# POLYMORPHISM AND THE ROLE OF HORMONES IN CASTE DIFFERENTIATION OF A HIGHER TERMITE SPECIES MACROTERMES MICHAELSENI (ISOPTERA, MACROTERMITINAE)

BY

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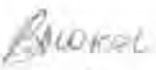
C O N T E N T S

# D E C L A R A T I O N

This thesis is my original work and has not been presented for a degree in any other University.



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# 1. GENERAL INTRODUCTION

Termites are social insects of the order Isoptera. They are predominant in the Tropics and dwell in dry or damp wood, underground in soil or in mounds or nest in trees. The basic food source for termites are cellulose materials like wood, grass etc. As a result of this, they pose a threat to mankind by being destructive to timber, buildings, boats and ships, forests, pastures, crops etc. Termites may also help in recycling organic materials in the soil. It becomes apparent, therefore, that some control measures need to be taken to regulate termite populations in an ecosystem in order to coexist with them. These cannot be achieved without the understanding of their biology. The present study was therefore designed to answer some basic questions regarding development and caste differentiation of a representative species of the *Termitidae*, one of the least studied families of the *Isoptera* predominant in Ethiopian Region. The basic questions posed were those regarding polymorphism and the mechanisms of caste formation. I will start by giving definitions of some fundamental terms used throughout the thesis. I will then outline the basic views of key authors which are related to the questions to which I have addressed myself.

## *1.1. POLYMORPHISM*

Polymorphism is a biological phenomenon exhibited by many animals particularly some groups of insects that are able to

appear in two or more forms. This phenomenon is especially common in social insects e.g. social wasps, ants, bees and termites. The different forms of these insects perform specific duties within the society or colony in which they live in harmony. *EMERSON* (1939) once described such social integration as a "superorganism". He expressed the idea that each caste functions for the benefit of the whole society, thus appearing like different organs or systems of a "superorganism". The various morphological forms are called castes. Three castes are known in termite colonies namely: Functional reproductives, workers and soldiers.

1.1.1. *Functional Reproductives*

These are of two kinds, primary and supplementary or replacement. *Primary reproductives*, the king and the queen, usually form a pair in a colony and usually head the colony. They are sclerotized and pigmented and develop from alates (macropterous) - winged adults. The *supplementary reproductives* or *replacement reproductives* are either slightly pigmented with short wing-pads (brachypterous) or very slightly pigmented without wing-pads (apterous). Both forms are termed *neotenic* because they become sexually matured without first reaching the imaginal form. Sometimes the primary reproductives are known as first-form reproductives or *imagoes* while the brachypterous neotenics are known as second-form reproductives and the apterous neotenics are known as third-form reproductives or *ergatoids*. The major function of the reproductive caste is reproduction that is, to

maintain the species through "nuptial" flights of alates which locate a site for new colonies. The first-form pair establishes a colony, feeds and takes care of their first progeny.

1.1.2. *Workers*

This is one of the sterile castes of a termite colony. They are apterous and usually blind. In some species the workers display polymorphism - large (major) and small (minor) types. True workers are considered absent in *Mastotermitidae, Kalotermitidae* and in the subfamily *Termopsinae* of the family *Termopsidae*. The duties of workers in these groups are carried out by immature individuals capable of moulting from time to time without appreciable change in size, or formed through regressive moults of nymphs (Pseudoworkers or pseudergates) (*GRASSE* and *NOIROT*, 1947). The role of workers or their false counterparts is to attend to the eggs and the young, gather food and feed the whole colony and maintain sanitation in the colony, also do the constructions and repairs of the nest. As a result, workers are by far the majority in a given colony. Sometimes among them there is a division of labour, thus polyethism. Differentiation occasionally occurs among the pseudergates. In that case, some individuals may moult into presoldiers or nymphs.

1.1.3. *Soldiers*

This is the other sterile caste. Like the workers, they are also wingless and usually blind. Polymorphism has been recorded among soldiers as well. There are three forms known, namely:

major (large), intermediate and minor (small) forms. The function of soldiers is to defend their societies, as such, they often have modified mandibles, long and strong or have other structural modifications for defence. Soldiers develop from a non-functional form known as a presoldier or white soldier.

In termite colonies one may also find young individuals usually of two types. The *apterous nymphs* known as "larvae" (without wing-pads) and the *brachypterous nymphs* known as "nymphs" (with wing-pads). These are non-functional and are, therefore, usually taken care of by the workers or the reproductives during the early stages of colony development.

## 1.2 *BRIEF CLASSIFICATION*

According to *WILSON* (1974) there are to-day about 2000 living and fossil termite species known. These species fall under 6 families namely: *Mastotermitidae, Kalotermitidae, Termopsidae, Rhinotermitidae, Serritermitidae* and *Termitidae*. The first five families are collectively referred to as "Lower termites". They are distinguished from "Higher termites", which form the family *Termitidae*, by the fact that they have symbiotic protozoans in the hind gut on which they depend for digestion of cellulose, while the latter do not posssess these protozoans. *Termitidae* is by far the largest, most advanced and diverse family. It is composed of four subfamilies, namely: *Termitinae, Apicotermitinae, Macrotermitinae* and *Nasutitermitinae* (*SANDS*, 1972).

The study presented here was based on a representative species from the sub-family *Macrotermitinae* (The species problem will be discussed below).

## 1.3. *THE PROBLEM OF CASTE DIFFERENTIATION*

### 1.3.1. *Lower Termites*

The pioneering work on caste differentiation was started in the last century with the work of *GRASSI* and *SANDIAS* (1893 - 1894) standing prominent. There were two schools of thought regarding mechanisms underlying caste differentiation. One was that caste differentiation is genetically controlled. This was the "intrinsic" school of thought headed by *IMMS* (1919); *THOMPSON* (1919, 1922) and *THOMPSON* and *SNYDER* (1920). *IMMS* (1919) believed that the question of caste differentiation was one of Mendelian inheritance, the soldier caste being accounted for by a loss of certain allelomorphs. *THOMPSON* and *SNYDER* (1920) interpreted the question rather as a series of fluctuating variations of mutations comparable to the mutations found in Drosophila. *THOMPSON* (1922) modified her view, considering the castes to be segregants, arising generation after generation by the splitting and recombination of the genes in a heterozygous parent form. She (*THOMPSON*, 1919, 1922) refuted the earlier work by *HEATH* (1903) who had set forth the idea that in the two species of *Zootermopsis* (*Z. angusticollis* and *Z. nevadensis*) the young are hatched without visible differences among them. She believed that there were two egg sizes and two types of newly hatched larvae distinguishable by differing brain sizes. She

did not, however, firmly correlate the sizes of the eggs with those of the types of larvae. This trend of affairs induced *HEATH* (1927, 1928) to collect a very large number of specimens of *Zotermopsis* from hundreds of colonies of varying sizes and ages which permitted him to conclude that there were not two distinct type of eggs or newly hatched larvae. He believed that there were modifications or transitional forms of the soldier-nymph line of development brought about by "extrinsic factors - probably feeding." He therefore refuted *Thompson's* claims that there were four stable adult castes, namely: first, second and third-form fertile or reproductive types and sterile or soldier type with no recognition of permanent worker caste. In fact, *HEATH* noted even that differentiation into the reproductive and the soldier castes did not appear until the close of sixth instar. This meant, therefore, that these forms were not pre-determined. Besides *HEATH* other authors, particularly *PICKENS* (1932), believed that all individuals were alike at hatching, posssessing equal potential to differentiate into any caste, and that specific caste development for a given proportion of the colony depended on "extrinsic" factors such as selective nutrition, influential exudates or environmental factors. He also suggested that in *Reticulitermes* a functional queen produced an inhibiting substance which regulated the development of nymphs in the colony.

*CASTLE* (1934) set to testing this hypothesis experimentally using *Zootermopsis*. He isolated individuals and found that

some of these individuals developed into replacement reproductives in higher proportion than could be expected by chance occurrence of genetically determined forms. He also fed alcohol or ether extracts of functional supplementary female reproductives to undifferentiated larvae. He found that in the former case, the differentiation was slower than in the latter. The extracts of female bodies did not hinder the development of males. In the presence of a functional reproductive of a given sex, no neotenic of that sex developed from the undifferentiated individuals, whereas in the control groups which lacked the functional reproductives, reproductives developed.

Similar experiments on the development of soldiers gave analogous results, namely:

Introduction of soldiers into an incipient colony before a presoldier appeared greatly delayed or inhibited the formation of a new soldier in that colony. On the other hand, removal of soldiers from these colonies induced production of more soldiers than normal. These were important findings supporting the theory of "extrinsic factors" and over-riding that of "intrinsic factors". *LIGHT* (1944 a, b) and *LIGHT* and *WEESNER* (1951) pursued *CASTLE's* work by preparing a variety of extracts of reproductives of *Zootermopsis* and feeding them to groups of termites. There was an inhibition of replacement reproductive differentiation though at a lower level than that induced by the primary reproductives. These findings thus supported *CASTLE's* theory.

Similar work had been going on with *Kalotermes* notable the work of *GRASSÉ* and *NOIROT* (1946 a). They found that all larvae in or past the fourth instar had the ability to transform into neotenic reproductives after a moult in the absence of an inhibitory influence of functioning reproductives. They also noted that an ophaned colony in the laboratory rapidly produced severely incipient reproductives, but only one pair was retained by the colony; the others were either eaten or neglected to death.

*GRASSÉ* and *NOIROT* (1946, b) attempted studies on the origin of soldiers in *K. flavicollis* and found that there were not two categories of early instar larvae thus also, rejecting *THOMPSON's* (1919) findings. They found that both sexuals and soldiers originated from a single stock and that soldiers could even originate from nymphs. The smallest soldiers were formed from third instar larvae and others from larger and older instars.

*GRASSÉ* and *NOIROT*,( 1947 )showed that while functioning as workers, pseudergates did not lose their ability to develop into soldiers or reproductives.

*LÜSCHER* (1952, a) pursued this work and found that depending on the composition and the size of the colony, pseudergates may:

1. undergo stationary moults without change in size.
2. moult into nymphs and, through two more instars into imagoes.

3. develop into replacement reproductives in the absence of primary reproductives.

4. in the absence of soldiers develop into soldiers.

He also noted that a nymph may undergo a regressive moult into a pseudergate or soldier. Replacement reproductives, soldiers and imagoes are terminal castes and, therefore, cannot regress or change. *LÜSCHER* later (1955) elaborated on the question of inhibition. He showed that the inhibiting factor for differentiation of the reproductives was given off through the abdominal end. The head did not. The inhibitor could be transmitted by larvae or pseudergates. Later experiments (*LÜSCHER*, 1956) showed that the inhibiting pheromone could be extracted from the head-thorax.

Not much attention had been previously paid to the mechanisms of soldier differentiation, until *LÜSCHER* (1958) first showed that implantation of corpora allata from replacement reproductives into larvae or nymphs would induce presoldier formation. Pursuing the question of soldier formation in *K. flavicollis*, *LÜSCHER* and *SPRINGHETTI* (1960) transplanted corpora allata from various donor castes into pseudergates. Corpora allata (CA) from reproductives, penultimate nymphs and from soldiers induced presoldier formation in the recipients. Corpora allata from pseudergates and other stages of nymphs did not.

These results stimulated further work on the mechanisms underlying caste differentiation. Particular interest was turned to the role of hormones. *LEBRUN* (1963 a, b) implanted

prothoracic glands from *Periplaneta americana* and replacement reproductives of *K. flavicollis* in soldiers. He obtained amazing results; the soldiers attempted to moult, but due to their sclerotized cuticle could not. These findings showed that soldiers and replacement reproductives which were once considered as terminal castes had actually not lost their moulting capability. *LEBRUN* got encouraged by his findings and in the following year (*LEBRUN*, 1964), he also transplanted CA from newly formed replacement reproductives into pseudergates at the beginning of their intermoult period and as a result induced presoldier or soldier intercastes. Transplantation of CA from roaches into pseudergates gave similar results. He also (*LEBRUN*, 1967 a, b) induced presoldier and soldier intercaste formation from nymphs by injecting them with CA from neotenics.

While much attention was given to *Termopsidae* some corresponding work was being done on *Rhinotermitidae*. Some of the notable contributions were those by *GRASSI* and *SANDIAS* (1893-1894) on *Reticulitermes lucifugus* who concluded that the regular development of *Termes (Reticulitermes)* up to the perfect insect may undergo a deviation at various periods of life which leads to the formation of : 1. workers.

2. complementary or substitute royal forms.

3. soldiers. *THOMPSON* (1917) strongly adhering to intrinsic or genetic theory of caste determination, claimed that in *R. flavipes* there were two types of newly hatched larvae: the reproductive type with large brains and

worker-soldier type with small brains. Studying *R. hesperus*, *PICKENS* (1932) suggested that, in the case of soldier, a difference in the egg is indicated. Hence, he had indicated intrinsic factors for separation of eggs into a reproductive-worker line and a soldier line, but an extrinsic factor for the differentiation of workers and reproductives.

*HARE* (1934) re-examined *THOMPSON's* materials on *R. arenincola* and *R. flavipes* and concluded that newly hatched larvae were not separable into reproductive and worker-soldier lines. *MILLER* (1942), reported that in *Prorhinotermes simplex* the distribution of head width and mesonotum width measurements failed to show any differences between larvae differentiating into reproductives, on one hand, and into soldiers or workers on the other hand until the fifth instar.

*MILLER* (1942) achieved remarkable results when he isolated wing-padded individuals and raised them in the laboratory. He found that these individuals developed into worker like forms, soldiers and supplementary reproductives all with variously reduced wing-pads. He also showed that caste specific inhibition occurs in this species. Soldiers if added into a colony inhibited the formation of other soldiers, and replacement reproductives inhibited formation of other replacement reproductives, while, the reproductives stimulate the production of soldiers. Therefore, the plasticity for differentiation depends on the composition of the society as suggested for *Kalotermes* and *Zootermopsis* cited above. *BUCHLI* (1958) after extensive

work on *R. lucifugus santonensis* concluded that it is the physiology of the whole colony which determines the destiny of the individual. *SHIMIZU* (1963) found that in artificial colonies of *R. speratus* the emergence ratio of soldiers increased with the number of larvae and that the development of supplementary reproductives also depended on the size of the colony; the larger the colony, the more conducive it is to development of the reproductives.

Mention must be made of the development in the most primitive family, *Mastotermitidae*. This family is represented by only one species, *Mastotermes darwiniensis*, found only in Australia. Little is known about caste differentiation of this species. *WATSON* (1971) examined the development of primary and neotenic reproductives and workers and concluded that there were three nymphal instars leading from medium sized workers to alates, and that neotenics developed from workers of about the same size. The developmental pathway was said to be rigid when compared with that of the other lower termites (*WATSON*, 1971, 1974; *WATSON*, et al., 1977). The unusual situation was also reported that field colonies are almost invariably headed by numerous apterous neotenics (*HILL*, 1942; *WATSON* and *HOWICK*, 1975). In the later years *WATSON*, et al., 1975 attempted some work on the control mechanisms of neotenic formation. He found that neotenics very rarely developed in laboratory groups of *Mastotermes* workers with or without soldiers, or in incipient colonies headed by primary reproductives. In half or fully orphaned incipient colonies, neotenics were produced. On the

hand, laboratory colonies headed by neotenic pairs produced numerous noetenics which were immediately destroyed by the workers. It was also established that female neotenics were the most potent inducers of neotenic development. This finding was unique since, in the previous studies on lower termites, it had been shown that members of one caste inhibit the development of more members of the same caste while here it does seem to be the opposite.

Recently *WATSON*, et al. (1977) re-examined the development of castes in *Mastotermes darwiniensis*. He rejected his earlier observation *(WATSON*, 1971) that there were three nymphal instars leading from medium sized workers to alates; instead he proposed a scheme showing that nymphs originated from first instar larvae and developed through eleven instars into alates and that workers develop from sixth instar larvae. In fact, he insisted that there is nothing like a pseudergate, but rather a true worker caste in the true sence of function and development of lower termites. Of course this is a question of terminology which I will not go into here.

About the development of soldiers of *M. darwiniensis*, *WATSON* (1974) reported that incipient colony soldiers develop from pseudergates (sixth instar) which is the same instar or higher than it occurs under field conditions.

*WATSON's* analysis of caste development here thus shows that even in this primitive termite species caste differentiation

is not genetically controlled.

### 1.3.2. *HIGHER TERMITES*

Turning to the problem of caste differentiation in higher termites, *Termitidae*, one point is clear: The various castes are much more distinct from one another than in the lower termites. Also there is less plasticity in development than in lower termites, therefore there are more common developmental features throughout the family.

As in lower termites, the newly emerged larvae are identical and it is only after the first moult that it is possible to recognize two categories of individuals:

1. larvae of the neuter line of development and
2. nymphs of the reproductive line with wing-pads.

Since not much has been done on the development in *Termitidae*, I will go through the basic findings in each sub-family. Much is owed in this respect to *NOIROT* who studied the development of castes in many of the sub-families (*NOIROT*, 1955, 1969).

#### 1.3.2.1. *Termitinae*

In *Amitermes* larvae pass through two instars to become workers. The workers may undergo stationary moults and some may moult into presoldiers which are of both sexes (*NOIROT*, 1955, 1969). While workers of *Termes hospes* pass through three stages; those of *Cubitermes*, *Noditermes*, *Orthotermes*, *Euchilotermes*, *Ophiotermes*, *Neocapritermes* and *Protohamitermes* pass through only one stage.

Soldiers develop from workers and they are almost always female (*NOIROT*, 1969). It seems that the production of replacement reproductives is readily accomplished in this sub-family. *WEYER* (1930) reported the formation of replacement reproductives in *Microcerotermes amboinensis*. Under his experimental conditions, *WEYER* reported having readily produced nymphoid reproductives from the last three nymphal stages and also ergatoids from the workers. *NOIROT* (1969) reports that nymphoids have been found in *Amitermes* as well and adultoids were found in *Microcerotermes parvus*. Most of the replacement reproductives which have been observed are adultoids.

1.3.2.2. *Apicotermitinae*

*NOIROT* (1969) notes that polymorphism in this sub-family is similar to that of the *Termitinae*. No sexual dimorphism is known in *Anophlotermes* with only one worker stage and no soldiers formed (*NOIROT*, 1969). So far no replacement reproductives have been found.

1.3.2.3. *Nasutitermitinae*

Two evolutionary lines are recognizable in *Nasutitermitinae* the *Nasutitermes* and *Subulitermes* lines. In the most primitive genus, *Syntermes* a marked sexual dimorphism is evident, males being the largest and passing through at least two instars. The majority of soldiers are males. In the *Nasutitermes* line the dimorphism of workers seems to be general and, in contrast, the females are the largest. Workers generally go through several stages of development and soldiers are males (*NOIROT*, 1969).

In *Leptomyxotermes dorine* the soldiers are both females and males. Soldiers usually arise from workers or from larvae of a special type as *WEESNER* found in *Tenuirostritermes tenuirostris* (*WEESNER*, 1953, *NOIROT*, 1955); and *SANDS* (1965) found in the development of minor soldiers of *Trinervitermes*. Soldier polymorphism is rare in the *Subulitermes* line, but common in the *Nasutitermes* line and a rule in *Trinervitermes* (*NOIROT*, 1969). In this genus, major soldiers develop from minor workers and minor soldiers from a special type of male larvae (*NOIROT*, 1955, 1969). In fact, male workers are not functional, but are rapidly transformed into soldiers; therefore, all the functional workers are females and all the soldiers are males.

Nymphoid neotenics have been most frequently encountered, particularly in *Armitermes*. Under experimental conditions *NOIROT* (1969) obtained adultoid reproductives in *Nasutitermes arborum*.

1.3.2.4. *Macrotermitinae*

This is a fungus growing sub-family and they have three larval instars, instead of two as described in the previous sub-families in the development of workers (*NOIROT*, 1969). In most species, major workers are males which is the opposite of what is known in most of the species in the other sub-families. Workers have only a single stage and soldiers are females except in *Sphaerotermes* where they are males. Two categories of soldiers, minor and major, are frequently found, major ones

derived from minor workers and minor ones from third instar larvae. These are common soldier types in *Macrotermes*, *Ancistrotermes*, and *Pseudocanthotermes*. In *Sphaerotermes*, *Protermes*, *Microtermes* and some *Odontotermes* only one type of soldier is present. However, in *Acanthotermes acanthothorax*, three types of soldiers are found.

Only adultoid replacement reproductives have been reported from *Macrotermitinae* (*HARMS*, 1927; *COATON*, 1949; *ROY-NOEL*, 1974; *BORDEREAU*, 1975).

Caste determination in higher termites seems to be under the control of extrinsic factors as in lower termites, although not much evidence is available to support this view. However, the fact that the soldiers are often formed from a portion of workers indicates that there is no genetical difference between the two castes. Also nymphal-soldier intercastes, described (*ADAMSON*, 1940; *GAY*, 1952, *NOIROT*, 1955; *BOUILLON* and *MATHOT*, 1964) show that no genetic differences exist between soldiers and the reproductives.

## 1.4. *MECHANISMS OF CASTE DETERMINATION*

Some important points have been mentioned above regarding mechanisms regulating the production of the reproductive castes and soldiers in lower termites. A pheromonal system has been implicated. Another factor which seems to be important should be mentioned here and that is the sensitive period within a given instar. For example, the development of replacement

reproductives is determined at the beginning of an intermoult in *Kalotermes* (LÜSCHER, 1952, b) while the determination of presoldier development generally occurs in *Kalotermes* (SPRINGHETTI, 1972) and *Zootermopsis* (LÜSCHER, 1974, a) only during the second half of the moulting interval.

Relatively constant proportions of castes are maintained in colonies, probably through inhibitory mechanisms whereby each caste inhibits the differentiation of the same caste, as discussed above. Castes may also have stimulatory effects upon the production of other castes. For instance, a significant stimulation of soldier production by reproductives was shown in *Kalotermes* (SPRINGHETTI, 1970), in *Prorhinotermes simplex* (MILLER, 1942) and in *Zootermopsis* LÜSCHER (1973). Soldiers were shown to have a stimulatory effect on the production of replacement reproductives in *Kalotermes* (SPRINGHETTI, 1969) and on a late production in *Zootermopsis nevadensis* (LÜSCHER, 1975).

While a pheromone system has been demonstrated in the reproductives by extraction, and feeding the extracts of parts of the female replacement reproductives to the larvae, with a subsequent inhibition of replacement reproductive development (LIGHT, 1944,b) and locating the source (LÜSCHER, 1974,a) of the pheromone. However, no direct evidence has been found showing that an inhibition pheromonal system exists in soldiers as well.

In a given individual larval determination is due to the influence of endocrine system. It has been shown in a number

of species of lower species of termites that juvenile hormones (JH) or their analogues induce soldier formation (*LÜSCHER*, 1969; *HRDY*, 1972; *HRDY* and *KRECEK*, 1972; *WANYONYI*, 1974; and *LENZ*, 1976, a) and that JH inhibits replacement reproductive development in the absence of reproductives (*LÜSCHER*, 1974, b). It seems therefore, that the pheromone must acts on the endocrine system to produce the desired effect.

Mechanisms underlying caste differentiation in higher termites are almost unknown. *KAISER* (1956) showed that prothoracic glands are more enlarged in nymphs than in larvae of *Anoplotermes pacificus*, and that this difference is already apparent at the end of the first larval stage which is composed of morphologically identical individuals. *NOIROT* (1969) ascertained similar facts in *Termes hospes*. The Prothoracic glands also have an effect on the formation of neotenics since they become enlarged at the time of the sexual moult (*KAISER*, 1956; *NOIROT*, 1969); but degenerate in the functional neotenics as they do in imagoes.

Very little is known on the role of corpora allata (CA) in the differentiation of soldiers in higher termites. Again *KAISER* (1956) showed that CA become enlarged at the time of the moult transforming workers into soldiers in *Neocapritermes*, *Microcerotermes* and *Nasutitermes*, thus implying that JH is required for soldier determination in higher termites as well.

## 2. GENERAL MATERIALS AND METHODS

The study was conducted on *Macrotermes michaelseni*. This species found in closed mounds is predominant in Kajiado area, Kenya some 80 Km. South-West of Nairobi and was earlier thought to be *M. subhyalinus* which in fact, inhabits mounds with open chimneys (Dr. *Ruelle* recently resolved the problem using samples sent to him from the area). Materials used in the study were larvae, nymphs, imagoes, workers and soldiers. Imagoes were used basically for establishing incipient colonies for various studies described in details below. Larvae and presoldiers, nymphs, workers and soldiers were used for studies on polymorphism and histological investigations of the endocrine system in relation to caste differentiation.

### 2.1. *COLLECTION OF MATERIALS*

All materials were collected as required from Kajiado. Mounds were dug until the nest was reached. Nest material containing larvae, workers, soldiers and nymphs during the right season was scooped out with a spade and carefully placed in large plastic basins ready for transportation to the laboratory. Imagoes (Alates) were collected during the swarming season (November - January) the period of short rains in that area. Light-traps were used to collect the alates near the mounds from which they swarmed. The alates were taken to the laboratory to be de-winged and colonies established as described below.

Materials for biometric studies were sorted out randomly from basins of field materials or from incipient colonies and the parameters required were measured with stereo-microscope in fresh or fixed form (80% alcohol was used for fixation).

2.2. *HISTOLOGICAL AND HISTOCHEMICAL PROCEDURES*

Some fresh materials were used for histological investigations. Alcoholic Bouin's solution was used for fixation which was generally for 24 hours. In order to allow quick and effective penetration of tissues by the fixative, the abdomens and head capsules of the individuals to be fixed were snipped. Tissues were dehydrated through graded alcohol up to xylene and embedded in paraplast before sectioning and subsequent hydrating. From distilled water, the sections were stained with Carazzi - Ehrlich's Hamalum (nuclear stain) and then, counter-stained using 1% erythrocin (eosin). Stained sections were subsequently dehydrated and then mounted in D.P.X. or Canada balsam, allowed to dry away from direct light and stored ready for examination and photography.

For the histochemical study, testing for glycogen in CA, materials were fixed in a cold saturated solution of picric acid in absolute ethanol. Subsequent procedures were exactly the same as described above up to the staining stage where sections were processed through periodic acid-schiff (PAS reaction) procedure as described by *PEARSE* (1968). Sections

were brought to water. One part, used as control, was pre-treated with saliva. The slides were finally dehydrated using the standard procedure and mounted in D.P.X. or Canada balsam ready for observations.

2.3. *DETERMINATION OF JUVENILE HORMONE TITRES*

The collection of haemolymph for juvenile hormone (JH) titres was conducted as follows: The haemolymph of larvae to be tested was collected by cutting a prothoracic leg and gently squeezing the abdomen. The haemolymph which oozed out was collected into capilary tubes of 5-10µl capacity, pulled into 30µl tubes. For juvenile hormone titre determination, the haemolymph was used straight away to avoid any break-down by the esterases.

The Galleria wax test of *SCHNEIDERMAN* and *GILBERT* (1958) as modified by *de Wilde* et al. (1968), was used in the present study. A local strain of *Galleria mellonella* was reared on a semi synthetic diet as described by *BECK* (1958). Fully grown larvae which occupied the glass tubes provided were kept at $30^{o}C$. Pupation usually occurred within 7 and 10 days. The larvae were checked daily and those which pupated on the same day (within 24 hours) were pulled and used for the test.

About one $mm^2$ of cuticle plus epidermis was excised from the median crest of the mesonotum. The wound was then sealed off with 1.8 mg. of the test material in a warm metal loop. The test material was prepared as follows:

A given volume of haemolymph was mixed with 2 pellets of bee-wax (melting point 52°C each weighing 12.5 mg. These were warmed until melted, stirred and mixed with 1 drop of olive oil, weight 12.5 mg.

Two controls were used. A known dilution of Cecropia JH II (a gift from the late *Professor LÜSCHER*), and pure wax mixed with olive oil after the same procedure as above. The treated pupae were then put in moist plastic boxes and kept at 30°C for seven days after which the reading was made. A positive result was indicated by a patch of pupal cuticle in the wound area, as judged by the brownish colour and the characteristic surface structure. A negative result was considered when normal adult-cuticle formed in the wound area.

2.4. *DETERMINATION OF ECDYSONE TITRE IN THE HAEMOLYMPH*

Part of the haemolymph collected as described above was used for the determination of ecdysone titre. Larvae of a local strain of *Musca domestica* were used for the test. Eggs which were laid on the same day were collected and put into jars containing food for larvae which hatched. Most larvae had reached final larval instar (fourth instar) by about the sixth day. Pupation started and when about 10% of the larvae had pupated, mature larvae were selected and ligated by the procedure of *KAPLANIS* et al. (1966) as modified by *STAAL* (1967). The ligation was carried out using rubber bands in which tiny holes had been burned with a hot needle The holes were stretched around a hollow tube connected to a T-suction tube. Larvae

were then inserted head down into this tube in succession for ligation and when the rubber band was removed from the tube, the ligature simply tightened over the 7th - 8th segment, thus, isolating the thoracic-head portion from the abdomen. No second ligation was required. Tens of larvae could be ligated using the same rubber band. The ligated larvae were kept in most plastic boxes at 30°C overnight. The following day, larvae which pupated at the anterior end were selected and injection followed.

The larvae were injected with 2µl of test material (sample extracted in 10% ethanol, or controls (i) 10% ethanol (ii) standard β-ecdysone) using a 50µl Terumo syringe mounted in a Hamilton repeating dispenser. The needle was inserted near-the mouth parts and pushed towards the posterior part of the abdomen where the test material was released. The treated larvae were then kept overnight under the same conditions mentioned above. The evaluation of the results was carried out the following day. The scoring was a graded one as for the Calliphora assay (*KARLSON*, 1956), i.e., 1.0 was given for complete sign of pupation; 0.75 was for marked; 0.50 was for slight, 0.25 for very slight and 0 for no pupation response at all. Positive response was therefore considered as a score of 0.5 or above. The results were expressed as a percentage of the number of larvae surviving.

2.5. *JUVENILE HORMONE ANALOGUE TREATMENT PROCEDURES*

The juvenile hormone analogue used for treating experimental

larvae was ZR - 515, (a gift from *Dr. Staal* of Zoecon Corporation, Palo Alto, California, U.S.A.). This chemical trademarked - "Altosid IGR" is a methoprene of the following chemical structure (*HENRICK, STAAL* and *SIDDALL*, 1976).

ZR - 515

The carbon skeleton of this structure is related to those of the three known natural juvenile hormones:

JH III $C_{16}$ (Judy et al.,1973)

JH II $C_{17}$ (Meyer et al.,1968)

JH I $C_{18}$ (Roller et al.,1967)

But Altosid is known to be more stable than juvenile hormones

and less expensive, therefore, it was considered the most suitable analogue to use in the study on caste differentiation as a JH. mimic.

The procedure used was either topical or vapour treatment of different stages of larvae collected from incipient colonies. Redistilled acetone was used as a solvent for the analogue. Details of the procedures are given in the methods of the appropriate chapter (see below).

# 3. INSTARS AND POLYMORPHISM OF CASTES FROM FIELD COLONIES

## 3.1. *INTRODUCTION*

Polymorphism has long been known in the lower termites. One can refer as far back as the days of *GRASSI* and *SANDIAS* (1893-1894) who recognized reproductive and soldier castes in *Kalotermes flavicollis* and attempted to explain the mechanism of their development. Basing his scheme on the works of *GRASSE* and *NOIROT* (1946 a, b; 1947) *LÜSCHER* (1952 a,b) presented a scheme of development in *K. flavicollis*. No sexual dimorphism was found in larvae or other stages of development. Five larval instars were established, the fifth instar larvae capable of moulting into a pseudergate, a presoldier, a replacement reproductive or a first instar nymph. There are two nymphal instars preceding the imaginal moult and one moult from presoldier to soldier. Soldiers can develop from any of the stages, starting from third instar larvae through second instar nymphs. Soldiers could be of either sex and polymorphism observed among them depends on the stage from which they develop.

*HEATH* (1927) worked on polymorphism of *Zootermopsis nevadensis* using biometrics. His results were later improved upon by *CASTLE* (1934) and later *MILLER* (1969) and *LÜSCHER* (1974, a) summarized them. The scheme of development in this species was shown to be basically the same as that of *Kalotermes*.

Within the genus *Reticulitermes* little difference in the

developmental scheme occurs, therefore, the development of *R. lucifugus* may serve as an example. *BUCHLI* first (1958) showed that true workers do not exist in this species. *LÜSCHER* (1974, a) gave a scheme of development for this species based on *BUCHLI's* findings. The nymphs develop from second instar larvae and go through six instars before the imaginal moult. From the same second instar larvae, pseudergates may develop or they may develop from any nymphal stage. Reversible development in the nymphs does not occur. Replacement reproductives may develop from any of the nymphal stages or pseudergates. Presoldiers develop exclusively from any stage of pseudergates.

*LÜSCHER* (1974), a) summed the work of *MILLER* (1942) and others on *Prorhinotermes* concerning the fact that the development of nymphs occurs much later than in *Reticulitermes*, in fact, first instar nymphs develop from seventh instar larvae and go through only two instars to the terminal caste (Imago). Nymphal development is also not reversible. Soldiers and replacement reproductives and soldiers may develop from third instar larvae or later up to the first instar nymphs. No true workers exist, except pseudergates which develop from either seventh instar larvae or first instar nymphs.

*CLEMENT* (1952) showed that in *Psammotermes hybostoma*, there are no true workers because the so called workers could moult into presoldiers.

Up to this level of development, no larval dimorphism has been shown. However, recently, *RENOUX* (1970, 1976) showed in *Schedorhinotermes lamanianus* that after the first larval moult two categories may be detected on the basis of head capsule measurements. Minor presoldiers develop from small third instar larvae. The so-called workers develop from large larvae of the third instar. Since these workers are capable of moulting, they may be considered pseudergates. Another striking difference between this species and those discussed above is the early appearance of the so called major soldiers. It seems also that soldiers may develop from later stages of pseudergates. All neuter castes are females and male larvae develop exclusively into male reproductives through 3 larval stages and four nymphal stages. The first 3 nymphal instars have also the capacity to develop into replacement reproductives. The development of reproductives was a hypothetical formulation of *LÜSCHER* (1974, a). This kind of early determination of castes and sex specialization shows a higher evolutionary level in this species tends towards that of the higher termites.

From fragmentary evidence based on work by *CLEMENT* (1956) it seems that in *Hodotermitidae*, as represented by *Anacanthotermes ochraceus* the developmental scheme is close to that of *Schedorhinotermes* except that all neuter castes are males. Therefore the development in *Hodotermitidae* appears also to resemble that of the higher termites.

Studies on termite polymorphism have also been extended to higher termites, as illustrated in the following examples of intensive work by *NOIROT* (1955) on polymorphism of *Termitidae; BOUILLON* and *MATHOT* (1964) on *Cubitermes exiguus; SANDS* (1965) on five species of *Trinervitermes; McMAHAN* and *WATSON* (1975), *WATSON* and *ABBEY* (1977) on *Nasutitermes exitiosus,* and a few others reviewed by *NOIROT* (1969) and in the general introduction. *NOIROT* has elaborated on a scheme of development for *Bellicositermes bellicosus* (now *Macrotermes bellicosus)* which shows an early differentiation between male and female larvae occuring at the moult to second instar. The larger larvae develop into major workers, the smaller ones which are females, develop into minor workers, minor or major soldiers. This scheme is modified by *LUSCHER* (1976). In view of work on caste differentiation carried out on *M. michaelseni,* it was necessary also to investigate the development in this species.

## 3.2. *MATERIALS AND METHODS*

Larvae, workers and soldiers were collected from five mounds near Kajiado, Kenya and kept in plastic basins with mound soil and fungus combs. Moisture was maintained by use of paper towels soaked in water. Nymphs usually first appear in the mounds shortly after the beginning of the long rains in April or May and swarming of alates occurs during the short rains (November-January).

Different instars of nymphs were therefore collected between April and October and the reproductives in December during swarming. Materials were randomly taken from the field collection for biometric work. The determination of larval and nymphal instars and polymorphism was then conducted as follows: Measurements were made of head capsule width, the posterior tibia length and the antennal length using a stereomicroscope with an ocular micrometer. The number of antennal segments was determined. In order to recognize the sexes of larvae in each of the groups defined biometrically, the abdomens were dissected medially and the halves were fixed in 70% ethanol for 1-2 days. They were then stained in matured alcoholic borax carmine prepared as described by *ROMEIS* (1968). The guts, malpighian tubules and any fat body obscuring the sex organs were removed before the material was dehydrated in 96% ethanol and cleared in xylene. The specimens were then mounted in D.P.X. and examined under a compound microscope. The sexes were distinguished as described by *NOIROT* (1955). The shape of the rudimentary sex organs and the position of their openings made it possible to distinguish between male and female individuals. Males have shorter but thicker sex organs, having lobes at the anterior end and opening between the ninth and tenth segments. Females, on the other hand, have rather thin ovaries of about 3-4 cell layers thickness extending over

almost the whole abdominal length and opening between the seventh and eighth segments.

## 3.3. *RESULTS*

### 3.3.1. *Larval instars and polymorphism in adult neuter castes*

The distribution curves of head capsule width, the posterior tibia length and the antennal length measurements of the same individuals are presented in Figures 1 a, b and c, respectively. These figures show that the larvae studied fall under 6 categories as represented by the histograms. The head capsule width measurements seem to give the most obvious separation between the larval groups since the dispersion of the measurements of individuals in each group is relatively small and there is a clear margin between groups except for groups 5 and 6. The other two measurements also give a good separation between larval groups.

Table 1 summarizes the mean values of the measurements taken of head capsule width, posterior tibia length, antennal length and the number of antennal segments of different groups of larvae and adult neuter castes. The measurements show a gradual, but definite increase in size of all parts of the animals measured throughout their development into the adult castes. They also show two apparent types of minor workers: (Fig. 2) one rare type being larger in all measurements (Table 2) which has proved to be a major presoldier precursor or fourth instar larva, as demonstrated below, and the other smaller type which remains as the true minor worker.

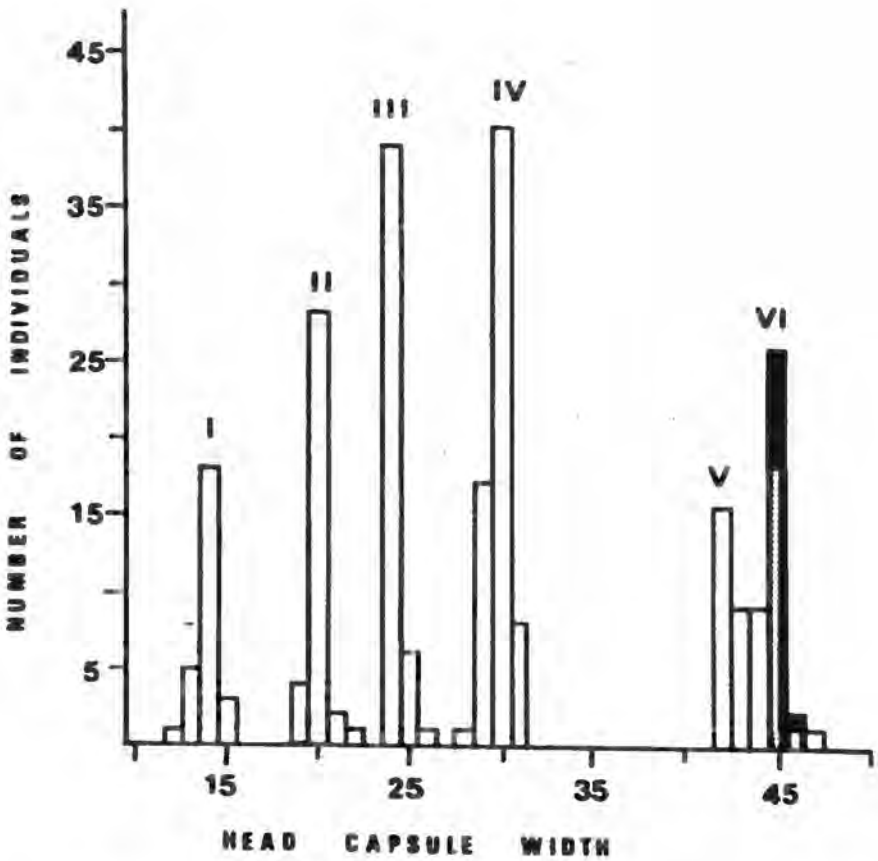



FIGURE 1a, shows the distribution histograms of head capsule width of larvae. I - VI are larval groups. Measurements were in arbitrary units, one unit = 0.04mm.

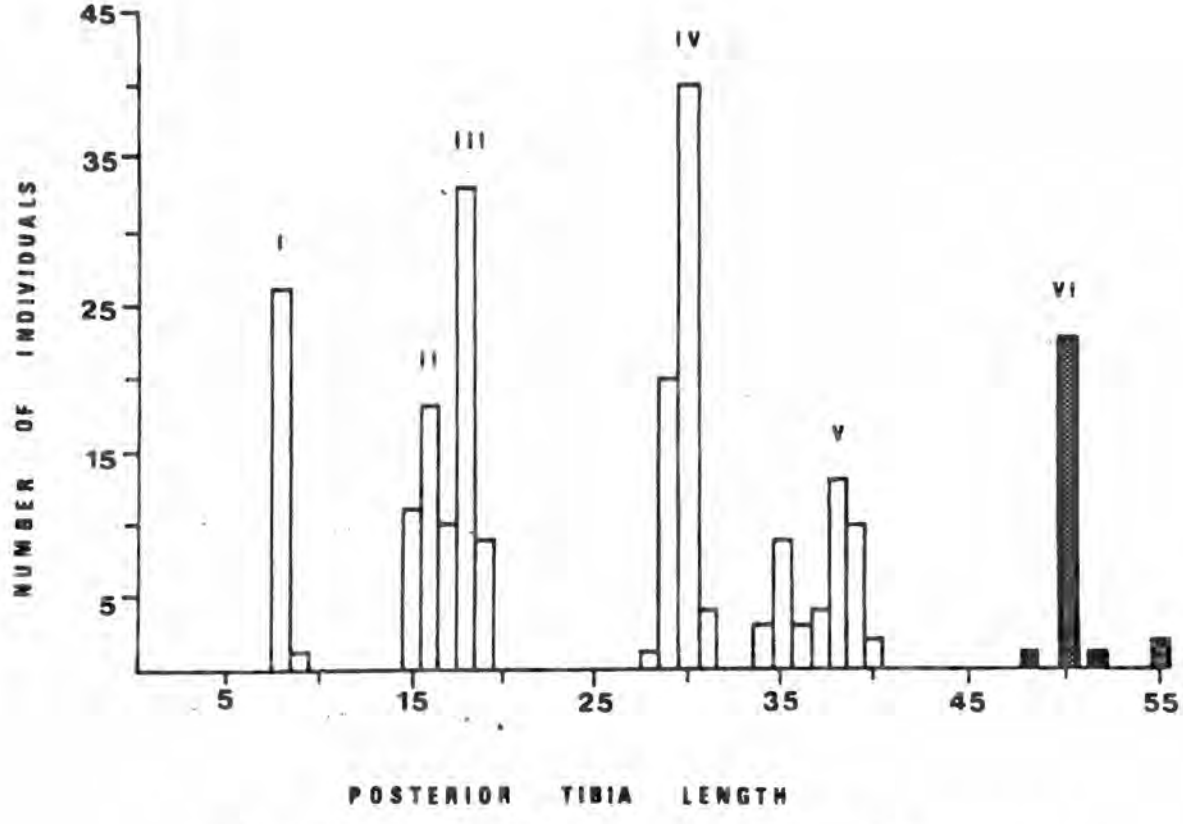



FIGURE 1b, shows the distribution histograms of posterior tibia length of larvae. I - VI are larval groups. Measurements were in arbitrary units, one unit = 0.04mm.

