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THE PRODUCTION AND ELIMINATION OF SUPPLEMENTARY REPRODUCTIVES IN *POROTERMES ADAMSONI* (FROGGATT) (ISOPTERA, HODOTERMITIDAE)

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SUMMARY

The factors that influenced the maturation and survival of supplementary reproductives in *Porotermes adamsoni* (Froggatt) were studied. It was found that the time taken for the first supplementary reproductive to develop decreased with increasing temperature. Supplementary reproductives also developed quicker in larger colonies.

The weight of larvae was not found to be a good index of the likelihood of a culture forming supplementary reproductives. The presence of functional supplementary prevented the formation of further supplementary reproductives but when there were no functional reproductives a number of larvae and nymphs developed into supplementary reproductives. All larvae had the potential to develop into supplementary reproductives.

The inhibitory effect of functional females on the sexual maturation of other females appeared to be stronger than the corresponding effect of functional males on other males. It was found that inhibitory activity of functional reproductives was ephemeral. The inhibition appeared to be entirely lost in about two to four days, after the reproductives were taken out of the colonies.

The time of formation of a supplementary reproductive did appear to influence its chances of elimination; the first ones that developed had higher survival rate than those that developed later. Disturbance of cultures did not seem to influence either the development or elimination of reproductives in cultures.

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RÉSUMÉ

La production et l'élimination des reproducteurs supplémentaires chez *Porotermes adamsoni* (Froggatt) (Isoptera, Hodotermitidae)

L'apparition des premiers reproducteurs supplémentaires est plus rapide quand la température augmente; le développement de ces reproducteurs est également plus rapide dans les grandes colonies.

Il n'y a pas de corrélation nette entre le poids des larves et l'apparition des sexués supplémentaires dans les élevages. Toutes les larves ont la potentialité de se sexualiser.

Lorsque, dans un élevage, il n'y a pas de sexués fonctionnels, un grand nombre de larves et de nymphs se transforment en sexués supplémentaires, mais les sexués supplémentaires fonctionnels inhibent la formation de nouveaux sexués. Toutefois, cette inhibition n'est pas totale. Le pouvoir inhibiteur des femelles sur la maturation d'autres femelles est plus élevé que le pouvoir inhibiteur des mâles vis-à-vis des mâles. Quand on supprime les sexués fonctionnels, l'inhibition disparaît au bout de deux à quatre jours. Le moment d'apparition des sexués supplémentaires paraît influer sur leur chance de survie : les premiers formés ont un taux de survie maximum. Les dérangements apportés aux élevages ne paraissent influencer ni le développement ni l'élimination des sexués.

INTRODUCTION

While there is no information on the differentiation of castes in species of *Porotermes* a great deal of research has been reported on such differentiation on the European Kalotermitid, *Kalotermes flavicollis* by LÜSCHER (1952 *a, b, c;* 1962, 1964 and 1974); GRASSÉ and NOIROT (1946, 1960); RÜPPLI (1969) and others and on *Zootermopsis* by CASTLE (1934), LIGHT (1942-1943); MILLER (1969) and others.

The aims of this study were to investigate the factors associated with supplementary reproductive differentiation in *Porotermes adamsoni.* The factors considered included the effect of some environmental factors.

MATERIALS AND METHODS

The insects used in the following studies were all taken from the Second Valley Forest Reserve in South Australia as elaborated in the previous paper (MENSA-BONSU, 1976).

Most cultures were kept in 9 cm petri dishes. The bottom of the dish was covered with filter paper discs. A block of wood from the appropriate infested log and measuring 8 cm × 4 cm × 4 mm was placed on the filter paper and drops of water added to the top of the wood. The supplementary reproductives were marked with nail polish on the head and thorax as they differentiated. Different colours of nail polish were used and the cultures were examined daily. This made it possible for individual supplementary reproductives to be observed as required.

These cultures were held in glass aquaria and maintained at 25 °C ± 1° unless otherwise stated.

RESULTS

1. The effects of temperature on caste formation.

7 groups of 100 larvae and nymphs were cultured in 83 cc. JUCCI-GRASSÉ tubes at each of the following temperatures : 15°, 20°, 25° and 28 °C. The date of appearance of each supplementary reproductive was recorded from the start of the experiment.

Results indicated a strong influence of temperature on the time taken for supplementary reproductives to differentiate (table I).

TABLE I. — The effect of temperature on the time in days taken to formation of first supplementary reproductives.

TABLEAU I. — Rôle de la température dans la formation des premiers reproducteurs supplémentaires (temps exprimé en jours).

Temperature °C	Mean days (7 replicates)	Range
15	33.6	14-39
20	24.1	22-27
25	19.9	17-24
28	15.9	13-19

A regression equation was calculated for the appropriate values and a regression line fitted by the method of Least Squares (fig. 1). The value for the regression was highly significant ($P. < .001$).

The results of these experiments showed that development of supplementary reproductives occurred at all temperatures tested but that the time taken for the first supplementary reproductive to develop tended to decrease with increasing temperature.

Within 24 hours of a termite moulting into a supplementary reproductive its cuticle has hardened and has turned yellowish-brown. As the reproductive aged its cuticle darkened further and many sclerites became dark brown. This was the colour of most mature supplementary reproductives collected in the field. Supplementary reproductives which developed from early alates or alate intercastes were frequently encountered in cultures.

