THE INITIATION OF FUNGUS COMB CONSTRUCTION IN LABORATORY COLONIES OF *ANCISTROTERMES GUINEENSIS* (SILVESTRI)

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Termites of the subfamily Macrotermitinæ have always proved difficult to rear under laboratory conditions and incipient colonies developing from a pair of dealated imagos have rarely survived more than a few months. Some observers have recorded young colonies beginning to construct fungus comb, but few of these were under conditions which permitted easy observation.

The colonies described here had survived for approximately six months (up to the conclusion of the experiment). The work was carried out at the Regional Research Station of the Northern Nigerian Ministry of Agriculture at Samaru, near Zaria.

Material.

Ancistrotermes guineensis (Silvestri) is not uncommon in Northern Nigeria, but is less frequently met with than its common congener *A. crucifer* (Sjöst.), possibly due to more cryptic foraging habits, since it frequently occurred in sampling pits in the guinean savannah woodland on red lateritic soils. It builds no mound or other visible evidence of its presence except occasional mud runways.

Methods.

The young colonies were reared in observation nests of a similar type to those described by Lüscher (1949 and 1951) and Williams (1959), consisting of two plates of window glass 6 inches by 4 inches, separated by two half-inch wide strips of glass to provide a chamber only slightly deeper than the bodies of the imagos. The upper glass plate was cut into three pieces 2 inches by 4 inches to facilitate removal for cleaning. The space between the plates was filled with sifted soil which was not otherwise modified either by sterilisation or addition of material. The soil was a slightly humic brown, loamy sand. At one end of each plate a small tunnel was made with a mounted needle to encourage the dealate imagos to enter in a central position to facilitate future observation.

Alates were collected at light. After they had shed their wings they readily entered the prepared tunnels, which they enlarged to form small chambers approximately 2 cm. in diameter. (Plate I, fig. 1).

The shape and size of the plates permitted regular examination under a binocular microscope. The space between the glass was narrow enough to prevent the termites covering it with mud, thus allowing observation and photo-micrography at all times. The plates were kept in a glass desiccator chamber over standing water, and the soil was also moistened from time to time, as seen to be necessary. The temperature was that of the laboratory and fluctuated between 70° F and 80° F during most of the experiment. Under these conditions the termites produced eggs and young in due course.

Results.

	Days
Pairing off and construction of chamber	0
First eggs deposited	3-4
40-50 eggs observed	11-12
Number of eggs 55-60, about 20 per cent of them swollen, opalescent, near hatching	28-30
First 4-5 eggs hatched	34
20-25 eggs hatched	40-42
First 2nd instars	41
First 3rd instars (worker); total of 30-35 eggs hatched	49-50
First 3rd instars (soldier)	52-53
First pigmented workers (minor)	58-60
First pigmented soldiers (minor)	60-62
First pigmented major workers	67
Small tunnel out of chamber begun	73-75
Fungus comb building commenced	78-80
Fungus comb of other species of *Ancistrotermes* (*A. crucifer* (Sjöst.) first introduced	94
Fungus comb of *Ancistrotermes guineensis* introduced	130
Appearance of *Termitomyces* hyphae on combs of own construction	131

Oviposition.

Three to four days after construction of the nest chamber the first eggs appeared. The rate of oviposition averaged 5 eggs per day until 40-50 eggs were present. After this the rate of egg laying fell off rapidly until only one a day or fewer was laid.

In the laboratory nests the rate was not observed to increase again to any marked extent. In nature it might be expected to rise again when mature workers take over the care of the brood, and begin to supply nourishment to the king and queen, which until then are the sole source of food supply for the developing young.

Development of brood.

The rate of egg laying and development of the young was comparable to that recorded by Light and Weesner (1955) in *Tenuirostritermes tenuirostris* (Desn.). It was more rapid than was observed by Grassé and Noirot (1955) for *Macrotermes* (*Bellicositermes*) *natalensis* (Hav.), by Kemp (1955) for *Noditermes* sp. and *Odontotermes badius* (Hav.), or by Williams (1959) for *Cubitermes ugandensis* Fuller. Of the species recorded by Lüscher (1951) only *Reticulitermes lucifugus* Rossi was comparable, and *Pseudacanthotermes spiniger* Sjost. was considerably slower to produce eggs and young. Five species of *Trinervitermes* which were reared at the same time as these *Ancistrotermes* under the same conditions showed equally rapid development, suggesting that it was due to a comparatively favourable environment rather than to major differences inherent in the species or families concerned.

