Insectes Sociaux, Paris.
Volume **XIX**, 1972, n° 1, pp. 39-61.

CASTE DETERMINATION IN *NEOTERMES JOUTELI* (BANKS) (1)

By Richard NAGIN (2)
The Rockfeller University, New York, N. Y. 10021.

RÉSUMÉ

Des études du mécanisme de la détermination des castes chez les termites ont été faites chez *Neotermes jouteli* (Banks), (Famille Kalotermitidae), de la Floride du sud. On a trouvé que les pseudergates de cette espèce peuvent répondre à l'isolement par une production rapide et importante de sexués néoténiques (SRs). De la même façon, la production de soldats s'observe chez des groupes dont on a enlevé cette caste.

L'intensité avec laquelle les SRs se sont produits (dénommée I_R) varie avec la colonie d'origine des pseudergates. Une I_R basse est trouvée chez des colonies qui avaient des grands pseudergates et qui produisaient des ailés. Une I_R haute est trouvée chez des colonies qui avaient des petits pseudergates et qui ne produisaient pas d'ailés. On conclut que l'I_R tombait avec l'âge de la colonie d'origine.

Chez les colonies d'I_R haute, tous les pseudergates étaient capables de se transformer en SRs. Chez les colonies d'I_R basse, les formes larvaires de la même taille que les pseudergates dans les colonies d'I_R haute étaient complètement incapables de se transformer en SR.

Les sexués mâles et femelles se sont trouvés jouer des rôles différents dans l'inhibition de la transformation en SR chez des groupes isolés. La femelle a bloqué complètement la formation de SRs femelles et, en partie, la formation de SRs mâles. Le mâle a bloqué, en partie, la formation de SRs mâles et de SRs femelles au même point. En plus, une paire de sexués du même sexe était aussi inhibitrice qu'une paire bisexuelle.

Le nombre de SRs produits par un groupe de pseudergates isolés croissait avec le nombre d'heures par jour d'absence des sexués. Les pseudergates femelles répondaient moins à l'absence des sexués que les mâles.

La transformation en SR ne pouvait pas être inhibée si on limitait l'alimentation des pseudergates isolés au papier de filtre imprégné de gouttes fécales obtenues de 25 paires de SRs par jour. Les sexués avec des anus bloqués étaient complètement inhibiteurs pour une semaine après que des témoins isolés commençaient à se transformer. On a donc conclu que l'alimentation proctodéale probablement n'était pas le mécanisme de transmission des phéromones inhibitrices postulées.

---

(1) This work was supported by a Rockefeller University Graduate Fellowship.

(2) Present address : Longview Publishing Co., 205 West 19th St., New York, N. Y. 10011.

SUMMARY

Studies of the mechanism of caste determination in termites were carried out on the southern Florida Kalotermitid, *Neotermes jouteli* (Banks). It was found that pseudergates of this species can respond to isolation by rapid and extensive production of supplementary reproductives (SRs). Similarly, soldier production was found in groups from which this caste was removed.

The intensity with which SRs were produced (I_R) was found to vary with the natural colony from which the pseudergates originated. Low I_R was found in colonies which had large pseudergates and produced alates. High I_R was found in colonies which had small pseudergates and did not produce alates. It was concluded that I_R declined as a function of colony age.

In high I_R colonies all pseudergates were found to be capable of SR transformation. In low I_R colonies larval forms with the same size characteristics as pseudergates of high I_R colonies were found to be totally incapable of SR transformation.

Male and female reproductives were found to play different roles in inhibiting SR transformation in isolated groups. The female reproductive totally blocked female SR formation and partially blocked male SR formation. The male reproductive partially blocked both male and female SR formation to the same extent. In addition, a pair of reproductives of the same sex was found to be as fully inhibitory as a normal bisexual pair.

The extent to which an isolated group of pseudergates underwent SR transformation was found to increase with the amount of time each day that reproductives were absent. Female pseudergates were less sensitive to the absence of reproductives than males.

SR transformation could not be inhibited by restricting isolated pseudergates to a diet of filter paper impregnated with freshly obtained fecal droplets from 25 pairs of supplementaries each day. Reproductives with sealed anuses were fully inhibitory for a week after isolated controls began transformation. It was concluded, therefore, that proctodeal feeding is probably not the mechanism of transmission of the postulated inhibitory pheromones.

ZUSAMMENFASSUNG

Studien des Mechanismus der Kastenbestimmung in Termiten wurden an Kalotermitid *Neotermes jouteli* (Banks) von suedlichen Florida durchgefuert. Es ergab sich, dass Pseudergaten dieser Art auf Isolierung mit schneller und extensiver Produktion von Ersatzgeschlechtstieren (EG) reagieren. In Gruppen von denen diese Kaste entfernt war fand man Soldatenproduktion.

Die Intensitaet mit der EG produziert wurde (I_R) war unterschiedlich in den natuerlichen Kolonien von denen Pseudergaten kamen. Niedrige I_R wurden in Kolonien gefunden die grosse Pseudergaten hatten und Imagines produzierten. Hohe I_R wurden in Kolonien gefunden, die kliene Pseudergaten hatten und keine Imagines produzierten. Daraus kann man schliessen, dass I_R abnahm als Funktion des Alters der Kolonie.

In Kolonien mit hohem I_R, fand man dass alle Pseudergaten faehig zur

EG Transformation waren. In Kolonien mit niedrigem I_R fand man, dass Larvenformen mit denselben Groessencharakteristiken wie Pseudergaten von Kolonien mit hohen I_R voellig unfaehig waren die EG Transformation zu vollbringen.

Maennliche und weibliche Geschlechtstiere spielten unterschiedliche Rollen in der Hemmung der EG Transformation in isolierten Gruppen. Das weibliche Geschlechtstier blockierte die weibliche EG Formation voellig und die maennliche EG Formation teilweise. Das maennliche Geschlechtstier blockierte maennliche und weibliche EG Formation teilweise zu demselben Masse. Ausserdem findet man ein Paar Geschlechtstiere desselben Geschlechts genauso hemmend wie ein normales Paar verschiedenes Geschlechts.