Differences in structure of the different sexes of supplementary reproductives were also noted. Usually the female has the styles reduced in size or absent although they were present in female larvae and nymphs. The seventh sternite of female reproductives is larger than in larvae and covers the U-shaped eighth sternite and most of the ninth sternite. The eighth sternite is entire in the male reproductives and the seventh is not enlarged. Males also retain the styles.

No soldiers were added to the original cultures but at the end of the expe-

rimental period which was 75 days, one culture each at 20 °C and 25 °C and two cultures at 28 °C had produced soldiers. None of the cultures at 15 °C produced soldiers.

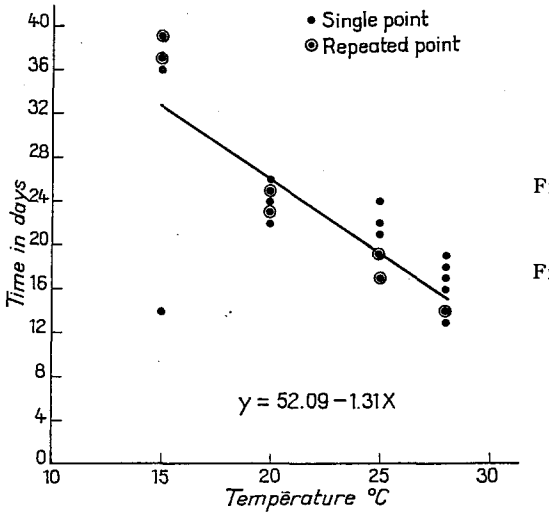

FIG. 1. — The effect of temperature on the development of supplementary reproductives.

FIG. 1. — Rôle de la température dans le développement de reproducteurs supplémentaires.

2. Effect of initial-size on the subsequent development of the colony.

Cultures with 6 replicates each of 20, 50, 100 and 200, large larvae and nymphs were set up in 25, 65 cc. and (for the large numbers) 85 cc. JUCCI-GRASSÉ tubes respectively. The cultures were maintained at 25 °C ± 1° and examined daily and the appearance of the first supplementary reproductives recorded as before. The number of supplementary reproductives surviving as well as the number of brood in each culture were recorded at the end of 4.5 months.

The results were analysed by regression (Table II).

TABLE II. — The effect of number of individuals per culture on the time taken for first supplementary reproductives to develop.

TABLEAU II. — Rôle du nombre des individus en élevage sur la durée de formation des premiers reproducteurs supplémentaires.

Number of termites per culture	Mean time for first supplementary reproductives to form (in days)	Range for first reproductive to form (in days)
20	15.5	13-21
50	14.2	12-19
100	13.5	11-16
200	10.3	6-13

The regression analysis (fig. 2) of the scatter diagram of the time supplementary reproductives took to develop in each culture-group provided the line of best fit and the difference in time of development proved significant. The F value was highly significant ($P. < .01$). It can be concluded therefore that the larger the culture the quicker the development of supplementary reproductives.

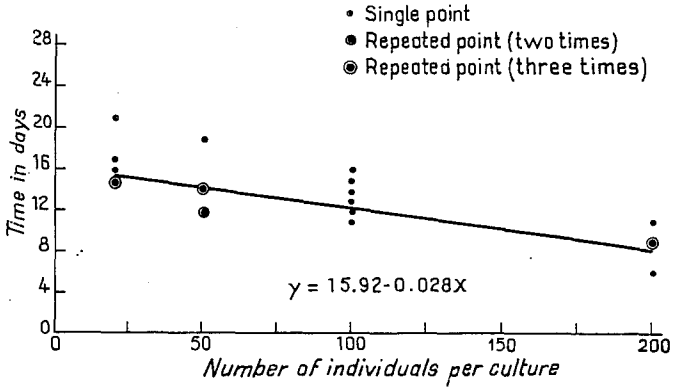

FIG. 2. — Effect of the initial number of individuals per culture on the time taken for first supplementary reproductives to form.

FIG. 2. — Rôle du nombre des individus en élevage sur la durée de formation des premiers reproducteurs supplémentaires.

No soldiers were added to the original cultures but some cultures had produced soldiers at the end of the experiment. One culture in the groups containing twenty had produced one soldier; three in the groups of fifty had produced an average of 1.3, four cultures in each of the groups of one hundred and two hundred had produced an average of 3.3 soldiers.

The size of the culture appears to affect the time taken for supplementary reproductives to develop and the number differentiated. Table III indicates that 50 or less individuals tend to sustain a single pair but more than one pair may be found in larger cultures. The inference is that the continuous development of these forms and their subsequent elimination tends towards the maintenance of

TABLE III. — Number of supplementary reproductives produced in cultures of different initial sizes.

TABLEAU III. — Nombre de reproducteurs supplémentaires obtenus dans des élevages d'importance initiale différente.

Initial number of individuals per culture	Period of experiment (months)	Mean number of supplementary reproductives
20	4.5	2.0
50	4.5	2.0
100	4.5	2.2
200	4.5	3.3

a single reproductive pair with the likelihood that more than one reproductive unit might be sustained at numbers above some undefined number of individuals per culture. In table III, the data suggest that this threshold may be somewhere above 200 larvae per culture.

3. Effect of the mean weight of individuals in a culture on the number of supplementary reproductives produced by the culture.

