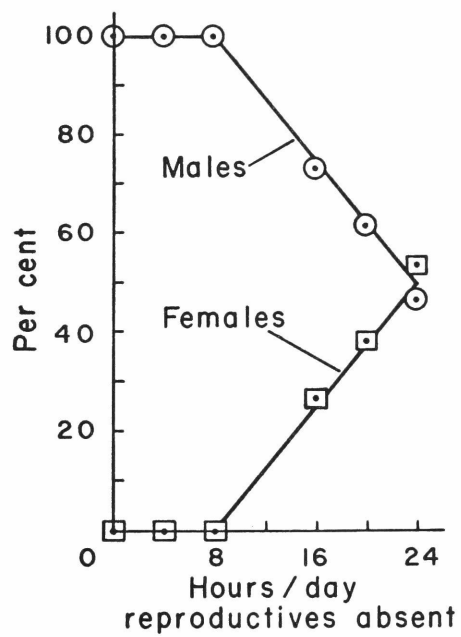


Figure 13. Sex ratio of supplementaries arising in partial orphaning experiment.

the inhibitory process would seem to imply such a threshold effect. For example, Grassé (1949) has proposed that the inhibition of termite larvae is due to a "group effect" by which he meant that the larvae are continuously aware of the composition of the group they are in because of communicatory signals of one type or another. This awareness would inhibit transformation. The present results, however, show that different colonies of the same composition (i.e., lacking reproductives) can respond differently. The pseudergates do not at some point suddenly all become aware of the absence of reproductives. Further, it would appear that when a pseudergate "perceives" the absence of the reproductives, that is, when it becomes determined physiologically to transform, it does not communicate this "perception" to other transformable pseudergates.

The data require, rather, a theory in which the pseudergates are not all equally inhibited or in which the pseudergates can have graded degrees of inhibition. For example, if the inhibition corresponds to the presence of a substance taken up by the pseudergates from the reproductives, as numerous authors have proposed, then different degrees of inhibition would correspond to different amounts of substance. Some pseudergates would run out of the substance sooner than other equally transformable pseudergates. If the reproductives are absent at that time these pseudergates would be determined to transform. The longer the reproductives are absent, the more pseudergates will run out of the substance.

Similarly, it is possible to understand why female pseudergates are less sensitive to part-time orphaning than males. As was seen in the previous chapter, females receive surplus inhibition. They are more inhibited by the reproductive pair than are males. A substance theory would therefore predict that a longer absence of reproductives would be necessary to stimulate female transformation, as was found. Such a finding, however, would not be expected on the basis of the "group effect" theory.

A difference in sensitivity to orphaning has been previously reported (Grassé and Noirot, 1960a). These workers, however, believed that the female larvae were more "reactive" than the males. They sexed

the reproductives that were present in K. flavicollis colonies 10, 20 and 35 days after orphaning and found more females than males at the first two time points. However, it should be pointed out that the sex ratio of the larvae was not determined and that furthermore, day 10 is rather late since reproductives can start appearing as early as day 6 in Kalotermes.

In the present work with Neotermes jouteli, censuses were carried out in orphaned experiments every day and it was found that on the first and second days that reproductives arose, those which appeared were overwhelmingly male. However, in the next few days the sex ratio rapidly evened out. As an example, in the half-orphaning experiment, it can be seen that the colonies headed by a female and therefore not producing any females responded more rapidly than colonies headed by a male (see Figure 10). In the colonies headed by a single male, male reproductives arose before females (see Table XVI) and the same is true of the totally orphaned colonies in that experiment for days 8 and 9.

Furthermore, it has been found throughout this work that under conditions of partial or nearly total inhibition it is always only males that arise. For example, it occasionally happens that supplementaries arise even though a functional reproductive pair is present. The new supplementary is usually killed but it is always a male. In the sex intensity experiment the two supplementaries that arose in the presence of two males or two females were both male (see Figure 11). Finally, even in Kalotermes flavicollis a similar situation has been found. Lüscher (1951a) removed the reproductives from laboratory colonies for 24 hours and then replaced them. Some of these colonies were thereby stimulated to produce one or two supplementaries, all of which were male.

Thus, many observations support the idea that male pseudergates are more readily transformable than females. This does not imply that males are inherently more reactive. It may simply result from males receiving less inhibition than females.

## IX. THE PHEROMONE THEORY OF CASTE REGULATION

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Introduction

It was Pickens (1932) who first proposed what is today the most widely accepted theory of caste regulation in the lower termites. He speculated that the reproductives produce a chemical substance that when picked up by larvae through grooming or trophallaxis, inhibits their development into supplementaries. In contemporary terminology such a substance would be known as a pheromone (Karlson and Butenandt, 1959; Karlson and Lüscher, 1959). While this theory is the only one so far conceived that is consistent with all the known facts, it is very far from being proved. In fact, the evidence that has been specifically brought forward to support this theory is extremely shaky and is based on experiments that in themselves are not only unconvincing but have also turned out to be unrepeatable. What follows is a discussion of these experiments together with others the present author has carried out in an effort to obtain evidence in support of the theory.