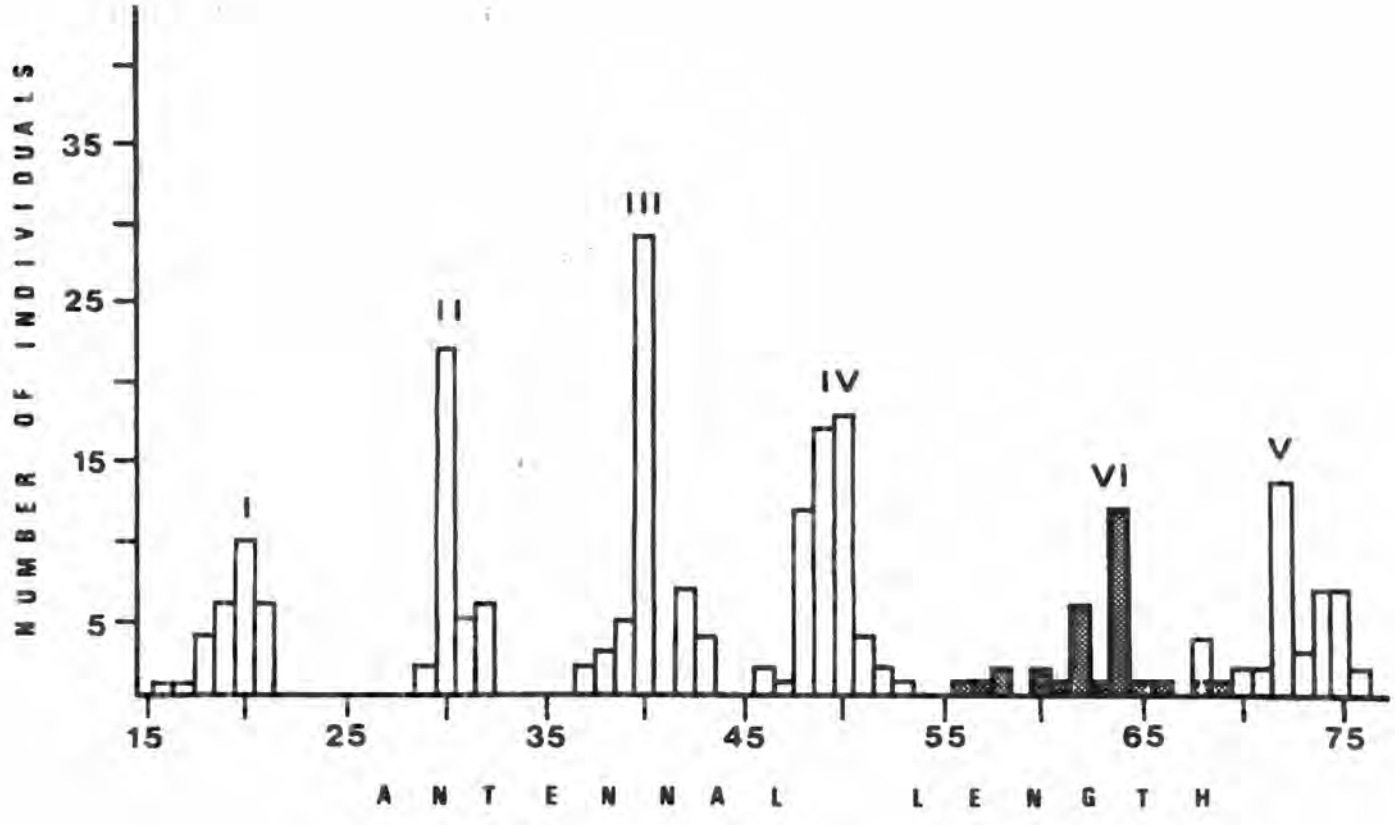



FIGURE 1c, shows the distribution histograms of antennal length of larvae. I - VI are larval groups. Measurements were in arbitrary units, one unit = 0.04mm.

| Group No. | Development Stage | Sample Size | Head Capsule Width(mm) ($\bar{x} \pm$ S.D.) | Posterior Tibia Length(mm) ($\bar{x} \pm$ S.D.) | Tibia Length/ Head Width | Antennal Length(mm) ($\bar{x} \pm$ S.D.) | No. of Antennal Segments |
|---|---|---|---|---|---|---|---|
| 1 | 1st Instar Larvae ♂♀ | 27 | 0.55 ± 0.03 | 0.32 ± 0.01 | 0.58 | 0.78 ± 0.06 | 13 |
| 2 | 2nd Instar Larvae ♀ | 35 | 0.80 ± 0.02 | 0.64 ± 0.03 | 0.80 | 1.24 ± 0.11 | 15 |
| 3 | 2nd Instar Larvae ♂ | 46 | 0.97 ± 0.02 | 0.72 ± 0.02 | 0.75 | 1.60 ± 0.04 | 15 |
| 4 | 3rd Instar Larvae ♀ | 67 | 1.19 ± 0.03 | 1.19 ± 0.02 | 1.0 | 1.97 ± 0.12 | 17 |
| 5 | 3rd Instar Larvae ♂ | 46 | 1.76 ± 0.05 | 1.49 ± 0.07 | 0.85 | 2.91 ± 0.12 | 17 |
| 6 | 4th Instar Larvae ♀ | 29 | 1.80 ± 0.01 | 2.02 ± 0.06 | 1.12 | 2.51 ± 0.11 | 17 |
| — | Minor Workers | 38 | 1.64 ± 0.08 | 1.70 ± 0.09 | 1.04 | 2.33 ± 0.13 | 17 |
| — | Major Workers | 29 | 2.66 ± 0.13 | 2.33 ± 0.09 | 0.88 | 3.83 ± 0.15 | 18 |
| — | Minor Presoldiers | 23 | 1.75 ± 0.10 | 1.99 ± 0.05 | 1.14 | 3.63 ± 0.09 | 17 |
| — | Major Presoldiers | 45 | 3.11 ± 0.36 | 2.79 ± 0.16 | 0.90 | 3.86 ± 0.23 | 17 |
| — | Minor Soldiers | 30 | 2.78 ± 0.11 | 2.94 ± 0.05 | 1.06 | 4.61 ± 0.17 | 17 |
| — | Major Soldiers | 30 | 4.55 ± 0.17 | 3.77 ± 0.11 | 0.83 | 5.37 ± 0.26 | 17 |

Table 1. Values of measurements made on larvae from different groups and terminal neuter castes.

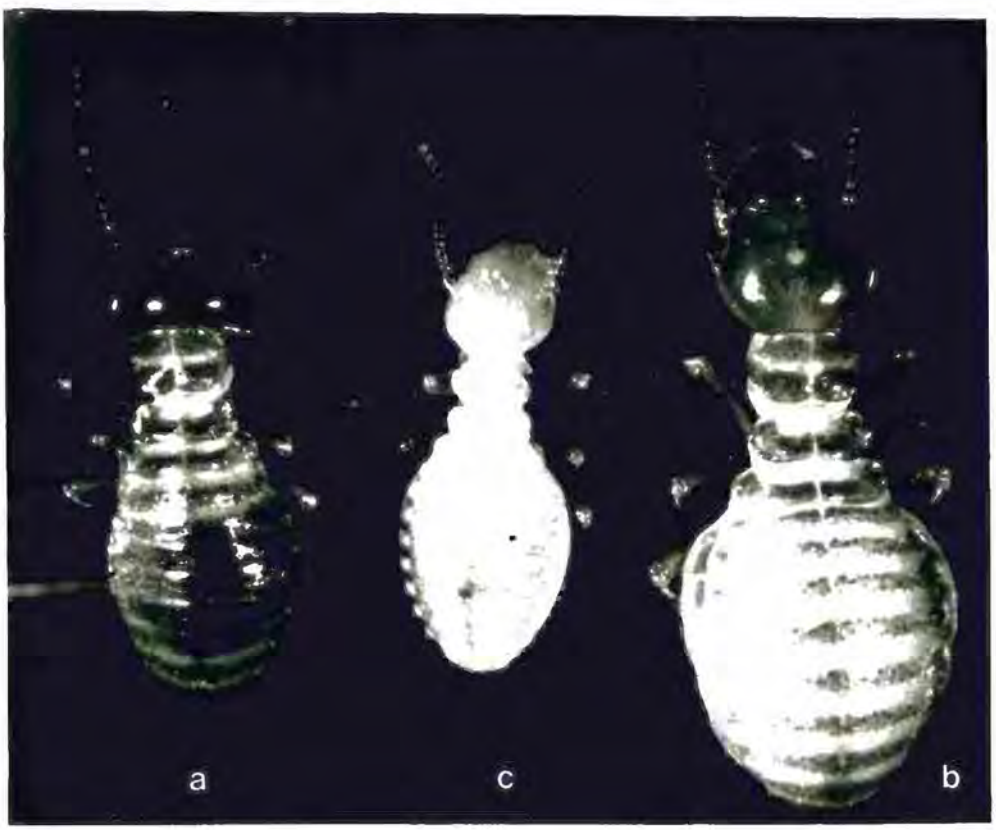



FIGURE 2. Two apparent types of minor worker, (a)- A normal functional type, smaller and cannot moult any further. (b) - Non-functional, larger, less pigmented and able to moult into major presoldier (4th. instar) (c) - Third instar female larva.

| Stage | n | Head Capsule Width (mm). | Posterior Tibia Length (mm) . | Antennal Length (mm) . | Mandibles | Pigmentation | Type of moult | Capacity to work |
|---|---|---|---|---|---|---|---|---|
| | | Mean ± S.D. | Mean ± S.D. | Mean ± S.D. | | | | |
| Minor Workers | 38 | 1.64 ± 0.08* | 1.70 ± 0.09** | 2.33 ± 0.13*** | Worker mandible without future presoldier mandible | Darkly pigmented | Do not moult | Functional |
| Special Type of Minor Workers (4th. Instar Larvae) | 28 | 1.80 ± 0.01* | 2.02 ± 0.06** | 2.51 ± 0.11*** | Worker mandible enclosing future presoldier mandible | Lightly pigmented | Moult into major presoldiers | Non-functional |

Students' Test

* P 0.001, ** P 0.001, *** P 0.001

Table 2. The Characteristics of a normal functional worker and a non-functional rare type (4th instar or major presoldier precursor)

The distributions of head capsule width, posterior tibia length and antennal length show that presoldiers from the field are of two sizes, small (minor) and large (major (Figs. 3 a, b and c). Similar observations were made in case of soldiers (Figs. 4 a, b and c). Figures 5 a, b and c also show bimodal distributions of the measurements from workers. It is clear therefore that soldiers and workers are of minor and major types.

A logarithmic plot of head capsule width against posterior tibia length shows an almost linear relationship between these two parameters (Fig. 6). The first, second, fourth, and sixth larval groups and minor workers fall within one curve (Curve A, Fig. 6). Group 1 larvae are morphologically identical. It appears that during their first moult some of these larvae grow and become larger than others, thus separating into two groups, 2 and 3. The second group individuals are smaller than those in the third. Subsequently, larvae in these two groups may moult into minor workers and a small proportion into group 6 larvae, while group 5 moult exclusively into major workers.

The antennae of larvae are of interest. Group 1 larvae have antennae with 13 segments, whereas those of groups 2 and 3 larvae have 15 each, while those of groups 4, 5 and 6 have the largest number of segments, 17 (Table 1). It seems that a degree of parallel development exists between first, second, fourth and sixth groups of larvae on one hand and first, third and fifth on the other as shown by the equal numbers of antennal segments in the paired groups of larvae (II and III, IV and V)(Fig. 7).

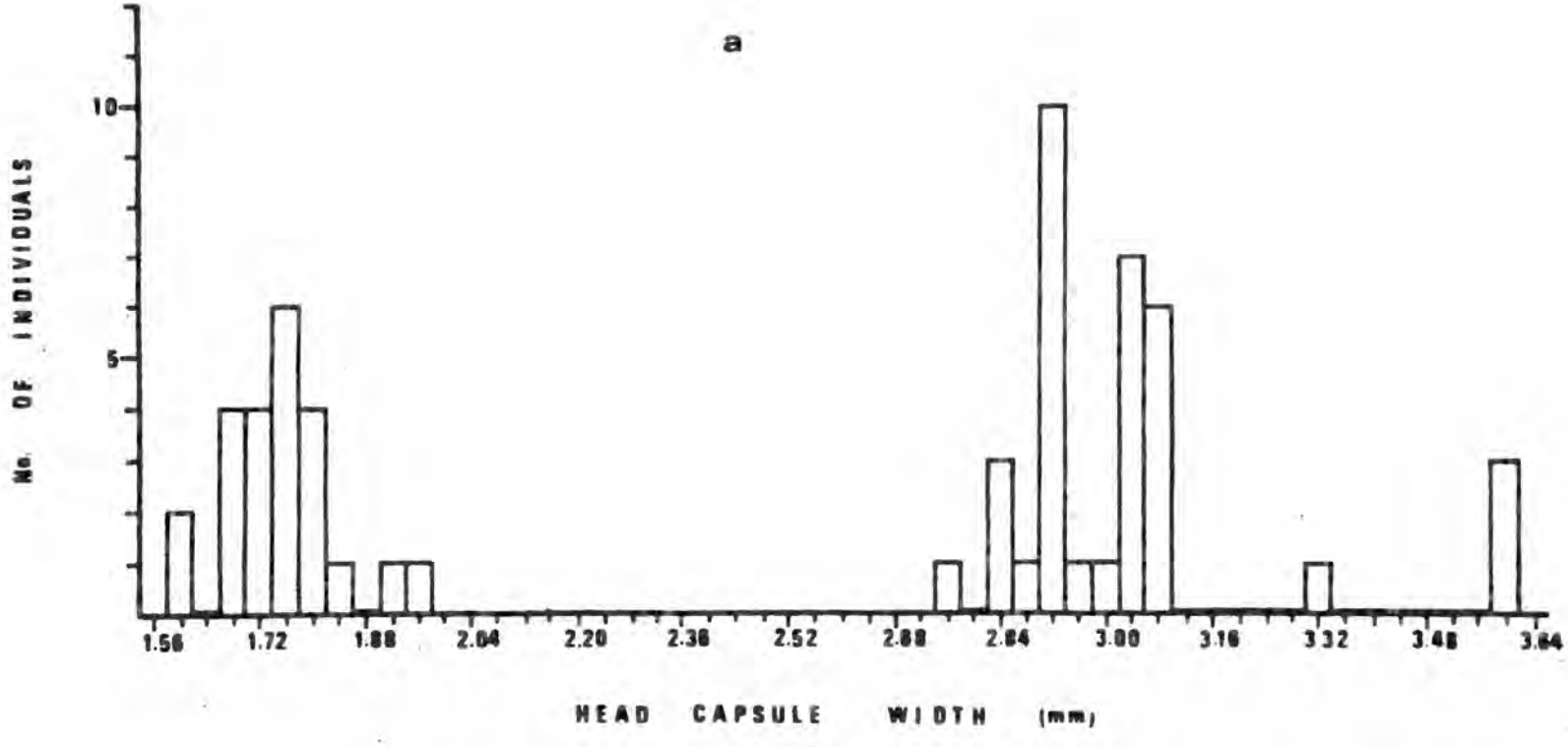



FIGURE 3a, shows a bimodal distribution of presoldier head capsule width.

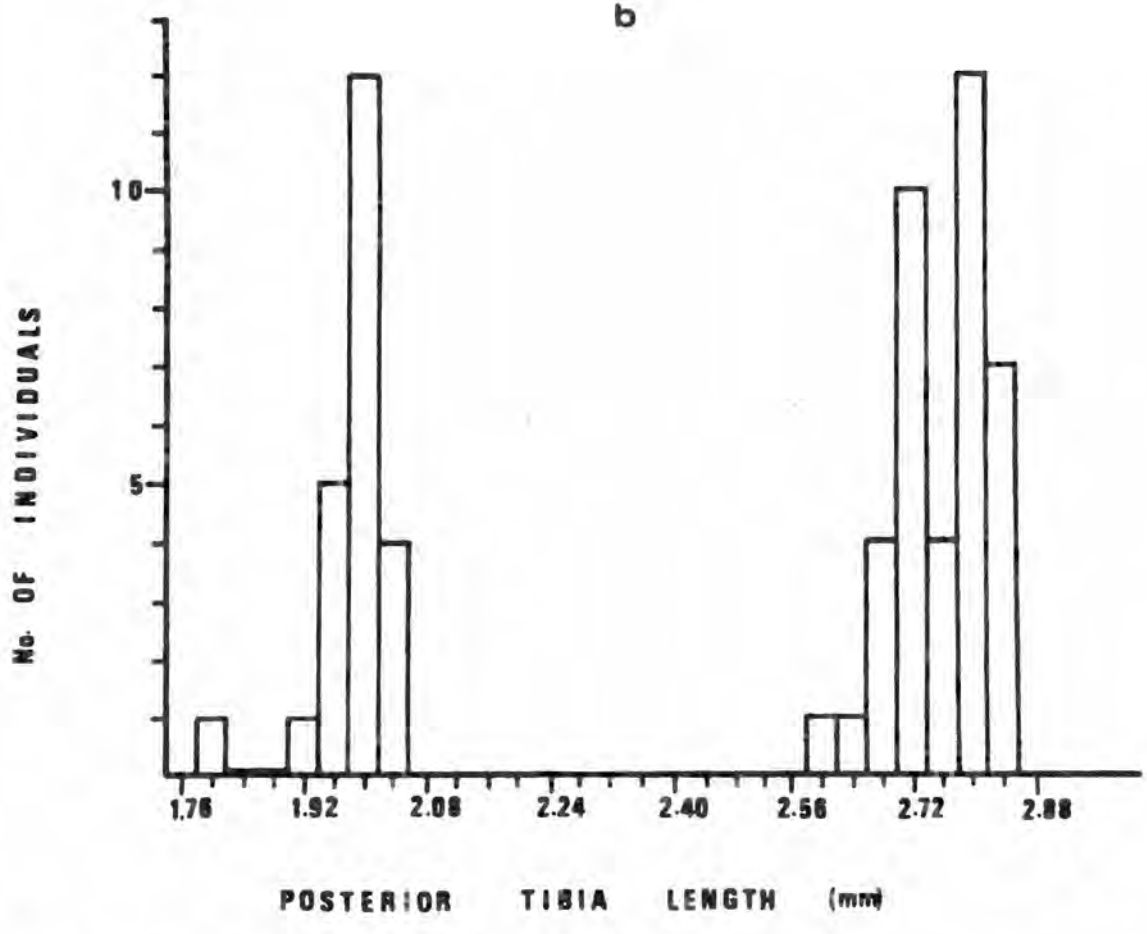



FIGURE 3b, shows a bimodal distribution of presoldier posterior tibia length.

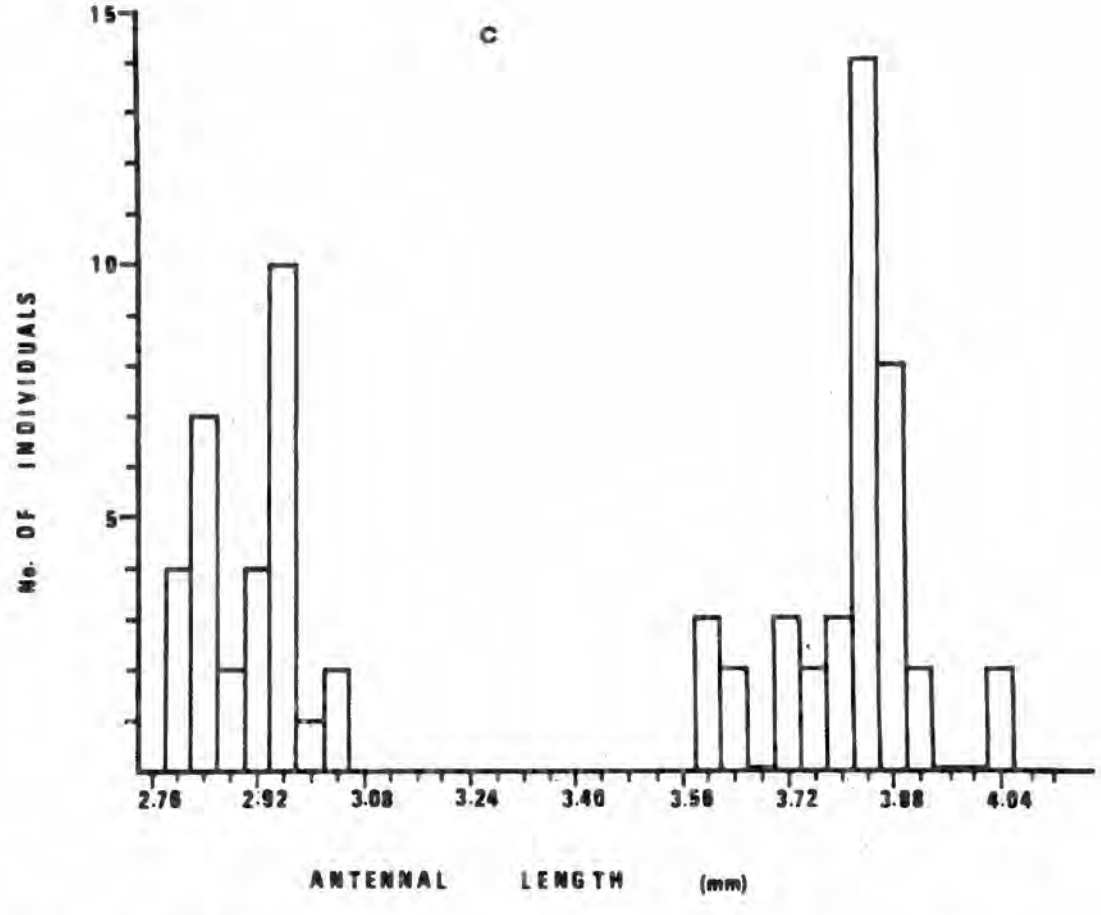



FIGURE 3c. Shows a bimodal distribution of presoldier antennal length.

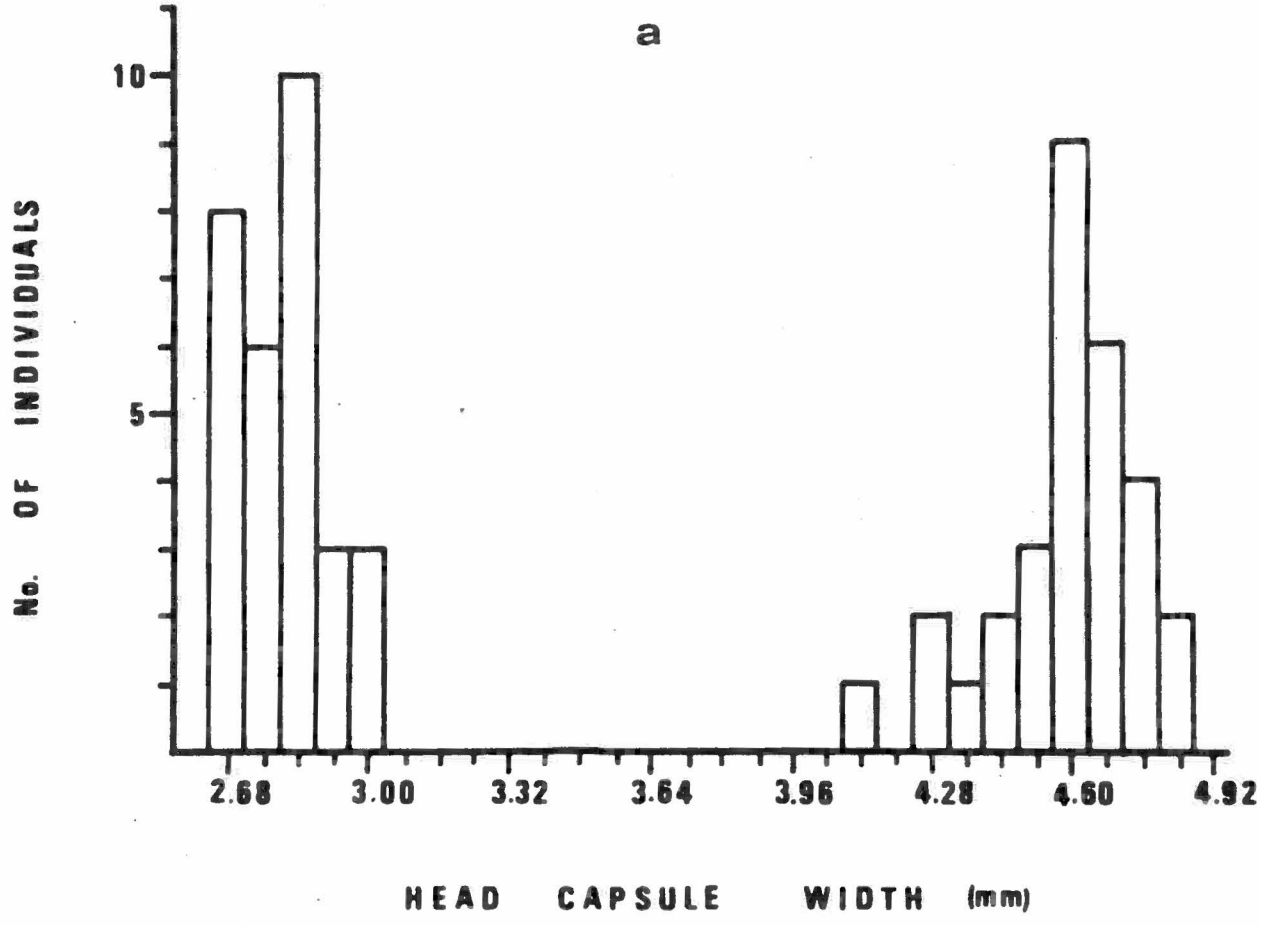



FIGURE 4a, shows a bimodal distribution of soldier head capsule width.

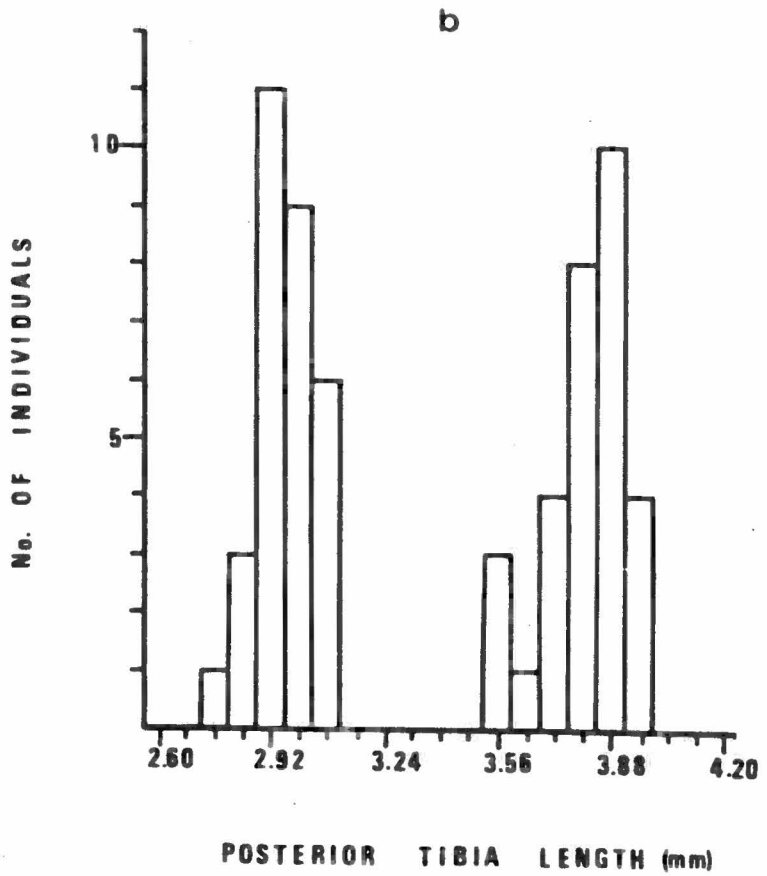



FIGURE 4b, shows a bimodal distribution of soldier posterior tibia length.

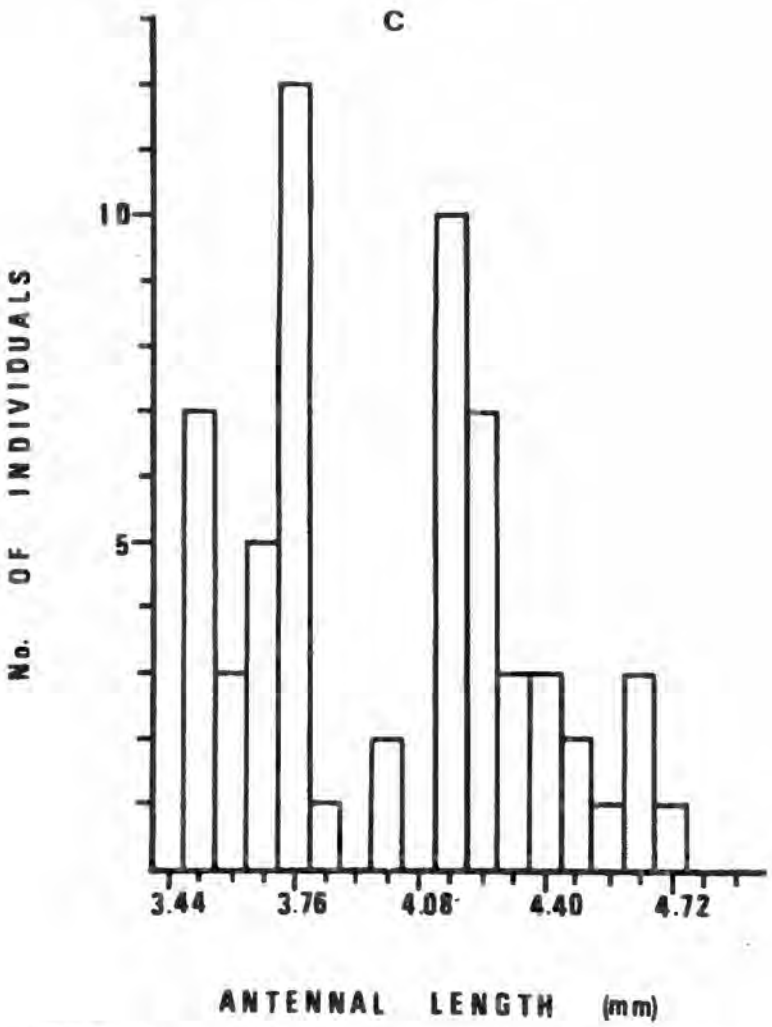



FIGURE 4c shows a bimodal distribution of soldier antennal length.

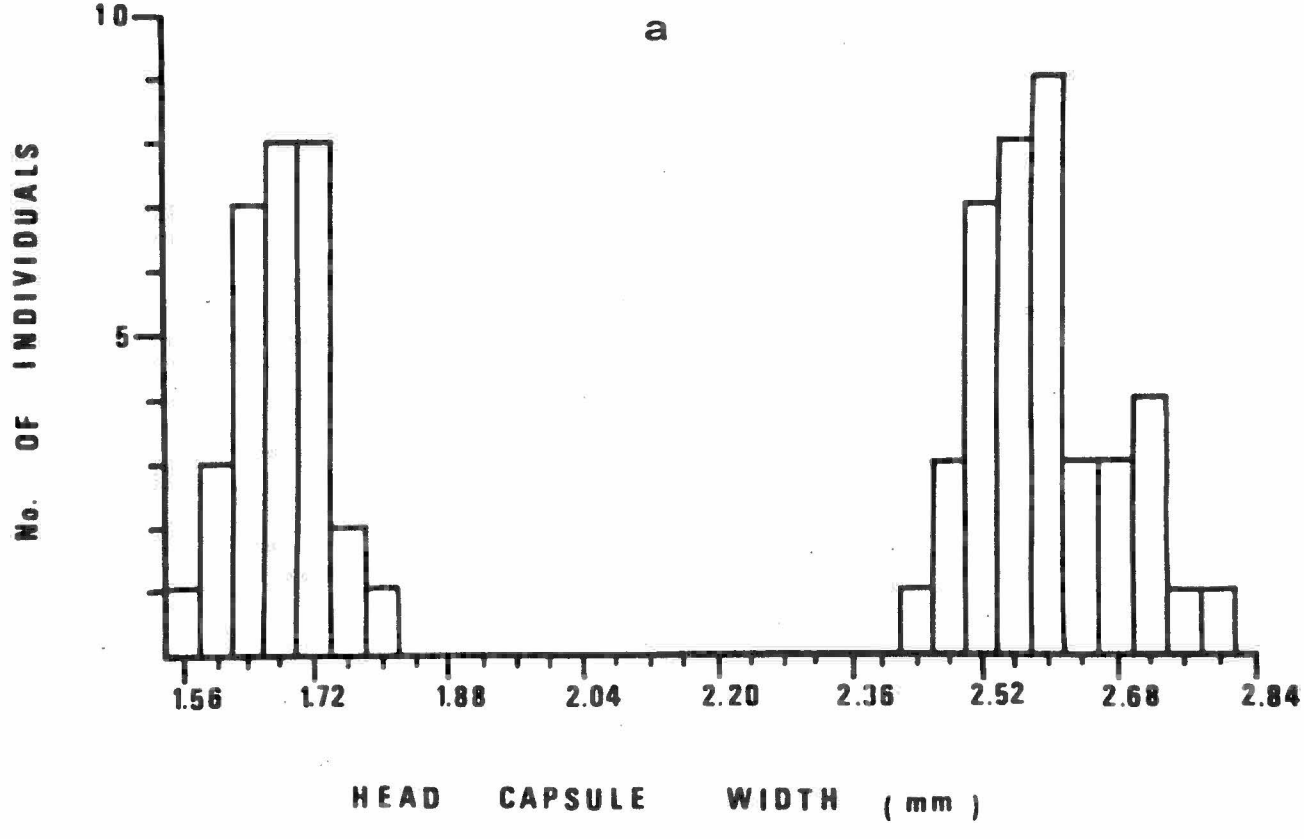



FIGURE 5a, shows a bimodal distribution of head capsule width of workers.

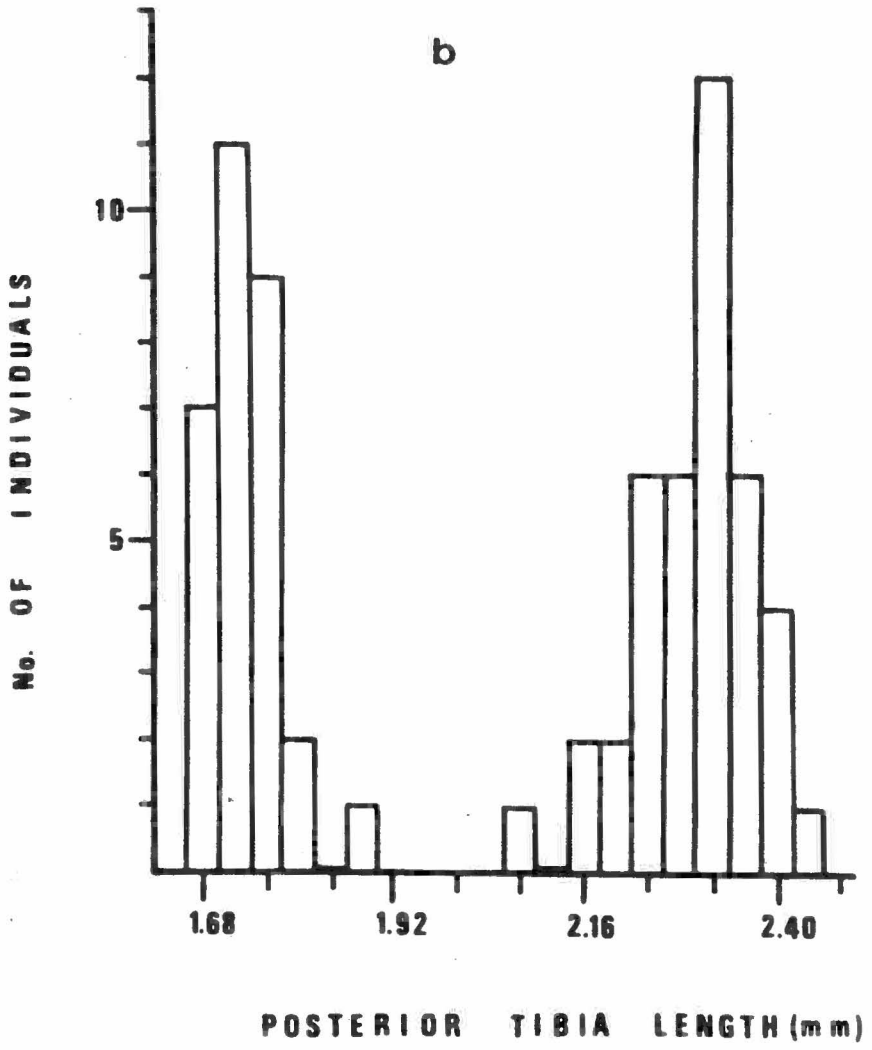



FIGURE 5b, shows a bimodal distribution of posterior tibia length of workers.

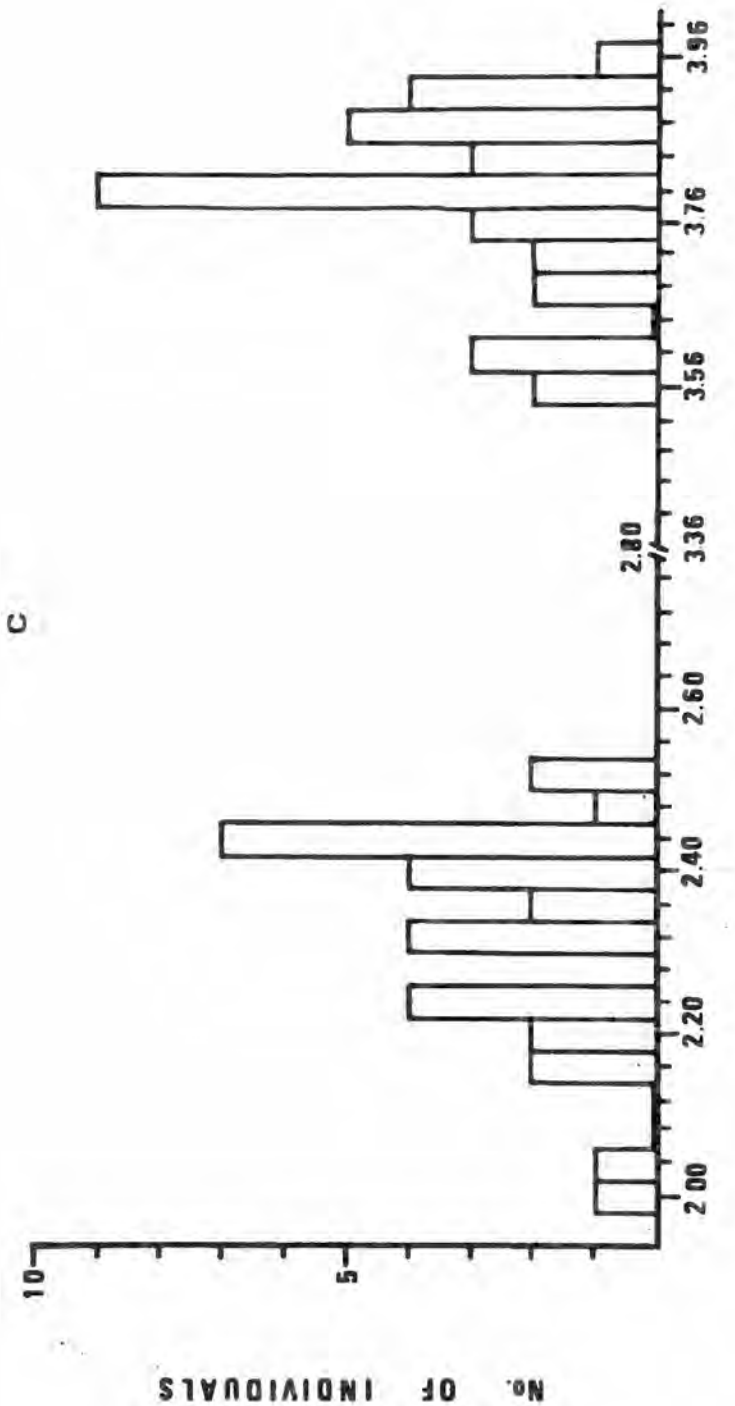



FIGURE 5c, shows a bimodal distribution of antennal length of workers.

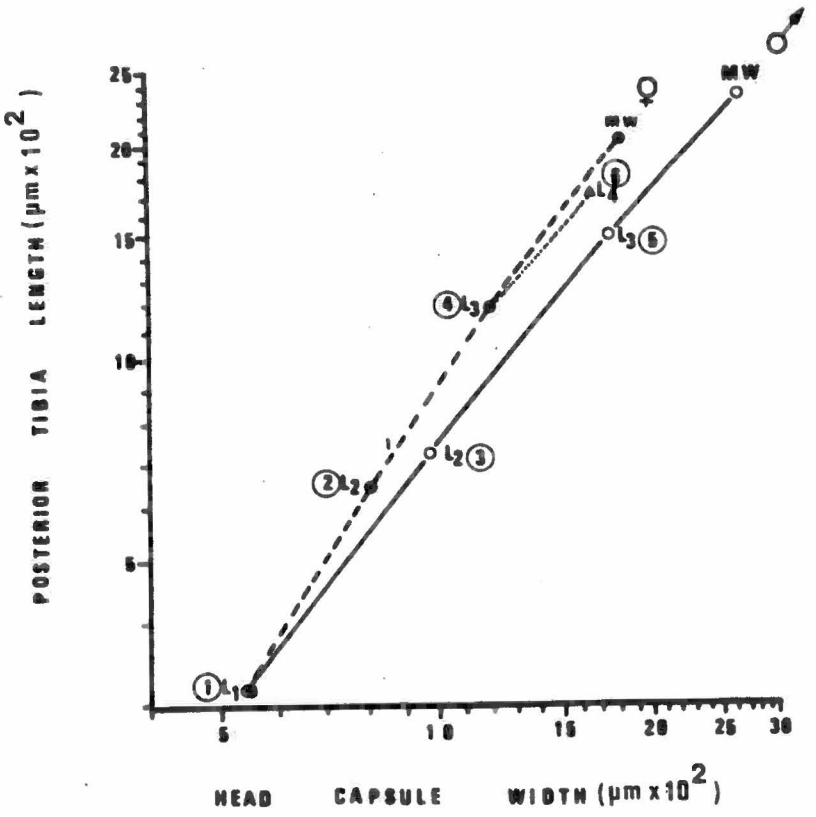



FIGURE 6. Logarithmic plot of head capsule width against posterior tibia length of larvae and workers. 1 - 6 are larval groups, $L_1$ - $L_4$ are larval instars, mw - minor workers, MW-major workers.