Colonies of *Zootermopsis nevadensis* and *Neotermes jouteli* vary in their ability to produce supplementary reproductives (LIGHT, 1942-1943; LIGHT and ILLG, 1945; NAGIN, 1972). NAGIN (1972) observed that the increased production of supplementary reproductives in *Neotermes jouteli* was correlated with the age of the colony. He used the size of pseudergates as an index of the ages of colonies examined. Because my studies on *P. adamsoni* indicated that these might be equally applicable to this species, I tested NAGIN's techniques in the following way.

Termites were collected from nine field colonies on the same day. Four cultures, each of fifty large larvae and nymphs from each of these colonies were weighed, the average weight of an individual being found.

Two soldiers were added to each of the cultures in an attempt to prevent the transformation of undifferentiated larvae and nymphs into soldiers. The numbers of soldiers used were determined by the soldier to non-soldiers ratio observed in the field colonies. The cultures were set up in 9 cm petri dishes and observed daily for 25 days. The supplementary reproductives were removed as soon as they were recognised and their antennal segments and molar plate ridges were counted.

The data (table IV) show no consistent trends relative to the increasing average weight of the larvae in the respective culture groups. Indeed the heaviest cultures produced fewer reproductives than some of the lighter ones. The variability in all groups was high. The medium group generally produced few reproductives and was more consistent than the other two.

TABLE IV. — The influence of larval weight on the number of supplementary reproductives formed in cultures of *P. adamsoni* (data from 12 replicates in each category).

TABLEAU IV. — Influence du poids des larves sur le nombre de reproducteurs apparus dans les élevages de *P. adamsoni* (résultats de 12 expériences de chaque catégorie).

Weight category	Weight of larva (mg)		Number of supplementary reproductives per culture	Mean number of molar ridges (Rt. mandible)	Mean number of antennal segment
	Average	Range			
Light	14.3	12.4-16.0	5.5	18.8	15.7
Medium	16.7	15.8-18.2	2.4	18.9	16.0
Heavy	18.2	17.0-20.0	6.9	19.4	17.4

All reproductives developed from the larger members of each culture and this is also indicated by the large number of molar ridges and antennal segments recorded for them. The results of the experiment differ from NAGIN's findings for *Neotermes jouteli* but as other factors may be involved, such as daily handling of cultures, clear cut conclusions at this stage are not possible. My results do suggest, however, that weight of larvae is not necessarily a good index of the likelihood of a culture forming reproductives and that other things such as isolation from reproductives and the nutrition/energy relationships in the colony are more likely to be important.

4. The influence of functional supplementary reproductives on the development of further supplementary reproductives in incipient colonies.

Cultures without functional supplementary reproductives appear to begin developing supplementary reproductives almost immediately after their establishment and the first mature forms may be recognised in two to three weeks. The presence of functional supplementary reproductives tends to inhibit the development of further supplementary reproductives in those species in which these processes have been studied.

The situation in *Porotermes* was unknown and was therefore examined in the following ways :

4.1. *The Inhibition of the development of new supplementary reproductives by matured supplementary reproductives.*

50 larvae and nymphs were cultured in 9 cm petri dishes. A pair of reproductives was taken from bulk cultures, each was marked and then smeared with gut contents from larvae of the culture to which it was added (CASTLE, personal communication).

Two soldiers were also added to each treatment and control and all were examined daily for 50 days. Each supplementary reproductive was removed from treatments and controls, as soon as it was recognised. The counts obtained for the experiment were analysed by the Mann-Whitney « U » test.

More supplementary reproductives were formed in control cultures started without functional reproductives. Initially reproductives developed rather rapidly, the first being distinguished in five days. The rate of formation increased for about a further ten days then declined. A total of 52 supplementary reproductives were formed in the cultures and compared with seven that formed in cultures started with functional reproductives. The difference between the numbers of supplementary reproductives produced in the two groups of cultures was highly significant ($P < .001$).

Three of the cultures started with functional reproductives did not produce

any new reproductives and none of the reproductives originally added was lost during the experiment.

Functional (matured) supplementary reproductives prevented the formation of further supplementary reproductives.

4.2. *Stabilisation of the production of new supplementary reproductives in orphaned colonies.*

In earlier experiments I had noticed that « stability » of the colony structure occurred about the 30th day after establishment of the cultures. « Stability » in this case is denoted by the fact that number of reproducives produced was about equal to the number eliminated.

The following experiment was designed to test the effect of removing reproductive units.

11 groups of 50 large larvae and nymphs plus two soldiers were cultured in 9 cm petri dishes. The cultures were observed daily and new supplementary reproductives produced were chilled at 0 °C and marked on head and thorax with different colours of nail polish as soon as they were recognised. After cultures had been observed for 55 days, the supplementary reproductives surviving in six cultures were taken out whilst those in five cultures were retained. New supplementaries in both cultures were recorded and marked as before.

New supplementary reproductives began to appear about 10 days after the reproductives were removed from the six cultures and increasing numbers appeared over the next 15 days. No new reproductives appeared in the controls.

It appears then that in stable colonies of small numbers of termites, existing reproductives either inhibit the maturation of other supplementary reproductives or stimulate their elimination soon after they appear. If the « controlling pair » is removed a number of individuals begin to mature and a period of maturation and elimination begins. Stability in this species occurs after some thirty days.

This experiment therefore shows that removal of the reproductives unit results in the same developmental processes as occur in isolated groups of larvae and nymphs. It provides an understanding of the processes which might occur in natural colonies when either of both of the reproductives are lost or where groups of larvae and nymphs become isolated from the influence of the reproductive unit in the colony.

