A pair of neotenic reproductives of *P. simplex*

SUMMARY

Ontogeny of soldiers in *Prorhinotermes simplex*

The soldiers were found to develop from larvae of the second up to the eighth instar, via a short (13–17 days) presoldier stage. The early soldier instars were found exclusively in incipient colonies while the mature colony contained late instar soldiers only. The first soldiers occur early in the incipient colonies; as soon as one year after the establishment of these colonies a high proportion of soldiers was observed, which is comparable to that in mature colonies. The abrupt change in the external anatomy occurs in two steps. During the larva-presoldier moult, the head increases in length but only slightly in width. During the presoldier-soldier moult, both the length and width of the head increase markedly and the typical cordate shape is attained; the relative size of the pronotum increases considerably. The long falcate mandibles develop mainly during the larva-presoldier moult. One antennal segment is added during these two moults. With increasing instar age of soldiers a relative increase of the pronotum and the head size in its posterior region was observed. No functional differences in external anatomy were found among the six soldier instars. The composition of the frontal gland secretion is similar in the six soldier instars, an extraordinarily high amount of the defensive substance, (*E*)-1-nitropentadec-1-ene, was detected together with (*Z,E*)-α-farnesene, presumably an alarm pheromone. *P. simplex* displays the typical mode of colony-age dependent strategy of soldier production. The observed patterns of soldier development and production in *P. simplex* well correspond to its ecological strategy of an advanced single-site nester able to migrate and form foraging groups.

Egg care by soldiers in *Prorhinotermes*

We observed an unusual non-combative mode of behaviour in soldiers of *P. simplex* and *P. inopinatus*, i.e. the handling and transportation of eggs after a nest disturbance. The soldiers, apart from performing alarm and defensive activities, tried to handle the eggs on the open surface. They often succeeded in lifting an egg or even a group of eggs, and then searched for a nest entrance. They either entered the nest to come out without the egg(s) within a while, or they passed the egg(s) onto a pseudergate waiting in the opening. As a result, all eggs

were rapidly evacuated. Laboratory experimentation showed that pseudergates and nymphs try to deposit the eggs in a dark shelter, whereas the soldiers tend to transfer the eggs to nestmates and drop them after a while if no other termite accepts them. The described activity of *Prorhinotermes* spp. represents a behavioural element of great adaptive value: it enables the saving of eggs without exposing the pseudergates to the risk of predation in the open. The soldiers, on the other hand, are well adapted to perform open-air activities (they are sclerotized, armed, and toxic). Moreover, the observed cooperation of soldiers with pseudergates (waiting in the openings) proves that the soldier collecting behaviour is not a simplified "worker-like" activity, but a highly specialized behaviour. This is also evidenced by the elementary differences between pseudergates and soldiers in the mode of egg deposition (formation of egg clusters in the case of pseudergates and transmission of eggs to another individual in the case of soldiers). This observation changes our view of the soldier caste as an exclusively defensive caste.

Alarm communication and alarm pheromone in *Prorhinotermes canalifrons*

Behavioural experiments showed that both the frontal gland secretion and (*E*,*E*)-α-farnesene triggered alarm reactions in *P. canalifrons*, whereas (*E*)-1-nitropentadec-1-ene did not affect the behaviour of termite groups. The alarm reactions were characterised by rapid walking of activated termites and efforts to alert and activate other members of the group. Behavioural responses to alarm pheromone differed between homogeneous and mixed groups, suggesting complex interactions. Antennae of both soldiers and pseudergates were sensitive to the frontal gland secretion and to (*E*,*E*)-α-farnesene, but soldiers showed quantitatively stronger responses. The dose responses to (*E*,*E*)-α-farnesene were identical for both soldiers and pseudergates, suggesting that both castes use similar receptors to perceive (*E*,*E*)-α-farnesene. Our study confirmed the complementary role of compounds contained in the frontal gland secretion of *P. canalifrons* soldiers: the toxic (*E*)-1-nitropentadec-1-ene is known to act as a contact poison, whereas the minor compound, the sesquiterpene (*E*,*E*)-α-farnesene, acts as an alarm pheromone.

Agonistic behaviour in *Prorhinotermes canalifrons*

Each of the three non-reproductive castes of *Prorhinotermes canalifrons* (pseudergates, presoldiers, soldiers of different ages) as well as artificial intercastes pseudergate-soldier displayed a specific behavioural repertoire when confronted with conspecific and heterospecific aliens. Pseudergates appeared to be non-negligible participants in termite-termite agonistic interactions, though the principle element of the striking power of the colony defence is the soldier caste. The typical defensive activities of the soldier start to appear during the second day after exuviation and the complete set of defensive behaviours can be observed on the third day, well before its defensive secretion is synthesized in the frontal gland. Reactions to heterospecific aliens were much faster and more violent than reactions to conspecifics, suggesting that individuals experience difficulties in identifying conspecific aliens. The behaviour of artificial intercastes induced by an analogue of JH is intermediate between that of pseudergates and soldiers presumably due to simultaneous expression of behavioural phenotypes of a pseudergate and a soldier in these individuals.