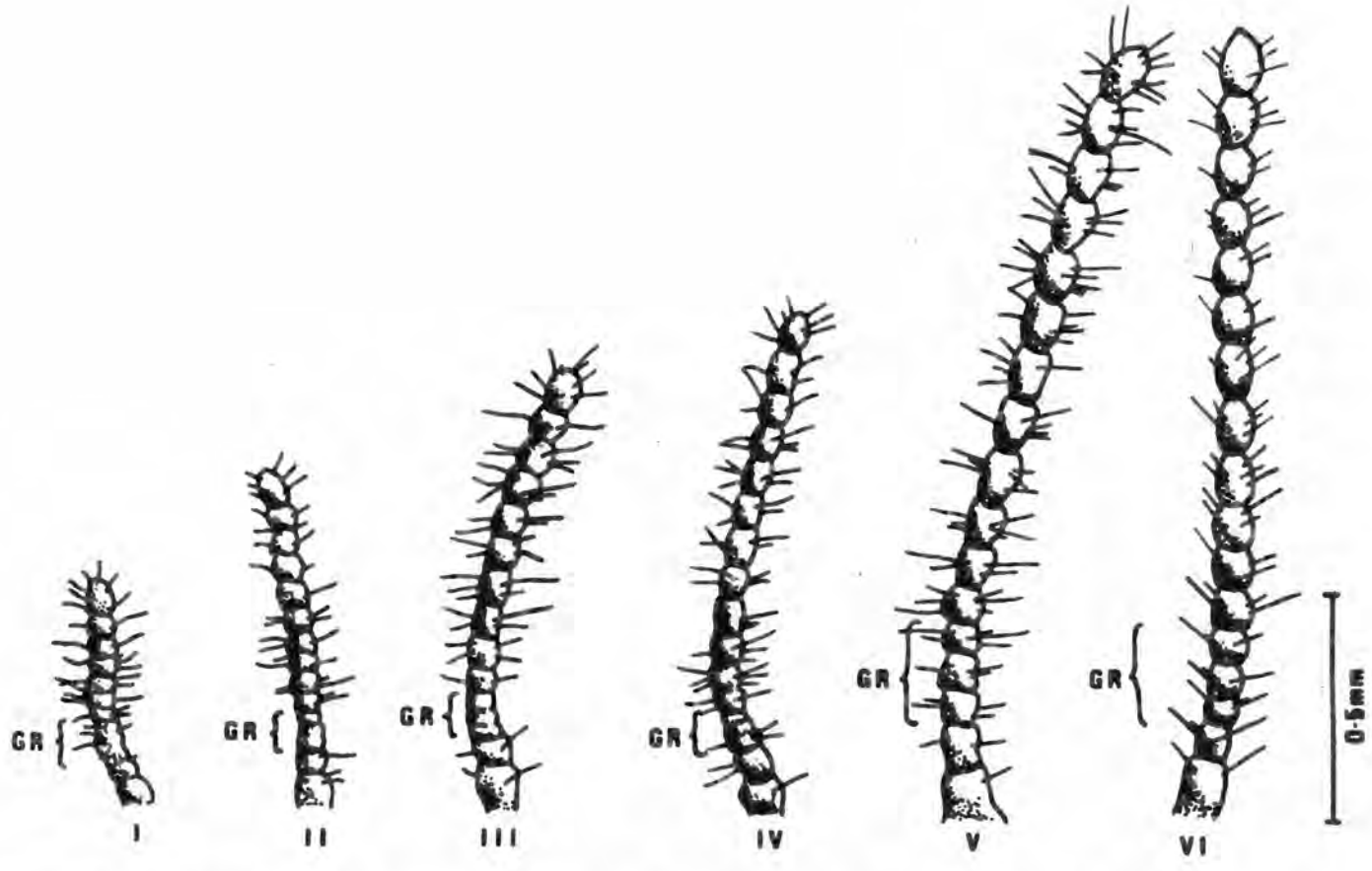
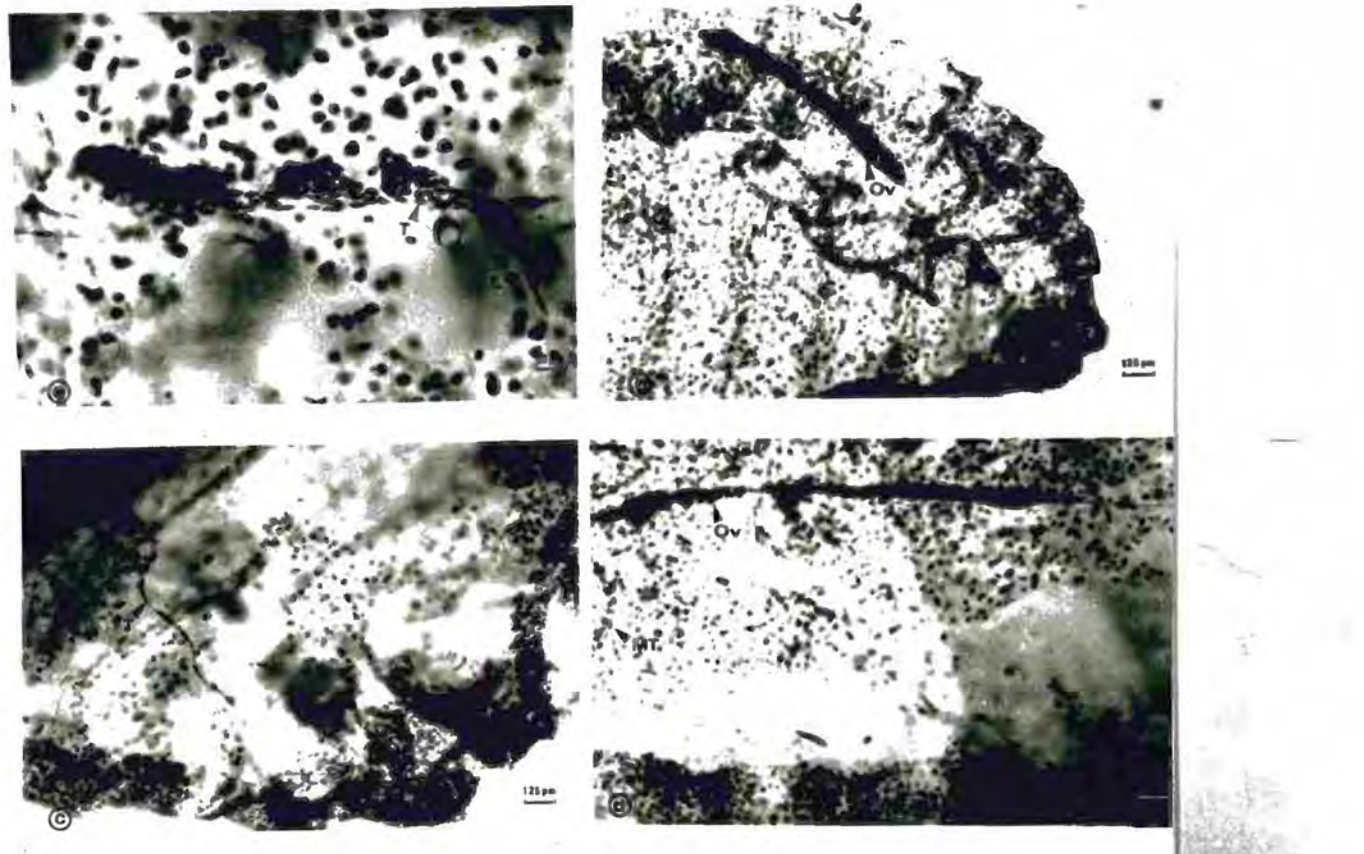



FIGURE 7. A drawing of antennae of 1st - 6th group larvae. GR - Growing region of antennae.

These results indicate a possible dimorphism in larval development which can only be detected after the first moult. Structurally, the third, fourth and fifth antennal segments are usually shorter than the others and boundaries between them are not well defined. This seems to be the growing region of the antennae (Fig. 7). Apparently the number of antennal segments increases by the budding of the third segment once it has attained a certain size during the developmental period. As the fifth segment grows and becomes the sixth, the third one divides, one part remaining a new third and the other becoming a new fourth as the old fourth becomes fifth and so on, until the maximum number of segments is attained during the final moult into either a worker or a soldier (Table 1).

Further studies were conducted to elucidate the possible dimorphic scheme of post-embryonic larval development into minor and major workers. Sexes of 30 larvae from each of the 6 groups were determined as described above. Group 1 is composed of female and male larvae. Larvae of groups 2, 4 and 6 are all females, whereas those of groups 3 and 5 are all males. This means that the larvae on the minor worker line of development are all females, whereas those on major worker line are all males. The minor and major pre-soldiers were found to be females. The male and female sex organs of some of the larvae are illustrated in Figs. 8a,b,c &d. From these results it is possible to detect three male larval instars and four female ones.




FIGURES 8a, b, c, d. THE RUDIMENTARY SEX ORGANS OF FIRST INSTAR LARVAE (a) MALE, (b) FEMALE, AND THIRD INSTAR LARVAE, (c) MALE AND (d) FEMALE. G.- GONADS. T- TESTIS, OV - OVARY, MT. - MALPIGHIAN TUBULE.

The sixth group of larvae is characterized by unique morphological features. These larvae are larger, less pigmented and less sclerotized than the usual minor workers. The appendages are larger, the abdomens in older individuals are more distended than those of the usual minor workers (Fig. 2). The posterior tibiae of these larvae tend to grow relatively faster than their head capsules (Table 2), unlike those of minor workers. In order to establish the fate of this interesting group of larvae, a study was designed in which ten one year old incipient laboratory colonies were used to support such individuals. A couple of days prior to the experiment, native soldiers and/or presoldiers were removed from the colonies to be used. This was done so as to circumvent influence which the soldier might exert on the development of the experimental larvae. The animals to be studied were then introduced singly into each of the recipient, incipient colonies. The colonies were checked daily and after an average of about 3 days all the larvae adopted had moulted into major presoldiers. Therefore they have to be considered as fourth instar larvae. This experiment was repeated several times using the same colonies as recipients and each time larvae of this type moulted into major presoldiers. However, it is worth noting that, on each occasion the experiment was performed, some of the newly emerged major presoldiers were eaten up in part or wholly by the workers or the young royal pairs of the recipient colonies. So far no explanation can be

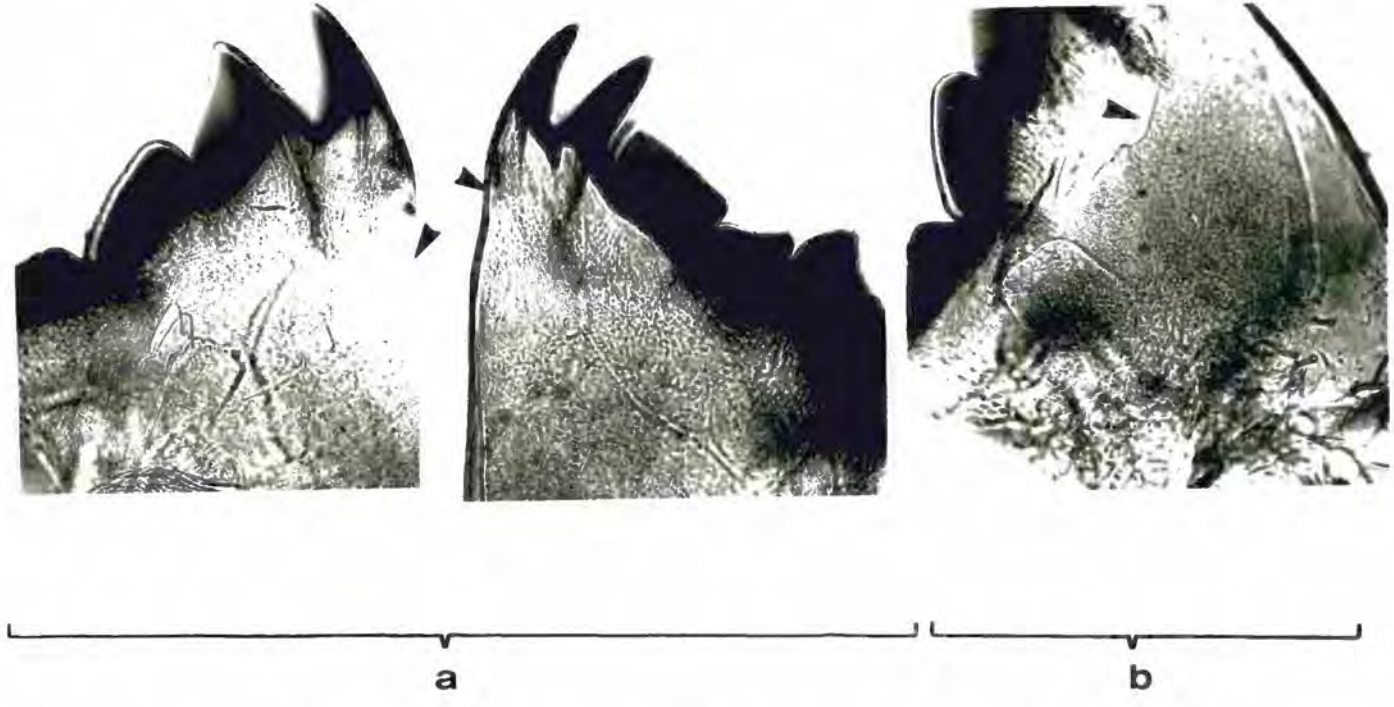



FIGURE 9. Mandibles of: (a) - a functional minor worker, (b) - a fourth instar larva.

Note - Mandibles of a functional minor worker do not show any signs of future presoldier mandibles while fourth instar mandibles do (arrows).

offered for this behaviour. In another experiment the mandibles of fourth instar larvae were fixed in alcohol (70%) overnight. It was possible to see under a dissecting microscope future presoldier mandibles within the worker-like mandibles of these individuals. The mandibles of newly moulted minor workers did not show these structures following similar treatment (Fig. 9). Casual observation of field material showed that some of the third instar female larvae moult directly into minor presoldiers.

3.3.2. *Nymphal instars and the reproductives*

The nymphal instars were recognized also by biometric studies. The measurements of head capsule width, posterior tibia and wing-pad length were carried out and the results are given in Figs. 10, a, b and c. The Figures show that during the development of nymphs from first instar larvae into adult reproductives, nymphs pass through five distinct instars. The results also show that any one of the parameters measured would be adequate to characterize an individual of a particular instar. These parameters are summarized in Table 3. The ratio tibia length/head capsule width remains almost constant throughout the development into the adult caste suggesting uniformity in the growth rates of both tibiae and head capsules of nymphs (Table 3). Wing-pads grow fast during the successive moults with a peak attained during the last moult. The antennae also increase in length as well as in the number of segments. The antennae of first, second, third, fourth, fifth

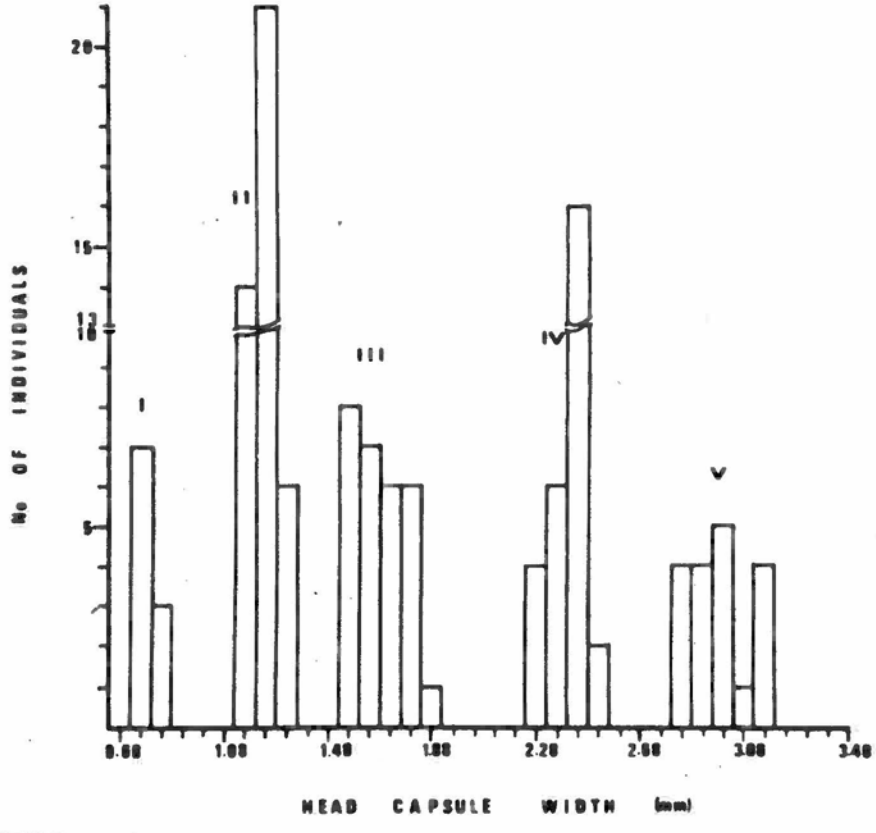



FIGURE 10a. A histogram showing the distribution of head capsule width of nymphs. I - V are the nymphal instars diagnosed.

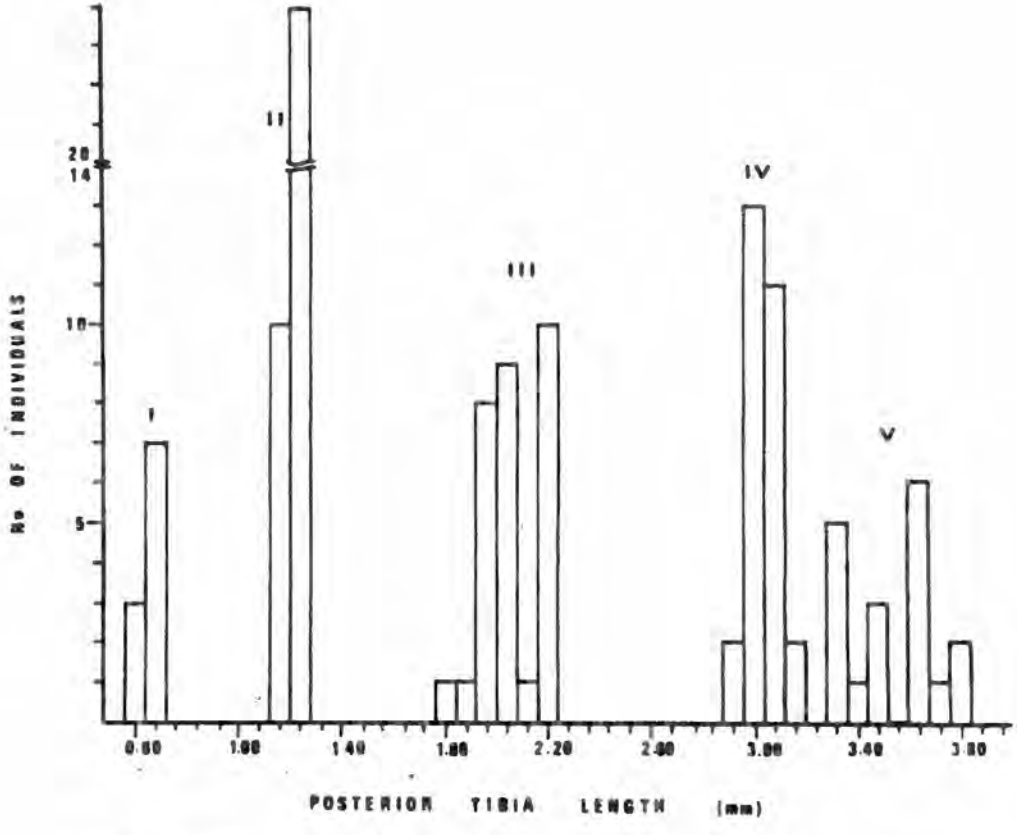



FIGURE 10b. A histogram showing the distribution of the posterior tibia length of nymphs. I - V are nymphal instars diagnosed.

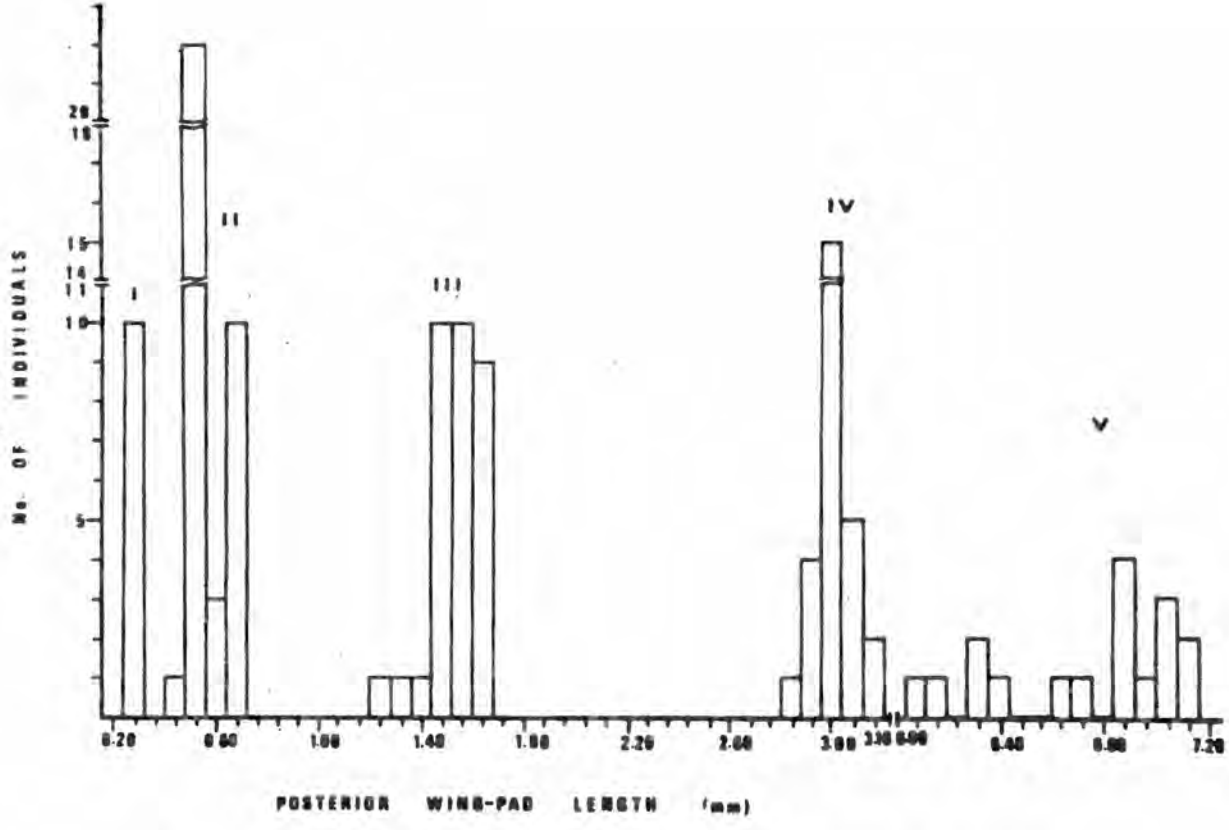



FIGURE 10c. A histogram showing the distribution of wing-pad length of nymphs. I - V are the nymphal instars diagnosed.

| Nymphal Instar | Sample Size(n) | Head Capsule Width(mm) ($\bar{X} \pm SE$) | Posterior Tibia Length (mm) ($\bar{X} \pm SE$) | Tibia Length/ Head Capsule Width | Wing-pad Length (mm) ($\bar{X} \pm SE$) | No. of Antennal Segments |
|---|---|---|---|---|---|---|
| 1st | 10 | 0.77 ± 0.01 | 0.64 ± 0.01 | 0.83 | 0.20 ± 0.01 | 14 |
| 2nd | 36 | 1.21 ± 0.01 | 1.19 ± 0.01 | 0.98 | 0.46 ± 0.01 | 16 |
| 3rd | 30 | 1.66 ± 0.02 | 2.03 ± 0.02 | 1.22 | 1.43 ± 0.02 | 18 |
| 4th | 48 | 2.26 ± 0.02 | 2.88 ± 0.02 | 1.27 | 3.11 ± 0.04 | 19 |
| 5th | 18 | 3.03 ± 0.03 | 3.57 ± 0.04 | 1.18 | 6.68 ± 0.10 | 19 |
| Adult | 35 | 3.54 ± 0.01 | 4.41 ± 0.02 | 1.25 | 37.90 ± 0.01 | 19 |

Table 3. The values of morphological measurements made on nymphs of different instars.

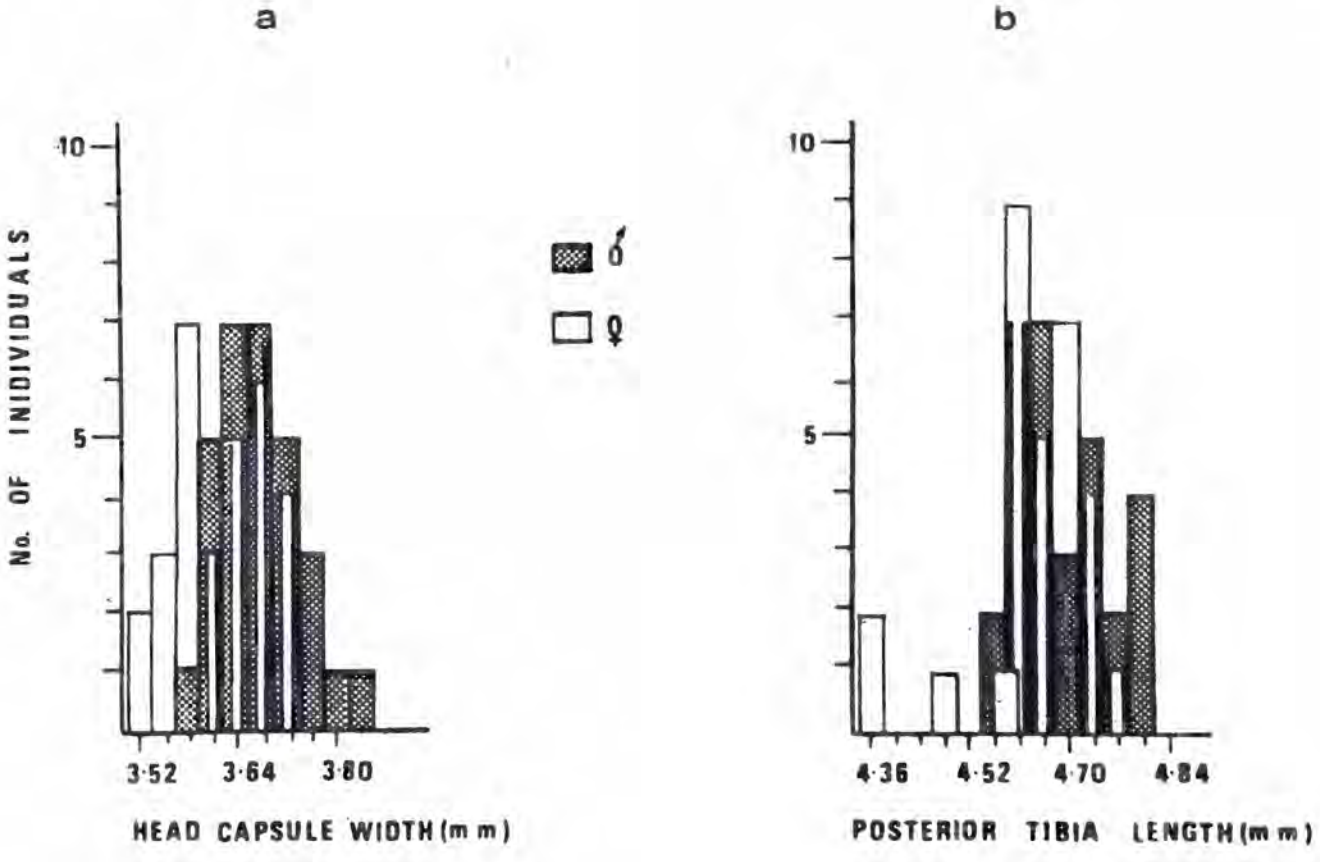



FIGURE 11. Histograms showing distribution of (a) - Head capsule width. (b) - Posterior tibia length of imagoes.

| Sex of Imago | n | Head Capsule Width (mm) | | Posterior Tibia Length (mm) | |
|---|---|---|---|---|---|
| | | $\bar{X} \pm$ S.E. | RANGE | $\bar{X} \pm$ S.E. | RANGE |
| Males | 30 | 3.72 ± 0.01* | 3.60 — 3.88 | 4.67 ± 0.01** | 4.56 — 4.80 |
| Females | 30 | 3.65 ± 0.01* | 3.52 — 3.76 | 4.63 ± 0.02** | 4.36 — 4.76 |

t — test of significance

* P 0.001, ** P 0.01

Table 4. Head capsule width and posterior tibia length of newly emerged imagoes.

instars consist of 14, 16, 18, 19 and 19 segments, respectively. Imagoes emerge from fifth instar nymphs retaining antennae with 19 segments, but after colony founding often lose 5 or more terminal segments thereafter.

A slight sexual dimorphism was found in the primary reproductives (imagoes). This is represented in Fig. 11. Males are a little larger than females and measurements in Table 4 show that the difference is statistically significant.

3.3.3. *Scheme of Development*

It is evident from these results that the royal pair produced eggs which hatch into first instar larvae identical in size but differ in sex. The second instar larvae are of two sizes depending upon sex the larger larvae being males and the smaller ones females. These in turn moult into larger males and smaller females, respectively. The third instar male larvae moult into adult major workers only, whereas female third instar larvae have 3 options, either to moult into minor presoldiers, fourth instar larvae or into minor workers, which form the majority. Fourth instar larvae in turn will develop into major presoldiers which subsequently will moult into major soldiers. So, among the sterile forms, the following castes are possible: minor and major workers and soldiers. Also, from first instar larvae, first instar nymphs may develop. These nymphs will then go through 5 successive moults into adult alates which are the reproductives. The following scheme of post-embryonic development is therefore proposed (Fig. 12).

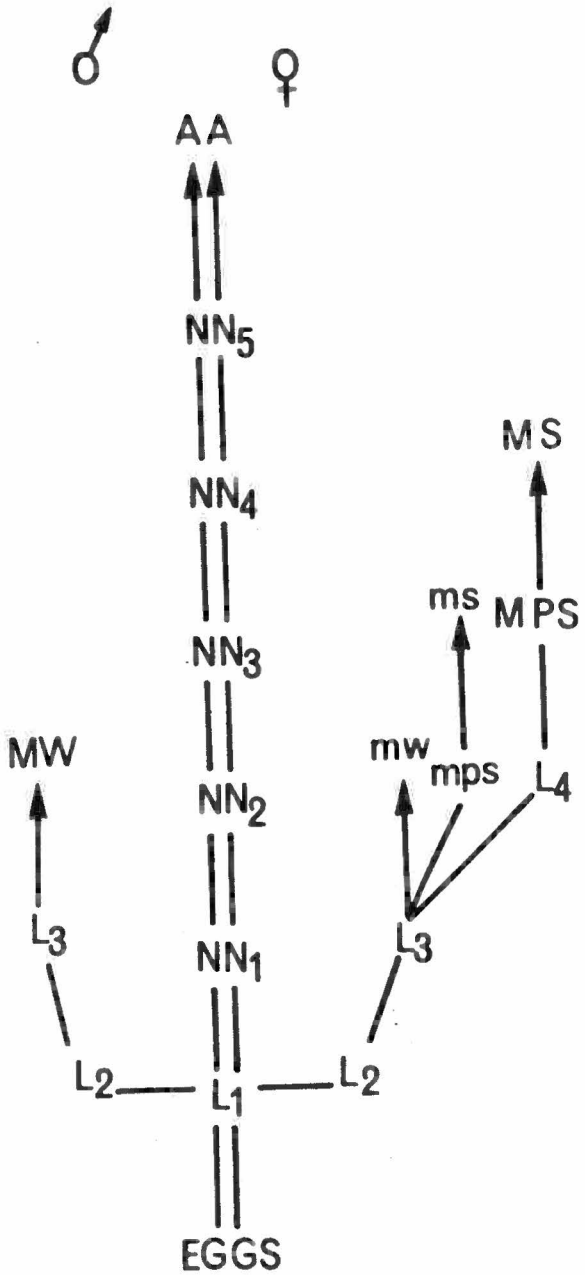



FIGURE 12. A scheme of post-embryonic development in Macrotermes michaelseni. $L_1$ - $L_4$ - Larval instars, MW - Major worker , mw - Minor worker, mps - Minor presoldier, ms - Minor soldiers, MPS - Major presoldier, MS - Major soldier, $NN_1$ - $NN_5$ - Nymphal instars and AA - Imagoes (Adult reproductives).

## 3.4. *DISCUSSION*

From these experimental findings it is apparent that the head capsule width, posterior tibia length, and the antennal length measurements are valuable parameters in the recognition of larval instars. The same technique has been used extensively by *NOIROT* while studying polymorphism in the *Termitidae* (*NOIROT*, 1955). *RENOUX* (1976) and other authors reviewed by *NOIROT* (1969) and *MILLER* (1969) have used the same tool successfully during their studies related in one way or another to termite polymorphism. In the present study it was, however, found that not all the parameters investigated are equally useful for the determination of larval instars. Head capsule width proved to be the most reliable parameter because it gave the most distinct picture on the distribution of larvae into 6 groups. The distribution of antennal length also separated larvae fairly well despite the breadth of the distribution. One therefore might need to use all the parameters described above in combination in order to achieve the best possible distinction between groups.

The number of antennal segments seems to increase by budding of the third segment. This was also observed by *FULLER* (1920) while studying post-embryonic development of the termites antennae and recently by *RENOUX* (1976) in his studies on *Schedorhinotermes lamanianus*. The observation that second and third groups of larvae have equal numbers of antennal segments, similarly fourth and fifth groups (Table 1) suggested parallel

development between first, second and fourth on one hand and first, third and fifth groups of larvae on the other hand. This was further indicated by the logarithmic plot of head capsule width against posterior tibia length (Fig. 6). Sexing larvae confirmed a sexual dimorphism in larval development.

The head capsule of female larvae tend to increase in size at a slower rate than those of males. Female head capsules increase in width at a rate of 1.3 x during the first moult, 1.5 x during the second moult and again 1.3 x during the adult moult. On the other hand, male larvae have higher growth rates of head capsules standing at 1.7 x, 1.8 x and 1.5 x, during respective moults. A similar picture holds for posterior tibiae though their growth rates are higher than those of head capsules. These results suggest that the highest rate of larval growth is attained during the second moult i.e. the last larval moult. These rates of growth fit well with the principles of allometric growth as explained by *HUXLEY* (1932). Similar observations were made for nymphal growth.

The experiment on rearing of larvae from sixth group by using incipient colonies devoid of soldiers and presoldiers clearly demonstrated that this group of larvae are actually fourth instar larvae. The fact that they are larger than the usual workers and have future presoldier mandibles within their worker-like mandibles suggests that the chain of events leading to major presoldier differentiation may be prompted before the

female third instar moult. *NOIROT* (1955, 169) on the other hand, found in *M. bellicosus* that major presoldier develop from minor workers which are morphologically similar to the others but incompletely sclerotized and non-functional, which suggests that in this species soldier differentiation occurs during the third instar female moult or shortly thereafter. While studying development in another genus of *Macrotermitiriae*, *Ancistrotermes*, he found that major soldiers are derived from normal functional minor workers suggesting an even later determination than in *M. bellicosus*. The development of minor presoldiers from third instar larvae is a common phenomenon in the above cited cases (*NOIROT*, 1955, 1969) as well as in the species in this study, suggesting that their fate is determined before the moult of this instar.

From the measurements conducted on the nymphs, it is certain that the first nymphal instar emerges from the first larval instar. Its size is comparable with that of the second instar larvae. However, the nymphal moult from first into second instar is accompanied by a drastic increase in size (by about 2 times) unlike what was observed for a larval moult into third instar. Five nymphal instars were found in the present study. These results agree with observations made by *BATHELLIER* (1927) on several species of higher termites in Indo-China. *NOIROT* (1952) made similar observations on several species of *Termitidae*, *WEESNER* (1953) on *Tenuirostritermes tenuirostris*, *BOUILLON* and *MATHOT* (1964) on *C. exiguus*, *HECKER*(1966) on *M. bellicosus* and more recently *N'DIAYE* (1977) quoting *NOIROT* and *BODOT*

and his own findings on *C. fungifaber*.

Five nymphal instars seems to be a common occurrence during the development of the reproductives in *Termitidae*, although *KAISER* (1956) and *SANDS* (1965) found only 4 nymphal instars in *Anoplotermes pacificus* and *Trinervitermes* sp., respectively. *NOIROT* (1969) however, reported that he observed in other species of these two genera five nymphal instars which makes them not unique in this respect.

Biometric studies of larvae and adult castes coupled with morphophysiological studies have made it possible to propose a scheme of post-embryonic development in this species of *Macrotermes*. A strong sexual dimorphism is expressed for workers, the majors being males, whereas the minor workers are females. Dimorphism also exists in soldiers, but it is not sex linked since both minor and major soldiers are females. This forms a link between soldier and minor worker castes. The proposed scheme of development is not unique to this species since *NOIROT* had described a similar scheme in other species of *Macrotermes* (1955, 1969). However, while *NOIROT* suggested that the minor worker could moult into the major presoldier, a specific fourth instar larva as a precursor of the major presoldier was detected.

# 4. HISTOLOGICAL AND SIZE CHANGES IN CORPORA ALLATA AND PROTHORACIC GLANDS DURING CASTE DEVELOPMENT

## 4.1. *INTRODUCTION*

From the early days of *HOLMGREN* (1909), *HANSTROM* (1940) and *CAZAL* (1948) work on the retrocerebral complex (corpora cardiaca + corpora allata) was purely anatomical description. Similar studies were conducted by *JUCCI* (1924) on prothoracic glands, which he called *tentorial glands* and *PFLUGFELDER* (1947) who named them *ventral glands*. *LÜSCHER* (1960) later called these glands *prothoracic glands* because in *Kalotermes flavicollis* they have two main parts: one in the head and the other in the prothorax. Since in *Macrotermes michaelseni* This seems also to be the case, I will follow the latter terminology throughout the text.

*Kalotermes flavicollis* was the main object of research into the role of corpora allata (CA) in termites during the fifties and sixties. The centre of the various investigations was on volume changes in the course of postembryonic development. Among the more important works were those of *SPRINGHETTI* (1957), *LÜSCHER* (1957, 1960, 1965) and *LEBRUN* (1967, a). The physiological role of these endocrine glands in termites was noted first by *LÜSCHER* (1958) on soldier differentiation. He then later (1960, 1965) followed up these studies. *LEBRUN* (1964, 1967 a,b) confirmed and expanded some of *LÜSCHER's* results on the role of the CA in soldier differentiation

*LÜSCHER* (1965) noted that they undergo growth up to the final moult into imagoes, but that these glands undergo a decrease in size soon after the moult of pseudergates (larvae) or nymphs. He also found that during the formation of neotenics (replacement reproductives) CA grow considerably. Therefore, enlargement of CA is known to occur in *K. flavicollis* during:

(i) maturation of imagoes

(ii) formation of presoldiers

(iii) formation of neotenics

While studying the morphology and histology of endocrine glands of *Zootermopsis angusticollis*, *GILLOTT* and *YIN* (1972) found that the CA of presoldiers and reproductives are much larger than those of larvae and soldiers. It seems, therefore, that changes in CA size during development of castes are not limited to *Kalotermes* alone but perhaps a general phenomenon in the whole of the lower termites.

In the higher termites, *Termitidae*, (*HOLMGREN* 1909) showed in *Nasutitermes chaquimayensis that the* CA become enlarged in the queen and King. *KAISER* (1956) and, *PASTEELS* and *DELIGNE* (1965) found similar results in *Anoplotermes pacificus* and *Microcerotermes parvus*, and in *Cubitermes heghi*, respectively.

*PFLUGFELDER* (1938) found that the CA of replacement reproductives of *Microcerotermes amboinensis* also become enlarged. *KAISER* (1956) showed in the same species that CA are progressively larger as neotenics are derived from more advanced nymphal instars.

Similarly, CA of ergatoid reproductives increase in size during development. *NOIROT* (1969) reported similar observations on *Termes hospes*.

During the post-embryonic development of imagoes, *KAISER* (1956) showed in *A. pacificus* that the CA slowly increase in size in the earlier stages of development, but sharply increase in size during the final stage. This seems to be the case also in *T. hospes* as noted by *NOIROT* (1969).

As far as workers are concerned, *PFLUGFELDER* (1938) and *KAISER* (1956) noted that CA are small in *M. amboinensis* and *A. pacificus*, respectively. According to *NOIROT* (1969) similar observations were made in *Nasutitermes arborum* and *T. hospes*, though less marked. A slight or no sexual dimorphism was observed in CA sizes of *Macrotermes bellicosus (Bellicositermes natalensis)* *(NOIROT*, 1969).

During soldier development it was noted that the CA undergo a marked size increase in *Neocapritermes* sp. and in a species of *Nasutitermitinae (KAISER*, 1956). *NOIROT* (1969) made similar observations in *M. natalensis* and *Mimeutermes giffardii*.

Changes in the prothoracic glands have been noted during development of *Kalotermes* by *LÜSCHER* (1960), *HERLANT-MEEWIS* and *PASTEELS* (1961) found that prothoracic glands degenerate in the replacement reproductives of *Kalotermes flavicollis*. Similar results were reported by *KAISER* (1956) in the nymphoid and ergatoid reproductives of *M. amboinensis* and by *NOIROT* (1969) on other species of *Termitidae*.

*SPRINGHETTI* (1957) found in *K. flavicollis* that prothoracic glands undergo regular growth during development of imagoes. *KAISER* (1956) and *NOIROT* (1969) made similar observations on *A. pacificus* and *T. hospes*, respectively. But, they noted that the growth in prothoracic glands is much more dramatic in these two species of *Termitidae*, although in the neuter line the increase is not so striking and in workers they remain poorly developed.

Prothoracic glands were also shown to be poorly developed and persist in soldiers in both lower and higher termites(*PFLUGFELDER*, 1947; *KAISER*, 1956; *LÜSCHER*,1960 *HERLANT-MEEWIS* and *PASTEELS*, 1961).

In the present study, an attempt was made to elucidate the pssible role of the endocrine glands discussed above in caste differentiation particularly of the soldiers of *Macrotermes michaelseni*.