During incubation the eggs increased in size, until immediately prior to hatching they had more than doubled their original volume. As other authors have observed, the imagos assisted the young in hatching and moulting. Instars lasted approximately 8 days each. It will be noted from the table of development that fully developed, pigmented, minor workers were present in the brood chamber for over two weeks before tunneling-out activity was begun, during which time they assisted in grooming the larvae and eggs.

Initiation of "fungus comb".

The first structures recognisable as the beginnings of "fungus comb" appeared in the chambers 78-80 days after the dealate imagos entered the plates. This occurred before the tunneling activities of the worker termites had brought them in contact with the wood chips with which they were supplied and the earliest combs therefore contained more mineral material than is usual. As soon as foraging on the wood began, the vegetable content rapidly increased. The first "combs" consisted of slender pillars of spheres, and later developed into aggregations of curiously serpentine shape, indicating in a rudimentary way the beginnings of the normal contours of the comb. These were closely similar to the early fungus combs of *P. spiniger*, described and photographed by Lüscher (1951). (Plate I, figs. 2 and 3).

The combs remained sterile with no trace of fungus at this stage. At about 94 days after setting up, the termites began to show signs of undernourishment in the form of white deposits in the fat body (Cf. Williams, 1950). The comb though normal in appearance still lacked fungus. Fungus comb of the commoner *Ancistrotermes crucifer* (Sjöst.) was obtained, and fragments including the white nodules of conidiophores and conidia were placed in the foraging chambers of the plates. Both comb and nodules were quickly consumed by the termites (c.f. Sands, 1956), but the combs of their own construction remained sterile with no trace of fungus.

The fungus comb of *Ancistrotermes guineensis* (Silv.) proved difficult to find and did not become available until 130 days after the beginning of the experiment (at which time the comb in the plates was still sterile). When placed in the foraging chambers the comb was again quickly consumed in large quantities.

Hyphæ of *Termitomyces* now appeared on the comb within 24 hours, and continued to grow until the conclusion of the experiment. Nodules of conidia and conidiophores were produced in the usual manner. (Plate I, figs. 4 and 5).

The fungus combs in the plate nests were constructed entirely from balls of faeces, partially moulded by the rectum. In the course of many hours under observation no chewed wood was ever added to the comb by the termites. On the other hand, the process of deposition of faecal pellets, resulting in the gradual building up of new comb, was observed many times. (Plate II, figs. 6 to 8).

When about to deposit a faecal pellet a worker walked over the comb to an area of recent defaecation. It then circled once or twice, curving the tip of the abdomen downwards. Simultaneously with the opening of the anus the abdomen was moved rapidly from side to side, finally rounding off the semi-liquid ball of faeces. The liquid content was rapidly absorbed by the rest of the comb, leaving a surface with an "oolitic" appearance as described by Grassé (1944) and Grassé and Heim (1950). Workers often remained motionless with their mouthparts in contact with the comb, presumably sucking up moisture. It was not uncommon for a worker to remove a newly deposited faecal ball and walk away eating it. Occasionally a ball picked up in the mouthparts would be replaced on the comb.

The new areas of faecal deposition were quickly covered with *Termitomyces* hyphae and, later, nodules. When well covered, the comb was eaten again, and was thus in continual circulation through the gut of the termites. This agrees with the observations of Grassé and Noirot (1957, 1958*a*) and their suggestion that the comb is part of the normal food cycle of the termites, in which the vegetable material is rendered more assimilable by the fungus, which also supplies other nutritive elements directly (see also Sands, 1956). (Plate II, figs. 9 to 11).

Conclusions and Discussion.

Many authors have accepted the theory first stated by Bathellier (1927) that the fungus combs of the Macrotermitinæ were constructed from chewed wood. Grassé and Noirot have repeated this statement in a number of papers, and Grassé and Heim (1950) described the process of comb construction in *Ancistrotermes latinotus* (Holmgren) in Oubangui-Chari.