5. Effect of continuous removal of supplementary reproductives that mature in cultures on the rate of development of reproductives.

In isolated cultures without a functional reproductive pair, a proportion of larvae and nymphs transform into supplementary reproductives in a given period (LIGHT and WEESNER, 1951; LÜSCHER, 1952 *a, b, c;* NAGIN, 1972). CASTLE (1934) and GRASSÉ and NOIROT (1946) have found that all newly emerged larva have the potential to differentiate into any caste. With time therefore all larvae

and nymphs in isolated cultures without a pair of functional reproductives should transform into supplementary reproductives and this was tested for *P. adamsoni.*

A pair of marked supplementary reproductives and two soldiers were introduced into each of 10 cultures of 40 large larvae and nymphs in the experiment described in 4.1. After one month, five surviving cultures in which all forty larvae and nymphs were alive and had not produced any new supplementary reproductives were each divided into two groups of 20 larvae and nymphs. One group was continued with the reproductives and each group kept one of the soldiers. The cultures were observed daily for 110 days and new supplementary reproductives formed were taken out as soon as they were recognised.

Results are shown in table V. Two cultures without supplementary reproductives and one culture with supplementary reproductives were lost. At the end of the experimental period, most larvae and nymphs had matured in those cultures from which continuous removal of reproductives occurred. This con-

TABLE V. — The effect of removing reproductives that developed in cultures on the maturation of larvae that remained, compared with larvae maturation in stable colonies (period of experiment 110 days).

TABLEAU V. — Résultat du retrait des reproducteurs se développant dans les élevages sur la maturation des autres larves, par comparaison avec des larves situées dans des colonies stables (durée de l'expérience : 110 jours).

Group	Mean number of larvae at start/ culture	Mean number of larvae at end/ culture	Number of reproductive formed during experiment/ culture
With reproductives removed	20	1.7	13.7
With reproductives retained (stable colonies).....	20	14.5	0.5

firms the results of CASTLE and NOIROT for other species and emphasies that all larvae in these « lower » termites retain the ability to mature sexually for the greater part of their lives. The results also support earlier data that indicate that functional reproductives in small colonies exert some control over maturation of larvae.

6. Effect of the sexes of functional supplementary reproductives on differentiation of further supplementary reproductives of the same sex.

When a pair of functional supplementary reproductives was introduced into cultures of larvae and nymphs further development of supplementary reproductive was usually prevented. It is not known, however, how the individual

sexes affect this inhibition of sexual maturation. It has usually been found that males inhibit the maturation of other males and females inhibit maturation of females. To provide evidence on this inhibition in *P. adamsoni* I designed the following experiment :

Cultures of 50 larvae and nymphs were established in 9 cm petri dishes. A supplementary reproductive was added in the way described in 4.1.

The following combinations of functional reproductives were added to the cultures :

one male, two males, one male and one female,
one female, and two females.

The control cultures had no reproductives.

There were six replicates of each treatment. The supplementary reproductives that developed were taken and their sex was determined. Due to increasing mortality in some cultures, the period of the experiment varied from 21 to 33 days.

The numbers of supplementary reproductives formed in the cultures of the control were compared with the following treatments : a single female, two females, and a male and female, using the Friedman two-way analysis of variance. Because some of the cultures with a single male and some with two males died, the surviving ones were compared separately with the control and with each other using the Mann-Whitney « U » test.

As found in earlier experiments, more larvae and nymphs transformed into supplementary reproductives in the control (having no reproductive unit) than in any of the treatments. There was a significant difference between the number of reproductives formed in the controls and those recorded for treatments having,

(i) a single female, (ii) two females and (iii) a pair of reproductives ($P < 0.1$ in each case).

The least number of transformations occurred in those treatments having one male and one female (a reproductive unit).

The differences in the number of reproductives formed in the controls and in the treatments having a single male or two males per culture were significant ($P < 0.5$). There was no difference between the number of reproductives formed in treatments beginning with a single male or with two males per culture, respectively. The only result that proved significant when sex ratio was considered was the number of reproductives formed in treatments having initially a single female or two females.

It is clear that functional reproductives inhibit the production of further reproductives to a certain extent although this inhibition does not appear to be initially complete. The inhibitory effect of functional females on the sexual maturation of other females appears to be stronger than the corresponding effect of functional males on other males (table VI).

TABLE VI. — The effect of functional reproductives of one sex on the sexual maturation of larvae of the same sex.

TABLEAU VI. — Influence des reproducteurs fonctionnels d'un même sexe sur la maturation des larves du même sexe.

Initial mature reproductives present		Number of cultures that survived	Number of reproductives matured per culture		Total reproductives matured	
Male	Female		Male	Female	Male	Female
1	1	6	0.33	0.33	2	2
1	0	5	1.60	1.60	5	8
0	1	6	2.33	0.17	12	1
2	0	4	1.25	1.50	5	6
0	2	6	1.67	1.0	10	6
0	0	6	3.33	1.67	20	10

7. The time required for larvae and nymphs to respond to the absence of a pair of functional supplementary reproductives.

Supplementary reproductives are produced in cultures soon after they have been isolated from the « influence » of their original reproductives.

