Biology of the soldier caste in the termite genus *Prorhinotermes* (Isoptera: Rhinotermitidae)

PhD. Thesis
Faculty of Science, Charles University in Prague

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Supervisor
Prof. RNDr. Pavel Štys, Csc.

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TABLE OF CONTENTS

View of a swarming colony of *P. simplex*.

PREFACE & ACKNOWLEDGEMENTS

If we forget for a moment the glorious tale of mankind being the dominant creature on earth, and look beyond the unbridgeable gap that humans tend to establish between themselves and other beings, we would realize that we are not alone in reaching the heights of intricate social organisation. Phenomena such as altruism, architecture, air-condition, agriculture, chemical weapons, self-sacrificial suicide, and slavery are known to occur in social insects as well. I am not particularly fond of over-simplified comparisons between humans and social insects; social engineers of all kinds have already abused enough this comparison. Contrary is the case, I consider the possibility of an insight into the biology of these fascinating creatures as an eventual source of respect for them and the environment they inhabit.

The social insects entered upon the way of social evolution long time ago and throughout the course of time, they became dominant elements in most terrestrial ecosystems in all senses of this term, i.e. the widespread geographical distribution, the abundance that manifold outnumbers that of humans, and functional role in the ecosystems with bees as principal pollinators, ants as major predators, granivores and seed dispersers, and termites as fundamental agents in the decomposition and turnover of dead plant material and soils. It is possible to distinguish the flow of materials and energy generated by social insects over the past 100 million years on many levels, including biogeochemical cycles of atmospheric gases.

The following text focuses on the biology of termites, social insects that resist our attempts to understand their biology, due to the obscure and cryptic lifestyle, hidden from our sight. Therefore, the termites are generally known only as significant pests, although the real pest species represent less than one percent of the almost 3000 species described so far. This negative attribute is included also in the Czech name for termites, i.e. *všekaz*. The meaning of this term is to destroy and to bring to an end. In this respect, I must recall the Czech parliamentarians' violent and indignant reactions when they were compared to termites in a TV news bulletin in 2003, due to their tendency to hide from the public control in the interiors of complex parliamentary palaces that are built from public resources.

The two fundamental reasons promoting the scientific interest in the biology of termites have little in common with the proximate aversion to termites as a pest of our crops and constructions. First, it is their role in tropical and subtropical ecosystems, where they represent an important portion of animal biomass and are hugely dominant among soil-living fauna. This abundance implies their fundamental role in the mineralization of carbon, production of carbon dioxide and methane, and modification of mechanical, chemical and microbiological properties of soils in savannas and forests. Therefore, termites are sometimes called ecosystem engineers. Second, termites represent the oldest eusocial insect group, with the most advanced social system in terms of morphological and behavioural diversity of castes, division of labour, and the level of reproductive altruism. These characteristics are reflected in the complexity of social tasks performed in cooperation by the members of termite societies. This results in abilities that are far beyond the scope of an individual or most other social animals, such as the construction of elaborate termite nests or their means of collective defence. This aptitude for defence is one of the most notable features proper to all eusocial animals and it often underlies the origin of eusociality itself by the evolution of specialized defensive castes. Thereby also in termites an observer can notice at the first glance the presence of individuals distinct of the majority of the work force: the soldiers.

My thesis is devoted to the biology of termite soldiers, one of the most fascinating features of termite societies and a topic that has been my passion and occupation during last few years. I addressed various aspects of the life history of the model genus *Prorhinotermes* with emphasis on the role of soldiers. This genus is attractive not only because of its biological peculiarities and phylogenetic position, but also its perfect viability in laboratory conditions, allowing us to study and compare several species from around the world. My approach to observational and experimental results was motivated by the interest in their biological significance, function, and proximate, as well as ultimate mechanisms of this function. Thereby, in this text, I try to accentuate the biological interpretation of the problem. I avoid deep insight into the details of chemistry related to the study of secretion of exocrine glands and into the ultrastructural anatomy of the described structures; this is the domain of my colleagues, specialists in diverse biological and chemical disciplines. I had the opportunity to cooperate with these specialists within a small team interested in biology and chemical ecology of social insects; therefore also the publications listed below present results of teamwork, describing the given topic from different points of view with various methodical approaches.