Ultrastructure of the frontal gland in castes of *Prorhinotermes simplex*

The frontal gland as a sac-like organ in *Prorhinotermes simplex* is present only in presoldiers, soldiers, and imagoes, but it develops also in nymph-soldier intercastes. The fully developed frontal gland of soldiers extends deep into the abdominal cavity. The secretory epithelium consists of a single type of secretory cells adhering directly to the cuticular intima. Secretory vacuoles originate in electron dense vesicles, which are transformed into large electron lucent vacuoles. Intermediate vacuoles frequently contain lipid droplets. The frontal gland cells in presoldiers reveal modifications connected with the production of a new cuticle; the new cuticle is thin and compact, whereas the old one is thick, porous, and wrinkled. None of these cuticles are present in soldiers. In soldiers, the cuticular intima is of endocuticular origin and is formed by dispersed dense material; the apical parts of secretory cells are formed by numerous irregular finger-like projections, true microvilli are completely lacking. In imagoes, the cuticle is composed of an epicuticle, a layer of epicuticular filaments, and one more basal layer; sexual differences were not observed. In nymph-soldier intercastes, the structure of the gland differs in the head and in the metathorax; the head part of the gland resembles the imaginal gland whereas the thoracic part resembles more that of the soldier; the development of secretory vacuoles stops at the stage of presence of lipid droplets. The frontal gland of *Prorhinotermes* soldiers belongs among the cases of the most pronounced development of these defensive weapons in termites.

Defensive secretion of the frontal gland in *Prorhinotermes* soldiers

Frontal gland contents of soldiers of three *Prorhinotermes* species, *Prorhinotermes canalifrons*, *Prorhinotermes inopinatus*, and *Prorhinotermes simplex*, consisted of two groups of compounds: nitroalkenes and sesquiterpene hydrocarbons. Analysis by gas chromatography-mass spectrometry revealed (*E*)-1-nitropentadec-1-ene as the major component of the secretion with mean values of 152, 207, and 293 µg/individual for *P. canalifrons*, *P. inopinatus*, and *P. simplex*, respectively. Four other 1-nitroalkenes (C13, C14, C16, and C17), and two nitrodienes (C15 and C17) were also detected in the three species. The C17:1 nitroalkene was identified as (*E*)-1-nitroheptadec-1-ene. The efficient protection of *Prorhinotermes* colonies provided by the nitroalkenes comes at a relatively high price: the use of large amounts of rare nitrogen.

The sesquiterpene composition of the gland was species-specific: *P. simplex* contained (3*Z*,6*E*)-α-farnesene (mean of 39 µg/individual), while *P. canalifrons* and *P. inopinatus* contained the same compound (means of 0.5 and 1.5 µg/individual, respectively) as well as the (3*E*,6*E*) isomer (means of 1.8 and 0.7 µg/individual, respectively). Two other sesquiterpenes, *trans*-β-bergamotene and (*Z*)-γ-bisabolene, were also found in low quantities in the frontal gland of *P. canalifrons*. In light of our findings identifying (3*E*,6*E*)-α-farnesene as a component of alarm signalling in *P. canalifrons*, we can hypothesize that the sesquiterpenes also act as alarm substances in other *Prorhinotermes* species. At the same time we cannot exclude their simultaneous role as irritants, which adopted secondarily the information role. This would partially explain the observed interspecific chemical diversity of sesquiterpenes.

Differential response of tissues and organs to JHA treatment in *Prorhinotermes simplex*

The experiment has proved that the given JHA can be transmitted by contact (trophallaxis and/or grooming) from individuals held on a treated substrate to untreated individuals. The parallel expression of soldier and reproductive traits appears to be due to this specific mode of JHA intake. After ingestion or topical application the intercastes never display any reproductive features despite their orphaning: they are pseudergate-soldier intercastes; they usually moult for the second time within two weeks. The neotenic-soldier intercastes obtained in our treatment moulted only once and their anatomy undoubtedly showed that they were intermediate between neotenics and soldiers. The basic neotenic traits were: well-developed gonads and outer genitalia, epidermal glands composed of numerous class 1 and 3 secretory cells; principal soldier traits were: prolonged sharp mandibles, and presence of developed frontal gland.

In general, the response of tissues, and especially of the epidermis, to JHA proved to follow the *all-or-none* rule of JH action: the individual phenotype was intermediary between two castes whereas particular cells could always be attributed to one of the two castes; they were not „intercaste". The variety of observed phenotypes can be explained by differential readiness and sensitivity of particular cells and tissues to JHA in the course of intermoult. The cells of the frontal gland epithelium, on the other hand, revealed a specific ultrastructure in the artificial intercastes, which cannot be compared to the ultrastructure of any of the described naturally occurring castes. This question is being addressed in our present-day research.