It seems likely that there is a critical time after larvae and nymphs are so isolated before the introduction of a pair of functional supplementary reproductives will inhibit further sexual maturation of larvae. This experiment was designed to determine whether such a critical time exists in *P. adamsoni,* for this inhibition to become effective.

Earlier experiments indicated that the addition of a « reproductive unit » to a larval culture significantly reduced the number of reproductives that transformed relative to controls having initially no reproductive unit. A test for the time taken for the inhibitory effect to become operative might be to find out how long it takes from establishment of a culture to addition of a reproductive unit before there is a difference between the number of reproductives formed by treatments and controls.

30 groups each of 50 larvae and nymphs and two soldiers were cultured in 9 cm petri dishes using described techniques. A pair of supplementary reproductives was added to each of five cultures on day zero (i.e. the day the experiment was started) another pair was added to each of five cultures on day one, two, four and eight. There was a control of five cultures without reproductives. The cultures were observed daily and reproductives removed as soon as they were recognised.

Results are presented in table VII.

The data indicates that the inhibitory activity of functional reproductives is ephemeral. Because of the similarity of reproductive formation in treatments and controls, inhibition appears to be entirely lost in about four days and pro-

TABLE VII. — The time taken for the inhibitory effect of the reproductive unit to become ineffective in cultures of P. *adamsoni*. Based on the similarity—numbers of supplementary reproductives formed by treatments and controls.

TABLEAU VII. — Durée de l'inhibition de l'unité reproductrice dans les élevages de *P. adamsoni*. Durées basées sur la similitude entre le nombre de reproducteurs supplémentaires dans les expériences et les témoins.

Time before reproductive unit added (days)	Number of new reproductives that developed per culture
0	1.4
1	2.6
2	4.0
4	6.2
8	10.2
Control	7.2

bably in as little as two or three days. Of the treatments which received a pair of functional supplementary reproductives each on day one, two out of five did not develop new reproductives but this could have been expected from the results of earlier experiments. While no attempt was made to identify the inhibitory factor, I believe that two aspects, « inhibitory substance » and « unused/feeding activity » or foraging energy which must be intense on the loss of reproductives which in small colonies are the main dependents are important. These effects may be involved either independently or together in the stimulation of sexual maturity in some of the remaining larvae or nymphs. Because, in the early stage any stimulus in the food regurgitated or excreted by foragers may be spread over a number of individuals in a more or less random manner, the maturation of more than one pair, and even differing numbers of each sex, might occur. This is the situation found experimentally in *P. adamsoni* and my observations reinforce the idea that attempts by larvae to feed each other might well precede the maturation of some and the secretion of the inhibitory substance by resultant sexually mature forms which then inhibit, with increasing effect, the sexual maturity of further larvae. Whatever the true situation is, there can be little doubt that this hypothesis satisfies the experimental and observed data in most respects.

8. The relationship between differentiation and elimination of supplementary reproductives.

Field colonies of *P. adamsoni* had been found to contain different numbers of supplementary reproductives and laboratory cultures also developed varying numbers of supplementary reproductives with time. The laboratory experiments described earlier in this section showed that there were more reproductives transformed than were sustained in true colonies of the same numerical size, or indeed were present in each culture at the end of the particular experiment.

Such reduction in the number of supplementary reproductives produced in cultures has also been reported in laboratory cultures of *K. flavicollis, N. jouteli* and *R. lucifugus* (GRASSÉ and NOIROT, 1960; LÜSCHER, 1952 *b*; NAGIN, 1972 and BUCHLI, 1956, 1958). These authors attribute cannibalism of extra reproductives to a defined behaviour pattern called « elimination ». This seems to be related to the relationship between the number of food-producing individuals and « dependent » individuals in any culture. It must also be influenced by the presence of « functional » reproductives and by the relative strength of their inhibitory activity. The trend towards the production of a certain number of supplementary reproductives in cultures of a standard size was examined to determine the method of elimination of the surplus individuals for *Porotermes*.

8.1. *The relationship between time and differentiation and elimination of supplementary reproductives.*

A certain proportion of larvae and nymphs in isolated cultures is transformed into supplementary reproductives in a given time and such cultures tend to « stabilise » about the thirtieth day. The following experiment was designed to examine the influence of time on differentiation of supplementary reproductives and elimination of those reproductives if indeed they are eliminated.

20 groups of 50 large larvae and nymphs plus two soldiers all from the same colony were cultured in 9 cm petri dishes. The cultures were examined daily for 55 days and the number of supplementary reproductives present on each day was counted. As the new supplementary reproductives were recognised, they were chilled at 0 °C, sexed and marked on the head and thorax. Different coloured nail polishes were used to separate newly matured insects from older ones.

Cultures were photographed at seven day intervals using positive films and the termites were counted on the film. This technique reduced the chances of injury to the termites.

Most cultures had developed their first supplementary reproductives by the seventeenth day while others varied up to twenty-nine days from establishment. Males outnumbered females as the first supplementary reproductives formed by seven to three.

Most supplementary reproductives appeared in cultures between the tenth and thirtieth days during which time further reproductives, additional to the first pair, developed. On the thirtieth day eggs were usually present in the cultures and it was at about this time that further development of reproductives markedly decreased until usually a reproductive pair was left in each (fig. 3). Eighty per cent of the cultures produced more than a pair of supplementary reproductives and 9 % of all larvae and nymphs cultured actually transformed into reproductives. More than 56 % of the reproductives formed were males but the male/female ratio approached one with increasing time.