The earliest authors in this field believed the combs to be faecal in origin, and this older theory is here shown to be correct for *Ancistrotermes guineensis* (Silv.). However, reconsideration of the observations published by Bathellier is required, because they would otherwise appear to contradict those described in the present paper. Bathellier pointed out that all termites other than *Cryptotermes* produce semi-liquid brown faeces which dry to produce a hard material known as "carton", containing less vegetable matter in more finely divided form than occurs in fungus comb. (This statement is itself incorrect: the Kalotermitidæ can produce a carton-like material when building partitions or sealing holes, and in *Zootermopsis angusticollis* (Hagen), though much of the structural work in the nest is derived from liquid faeces, deposits of faecal pellets also frequently occur. Coaton (1946) describes the nest of *Hodotermes mossambicus* (Hagen) which builds the hive from fragile black carton of faecal origin, and expels faecal pellets along with the soil particles around the entrances of the foraging holes). Bathellier also stated that the contents of the rectal pouch of all the castes of *Macrotermes gilvus* which he examined were of a similar nature.

In the observation nests the production of fungus comb was not carried out by the entire population. The semi-liquid brown faeces produced by many of the termites were deposited anywhere, sometimes on the comb, but often on the walls of the chambers and galleries. Grassé (1937) noted the similarity between the contents of the rectal pouch of *Macrotermes* (*Bellicositermes*) *natalensis* (Hav.) and the fungus comb, but proceeded to restate Bathellier's theory on the grounds that the "normal" faeces were dark brown and semi-liquid. In fact the material obtained by dissection from the rectal pouch of a sample of workers from a mound might be expected to resemble fungus comb in relatively few cases.

It may be suggested that an individual termite only occasionally produces a faecal pellet, when it has fed directly on vegetable material, and that consumption of fungus comb or other pre-digested food gives rise to the brown semi-liquid type of faeces. Alternatively it is possible that the behaviour of workers changes with advancing age, resulting in a form of division of labour approximately analogous to that which occurs in some Hymenoptera. The latter theory is supported by the fact that apparently mature pigmented workers remained in the brood chamber for over two weeks before beginning to tunnel out. A similar comparatively sudden change of behaviour was recorded by Grassé and Noirot (1955) in young colonies of *Macrotermes* (*Bellicositermes*) in connection with the very rapid construction of the "hive".

The account given by Grassé and Heim (1950) also requires further comment, since these authors describe the fungus comb as being made of—"little balls of chewed wood, perfectly spherical, stuck together, one on another, and always maintaining a very distinct 'oolitic' appearance"—stating: « Nous avons assisté à leur confection et suivi les étapes de leur développement ». The description they give of the early stages of development of the comb agrees exactly with the observations from the plate nests, with the exception of the faecal nature of the comb. It may therefore be presumed that though Grassé and Heim observed various stages in the construction of the comb by examination of newly-opened nests, they were not able to witness the actual deposition of faecal pellets. Worker termites carrying pellets in the mouthparts may have led to the conclusion that they were of chewed wood: this point has already been covered in the previous section. Later in their paper, the authors note that the more liquid type of faeces are often deposited on the comb, and that the reddish spots so formed are soon covered with hyphae like the rest. Grassé and Noirot (1958*a*) state in a footnote that in their young colonies of *Bellicositermes* (paper of 1955) the rapid construction of the comb did not permit them to follow it in detail.

In the inhabitants of a normal colony, the contents of the rectum vary from a yellowish fluid in very young workers and older nymphs to the copious material used by workers to build the fungus comb. The deposition of different types of faeces in different parts of the nest is not uncommon in the Isoptera, from Kalotermitidæ upwards. In many carton nest builders (e.g. *Cephalotermes*, *Microcerotermes*) the outer parts of the nest are made of dark brown carton of a very different structure and consistency from the inner, a difference which is inherent in the material and not merely due to the prevailing higher humidity in the interior. The occurrence of a similar habit in the Macrotermitinæ is therefore not in any way abnormal or unusual.

It has already been shown (Sands, 1956) that the fungus comb is an essential part of the diet of *Odontotermes badius* (Hav.). These results with *Ancistrotermes guineensis* (Silv.) indicate that a very close symbiosis exists between termite and *Termitomyces*. The failure of the alien fungus to become established on the comb, and the immediate growth of the correct one suggests that there is only one species or strain of *Termitomyces* associated with this termite species.