Castle (1934) was the first to seek experimental evidence for Pickens' theory. His procedure, which was followed by all later authors, was to feed orphaned larvae filter paper impregnated with various extracts of supplementary reproductives. He reported that ether and 70% ethanol extracts of female supplementaries retarded egg production in supplementaries that arose in orphaned colonies of Zootermopsis angusticollis. However, the rate of appearance of pigmented forms was the same as in control colonies fed plain filter paper. Castle was unaware of the fact

that supplementaries arise from larvae via a special molt during which they become pigmented. Thinking rather that they were simply larvae that became sexually mature without molting, he took his results as evidence for the isolation of an active inhibitory extract.

Light (1944b) attempted to repeat Castle's experiments. He described three experiments in which orphaned Z. nevadensis larvae were fed weekly ether extracts of female supplementaries. In two of the experiments there was no effect either on the production of eggs or of pigmented forms. In the third the production of both was reduced relative to controls but the reduction was slight and did not appear at all until six and a half weeks. Since by this time negative feedback inhibition and, possibly, caste elimination would have come into play, it is difficult to evaluate these results. They could certainly, in any case, have been due to random fluctuations.

Light (1944b) then carried out a different sort of experiment whose results have generally been taken as the strongest support for the pheromone theory. In this experiment Light extracted separately from three portions of the bodies of 33 female supplementaries. The portions were the head-thorax, the viscera and the abdominal body wall, and each portion was first extracted with water and then with absolute methanol. Aliquots of the six filtrates were then fed to small colonies of orphaned larvae of Z. angusticollis arranged in six groups. The extraction and feeding were repeated each week. One control group of colonies was fed blank filter paper. Lüscher (1956a) has summarized Light's results as of three weeks in Table XXII.

Light held serious reservations about these results, the most important of which was that the experiment was carried out before he became aware of colony variations in regenerative intensity. The larvae used in the experiment came from different natural colonies and no record was kept of which experimental colonies were derived from which natural colonies. In fact, Light suspected that a sizeable number of the colonies came from a different species, Z. nevadensis. He also felt that physiological and health differences between different groups of colonies could have affected the results.

TABLE XXII

1937 Extract-Feeding Experiment of Light (1944b) in Zootermopsis

(results after three weeks)

Extract	Extracting solvents	Total no. of larvae present in all colonies	Total no. of reproductives present in all colonies
Control		162	17 = 9.5%
Abdominal wall	H <sub>2</sub> O	166	9
	CH <sub>3</sub> OH	<u>163</u>	<u>19</u>
	Total	329	28 = 7.8%
Viscera	H <sub>2</sub> O	179	11
	CH <sub>3</sub> OH	<u>159</u>	<u>4</u>
	Total	338	15 = 4.25%
Head-thorax	H <sub>2</sub> O	189	0
	CH <sub>3</sub> OH	<u>196</u>	<u>1</u>
	Total	385	1 = 0.25%

Many authors, citing these results as strong evidence for the inhibitory pheromone theory, have ignored Light's criticisms of his own experiment. This is especially unjustified since in the same paper Light presented the wholly negative results of a repetition of the experiment, but with its previous deficiencies corrected.

In the repeated experiment, besides taking all larvae from the same natural colony, Light also increased the dosage of extract fed to experimental colonies and added new controls in which larvae were fed identical extracts of larvae. The results are presented in Table XXIII as of the fourteenth week. The overall mortality for the experimental and control colonies was about the same. All were in the range  $29 \pm 6\%$  as of 14 weeks.

Fourteen weeks is very late to be looking for an inhibitory effect, but this was the earliest data given by Light. If this data means anything, it is that aqueous extracts of reproductives or larvae stimulate reproductive production in orphaned colonies compared with filter paper-fed controls. In any case this experiment, which is superior in design and better controlled than Light's 1937 experiment, gives no evidence that reproductives contain a substance inhibiting supplementary formation.

TABLE XXIII

Data Calculated from SE-HX Extract  
Feeding Experiment of Light (1944b)  
(results after 14 weeks)

Extract (fed weekly)	Total initial no. of larvae in all colonies	Percent of initial no. which trans- formed
Aqueous surface extract of 50♂ + 50♀ supple- mentaries	500	21
Aqueous surface extract of 250 larvae	500	19.4
Aqueous extract of ground-up heads of 40♀ supplementaries	400	22.8
Aqueous extract of ground-up heads of 100 larvae	400	20.5
Control (filter paper)	400	14.8



In some of Light's experiments a small reduction in egg production in colonies receiving extracts of reproductives was observed. Partly because of this effect Light felt that the overall trend of the evidence obtained by Castle and himself supported the pheromone theory. Like Castle, Light was unaware that supplementary formation involves a molt and so did not consider pigmentation to be of decisive importance. Even if the effect on egg production were real and repeatable, which is by no means certain, the extracts could have been interfering with other processes such as mating or fertility rather than transformation. In the present author's view, the evidence produced by Light and Castle is extremely frail and it is, in fact, disturbing that stronger evidence was not obtained.