In most cultures observed, reproductives of the same sex, additional to the first formed, were eaten by the larvae and nymphs. Elimination of male repro-

ductives usually proceeded much more quickly than did elimination of females. In a few cultures only one reproductive pair was developed and so there was no elimination recorded in these. In four of the cultures in which reproductives continued to develop, sometimes all were eliminated and when the experiment was completed there was still only a single reproductive in each of these cultures. The population of three of these cultures had decreased to less than half of their original number indicating that conditions were unsatisfactory. This could have been the cause of the excessive elimination noted in these cultures which died out ten days later. A culture which developed a single female and three male reproductives eliminated the female leaving the three males, whilst

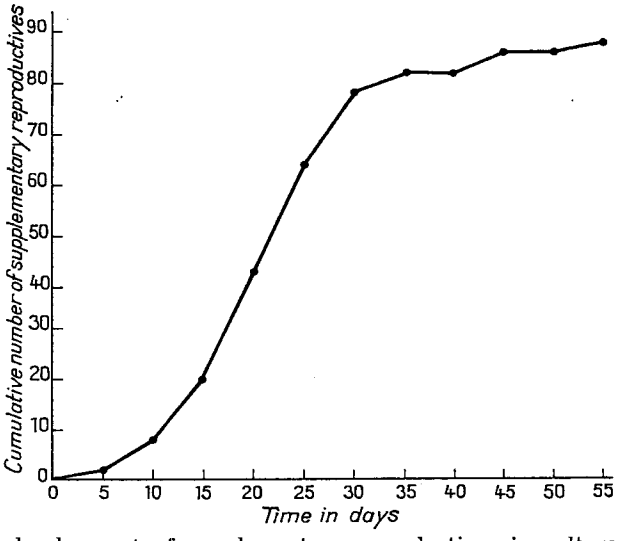

FIG. 3. — The development of supplementary reproductives in cultures of *Porotermes adamsoni* with time (data from 20 cultures).

FIG. 3. — Développement des reproducteurs supplémentaires dans les élevages chez *Porotermes adamsoni* en fonction du temps.

another produced four females and one male, but none were eliminated. One culture produced only male supplementary reproductives.

The time of formation of a supplementary reproductive did appear to influence its chances of elimination (table VIII); the first ones that developed had higher survival rate than those that developed later.

The formation of supplementary reproductives in most isolated cultures of larvae and nymphs of *Porotermes adamsoni* is initially continuous with elimination by cannibalism or starvation usually of the later ones matured.

There is, in these results therefore, some evidence for both inhibition and elimination of reproductives in *P. adamsoni*. In some cases these phenomena do not appear to be as strongly developed as has been reported for other species and there is little doubt that the variation reported for *Porotermes* may *sometimes*

TABLE VIII. — The relationship between the survival of initial and subsequent supplementary reproductives in cultures of larvae and nymphs maintened for fifty-five days (data for twenty cultures).

TABLEAU VIII. — Relation entre les reproducteurs supplémentaires initiaux et suivants dans des cultures de larves et de nymphes d'une durée de 55 jours (résultats par 20 cultures).

% Initial supplementary reproductives that survived			% Subsequent supplementary reproductives that survived		
♀	♂	Total	♀	♂	Total
33	29	62	18	20	38

be due to the conditions existing within particular culures. At least some of the problems in cultures appear to be due to the disturbance necessary for experiments of the kind I have designed.

TABLE IX. — The effect of disturbance on survival of cultures of *P. adamsoni* (Mean number of individuals/culture assessed 12 weeks from establishment. Each culture originally of 62 individuals).

TABLEAU IX. — Effets du dérangement dans la survie des cultures de *P. adamsoni* (moyenne du nombre d'individus/élevages de 12 semaines. Chaque culture comprenant à l'origine 62 individus).

Control not disturbed	Disturbed daily	Disturbed daily after forty-seventh day of establishment	Disturbed weekly after forty-seventh day of establishment
55	24	32	48

9. Effect of disturbance of cultures on the survival of their reproductives.

In some of the cultures in previous experiments all reproductives were at times eliminated, and some decreased in number of individuals relative to their initial numbers. Daily handling appeared to place some cultures under stress and an experiment to test this possibility was carried out.

38 cultures each containing 60 larvae and nymphs and two soldiers from the same colony were cultured in 9 cm petri dishes. The cultures were randomly assigned to four groups which were subjected to the following treatments : Group I : 10 cultures were daily disturbed (disturbance consisted of opening the dishes to observe the culture under a dissecting microscope); Group II : 10 cultures were left undisturbed until eggs had appeared in all cultures of Group I (on the forty-seventh day). Then they were disturbed daily; Group III : eight cultures were treated the same way as Group II but were disturbed at weekly intervals after the forty-seventh day; Group IV : ten cultures were left undisturbed for the full three months of the experiment.

The number of supplementary reproductives and undifferentiated termites

in each of the surviving cultures in all the treatments and controls were then assessed.

Though all colonies decreased in number of individuals during the experiment, clearly the highest survival rates were in those which were disturbed least (tables IX, X and XI). There did not appear to be a similar significant effect of disturbance either on the number of reproductives found per culture or on the numbers of cultures that developed reproductives. However, only in the group not disturbed at all, did all cultures survive the three months of the experiment.

Survival of cultures was analysed by the analysis of variance and was found to be significant ($P < .01$) (table X).