The early sterility of the comb in the plates also suggests that the swarming alates did not carry with them an inoculum of viable spores from the parent colony. Grassé and Noirot (1955) reported the construction of fertile combs in their laboratory colonies of *Macrotermes* (*Bellicositermes*) *natalensis*, but considered that the origin of the fungus could not with certainty be attributed to inoculation by the young king and queen. In view of the great abundance of *Macrotermes* (*Bellicositermes*) *natalensis* the basidiospores of its associated *Termitomyces* may be expected to be equally common, and were probably introduced with the wood included in their laboratory nests. Lüscher (1951) reared young colonies of *Pseudacanthotermes spiniger* (Sjöst.) in which the fungus combs remained sterile. It seems more probable that the development of fungus on the comb of the young colony is dependant on the introduction, by chance or otherwise, of basidiospores by the worker castes during earliest foraging among dead wood above ground level. Failure to obtain basidiospores might result in the premature death of many young colonies of Macrotermitinæ, and thus be an important factor controlling their distribution and abundance.

The significance of fungus comb in the phylogeny of the Macrotermitinae as a whole may now be reviewed in the light of the observations described here. The diversity of appearance and texture of the combs is well known, from the descriptions of the majority of the genera by Kemner (1934), Grassé (1944-45) and Grassé and Noirot (1951). This variation in the production of the combs is not readily explained on the basis of their hypothetical construction from chewed wood. The more primitive genera such as *Pseudacanthotermes* and *Acanthotermes* produce combs of a denser, more homogeneous, and less granular or "oolitic" structure than the rest of the group. They are relatively simple being of convoluted lamellae rather than sponge-like form, though in *Acanthotermes* they are rather more variable than in *Pseudacanthotermes*. In other genera the combs are more complex in their architecture and more granular in texture. The greatest complexity of form is found in *Odontotermes* and *Protermes*. The smaller genera, *Ancistrotermes* and *Microtermes*, have combs in which the granular texture is most strongly developed, and the loss of cohesion between the pellets may be responsible for the reduction in the complexity of form. The end point in this respect appears to have been reached in some *Microtermes* in which the "combs" are mere crumbling aggregations of pellets with a few pits in the surface.

It is suggested that the differences of form and texture of the fungus comb can best be explained by differing degress of symbiosis of the termites with the fungi of the genus *Termitomyces*. The more primitive genera are not far removed from their Rhinotermitid-like ancestors in which the faeces produce "carton". The fungus comb of *Pseudacanthotermes* closely resembles the soft carton found in the nests of some *Coptotermes*. According to Grassé (1944) this similarity was a fortuitous convergence with no phytogenetic significance, on the grounds that the origins of the two structures were different. Since the faecal nature of comb has been demonstrated, his objection is removed. It is reasonable to suggest that fungus combs have evolved from soft carton similar to that which occurs in the Rhinotermitidæ, because these complex structures must have had some simpler precursor in more primitive groups without any symbiosis with basidiomycete fungi. As the latter habit has evolved, and the dependance of the termites on the fungus for their nutrition has increased, the structure of the comb has become more adapted to its use as a fungal substrate. The reduction of the ability to digest woody material might result in a higher vegetable content and more pellet-like structure in the faeces, such as seen in *Microtermes*. The greater dependance of *Microtermes* on the comb is to some extent indicated by the completeness with it is eaten by the termites, as was noted by Grassé and Noirot (1957). Similar behaviour was observed in *Ancistrotermes guineensis* in the plate nests described above.

Summary.

Ancistrotermes guineensis (Silv.) colonies were reared in observation nests The timetable of development of the young colony is given. The construction of fungus comb from the faeces of the worker caste was observed; chewed wood was not used is constructing the comb. The fungus comb remained sterile until the correct species of *Termitomyces* was introduced. The symbiosis between termite and fungus would appear to be specific. It seems that the alates do not carry an inoculum of viable spores of the fungus from the parent colony, but that workers introduce basidiospores in early foraging. This may be an important factor controlling the abundance of the termites. The phylogeny of the Macrotermitinæ is reviewed in the light of the new evidence.

Résumé.