In the last two decades Lüscher has been the major exponent of the pheromone theory of caste regulation in termites. Lüscher (1951b, 1952b) first repeated an unpublished experiment of Grassé and Le Masne in which an orphaned laboratory colony of Kaloterms flavicollis pseudergates was separated from a normal colony by a metal screen through which antennal contact could be maintained. The results were that supplementaries arose in the orphaned colony but were eliminated, and so new supplementaries continued to arise and be killed. If the colonies were separated by two screens so that antennal contact was no longer possible, then new supplementaries were not eliminated. Lüscher concluded that in the single screen experiment the orphaned pseudergates perceived the presence of the reproductives across the screen by antennal contact and were thereby led to eliminate new supplementaries. However, neither antennal contact nor odors were adequate to inhibit larval transformation. For this, actual cohabitation was necessary and Lüscher (1952b) proposed that the reproductives produced an inhibitory substance that the pseudergates obtained either by ingesting the reproductives' feces, saliva or some exudate secreted through the cuticle.

To test these latter possibilities Lüscher (1955) conceived the idea of placing a reproductive in a screen separating two colonies so that its head was in one colony and its abdomen in the other. Unfortunately only two female reproductives survived this treatment. A male reproductive was present on the head side of one female and on the abdomen

side of the other. Lüscher found complete inhibition in the colony containing a male reproductive plus a female abdomen. In the other three cases, as many reproductives arose as in totally orphaned control colonies, although more males arose than females. Since Lüscher was convinced that Light (1944b) had demonstrated the presence of an inhibitory substance in the head-thorax of female reproductives, he proposed that the substance produced in the head was secreted into the alimentary tract and was ingested by larvae at the reproductive's anus. Such proctodeal feeding is very commonly observed in termites and so the theory seemed plausible. Lüscher later stated (1960b) that if the anus of the female reproductive was varnished, then the inhibition was released. However, he presented no data. The data in the previous experiment (1955) are so meager that the results are not fully convincing. Furthermore, it is not clear from Lüscher's paper whether he used pseudergates from the same stock colony in all parts of the experiment.

Lüscher (1956b) then attempted to show that larvae could transmit the inhibitory substance they obtained from reproductives to other larvae. He placed larvae in screens separating two colonies. If a pair of reproductives was present on the head side then fewer supplementaries arose on the abdomen side than if the pair was absent. It is difficult to evaluate these results since there was great variation in the effect from colony to colony and the overall effect was not strong.

Lüscher (1956b) then attempted to obtain an active inhibitory extract as Light and Castle had tried to do. He believed that Light had failed to obtain complete inhibition because he had only provided larvae with extracts from female reproductives. Lüscher therefore homogenized the heads of 100 male and 100 female reproductives in methanol and fed portions absorbed on filter paper daily to orphaned larvae. Control colonies received filter paper plus methanol. No inhibitory effect was found. In fact, more reproductives arose in the experimental colonies than in the controls. Lüscher (1961a) later stated that he had tried several times to obtain active inhibitory extracts but had each time failed.

The present author had also failed to obtain direct evidence in support of the pheromone theory. In addition, the evidence that has been obtained casts doubt on Lüscher's idea that the hypothetical substance is obtained from reproductives via proctodeal feeding.

Three experiments are described. The first involved feeding orphaned pseudergates a dead pair of reproductives each day. In the second, orphaned pseudergates were fed filter paper each day that had absorbed a large quantity of reproductive fecal material. In the third, pseudergates were maintained with reproductives whose anuses were sealed.

#### Dead Pair Experiment

Four laboratory colonies of Neotermes jouteli were established, each containing 50 pseudergates, 5 soldiers and a pair of supplementary reproductives. All termites originated in the same stock colony. After two weeks of incubation at 26°C in the dark the reproductives were removed from all colonies on day 0. Starting on this day and repeating each day thereafter, two of the colonies were given a piece of filter paper on which were freshly crushed a pair of supplementary reproductives. A pestle was used to crush the heads of the reproductives onto the paper. The other two colonies received daily a pair of pseudergates whose heads had been crushed with a different pestle. The crushed termites were always immediately attacked by the pseudergates, who rapidly and totally ate them.

Results and discussion New supplementaries appeared in all colonies by day 8 or 9. They were allowed to remain and the normal caste elimination process set in. Daily censuses were made so that careful track could be kept of the number of reproductives that arose. By day 19 a total of 12 supplementaries had arisen in the experimental colonies and 13 in the control colonies. Thus, there is no evidence that transformation is inhibited if pseudergates eat a single pair of reproductives each day. It is possible that an effect would be observed with a higher dose.

#### Feces Experiment

Six laboratory colonies were established, each containing 50 pseudergates, 5 soldiers and a pair of supplementary reproductives, all

originating from the same stock colony. The colonies were maintained in small plastic petri dishes containing a thin layer of agar for moisture and a piece of #597 filter paper as the only source of food. On day 0 the reproductives were removed. Each day the colonies were removed from their dishes and a census was taken. Each empty dish was then treated as follows: To two of the dishes 25 pairs of supplementaries were added and anaesthetized with  $\text{CO}_2$ . This caused each supplementary to deposit a droplet of rectal fluid and fecal material on the filter paper. Two other dishes similarly received droplets from 50 pseudergates. These pseudergates were from colonies that were maintained without reproductives so that the pseudergates could not contain any inhibitory substance obtained from reproductives. The remaining two dishes were untreated. The droplet-giving supplementaries and pseudergates were removed and replaced by the original colonies. This process was repeated daily. New filter paper was added as needed. In addition, for six days prior to day 0 the droplets were added to the first four dishes. This was in an effort to increase the level of the hypothetical inhibitory substance so as to minimize the effect of sudden orphaning. The experiment was also repeated with six colonies of termites taken from another stock colony. The procedure was identical except that droplets were added for 30 days prior to day 0.