Das Mass, in dem eine isolierte Gruppe von Pseudergaten die EG Transformation durchmachte, erhoete sich mit der taeglichen Zeitspahne in der die Geschlechtstiere abwesend waren. Weibliche Pseudergaten waren weniger empfindlich gegen die Abwesenheit von Geschlechtstieren als maennliche.

EG Transformation en konnte nicht gehemmt werden indem man die isolierten Pseudergaten taeglich auf eine Diat von Filterpapier mit frischen Tropfen von Darmextraken von 25 Paaren von Ersatzgeschlechtstieren beschraenkte. Ersatzgeschlechtstiere mit versiegelten Daermen waren eine Woche voellig gehemmtend, waehrend isolierte Kontrolltiere die Transformation begannen. Daraus ist zu schliessen, dass Darfuetterung wahrescheinlich nicht der Mechanismus ist zur Uebertragung hypotetischer hemmender Pheromons.

INTRODUCTION

Considerable progress towards understanding the mechanism of caste determination in termites has been made through the work of LÜSCHER, GRASSÉ and NOIROT and others on the European Kalotermitid, *Kalotermes flavicollis.*

In an effort to confirm and extend their findings, an investigation was made of a species of the same family, native to the Caribean region, *Neotermes jouteli* (Banks).

The phenomena studied included caste formation in isolated groups of larvae, its variation in different natural colonies, the competence of larval forms to transform to supplementary reproductives, the effects of each reproductive sex in regulating such transformation, and the effect of varying the time reproductives were absent from experimental groups. In addition, an attempt was made to test whether proctodeal feeding is the means by which pheromones, believed to be involved in caste determination, are transmitted.

MATERIALS AND METHODS

Choice of species. — A survey of the readily available southern Florida Kalotermitidae indicated that *Neotermes jouteli* (Banks) was the most promising material for studies of caste determination (NAGIN, 1970). Pseudergates of this species can respond vigorously to isolation by rapid and large-scale production of supplementary reproductives. In addition, the stages of the transformation of an individual termite are well defined. Besides southern Florida, this species is native to the West Indies, the Bahamas and the eastern coast of Mexico (WEESNER, 1965).

Collection and extraction of colonies. — Logs containing whole colonies of *N. jouteli* were collected in woods in Virginia Key and Key Largo, Florida in February, 1967; January, 1968 and January, 1969. The logs were transported to New York and the colonies were extracted within a month in the laboratory.

By carefully breaking open the logs entire natural colonies were obtained with only a few per cent mortality. Immediately upon extraction a census was carried out, recording the numbers of pseudergates, soldiers. white soldiers and reproductives.

Definition of pseudergate. — The term pseudergate (GRASSÉ and NOIROT, 1947), is used in the present work to describe the largest apterous larvae functioning as workers in any given natural colony. With this definition, the pseudergate population may include forms with different numbers of antennal segments, particularly in immature colonies. However, it was found (NAGIN,. 1970) that in such colonies larvae with 15, 16 or 17 antennal segments had essentially identical head width distribution curves and that their potentials to become soldiers or supplementary reproductives were indistinguishable.

Stock colony nests. — Freshly extracted colonies were transferred to stock colony nests consisting of glass battery jars 15 cm in diameter and 20 cm high in which a layer of stiff ($\sim$4 %) Difco Bacto agar 1.5 cm thick containing $\sim$0.5 % Drosophila mold inhibitor had been poured. The agar gel, which the termites ingest and burrow into, provides a long term source of moisture. Strips of wood from the colony's natural nest were placed on top of the agar. These included short strips lying on the agar layer and long strips which stood on the agar leaning against the wall of the jar. The strips were broad and thin so there was enough wood to cover and feed the termites but not enough that they became inaccessible upon burrowing. The strips, to which the termites clung, were then removed whenever experimental organisms were needed.

The battery jars were covered with glass tops and stored in a dark incubator at 26 °C. The nests required minimal maintenance. When the agar began to dry out, fresh strips were added. Fresh wood was added every few months.

Whole colonies could be maintained in such nests indefinitely. Colony growth continued and alates arose each summer in mature colonies.

Experimental nests. — Small, standardized nests in which termites were readily accessible were used for experiments. These consisted on 60 × 20 mm plastic petri dishes into which a thin layer of stiff agar containing mold inhibitor was poured. A few flat pieces of wood from the termites' natural log were placed on the agar gel. The wood could be easily removed and the termites tapped out of the dish. After carrying out whatever experimental manipulation was required on the termites, the wood strips were replaced in the positions from which they were taken and the termites were returned to the nest. They rapidly reorganized themselves and repaired any damage done.

Groups of approximately 50 individuals fitted comfortably in these dishes. All normal activities includinq mating, egglaying, hatching, molting, alate production and trophollaxis have been observed in such nests.

RESULTS

1. Caste formation.

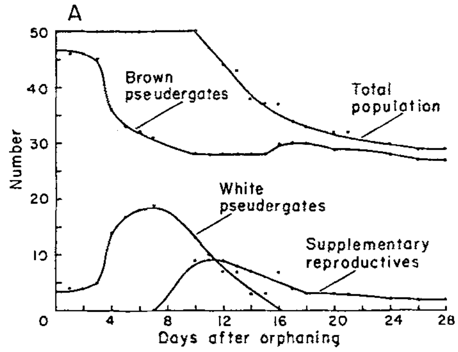
1.1. Production of supplementary reproductives (SRs)

On day zero fifty pseudergates were removed from a nest of a stock colony headed by a single pair of supplementary reproductives. The pseudergates were placed in an experimental nest together with a few (2-5) soldiers from the same stock to supress the tendency towards soldier regeneration which 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.

Prior to a molt, pseudergates, normally brownish, empty their guts and appear totally white (Grassé, 1949). The numbers of white and brown pseudergates were noted in the daily census which also included the number of SRs, white soldiers and soldiers.

The census as a function of time is presented in figure 1 *a* for a single experimental group established in 1967 and in figure 2 for the average of eight experimental groups in 1969. Figure 1 *b* shows the behavior of a control group which contained a pair of SRs from the beginning

It can be seen that the process of SR production began very soon after isolation from the main colony. White pseudergates began to appear in 1-3 days and the white pseudergate population built up at a very rapid rate. Starting on day 4 this rate began to level off but the population of white pseudergates continued to grow until day 8 or 9 when the first white pseudergates molted to become SRs. Thereafter the brown pseudergates stopped becoming white.