## 4.2. *MATERIALS AND METHODS*

Specimens of *Macrotermes michaelseni* larvae, nymphs and adults were collected from closed mounds near Kajiado, in Kenya. In the laboratory, the larvae and nymphs were sorted out into instars as described in Chapter Four. For the histological studies teneral minor and major workers, minor and major soldiers and presoldiers, nymphs, alates and larvae were fixed in alcoholic Bouin's solution for about 24 hours, embedded in paraplast, sectioned and stained with haematoxylin and eosin.

An ocular micrometer was used to measure directly under a compound light microscope the diameter of the largest of the serial transverse sections of both CA of each specimen of the adult castes, larvae, nymphs and presoldiers. Assuming that the glands were practically spherical, the cross-sectional areas were calculated using the formula: $A=\pi r^2$ . Nuclear diameters of CA and prothoracic glands (PG) were also measured. Using camera lucida (drawing tube) serial sections of each PG of nymphs were traced on paper for a more detailed analysis of size. A planimeter was then used to measure the surface area of the sections. The volume of each prothoracic gland was calculated by using the following formula:

$V = ab.$, where $V$ = gland volume , $a$ = thickness of sections, $b$ = total surface area of the glands.

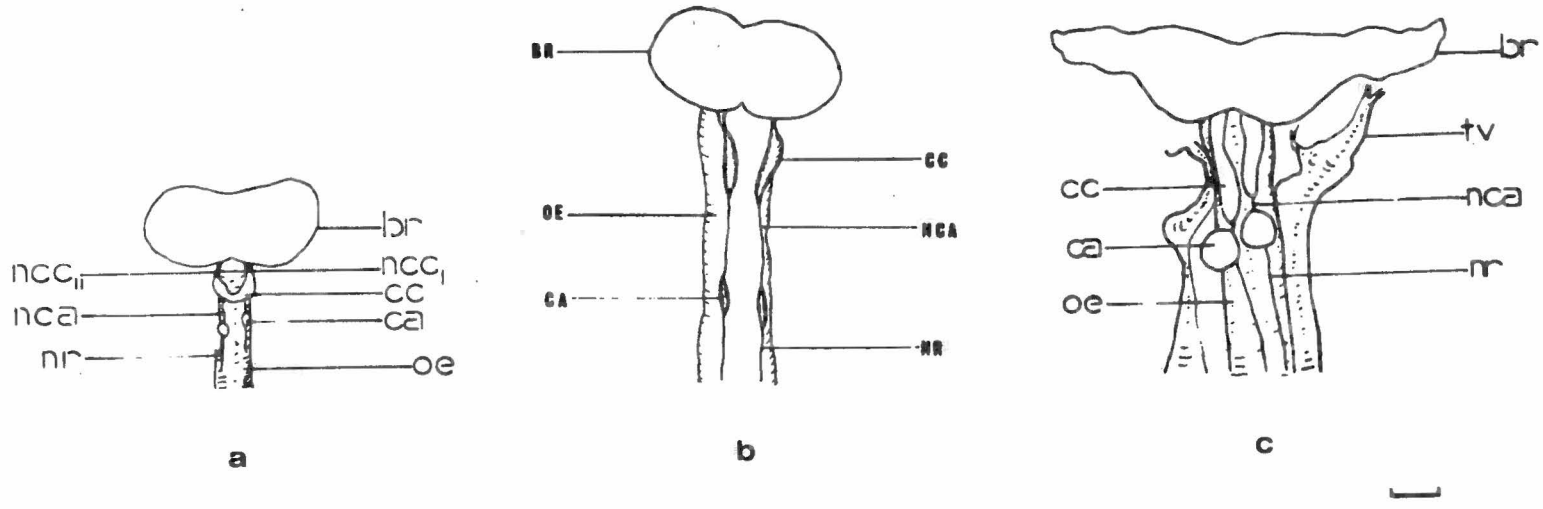
Corpora allata of major presoldiers were analysed for glycogen distribution since vacuolation was more evident in them. Periodic acid Schiff (PAS) reaction as modified by *PEARSE* (1968) was used as a test for glycogen.

## *4.3 RESULTS*

### *4.3.1 Larvae and neuter castes*

#### *4.3.1.1 Corpora cardiaca, Corpora allata and Prothoracic gland morphology*

Corpora allata of larvae (including fourth instar larvae which




FIGURES 13a, b and c. Illustrations of the morphology of retrocerebral complex of: (a) a worker (b) a soldier and (c) an Imago. br. - Brain, c.c. - Corpus cardiacum, c.a. - Corpus allatum, n.c.c. I, II - Nervi corporis cardiaci, nca I, II - Nervi corporis allati, nr - nervi recurrens , oe -Oesophagus. S c a l e = 150µm.

normally moult into major presoldiers) and of teneral workers are paired and spherical in shape, whereas those of presoldiers and soldiers are somewhat oval-shaped. In all cases CA lie posterior to a pair of large egg-shaped corpora cardiaca (CC) which are located immediately behind the brain (Figs. 13, a, b and c). Corpora allata nerves connect the two pairs of glands. Transverse sections show that CA are in close contact dorso-laterally with the aorta and that there is apparently a nerve leading ventrally from each of the glands passing on either side of the oesophagus to join the PG (Fg. 14). The prothoracic glands lie in the head ventro-laterally to the oesophagus, extend posteriorly towards the neck region and anteriorly to a position beneath the posterior end of CC. They are attached to the two ventral tracheae which penetrate the head.

4.3.1.2. *Histological appearance of Corpora cardiaca, Corpora allata and Prothoracic glands*

The corpora allata cell boundaries are difficult to see, though in some cases, like those of the older fourth instar larvae and sometimes in the presoldiers, the boundaries are fairly prominent (Fig. 15). The cells, which are arranged radially, have spherical nuclei usually positioned close to the periphery of the glands. Depending on the size of the glands, the nuclei may be either closely packed, as in the small glands of first instar larvae and teneral workers, (Figs. 14, 16) or sparsely distributed, as in those of third instar larvae and more so in fourth instar larvae (Figs. 15, 17) and presoldiers (Fig. 18).

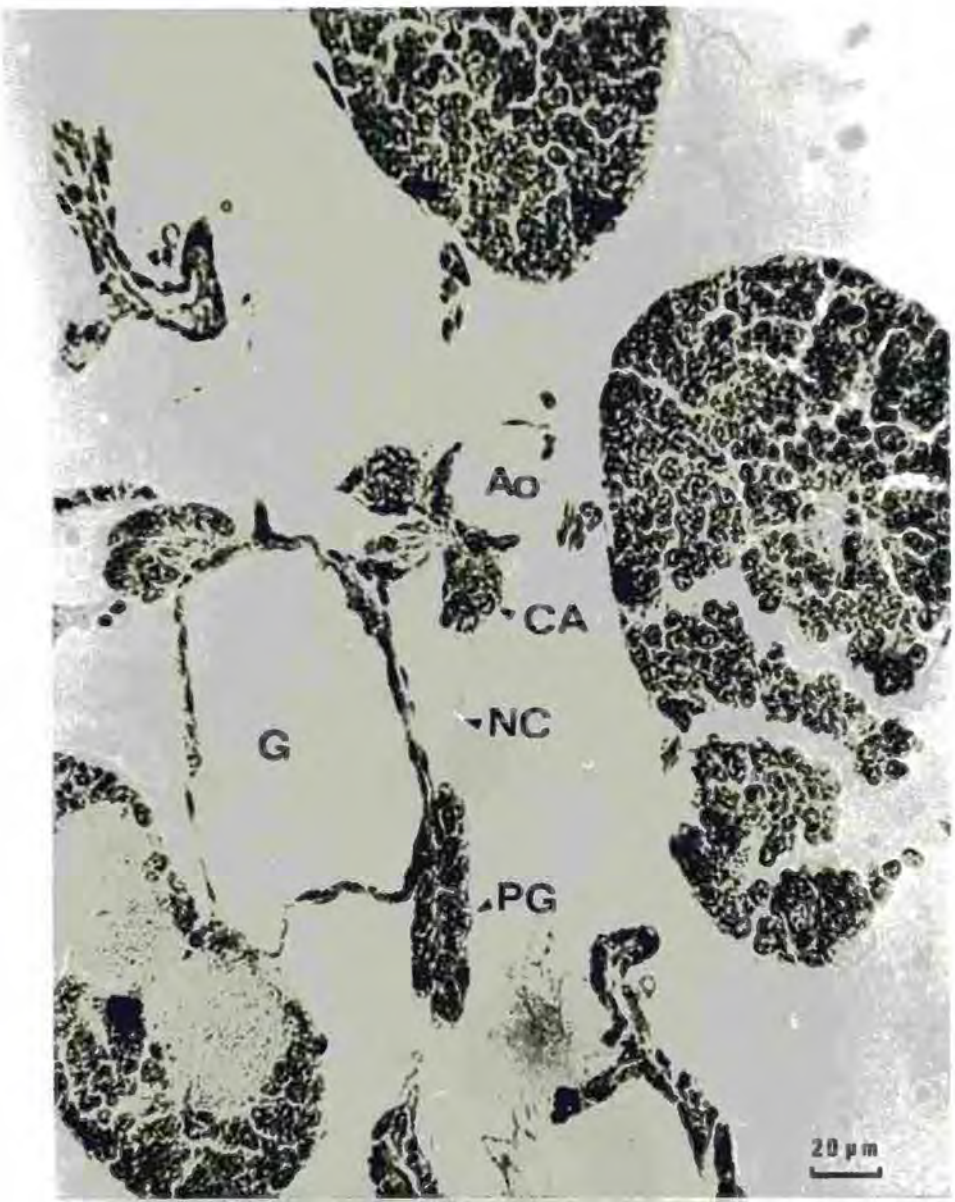



FIGURE 14. A cross-section of corpora allata (CA) and prothoracic glands (PG) of first instar larva showing a possible nerve connection (NC) between the two glands. Note also the closely packed nuclei . G - Oesophagus, AO - Aorta.

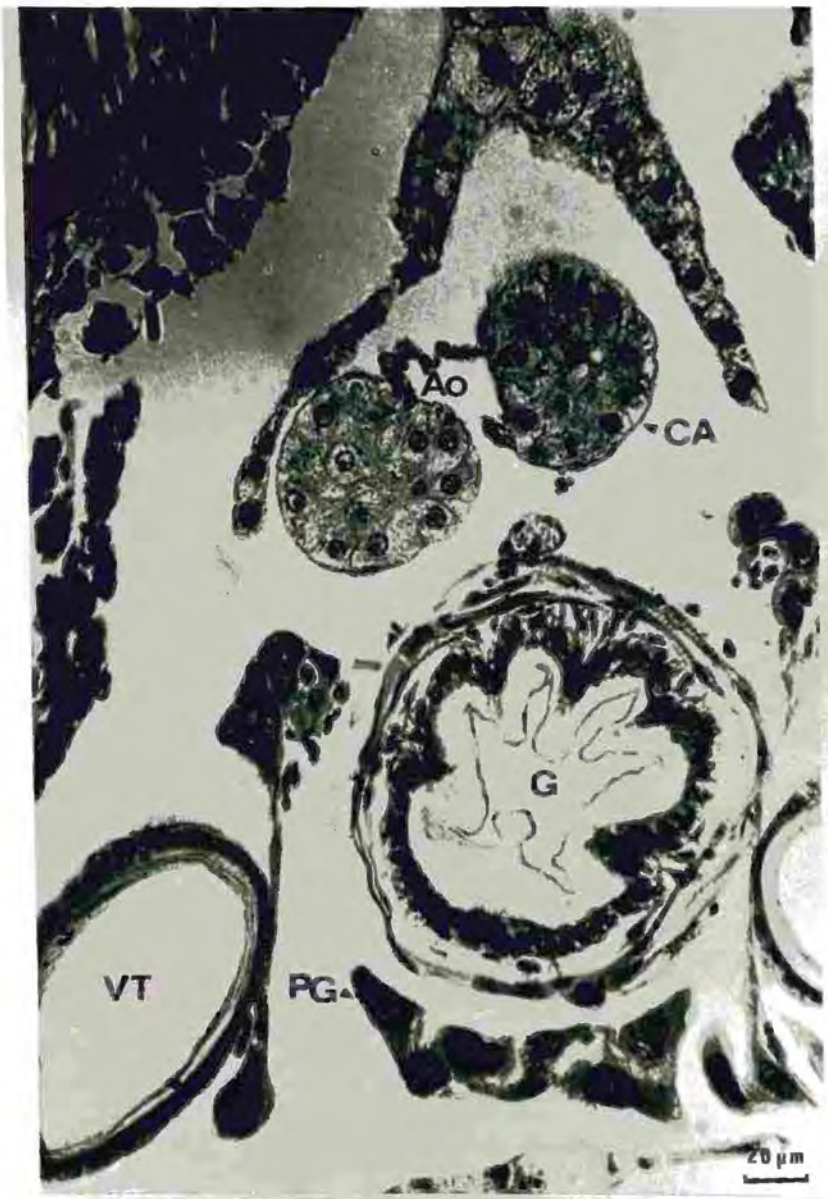



FIGURE 15. A transverse section of corpora allata (CA) of fourth instar larva showing fairly distinct cell boundaries and sparsely distributed nuclei .

AO - Aorta; G - Oesophagus; VT - Ventral trachea; PG - Prothoracic gland


FIGURE 16. A transverse section of corpora allata (CA) of a minor worker showing closely packed nuclei Ao - Aorta


FIGURE 17. A transverse section of corpora allata (CA) of a fourth instar larva showing sparsely distributed nuclei , minor vacuolation of nuclei and cytoplasm.

Ao - Aorta; G- Oesophagus, PG - Prothoracic gland

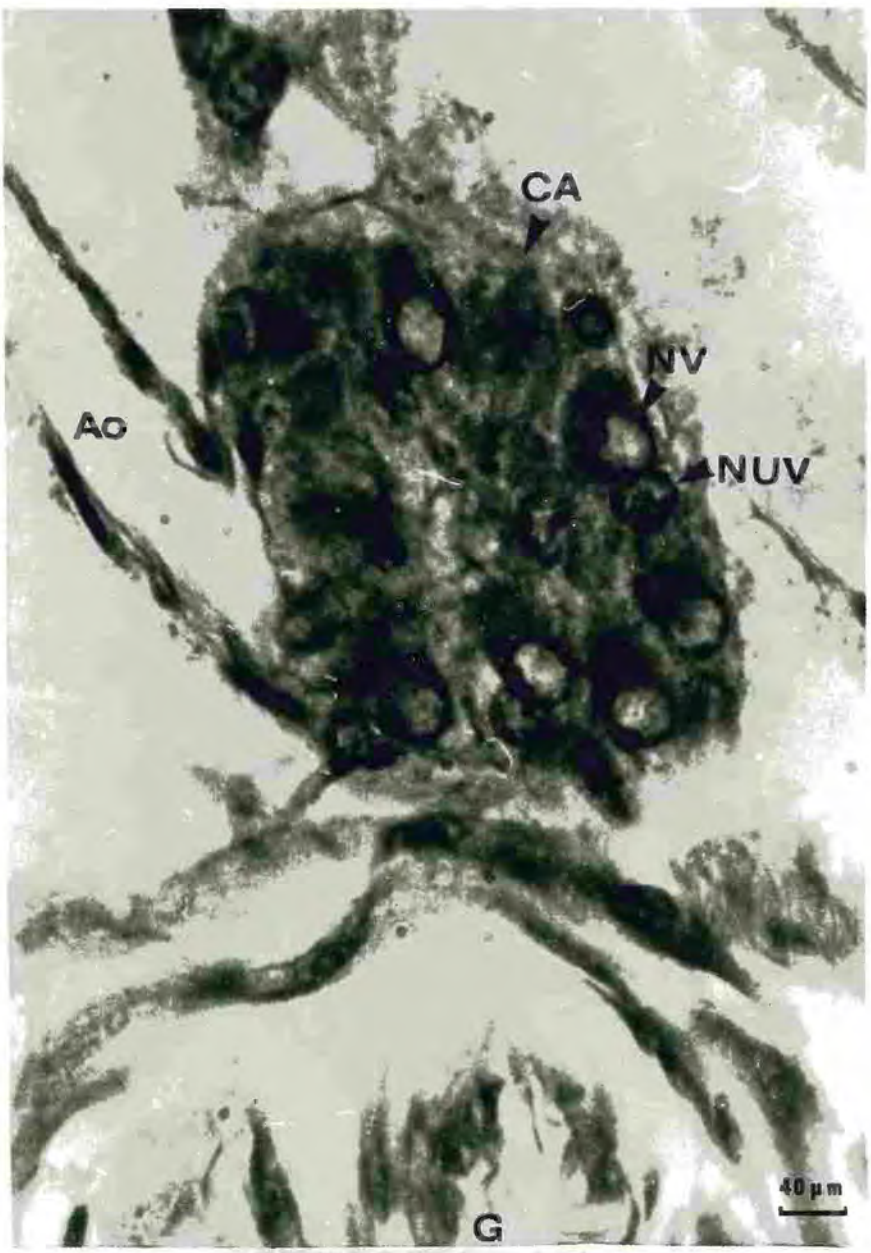



FIGURE 18. A transverse section of corpora allata (CA) of a major presoldier showing a sparse distribution of nuclei which are vacuolated (VN). Note that the cytoplasm is also vacuolated. NUV - Nucleus unvacuolated; G - Oesophagus; AO - Aorta

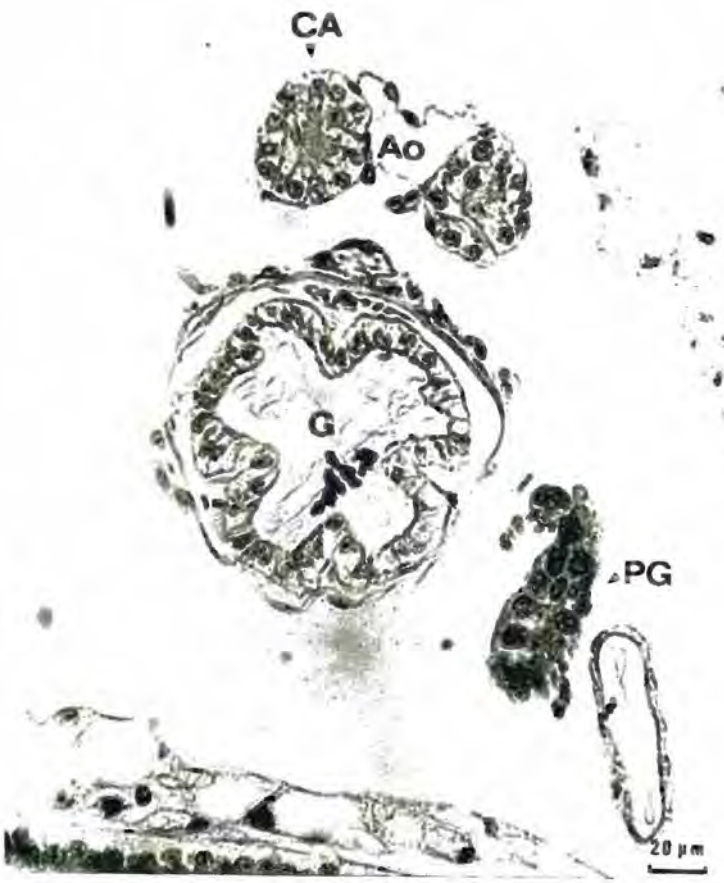



FIGURE 19. A transverse section through the corpora allata (CA) of a third instar larva having vacuolized cytoplasm (Arrow). Note a well developed prothoracic gland (PG). Ao - Aorta; G - Oesophagus

Some of the late stage fourth instar larvae and particularly presoldiers have CA nuclei bearing characteristic and histologically interesting features. Some of these nuclei have large portions which apparently do not take up the stains; they therefore appear highly vacuolated with only a thin layer of karyoplasm along the nuclear membrane and a little denser at one end where the nucleoli are located (Fig. 18). The corpora allata nuclei of the younger fourth instar larvae, however, stain like those of the other instars i.e. they pick up the stains fairly uniformly, have only smaller vacuoles and randomly distributed nucleoli (Fig. 17). Vacuolation is also seen in the cytoplasm of some CA cells. Some larvae from different instars have CA with intensely vacuolated cytoplasm, for example, that of third instar (Fig. 19). However, there are other larvae, presoldiers and adult neuter castes that have less vacuolation of CA cytoplasm (Figs. 16, 17 and 18).

4.3.1.3. *Glycogen distribution in the nuclei and cytoplasm*

The PAS test has shown that glycogen is randomly distributed throughout the cytoplasm as well as in the nuclei of most cells of CA. Glycogen deposits appear as large clumps in some areas of cytoplasm and in the centre of the nuclei (Fig. 20 a). The glycogen clumps, when present, appear to displace the nucleoli to the periphery of the nuclei. These clumps apparently occupy those areas where vaculation was observed in the histological preparations discussed above.

Glycogen deposits were also observed in the fat body strands in

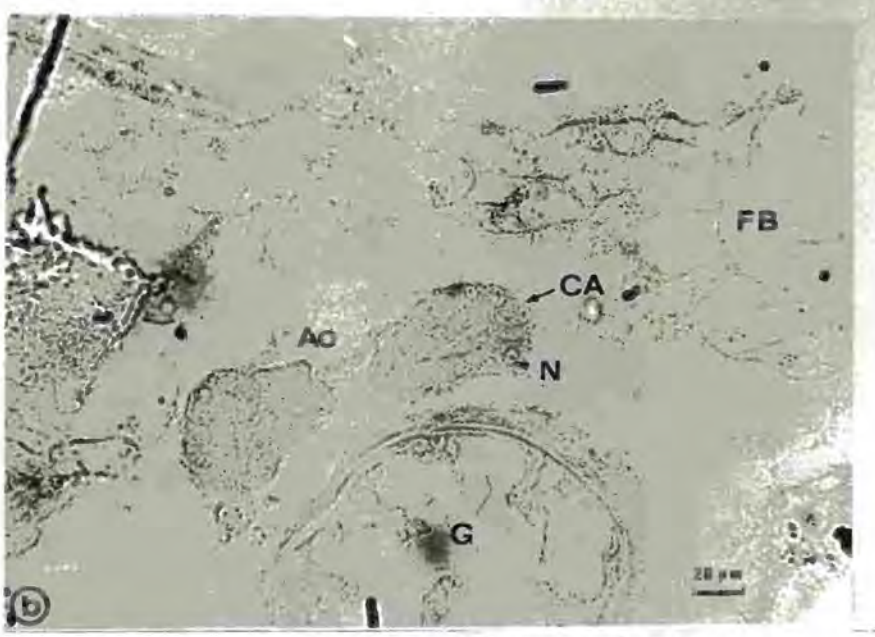




FIGURE 20a and b. (a) The distribution of glycogen deposits in corpora allata (CA), fat body tissue (FB) and frontal gland (FG) of a major presoldier. (b) A negative control following pretreatment of sections with saliva. Ao - Aorta, UN - Unstained nucleus, SN - Stained nucleus and G. - Oesophagus.

the vicinity of the CA, but not in the corpora cardiaca and occasionally in the cytoplasm of prothoracic glands never in their nuclei.

No clumps of glycogen were observed in the controls following treatment of specimens with saliva before carrying out the glycogen test (Fig. 20 b). This observation confirmed the presence of glycogen in those areas described above.

4.3.1.4. *Changes in corpora allata size during larval development*

During larval development, the nuclei barely increase in size until the later stage of fourth instar larval development when a drastic increase in the size can be observed (Table 5). Nuclei of the CA actually double in cross-sectional area when compared with the nuclear size of CA in the preceding instar. In workers, the size remains the same as that observed for the larvae. The number of nuclei within the largest CA transverse sections hardly changes throughout larval development. This indicates that there is no proliferation of the cells during the whole development.

Changes in size of CA take place during the development of larvae. Glands are smallest in the first instar larvae and gradually increase in size during the second instar. The increase attained is a significant one ($P<0.001$). The largest size is realized during the third larval instar of both minor and major worker lines of development. A subsequent size decrease which is highly

| Development Stage | Sample Size(n) | Max. Cross-sectional Area of CA($um^2$) ($\bar{X} \pm$ S.E.) | Max. number of Nuclei in the Cross-section of CA($\bar{X} \pm$ S.E.) | Max. Cross-sectional Area of Nuclei ($um^2$) ($\bar{X} \pm$ S.E.) | Area of Nuclei/area of Cytoplasm ($\bar{X} \pm$ S.E.) | Diameter of PG Nuclei.(um) ($\bar{X} \pm$ S.E.) |
|---|---|---|---|---|---|---|
| 1st Instar Larvae ♂♀ | 30 | 460.65 ± 25.57 | 12.40 ± 0.31 | 20.05 ± 0.53 | 1.70 ± 0.26 | 5.69 ± 0.21 |
| 2nd Instar Larvae ♀ | 28 | 572.16 ± 27.09 | 13.64 ± 0.35 | 19.46 ± 0.20 | 1.20 ± 0.25 | 5.98 ± 0.21 |
| 2nd Instar Larvae ♂ | 24 | 749.23 ± 34.20 | 13.62 ± 0.58 | 19.63 ± 0.01 | 0.71 ± 0.13 | 7.12 ± 0.34 |
| 3rd Instar Larvae ♀ | 18 | 760.22 ± 36.97 | 14.35 ± 0.51 | 20.53 ± 0.86 | 0.67 ± 0.09 | 6.37 ± 0.70 |
| 3rd Instar Larvae ♂ | 24 | 879.70 ± 44.59 | 12.76 ± 0.33 | 19.16 ± 0.46 | 0.43 ± 0.04 | 7.71 ± 0.46 |
| 4th Instar Larvae (early stage). ♀ | 11 | 1425.71 ± 172.92 | 14.73 ± 0.52 | 21.02 ± 1.09 | 0.41 ± 0.08 | 11.21 ± 0.53 |
| 4th Instar Larvae (late stage). ♀ | 40 | 2864.46 ± 110.13 | 12.57 ± 0.3 | 43.38 ± 1.43 | 0.27 ± 0.03 | 13.95 ± 0.43 |
| Pigmenting Minor Workers ♀ | 12 | 628.49 ± 17.23 | 14.86 ± 0.78 | 20.36 ± 0.96 | 1.05 ± 0.20 | 5.76 ± 0.38 |
| Pigmenting Major Workers ♂ | 6 | 653.86 ± 37.40 | 13.64 ± 0.42 | 21.02 ± 1.88 | 0.76 ± 0.08 | 6.25 ± 0.29 |

Table 5. Measurements of size changes in corpora allata and prothoracic glands during larval development.

significant ($P<0.001$) is experienced during the pigmentation of moulted major and minor workers(Fig. 21). A sexual dimorphism is obvious during the development of the larvae, male larvae having larger CA ($P<0.001$) than females of the same instar. In Figure 22 the size of CA of different instars is expressed as a ratio of their head capsule width. This is to minimise CA size differences between male and female individuals of the same instar and between instars caused by size differences among these categories of individuals. During pigmentation the glands of both minor and major workers decrease in size almost to the same level, and the difference is not significant ($P<0.5$) despite the differences in head capsule size. When CA of the third instar are compared with those of the fourth instar in their later stage of development, an increase in size by a factor of about 4 (Fig. 21) is evident. This increase is highly significant ($P<0.001$), despite the fact that the head capsule size of third instar males is comparable with that of the fourth instar individuals (Table 1).

The changes in the relationship between CA nuclear and cytoplasmic cross - sectional areas in various larval instars are illustrated in Figure 23. During larval development, there is a relatively more rapid increase in the amount of cytoplasmic material than in the size of the nuclei. This is most pronounced in the CA of the later stage of fourth instar larvae.

*4.3.1.5 Changes in corpora allata during presoldier development*

A study was conducted to investigate size changes that may occur

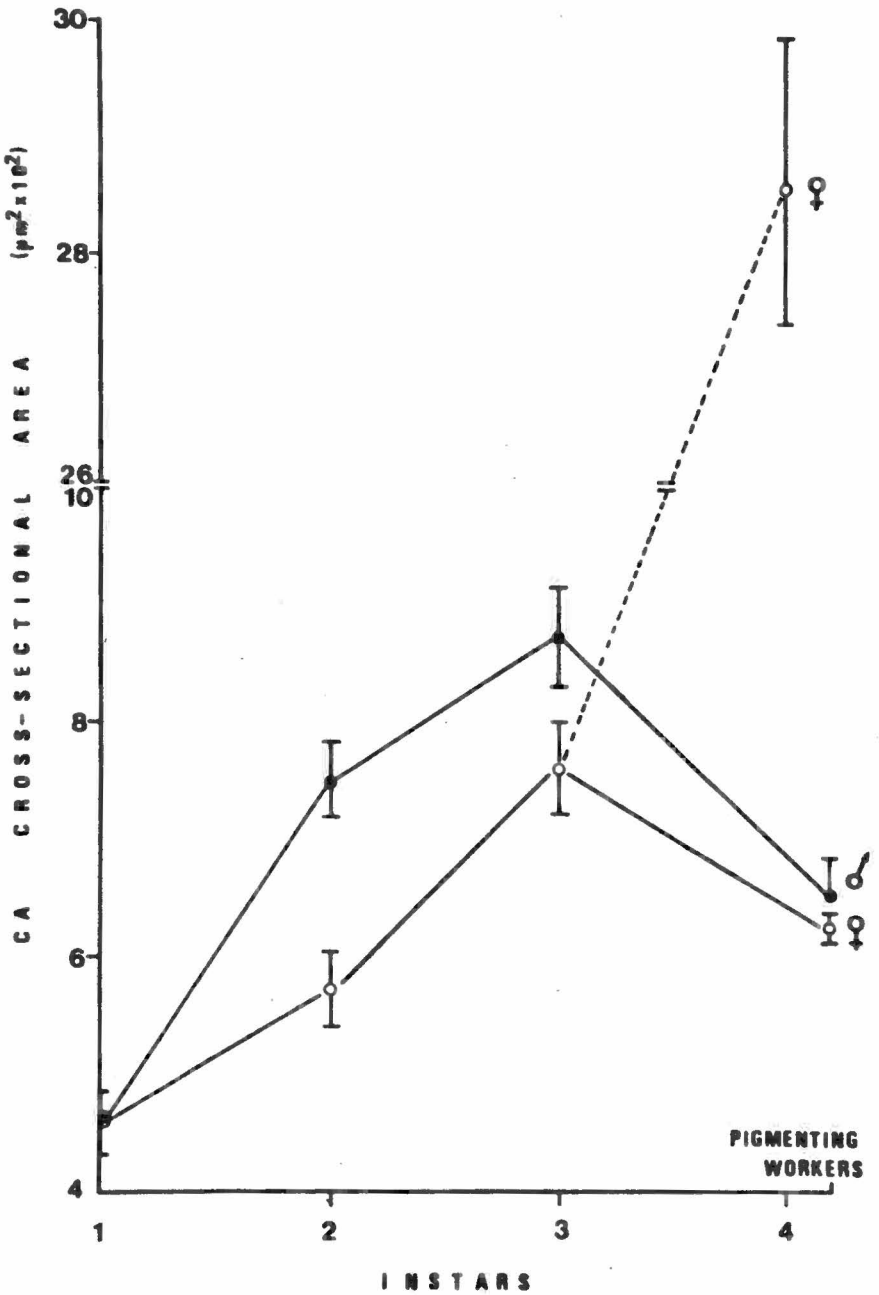



FIGURE 21. A profile of changes in corpora allata cross-sectional areas during larval development.

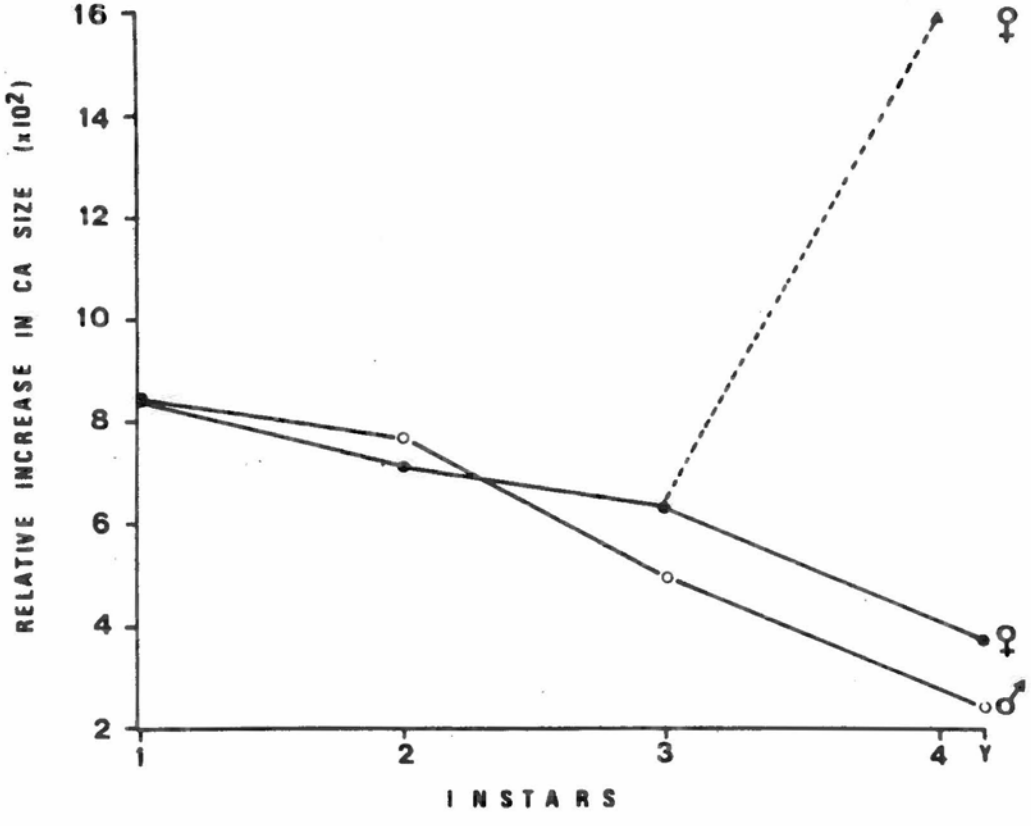



FIGURE 22. Relative corpora allata sizes with respect to head capsule width. Y - teneral workers.

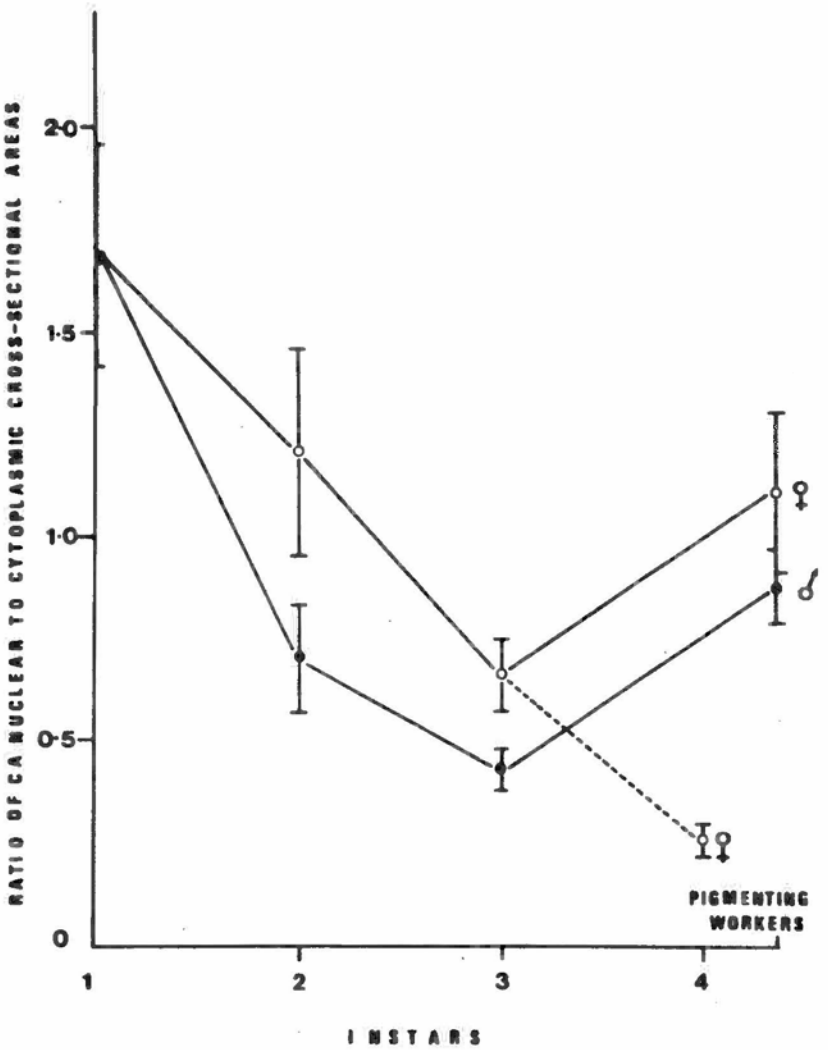



FIGURE 23. Changes in the relationship between corpora allata nuclear and cytoplasmic cross-sectional areas of individuals from various instars.

in CA during the intermediate stages of both minor and major soldier development. The corpora allata of the following stages were studied Undifferentiated third instar female larvae, third instar larvae in process of moulting into minor presoldiers, early and late stages of minor presoldiers and soldiers , as well as early, late and moulting stages of fourth instar larvae, early and late stages of major presoldiers, and soldiers. The results of these investigations are expressed in Figs. 24 and 25. They show that, although there are changes in the size of the glands during soldier development through these stages, these changes are not significant except during the early stages of development. Before third instar larvae moult into either fourth instar or minor presoldier, CA are relatively small. However, during the moult into minor presoldiers or fourth instar, these glands were found to have enlarged in cross-sectional area considerably by a factor of 2 which is highly significant ($P<0.001$). During major soldier development, CA continue, increasing in size in the fourth instar , until they have doubled in cross-sectional area by the end of the instar. After the final moult into soldiers the CA decrease dramatically in size by about four times. In addition, changes in the nuclear sizes and nuclear/cytoplasmic ratios take place as well. In Table 6 it is shown that, during minor soldier development, CA nuclei are largest at the early presoldier stage of development, i.e. soon after differentiation has occurred. On the other hand, the nuclear/cytoplasmic ratio is smallest during moulting of this instar

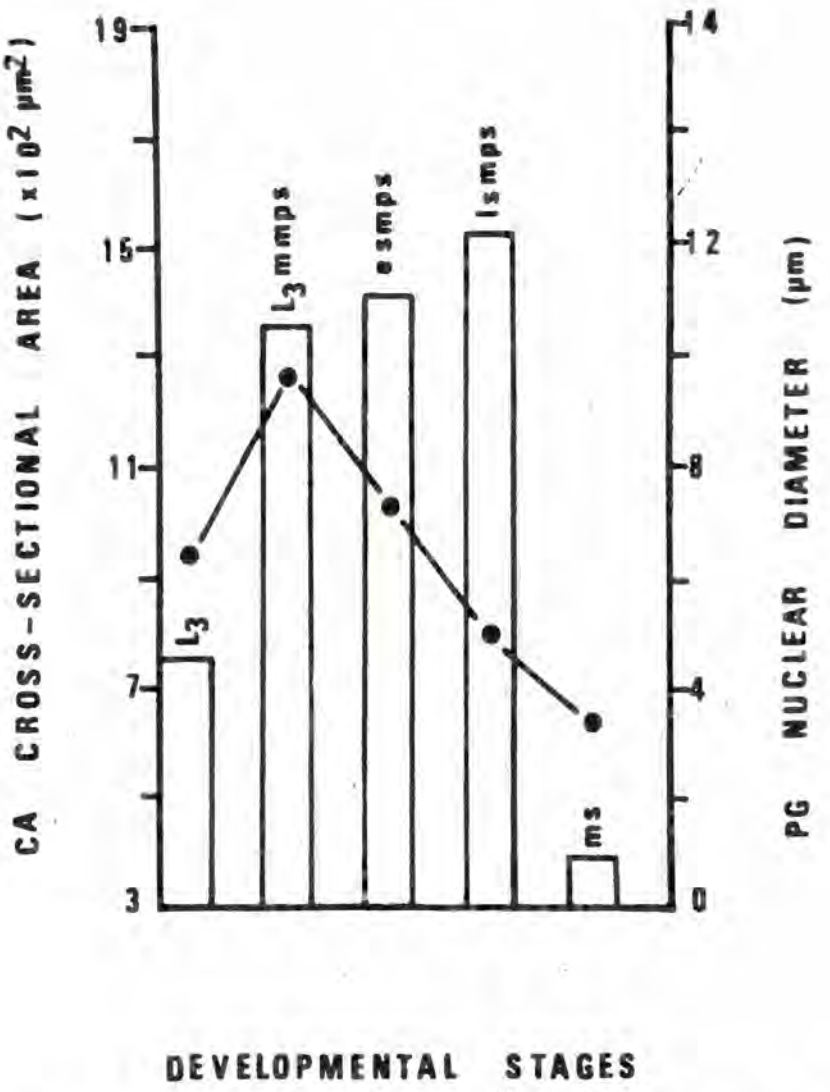



FIGURE 24. Changes in corpora allata size (Columns) and prothoracic gland nuclear diameter (Line) during different stages of minor presoldier development. $L_3$ - third instar female larva, $L_3$ mmps - Minor presoldier moulting from third instar larva, esmps - Early stage of minor presoldier, lsmps - Late stage of minor presoldier and ms - Minor soldier

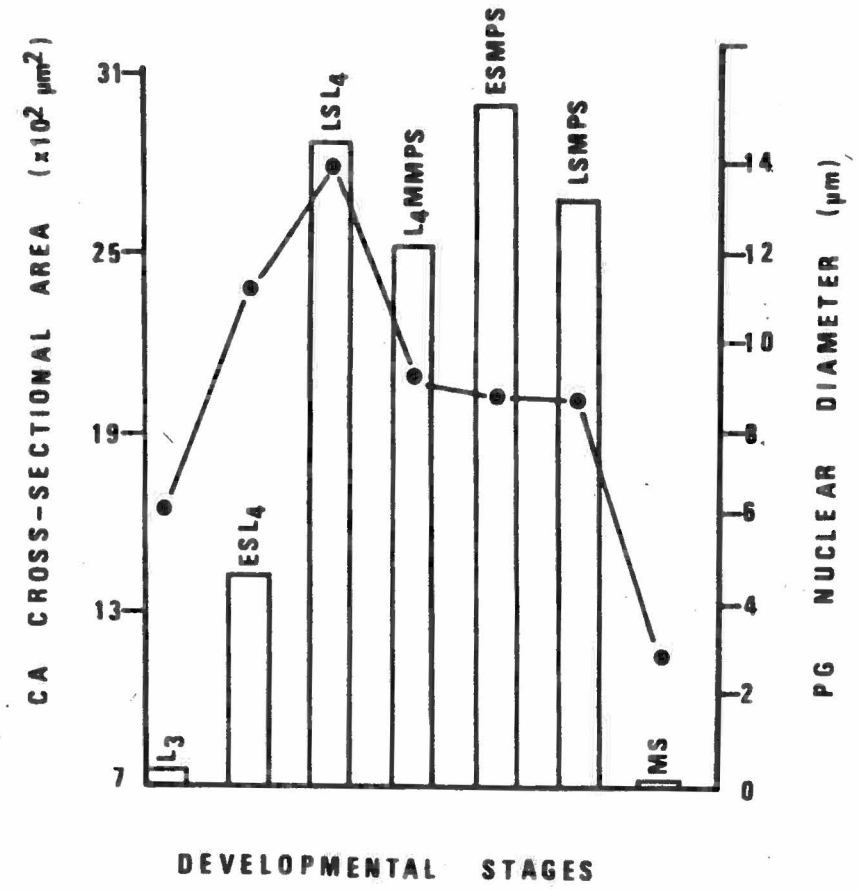



FIGURE 25. Changes in corpora allata size (Columns) and prothoracic gland nuclear diameter (Line) during different stages of major presoldier development. $L_3$ - Third instar female larva $ESL_4$ - Early stage fourth instar larva . $LSL_4$ - Late stage fourth instar larva . $L_4MMPS$ - Fourth instar larva moulting into major presoldier . ESMPS - Early stage major presoldier . LSMPS - Late stage major presoldier . MS - Major soldier.

| Stage | Sample Size (n) | CA Max. Cross-sectional Area ($um^2$) ($\bar{X} \pm$ S.E.) | CA Nuclear Cross-sectional Area ($um^2$) ($\bar{X} \pm$ S.E.) | PG Nuclear Diameter (um) ($\bar{X} \pm$ S.E.) | Number of Nuclear in max. Cross-section (n) ($\bar{X} \pm$ S.E.) | CA Nuclear Cross-sectional Area/ Cytoplasmic Area |
|---|---|---|---|---|---|---|
| 3rd Instar | 18 | 760.22 ± 36.97 | 19.63 ± 0.01 | 7.12 ± 0.34 | 13.62 ± 0.58 | 0.71 |
| 3rd. Instar Moulting into Minor Presoldiers | 11 | 1360.91 ± 63.44 | 21.02 ± 1.09 | 9.70 ± 0.22 | 13.45 ± 0.44 | 0.29 |
| Early Stage Minor Presoldiers | 18 | 1416.50 ± 117.97 | 32.62 ± 2.22 | 7.32 ± 0.31 | 12.56 ± 0.36 | 0.49 |
| Late Stage Minor Presoldiers | 10 | 1529.60 ± 136.81 | 28.35 ± 1.59 | 5.00 ± 0.19 | 13.00 ± 0.62 | 0.34 |
| Minor Soldiers | 4 | 394.08 ± 31.85 | 19.48 ± 0.01 | 3.33 ± 0.27 | 13.16 ± 0.24 | 0.54 |

Table 6. Measurements of size changes in corpora allata and prothoracic glands during minor soldier development.

into minor presoldier, suggesting that the peak of C.A. activity important for differentiation occurs about this time or long before the ecdysis ensues. From the same Table, it is also evident that the number of CA nuclei does not change during the development of minor soldiers.