TABLE X. — Analysis of variance for the effect of disturbance on survival of cultures.

TABLEAU X. — Analyse de variance du rôle du dérangement sur la survie des élevages.

Variation due to	d.f.	S.S.	M.S.	V.R.	P.
Disturbance	3	6316.68	2105.56	6.1.	.01
Error	34	11735.87	345.17		
Total	37	18052.55			

TABLE XI. — The effect of disturbance on the survival of supplementary reproductives that developed in cultures of 60 larvae and nymphs (data from 32 cultures assessed at 12 weeks from establishment).

TABLEAU XI. — Rôle du dérangement dans la survie des reproducteurs supplémentaires obtenus depuis des élevages formés de 60 larves et nymphes (résultats de 32 élevages, durant 12 semaines).

Group of cultures	Status of cultures at assessment Number of cultures with :		
	Less than one pair of reproductives	One pair of reproductives	More than one pair of reproductives
Control (not disturbed).....	0	7	3
Disturbed daily	0	4	3
Disturbed daily after 47 days..	1	7	0
Disturbed weekly after 47 days.	0	5	2

Records on formation and elimination of reproductives (table XI) strongly supported previous data, as did indications of suppression of reproductive development at about the time eggs appeared in cultures. There were no unusual effects on the sex ratio of reproductives that developed.

10. The influence of sex ratio on the development and elimination of supplementary reproductives.

Most of the cultures in previous experiments had produced more than a single pair of supplementary reproductives and usually males outnumbered females, at least initially. In the most vigorous cultures, the number of supplementary reproductives fluctuated with a tendency toward the survival of a pair. In one

case a culture produced only male supplementary reproductive. As this culture included both sexes of larvae and nymphs, the ratio of the sexes initially present may have influenced the result. An experiment was therefore designed to test the influence of sex ratio on the formation and survival of reproductives.

Using the 9 cm petri dish culturing technique, I established cultures of suitable larvae and nymphs from the same colony. These were all sexed and assigned to cultures each of 50 individuals according to the following male to female sex ratios : 4 : 1; 1 : 4; 1 : 1; 50 : 0; 0 : 50.

Each culture was replicated four times. Two soldiers were added to each culture and the number of supplementary reproductives formed assessed from daily observations.

Results are presented in table XII.

TABLE XII. — The influence of the sex ratio of larvae in initial cultures on the formation of supplementary reproductives in them (culturing time = 50 days).

TABLEAU XII. — Influence du sexe ratio des larves en culture sur la formation de reproducteurs supplémentaires (temps de culture : 50 jours).

Initial sex ratio of larvae/culture		Number of cultures that survived/ group	Number of reproductives *produced* per culture		Number of individuals that survived per culture (Sex ratio in brackets) (female to male)
Female	Male		Female	Male	
50	0	4	4	0	42.00 (42:0)
0	50	4	0	2.5	36.25 (0:36)
40	10	4	3.25	1	34.25 (4:1)
10	40	4		3.25	39.0 (2:9)
25	25	3	2.3	1.3	35.0 (1:1)

There was a distinct trend toward the development of four reproductives in each culture except those of males only. In those cultures with both sexes present, the numerically dominating sexual form developed most reproductives.

Where the sexes were initially equal, females tend to outnumber males among the reproductives matured.

Observations during this experiment indicate that the rate of development of new supplementaries tend to be offset by the rate of their elimination. Inhibition may also have been involved. The interesting point was that the development of this stabilisation in cultures again occurred about the 30th day of culture.

DISCUSSION

In attempting to define the biological parameters of the supplementary reproductives (such as initial developmental period and abundance), I have examined environmental and social factors (such as inhibition and elimination) that may influence both their development and survival. Though these factors, espe-

cially social factors, have been reasonably investigated, no attempt has been made here to identify precursors for any of the behavioural responses noted. However observations and quantitative data in certain of my experiments have indicated that such factors and the behaviour they stimulate are operating in the colonies of this species.

The rate of transformation of larvae and nymphs into supplementary reproductives is similar to that reported for *Zootermopsis* but the influence of the number of individuals in a culture on the number of supplementary reproductives maintained by it appears to differ (see LIGHT and ILLG, 1945). This could be due to difference in the elimination-potential of the two species. The ability of larvae and nymphs to transform into supplementary reproductives appears to be influenced by their respective intermoult periods. This assumed, the potential of colonies to develop supplementary reproductives would then depend on the availability of recently moulted larvae and nymphs. This would be an adequate explanation for the observed difference in the number of supplementary reproductives formed in different colonies of *P. adamsoni*. It may also explain why this varied with time for the same colony as has also been reported for other species by LÜSCHER (1952 *b*) and LIGHT and ILLG (1945). My results for *P. adamsoni* differ greatly from those of RÜPLI (1969) for *K. flavicollis*,, but this could be expected not only on ecological grounds but also on the fact that the two species are not closely related. The variability in the number of supplementary reproductives developed by different colonies of *N. jouteli* (NAGIN, 1972) may not be due to the differences he reported in the weight of individuals of those colonies. He used only one replication and, if my results with *P. adamsoni* are any indication of the variability that might occur in experiments of this kind, NAGIN's results should be considered tentative until confirmed. The same may be said about his reference to the effect of age of individual on the formation of supplementaries by cultures of *N. jouteli*. The inhibitory influence of supplementary reproductives which appeared in cultures of *P. adamsoni* initially with functional reproductives, appeared to increase with time. This has also been reported for *K. flavicollis* (GRASSÉ and NOIROT, 1960; RÜPLI, 1969).