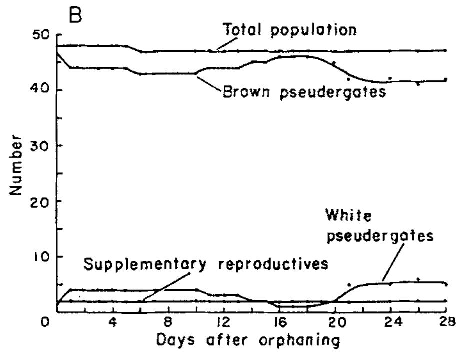


Fig. 1 *a*. — SR production in a single group of isolated pseudergates, 7-8/67. Group maintained at 26 °C in the dark. All pseudergates with 16 antennal segments.

Fig. 1 *b*. — Control group from same stock run concurrently. All pseudergates with 15 antennal segments.

Starting on day 10 or 11 the remaining brown pseudergates began to cannibalize SRs, as reflected in the fall of the total population. In some cases SRs were forced outside of the nest's galleries where they apparently starved to death.

The process continued until all the white pseudergates became SRs and the SR population was reduced to a single male and female. By approximately day 24 the group had been stabilized. Within the next four days, the first eggs appeared. The entire process of restoring a reproductive pair, therefore, cost the group four weeks plus 40 % of its pseudergate population.

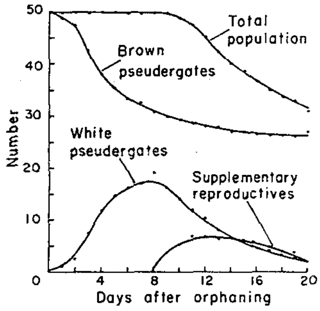
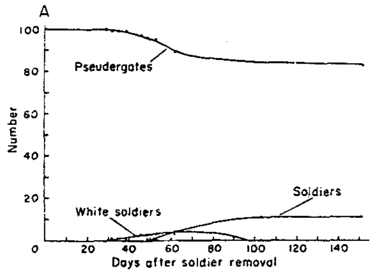


FIG. 2. — SR production in *Neotermes jouteli*. Average of eight groups of pseudergates isolated from Stock 69-2. Groups maintained in dark at 26 °C, 1-4/69.

1.2 SOLDIER FORMATION

On day zero, fifty pseudergates were removed from a stock colony nest and placed in an experimental nest together with a pair reproductives. In the initial stock colony the soldier : pseudergate ratio was approximately 1 : 10. Control groups were also set up which contained 50 pseudergates plus five soldiers.

The results for two experimental groups are shown in figure 3 *a*. It can be seen that it took 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. By fifteen weeks the groups had restored their normal complement of soldiers and were thereafter stable.



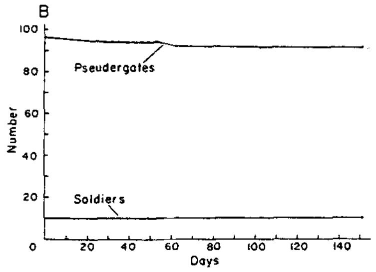


FIG. 3 *a*. — Soldier production. Sum of two groups, 6-11/67.

FIG. 3 *b*. — Sum of two control groups with full complement of soldiers, 6-11/67.

Control groups (fig. 3 *b*) showed no change in soldier number.

Although soldier formation did not involve the massive overshoot seen in the case of SR formation, situations can arise in which excess soldiers are present. Soldier elimination then occurs.

For example, in the experimental group shown in figure 1 *a*, the five original soldiers constituted an excess when the total population fell. Two soldiers were then killed, thus restoring the original caste ratio.

2. Colony variations.

2.1. Colony survey

On January 6 and 7, 1969, 18 natural colonies of *Neotermes jouteli* were collected in the same woods on Virginia Key, Florida. They were brought to New York, January 9 and extractions were carried out beginning January 10 and ending February 12.

Immediately upon extraction 25 pseudergates plus a proper number of soldiers were isolated from each stock colony, transferred to experimental nests and incubated at 26 °C in the dark. SR formation was monitored through daily censuses. In two cases (colonies 69-14 and 69-15) SRs were removed as they arose but in the other groups the normal course of events was allowed to occur.

A simple index I_R, was used as a measure of a group's ability to produce new reproductives :

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

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

The weights and antennal segment numbers of the pseudergates used were determined at the beginning of the experiment. Ten soldiers from each stock colony were also weighed.

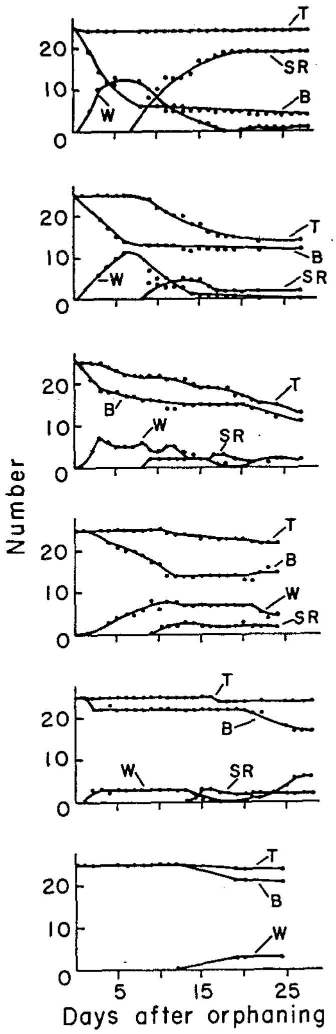
Finally, it was noted whether or not alates arose in the stock colony nests in the following summer.

The measurements carried out are summarized in table I, where the data are pre-

Table I. — 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_R	T-day	Ave. wt. PEs (mg)	Ave. no. antennal segments	Ave. wt. soldiers (mg)	Population (PE)	Ratio PE : (S+WS)	Reproductives	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 13.2	14.2 14.8	13.0 22.0	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 23.1	16.6 16.4	37.9 42.8	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 reproductive arose; PE = pseudergates; SR = supplementary reproductive ; PR = primary reproductive; S = soldiers; WS = white soldiers; I_R = index of regeneration.