Quelques colonies d'*Ancistrotermes guineensis* (Silv.) ont été élevées dans des nids sous verre pour en faciliter l'observation. La table chronologique du développement de la nouvelle colonie est donnée. On a observé que seule la matière fécale était utilisée pour la construction des meules à champignons et que les termites ne se servaient pas du bois mâché. Les meules demeurèrent stériles jusqu'à l'introduction de l'espèce correcte de *Termitomyces*. La symbiose entre les termites et les champignons paraît être spécifique. Il semble que les essaimants ailés ne transportent pas d'inoculum de spores vivantes de la colonie d'origine et que les ouvriers les introduisent lors des premières récoltes à l'extérieur. C'est peut-être là un facteur important qui règle l'abondance des termites champignonnistes. La phylogénie des Macrotermitinæ doit être reconsidérée en fonction de cette nouvelle évidence.

Zusammenfassung.

Die Kolonien von *Ancistrotermes guineensis* '(Silv.) waren in Beobachtungsnestern gezüchtet. Die wichtigsten Daten in der Entwicklung der jugen Kolonie ist gegeben. Die Konstruction der Pilzgarten aus dem Kot der Arbeiter wurde beobachtet ; gekautes Holz wurde nicht für den Bau benutzt. Der Pilzgarten blieb sterile bis die richtige Art von *Termitomyces* eingeführt wurde. Es ist wahrscheinlich, dass dei Symbiose zwischen Termiten Art und Pilz specifich ist. Es scheint als ob die Geflügelten beim Ausschwärmen von der ursprünglichen Kolonie nicht Pilz Basidiosporen mitnehmen, sondern dass die Arbeiter sie beim ersten furagieren einschleppen. Diese Tatsache könnte in der Kontrolle des Ueberflusses von Termiten sehr wichtig sein. Die Phylogenese der Macrotermitinae ist im Lichte dieser neuen Evidenz besprochen.

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PLATE I

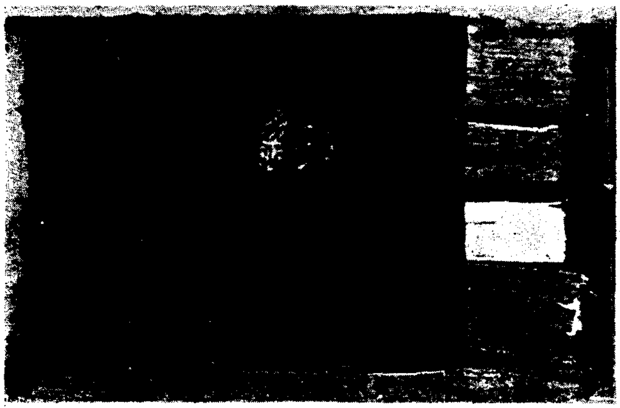
Fig. 1. — Entire plate nest showing brood and royal pair in chamber, and wood chips inserted for food.

Fig. 2. — Brood chamber of another plate nest enlarged to show the beginnings of fungus comb towards the left side of the chamber. Eggs and second instar larvae are visible, also a few mature workers.

Fig. 3. — Sterile fungus comb before the introduction of the *Termitomyces* sp. associated with the comb of the *Ancistrotermes guineensis* (Silv.) showing the granular structure of the comb.

Fig. 4. — Fungus comb 24 hours after introduction of the correct species or strain of *Termitomyces*—hyphae have spread over all except the most recently deposited pellets.

Fig. 5. — Fungus comb some time later, nodules of conidia and conidiophores developing in a typical manner.



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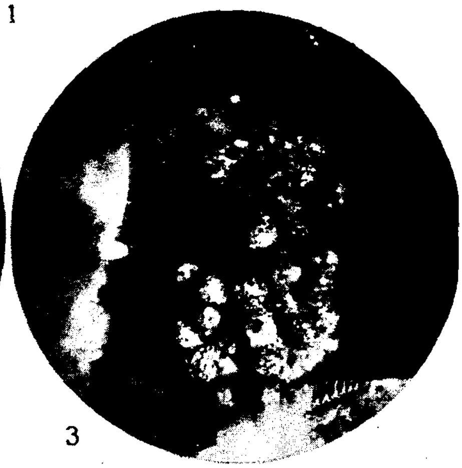