Results and discussion The total number of reproductives that arose in each of the 12 colonies as of day 19 is presented in Table XXIV. The data could be interpreted as suggesting a slight inhibitory effect of reproductive feces and a slight stimulatory effect of larval feces. Probably, however, the differences are not significant.

TABLE XXIV

Reproductives Arising in Feces Experiment as of Day 19

Colonies receiving	Stock 683 colonies	Stock 688 colonies	Total
reproductive feces	7	11	18
pseudergate feces	14	13	27
nothing	12	9	21

### Sealed Anus Experiment

If anal droplets are the source of the inhibitory substance(s) then reproductives with sealed anuses should not be inhibitory. Six laboratory colonies were established containing 60 pseudergates, 6 soldiers, and a pair of supplementary reproductives, all originating from the same stock colony of N. jouteli. The colonies were incubated for 19 days at 26°C in the dark. At the end of this time the reproductives from two of the colonies were removed, placed in a dish under a dissecting microscope, and anaesthetized with CO<sub>2</sub>. This caused them to excrete a small droplet of fecal fluid which was cleaned away with a broken wooden applicator. A small drop of lucite dissolved in ethylene dichloride was then applied to their anuses with a metal probe. The lucite rapidly dried, forming a cap over the anus, and the reproductives were returned to their original nests. A number of other sealing agents, including rubber cement, Duco cement, Elmer's Glue, Shellac and Canada Balsam, were tested in preliminary experiments, but none dried as rapidly, were as easy to apply and provided as good a seal as lucite.

The reproductives were removed permanently from two of the other colonies and were maintained permanently in the remaining two colonies. Each day after the initial sealing (day 0) the sealed reproductives were removed and anaesthetized with CO<sub>2</sub>. This provided a test for the seal since one could see whether the reproductives were capable of excreting any fluid. Occasionally the seal had been broken and this was noted. In any case a new drop of lucite was added each day. Daily censuses were carried out on all colonies and new reproductives were removed as they arose. Sealed reproductives often did not survive and so replacements were added.

Results The cumulative production of reproductives in the three types of colonies is presented graphically in Figure 14. The survival and daily state of the seal of the sealed reproductives is presented in Figure 15. It can be seen that reproductives did not arise in the experimental colonies until considerably later than in orphaned colonies and then at a lower rate.

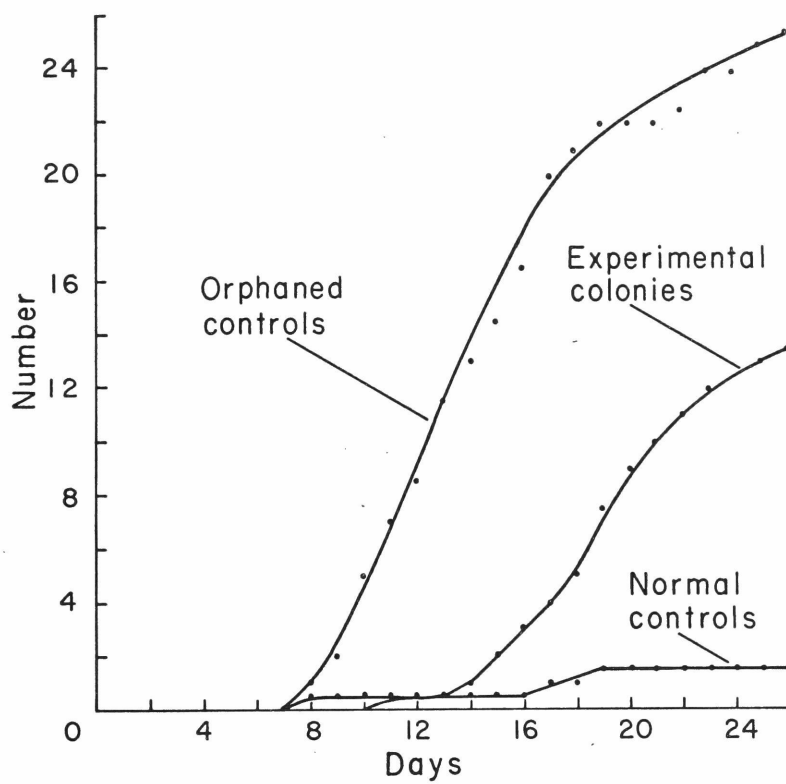


Figure 14. Supplementary production in laboratory colonies of N. jouteli in the presence of reproductives with lucite-sealed anuses. Each curve is the average of two colonies.

Colony	day sex	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
A	♂	S	S	U	S	S	S	?	U	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	♀	S	U	?	S	S	U	S	S	S	S	S	S	S	S	U	S	S	S	S	S	S	S	U	S	S	S	S
B	♂	S	U	U	S	S	S	S	S	S	S	S	S	S	U	S	U	S	S	S	S	S	S	S	?	S	S	S
	♀	S	S	S	S	S	S	S	U	S	S	S	S	S	S	S	S	S	U	S	U	S	S	S	S	?	U	S

Figure 15. State of sealed reproductives in sealed anus experiment. Straight line indicates survival. S = sealed; U = unsealed.