Colony 69-	I_R	Ave. wt. PE (mg)	Alates
15	6.5	9.7	-
2	5.5	13.3	-
16	2.7	28.5	+
7	1.6	12.7	-
13	0.86	21.1	+
4	0.0	22.8	+

FIG. 4. — SR production in groups isolated from colonies collected in the same woods 1/6-7/69. T = total population (sum of curves), B = brown pseudergates, W = white pseudergates, SR = supplementary reproductives.

sented in order of decreasing I_R. It can be seen that I_R varied continuously from 0 (colony 69-4) to 6.5 (colony 69-15). Graphs of sample groups are presented in figure 4.

In the cases of colonies 69-14 and 69-15, from whose test groups 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 SRs appeared. 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.

When the data are presented as in table I it is seen that the colonies fell into two groups : those in which the pseudergates and soldiers were large and those in which they were small. The large individuals weighed almost twice as much as the small ones and usually had more antennal segments.

The colonies with large individuals tended to have low I_R's and to produce alates. The colonies with small individuals tended to have high I_R's and did not produce alates. By definition, colonies which do not produce alates are immature. In addition, colonies with small individuals began to produce SRs sooner than colonies with large individuals.

There are a few exceptions to these generalizations. 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 69-11 with very small individuals began SR production rather late (day 11) and produced few SRs ($I_R = 1.5$). However, the test group used in this case was pathological. Five pseudergates died before any SRs arose; by day 20, ten pseudergates had died; by day 35, only six pseudergates remained and all the SRs had died.

Table I also shows the average weights and numbers of antennal segments for colonies with $I_R \geqslant 1.5$ 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, the results were essentially the same and are presented in table II.

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 is known about nest development in the Kalotermitidae.

The populations of the natural colonies, as determined by censuses immediately upon extraction, did not seem to be correlated with I_R. The same negative result obtained for the pseudergate to soldier plus white soldier ratio and for the type of reproductives which were present in the natural colony.

TABLE II. — DATA AVERAGED ON THE BASIS OF PRESENCE OR ABSENCE OF ALATES

	AVE. WT. (mg) PSEUDERGATES	AVE. NO. ANTENNAL SEGMENTS	AVE. WT. (mg) SOLDIERS
Colonies not producing alates ...	11.5	14.6	19.7
Colonies producing alates	22.6	16.3	40.5

2.2. NON-PSEUDERGATE LARVAE OF LOW I_R COLONIES

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 appropriate numbers of soldiers from two colonies, which 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 an SR (T-day) was noted as was the total number of SRs produced by each group.

The results, presented in table III, 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 SRs.

TABLE III. — SR PRODUCTION 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

3. Pseudergate competence.

It has been shown that if groups of pseudergates are isolated from immature colonies, a large percentage transform to SRs. This experiment was set up to find out if all such pseudergates can transform.

Two groups of termites, each containing 100 brown pseudergates and 6 soldiers were taken from the same stock colony, 68-5 (1) and transferred to experimental nests on day zero. The groups were maintained at 26 °C in the dark. A census was taken each day. Whenever SRs arose they were removed.

The results for the average of the two groups are shown in figure 5. The curves for white and brown pseudergates represent daily censuses. The SR 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 which survived (i.e., 66 %) had transformed to SRs.

Many of the features of normal SR formation seen in experiment 1.1 are repeated here. By day two the bloom of white pseudergates had begun in full force, by day four it had begun to level off and by day seven it had reached its peak. By seven days later essentially all the white pseudergates in this initial bloom had transformed to SRs.

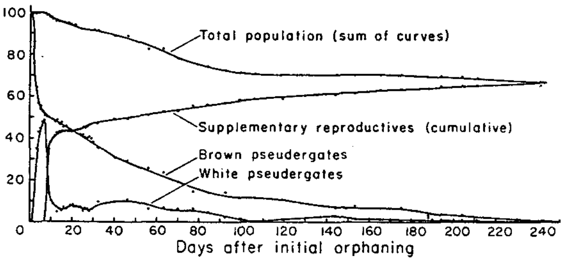


FIG. 5. — Effect of continual removal of SRs from groups of isolated pseudergates. Average of two groups, 5/68 - 1/69.

There were about five more white pseudergates in the day seven peak than cumulative SRs on day fourteen. It is possible that these white pseudergates did actually molt into SRs but were injured and then cannibalized by larvae attempting to groom them.

What is most interesting is that after the initial bloom of SRs the cumulative SR curve entered a new phase in which new SRs appeared at a much slower rate until the groups had been totally depleted of pseudergates.

(1) Characteristics of stock colonies referred to in the rest of this paper are listed in table IV.

4. Sex specificity.

4.1. Effect of single reproductives

This experiment was set up to determine the separate effects of the male and female reproductives in inhibiting isolated pseudergates from transforming to SRs.

480 pseudergates and 16 soldiers were removed from stock colony 69-14 and 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 and was then maintained at 26 °C in the dark 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 subgroups, one of which kept the male reproductive, one the female and one was totally orphaned.

The composition on day 0 for each subgroup is presented in table V. Thus, the results from the subgroups in each group can be compared as can the results from the subgroups of the same experimental type since subgroups from different groups came initially from the same stock. Censuses were made each day in each colony and all new SRs were removed and sexed. The termites were maintained in the dark at 26 °C.

The results at a number of time points are given in table VI. The sum of the daily results for all three groups are presented graphically in figure 6. The results can be summarized as follows : in the presence of a single male reproductive equal numbers of males and females arose. In the presence of a single female reproductive as many males arose as with a single male reproductive but essentially no females arose. In the absence of both reproductives equal numbers of males and females arose but the numbers were approximately twice as high as with a single male.

4.2. Effect of two reproductives of the same sex

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

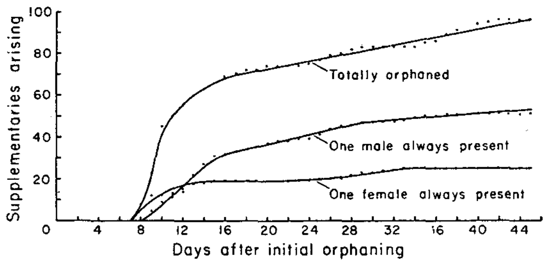


Fig. 6. — Cumulative SR production in continually orphaned and half-orphaned pseudergates. Each curve represents sum of three experiments, 4-6/69.