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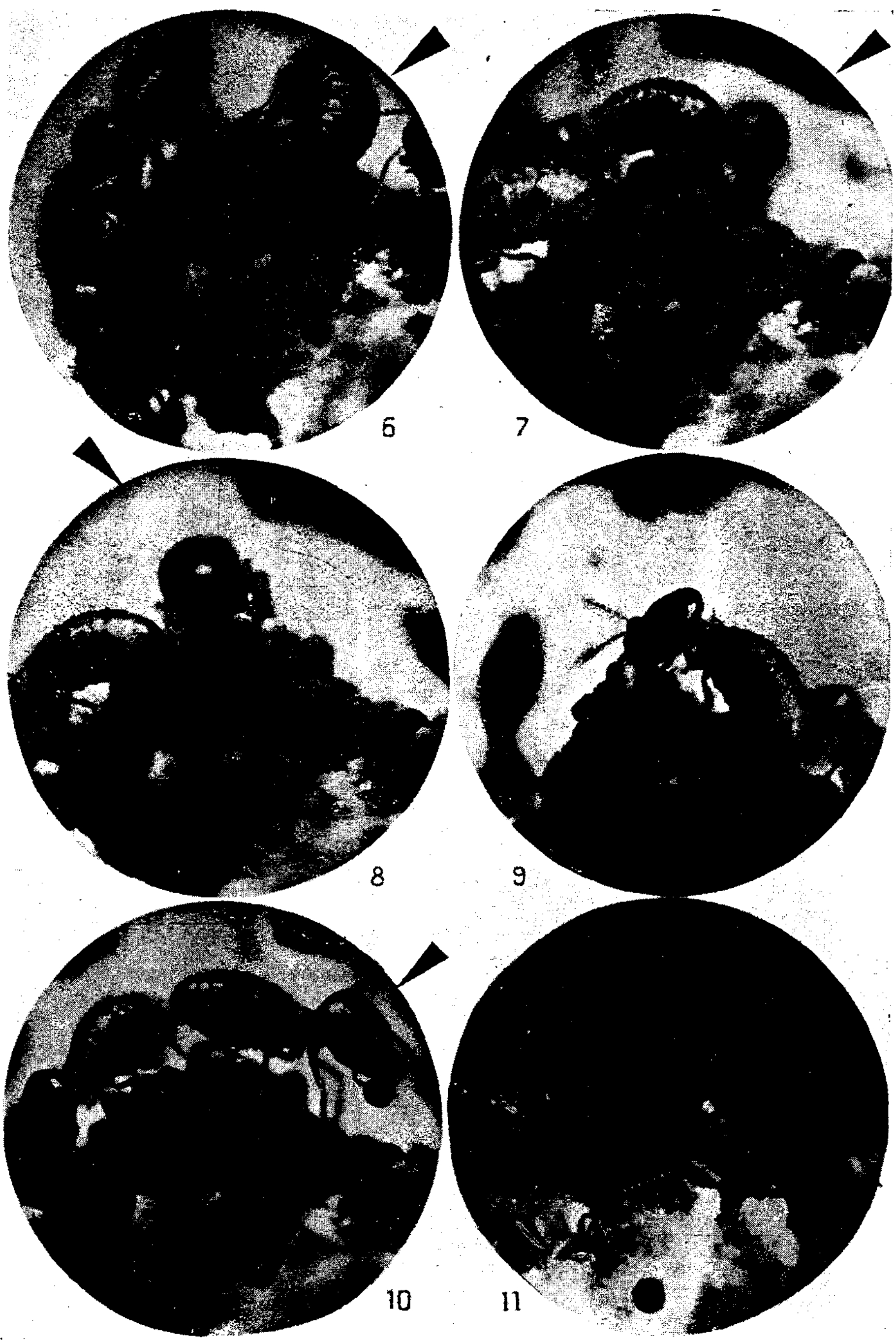
PLATE II

FIG. 6, 7, 8.—Worker termites in the act of depositing faecal pellets. That in 6 is positioning itself; that in 7 is expelling the pellet, and, is, therefore, in rapid side-to-side motion; in 8 the worker has deposited a pellet.

FIG. 9.—Worker termite sucking up moisture from the comb. The abdomen of the same individual appears in Figures 7 and 8, showing that several minutes were occupied in this manner by this termite whilst comb construction was taking place all around it.

FIG. 10.—A worker having removed a newly-deposited faecal pellet from the comb, is shown moving away it held in the mouthparts.

FIG. 11.—The older parts of the comb, well covered with hyphae, are being eaten by the termites, one of which is shown at work.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