Discussion The sealed anus experiment indicates that reproductives with sealed anuses are strongly if not completely inhibitory. The supplementaries that did arise in this experiment can probably be accounted for by the release in inhibition that occurred when sealed reproductives died. Grassi and Sandias (1893-1894) showed that the removal of reproductives for 24 hours was sufficient to induce some transformation in Kalotermes flavicollis. We have seen from the partial orphaning experiment that the continuous presence of reproductives is necessary to fully inhibit transformation. For the same reason it is unlikely that the occasional breaking of the seal could account for the inhibition observed. That is, there were too many days on which the seal was unbroken. Thus, this experiment casts doubt on Lüscher's idea that larvae obtain an inhibitory substance from reproductives via proctodeal feeding, although one could possibly argue that with the anus blocked, the substance is forced out some other place such as the mouth.

It seems, therefore, that proctodeal feeding has primarily a nutritive function and is essential for transferring symbiotic protozoa to young larvae and freshly molted pseudergates. It is of interest that the fungus-growing termites, which lack protozoa, do not engage in proctodeal feeding (Harris and Sands, 1965).

#### General Discussion

Despite the fact that no direct evidence for an inhibitory substance has been obtained, the pheromone theory remains the most plausible explanation of caste regulation in lower termites. Thus, genetic or blastogenic theories of caste determination (Thompson, 1917; Imms, 1919) are contradicted by the finding that all larvae are capable of transformation (see Chapter VI). Trophic theories, which hold that larvae are determined to become reproductives because of special food or stimulatory substances obtained from other members of the colony, are contradicted by the finding that totally isolated larvae invariably transform to supplementaries (Castle, 1934; Grassé and Noirot, 1960b). Simple behavioral theories that hold that larvae are inhibited by being kept aware of the presence of reproductives through sensory stimuli (Grassé, 1949) are made doubtful



in the light of the part-time orphaning experiment presented in this work (Chapter VIII) and the experiments in which orphaned colonies were separated from normal colonies by a single screen (Lüscher, 1951b, 1952b). In these latter experiments information about the existence of reproductives crossed the screen but the inhibitory influence did not. The pheromone theory seems to be the only one that fits all the known facts. It can also be easily elaborated to account for the different inhibitory effects of the male and female reproductives.

Why then has strong direct evidence been so far unobtainable? There are many possible reasons. The substance could be very labile and easily destroyed either during extraction or when exposed to the air on filter paper. It could also be that the proper extracting solvent has not been tried. Furthermore, it could also be that several substances are involved and all are necessary for inhibition.

It seems, however, that it would be a mistake to plunge into further extraction studies. The inhibitory process has got to be defined much more closely by biological techniques before it is worth investing time in chemical ones. For example, an approach such as Lüscher (1955) has initiated, in which reproductives are immobilized between two colonies, should be pursued. Such experiments are extremely difficult (the present author has invariably failed) because the immobilized reproductives rarely survive long enough. However, such experiments could lead to localizing the source of the pheromone. In addition, experiments in which orphaned larvae are fed freshly killed reproductives at higher doses than reported here should be tried. If an effect were found it could be localized by feeding only parts of the reproductives. Finally, more behavioral experiments have to be carried out in which the interactions between the reproductives and the pseudergates are accurately defined and quantitated. For example, the author has observed that although all individuals in a colony engage in proctodeal feeding, only advanced larvae, pseudergates and nymphs engage in licking behavior. These transformable forms lick or groom the entire bodies of each other and those of the two fixed castes. Therefore, this could be a mechanism of pheromone transmission.

## X. DISCUSSION: THE SOCIETY AS AN ORGANISM

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Introduction

Termite colonies are very complicated systems. They are clearly not simple aggregations of individuals like bacterial or fungal colonies. Nor are they loosely integrated groupings like flocks of birds, swarms of locusts or herds of reindeer. Rather their degree of integration is so high that the behavior and other characteristics of individuals are intelligible only in relation to the overall society. It is for this reason -- the clear subordination of individuals to their society -- that termite and other social insect societies have been considered to be organisms, or rather higher forms of biological organization above the organism -- "superorganisms."

This view of insect societies is a very old one, going back at least to the Greeks. In modern times the superorganism idea has been propounded and developed on the basis of studies of ants (Wheeler, 1911), termites (Emerson, 1939, 1952, 1956) and bees (Chauvin, 1968). It is the purpose of this concluding section to relate what has been learned in the course of this work as well as in other studies to the concept of the society as an organism.

The Castes

In Chapter VI it was shown that all larval forms of Neotermes jouteli possess the genes for supplementary reproductive development. This is probably also true with respect to the soldiers and alates. The larvae are like totipotent cells of a developing organism. The castes are its differentiated parts.