300 pseudergates and 30 soldiers were removed from stock colony 69-21 and 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

TABLE IV. — STOCK

STOCK COLONY	68-3	68-5	68-8
Location collected	Virginia Key, Florida.	Virginia Key, Florida.	Virginia Key, Florida.
Date collected	1/30/68	1/30/68	1/30/68
Date extracted	2/68	2/68	2/68
Population upon extraction :			
pseudergates			
soldiers			
white soldiers			
reproductives		Supplementary pair.	
small larvae			
eggs			
Date pseudergates and soldiers removed for experiment	7/16/68	5/2/68	8/3/68
Average weight pseudergates used in experiment	11.2 mg	9.5 mg	9.9 mg
Aevrage weight soldiers used in experiment	16.6 mg	12.4 mg	10.4 mg
I_R	1.3	6.1	1.0

getting one of the two reproductives. The other large group was similarily 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 experimental subgroups of about the same size with different numbers of reproductives. The initial (day 0) censuses of these subgroups are presented in table VII.

Censuses were carried out each day in each subgroup and all new SRs were removed as they arose and were sexed. Also, if any of the initial reproductives was killed, a replacement was added to the subgroup. Thus, it was necessary to add a male repro-

TABLE V. — DAY 0 CENSUSES FOR SUBGROUPS IN SEX SPECIFICITY EXPERIMENT 4.1

REPRODUCTIVE PRESENT (subgroup)	FEMALE			MALE			NONE		
Group	*Pseudergates*		*Soldiers*	*Pseudergates*		*Soldiers*	*Pseudergates*		*Soldiers*
	white	brown		white	brown		white	brown	
I	8	40	5	8	40	5	8	40	5
II	11	39	6	11	39	6	11	39	6
III	5	32	4	5	32	4	5	32	4
Total	24	111	15	24	111	15	24	111	15

COLONY CHARACTERISTICS

69-1	69-7	69-14	69-20	69-21
Virginia Key, Fla.	Virginia Key, Fla.	Virginia Key, Fla.	Virginia Key, Fla.	Virginia Key, Fla.
1/6/69	1/6/69	1/7/69	7/30/69	7/30/69
2/5/69	2/6/69	1/12, 13/69	8/5, 6/69	8/7/69
1 780	671	1 043	421	313
99	80	58	48	107
7	2	12	6	12
Primary female, supplementary male.	Primary pair.	supplementary pair.	supplementary pair.	Primary pair.
+	+	+	+	+
+	+	+	+	+
3/13/69	4/2/69	3/2/69	9/2/69	9/3/69
13.5 mg	12.7 mg	12.8 mg	11.3 mg	8.6 mg
29.3 mg	30.4 mg	16.6 mg	14.5 mg	11.8 mg
3.8	1.6	5.5	2.3	2.2

ductive to subgroup « M » on day 4 and another to subgroup « 2 M » on day 8. The other reproductives survived the course of the experiment.

The cumulative total of reproductives which had arisen in each subgroup as of day 31 is presented in figure 7. The results for the first three subgroups are similar to what was found in the previous experiment. More SRs arose in totally orphaned subgroups than in half-orphaned subgroups. Only males arose in the presence of a female whereas both sexes arose in the presence of a male. In this experiment the sex ratio of the SRs wich arose in the « M » and the « 0 » subgroups was not even as previously found. This could reflect an uneven pseudergate sex ratio.

The important new result is that SR formation is strongly suppressed in the presence of either two males or two females. Only a single male SR arose in each of the « 2 F » and « 2 M » subgroups.

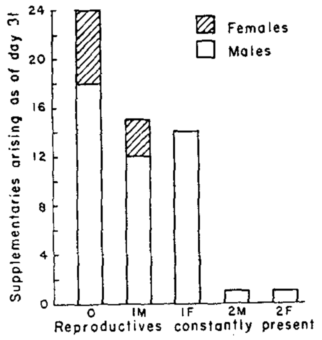


FIG. 7. — Effect of two reproductives of the same sex on SR production.

5. Effect of absence of reproductives.

The following experiments were carried out to determine how isolated groups respond if the reproductive pair is absent a certain number of hours per day.

Table VI. — SRs of each sex arising in continually half orphaned and totally orphaned groups
(cumulative production as of various dates).

Day 10.

Reproductive present	Female		Male		None	
Group	males	females	males	females	males	females
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

Day 20.

Reproductive present	Female		Male		None	
Group	males	females	males	females	males	females
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

Day 30.

Reproductive present	Female		Male		None	
Group	males	females	males	females	males	females
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

Day 70.

Reproductive present	Female		Male		None	
Group	males	females	males	females	males	females
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

TABLE VII. — DAY 0 CENSUSES OF SUBGROUPS IN EXPERIMENT 4.2

SUBGROUP	PSEUDERGATES		SOLDIERS	WHITE SOLDIERS	SUPPLEMENTARY REPRODUCTIVES
	white	brown			
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

Groups of 120-130 pseudergates together with some soldiers were removed from two stock colonies (69-1 and 69-20). The groups were each provided with a pair of foster supplementary reproductives and were maintained 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 equal subgroups. For each group one subgroup kept its reproductive pair for a fixed period each day and the other subgroup kept the reproductives for the rest of the day.

The day the groups were divided and the

TABLE VIII. — EFFECT ON SR PRODUCTION OF VARYING THE TIME PER DAY REPRODUCTIVES ARE ABSENT FROM ISOLATED GROUPS

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 TRANSFORMED
					F	M	Unknown	
24	Always absent.	69-1	50	11	7	4	0	22.0
		69-20	71	17	8	9	0	23.9
		Total.	121	28	15	13	0	23.1
20	2 PM - 10 AM	69-1	55	17	4	13	0	30.9
		69-1	52	13	8	4	1	25.0
		69-20	53	5	1	4	0	9.4
		Total.	160	35	13	21	1	21.9
16	6 PM - 10 AM	69-1	49	13	3	9	1	26.6
		69-20	50	3	1	2	0	6.0
		Total.	99	16	4	11	1	16.2
8	10 AM - 6 PM	69-1	49	8	0	7	1	16.3
		69-20	51	2	0	2	0	3.9
		Total.	100	10	0	9	1	10.0
4	10 AM - 2 PM	69-1	56	3	0	3	0	5.4
		69-1	52	6	0	6	0	11.5
		69-20	52	2	0	2	0	3.8
		Total.	160	11	0	11	0	6.9
0	Never absent.	69-1	49	1	0	1	0	2.0

reproductive transfers begun was considered day 0. A census was taken each day thereafter and all new SRs were removed as they arose and were sexed. The termites were otherwise maintained in a dark incubator at 26 °C.