Results showing changes in the size of CA nuclei and the nuclear/cytoplasmic ratios during major soldier development are given Table 7. The CA nuclei are largest soon after major soldier determination. The nuclei seem to remain active throughout the soldier developmental interval. Also the nuclear/cytoplasmic ratio is smallest at the stage close to the interval of the soldier emergence, suggesting high activity during this period. In this case, like during minor soldier development, the number of CA nuclei is almost constant throughout major soldier development. In both cases of development, it seems that CA activity is more correlated with the amount of cytoplasm than the size of the nuclei.

4.3.1.6. *Histology of prothoracic glands of neuter castes*

The prothoracic glands are paired; each comprising double strands of cells attached to muscle fibres on both sides within the head capsule. Some of the PG cells have elongated nuclei barely surrounded by any cytoplasm. This appearance is common among the teneral workers and is occasionally observed among the PG of some larvae (Fig. 18). Other specimens studied have PG with generally oval-shaped nuclei of varying sizes surrounded

| Stage | Sample Size (n) | C.A. Max. Cross-Sectional Area ($u^2$) $\bar{X} \pm$ S.E. | C.A. Nuclear Cross-Sectional Area ($u^2$) $\bar{X} \pm$ S.E. | PG Nuclear Diameter (u) $\bar{X} \pm$ S.E. | Number of CA Nuclei (n) $\bar{X} \pm$ S.E. | C.A. Nuclear Cross-Sectional Area/ Cytoplasmic Area. |
|---|---|---|---|---|---|---|
| 3rd Instar | 18 | 760.22 ± 36.97 | 19.63 ± 0.01 | 7.12 ± 0.34 | 13.63 ± 0.58 | 0.71 |
| Early stage of 4th. instar | 11 | 1425.71 ± 172.92 | 21.02 ± 1.09 | 11.21 ± 0.53 | 14.73 ± 0.52 | 0.41 |
| Late stage of 4th instar | 40 | 2864.46 ± 110.13 | 43.38 ± 1.43 | 13.95 ± 0.43 | 12.57 ± 0.35 | 0.27 |
| Moulting 4th instar | 6 | 2525.09 ± 140.91 | 51.24 ± 1.88 | 9.17 ± 0.54 | 12.83 ± 0.23 | 0.38 |
| Early stage of Major Presoldiers | 13 | 2989.00 ± 192.97 | 49.99 ± 2.02 | 8.84 ± 2.08 | 13.46 ± 0.49 | 0.33 |
| Late stage of Major Presoldiers | 9 | 2678.78 ± 188.37 | 51.97 ± 3.38 | 8.70 ± 0.17 | 15.56 ± 0.91 | 0.45 |
| Major Soldiers | 31 | 706.86 ± 63.70 | 19.48 ± 0.01 | 3.33 ± 0.28 | 13.80 ± 0.47 | 0.61 |

Table 7. Measurements of size changes in corpora allata and prothoracic glands during major soldier development.

by appreciable amounts of cytoplasmic material. The amount of the PG cytoplasm of larvae also varies from one larval instar to another, being most bulky in some final instar larvae. It seems that the animals which have vacuolated CA have PG containing rounded, well defined nuclei in abondant cytoplasm (Fig. 19). On the other hand, those with barely vacuolated CA have PG that look poor in cytoplasmic material and nuclei that seem to be partly degenerated (Fig. 26).

The nuclear diameter which was measured to indicate the activity state of PG from individuals of different instars shows that, apart from individual variations, there is a general increase in the size of PG nuclei from the first larval instar to a maximum diameter during the final larval instar. The PG nuclei of late fourth instar larvae are the largest of all the larvae studied (Table 5). Cytoplasm, nuclei and nucleoli are deeply stained and well differentiated (Figs. 15 and 17). The nuclei are about twice the size of those of third instar larvae (Table 5) which normally develop into workers. During major soldier development the PG nuclei achieve maximum size just before fourth instar larva moults into a major presoldier (Fig. 25). In the case of minor presoldiers, the maximum nuclear size is noted during the moult of third instar larvae into minor presoldiers (Fig. 24). There are no data on the PG nuclei before this stage since one cannot detect the third instar individuals which will moult into minor presoldiers or fourth instar. After these decisive moults into presoldiers, the PG decrease in size gradually throughout the subsequent developmental stages

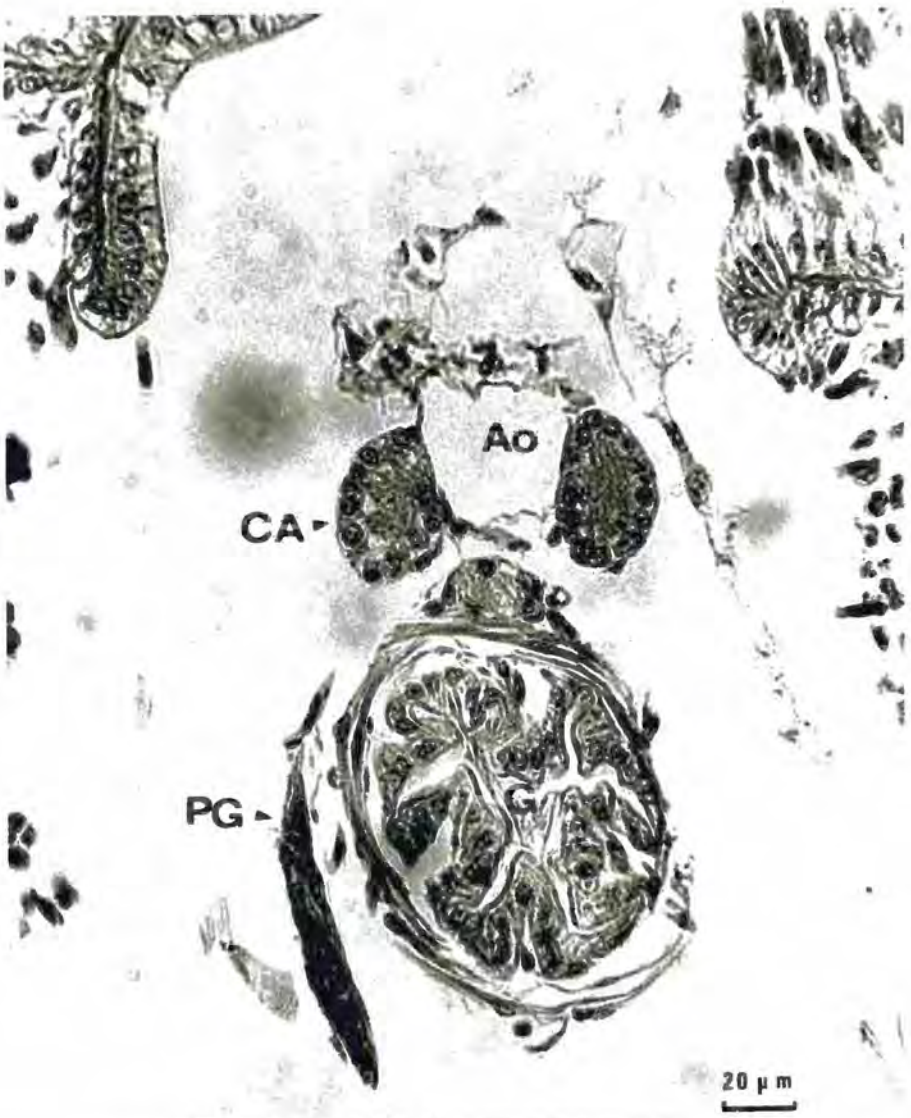



FIGURE 26. Transverse section of corpora allata (CA) and prothoracic glands (PG) of third instar larva showing little vacuolation of CA cytoplasm, but apparently degenerating PG. Ao - Aorta, PG - Prothoracic gland, G - Oesophagus

These glands persist in rudimentary form even in soldier and worker castes which are considered to be adult or terminal castes.

## 4.3.2. *NYMPHS AND REPRODUCTIVES*

### 4.3.2.1. *Changes in corpora allata and prothoracic glands during nymphal development.*

Corpora allata of nymphs and reproductives are positioned within the head capsule and are arranged just like those of neuter castes described above. The shape of these glands is approximately spherical therefore, their volumes to be described were worked out from the histological preparation of serial sections (see methods). The diameters of the glands were measured and the volumes calculated by the formula:

$V = 4/3\pi r^3$, where, V = gland volume, r = gland radius.

The calculated volumes are shown in Fig. 27. The cross-sectional nuclear areas were also worked out from their diameter (Table 8). Nuclear/cytoplasmic ratios of CA and the number of nuclei within the cross-sections are given in the same Table. It is evident from these results that CA change in size during nymphal development as well. One must note that although these changes are significant ($P<0.001$) throughout development, they are rather minimal during the developmental period covering the first through fourth nymphal instar (Fig. 27). By that time, the glands have hardly doubled their size. However, during fifth instar (late stage) these glands increase in size about 10 fold compared with those of fourth instar nymphs. Finally, CA increase in volume another five times soon after moulting into adults.

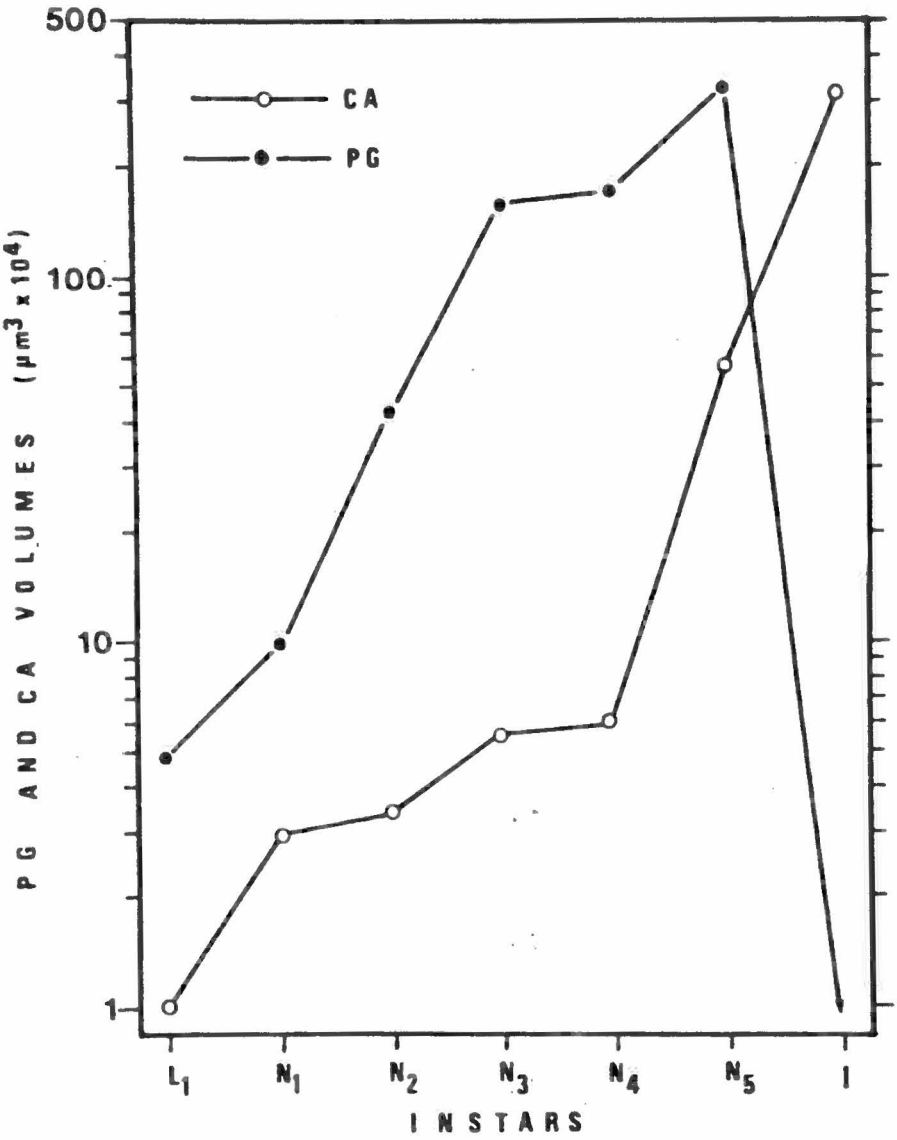



FIGURE 27. Changes in the corpora allata and prothoracic gland volumes during nymphal development. $L_1$ - First instar larva, $N_1$ - $N_5$ - Nymphal instars, I - Imagoes.

The increase in size of CA during nymphal development is reflected in the increase in the number of nuclei within the largest cross-section of each gland. This is in contrast to what was observed during the development of neuter castes. There is a gradual increase in the number of nuclei in the sections a great jump after the moult into the first nymphal instar (Table 8), then another during later fifth instar and early adult stage.

It is evident from Fig. 27 that PG increase in volume more steadily during nymphal development than CA. From first instar larvae to first instar nymphs, the PG simply double in volume, but after the first nymphal moult, they increase in size by a factor of 4.5. After the following moult, the size is again significantly increased ($P<0.001$), 3.5. fold. Curiously enough, there is hardly any increase in PG size between third and fourth instar, but a significant one is observed in the fifth instar. Finally, following a moult into the adult stage, these glands disappear.

From Table 9 one may notice that a large variation in PG volume exists among the samples studied. The reasons for this might be due to the fact that samples used in the study were heterogenous, particularly when one considers the long instar duration (some instars may last longer than a month).

The volumes of nymphal CA and PG were also expressed in relation to their head capsule width (in case of CA) and to weight (in case of PG) so as to minimize the influence of the growth of the animals. These relationships are given in Figure 28.

| Instars | Sample Size (n) | Corpora Allata Volume ($um^3$)(X $x10^5$ ± S.E.) | No. of Nuclei Within Largest Cross-sectional area(X ± S.E.) | Corpora Allata Cross-sectional Area ($um^2$) (X ± S.E.) | Max. Cross-sectional Area of CA Nuclei ($um^2$) (X ± S.E.) | Nuclear Area/ Cytoplasmic Area |
|---|---|---|---|---|---|---|
| 1st Instar Larvae | 10 | 0.099 ± 0.020 | 13.20 ± 0.29 | 535.47 ± 72.79 | 13.91 ± 1.02 | 0.76 |
| 1st Instar Nymphs | 8 | 0.296 ± 0.026 | 17.38 ± 0.63 | 1114.81 ± 80.29 | 20.25 ± 3.89 | 0.46 |
| 2nd Instar Nymphs | 12 | 0.339 ± 0.040 | 16.67 ± 0.70 | 1246.94 ± 97.29 | 20.48 ± 2.52 | 0.38 |
| 3rd Instar Nymphs | 10 | 0.557 ± 0.039 | 21.56 ± 0.72 | 1754.61 ± 82.31 | 16.60 ± 1.78 | 0.26 |
| 4th Instar Nymphs | 8 | 0.577 ± 0.069 | 23.88 ± 1.32 | 1784.55 ± 145.87 | 13.22 ± 1.03 | 0.21 |
| 5th Instar Nymphs | 3 | 5.663 ± 0.783 | 452.33 ± 29.73 | 8238.04 ± 771.50 | 5.56 ± 0.58 | 0.44 |
| Adults (Imagoes) | 10 | 31.0 ± 2.17 | 546.0 ± 33.98 | 26532.72 ± 1022.52 | 5.56 ± 0.35 | 0.13 |

Table 8. Measurements of corpora allata sizes during nymphal development.

| Developmental Stage | 1st Larval Instar | 1st Nymphal Instar | 2nd Nymphal Instar | 3rd Nymphal Instar | 4th Nymphal Instar | 5th Nymphal Instar | Adult |
|---|---|---|---|---|---|---|---|
| No. observed (n) | 10 | 9 | 9 | 8 | 10 | 3 | 10 |
| Prothoracic Gland volume ($um^3$) ($\bar{X} \times 10^4 \pm$ S.E.) | 4.77 ± 0.50 | 9.61 ± 1.44 | 43.0 ± 7.25 | 155.70 ± 13.43 | 168.70 ± 14.67 | 325.23 ±72.81 | 0 |
| Range ($um^3$) ($\bar{X} \times 10^4$) | 3.47 — 8.35 | 2.86 — 15.54 | 20.94 — 77.02 | 95.55 — 222.24 | 98.46 — 241.39 | 237.28 — 469.72 | 0 |

Table 9. Measurements of prothoracic gland sizes during nymphal development

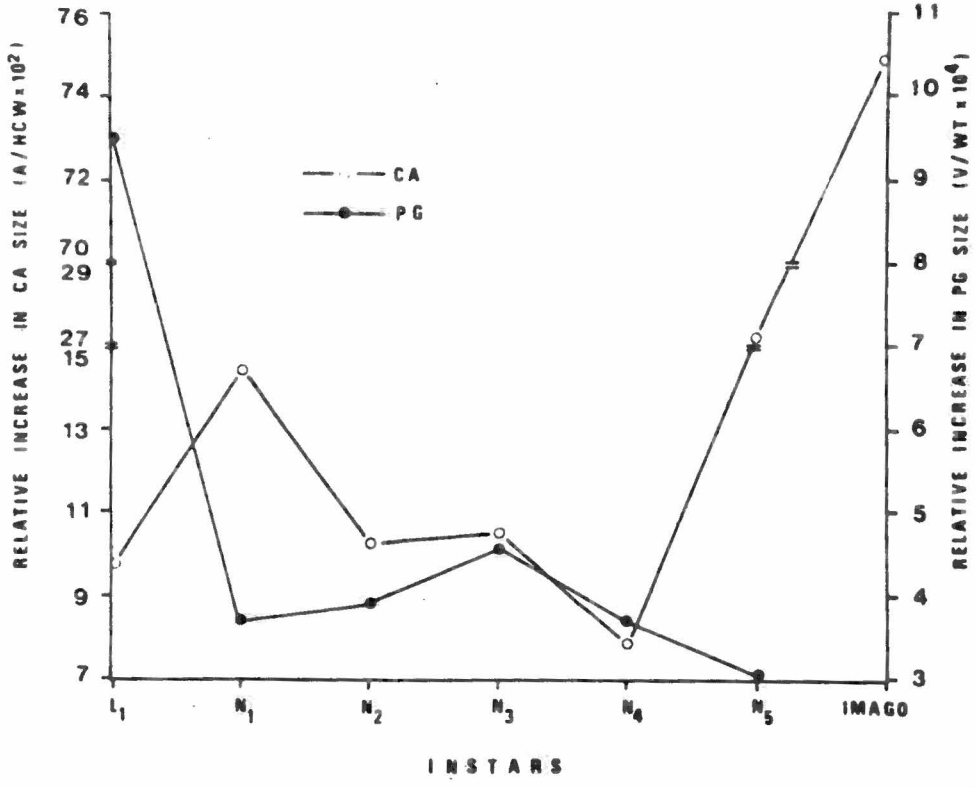



FIGURE 28. Relative changes in corpora allata and prothoracic gland sizes during nymphal development. $L_1$ - First instar larvae, $N_1$ - $N_5$ - Nymphal instars, A - CA Cross-sectional area, V - PG volume, Hcw - Head capsule width, Wt - Nymphal weight.

It becomes apparent that CA decrease in relative size after the initial increase which occurs during the first nymphal instar. The minimum level occurs during the fourth nymphal instar after which it shoots through the fifth instar to attain a peak in the imaginal stage. The relative size of the PG, however, keeps decreasing until the glands degenerate altogether after the imaginal moult.

## 4.4. *DISCUSSION*

The use of CA size as an indication of their activity has been wide. *STRONG* (1965) and *ODHIAMBO* (1966) measured the volume of CA as an indication of glandular activity in *Schistocerca gregaria*. *WHITE* (1965, 1968, 1970) determined the size of the glands while working with cabbage aphids and *LECKSTEIN* (1976) also used the same criterion in estimating the activity of CA in his investigation of the role of CA in prenatal wing determination in *Megoura viciae*. Other author (see introduction) in specific physiological studies on termites used the CA volume as a criterion of activity. Therefore, in the present study, the use of CA size as an indication of activity seemed appropriate for investigating the role of CA in the mechanisms involved in caste differentiation of *M. michaelseni*. However, such measurements have been doubted by *MORDUE* (1967), *LEA* and *THOMSEN* (1969), *GILLOTT* and *DOGRA* (1972), and *JOHNSON* and *HILL* (1973). The latter attributed changes in corpora allata volume in the fourth and fifth instars of the migratory locust to a general somatic growth of the animals, rather than to activity. The

relatively equal sizes of CA in second instar larvae and the different sizes in teneral major and minor workers tend to make it difficult to attribute the changes in CA size during larval development to somatic growth alone. Moreover, it has been recently demonstrated that the haemolymph of *M. subhyalinus* (now *M. michaelseni*) contains high titres of JH (*MEYER* and *LÜSCHER*, 1973) which correlates well with the findings (*LÜSCHER* 1976; *OKOT-KOTBER*, 1977) that the queens of this species and others (*NOIROT*, 1969) have enormous glands. This kind of evidence makes the size of CA, coupled with measurements of nuclear and cytoplasmic sizes a useful tool for indirectly determining glandular activity.

Histological studies have revealed that CA and PG undergo structural and size changes during post-embryonic larval development. Vacuolation of CA nuclei and cytoplasm might indicate a period that follows intensive glandular activity i.e., a phase before moulting events ensue, since it coincides with the interval during which the PG become enlarged and stain best. Vacuolation was found also in the PG cells of *Hyalophora cecropia* (*HERMAN* and *GILBERT*, 1966) and was considered a sign of glandular activity.

The finding that clumps of glycogen are deposited in the cytoplasm and nuclei may be the cause of the vacuolation observed in CA during the histological study. Nuclear glycogen to the best of my knowledge, has never before been reported in any tissue of any insect species, except in the form of glycoprotein

in the nuclei of polyhedral virus infested lepidopteran *Mamestra brassicae* (GRÖNER, 1979). The function of glycogen in the nuclei is still unknown, but might be related to special energy requirements of the CA during the critical interval of soldier development.

The size changes of the CA are not very striking during the larval development towards the formation of workers. The increase in size of the nuclei and cytoplasm is slow, suggesting only a gradual increase in the rate of CA activity. It seems, therefore, that the slow increase in the size of CA is adequate for the basic maintenance of juvenile characters during larval development. On the other hand the CA of fourth instar larvae undergo major changes. The nuclei become extensively enlarged and the cytoplasmic material increases enormously during a later period of differentiation, indicating a much higher glandular activity. It is probable, therefore, that the older fourth instar larvae have the most active CA within the instar, as well as during overall larval development since they have CA with the largest cytoplasmic material and nuclei. The prothoracic glands, like the CA in larvae, also increase gradually in size, apparently sufficient to maintain the hormonal balance for normal development. It is not surprising to note that CA and PG greatly decrease in size during the pigmentation of newly moulted workers and soldiers. These glands would not generally be required for any other purpose during the adult life of the workers which are, afterall sterile. It is difficult even to speculate as to why PG are maintained

in these adult castes. Other workers review by *NOIROT*, (1969) have made similar observations while working with other termite species.

The role of JH (CA) in soldier differentiation in lower termites has been demonstrated with overwhelming evidence *(MILLER*,1969; *LUSCHER*, 1974,a and others (see introduction).

The enlarged CA of fourth instar larvae reported here would seem, therefore, to be playing some vital role in soldier differentiation. The increase in the size of CA is shown to occur in the fourth instar larva as it develops into the major presoldier. *KAISER* (1956) noticed that soldier differentiation in *Neocapritermes* and some unknown species of *Nasutitermitinae* (both are higher termites) is accompanied by an enlargement of CA. In our *Macrotermes* species minor and major presoldiers also have enlarged CA. However, those of the fourth instar larvae unlike the presoldier seem to be more active as evidenced by the abundant cytoplasm and enlarged nuclei. Apparently the initial events leading to the differentiation of major soldiers start during the third instar since it was demonstrated in the earlier studies that fourth instar larvae already have future presoldier mandibles within the existing ones which are superficially like those of minor workers (Chapter Three).

It seems that a certain level of JH is required throughout soldier development following the initial determination. This has been shown by the fact that CA change in size only slightly during the developmental stages of soldiers. *LUSCHER* and *VAN*

*DOORN* (1976) showed that the differentiation characteristics of soldiers in *Zootermopsis* is dependent on the duration of JH or JHA treatment.

Whereas prothoracic glands are largest during the fourth instar development, they become reduced in the presoldiers. This suggests that a certain threshold of ecdysone is required for early soldier development, and that this is higher than that required for ordinary larval development. This may be correlated with the activity of CA as shown by *SIEW* and *GILBERT* (1971) that JH activates PG in *Philosamia cynthia*. *WANYONYI* (1974) also found that, after JHA treatment of *Zootermopsis nevadensis* larvae, prothoracic glands increase much more in the individuals developing into soldiers.

The mechanism involved in soldier differentiation in higher termites is still far from resolved. However, the present findings give some important clues, at least, as to the initiation of soldier development and that CA play a vital role in determining this development. More evidence will be given below in support of CA (JH) as a major component of soldier differentiation.

During nymphal development the results have shown that CA increase in size slowly until the enormous increase late in the fifth instar. However, this increase seems to be caused mainly by somatic growth of the glands as indicated solely by the sudden increase in the number of nuclei and has no relevance

to activity at this stage. This seems to be a preparation for future events in the life of the adult when reproduction ensues. The inactive nature of the gland from first through fourth instar suggests that unlike the soldier, JH may not be necessary for the determination of the reproductive.

There are two possible explanations for the enlargement of PG during nymphal development. These are: either PG are necessary for the differentiation of nymphs or they are needed to meet the requirement for high growth rates that occur during nymphal development particularly in the earlier stages. The role played by PG in the differentiation of the reproductive still remains obscure. There are however, suggestions by *KAISER* (1956) and more recently, by *NOIROT* (1977) that ecdysone may be necessary for differentiation of reproductives. However, there has been no direct evidence reported to support this view.

# 5. LEVELS OF JUVENILE HORMONES AND ECDYSONE IN THE HAEMOLYMPH OF LARVAE DURING SOLDIER DEVELOPMENT

## 5.1. *INTRODUCTION*

It has been stated in Chapter Four that some authors have expressed doubt about the validity of using gland size as an indicator of endocrine activity (*LEA* and *THOMSEN*, 1969; *GILLOTT* and *DOGRA* 1972; and *JOHNSON* and *HILL*, 1973), despite *NOVAK's* earlier proposal which stated to the contrary (1966).

However, other authors have very well correlated CA sizes with various physiological conditions in a number of insect species (*STRONG*, 1965; *ODHIAMBO*, 1966; *WHITE*, 1965, 1968, 1970 and *LECKSTEIN*, 1976).

A number of investigations on the role of CA in caste determination have been carried out (*LÜSCHER*, 1957, 1960, 1965; *SPRINGHETTI*, 1957, *LEBRUN*, 1967, a,b and *GILLOTT* and *YIN*, 972) These investigators used size as a criterion for determining glandular activity. I have also found a good correlation between enlarged CA and soldier formation (Chapter Four).

Since the issue of correlating glandular size with activity continues to be a controversial one, I have conducted a study to measure the titres of juvenile hormones and ecdysone in the haemolymph of fourth instar larvae and presoldiers. This was an attempt to correlate haemolymph hormonal content with glandular sizes summarized in Chapter Four.

## 5.2. *MATERIALS AND METHODS*

Larvae of *Macrotermes michaelseni* were collected from the same area as the rest of the materials used in the previous studies (see Chapters Three and Four). Fourth instar larvae and major presoldiers were sorted out. Thirty fourth instar larvae were pooled into one group. Major presoldiers were sub-divided into three groups namely:

1. Early stage presoldiers.
2. Intermediate stage presoldiers.
3. Late stage presoldiers.

These three stages of major presoldiers were characterized as follows:

Early stage presoldiers were those individuals with whitish almost clear slender abdomens apparently with no food indicating that they had recently moulted. The intermediate stage consisted of individuals with yellowish, translucent, distended abdomens, apparently well fed. The late stage was made up of individuals of a darker yellowish coloration, almost transparent abdomens, apparently empty, and probably preparing to moult. The tips of their mandibles were more heavily pigmented, a kind of brownish colour.

Haemolymph was collected from these various groups of individuals as described in the general materials and methods (Chapter Two). Each sample of haemolymph was divided into two equal parts. One part was for JH titre determination and the other for ecdysone.

The details of the procedures for these determinations have been given in Chapter Two.

## 5.3. *RESULTS*

### 5.3.1. *Juvenile hormone titres*

The haemolymph juvenile hormone titres are given in Fig. 29. The highest titres were found in the fourth instar larvae followed by a progressive decline during subsequent stages of soldier development. The second highest titre was that of early stage presoldiers when it had dropped by about a half. Intermediate stage showed further decline by about 0.7 times that of the preceding stage. The lowest titre was recorded in the late stage of presoldier development.

Since $JH_{II}C_{17}$ was used as a control, the JH titres are expressed in µg $JH_{II}$ equivalent per ml of haemolymph (Table 10). One galleria unit (GU) being the amount of JH that would give positive result in 50% of treated pupae. In the present case where $JH_{II}$ was used as standard, 1GU is equivalent to $4x10^{-5}$ µg of JH.

### 5.3.2. *Ecdysone titres*

The levels of ecdysone found in the haemolymph of different stages of soldier development are presented in Fig. 30. These levels were calculated from a standard curve of β-ecdysone. The levels follow closely the pattern of JH decline during the development. The highest was registered in fourth instar larvae but a very sharp decline followed after the moult into the

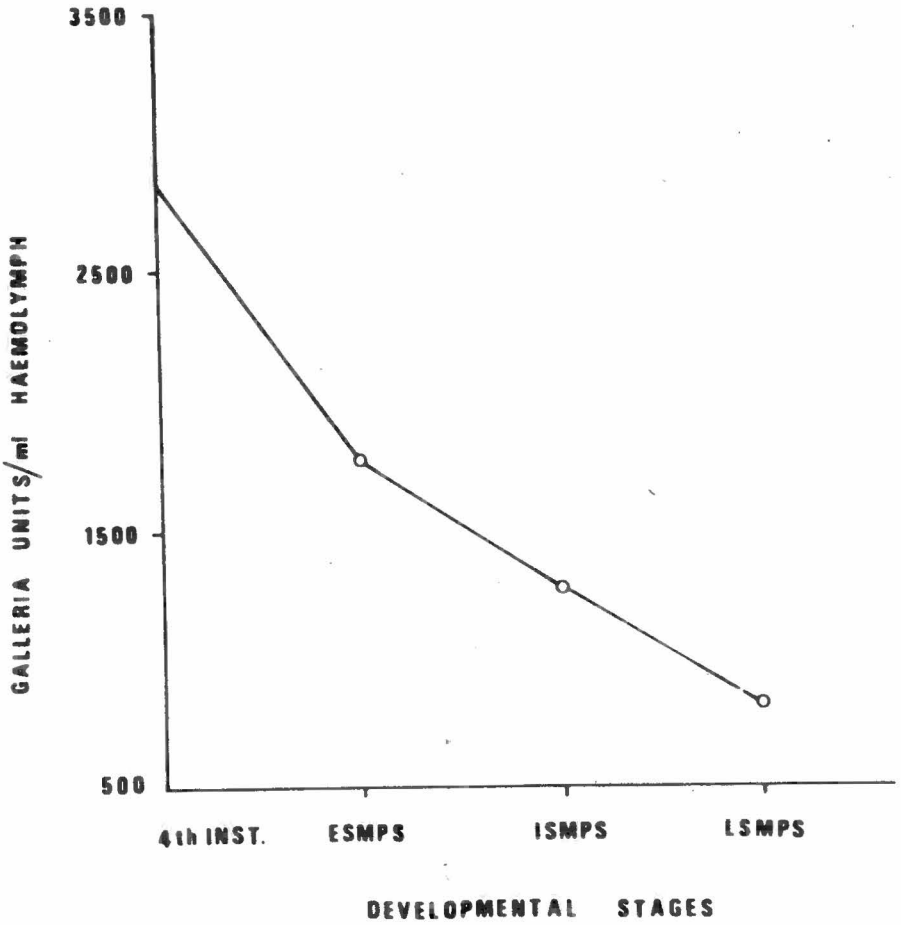



FIGURE 29. Changes in juvenile hormone titres in the haemolymph during soldier development. 4th. INST.-Fourth Instar, ESMPS - Early stage major presoldiers, ISMPS - Intermediate Stage major presoldiers, LSMPS - Late stage major presoldiers.

| Stage | Fourth Instar Larvae | Early Stage Major Presoldiers | Intermediate Stage Major Presoldiers | Late Stage Major Presoldiers |
|---|---|---|---|---|
| No. of Individuals Used | 80 | 40 | 35 | 24 |
| Total Amount of Haemolymph Collected (ul). | 155 | 185 | 280 | 160 |
| Juvenile Hormone Titre (ng/ml h) | 113.8 | 71.5 | 51.3 | 32.6 |

Table 10. Juvenile hormone levels in the haemolymph during soldier development

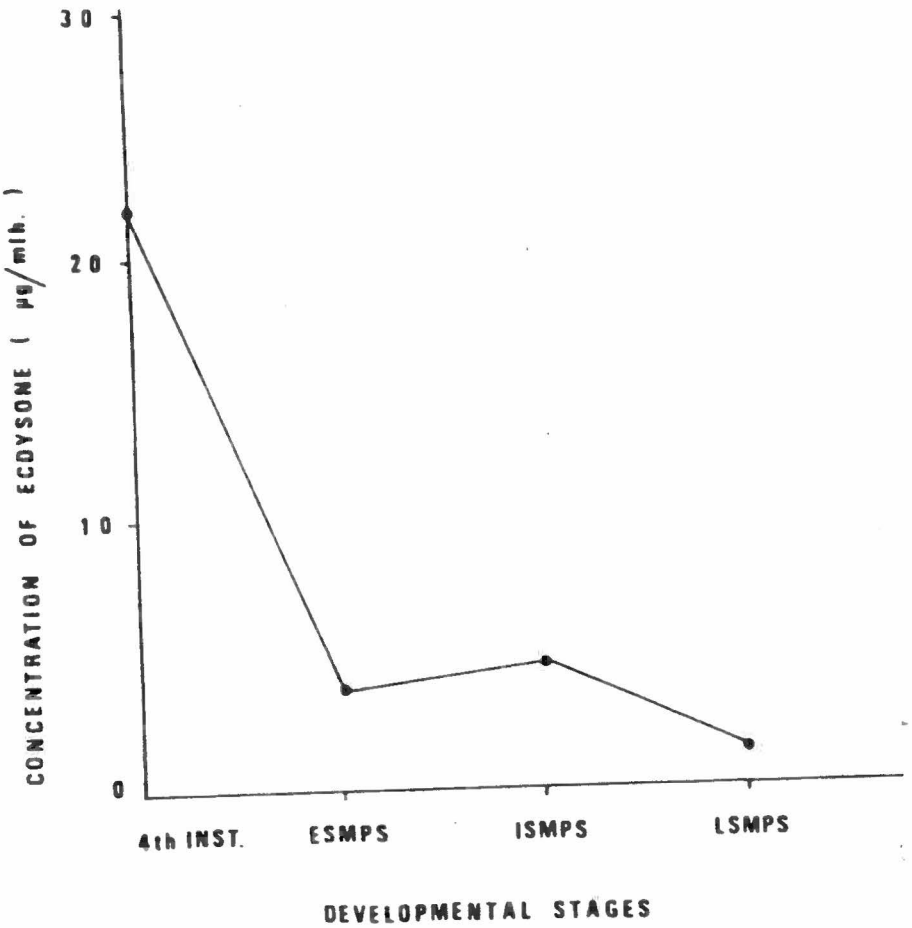



FIGURE 30. Changes in ecdysone titres in the haemolymph during soldier development. 4th. INST.-Fourth Instar larvae, ESMPS. - Early stage major presoldiers, ISMPS- Intermediate stage major presoldiers, LSMPS - Late stage major presoldiers.

presoldier. The level of ecdysone during this time had dropped by almost 7 fold. During the intermediate stage a slight rise was recorded with a further drop (by about 3.5 fold) observed in the late presoldier stage. This last drop was about 3.5 fold the level observed in the intermediate stage. These observations also closely agree with the histological investigations (Chapter Four) where PG activity was shown to be highest in fourth instar larvae followed by a steep decline in the subsequent stages.

## 5.4. *DISCUSSION*

Changes in the levels of juvenile hormones found in the present study agree with the findings on size changes of corpora allata reported in Chapter Four. There is quite a good correlation between CA size and the JH titres in the haemolymph during different stages of soldier development. The changes in both CA size and JH titres are not drastic as the presoldier develops from one stage to another. Similar results were found by *DE WILDE* and *OOSTRA* (see *de WILDE* et al., 1968) while studying hormonal conditions in the Colorado potato beetle during diapause and reproduction. They found that increased titres of JH were correlated with increased volume of the corpora allata.

The high levels of JH which were maintained throughout most stages of soldier development suggest a requirement for a constant supply of JH for normal soldier development. *LÜSCHER* and *VAN DOORN* (1976) showed in *Zootermopsis* that JH or JHA is required for prolonged periods, even after differentiation, in the formation of normal soldier characteristics. It seems that this

requirement progressively declines after differentiation since these results show that the highest JH levels are realized after or before differentiation and thereafter, a progressive decline to the lowest level just before the soldier moult.

The pattern of changes in ecdysone titres follows closely that of JH. This suggests a close interplay between JH and ecdysone during soldier development. In the fourth instar, when JH titre is highest, ecdysone level is also highest. After the moult, ecdysone titre decreases very sharply (7 fold) compared with JH (only about 2 fold) this may suggest that the premoult interplay between JH and ecdysone differs from that in the postmoult presoldier. A small peak in ecdysone titre is realized in the intermediate presoldier stage and it may be responsible for a final moult into the soldier.

# 6. POLYMORPHISM AND THE DEVELOPMENT OF THE FIRST PROGENY IN INCIPIENT COLONIES

## 6.1. *INTRODUCTION*

When studying the biology of termites, it is imperative first to establish the life cycle of the colony. Studies in this line have been conducted on all families covering a wide range of genera of this order of insects. *GRASSE* and *NOIROT*(1946, 1958) and *LÜSCHER* (1952, b) have reared *Kalotermes flavicollis* and established developmental pathways in this species. *BUCHLI* (1950, 1956, 1958) carried out similar studies in several species of rhinotermitid, which enabled him to determine polymorphism in *Reticulitermes lucifugus* and *R. l. santonensis*. *LIGHT* and *WEESNER* (1955), working with *R. hesperus*, investigated the regulation of soldier numbers and development of primary colonies. *KING* and *SPINK* (1974) also reported on young colony development in another genus of rhinotermitid, *Coptotermes*.

Similar work has been done on higher termites as well, but this has not been comprehensive, probably due to the difficulties involved. Notable reports which can be cited are those of *LIGHT* and *WEESNER* (1947) on development of the neuter castes in *Tenuirostritermes tenuirostris* and *Gnathamitermes perplexus*. *NOIROT* (1949) followed the development of *Nasutitermes arborum* in the Ivory Coast and established a developmental scheme for this species. *GRASSE* and *NOIROT* (1955) carried out pioneering work on colony founding in *Bellicositermes natalensis* (now

*Macrotermes bellicosus)* a species related to the one in this study.

Other reports include those of *WILLIAMS* (1959) on *Cubitermes ugandensis* and *SANDS* (1965) on alate development and colony foundation in five species of *Trinervitermes* in Nigeria, W. Africa.

It became necessary to observe closely the initial stages of development of incipient colonies of the *Macrotermes* since the mechanisms underlying caste differentiation cannot be studied without the knowledge of polymorphism and instar duration.

## 6.2. *MATERIALS AND METHODS*

Reproductives (alates) were collected from the Kajiado area, Kenya, during the swarming season of the species of *Macrotermes* under study. Alates were attracted to a light trap and collected in small plastic basins with a thin layer of moist soil at the bottom. They were dewinged in the laboratory and heterosexual pairs were introduced into plastic petri dishes about 11 cm in diameter. These dishes had previously been filled with about 70 gm of sterilized, sifted mound soil and moistened with boiled water. An adequate stream of water poured into the centre of the dish moistened the soil and created a hole which later facilitated the formation of copularium by the pair of the reproductives. The established colonies were kept in the insectary at $30^{\circ} \pm 1^{\circ}C$ and watered once or twice a fortnight to maintain the high humidity required.