Three basic forms are found in termite colonies: soldiers, reproductives and workers. The soldiers and reproductives are highly special-

ized for carrying out their respective colony functions: defense and growth. The workers or pseudergates in lower termites are morphologically and behaviorally less specialized. They carry out a number of essential colony functions including nutrition, brood rearing and nest maintenance and construction. It is natural that these functions should be performed by the same individual. In the lower termites the nest is food and the brood rearing consists essentially of feeding the young larvae and insuring that the eggs are kept in the region of the nest with the optimum microclimate. The ants and bees that do not inhabit their food allocate these different functions to different "temporal castes." That is, young workers generally engage in nursing, then at a later time switch to nest work and finally engage solely in food gathering.

One final form encountered in termite colonies is the alate. It is not integrated into the life of the colony but rather is tolerated and fed until the proper meteorological conditions arise that trigger its escape. The alates are the sex cells of the superorganism. The colony is hermaphroditic. Inasmuch as the same conditions trigger simultaneous swarming in all colonies in a given area, interbreeding is assured.

It is interesting to consider the differentiated zooids of a polymorphic coelenterate. In the simple hydrozoan Hydractinia, and in the highly complex siphonophore Physalia, three basic forms are found: the gastrozooids, the dactylozooids and the gonozooids fulfilling the functions of colony nutrition, defense and reproduction. The same colony functions are performed by the three polymorphic individuals composing Ectoproct colonies. Colony growth, on the other hand, is not the function of any differentiated form as it is in termites.

In "The Origin of Species" Darwin (1859) stated that the existence of sterile castes in social insects posed "the most serious special difficulty...which at first appeared...insuperable, and actually fatal to [the] whole theory [of natural selection]." How could termite soldiers that do not reproduce ever evolve with their unique morphology and behavior? How can one account for polymorphism among the sterile castes, or the fact that higher termites and ants have sterile soldiers and workers?

These difficulties disappear when one looks at a colony as an organism. As Darwin recognized, natural selection acts on the society as a whole. Colonies lacking soldiers would not reproduce. Other factors being equal, those colonies with the most fit soldiers would produce the largest number of alates who would thus tend to propagate any particular modification those soldiers possessed.

An example of soldier evolution in termites that shows these forms are simply differentiated parts of a superorganism is the regression of compound eyes (Emerson, 1952). This has occurred along several independent phylogenetic lines. The loss of eyes in soldiers of advanced termites cannot be due to any loss of the genetic basis of the compound eyes since the soldier is the progeny of primary reproductives with fully developed compound eyes. What evolved, rather, is that inhibition of eye development became associated with the turning on of the genes controlling soldier development.

As indicated in Chapter I, this thesis basically represents an attempt to investigate the social homeostasis of castes. We have seen that mechanisms exist for regenerating lost castes and eliminating excess castes. At least in the case of reproductive regeneration the evidence indicates that the mechanism involves social substances analogous to hormones. In insect development sexual maturation is inhibited by the presence of juvenile hormone. This would be comparable to the inhibitory activity of the supposed pheromone that prevents the development of supplementary reproductives.

### Grooming and Trophallaxis

Grooming and trophallaxis are behavioral systems of major importance in the homeostasis of insect societies. Grooming of queens is the mechanism by which pheromones inhibiting queenless behavior in bees are transmitted from queen to worker (Butler, 1954). The same mechanism may operate in termites. Other special substances can also be transmitted through grooming. In Odontotermes redemanni the exudate of physogastric queens contains fatty acids with strong antibacterial and antifungal properties (Sannasi and Rajulu, 1967). Molds are a great danger to

termites whose nests generally have high humidities. Grooming also undoubtedly plays an important role in the recognition of colony members and in their distinction from foreign intruders, which are generally attacked.

Trophallaxis or mutual feeding is found among all social insects and is probably the most important mechanism of social integration. Trophallaxis is the means by which symbionts, food and other substances are socialized. For example, the pheromones secreted by a queen honeybee which workers obtain by grooming are exchanged through stomodeal feeding (Butler, 1956).

The non-foraging castes depend upon trophallaxis for nourishment. Looking at the same thing in another way, one can say that trophallaxis permits the extreme specialization associated with these castes. Thus the mandibles used for gnawing at wood in pseudergates become greatly elongated and specialized for slashing in soldiers, which are then incapable of attacking wood. Similarly, the giant physogastric queens of the higher termites are unable to move, let alone forage, but can produce eggs at a prodigious rate.

The pseudergates also feed each other. There is at least one reason why this is necessary. As has been mentioned, molting involves gut-emptying and loss of symbionts. Post-molt pseudergates are refaunated by proctodeal feeding. This is also the means by which early instar larvae first obtain their protozoa as well as receiving nourishment.

Thus it is clear that for many reasons pseudergates must engage in trophallaxis or else the colony will perish. The same is also true of the foraging castes in social hymenoptera. In one species a biochemical mechanism enforcing this kind of social integration has been demonstrated. Workers of the social hornet Vespa orientalis are predatory on other insects. After foraging they present the grub-like larvae with bits of meat and flower-nectar, and receive in exchange a droplet of saliva. It has been found (Ikan et al, 1968) that larval saliva contains an abundance of proteolytic enzymes which the workers totally lack. Thus the workers

must forage and engage in trophallaxis in order to nourish themselves. If all larvae are removed from a nest, it is abandoned by the workers.