The number and sex of SRs which arose as of day 26 for the time points studied are presented in table VIII. In addition, the overall per cent of pseudergates which transformed is plotted in figure 8 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 the subgroups produced SRs varied directly with the amount of time per day a functional reproductive pair was absent.

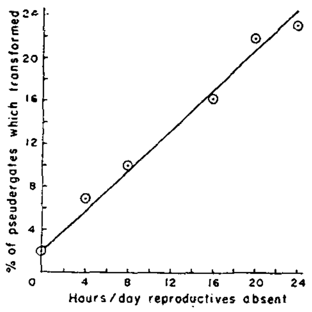


Fig. 8. — SRs arising as a function of the number of hours per day reproductives were absent from groups of pseudergates. SRs expressed as a per cent of pseudergates present on day 0. Sum of two experiments. Results as of day 26.

While this variation was more or less continuous for the production of male SRs, female pseudergates could be orphaned for at least eight hours a day without being stimulated to transform. Even when reproductives were absent twenty hours each day many fewer females transformed than males. The sex ratios of the SRs whose sex could be determined are shown for each time point in figure 9.

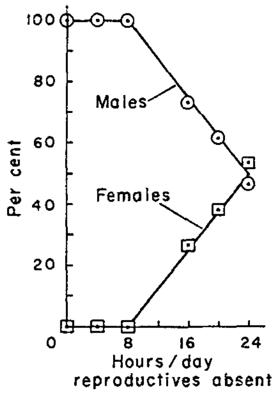


Fig. 9. — Sex ratio of SRs arising in groups from which reproductives were absent for varying numbers of hours per day.

6. The source of the pheromone(s).

Lüscher (1955) has hypothesized that the inhibition of pseudergates from transforming to SRs in the presence of a reproductive pair results from pheromones produced in the heads of the reproductives and passed to the pseudergates by proctodeal feeding. The following experiments were carried out in an effort to obtain evidence for this hypothesis.

6.1. Feces experiment

In this experiment isolated groups of pseudergates were fed massive doses of feces from reproductives.

Six experimental groups were established each containing 50 pseudergates, 5 soldiers and a pair of supplementary reproductives, all originating from the same stock colony, 68-3. A replicate experiment using termites from stock 68-8 was also set up. The groups 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 termites were removed from their dishes and a census taken. Each empty dish was then treated as follows : to two of the dishes 25 pairs of supplementaries were added and anaesthetized with 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 maintained without reproductives so that they 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 groups of termites. This process was repeated daily. New filter paper was added as needed.

In addition, starting from the day groups were isolated from their stocks, 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. That is, it was feared that some pseudergates might otherwise have a low level of pheromone at the time of orphaning and might not eat impregnated filter paper soon enough to prevent their being determined to transform. This « presaturation » went on for six days for the 68-3 groups and 30 days for the 68-8 groups.

SRs were recorded when they arose but not removed. The total number which arose in each of the 12 colonies as of day 19 is presented in table IX. 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 were not significant.

TABLE IX. — SRs ARISING IN GROUPS RESTRICTED TO DIETS OF FILTER PAPER IMPREGNATED WITH FECAL DROPLETS

DIET	68-3 GROUPS	68-8 GROUPS	TOTAL
Reproductive feces	7	11	18
Pseudergate feces	14	13	27
Control (filter paper alone)	12	9	21

6.2. SEALED ANUS EXPERIMENT

This experiment was set up to see whether reproductives with sealed anuses fail to inhibit as would be expected with Lüscher's hypothesis.

Six experimental groups were established containing 60 pseudergates, 6 soldiers and a pair of supplementary reproductives, all originating from the same stock colony (69-7). The groups were incubated for 19 days at 26 °C in the dark.

At the end of this time the reproductives from two of the groups were removed, placed in a dish under a dissecting microscope, and anaesthetized with CO_2. 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.

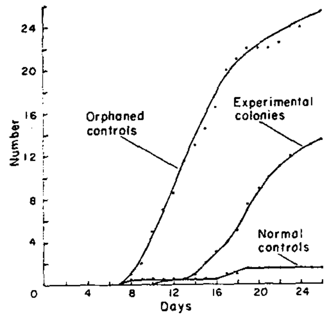
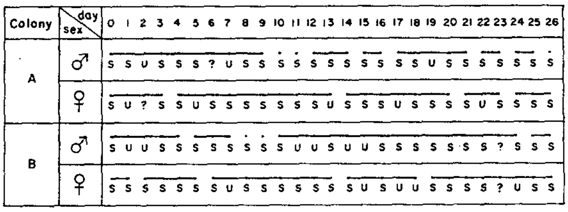


FIG. 10. — SR production by pseudergates in the presence of reproductives with lucite-sealed anuses. Each curve is the average of two groups.

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, was as easy to apply and provided as good a seal as lucite.

The reproductives in two of the remaining four groups were removed permanently, but kept in the other two. Each day after the initial sealing (day 0) the sealed reproductives were removed and anaesthetized with CO_2. 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 groups and new SRs were removed as they arose. Sealed reproductives often did not survive and so replacements were added.

The cumulative production of SRs in the three types of groups is presented graphically in figure 10. The survival and daily state of the seal of the sealed reproductives is presented in figure 11. It can be seen that SRs did not arise in the experimental groups until considerably later than in orphaned groups and then at a lower rate. Thus, at least initially, reproductives with sealed anuses are strongly, if not completely, inhibitory.



Colony	sex \ day	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	U	S	S	S	S	S	S	S
	♀	S	U	?	S	S	U	S	S	S	S	S	S	S	U	S	S	S	U	S	S	S	S	U	S	S	S	S
B	♂	S	U	U	S	S	S	S	S	S	S	S	U	U	S	U	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	U	S	U	U	S	S	S	S	?	U	S	S

FIG. 11. — State of sealed reproductives in sealed anus experiment. Straight line indicates survival, S = sealed, U = unsealed.