The incubation period of the eggs was determined by checking daily for 6 weeks 90 colonies which had been previously chosen for their good condition and having laid their first batch of eggs on the same day. Daily observations were continued for another 6 weeks during which the types of moult occurring were recorded.

Samples of larvae in different instars, presoldiers, soldiers, minor and major workers were taken and fixed in 80% alcohol for subsequent biometric studies. The following parameters were measured with a dissecting stereomicroscope with calibrated ocular: maximum head capsule width, posterior tibia length and antennal length. The number of antennal segments was also counted.

## 6.3. *RESULTS*

Female reproductives in the colonies chosen for these studies had laid their first batch of eggs by the first week following colony establishment. The mean incubation period of eggs was 36.2 $\pm$ 0.3 (one standard error of the mean) days. The incubation period ranged from 33-42 days. On the 33rd. day the greatest number of colonies (33%) had eggs hatching into first instar larvae of any single day. There was a gradual increase in the number of colonies with eggs hatching between 34th and 41st day, summing to an additional 65%. The lowest point was observed on the last day of the investigation which constituted the remaining 2%.

Fifteen colonies (17%) had to be eliminated from the experiment after noting the date of first instar emergence. This was necessary as a result of their poor condition expressed as general weakness of the parents, disappearance of eggs and/or larvae, or in their eventual death.

Fig. 31 differentiates between groups of larvae on the basis of head capsule width versus the ratio of antennal length to head capsule width. Five groups are detectable by such a plot. However, one may note that the separations between the first three groups of larvae are not so distinct, while the last two are quite evident. The first group represents a mixture of first instar female and male individuals. The second group contains female second instar larvae, the third group consists of male second instar, while the fourth and fifth represent female and male third instars, respectively. Measurements of larvae are summarized in Table 11. Five larval groups were distinguishable based on these measurements as well. The data also show that there are only minor size differences between the first two groups of larvae. However, a size difference was obvious between larvae of fourth and fifth groups. Although antennal length showed differences between the groups, the number of antennal segments varied little Table 11. The antennae from the first three groups of larvae consisted of 13 segments while those of the last two groups had 15 segments each.

Polymorphism in worker and soldier castes was diagnosed by measurements of head capsule width, posterior tibia length

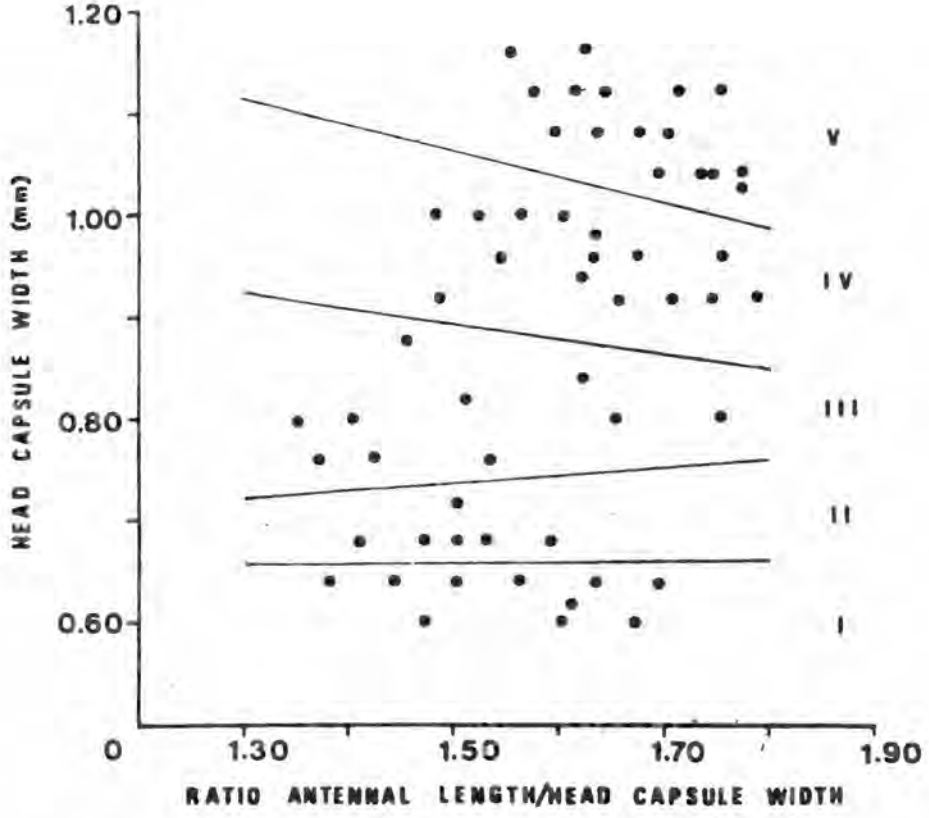



FIGURE 31. A plot of head capsule width versus the ratio of antennal length to head capsule width of larvae from incipient colonies. I-V - Larval groups.

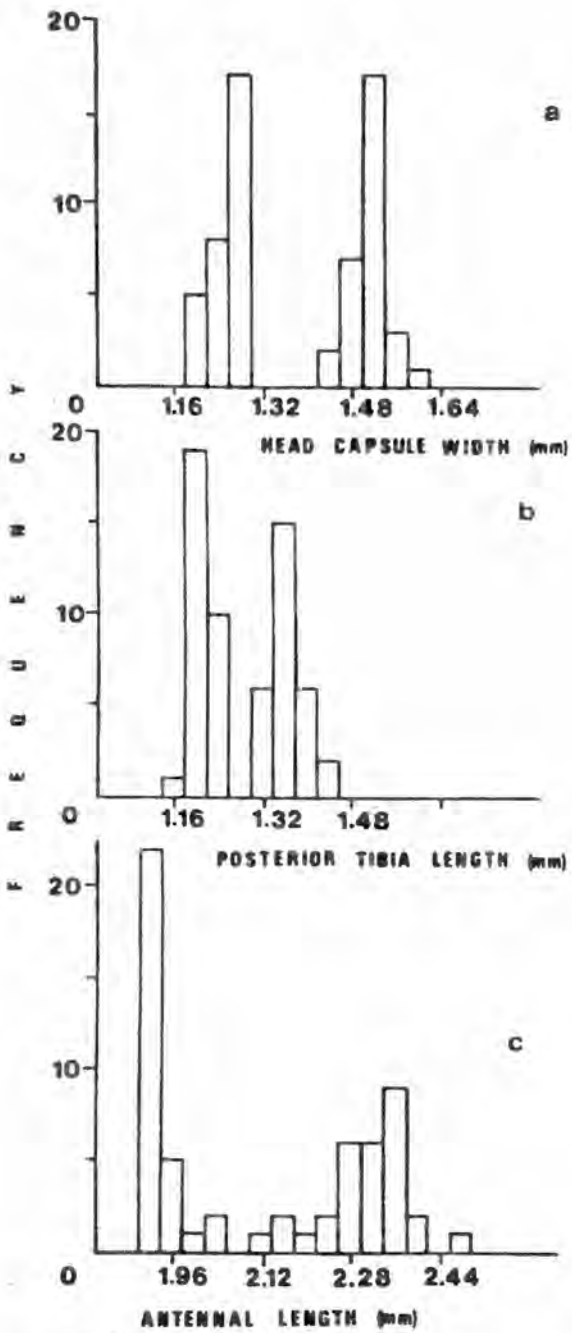
| Larval Instar (L) | n | Head Capsule Width (mm) | | Posterior Tibia Length (mm) | | Antennal Length (mm) | | Number of Antennal Segments. |
|---|---|---|---|---|---|---|---|---|
| | | Mean ± S.E. | Range | Mean ± S.E. | Range | Mean ± S.E. | Range | |
| 1 First $L_1$ | 27 | 0.63 ± 0.01 | 0.60-0.64 | 0.42 ± 0.01 | 0.36-0.48 | 0.98 ± 0.01 | 0.98-1.08 | 13 |
| 2 Second $L_2$ | 28 | 0.68 ± 0.01 | 0.68-0.72 | 0.43 ± 0.01 | 0.40-0.48 | 1.02 ± 0.04 | 0.96-1.08 | 13 |
| 3 Second $L_2$ | 12 | 0.80 ± 0.01 | 0.76-0.88 | 0.53 ± 0.02 | 0.44-0.64 | 1.21 ± 0.04 | 1.04-1.40 | 13 |
| 4 Third $L_3$ | 34 | 0.95 ± 0.01 | 0.94-1.00 | 0.81 ± 0.01 | 0.76-0.88 | 1.57 ± 0.01 | 1.36-1.68 | 15 |
| 5 Third $L_3$ | 33 | 1.09 ± 0.01 | 1.04-1.16 | 0.88 ± 0.01 | 0.80-0.96 | 1.81 ± 0.01 | 1.72-1.96 | 15 |

Table 11. Measurements of larvae from incipient colonies

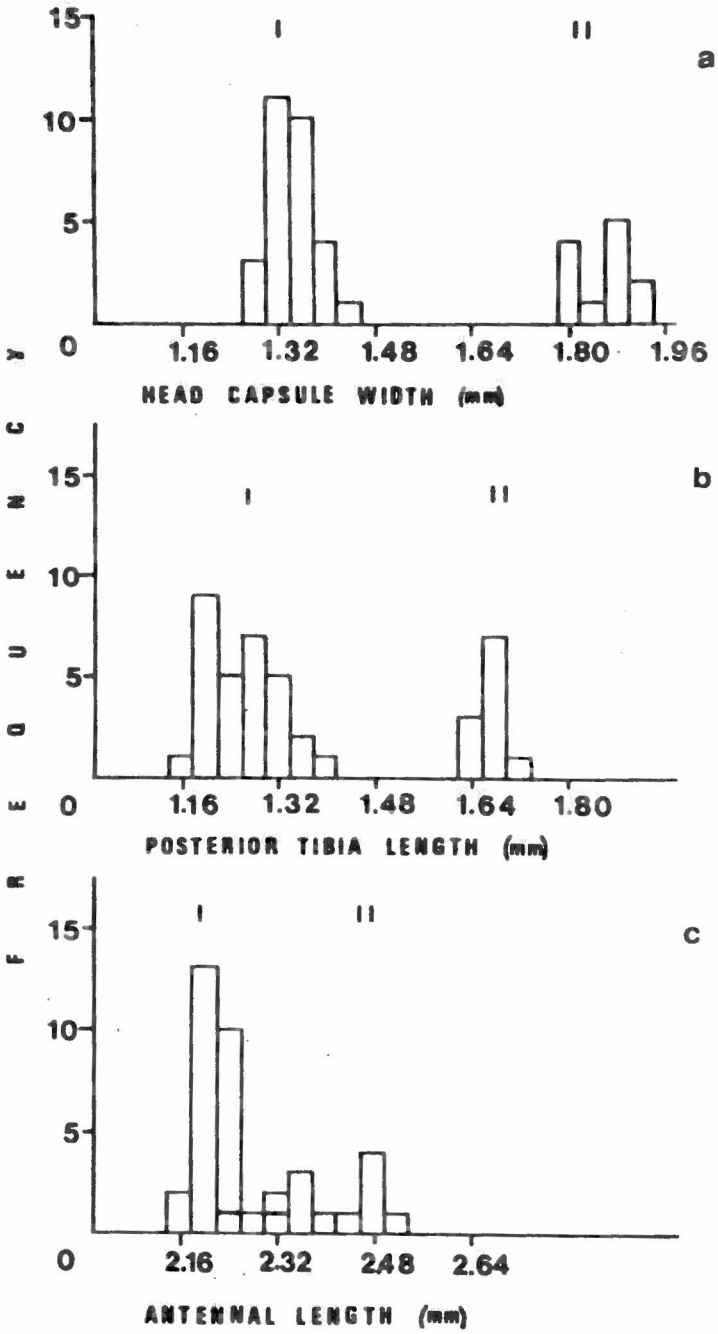
and antennal length. A bimodal distribution in the histogram of these measurements shows that two types of workers already exist among the first progeny of incipient colonies (Figs. 32, a, b and c). However, only one category of presoldier and soldier was diagnosed by the same procedure (Figs. 33, a, b and c) as evidenced by the single normal distribution.

Mean values of these terminal caste measurements along with those of minor presoldiers are presented in Table 12. Workers could be separated into minors and majors while only one type of soldiers was detectable. Also only one type of presoldier could be diagnosed. The differences between the head capsule width, posterior tibia length and antennal length of minor and major workers were all statistically significant at ($P<0.01$). Workers, presoldiers and soldiers all had the same number of antennal segments just as in third instar larvae (Tables 11,12).

Although it was difficult to distinguish visually between newly emerged female and male second instars, with some practice, it became possible to recognize them by size alone in at least 24 (32%) of the colonies. Subsequent moults into third female and male instars were accurately recorded in 20 of the 24 colonies. It was easier to detect moults leading to minor and major workers, or to minor presoldiers since these three forms are distinct on the basis of size and form. The mean duration of each instar was therefore determined and is summarized in Table 13. The shortest period covers development of first instar larvae into second instar females or males. Second instar female larvae




FIGURES 32 a, b and c. Bimodal distribution of (a) - Head capsule width, (b) - Posterior tibia length, and (c) - Antennal length of workers from incipient colonies.




FIGURES 33 a, b and c. Histograms showing distribution of: (a) - Head capsule width, (b) - Posterior tibia length, and (c) - Antennal length of presoldiers (I) and soldiers (II) from incipient colonies.

| Instar | n | Head Capsule Width (mm) | | Posterior Tibia Length (mm) | | Antennal Length (mm) | | Number of Antennal Segments |
|---|---|---|---|---|---|---|---|---|
| | | Mean ± S.E. | Range | Mean ± S.E. | Range | Mean ± S.E. | Range | |
| Minor Worker | 30 | 1.26 ± 0.01 | 1.20 - 1.28 | 1.21 ± 0.01 | 1.16 - 1.24 | 1.94 ± 0.01 | 1.92 - 2.04 | 15 |
| Major Worker | 30 | 1.51 ± 0.01 | 1.44 - 1.60 | 1.37 ± 0.01 | 1.32 - 1.52 | 2.31 ± 0.01 | 2.12 - 2.48 | 15 |
| Minor Presoldier | 29 | 1.35 ± 0.01 | 1.30 - 1.44 | 1.26 ± 0.01 | 1.16 - 1.36 | 2.23 ± 0.01 | 2.14 - 2.40 | 15 |
| Minor Soldier | 12 | 1.86 ± 0.01 | 1.80 - 1.92 | 1.65 ± 0.02 | 1.40 - 1.72 | 2.42 ± 0.030 | 2.24 - 2.52 | 15 |

Table 12. Measurements of minor presoldiers, minor soldiers, minor and major workers from incipient colonies.

| Instars | Number of colonies observed | Instar duration (days) ♀ | | Instar duration (days) ♂ | |
|---|---|---|---|---|---|
| | | (Mean ± S.E.) | Range | (Mean ± S.E.) | Range |
| First ♂♀ | 24 | 5.5 ± 0.2 | 4 — 7 | 5.6 ± 0.2 | 4 — 7 |
| Second ♂♀ | 20 | 5.2 ± 0.4 | 3 — 11 | 8.0 ± 0.5 | 5 — 12 |
| Third ♀ | 54 | 10.2 ± 0.3* | 7 — 18 | — | — |
| Third ♂♀ | 54 | 14.5 ± 0.3** | 7 — 19 | 13.9 ± 0.4 | 9 — 17 |
| Minor Presoldiers | 63 | 11.7 ± 0.2 | 9 — 16 | — | — |
| Total developmental period after hatching (days) | | 25.2 mw<br>32.6 ms | | 27.5 MW | |

* Duration of female 3rd. Instar developing into a minor presoldier.
** Duration of female 3rd. Instar developing into a minor worker.
mw — minor worker, MW — major worker, ms — minor soldier

Table 13. Duration of larval instars during worker and soldier development in incipient colonies

| Order of appearance | Colonies with minor presoldiers n(%) | Colonies with minor workers n(%) | Colonies with major workers n(%) | Colonies with minor soldiers n(%) |
|---|---|---|---|---|
| 1 | 63(94.0) | 3(4.5) | 0 | 0 |
| 2 | 4(6.0) | 61(91.0) | 9(13.4) | 0 |
| 3 | 0 | 3( 4.5) | 55(82.1) | 10(14.9) |
| 4 | 0 | 0 | 3( 4.5) | 57(85.1) |
| Total number of colonies observed | 67(100) | 67(100) | 67(100) | 67(100) |



Table 14. The frequency distribution of the sequence of appearance of first minor presoldier, and soldier, minor and major workers in incipient colonies.

require the same length of time to develop into third instars, whereas it takes much longer for second instar males to develop into third which subsequently develop into major workers. Female and male third instar larvae took practically the same length of time to develop into respective types of workers (Table 13).

The frequency of chronological appearance of the first minor presoldier, soldier, minor and major workers, is shown in Table 14. In the bulk of the colonies presoldiers were the first to appear, followed by minor workers, then major workers and finally soldiers. A small proportion of the colonies had the first presoldiers much later, in fact, at the same time as most colonies were producing minor workers. However, no presoldiers had appeared at the time when the majority of colonies had their first major workers. On the other hand, a few colonies had their first soldiers at the time the first major workers were appearing in most of the colonies. Some colonies also had their minor workers at this time. In a few colonies minor worker moults were observed at the same period when presoldier moults were occurring in the majority of the colonies. In a limited number of colonies, major workers appeared at the same time as minor workers.

The sequence of appearance of terminal neuter castes in the 80-day-old incipient colony may be summarized as follows: In most colonies (91%), minor workers were the first to appear, in 82% of the colonies, the major workers soon followed and, finally, in 85% of colonies the minor soldier caste was the last to emerge.

## 6.4 DISCUSSION

The data presented here show the sequence of events occurring in incipient colonies of *Macrotermes michaelseni*. These events are similar to what *NOIROT* (1955) and *GRASSE* and *NOIROT* (1955) have reported on the development of incipient colonies of *M. bellicosus*. However, there are differences in some details which might reflect species specificity. For example, in the present study, the mean incubation period for eggs was slightly shorter, but the time taken between hatching of eggs and the appearance of minor presoldiers or minor workers is about the same in both cases. They reported much longer intervals for development into major workers and minor soldiers than what we found for *M. michaelseni*. However, the number of larval instars in both cases are the same i.e. three larval instars for male and female lines of development into workers and/ or presoldiers in case of female larvae.

The individuals produced in the incipient colonies studied were much smaller than those from field colonies as was also reported for *M. bellicosus (NOIROT*, 1955), and other termite genera, particularly *Reticulitermes (PICKENS*, 1932; *BUCHLI*, 1950), *Mastotermes darwiniensis (WATSON*, 1974). This phenomenon seems therefore, a common feature in termite colony development. The only individuals found here to be larger in incipient colonies than their counter-parts from mature field colonies, were first instar larvae with head capsule width of about 0.63mm as compared to 0.55mm for those from field colonies. *NOIROT* (1955) did not note any size differences between first instar larvae from

incipient colonies and those from field colonies of *M. bellicosus*. The small size differences found between first, second and third groups of larvae were also reflected in the closeness in instar duration of male or female first instar larvae, moulting into respective second instars. This may mean that having undergone similar intermoult duration, these larvae received comparable quantities of food, thus the similar tendencies in growth rates. In field colonies, first, second and third group larvae have small differences in head capsule width and posterior tibia length (see Chapter three). It would therefore be expected that even smaller differences in individuals from the incipient colonies would prevail.

It was also observed that individuals from the first progeny of incipient colonies generally have antennae with fewer numbers of segments as compared with those of mature field colonies. The exception are first instar larvae, where in both cases the larvae have antennae with 13 segments. Second and third instar larvae, minor and major workers, minor presoldiers and soldiers have antennae with 13, 15, 15, 15, 15 and 15 segments, respectively, while their counterparts from field colonies have 15, 17, 17, 18, 17 and 17 segments, respectively. It is not clear why these differences occur. The type of polymorphism observed in mature colonies of *M. michaelseni* (Chapter three) is very similar to what is found under incipient laboratory conditions with the exception that, individuals in the latter are smaller and lack fourth instar larvae and subsequently, major soldier.

Also, under these conditions no reproductives of any type are produced in the first progeny. No data to compare instar duration between immature individuals from an incipient colony and those from mature field colonies where development is within a closed mound could be collected.

# 7. THE INFLUENCE OF JUVENILE HORMONE ANALOGUE ON CASTE DIFFERENTIATION

## 7.1. *INTRODUCTION*

In Chapter four evidence on the role of corpora allata and prothoracic glands on caste differentiation was presented. There was every indication that the increase in size of corpora allata is associated with soldier development. Bearing this in mind, the work being reported in this chapter was initiated with an aim of obtaining direct evidence on the role played by juvenile hormones (JH) on caste differentiation in *Macrotermes michaelseni*.

Evidence has accumulated in the literature supporting the view that JH play a vital role in the determination of soldiers in lower termites. *LÜSCHER* (1969) showed that he could induce soldier formation in *Kalotermes flavicollis* by feeding isolated larvae with food impregnated with a juvenile hormone analogue (JHA) or by injecting them with synthetic JH. Later *WANYONYI* (1974) showed that JHA administered in vapour form had a similar effect on pseudergates and nymphs of another termite, *Zootermopsis nevadensis* belonging to the family of *Termopsidae*. Similar investations were carried out on a representative species of *Rhinotermitidae*, *Reticulitermes lucifugus santonensis* (*HRDY*, 1972; *HRDY* and *KRECEK*, 1972) whereby filter paper was impregnated with a JHA or synthetic JH and fed to groups of pseudergates or larvae. This treatment also induced development of

superfluous soldiers. Recently *LENZ* (1976a) showed that JHA has a soldier inducing effect in another species of *Rhinotermitidae, Coptotermes amanii.* (*LENZ*, 1976b) also demonstrated a similar effect of JHA on *K. flavicollis, C. niger, R. lucifugus* and *Heterotermes indicola* while investigating the dependence of hormone action on external factors. *HRDY* (1976) reached the same conclusion in his work on a number of genera of lower termites.

However, little is known about the mechanisms regulating on castes in higher termites. We have to date only fragmentary evidence supporting the view that JH play a major role in caste differentiation. *FRENCH* (1974) reported that he could induce soldier formation in *Nasutitermes exitiosus* using JHA. Similar results were later obtained by *LENZ* (1976, b) also on *N. nigriceps.*

## 7.2. *MATERIALS AND METHODS*

*General*

First, second and third instar larvae were used in the experiments to be described. Pairs of reproductives from incipient laboratory colonies were used. The reproductives were first kept in 11cm plastic petri dishes with moist soil together with their brood. They were later transferred as required into smaller (6-cm diameter) plastic dishes for experiment. Alternatively, the colonies were established in the small plastic dishes until required; therefore the experiments were carried out without transferring the reproductives.

Preliminary experiments had shown that reproductives of only a particular age were capable of adopting larvae, therefore all the reproductives used were of this "adoptive" age. This was usually about the time when the first workers and soldiers were formed in the incipient colonies.

Experimental larvae were placed with adoptive pairs in groups of 20 as necessary and treated topically with either redistilled acetone alone (control) or acetone plus JHA (ZR-0515, Altosid IGR, a gift from *DR. G.B. STAAL* of Zoecon Co-operation Palo Alto, California, USA). A minute volume (0.5µl) of the solvent and compound was released onto the dorsum of the larval abdomen with a micro-applicator (Instrumentation Specialities Company, USA) fitted with a syringe and a fine glass needle. The acetone was allowed to evaporate before the treated individuals were placed back into the colonies with a fine brush. For the vapour treatment, clean dish covers were modified by sticking round cover slips onto their inner side. The JHA solutions of a required concentration in acetone or acetone controls 50µl were carefully placed on the glass surface. After the acetone had evaporated, the lids were then replaced on the treated colonies.

The treated colonies were kept at 30$^{o}$C in an incubator throughout the experimental period. They were checked at intervals according to the requirement of the experiments. Development of individuals was noted and where reproductives died they were replaced by an individual of the same sex and age from the stock colonies.

## 7.3. *RESULTS*

### 7.3.1. *Responsiveness of larvae of different instars to JHA*

It was of paramount importance to establish whether larvae of different instars would respond to JHA and, if so, what kind of response, since subsequent experiments required precise data on timing of larval differentiation.

Reproductives from about 70-day-old colonies deprived of their own brood were used as adoptive parents of the experimental larvae: First instar, females of second and third instars, all collected from incipient stock colonies. Only 15 individuals were used in each of the control colonies. Four topically administered doses of JHA were tested, namely, 1.0µg, 6.25µg, 12.5µg and 25.0µg per animal. Observations were made once every three days, save at the start when the first checking was made after 9 days, since earlier experiments had shown that presoldiers to not appear until at least 9 days after treatment.

The results showed that survival rates of treated individuals were lowest at JHA concentrations of 6.25µg/animal and above. The most vulnerable were first instars followed by second and finally third instars. The results, therefore, will refer only to individuals treated with 6.25µg JHA or less. The first presoldiers appeared by the ninth day following treatment in most colonies which had adopted third instar larvae. No presoldiers had been formed by then in the colonies containing first or second instar larvae. The peak of presoldier emergence was

| Treatment (JHA in ug) | Instars Treated | Replicates | Total Maximum No. (%) of Presoldiers Differentiated | No. (%) of Workers at the Time | | No(%)of Unpigmented Workers + Intercastes | Max. No.(%)of Soldiers Formed | No. (%)of Workers at the time | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Minor | Major | | | Minor | Major |
| CONTROL | 1st | 5 x 15 | 4(13.3) | 6(20) | 0 | 0 | 4(16.0) | 10(40.0) | 11(44.0) |
| (ACETONE) | 2nd | 5 x 15 | 4(15.0) | 2(7.7) | 13(50) | 0 | 4(16.0) | 3(12.0) | 17(70.8) |
| | 3rd | 5 x 15 | 2(8.0) | 22(40) | 12(21.8) | 0 | 1( 2.0) | 33(60.0) | 20(36.4) |
| | 1st | 5 x 20 | 6(17.1) | 15(35.9) | 5(14.3) | 1(3.3) | 5(16.7) | 15(50.0) | 9(30.0) |
| 1.0 | 2nd | 5 x 20 | 3( 9.1) | 5(15.2) | 17(51.5) | 0 | 3(10.0) | 8(26.7) | 19(63.3) |
| | 3rd | 5 x 20 | 7(16.3) | 19(38.8) | 10(20.4) | 6(12.2) | 7(14.5) | 24(50.0) | 11(22.9) |
| | 1st | 5 x 20 | 1(14) | 2(28) | 2(28) | 1(11.1) | 1(20.0) | 1(20.0) | 3(60.0) |
| 6.25 | 2nd | 5 x 20 | 2(12.5) | 0 | 0 | 2(18.2) | 2(18.2) | 2(18.2) | 5(45.5) |
| | 3rd | 5 x 20 | 15(30.6) | 15(30.6) | 13(26.5) | 5(11.1) | 11(27.5) | 13(32.5) | 12(30) |
| | 1st | 5 x 20 | 0 | 0 | 0 | | 0 | 0 | 0 |
| 12.5 | 2nd | 5 x 20 | 3(16.7) | 4(22.2) | 5(27.8) | 0 | 4(22.2) | 5(27.8) | 9(50.0) |
| | 3rd | 5 x 20 | 6(54.6) | 0 | 4(36.4) | 1(9.1) | 6(66.7) | 1(11.1) | 2(22.2) |
| | 1st | 5 x 20 | 0 | 1(100) | 0 | 0 | 0 | 1(100) | 0 |
| 25.0 | 2nd | 5 x 20 | 4(66.7) | 0 | 0 | 0 | 0 | 0 | 0 |
| | 3rd | 5 x 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 15. The peak of presoldier formation in colonies containing JHA-treated adopted larvae of various instars

reached earliest (by day 12 - 15) in the colonies with third instar larvae and latest in the colonies with first instar larvae (by day 33).

Similarly, soldiers first appeared in colonies which started with third instar larvae (by day 15). The peak of soldier emergence also was achieved earliest in the colonies which had started with third instar larvae (by day 23) and latest in colonies started with first instar larvae (by day 34). The timing of events in presoldier formation seems therefore to be independent of treatment, but rather dependent on the instar treated. Table 15 summarises the rates of presoldier formation under different conditions. It is clear from the table that the JHA has no influence on first and second instar larvae as far as presoldier formation is concerned. However, the analogue induces presoldier formation in third instar female larvae, especially at doses greater than 1.0µg/animal. It became evident therefore, that subsequent experiments exploring mechanisms of soldier formation should be based on only third instar larvae.

7.3.2. *Dose response of third instar larvae to JHA (Topical treatment).*

It was necessary to obtain some data on the optimal dose reponse for JHA to be used in the subsequent experiments, particularly after determining that excessively high doses cause high mortality of treated larvae. Third instar female larvae were used in these experiments. As in the previous experiments groups of 20 treated larvae were adopted by pairs

| Amount of JHA Applied (ug) | Number Treated | Total No. of Survivors | No. of Presoldiers Formed n(%) | No. of Individuals With Presoldier Characteristics n(%) | Total n(%) | Total No. of Survivors n(%) | No. of Soldiers Formed n(%) | No. of Individuals With soldier Characteristics n(%) | Total n(%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | BY 12 DAYS AFTER TREATMENT | | | | BY 25 DAYS AFTER TREATMENT | | |
| 0 | 200 | 110 | 16(14.6) | 0(0) | 16(14.6) | 95 | 12(12.6) | 0(0) | 12(12.6) |
| 1.25 | 200 | 126 | 30(23.8) | 19(15.1) | 49(38.9) | 88 | 23(27.7) | 2(2.4) | 25(30.1) |
| 2.5 | 200 | 91 | 23(25.3) | 12(13.2) | 35(38.5) | 75 | 15(20.0) | 13(17.4) | 28(37.4) |
| 5.0 | 200 | 35 | 6(17.1) | 3(8.6) | 9(25.7) | 24 | 4(16.7) | 2(8.3) | 6(24.0) |
| 10.0 | 200 | 22 | 5(22.7) | 2(9.1) | 7(31.8) | 8 | 3(37.5) | 1(12.5) | 4(40.0) |

Table 16. Rates of presoldiers and presoldier-like individual formation following topical treatment of third instar female larvae with different concentrations of JHA. The rates of formation of soldiers and soldier-like individuals and the rate of survival of treated individuals are also given.

FIGURE 34. The types of individuals produced under the influence of JHA.

of reproductives and the colonies were checked at 3-day intervals, and the type of development noted. Four dosages applied topically were: 1.25µg; 2.50µg, 5.0µg and 10µg JHA/Animal following the procedure described above. Controls were treated with acetone. The results are summarized in Table 16.

It is evident from the Table that survival rate of larvae was inversely proportional to the concentration of the hormone applied as was the case in the previous experiment. The maximum number of presoldiers produced was on the 12th. day post treatment. The best production was achieved when 1.25-2.5µg. JHA were used. Other concentrations were detrimental to the whole colony, usually causing high larval mortality. On some occasions the reproductives which tend to eliminate treated larvae also died. Besides soldiers, intermediate forms were also formed, but only under JHA influence. None was found in the control groups. They ranged from worker-like to soldier-like individuals (Fig. 34). A more detailed description of these individuals will be given below. The present results indicate that production of intermediate forms was inversely proportional to JHA concentration applied (Table 16). Fewer presoldiers mature than were formed as shown in Table 16, indicating partial elimination or death of the total number which had differentiated.

7.3.3. *The Influence of JHA on male and female third instar larvae (Topical treatment).*

An experiment was designed to determine whether male third

instar larvae, which normally develop exclusively into major workers, could be induced under the experimental influence of JHA to develop into presoldiers.

Third instar larvae were sorted out into males (260 control and 340 treated larvae). Two µg of JHA in 0.5µl of acetone were used for treating experimental larvae. They were then introduced into adoptive colonies with pairs of reproductives and their development followed closely and recorded every other day.

Figure 35 illustrates survival rates of individuals in the experimental as well as control groups. The survival rates for all groups were between 60% and 80% after stabilization had occurred (3 days following the start of the experiment). A good survival rate continued to be observed throughout the experimental period.

Female control larvae developed either into minor workers or presoldiers. The first presoldiers emerged 5 days after the start of the experiment (Fig. 36a) and reached a peak on the 14th day, making about one presoldier/per colony (7%) of the total individuals which moulted. On about day 10, about seven (47%) of the larvae had transformed into workers and just less than one (3%) into presoldiers per colony. Thereafter, the total transformation of larvae (about 9 individuals) was tending towards 90% or more of minor workers and 7-10% of minor presoldiers. Female larvae treated with JHA had several options for their differentiation (Fg. 36b). They could be

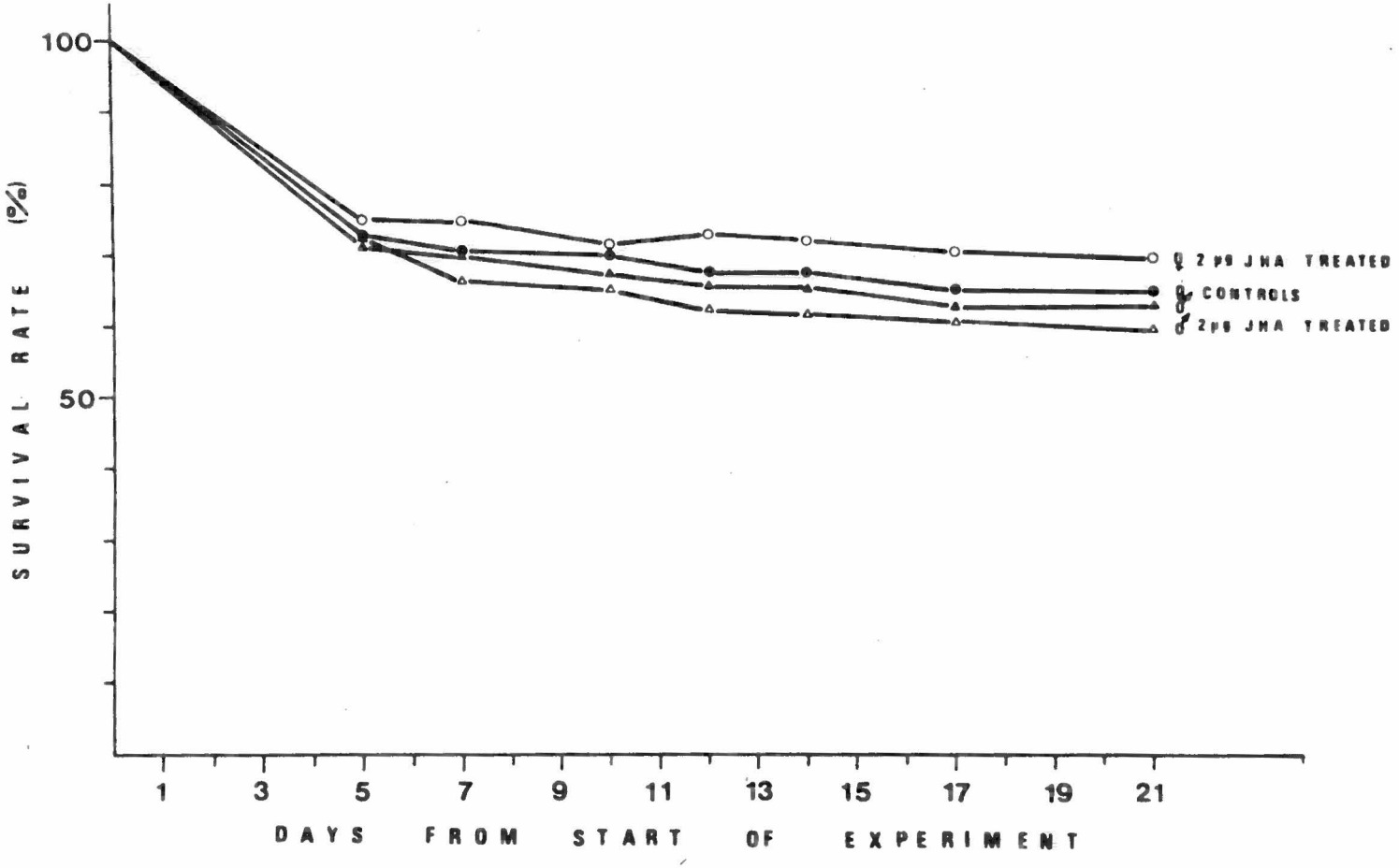



FIGURE 35. Survival rates of control and treated male and female third instar larvae.

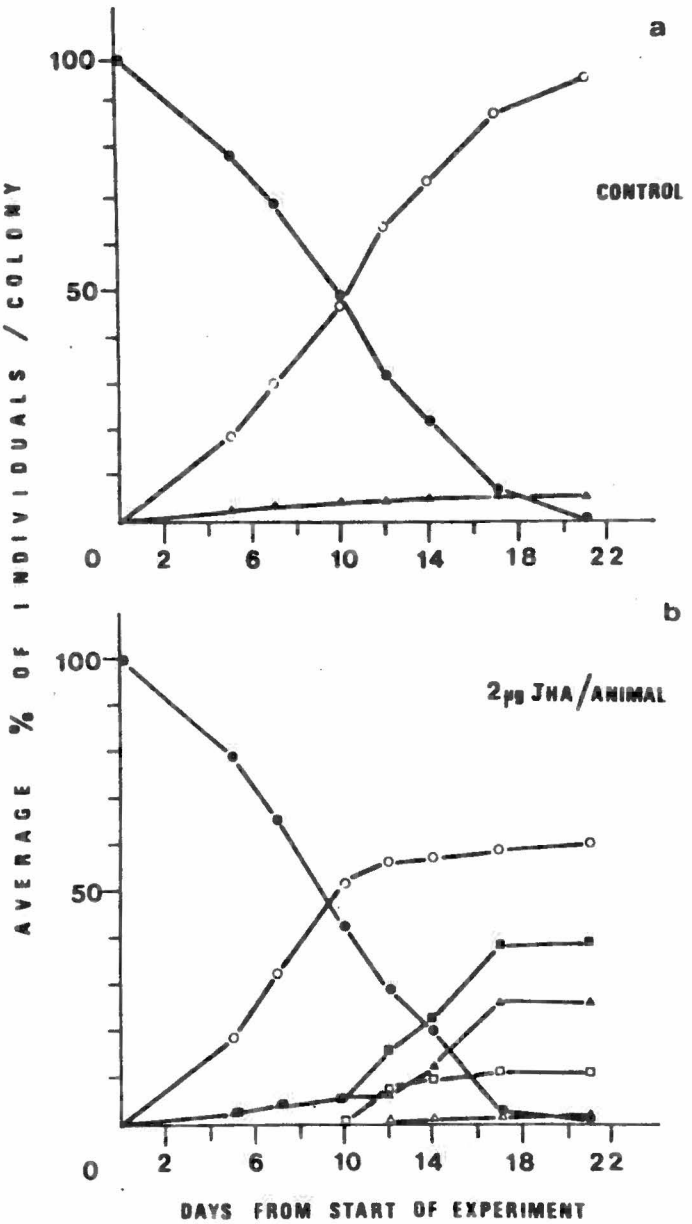



FIGURE 36a. Rate of presoldier formation in control colonies which had adopted third instar female larvae.

FIGURE 36b. Rate of presoldier formation and other options in topically JHA treated third instar female larvae. Workers ( o ), Larvae ( ● ), Presoldiers ( ▲ ), Presoldier-like ( □ ), Worker-like ( △ ), total of affected individuals ( ■ ).

described briefly as follows:

1. Presoldiers (which have normal presoldier morphology).
2. Presoldier-like individuals (which have features close to presoldiers, but have at the same time a small degree of worker characteristics - mandibles and shape of the head).
3. Worker-like individuals (they have predominantly features of workers but a bit of "soldierness".
4. Finally, true workers having all the characters of workers (Fig. 34).

These divisions are arbitrary since it is difficult to classify some of the intermediate individuals. The intermediates are sometimes referred to as intercastes, but the differences between various forms are almost infinite even if one considers only the mandibular structures (Fig. 37). There is a whole range of intermediate forms between worker and soldier castes (Fig. 38) as shown by mandible and clypeus.

During the transformation of the first half of JHA treated larvae, the development was very similar to what was observed in the control groups. After the 10th day, the formation of workers stagnated and a shift occurred towards presoldier and intermediate forms (Fig. 36b). The peak in presoldier form-

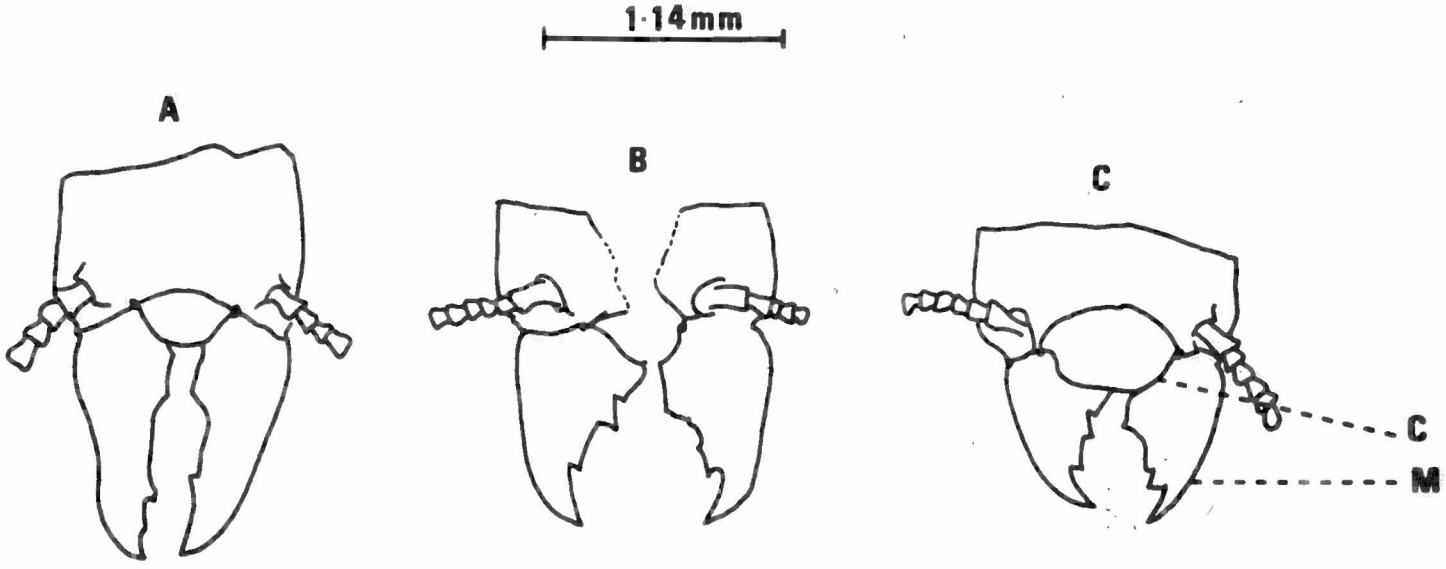



FIGURE 37. An illustration of normal presoldier (A) and presoldier-like (B,C) mandibles and clypeus from individuals formed under the influence of JHA. M. - Mandibles, C. - Clypeus. The drawing was made with a camera lucida.