### Termite Nests

One of the most remarkable examples of organized social behavior in termites is the construction and maintenance of nests. The lower termites typically do not have well-defined nest structures since the design of their dwellings is determined to a great extent by the characteristics of the wood they are in. It is in the higher termites that characteristic nest constructions appear. In fact, the ability of these termites to construct their own shelters and attain some control over their microclimate probably accounts for their tremendous success. Their nests range from small carton balls built around the branch of a tree by species such as Nasutitermes arboreus to complex gigantic mounds rising as much as 6 meters above the ground and having diameters of 25-30 meters built of a hard cement by certain African species of Macrotermes (Grassé, 1949). These latter nests house tens of millions of termites.

Nest designs are usually well defined and can be used as a species characteristic. Various forms of symmetry are found including spherical (Nasutitermes, Microcerotermes), radial (Cubitermes) and bilateral (Amitermes, Procubitermes) (see Emerson, 1939). In Amitermes meridionalis, nest bilateral symmetry is believed to have a temperature regulating function (Allee et al, 1949). The mounds of this species, which inhabits tropical Australia, are shaped approximately like isosceles prisms. The long axes are always oriented north and south. Thus the broad sides face the rising and setting sun while the narrow top edge faces the vertical rays.

The external surface may have specialized parts including rain-shedding nest caps (Cubitermes intercalatus), radial ridges (Macrotermes natalensis), repeating arrays of funnel-shaped ventilation pores (Apicotermes lamani). From studies of the pores and other nest structures of various species of Apicotermes, it has been possible to derive a phylogenetic sequence (see Schmidt, 1958).

In addition the internal structure of the nests may be highly complex. The giant mounds of Macrotermes natalensis contain specialized compartments for reproductives, nurseries for young larvae, chambers containing fungus combs and a complex ventilation system consisting of hollow spaces in the bottom and top of the nest connected by channel systems running through the external ridges. Lüscher (1961b) has shown that air circulates in the channel system in such a way as to maintain constant temperature and CO<sub>2</sub> pressure in the center of the nest. Heat is released at the top of the nest and the internal temperature is unaffected by normal diurnal fluctuations. CO<sub>2</sub> is exchanged for oxygen in the ridges. This "air conditioning" system has enabled Macrotermes to attain the widest distribution of any African termite.

Although termite nests have a stable appearance they are actually homeostatic structures in a state of dynamic equilibrium. Grassé (1944-1945) has found that in nests of M. natalensis construction and destruction constantly take place without any change in the overall structure. Furthermore, this constant activity proceeds without any obvious stimuli.

If a nest wall is broken a defensive ring of soldiers forms around the edge of the nest opening like "phagocytes in the vicinity of the wound in a mammal" (Emerson, 1956) and the nest is rapidly repaired. Hingston (1932) showed that if one removes the rain-shedding ridges from a nest of Constrictotermes cavifrons regeneration will occur over the course of the next few months. Grassé (1944-1945) has also described nest regeneration in Macrotermes.

Although less is known about the situation in lower termites, it is clear that they also must monitor and regulate the structure of their nests. For example, they never hollow out the trunk of a tree in such a way that the tree collapses. Thus, they must respond to the stresses created as they eat out galleries.

#### Colony Life History

The colonies of social insects undergo a developmental process of their own that is analogous to the life history of organisms in a number of fundamental ways. From surveying population growth in colonies of a

variety of social insects Bodenheimer (1937) concluded that the growth curves of colonies and organisms were the same. He divided colony development into three periods: 1) foundation, 2) expansion and 3) senescence. The first period is characterized by slow growth with the reproductives being forced to carry out non-reproductive work involved in foraging, brood-rearing and nest construction and maintenance. Finally, sufficient workers and soldiers are raised that the reproductives can devote themselves fully to egg production and the main organizational features of the colony are established. The colony then enters period two characterized by logarithmic growth. The point at which maximum growth is attained generally coincides with colony maturity, i.e., alate production. When the growth curve levels, the colony enters period three and then the population declines until the colony dies. Bodenheimer's three periods correspond to the periods of embryogenesis, growth and sexual maturity in an organism.

The small initial colonies were not studied in the present work. Studies by a number of workers on six species in three of the lower families of termites have shown that incipient colonies invariably produce at least one soldier (see Castle, 1934). It is not clear whether such colonies can produce supplementaries. The present work has shown that there are striking physiological differences between colonies in period two and those in period three. Period two colonies vigorously regenerate lost reproductives and minimize pseudergate size. Both of these things favor maximal population growth. Period three colonies regenerate reproductives weakly, sometimes not at all. Given that the population has reached its height, this maximizes the number of alates produced. Furthermore, the pseudergates are at a maximum size, enhancing the success of the alates into which they develop.

In summary, a termite society is an organism composed of organisms. It contains a variety of forms: some are differentiated for specialized functions and some are undifferentiated and carry out generalized functions. All are integrated together primarily through chemical interactions mediated by the behavioral systems of grooming and trophallaxis. The construction and maintenance of elaborate and finely regulated nests is only



the most spectacular example of a termite colony's integrated social behavior. Finally, the termite social organism has a life history with characteristic stages analogous to the embryonic, juvenile and sexually mature stages of an individual organism. To take Haeckel one step further, sociogeny recapitulates ontogeny.