DISCUSSION

1. SR formation in *Neotermes jouteli* is very similar to that in *Kalotermes flavicollis*. In the latter species LÜSCHER's data (1952 *a*) indicate a slightly shorter white premolt period and possibly also a shorter time before the first pseudergates are activated. Egg-laying, on the other hand, does not occur until two — four weeks in *K. flavicollis* (GRASSÉ and NOIROT, 1946 *a*).

The data presented here support LÜSCHER's conclusion (1952 *a*) that the SR molt is specially induced by removal of reproductives. Thus the number of white pseudergates in control groups fluctuates around a constant level of 6 % (fig. 1 *b*). The fact that a large bloom of white pseudergates occurs in experimental groups shows that SRs are not simply arising from pseudergates which are at the end of the normal intermolt cycle.

One major difference between *N. jouteli* and *K. flavicollis* appears to lie in the intensity with which *N. jouteli* pseudergates are capable of producing SRs. Judging from the data presented by GRASSÉ and NOIROT (1960), approximately 5 % of the larvae transform to SRs in groups of *K. flavicollis* isolated for ten days. In some colonies of *N. jouteli* in this study about 15 % of isolated pseudergates transformed in ten days and 40 % in twenty days. Lower levels of production were found to be correlated with alate production. It is possible that the studies of *K. flavicollis* dealt with mature colonies.

Much less attention has been paid to the problem of the production of soldiers than to that of reproductives. The present findings are fully consistent with the reports of CASTLE (1934), MILLER (1942) and LÜSCHER (1961) regarding both production and elimination. Soldier formation is slower and much less vigorous than for SRs. By the same token, it is less wasteful of pseudergates since overshoot is lacking.

2. The fact that colonies vary in their ability to produce SRs was first noticed in *Zootermopsis nevadensis* by LIGHT (1942-1943) who proposed three possible explanations. He proposed that the variations might result 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 progressive, regressive or cyclic. LIGHT doubted that the differences had a genetic basis but offered no evidence related to any of the three possibilities.

The present finding that low I_R is associated with the ability to produce alates suggests that the colony variations can at least partly be accounted for as a regressive shift in I_R with increased colony age. The fact that high I_R colonies often had also rather undeveloped gallery systems in their natural logs further suggests that these colonies were new.

Some observations of LIGHT and ILLG (1945) also support this idea. These authors carried out censuses of natural colonies of *Z. nevadensis* with different abilities to produce SRs in an effort to see whether the differences could be related to differences in colony composition. Although they concluded that such correlations were lacking, it seems to the present author that a correlation existed between low productivity and the presence of large numbers on nymphs, the precursors of alates.

The idea that the differences in pseudergate size and SR productivity might be due to differences in colony age was suggested to the author by Alfred EMERSON to whom specimens from low and high I_R colonies were sent for the purpose of excluding the possibility that the differences had a taxonomic basis.

Such changes in I_R and pseudergate size with colony age would make biological sense. 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 which are devoted primarily to transforming larvae into alates. Low I_R in fully developed colonies, which presumably have begun to exhaust the food supply in their log, tends to preserve pseudergates for alate production.

Similar reasoning applies in the case of pseudergate size. 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 to that they will be successful sexuals.

N. jouteli larvae in immature colonies have been observed to molt without any significant change in size after about the fourth instar (Nagin, 1970). Molting without growth has also been observed in other species (Miller, 1942; Lüscher, 1952 *b*). Thus, it appears that the pseudergates « mark time » while the pseudergate population builds up.

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.*

The loss of the ability to produce SRs together with decline of egg-production with colony age would explain why it is that termite colonies die. If vigorous SR production were a constant possibility, then termite colonies should be immortal. In fact, Kalshoven (1930) found that the longevity of *N. tectonae* colonies was never more than 15 years and that a natural death might occur in as few as six years.

The existence of « physiological » changes in a colony at different stages of its life history is also seen in experiment 2.2 which showed the loss of differentiation abilities of early larval instars in mature colonies. Similar findings have been reported for other species (Kalshoven, 1930; Light, and Illg, 1945; Grassé, 1949) and castes (Grassé and Noirot, 1946 *b*).

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 fact that in a given species of termite the morphological and developmental characteristics of pseudergates are colony-specific leads to some conclusions regarding methodology. 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 for the experiment. In addition, the characteristics listed in table I should be given.

3. Two conclusions can be drawn from the experiment in which SRs were removed as they arose. First, the two-phase nature of the cumulative reproductive curve shows that at any given time only a certain proportion of pseudergates is transformable. Lüscher (1952 *a*, 1953, 1956) has found this to be true also in *K. flavicollis.*

Second, all pseudergates in colonies with high I_R are genetically capable of SR development. The only escape from this conclusion would be if one could show that all pseudergates which 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.

4. From the results of the sex specificity experiments it can be readily seen why a pair of reproductives should totally inhibit the production of SRs. The male and female reproductives in isolation each partially inhibit pseudergate transformation. The separate partial effects seen in isolation, would, if combined in the same pseudergate population, produce total inhibition.

The male reproductive indiscriminately half-inhibits both male and female pseudergates. The female reproductive also half-inhibits male pseudergates but additionally inhibits female pseudergates totally. Thus, female pseudergates experience surplus inhibition.

If it is asumed 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 were 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.

For example there could be a female-produced substance and a different male-produced substance and the female-produced substance would affect 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.

It is interesting to compare these results with what has been found in other species. Two points of universal agreement (LIGHT and WEESNER, 1951, in *Zootermopsis angusticollis;* GRASSÉ and NOIROT, 1960 and LÜSCHER, 1964, in *K. flavicollis*) 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.

The strong suppression of SR production by two reproductives of the same sex is in accord with the finding that a single reproductive of either sex partially suppressed SR production. 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.

The fact that two male reproductives strongly inhibited SR production in this experiment disagrees with LÜSCHER's result (1964) that in *K. flavicollis* two males had no effect on the number of SRs which arose but only shifted the sex ratio in favor of more females and fewer males. On the other hand, GRASSÉ and NOIROT's finding (1960) that, in colonies of *K. 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.