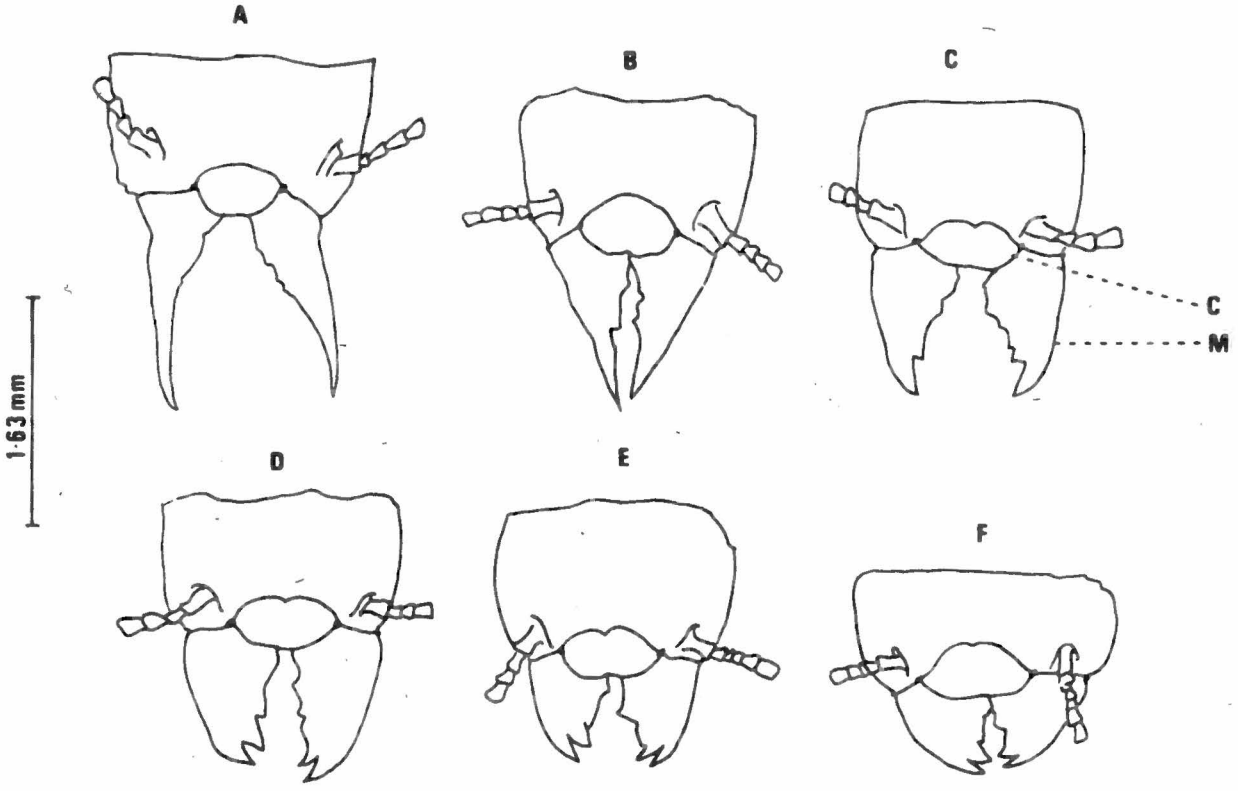



FIGURE 38. An illustration of mandibles and clypeus from major groups of individuals formed under JHA influence. They range from minor soldier type to worker type (A-F).

ation was achieved on about the 16th day. Presoldiers then formed about 26% (about 4 per colony) of moulted individuals while presoldier-like and worker-like individuals formed 10% and about 2%, respectively.

The formation of presoldiers had already started around 5th day from the beginning of the experiment in some colonies but it was minimal. It seems that presoldiers formed before the 10th day were spontaneous (previously determined) presoldiers as the JHA influence was evident only after the tenth day (Fig. 36b).

The untreated male third instar larvae developed exclusively into major workers (Fig. 39a). By about the 10th day, as in female larvae, about 50% of male larvae (about 8 individuals per colony) had moulted into major workers and by about 20 days all had moulted into workers. The most interesting results were achieved from the groups of male larvae treated in a similar manner as female larvae with JHA. Presoldier formation was observed starting from about the 12th day following treatment and reaching a peak also on about the 16th day. Presoldier-like as well as worker-like individuals were formed in the same proportions as was the case with the female larvae (Fig. 39b). However, far fewer presoldiers were formed than in the case of females (only about two individuals per colony (18%). The overall percentrage of affected males was smaller (30%), about 4 per colony compared with 38% (about 6 per colony) in case of female larvae.

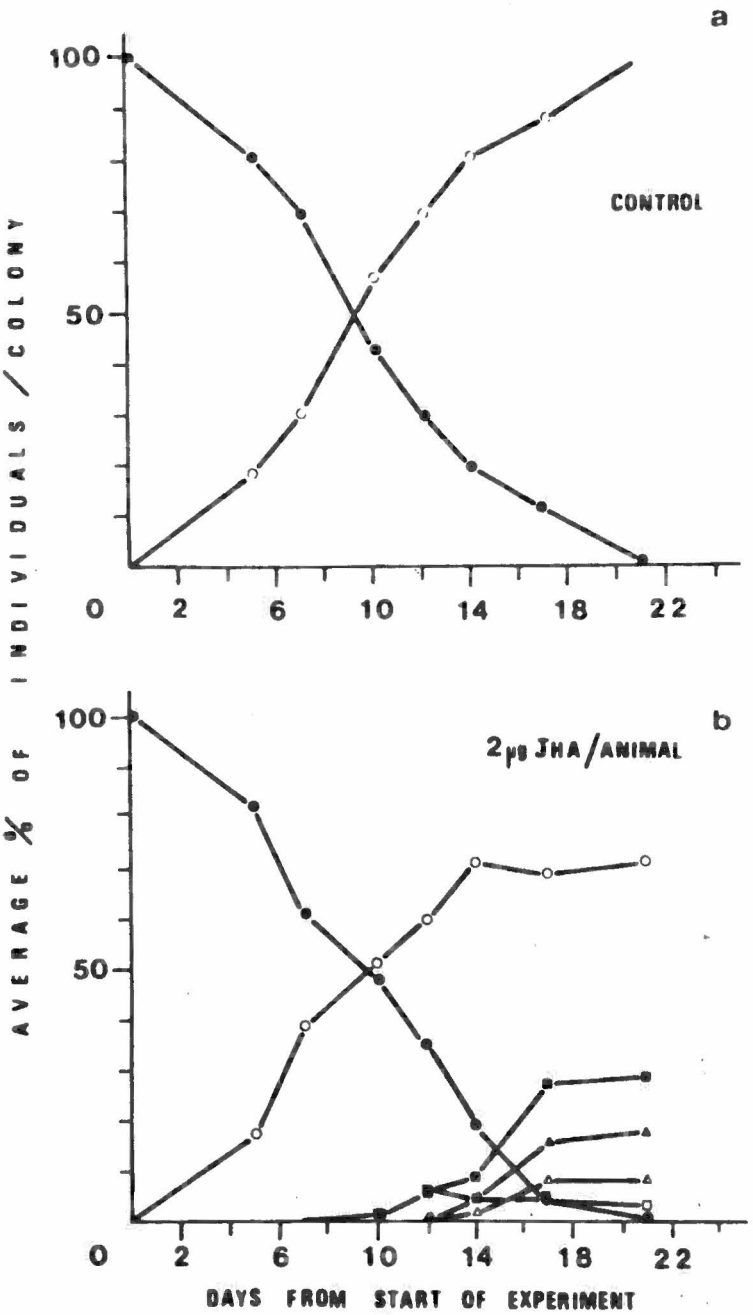



FIGURE 39a. The developmental trend in control colonies containing adopted male third instar larvae.

FIGURE 39b. Rates of presoldier ( ▲ ), presoldier-like ( △ ), worker-like ( □ ) and worker ( ○ ) formation in adopted third instar male larvae topically treated with JHA ( ● ). Total affected individuals ( ■ ).

7.3.3.1. *Biometric studies*

Biometric studies were conducted on both presoldiers as well as the soldiers and workers obtained by treating male and female larvae with JHA. The following parameters were measured.

1. Maximum head capsule width.
2. Head capsule length.
3. Maximum mandibular length.
4. Number of antennal segments.

The head capsule length is defined as the length of the head capsule from the posterior most end to the anterior tip of the clypeus in workers and intermediate castes and to the level of the mandibular base in case of presoldiers and soldiers. Maximum mandibular length was the straight-line measurement of the mandible from the base to the tip (apical tooth). Both left and right mandibles were measured and the measurements averaged.

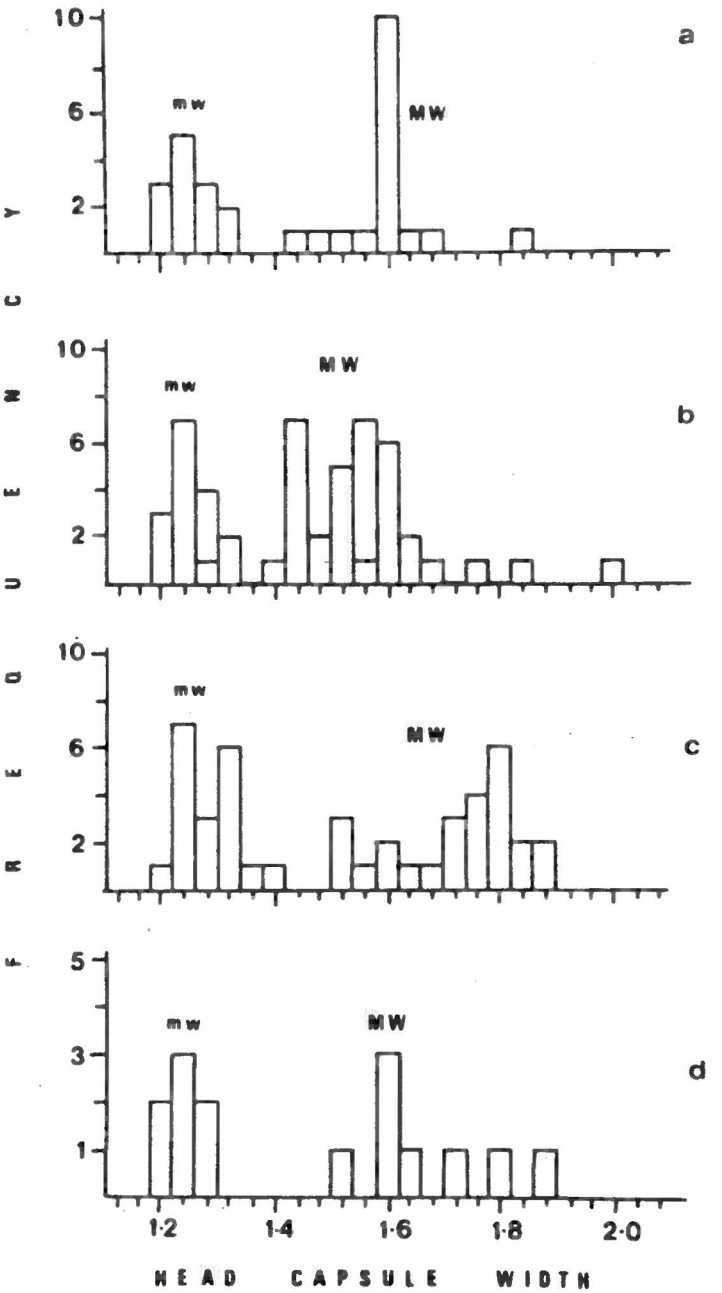
This study was conducted in order to determine whether:

1. Male presoldiers and soldiers were larger than their female counter-parts (when the head capsules were compared).
2. Mandibular sizes differed between male and female presoldiers and soldiers.
3. JHA-formed presoldiers and soldiers were biometrically identical with spontaneously formed individuals.

4. Workers formed after JHA treatment were identical with those formed in the control groups.

In Figures 40 a and b a bimodal distribution of head capsule width of workers from both control and treated larvae is evident. Table 17 shows that the mean head capsule widths of workers from treated and untreated larvae are comparable although the head lengths of minor workers from JHA treated larvae are slightly smaller than those from control ($P<0.05$), thus causing a slight difference in the head capsule indices (the ratio of head capsule length to width). Head capsule widths and legnths of major workers from untreated larvae on the other hand, are larger than those from groups of treated individuals ($P<0.05$); while the head capsule indices remain comparable ($P<0.1$) (Table 18). The mandibular lengths of minor workers from experimental and control groups are comparable ($P<0.1$) although, mandibular indices (the ratio between head capsule length and mandibular length) differ ($P<0.05$) due to differences in the head capsule lengths (Table 17). However, the two parameters are reasonably close ($P<0.1$) major workers formed from treated and untreated larvae (Table 18). The number of antennal segements did not change whether the individuals were from treated or untreated larvae. In major workers they ranged from 15 to 17 while in minor workers they were consistently 15.

The following observations were made for minor presoldiers. The




FIGURES 40 a and b. Bimodal distribution of head capsule width of minor and major workers from (a) - Control groups (b) Treated larvae (JHA topical treatment).

FIGURES 40 c and d. A distribution of head capsule width of workers from control groups (c) and from JHA vapour treated groups, (d).

| Type of Individuals | n | Head Capsule Width (mm) Mean ± S.E. | Head Capsule Length (mm) Mean ± S.E. | Head Capsule Index Mean ± S.E. | Mandibular Length (mm) Mean ± S.E. | Mandibular Index Mean ± S.E. | Number of Antennal Segments |
|---|---|---|---|---|---|---|---|
| Minor Workers From Acetone Topically Treated Larvae (Control) | 17 | 1.29 ± 0.02 | 1.24 ± 0.02 | 0.96 ± 0.01 | 0.70 ± 0.01 | 0.54 ± 0.03 | 15 |
| Minor Workers From JHA Topically Treated Larvae | 18 | 1.29 ± 0.02 | 1.13 ± 0.04 | 0.86 ± 0.02 | 0.69 ± 0.02 | 0.61 ± 0.02 | 15 |
| Minor Workers From Acetone Vapour treated Larvae (Control) | 22 | 1.32 ± 0.02 | 1.18 ± 0.03 | 0.90 ± 0.02 | 0.67 ± 0.02 | 0.57 ± 0.02 | 15 |
| Minor Workers From JHA Vapour Treated Larvae | 7 | 1.24 ± 0.02 | 1.10 ± 0.02 | 0.89 ± 0.01 | 0.69 ± 0.02 | 0.62 ± 0.02 | 15 |

Table 17. Measurements of minor workers produced from female third instar larvae under various experimental conditions

| Type of Individuals | n | Head Capsule Width (mm) | Head Capsule Length (mm) | Head Capsule Index | Mandibular Length (mm) | Mandibular Index | Number of Antennal Segments |
|---|---|---|---|---|---|---|---|
| | | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | |
| Major workers from Acetone Topically Treated Larvae (Control) | 14 | 1.62 ± 0.02 | 1.47 ± 0.02 | 0.91 ± 0.01 | 0.80 ± 0.01 | 0.55 ± 0.01 | 15 — 16 |
| Major workers from JHA Topically Treated Larvae | 37 | 1.54 ± 0.02 | 1.43 ± 0.03 | 0.93 ± 0.01 | 0.75 ± 0.02 | 0.53 ± 0.01 | 15 — 17 |
| Major workers from Acetone Vapour Treated Larvae | 22 | 1.71 ± 0.05 | 1.62 ± 0.03 | 0.93 ± 0.01 | 0.78 ± 0.02 | 0.48 ± 0.01 | 15 — 17 |
| Major workers from JHA Vapour Treated Larvae | 8 | 1.67 ± 0.04 | 1.55 ± 0.05 | 0.93 ± 0.01 | 0.77 ± 0.03 | 0.49 ± 0.02 | 15 — 17 |

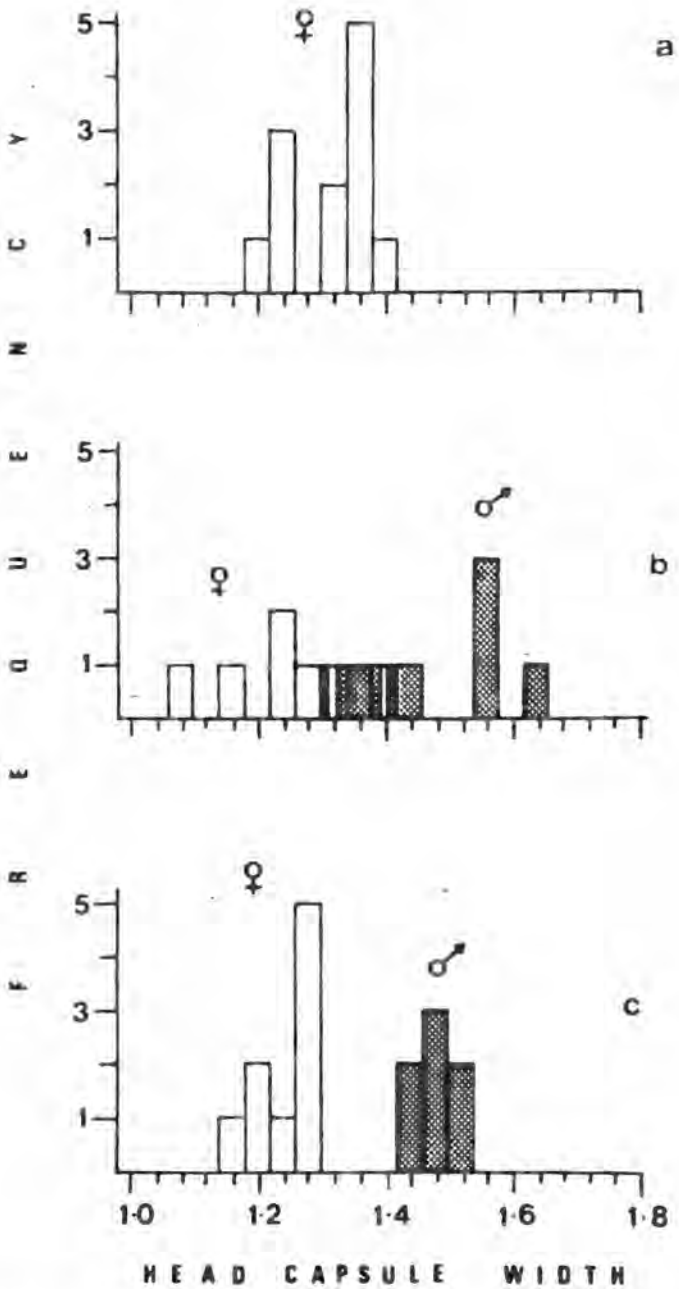
Table 18. Measurement of major workers produced from male third instar larvae under various experimental conditions

| Type of Individuals | n | Head Capsule Width (mm) Mean ± S.E. | Head Capsule Length (mm) Mean ± S.E. | Head Capsule Index Mean ± S.E. | Mandibular Length (mm) Mean ± S.E. | Mandibular Index Mean ± S.E. | Number of Antennal Segments |
|---|---|---|---|---|---|---|---|
| Spontaneously Produced minor Presoldiers | 12 | 1.31 ± 0.02 | 1.46 ± 0.03 | 1.11 ± 0.01 | 1.37 ± 0.02 | 0.95 ± 0.02 | 15 |
| JHA Topically Induced Minor Presoldiers ♀ | 7 | 1.25 ± 0.04 | 1.30 ± 0.05 | 1.04 ± 0.04 | 1.22 ± 0.05 | 0.96 ± 0.06 | 15 — 16 |
| JHA Topically Induced Minor Presoldiers ♂ | 8 | 1.51 ± 0.05 | 1.56 ± 0.04 | 1.04 ± 0.04 | 1.36 ± 0.01 | 0.88 ± 0.02 | 15 — 16 |
| JHA Vapour Induced Minor Presoldiers ♀ | 9 | 1.24 ± 0.02 | 1.32 ± 0.02 | 1.06 ± 0.01 | 1.20 ± 0.01 | 0.92 ± 0.01 | 15 |
| JHA Vapour Induced Minor Presoldiers ♂ | 7 | 1.49 ± 0.01 | 1.58 ± 0.02 | 1.07 ± 0.02 | 1.36 ± 0.02 | 0.85 ± 0.01 | 15 |

Table 19. Measurements of minor presoldiers produced under different experimental conditions

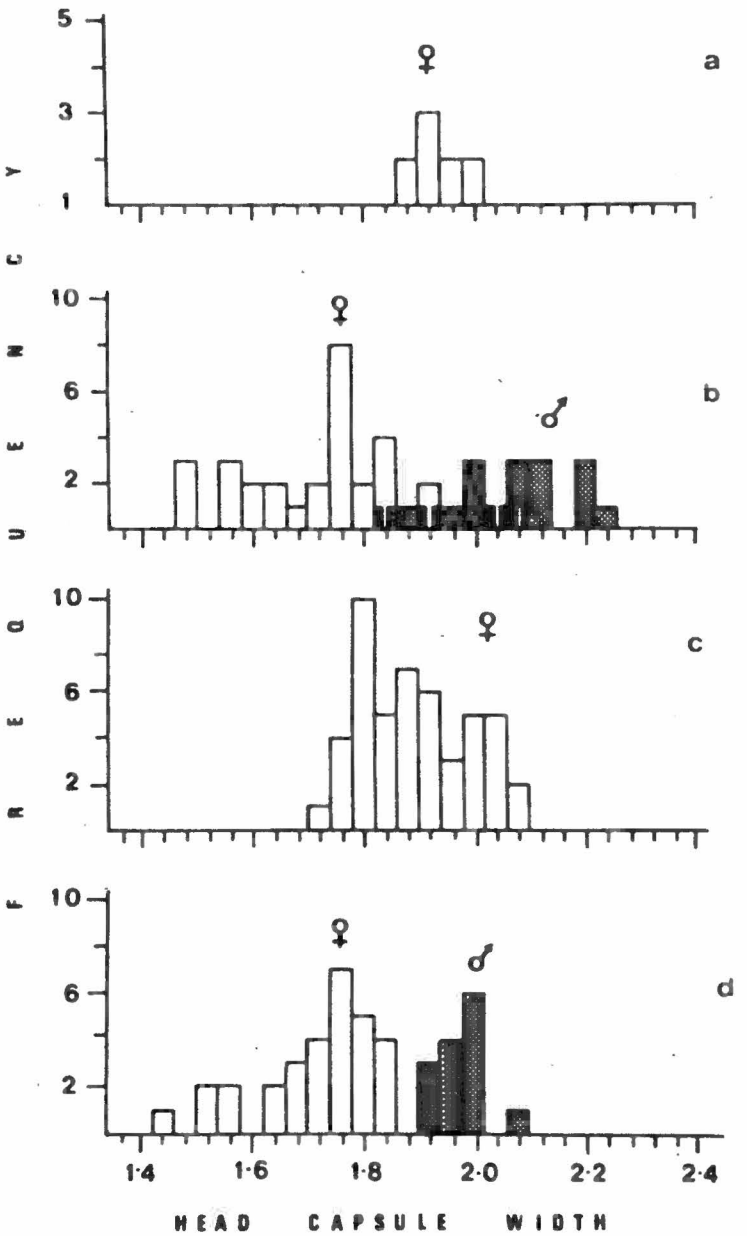
head capsule widths and lengths were slightly greater in control groups than in the experimental groups, subsequently the head capsule index was also slightly greater in the former ($P<0.05$) (Table 19). The same applied to mandibular lengths. However, the mandibular indices were practically the same ($P<0.1$). There was a more significant difference between these parameters measured in male presoldiers as compared with controls or female presoldiers formed after JHA treatment (Table 19). The exception was in head capsule index where that of male presoldiers was comparable with that of female soldiers formed under JHA influence ($P<0.1$). Furthermore, the mandibular index of male presoldiers was smaller ($P<0.05$) than those of spontaneous presoldiers and female presoldiers formed under JHA topical treatment (Table 19). The number of antennal segments also differed. Spontaneous presoldiers had 15-segmented antennae while both male and female JHA topically induced presoldiers had antennae with 15-17 segments. Figure 41 a. shows a single distribution of head capsule measurements made on presoldiers from control groups. However, the measurements of head capsule width of male and female presoldiers formed a bimodal distribution (Fig. 41 b).

Minor soldiers formed from control groups had also larger head capsule widths and lengths, and longer mandibles than minor soldiers formed from JHA treated larvae (Table 20). This difference was significant for head capsule widths and lengths ($P<0.05$); however, for the head capsule and mandibular indices no statistical difference was found ($P<0.1$).




FIGURES 41a and b. Histograms showing head capsule width distributions of: (a) - Control presoldiers, (b) - Experimentally induced female and male presoldiers.

FIGURE 41c. A bimodal distribution of presoldier head capsule width from JHA vapour treated colonies.




FIGURES 42 a and b. Histograms showing head capsule width distributions of: (a) - Soldiers formed in control groups, (b) Experimentally induced female and male soldiers.

FIGURES 42 c and d. Distribution of head capsule width of soldiers: (c) - from control groups, (d) - from JHA vapour treated groups.

As far as soldiers are concerned, male soldiers had larger heads ($P<0.05$) and longer mandibles than either the control group, soldiers, or female soldiers which had developed from JHA topically treated individuals. However, both mandibular and head capsule indices were quite comparable ($P<0.1$) with those of the control group soldiers. Spontaneously produced soldiers had antennae with 15 segments while both male and female soldiers produced under JHA had 15-17. Figures 42 a. shows a single distribution of head capsule width of female soldiers formed from spontaneous presoldiers (untreated control groups), while both male and female soldiers formed under experimental conditions exhibit a bimodal distribution (Fig. 42 b).

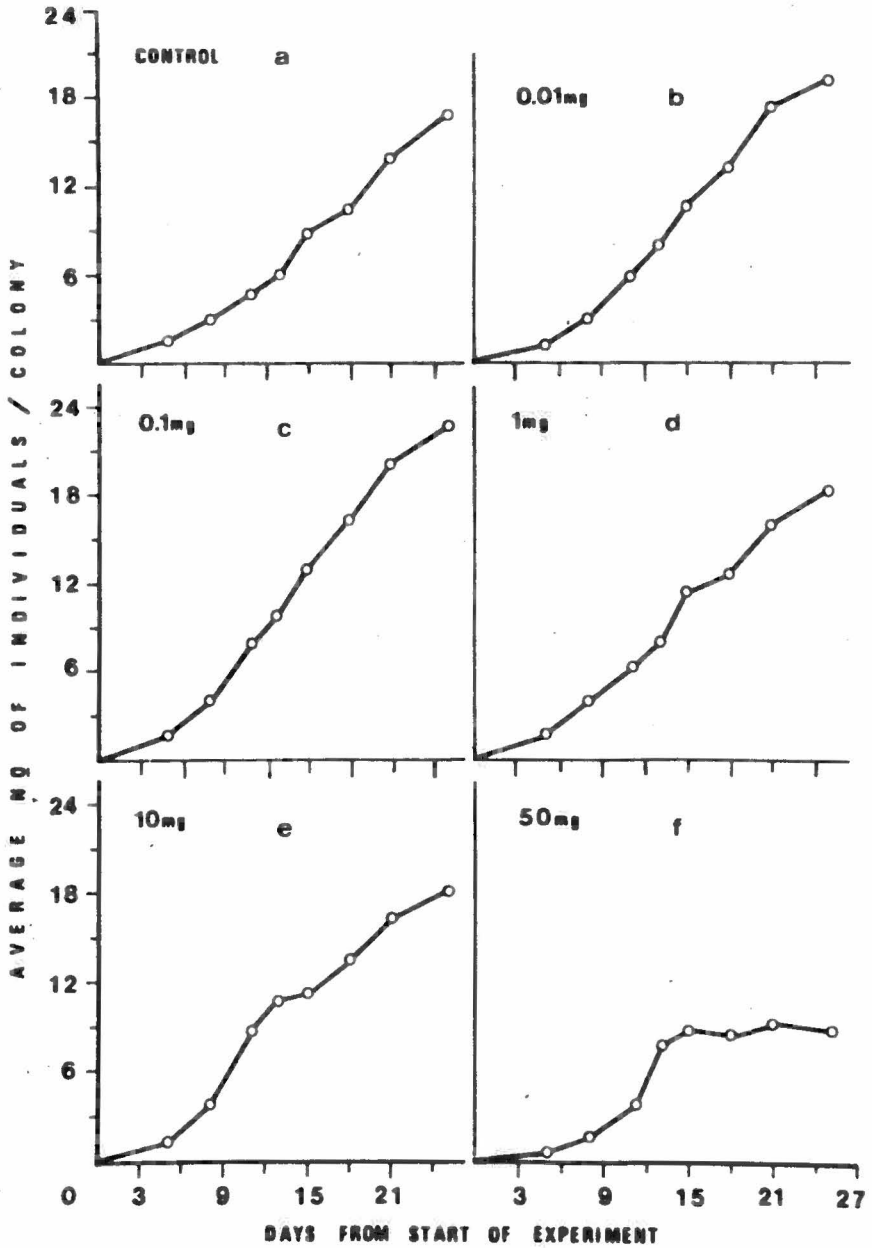
### 7.3.4. *Prolonged effects of JHA vapour on normally developing incipient colonies*

In another set of experiments performed in parallel with the ones described above, whole colonies of the same age (about 70 days old) as those used in the experiments on JHA topical application were treated with JHA vapours of different concentrations. The doses used were 0.01, 0.1, 1, 10 and 50mg/colony. Incipient colonies in plastic dishes measuring 6 cm - diameter were used for these experiments as well. The first soldiers, presoldiers and workers were removed from the colonies to be used leaving behind only larvae of different instars and sexes. Twenty five colonies were used for each treatment. Treatment was carried out as described in the general methods at the beginning of this chapter. The lids of petri dishes with JHA were not replaced

throughout the experimental period. Observations were made at 3-day intervals and the development recorded. Any dead reproductives were replaced with individuals of the same sex from stock colonies of the same age. High mortality was observed among those colonies treated with 50mg JHA. The larvae started dying early in the experiments, therefore, the formation of workers and spontaneous presoldiers was retarted. In some colonies, the reproductives also died, so that by mid-way in the experiments half the colonies had died. These were eliminated from the study. The remaining colonies fared well, so they formed the group to be discussed together with the rest of the other colonies.

The pattern showing the progress in replacement of workers, presoldiers and soldiers previously removed from the colonies is presented in Figs. 43a, b, c, d, e, and f. The rate of replacement was quite comparable in the first four experimental groups (Figs. 43a, b, c and d) since the slopes of the graphs are nearly the same. However, the picture looks different in the fifth group (colonies treated with 10mg. JHA) and more so in the sixth group (colonies treated with 50mg. of JHA) (Figs. 43e and f). There was a slight overall slow-down in the replacement of the individuals which had been removed. This was mainly due to mortality of larvae or even workers that had been formed in these colonies.

While considering the rate of replacement of workers, it was found that both minor and major workers were replaced at relatively the same rate (Figs. 44a, b, c, d, e and f), although replacement of

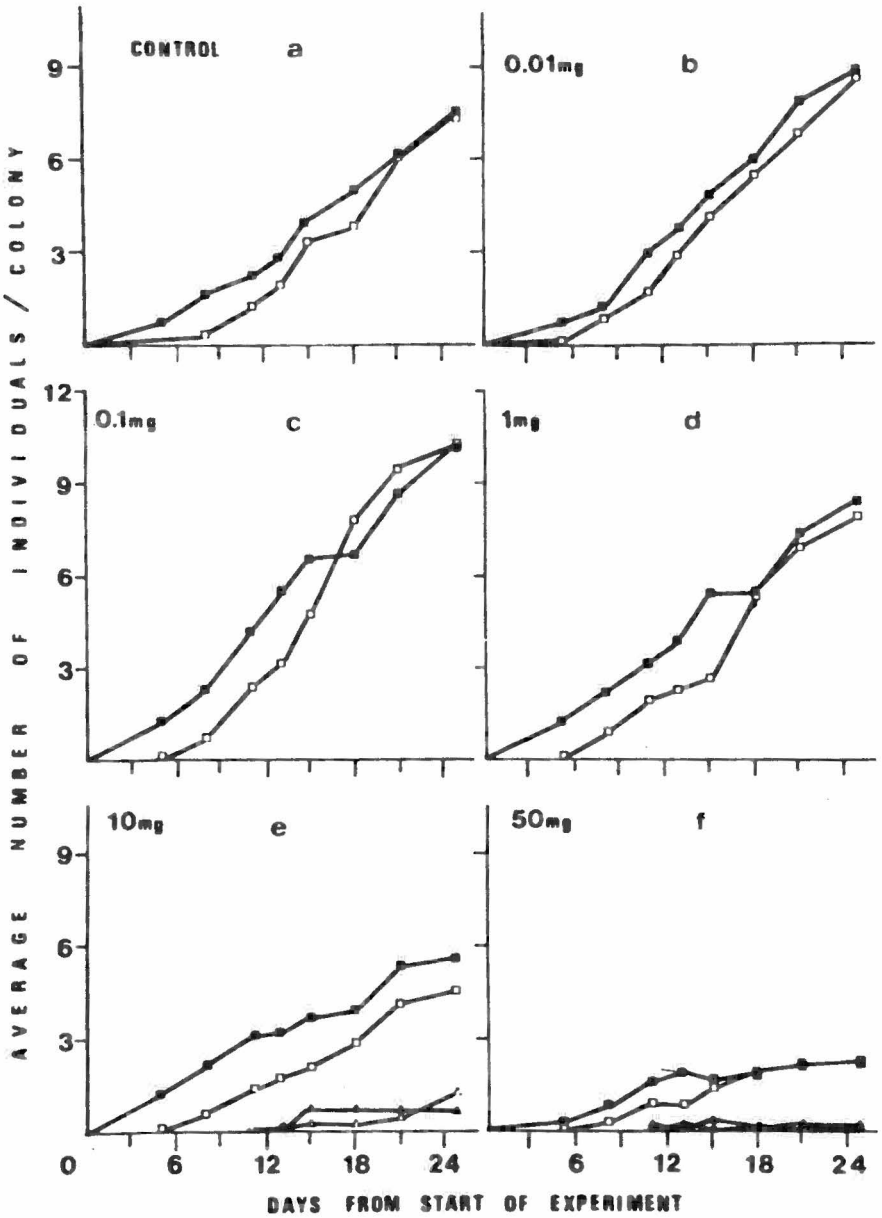



FIGURES 43 a, b, c, d, e and f. The average rates of replacement of all workers, presoldiers and soldiers together under different levels of JHA vapour treatments.

major workers generally lagged behind. Nevertheless, the two types of workers in most of the groups approached the same rate of development on about the 18th day. It is worth noting that, while the rates of worker production in the first four groups (Figs. 44a, b, c and d) are comparable and follow trends similar to those of the entire brood being replaced(Figs. 43a, b, c, d, e and f), the picture is again different in cases of colonies treated with 10mg and 50mg of JHA. In the latter cases, the rate of worker production is much lower, especially where the colonies were treated with 50mg of the analogue. This result may be attributed to both mortality incurred and the higher rate of presoldier formation in these two groups of treated colonies, as will be shown below.

Besides the replacement of workers in the colonies treated with 10 and 50mg JHA, worker-like individuals were formed. Their formation started between the 11th and 13th day following treatment and included both minor and major worker types. More of these individuals were formed in the colonies treated with 10mg than in those treated with 50mg. However, in each case, their formation rates were comparable (Figs. 44e and f).

The rate of presoldier replacement is of special interest. Presoldiers were replaced faster in the first four groups of treated larvae (including control group) (Figs. 45a, b, c and d) during the first 9 days following the start of the experiments. During this period, most colonies of these groups already had an average of one presoldier, while the last two groups (10 and 50mg.

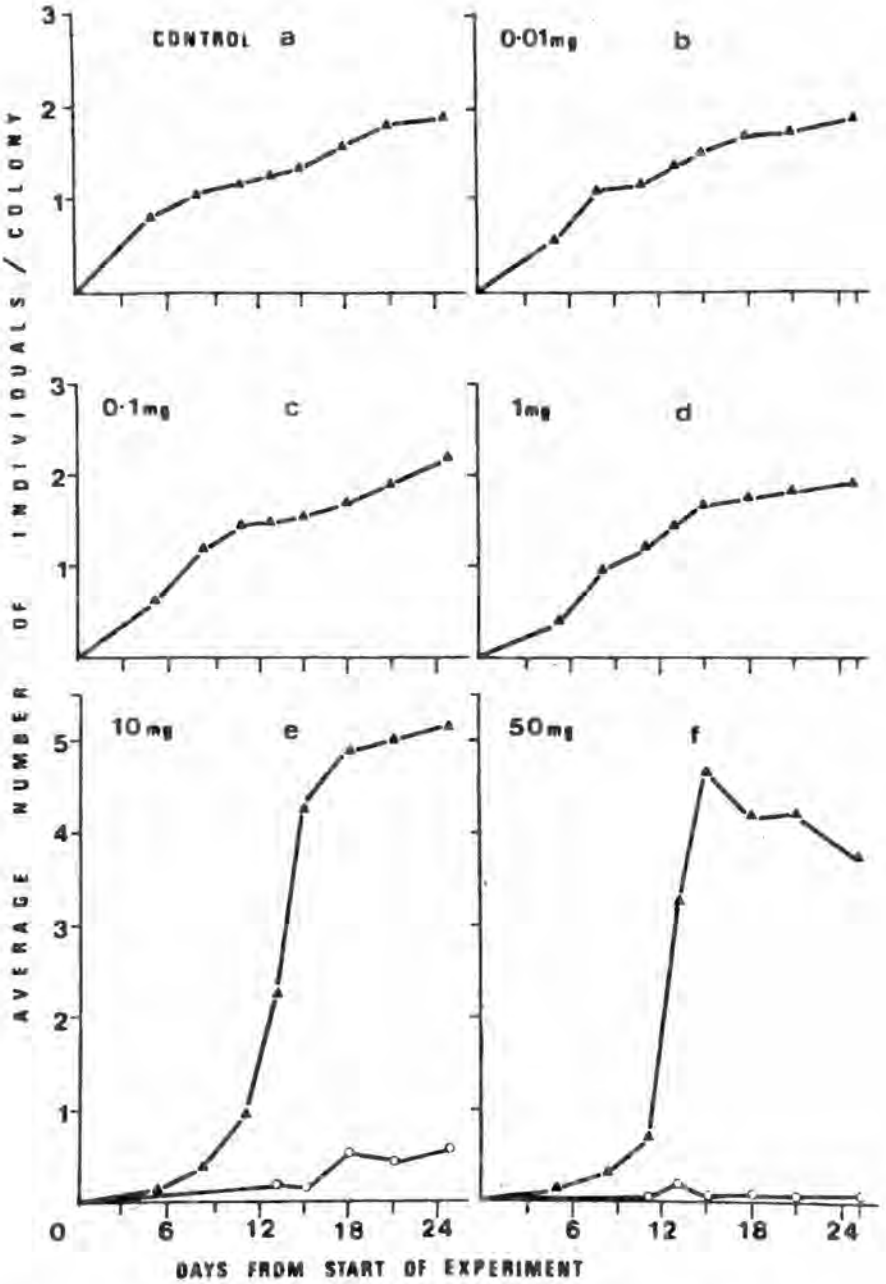



FIGURES 44 a, b, c, d, e and f. Rates of minor ( ■ ) and major ( □ ) worker replacement in incipient colonies where workers, presoldiers had been removed and the colonies treated with different levels of JHA vapour. Note that worker-like individuals ( ( ▲ ), minor worker-like; ( △ ), major worker-like) were also formed under 10-50 mg. JHA vapour.

JHA treated) had an average of less than a half pre-soldier per colony. This means that more than a half of these colonies had no presoldiers formed until after the 9th day when there was a drastic change of events. A burst of presoldier production occurred in the last two groups, reaching a peak between the 15th and 18th day. An average of about 5 presoldiers per colony was observed as opposed to about 2 per colony in the first four groups (Figs. 45a, b, c, d, e and f). Soon after this period, a plateau was reached, especially in the case of the 10mg. JHA treated colonies and in the other first 4 groups of colonies. A slight drop in the number of presoldiers was observed in the colonies which were treated with 50mg. JHA following this peak period, due to slight mortality in some of the colonies.

As in the previous experiments, presoldier-like individuals were also formed in the colonies treated with 10 and 50mg. of JHA (Figs. 45e, f). Again as was the case for worker-like individuals, more presoldier-like individuals were formed under 10mg. than 50mg. JHA treatment (Fig. 46).

In summary, the responses of larvae to different doses of JHA vapour are illustrated in Figure 46. Larvae treated with 0.01mg. JHA vapour responded in a manner comparable to that observed in the control groups. The percentage of presoldiers formed by the 15th day was approximately 15% while minor and major workers formed 45% and 40%, respectively (Fig. 46). The picture was quite different at concentrations of 10mg. or 50mg. JHA/colony. As mentioned above, more presoldiers were formed under these




FIGURES 45 a, b, c, d, e and f. Rate and level of presoldier replacement in incipient colonies under the influence of various levels of JHA vapour. Presoldiers ( ▲ ) and Presoldier-like ( o ).