There appears to be a relationship between two major factors in the process of colony development and stabilisation (in the sense I have used it in this text) in *Porotermes*. These factors are :

(i) The relationship between feeders and dependents involving an imbalance towards « feeding energy » in the colony when the reproductives are lost.

(ii) When « feeding energy » is dominated by dependency, because of the number of reproductives that are stimulated to mature, cannibalism provides the main way of eliminating the extra dependents. The development and elimination of reproductives stabilises at about the onset of oviposition by the young queen. This strongly suggests that the inhibitory substances (whatever it may be) is produced and distributed from about the time of ovarian maturity. If this is correct, it stresses the importance of using functional reproductives in all experiments on inhibition. The inhibitory substance in *P. adamsoni* appears to be sex-specific, but female reproductives are apparently more effective in inhibiting female maturation than males are in inhibiting the maturation of other males. This suggests that there may be a quantitative difference either in

the responses of the sexes or in the potency of the inhibitory substance produced by each sex. The inhibition of the development of reproductives was more effective when both male and female reproductives were present which may indicate a synergistic effect of the substances produced by one sex on those produced by the other. This has also appeared to be the case in other termites (see LIGHT and WEESNER, 1951; GRASSÉ and NOIROT, 1960; LÜSCHER, 1964 and NAGIN, 1972).

My results with unisexual cultures of *P. adamsoni* where stabilisation occurred with time, cast some doubt on LÜSCHER's (1962) hypothesis that the distribution of the « inhibitory pheromone » is sex specific and that male pseudergates « collect and distribute » female pheromone and vice-versa. Other research on *K. flavicollis* (GRASSÉ and NOIROT, 1960) is supported by my results and unless LÜSCHER's theory is subsequently confirmed with quantitative data, it would seem that the type of pseudergate transmission of the inhibitory substance postulated by him must be considered doubtful.

In *Zootermopsis* the inhibitory effect of functional reproductives is incomplete unlike in *Kalotermes* where it is completely effective. Usually more than a pair of supplementary reproductives are found in colonies of *Zootermopsis* whilst a pair is usually found in colonies of *Kalotermes* indicating that the « eliminatory factor » may be weaker in *Zootermopsis* (MILLER, 1969). The intensity of « inhibitory » and « eliminatory » factors in *Porotermes* appears to fall between those of *Kalotermes* and *Zootermopsis*. In *Reticulitermes* the inhibitory effect is very weak. Supplementary reproductives are produced in primary colonies with functional primary reproductives if the nutritional equilibrium of the colony is favourable (BUCHLI, 1956, 1958). In *Porotermes* the influence of the inhibitory substance is lost with the loss of the reproductive unit. This loss of influence occurs more quickly in cultures of *P. adamsoni* from which supplementary reproductives are removed than in those of *Z. angusticollis* from which primary reproductives are removed (LIGHT and WEESNER, 1951). But it was quickest for cultures of *K. flavicollis* from which primary reproductives were removed (LÜSCHER, 1952 *a, b*). However these temporal differences could be due to innate characteristics of these species, or differences in reproductives involved (LIGHT, 1942-1943).

The disturbance of colonies of *P. adamsoni* affected the survival of the nymphs and larvae but did not influence the stabilisation of the colony or the survival of the reproductive unit. Furthermore the development and elimination of supplementaries appeared to proceed similarly in disturbed and undisturbed cultures. In other words the end results in various cultures of fairly short duration were similar whether disturbed regularly or not. The pattern of elimination of the extra reproductives in cultures of *Porotermes* compared well with that reported for *Kalotermes* and *Neotermes* but differed from that of its closer relative, *Zootermopsis* (see CASTLE, 1934; LÜSCHER, 1952 *a, b, c;* GRASSÉ and NOIROT, 1946 and STUART, 1970; NAGIN, 1972).

Supplementary reproductives are very common in the galleries of field colonies or sub-colonies. However, the field situation is extremely complex and difficult to unravel. There were instances where supplementary reproductives

were present with older larvae, nymphs and soldiers but eggs and young larvae were absent, giving the impression that brood production by such reproductives is spasmodic or seasonal. Another explanation for this common situation is that the supplementaries wander about the galleries with older larvae and soldiers and that eggs laid are carried to nurseries and cared for by the larvae or pseudergates. This may involve common nurseries for several reproductives units or separate nurseries for each sub-colony. Certainly nurseries with resident reproductives are found and, on the other hand other reproductives attended only by older larvae, nymphs and soldiers. Sometimes both of these situation are present in the same log.

Porotermes may therefore exist either as separate primary and secondary colonies or as complex colonies, the sub-units of which might be located in different logs and stumps or in different parts of the same log or stump. Occasionally underground galleries, in use by larvae and soldiers and linking adjacent infested logs, have been discovered during this study. This behaviour is similar to that of higher termites of subterranean groups. It should be remembered, however, that *Hodotermes*, in the same family as *Porotermes*, is a subterranean termite. Perhaps dispersal by what HARRIS (1956) has called « colony-budding » occurs in *Porotermes*. The colony-supplementary colony (sub-colony) relationship in *Porotermes adamsoni* however remains uncertain.

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