## ADDENDUM

A. The Presence of the Flagellate, Staurojoenina, in the Hindgut of Neotermes jouteli Pseudergates.

When the protozoan fauna of pseudergates of the stock colonies of Neotermes jouteli was surveyed, it was discovered that the most prominent and unusual organism present was a species of Staurojoenina. In some colonies, however, the pseudergates lacked Staurojoenina but otherwise possessed the normal range of symbionts. These colonies were all mature (alate-producing) and could regenerate reproductives only weakly. Previous studies have stated that Staurojoenina is absent from N. jouteli.

Staurojoenina is a large flagellate, approximately 150 microns in length, whose several hundred flagella are arranged in four symmetrical bundles at the anterior end of the cell. It was first discovered by Grassi (1917) in the hindgut of an East African Kalotermitid, Epicalotermes aethiopicus. The new species, which he named Staurojoenina mirabilis, was the unique member of a new family, Staurojoeninidae, of the order Hypermastigina.

Kirby (1926) later found Staurojoenina in two Kalotermitids native to California, Arizona and northern Mexico, Incisitermes minor and Marginitermes hubbardi. Kirby did not have the East African termite at hand, but concluded from Grassi's drawings that the size range and number of flagella of the flagellate in I. minor were greater than in Grassi's species. For these reasons and because of the geographical separation, he described the flagellate from I. minor as a new species, Staurojoenina assimilis. He believed that the flagellate in M. hubbardi was more similar and possibly identical to S. mirabilis.

Finally, Staurojoenina was found in the Kalotermitid Postelectrotermes praecox from the island of Madeira by Grassé and Hollande (1942, 1945). Once again, the newly found flagellate was very similar to the to the previously described species, but, on the basis of a number of cytological differences, Grassé and Hollande considered it to be a new species, S. Caulleryi. No attempt has been made in the present work to

describe Staurojoenina from N. jouteli in detail or to compare it with previously described species.

Kirby (1926) stated that Staurojoenina was absent from Neotermes jouteli from Florida. Furthermore, in later work (1942, 1945) he studied the protozoa in N. jouteli in detail and described three new species without mentioning Staurojoenina. It was therefore surprising to find this species in most of the stock colonies of N. jouteli studied in the present work.

Of twelve colonies collected in 1968, nine had Staurojoenina and three did not. These latter colonies all had large pseudergates and soldiers, produced alates and regenerated lost reproductives weakly. However, three other similarly mature colonies possessed the flagellate. Nothing is known about the nature of the termites used in Kirby's studies.

Since the 1968 stock colonies had been maintained in the laboratory for ten months before their protozoa were surveyed, it is possible that the loss of Staurojoenina in some colonies was a laboratory-induced artifact. Therefore, it was decided that the protozoa should be surveyed immediately upon extraction of N. jouteli colonies collected in the future. As stated in Chapter V, this was in fact done with the colonies collected in January, 1969 and pseudergates from all colonies, both mature and immature, contained Staurojoenina. Still the possibility remains that a new physiological characteristic of pseudergates from mature colonies has been detected.

## ADDENDUM

## B. The Families of Termites together with the Species Referred to in the Present Work.

The division of Isoptera into six families follows Emerson (1965). The nomenclature of the species of Kalotermitidae follows Krishna's generic revision (1961) of that family. All other species names follow the taxonomic scheme of Snyder (1949). Numerals following a species name refer to chapters of the present work in which it is mentioned.

Family I	Mastotermitidae	<u>Mastotermes darwinensis</u> (I)
Family II	Kalotermitidae	<u>Calcaritermes nearcticus</u> (II)
		<u>Cryptotermes brevis</u> (II)
		<u>Cryptotermes cavifrons</u> (II)
		<u>Epicalotermes aethiopicus</u> (Addendum A)
		<u>Incisitermes milleri</u> (II)
		<u>Incisitermes minor</u> (Addendum A)
		<u>Incisitermes schwarzi</u> (II)
		<u>Incisitermes snyderi</u> (II, IV)
		<u>Kalotermes approximatus</u> (II)
		<u>Kalotermes flavicollis</u> (III - IX)
		<u>Marginitermes hubbardi</u> (Addendum A)
		<u>Neotermes castaneus</u> (II, IV)
		<u>Neotermes jouteli</u> (I - X, Addendum A)
		<u>Neotermes tectonae</u> (IV, V)
		<u>Postelectrotermes praecox</u> (Addendum A)
Family III	Hodotermitidae	<u>Zootermopsis angusticollis</u> (III, IV, VI, VII, IX)
		<u>Zootermopsis nevadensis</u> (IV, V, VI, VII, IX)
Family IV	Rhinotermitidae	<u>Prorhinotermes simplex</u> (III, IV)
Family V	Serritermitidae	

## Family VI

## Termitidae

## Subfamily Amitermitinae

Amitermes meridionalis (X)Microcerotermes (X)

## Subfamily Termitinae

Apicotermes lamani (X)Cubitermes intercalatus (X)Procubitermes (X)

## Subfamily Macrotermitinae

Macrotermes natalensis (IV, V, X)Odontotermes redemanni (X)

## Subfamily Nasutitermitinae

Constrictotermes cavifrons (X)Nasutitermes arboreus (X)Nasutitermes triodiae (V)

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