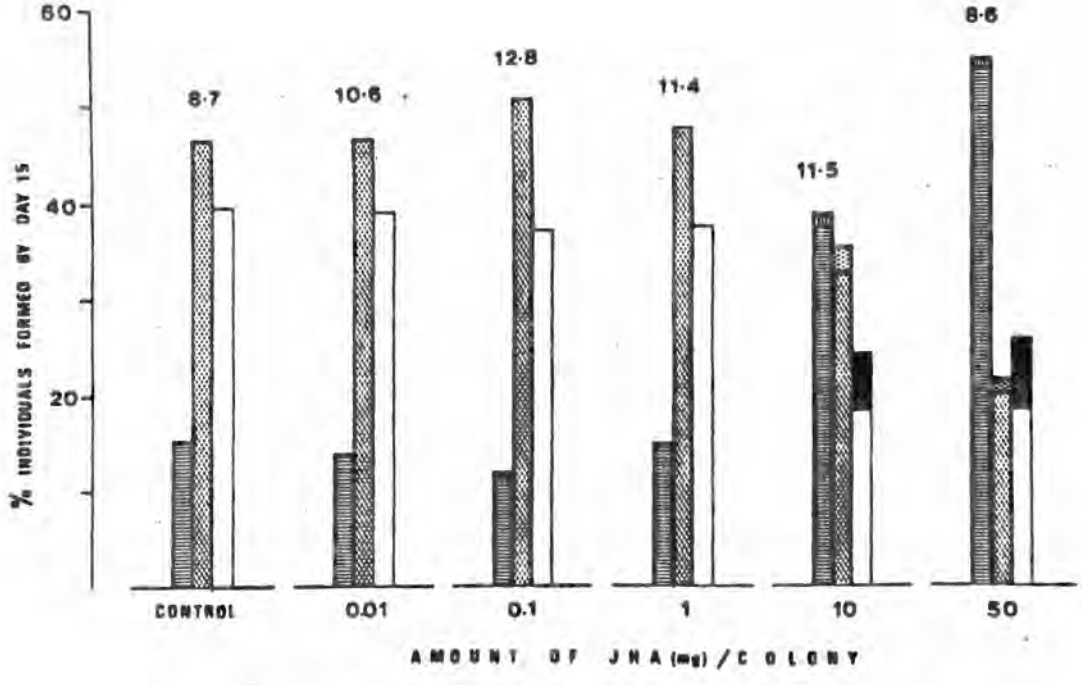



FIGURE 46. A summary of the formation of, presoldiers ( ▒ ) presoldier-like ( ▓ ), minor workers( ▒ ) minor worker-like ( ▒ ), major workers ( □ ) and major worker-like ( ▒ ) individuals in incipient colonies treated with various concentrations of JHA vapour. Figures above the columns represent the average number of individuals observed per colony.

conditions by day 15. At the 10mg. concentration, about 40% of the differentiated individuals were presoldiers while 8% were presoldier-like; 32% were minor workers and 3% were minor worker-like; about 18% were major workers and 5% were major worker-like individuals. At a concentration of 50mg. JHA/colony an even higher proportion of pr soldiers was recorded. About 53% of the total number of individuals formed were presoldiers and about 1% were presoldier-like. Minor workers formed only about 20% while minor worker-like intermediate forms contributed about 2%. Major workers constituted about 18% while their intermediate forms (worker-like) were 6%. In both cases the proportion of presoldiers formed, as opposed to workers, was statistically higher ($P<0.01$) than observed in the control group, however, between the controls and the groups treated with 0.01mg., 0.1, and 1.0mg. there was no difference ($P<0.1$) in presoldier formation.

7.3.4.1. *Biometric studies*

It was important to know whether male presoldiers were also formed under JHA vapour conditions, since it was noticed that the proportion of major workers formed under 10 and 50mg. JHA per colony was lower than in the control group. This observation suggested a shift in development towards male presoldier formation. It was also necessary to find out whether JHA vapour treatment could influence sizes of major and minor workers. This study might also permit a size comparison between individuals formed under JHA topical and vapour treatments. The parameters measured were the same as for those individuals topically treated with JHA

described above.

As results show, worker head capsule widths fall in a bimodal distribution (Figs. 40c, d), i.e., minor and major workers were distinguishable under both control and experimental conditions. A normal distribution was characteristic of similar measurements of presoldiers formed under control conditions (Fig. 41a), however, measurements of those formed under JHA vapour show a bimodal distribution (Fig. 41c). This indicated that, under these conditions, male presoldiers could be produced as well. In Figure 42d. a distinct bimodal distribution is also evident representing two types of minor soldiers, males and females, while in the control group only a single distribution representing male soldiers appeared (Fig. 42c). These results confirm those found for presoldiers which were produced under the same conditions.

Further analysis of workers gave the following results: minor workers from the control group had larger heads than those from JHA vapour treated groups ($P<0.05$ for both head capsule widths and head capsule lengths). However, the head capsule indices for workers from the two groups did not differ ($P<0.01$). The mandibular lengths in the two cases were comparable ($P<0.1$), although the mandibular indices which differed slightly, were not significantly different ($P<0.1$). The number of antennal segments in minor workers from both control and JHA vapour treated groups was the same (Table 17). Biometric analysis of major workers showed results similar to that for minor workers (Table 18).

The analysis of presoldiers revealed the following: spontaneously produced presoldiers were larger (in all parameters measured as well as the two indices) than female presoldiers produced under JHA vapour conditions ($P<0.05$)(Table 19). However, the situation is reversed if one compares control with male presoldiers from the JHA vapour treated colonies. Presoldiers formed in the control group (spontaneously produced presoldiers) had smaller head capsules than those of experimentally produced male presoldiers ($P<0.05$) while the mandibular and head capsule indices remained higher in the latter than in th former ($P<0.01$) (Table 19). The number of antennal segments was the same in the spontaneous presoldiers, and the female and male presoldiers formed under JHA vapour (Table 19).

The soldiers studied biometrically showed that female soldiers formed under JHA vapour had narrower ($P<0.05$) and shorter ($P<0.5$) head capsules than spontaneous soldiers (Table 20). However, the head capsule indices were comparable ($P<0.1$). Mandibular lengths of these soldiers were not significantly greater than ($P<0.1$) those of spontaneous soldiers. Nevertheless, the mandibular index for female soldiers produced by JHA vapour treatment was larger ($P<0.001$). As far as male soldiers are concerned, again their head capsule width and length were much greater than those of control soldiers (in both cases $P<0.001$). The mandibular and the head capsule indices turned out to be comparable in both cases ($P<0.1$) (Table 20).

A general analysis of the mandibles of the intermediate forms

| Type of Individuals | n | Head Capsule Width (mm) | Head Capsule Length (mm) | Head Capsule Index | Mandibuiar Length (mm) | Mandibular Index | Number of Antennal |
|---|---|---|---|---|---|---|---|
| | | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | Segments |
| Minor Soldiers From Acetone Topically Treated Larvae (Control) ♀ | 5 | 1.94 ± 0.02 | 2.42 ± 0.05 | 1.25 ± 0.03 | 1.48 ± 0.05 | 0.61 ± 0.02 | 15 |
| Minor Soldiers from JHA Topically Treated Larvae ♀ | 31 | 1.72 ± 0.02 | 2.15 ± 0.04 | 1.20 ± 0.01 | 1.45 ± 0.03 | 0.68 ± 0.02 | 15 — 16 |
| Minor Soldiers From JHA Topically Treated Larvae ♂ | 18 | 2.06 ± 0.04 | 2.56 ± 0.04 | 1.24 ± 0.01 | 1.58 ± 0.03 | 0.62 ± 0.01 | 15 — 16 |
| Minor Soldiers From Acetone Treated Colonies (Control) ♀ | 48 | 1.89 ± 0.02 | 2.34 ± 0.02 | 1.22 ± 0.02 | 1.47 ± 0.02 | 0.63 ± 0.01 | 15 — 16 |
| Minor Soldiers From Vapour Treated Colonies ♀ | 30 | 1.76 ± 0.02 | 2.18 ± 0.04 | 1.24 ± 0.01 | 1.52 ± 0.02 | 0.71 ± 0.01 | 15 |
| Minor Soldiers from JHA Vapour Treated Colonies ♂ | 14 | 1.98 ± 0.01 | 2.45 ± 0.02 | 1.24 ± 0.01 | 1.59 ± 0.02 | 0.65 ± 0.01 | 15 |

Table 20. Measurements of minor soldiers produced under various experimental conditions.

(Figs. 37, 38) shows beyond doubt that the mandibles of these individuals are shorter than those of presoldiers or soldiers. It is worth noting, however, that these intermediate forms vary considerably in many features: mandibular morphology, state of pigmentation, shape of head, size and shape of clypeus. In fact, morphological variations may range from being very close to those of workers to those of soldiers (Fig.34). Pigmentation also ranges from very light to very dark.

7.3.5. *Studies on corpora allata from presoldiers formed under different conditions*

The aim of these investigations was to establish the role of corpora allata (CA) in soldier differentiation under varying conditions of their formation, namely:

1. spontaneous formation (in control groups)
2. determination under the influence of a single topical application of JHA

Presoldiers were obtained from control colonies (untreated) as well as from JHA treated colonies (Topical). They were processed for histology as described in the general methods (Chapter Two). The analysis for activity was conducted as in Chapter Four.

The results showed that the histology of the CA was very similar to that described for presoldiers from field mounds (see Chapter Four). However, these glands differed in size between spontaneously produced individuals from incipient colony larvae and those produced under JHA topical treatment (Table 21). The difference between the cross-sectional areas between the two was significant

| Type of Individuals | n | Corpora allata Cross-Sectional Area ($um^2$) | Number of Nuclei/Cross-Section | Nuclear Cross-sectional Area ($um^2$) | Nuclear Area / Cytoplasmic Area |
|---|---|---|---|---|---|
| | | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. |
| Field Minor Presoldier | 9 | 1568.89 ± 216.87 | 11.47 ± 0.23 | 34.18 ± 4.51 | 0.26 |
| Spontaneous Laboratory Minor Presoldiers (Produced from adopted Field Larvae) | 12 | 1939.94 ± 409.52 | 11.58 ± 0.31 | 36.06 ± 2.28 | 0.27 |
| Spontaneous Laboratory Minor Presoldiers (Produced from Incipient Colony Larvae) | 3 | 2531.64 ± 288.88 | 13.0 ± 0.68 | 41.46 ± 6.52 | 0.27 |
| Minor Presoldiers Induced by JHA Topical Application (Incipient Colony Larvae) | 4 | 1199.19 ± 104.59 | 12.38 ± 0.38 | 34.94 ± 0.01 | 0.56 |

Table 21. Measurement of corpora allata of minor presoldiers formed under various conditions.

($P<0.01$). Both types had approximately equal number of nuclei per maximum cross-sectional area and their cross-sectional areas did not statistically differ ($P<0.1$) (Table 21). The ratio of nuclear to cytoplasmic areas was nevertheless smaller in spontaneously produced presoldiers (Table 21). This suggests strongly a higher activity of CA in spontaneous presoldiers than those of JHA produced individuals.

In another set of investigations, CA of presoldiers collected from the field were compared with those formed spontaneously in the laboratory. (These presoldiers had developed from third instar larvae which had been previously adopted as second instars in the laboratory colonies, and had subsequently moulted into third instars ). Although corpora allata of field presoldiers were smaller (Table 21) than those of presoldiers spontaneously produced under laboratory conditions from field adopted larvae, the difference is not significant ($P<0.1$). This is despite the fact that the latter individuals were smaller, if the parameters measured and summarized in Table 22 may be taken as standards. However, CA of field presoldiers were larger than those of minor presoldiers induced in the incipient colonies by JHA topical application (Table 21) but the difference is not statistically significant ($P<0.1$). Although CA of the three types of presoldiers had comparable numbers of nuclei per maximum cross-sectional area, and practically equal ($P<0.1$) nuclear cross-sectional areas; the nuclear/cytoplasmic area ratio of CA from minor presoldiers produced under JHA topical influence was larger than those of CA from the other two types of presoldiers (Table 21). These differences

| Type of Individuals | n | Head Capsule Width (mm) | Head Capsule Length (mm) | Head Capsule Index | Mandibular Length (mm) | Mandibular Index | Number of Antennal Segments |
|---|---|---|---|---|---|---|---|
| | | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | | |
| Field Minor Presoldiers | 20 | 1.82 ± 0.01 | 2.07 ± 0.02 | 1.14 ± 0.01 | 2.01 ± 0.02 | 0.97 ± 0.01 | 17 |
| Laboratory Produced Minor Presoldiers from Field Larvae | 10 | 1.58 ± 0.05 | 1.74 ± 0.05 | 1.09 ± 0.01 | 1.68 ± 0.04 | 0.98 ± 0.01 | 17 |

Table 22. Measurements of minor presoldiers from the field and those produced in the laboratory from field collected female third instar larvae.

again were not statistically significant.

### 7.3.6. *Competence of larvae to differentiate into soldiers under the influence of JHA*

This study was conducted to elucidate the capacity of third instar larvae to differentiate into presoldiers when treated with JHA at different ages. Homogeneous groups of larvae were obtained by rearing second instar larvae in adoptive colonies. The second instar larvae were collected from incipient colonies of the same age (4 months old). They were maintained by pairs of reproductives as old as their parents. These colonies were checked daily for 10 days. Individuals which had moulted into third instar, each day were collected, pooled and re-adopted by another series of recipient reproductive pairs, thus forming relatively homogeneous groups of individuals. The individuals in each group did not differ from one another in age by more than 24 hours. The ages were designated as follows: The last group of individuals to be collected (on the day of treatment) was considered as "day 0" group. The ones which had been collected the previous day, were designated as "day 1" group and so on. All groups were finally treated topically on the same day (last day of collection) with 2µg JHA in 0.5µℓ redistilled acetone per larva. Observations were made daily and the type of moult which ensued was recorded.

The results of these studies are summarized in Figure 47. The individuals which responded moulted into presoldiers and the array of intermediate forms described above. The rate of presoldier and soldier-like formation was high when larvae five or fewer days old

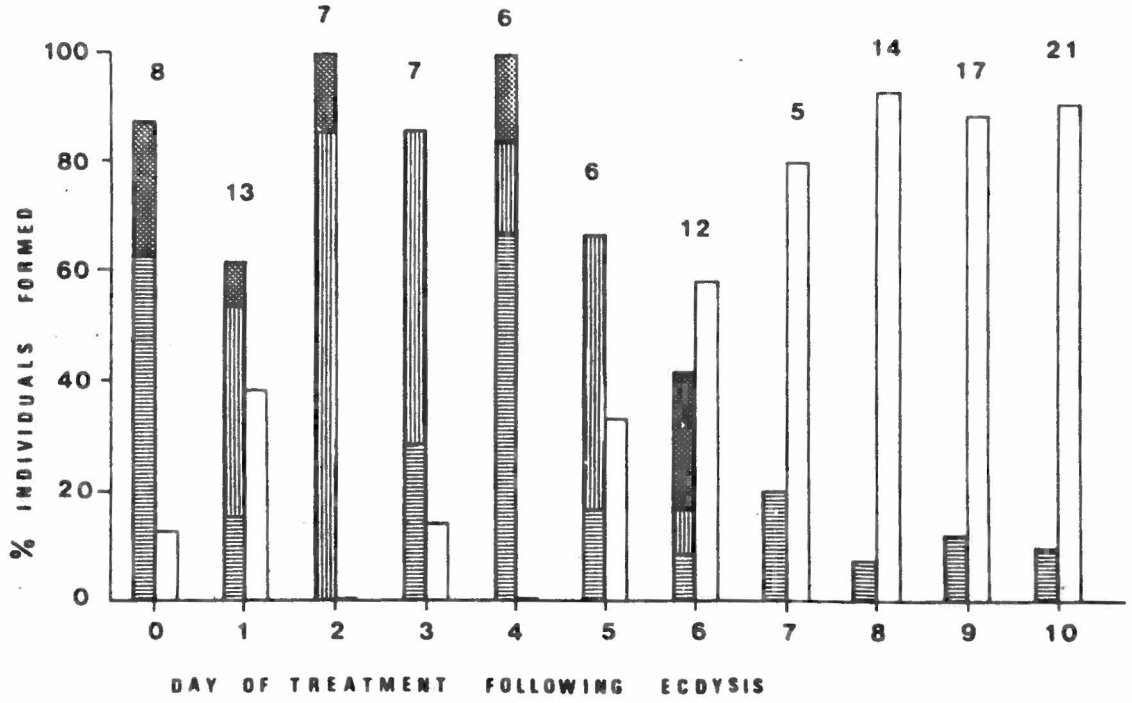



FIGURE 47. Formation rate of presoldiers and other individuals from larvae of different ages topically treated with JHA. ( ▒ ) presoldiers, ( ▓ ) presoldier-like, ( □ ) workers, ( ▩ ) worker-like. Figures above the columns represent the total number of individuals observed.

were treated. Six day old larvae also responded to the treatment, but at a relatively lower rate (Fig. 47). However, some larvae of this age had still a high capacity for forming worker-like individuals. No response to the hormone analogue treatment was shown by 7 day or older larvae. All the presoldiers formed during the first 7 - 10 days, were spontaneously induced presoldiers and were formed between 12 and 14 days following the collection of third instar larvae from donor colonies. While those formed under JHA influence emerged during the 14 - 16 day interval following treatment, irrespective of the age of the larvae treated.

## 7.4. *DISCUSSION*

Results on the responsiveness of larvae at different instars to varying doses of JHA showed that doses higher than 6µg/animal were toxic to larvae of all instars. First and second instars were most affected apparently due to their relatively smaller sizes.

The following observations strongly suggest that presoldier formation occurs only in the third larval instar: First that the first presoldiers appeared in colonies containing third instar larvae and second, that the peak in presoldier formation was reached earliest in these colonies and latest in colonies containing first instar larvae. This observation is compatible with the earlier one made on minor presoldier formation under field conditions (Chapter Three). *NOIROT* (1955) also established the

same scheme for minor presoldier formation in a number of species of *Termitidae*. The results here have also showed that exogeneous JHA at any dose would not induce presoldier formation at an instar earlier than third. This indicates a very rigid timing mechanism in presoldier formation.

Experiments on JHA dosage response clearly showed that there is a JH threshold that is required for soldier determination. If the JH concentration is too low, the larvae do not respond to it and if too high, the hormone becomes toxic and death ensues. It was also found that apparently higher does of JHA above the lower limit which elicits response can induce the production of more presoldiers and fewer intermediate forms. This suggests that the formation of intermediate forms is partly related to the JHA dosage. Further analysis of this will be given below.

When both female and male third instar larvae were treated topically with JHA in separate groups, it was evident that both sexes had the capacity to differentiate into presoldiers. In the control groups, however, only female larvae were capable of presoldier differentiation. it is known that soldier castes (both minor and major) in *Macrotermes bellicosus (NOIROT*, 1955, 1969) under natural conditions develop only from third instar female larvae. It was also found here (Chapter Six) that the same is true for *Macrotermes michaelseni*. It was therefore surprising that presoldiers may also develop from male third instar larvae as well under the influence of exogeneous JH analogue. This finding has important significance in that it goes far toward giving more solid direct

evidence supporting the theory of *CASTLE* (1934) on caste determination. The theory holds that termite castes are determined not genetically (intrinsic factors), but rather by external factors (extrinsic factors). If soldier castes in *Macrotermes* were genetically determined, then our treatment of male larvae should not have triggered presoldier development. (The gene set for soldier development would have been lacking in male larvae as they do not form soldiers in the field under natural conditions ). Since it was possible to induce male presoldier formation by JHA treatment, this should mean that the set of genes for soldier formation is also present in males, but normally dormant. This apparently dormant set of genes has been shown to be activated by exogeneous JHA.

Female and male larvae treated with JHA responded at about the same time in presoldier formation indicating that the mechanism of action of the hormone analogue is probably similar in both cases. There was a differences, however, in the proportion of presoldiers formed. More presoldiers were formed from female larvae than from males. This suggests that male soldier genes are more rigin in responding to the hormone analogue than those of females. Alternatively, male larvae might have evolved a more efficient JH - specific esterase system than females, hence a possibly faster degradation of the analogue before it reaches the target tissues.

Biometric investigations have shown that workers which were formed under JHA topical treatment have smaller head capsules than those from control colonies. This implies that JHA had a growth retarding

effect on the head capsules of workers. There seemed to have been no noticeable effect of JHA on the mandibles of either minor or manor workers formed from larvae incompetent of developing into presoldiers after JHA treatment. As with the workers, presoldiers formed in the control groups had larger head capsules as well as longer mandibles than female presoldiers formed under the influence of JHA topical treatment. This suggests that JHA also influenced the size of the presoldiers formed. Male presoldiers formed under JHA were much larger than presoldiers formed in the control groups. This was expected, since those from the control group were only females and developed from female third instar larvae. They are naturally much smaller than the males which had been induced to differentiate into presoldiers. Although head capsule indices in all three types of presoldiers were similar, the mandibular index of the males was smaller than those of the female and spontaneously produced presoldiers. This observation suggests a differential effect of JHA on male and female larvae as far as soldier determination is concerned.

The results of biometric *analyses* of soldiers and presoldiers formed under different conditions were similar. However, the mandibular indices, unlike those of the presoldiers, were comparable. This observation prompts the assumption that JHA triggers the CA of the treated individuals. The corpora allata may then become self sufficient in JH production apparently required for mandible formation. *LÜSCHER* and *VAN DOORN*,(1976) showed that a constant supply of JH may be necessary for the formation of normal mandibles in the presoldiers of *Zootermopsis*.

Experiments on the rates of replacement of individuals removed from colonies under different concentrations of JHA vapour showed that the rates for replacement of both minor and major workers in colonies treated with 0-1.0mg/colony were practically the same. It was noticed, however, that the replacement of minor workers was slightly higher and started a few days earlier than for major workers. The rate of worker replacement was lower in the colonies treated with higher doses of JHA (10-50mg/colony). This result may be attributed partly to death of some workers formed at these high JHA concentrations and partly to the fact that a large number of worker precursors went to forming presoldiers.

Within the first 9 days following treatment presoldiers were replaced faster in the first four groups of colonies than in the last two. This suggests partial inhibition of moulting capacity by larvae in these latter groups. *MASNER* and *HANGARTNER* (1973) showed that JHA - geranylphenylester or JH may inhibit moulting when continuously supplied to the nymphs of the cockroach *Blattella germanica*. *WANYONYI* (1974) also noted a similar effect on groups of larvae and nymphs of *Zootermopsis nevadensis*. However, under conditions being reported here; the inhibition effect seems to be temporary because soon afterwards, there was a sudden burst of moulting, and between 15 and 18 days a peak in presoldier formation was achieved in the two groups of colonies treated with the highest JHA doses. It is probable, therefore, that the PG go through two phases under these conditions. First they are inhibited by the exogenous JHA, then re-activated just before the burst

of presoldier moults. This induces a well synchronized moult of larvae which suggests a re-programming of soldier forming genes, probably under the control of JH.

These experiments have also shown that the formation of pre-soldiers is JHA dose dependent since 0.01 - 1mg. JHA/colony did not stimulate soldier formation while 10-50 mg. JHA/colony did. Similar findings were made by *SPRINGHETTI* (1974) who fed different concentrations of farnesenic acid ethyl ester to induce soldier formation in *Kalotermes flavicollis*. *HRDY* (1972) also made similar observation while working with *Reticulitermes lucifugus santonensis*.

Biometric analysis of individuals produced here showed that the differentiation of male presoldiers and subsequently soldiers can be induced by JHA vapour as well as topical treatment. The results also showed the following: As with topical treatment, workers produced in control groups were larger than those from treated groups. An explanation for this phenomenon remains obscure. The mandibular index of male presoldiers was less than those of JHA produced female and spontaneously induced presoldiers. Nevertheless under these conditions, JHA induced female presoldiers also had a smaller mandibular index than those spontaneously produced. This suggests that, apart from a continuous supply of JH, other factors may be necessary as suggested by *LUSCHER* and *VAN DOORN* (1976) in their work on mandibular formation in presoldiers of *Zootermopsis*.

The formation of intercastes following CA implantation or JHA treatment has been observed by many authors (*LÜSCHER* and *SPRINGHETTI*, 1960; *LEBRUN*, 1967a, b; *LÜSCHER*, 1969; *HRDY*, 1972; *SPRINGHETTI* 1974) working on lower termites, as well as *FRENCH* (1974) and *LENZ* (1976a, b) while experimenting with *Nasutitermes*, *C. amanii* and *K. flavicollis*. None of these authors have given any satisfactory explanation for the formation of the intermediate forms. Present results seem to implicate JHA dosage to be partly responsible for the phenomenon. Of course, it would be simpler to explain this in such vague terms as physiological conditions not allowing some of the treated individuals to differentiate into proper presoldiers and subsequently into soldiers. I have chosen to consider this as still an open question.

Studies on CA of presoldiers formed under varying conditions have shown that these glands may differ in size, hence the activity depends on the conditions under which the presoldiers are formed. The most active glands are those from spontaneously formed presoldiers in laboratory incipient colonies. The next largest are those of minor presoldiers also formed under laboratory conditions but from field adopted larvae. Corpora allata of minor presoldiers collected from the field are third largest while those from minor presoldiers formed under the JHA topical treatment are the smallest. *LÜSCHER* (1969) reported that CA from spontaneous presoldiers of *K. flavicollis* were larger than those of JHA induced presoldiers. This observation agrees well with what has been found in the present study. However, the most interesting finding here is that

CA of minor presoldiers, spontaneously formed in the laboratory from incipient adopted third instar larvae, are much larger than those of presoldiers from field larvae adopted by laboratory incipient colonies or even of field collected minor presoldiers.

The phenomenon of royal couple influence on the differentiation of soldiers has been reported by *SPRINGHETTI* (1969, 1970) in *K. flavicollis*, *MILLER* (1942) on *Prorhinotermes simplex* and by *LÜSCHER* (1973) in two species of *Zootermopsis*. This influence must be through CA activation since it is known that JH is required for soldier formation (*LÜSCHER*, 1969, and others cited above). The mechanism of presoldier induction by the reproductives would seem therefore to be via a pheromone activating the CA rather than a direct JH influence on the larvae. Supporting evidence here lies in the fact that no intermediate forms were found in untreated colonies, only in JHA treated ones. *LÜSCHER* (1975) also came to the same conclusion from his experiments on *Zootermopsis*.

This observation therefore suggests that the royal influence in the activation of CA is greater under laboratory conditions than in the field where communication between the royal couple and the larvae may be more tenuous than in laboratory colonies. Since under the present conditions it is not possible to rear orphaned larvae of *Macrotermes* in the laboratory, this hypothesis cannot be verified. It seems also that the CA of presoldiers formed under JHA influence became slightly activated, either by the adoptive royal couples or by the JHA itself. This may explain in part why the differences in mandibular size between presoldiers formed spontaneously and those formed under JHA was not very significant.

The cumulative effect of a single dose of JHA plus the influence of the royal pair might enhance mandible development. This view is further supported by the observation that soldiers formed under JHA vapour (mimicking continued JH influence) had slightly longer mandibles than those formed under control conditions (untreated). This suggest that JHA vapour concentration, as perceived by the presoldiers, is even greater than the amount of JH produced in the spontaneous presoldiers.

While working on the development of supplementary reproductives in *K. flavicollis*, LÜSCHER (1952,b) introduced the idea of competence. He found that larvae and nymphs differentiate into supplementary reproductives when given the right conditions but only during specific periods within an intermoult. This period he called the sensitive or competence period. *SPRINGHETTI* (1972) has shown that pseudergates of *K. flavicollis* also have varying competence to differentiate into soldiers. He showed that pseudergates display a rather short competence period for differentiation into soldiers during the second half of the moulting interval, which occurs between 45th and 60th days of the approximately 70-day moulting interval. LÜSCHER (1974,b) arrived at the same conclusion when he studied presoldier competence in *Z. angusticollis* by applying vapours of JHA or farnesyl/Methyl/ester to groups of larvae of known age within the moulting interval. However, my studies on competence of third instar larvae to form presoldiers have shown that in *Macrotermes michaelseni*, this sensitive period occurs during the first half of the moulting interval. The loss of

competence is relatively sudden. Present results also showed that intermediate forms are formed only during the competence period and that their formation is not correlated with any particular period of the competence interval.

# 8. GENERAL DISCUSSION AND CONCLUSIONS

Results have been presented on polymorphism and the mechanisms underlying caste differentiation in a species of a higher termite, *Macrotermes michaelseni*. Polymorphism in this species does not differ from what *NOIROT* (1955) has found in a related species, *Macrotermes bellicosus* with a minor exception. However, concerning the origin of major presoldiers, according to his findings *NOIROT* (1955, 1969) found that major presoldiers originated from incompletely sclerotized, non-functional minor workers otherwise morphologically identical with functional minor workers, while I have identified a specific fourth instar larva which moults into a major presoldier. It is also incompletely sclerotized, and non functional, resembling a very young minor worker except that it is much larger than a minor worker and capable of moulting into a major presoldier. As its development from third instar female larvae must be controlled differently from that of a minor worker it is, therefore, designated as fourth instar. It is thus probable that initiation of soldier determination occurs in the third instar. While minor presoldiers develop directly from female larvae of the third instar, major presoldiers must pass through a fourth instar in their development, undergoing a kind of "supernumerary" moult to gain in size.

On the development of reproductives, *KAISER* (1956) and *SANDS* (1965) have found that nymphs pass through four nymphal instars before the imaginal moult in *Anoplotermes pacificus* and *Trinervitermes* sp., respectively. However, in the present study, I have found that *Macrotermes* nymphs undergo five moults before the final one. This agrees with *BATHELLIER's* (1927) work on several species of

higher termites in Indo-China. *NOIROT* (1952) made similar observations on several species of *Termitidae*, *WEESNER* (1953) on *Tenuirostritermes tenuirostris*, *BOUILLON* and *MATHOT* (1964) on *Cubitermes exiguus*, *HECKER* (1966) on *M. bellicosus* and *N'DIAYE* (1977) quoting *NOIROT* and *BODOT*, plus his own findings on *C. fungifaber*. It seems therefore that the five nymphal instars leading to the formation of primary reproductives in higher termites is quite a common pattern in higher termites. The findings of *KAISER* (1956) and *SANDS* (1965) are open to question, since *NOIROT* (1969) noted five nymphal instars in other species of the same genera.

Having established the pattern of polymorphism in field materials of *M. michaelseni*, studies were initiated to unravel the mechanisms of caste formation. Histological procedures were used to establish the activity of endocrine glands suspected of being involved. Similar studies have previously been conducted by a number of authors (*LÜSCHER*, 1957, 1965; *LEBRUN*, 1967 a, b; *GILLOTT* and *YIN*, 1972) on lower termites, and by *KAISER* (1956), *PASTEELS* and *DELIGNE* (1965) and *NOIROT* (1969), to mention but a few, on higher termites. The present results strongly suggest that highly active CA play a role in soldier differentiation as already demonstrated in lower termites (*LÜSCHER*, 1958; *LÜSCHER* and *SPRINGHETTI*, 1960, *LEBRUN*, 1965). On the other hand CA having low activity permit the worker caste to develop. As far as the development of the reproductives is concerned, they also require low levels of CA activity and relatively more active prothoracic glands than the neuter castes. *NOIROT* (1977) has suggested that ecdysone may be

necessary for the differentiation of the reproductives, hence supporting an earlier suggestion made by *KAISER* (1956) that more active prothoracic glands are associated with nymphal development.

This argument may be valid, but other roles for ecdysone have been reported in insects. Ecdysone has been implicated in ovarian growth in the larvae of *Tenebrio Molitor (LAVERDURE,* 1971), oocyte maturation in *Malacosoma pluviale (SAHOTA,* 1969) or in ovarian competence to undergo maturation *(BELL* and *SAMS,* 1975) in a cockroach. Therefore, the larger size of prothoracic glands during nymphal development may well be related to the development of the gonads. Until direct evidence becomes available it remains unclear why prothoracic glands become activated during nymphal development.

It may be recalled that many authors have suspected any correlation between corpora allata size and the level of their activity on various species of lower termites and higher termite-species. In view of the findings presented here, I find it important to attempt a clarification of this controversy.

A correlation has been shown between CA size and juvenile hormone titre in the haemolymph of fourth instar larvae and various stages of major presoldier development. No similar work has been reported in any other termite species in connection with soldier differentiation. *DE WILDE* and *OOSTRA* (see *DE WILDE et al.,* 1968) while studying hormonal levels in homogenates of Colorado potato beetles during diapause and reproduction, also found JH levels well correlated with CA size.

Ecdysone levels found in the haemolymph of the fourth instar *Macrotermes* larvae and major presoldiers, follows the pattern of JH levels during the various stages of development, although the drop in the ecdysone level is more pronounced. This observation suggests an interplay between the two hormones during soldier development.

During the development of incipient colonies, it was found that in *M. michaelseni* egg incubation period was shorter than what was found for *M. bellicosus* by *GRASSE* and *NOIROT* (1952) and *NOIROT* (1955) although the overall developmental period from the hatching of eggs to presoldier, or minor worker formation is practically the same in the two species of *Macrotermes*. While they have reported much longer intervals for the development of major workers and minor soldiers than those found in the present study, we have both agreed that there are three larval instars in both male and female lines of development into major and minor workers or presoldiers, respectively. Any small differences betewen the two species are probably due to species specificity.

It was also found that individuals produced in the incipient laboratory colonies are much smaller than their counterparts from mature field colonies. This also was true for *M. bellicosus* (*NOIROT*, 1955), and even for lower termites, for example, similar observations were made in *Reticulitermes* (*PICKENS*, 1932; *BUCHLI*, 1950) and even in the most primitive species, *Mastotermes darwiniensis* (*WATSON*, 1974). This phenomenon seems therefore to be a characteristic feature in the development of young colony in

the *Isoptera*. A possible explanation for this lies in the quality or quantity of food on which the colony is fed during the initial stages of its development. This assumption may also explain the absence of fourth instar larvae and nymphs in incipient colonies as observed here.

After determining the post-embryonic developmental pathways and the duration of each instar, investigations were conducted in search of direct evidence for hormonal involvement in caste differentiation. The juvenile hormone analogue, ZR 515, was used to manipulate the development of larvae from incipient colonies. The results have shown that the analogue induces soldier development in treated larvae as has been shown in a number of lower termite species (*LÜSCHER*, 1969; *HRDY*, 1972; *HRDY* and *KRECEK*, 1972; *WANYONYI*, 1974; *LENZ*, 1976 a,b) and one higher termite species from one genus, *Nasutitermes* (*FRENCH*, 1974; *LENZ*, 1976 b). The induction of soldier formation is also instar specific. It was possible to induce soldier formation only in third instar larvae. This agrees with what has been observed during larval development in field colonies, as well as in incipient colonies (Chapters Three and Six) and in *NOIROT's* (1955) findings on *M. bellicosus*. This is a very strong indication of a rigid timing mechanism of caste formation in this species quite unlike the situation in the lower termites where soldier formation may be accomplished at several instars during the development (*MILLER*, 1969; *LÜSCHER*, 1974, a).

The larval response to JHA also depends on dosage and mode of

application. Low dosages have no effect, while too high a dosage proves toxic. *SPRINGHETTI* (1974) made similar observations on the induction of soldier formation in *Kalotermes flavicollis*. Vapour application of the JHA yielded more presoldiers in incipient colonies devoid of soldiers, presoldiers and workers. This might well have been due in part to the prolonged presence of the hormonal analogue vapour in these colonies.

It has also been demonstrated that soldier formation can be induced in male third instar larvae by either JHA topical application or vapour treatment. This observation is contrary to what has been observed under natural conditions. However, this indicates that male larvae have not lost their capacity to develop into soldiers. Two possible mechanisms are suggested. Either a more dormant gene set for soldier formation has evolved which can be reactivated by exogenous JHA. Alternatively, under natural conditions, there are no agents capable of activating the modified CA of male larvae to the threshold for soldier formation. This finding further supports the theory of "extrinsic factors" involvement in caste differentiation as opposed to that of "intrinsic factors". If soldier determination were genetic and sex-linked, one would not expect any male larvae treated with JHA to develop into soldiers, since one finds only female soldiers under natural conditions.

Other results have shown that workers formed under JHA treatment are smaller than those formed under control conditions. No explanation can be offered for this, but one may only speculate

that this phenomenon is probably related to toxic effect of JHA on the developing individuals. Similar results have been noted when sizes of spontaneously formed presoldiers are compared with those formed from JHA treated female larvae.

The mandibular index of male presoldiers formed under JHA topical treatment is smaller than that of female presoldiers whether they are formed under the same conditions or spontaneously. This observation suggests a differential effect of JHA on male and female larvae.

Biometric analysis of soldiers shows relationships similar to those of presoldiers; however, the mandibular indices are also comparable. This suggests a reactivation of CA in JHA topically treated individuals, which should boost the level of circulating JHA to the stage of presoldier development into soldiers. Since *LÜSCHER* and *VAN DOORN* (1976) had shown that a constant supply of JH is required for normal mandibular formation of presoldiers in *Zootermopsis*, therefore the same could well be true for soldier formation.

The formation of intercastes proved to be a common phenomenon under all conditions of JHA treatment described above. Other authors (*LÜSCHER*, 1969; *HRDY*, 1972; *SPRINGHETTI*, 1974, *WANYONYI*, 1974) have made similar observation while working with lower termites. *FRENCH* (1974) and *LENZ* (1976,b) also produced intercastes in *Nasutitermes* by JHA treatment. The explanation for this phenomenon remains obscure.

Size/activity relationships determined on corpora allata from presoldiers formed under varying conditions show that CA from spontaneously produced presoldiers are the largest and those of JHA induced presoldiers are smallest. Those prepared from field presoldiers are intermediate in size. *LÜSCHER* (1969) has made similar observations although he did not include spontaneous presoldiers collected from the field in his study. The possibility of a royal couple influence is suggested by the fact that the CA of presoldiers formed from field larvae, and adopted by incipient colonies, are larger than those of field collected presoldiers. The influence of royal pairs on soldier differentiation has been reported by *MILLER* (1942), *SPRINGHETTI* (1969, 1970) and *LÜSCHER* (1973). It seems probable from the present study that this influence is through CA activation.

All these facts together strongly suggest that the mechanism of soldier formation in higher termites is through CA activation rather than direct transmission of JH from the royal pair to competent larvae. If the latter is true one might expect to observe inter-caste formation under natural conditions as well. *LÜSCHER* (1975) reached similar conclusions in his review on pheromones and polymorphism in bees and termites.

Studies on the competence of third instar larvae for developing into presoldiers have shown that only young larvae (no older than six days) are capable of responding to JHA treatment. This observation is contrary to the findings of *SPRINGHETTI* (1972)

and *LÜSCHER* (1974, b) who showed that the competence period for soldier formation in *K. flavicollis* and *Zootermopsis angusticollis* is during the final days before a larval moult. The discrepancy here might well be due to evoluticnary level of the species involved and consequent different developmental patterns.

The present studies have provided answers to many difficult questions regarding polymorphism and mechanism cf caste differentiation in a higher termite. To date we have fragmentary evidence that high levels of JH play a role in the formation of soldiers. Much of it is indirect *(KAISER*, 1956; *NOIROT*,1969) and only two pieces of work *(FRENCH*, 1974, and *LENZ*, 1976) on two species of *Nasutitermes* have given any direct evidence regarding the role of JH in soldier formation. I have here supported this view with a considerable body of rather strong evidence on a species with a more rigid scheme of development.

A thorough analysis of the mechanism of caste determination has been presented which should bring us to the level of the extensive work already done on the differentiation of castes in lower termites. These lines can of course be explored further to give deeper insight to the problem of caste differentiation, a phenomenon which could be manupulated for control and at the same time, pursued simply for the answers to some of the most interesting questions in the biology of differentiation.

# 9. S U M M A R Y

Biometric studies coupled with physiological investigations have made it possible to propose a scheme of post-embryonic development and polymorphism in *Macrotermes michaelseni*. A hitherto undescribed fourth instar has been found. This instar is comprised of female larvae which moult into major presoldiers. Minor presoldiers moult from some of the third instar female larvae which are morphologically identical to the female worker larvae. Three instars were detected in the course of both minor and major worker development. Workers and soldiers are sterile castes. Nymphs develop from first instar larvae and pass through five instars before the imaginal moult.

Studies on sizes of corpora allata (CA) and prothoracic glands (PG) have indicated that the CA play a role in the differentiation of soldiers in this species of higher termites. This was evidenced by enlargement of the glands in the fourth instar larvae which were found to moult into major presoldiers. Such glandular enlargement was not dramatic in the larvae which develop into workers. There was also no appreciable enlargement of the CA during nymphal development until just before the adult moult. Therefore it seems that the CA play no role in the differentiation of workers or reproductives. On the other hand, nymphs were shown to have very much enlarged PG as compared to those of larvae. It is not known whether this enlargement of

PG in nymphs is associated with the differentiation of the reproductives or simply a normal requirement for growth which was marked in this caste.

In further investigations, haemolymph titres of both juvenile hormone and ecdysone were measured in immature individuals during soldier development. Results showed that the highest titres of juvenile hormone is reached during the fourth larval instar. A progressive decline then follows throughout subsequent presoldier development reaching the lowest point before a soldier moult.

Changes in the ecdysone titres followed closely the pattern of JH. However, a small rise in ecdysone titre was observed during the intermediate stage of presoldier development, with a further fall just prior to a soldier moult. These observations are in close agreement with the results obtained by histological investigations of CA and PG activities during these stages of development.

Studies were conducted on the development of incipient colonies. Most females produce their first batch of eggs within the first week following the pair formation. The incubation period for these eggs was found to be about 36.2 days. Three larval instars, both males and females, minor and major workers and minor soldiers were found among the first progeny. No fourth instar larvae or major soldiers were detected. Neither was there any form of reproductives.

The instar duration for each stage of development was determined. The longest was that of third instar larvae developing into either minor or major workers. The shortest duration was that of first instar larvae moulting into either male or female second instars. The first neuter adults to appear in the colonies in most cases were minor workers followed by major workers and soon after by minor soldiers.

A juvenile hormone analogue (ZR 515) was used to investigate the role of JH in caste differentiation. The analogue influences soldier differentiation only during the third larval instar and is independent of the dosage used above the required threshold. Therefore, subsequent experiments were conducted using only third instar larvae.

The results also showed that a hormonal threshold exists, above which soldier differentiation occurs. A dose far above threshhold increases mortality drastically. When female larvae were used, survival rate of treated larvae was proportional to the dosage of the analogue administered. It was also shown that the formation of intermediate forms seems to be correlated with the dose of the analogue administered. The influence of JHA on male third instars was also investigated. The results have shown that soldier formation can be induced in male third instar larvae as well, although no soldiers are formed in control groups. The pre-soldiers and resultant soldiers were larger than female ones produced under the same conditions.

Intermediate forms were produced from JHA treated male larvae as well. The pattern of soldier formation in both cases was similar and the peak, though lower for males was reached at about the same time.

Further experiments were conducted to determine whether prolonged treatment of larvae with JHA was necessary for normal formation of soldiers. The results showed that, even under prolonged treatment (vapour), a certain threshhold of JHA is necessary for the induction of male or female soldier formation. It was also observed that more presoldiers were formed when JHA was administered in vapour form than by topical treatment. Presoldiers and soldiers formed under vapour treatment had more normal mandibles than those produced under topical treatment and compared well with those formed naturally (spontaneously). Intermediate forms were produced even under these conditions, although at a much lower rate.

The influence of exogeneous JHA on the size of corpora allata during soldier formation was also investigated. The largest glands were found in spontaneously produced presoldiers from incipient colony larvae. Corpora allata from field-collected minor presoldiers were comparable in size with those of spontaneously produced minor presoldiers from field-adopted larvae. However, those of presoldiers produced under the influence of topically administered JHA were much smaller than those from individuals of other groups.

Competence of third instar female larvae to differentiate into presoldiers following JHA treatment was studied as well. Results showed that larvae of five days old or less responded well to the treatment while those of seven or more days did not. Therefore, the competence period must extend from day 0 - 6 in the third instar. Intermediate forms were produced only during the first 5-6 day period.

These results permit us to make the following conclusions: Caste development of *Macrotermes michaelseni* is rigidly controlled and thus unlike the flexible situation in lower termites. Highly active corpora allata and subsequently high juvenile hormone titres are required for soldier differentiation and development, while less active CA and more active PG may be needed for the differentiation of reproductives. A period of competence exists when third instar larvae can be induced to develop into presoldiers. There is a possibility that the CA of competent larvae are normally activated by some (unknown) pheromonal system, other than the circulation of JH through the colony by the reproductives and/or workers. The evidence provided here supports the extrinsic theory of caste differentiation in a higher termite.

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