5. The fact that the extent of SR production varies directly with the time reproductives are absent 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 transformation.

GRASSÉ's « group effect » theory (1949) would seem to imply such a threshold effect. This theory holds that 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 groups 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, 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 absences than male. As shown by the sex-specificity experiments, 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. This would not be expected on the basis of the « group effect » theory.

6. The present findings, although not conclusive, cast doubt on the idea proposed by Lüscher (1955) that inhibitory pheromones are obtained from reproductives via proctodeal feeding. Orphaned pseudergates were not significantly inhibited from transforming to SRs when fed large amounts of rectal fluid from reproductives. It was also found that reproductives with sealed anuses were inhibitory for a week after SRs arose in orphaned controls.

Supplementaries which eventually arose in the sealed anus experiment may have resulted from the release in inhibition which 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 *K. flavicollis*. We have seen in experiment 5 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.

Proctodeal feeding may therefore have primarily a nutritive function and be 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).

In *N. jouteli* 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.

ACKNOWLEDGEMENTS

The work presented in this paper was part of a Ph.D. thesis submitted to the faculty of The Rockefeller University. I am very grateful to my adviser, Prof. William Trager, for his encouragement, criticisms and support. I am also grateful to Prof. Alfred Emerson for his advice. Finally, I would like to thank Dr. Margaret Collins of the Department of Zoology, Howard University, for instructions in collection, extraction and culturing techniques for the Florida Kalotermitidae.

BIBLIOGRAPHY

Castle (G.), 1934. — The damp-wood termites of western United States, genus *Zootermopsis* (formerly *Termopsis*). In *Termites and Termite Control* (C.A. Kofold, ed.), 2nd. ed., pp. 273-310. Univ. of California Press, Berkeley, California.

Grassé (P. P.), 1949. — Ordre des Isoptères ou Termites. In *Traité de Zoologie* (P. P. Grassé, ed.), vol. **IX**, 408-544. Masson, Paris.

Grassé (P. P.) and Noirot (Ch.), 1946 *a*. — La production des sexués néoténiques chez le termite à cou jaune (*Calotermes flavicollis* F.) : inhibition germinale et inhibition somatique. *Compt. Rend. Acad. Sci.*, **223**, 869-871. — 1946 *b*. Le polymorphisme social du termite à cou jaune. (*Calotermes flavicollis* F.). La production des soldats. *Compt. Rend. Acad. Sci.*, **223**, 929-931. — 1947. Le polymorphisme social du termite à cou jaune (*Calotermes flavicollis* F.). Les faux ouvriers ou *pseudergates* et les mues regressives. *Compt. Rend. Acad. Sci.*, **224**, 219-221. — 1960. Rôle respectif des mâles et des femelles dans la formation des sexués néoténiques chez *Calotermes flavicollis. Insectes Sociaux*, **7**, 109-123.

Grassi (B.) and Sandias (A.), 1893-1894. — Costituzione e sviluppo della societá dei Termitidi. Atti Accad. Gioenia, Catania 6, 7, 1. English translation in Quart. *J. Micros. Sci.*, **39**, 245-322, 1897; **40**, 1-75, 1898.

Harris (W. V.) and Sands (W. A.), 1965. — The social organization of termite colonies. In *Social Organization of Animal Communities* (P. E. Ellis, ed.). *Symp. Zool. Soc. Lond.*, **14**, 113-131.

Heath (H.), 1931. — Experiments in termite caste development. *Science*, **73**, 431.

Kalshoven (L. G. E.), 1930. — De biologie van de Djatitermit (*Kalotermes tectonae* Damm) in verband met zijn bestrijding (English summary). *Mededeelingen van Het Institut voor Plantenzeikten*, no. 76, 1-153 Wageningen.

Light (S. F.), 1942-1943. — The determination of the castes of social insects. *Quart. Rev. Biol.*, **17**, 312-326; **18**, 46-63.

Light (S. F.) and Illg (P. L.), 1945. — Rate and extent of development of neotenic reproductives in groups of nymphs of the termite genus *Zootermopsis. Univ. Calif. Publ. Zool.*, **53**, 1-40.

Light (S. F.) and Weesner (F. M.), 1951. — Further studies on the production of supplementary reproductives in *Zootermopsis* (Isoptera). *J. Exptl. Zool.*, **117**, 397-414.

Lüscher (M.), 1952 *a*. — Die Produktion und Elimination von Ersatzgeschlechstieren bei der Termite *Kalotermes flavicollis* Fabr. *Zeitsch. vergl. Physiol.*, **34**, 123-141. — 1952 *b*. Untersuchungen über das individuelle Wachstum bei der Termite *Kalotermes flavicollis* Fabr. (Ein Beitrag zum Kastenbildungsproblem). *Biol. Zbl.*, **71**, 529-543. — 1953. Kann die Determination durch eine monomolekulare Reaktion ausgelöst werden ? *Rev. suisse Zool.*, **60**, 524-528. — 1955. Zur Frage der Uebertragung sozialer Wirkstoffe bei Termiten. *Naturwissenchaften*, **42**, 186. — 1956. Die entstehung von Ersatzgeschlechtstieren bie der Termite *Kalotermes flavicollis* Fabr. *Insects Sociaux*, **3**, 119-128. — 1961. Social control of polymorphism in termites. In *Insect Polymorphism* (J. S. Kennedy, ed.), pp. 57-67. *Royal Entomol. Soc., London*. — 1964. Die spezifische Wirkung mannlicher und weiblicher Ersatzgeschlechtstiere auf die Entstehung von Ersatzgeschlechstieren bei der Termite *Kalotermes flavicollis* (Fabr.). *Insectes Sociaux*, **11**, 79-90.

Miller (E. M.), 1942. — The problem of castes and caste differentiation in *Prorhinotermes simplex* (Hagen). *Bull. Univ. Miami*, **15**, 1-27.

Nagin (R. D.), 1970. — *Caste regulation in the termite, Neotermes jouteli (Banks)*. Doctoral Dissertation, The Rockefeller University, New York.

Weesner (F. M.), 1965. — *Termites of the United States. A Handbook*. National Pest Control Association. Elizabeth, New Jersey.