The following text consists of an *Introduction* to the basic traits of the biology of termite soldiers and defence. Its last chapter describes the biology of our model genus *Prorhinotermes*. The aim of the introduction is to highlight the most pointed facts and hypotheses concerning the soldier caste and diverse aspects of defence in termites, creating a framework for my own investigations. This introduction is followed by the presentation of particular *Results* on the subject of the soldier caste in the genus *Prorhinotermes* including reprints of articles published in peer-reviewed scientific journals, one manuscript accepted for publication, and one poster presentation. The results are then summarized in *Summary* and *Conclusions* sections, followed by the list of *References*. As an *Appendix*, the reader can find few of our other publications about *Prorhinotermes*. These do not concern the soldier caste itself, but various other aspects of this genus' biology.

The list of people to whom I feel indebted when finishing this thesis would be very long. Among these people there were few who influenced fundamentally the course of my studies and/or contributed essentially to my scientific activities. At the very first place there are four of them without whom I surely would not be where I am today. The first of them, in order of appearance, was Daniel Frynta who helped me to find a way back to the science when I was decided to leave my studies in their early stage. Later, he was the one to propose me the topic of my future scientific interest, social insects, and who had the idea to introduce me to two specialists in the field of termite biology, Ivan Hrdý and Jan Šobotník. In the course of time this decision revealed to be irreversible as the termites became my passion and source of fascination. I am deeply grateful to Ivan Hrdý for introducing me to the biology of termites and for being the supervisor of my master studies. By providing me with the fundamental termite literature, namely the Grassé's *Termitologia* and the *Biology of termites*, he allowed me to discover the world of these "dwellers in the dark". Although he was not the formal supervisor of my thesis, he was disposed to help at any moment with an experienced advice. And also another point must be underlined here: I remember him to be almost always in a good mood. It is not simple to express my gratitude to Jan Šobotník who played several principle roles in my studies and my life with termites. First of all he was the one to show and teach me the principles of termite biology; he shared with me his experiences "how to make science", and assisted my own first steps in the science. He motivated me to start my PhD. studies and always stand by me in

decisive moments. We created together a vision of common scientific projects concerning termite biology. Up to now we already succeeded to accomplish a part of this vision despite of the difficulties inherent to the intentions to study tropical termites in a temperate region far from their natural occurrence, and at the same time in a chemical institute. Although we both start to realize that our union does not have to persist forever, and one day maybe we will decide to go each his own way, I feel fortunate that I was allowed to meet him as a colleague, friend, and teacher. Also his participation on this thesis was crucial in all aspects, i.e. the choice of the topic, the contribution to particular articles, especially in the field of anatomy of endocrine glands and electron microscopy, and finally the proofreading of the whole text. In other words, he did not become the supervisor of my thesis only due to his age, which does not differ much from mine. Thanks again. I must sincerely acknowledge my thesis supervisor Pavel Štys who proved to be far from being the redoubtable professor as he is viewed by young students of his basic lessons of zoology, evolutionary biology, and general entomology. The contrary is the case, I will always be grateful for the confidence he had in me when allowing me to choose myself the topic of my thesis. He was helping me to stay in contact with my faculty, which I visited scarcely due to my engagement at the Academy of Sciences, and he was fighting in my name with the bureaucratic necessities accompanying the PhD. studies. I would like to thank him for the invaluable help with reading the manuscripts of my articles and sharing with me constructive and experienced comments and suggestions. He is for me a great example of a worker of science wholly devoted to his passion, which became his profession.

Natural science is not a solitary discipline and I was dependent on a constant help from my colleagues experienced in particular methods. I wish to thank here all co-authors of the articles included in this thesis, particularly Jan Šobotník, František Weyda, Irena Valterová, Blanka Kalinová, Rafal Piskorski, and Yves Roisin for their contribution. I am grateful to Christian Bordereau for his willingness to help at any moment and to all anonymous reviewers of the articles.

During more than eight years that I visit the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, I had the opportunity to meet many people who helped me more or less in my work. The following of them deserve special mention: Jan Žďárek for being a real ideal of a professor, willing to help his students to get higher, and a passionate teacher. Irena Valterová for being a generous team leader, endowed with a charming maternal approach to our common problems. I am grateful to František Weyda for his tireless full-time activity in realizing his scientific ideas and dreams. I should not forget Zdeněk Wimmer who helped me as an experienced boss in my beginnings at the Academy. And, of course, I thank Pavel Jedlička, my close colleague who pushed me to finish the studies along with him and became my companion also outside the walls of our institute.

I would like to thank those who helped me with this text, above all Thomas Bourguignon, Rafal Piskorski, Krystle Carrara, and Jusuf Čumlivski. Last but not least, I must acknowledge my immediate family to support me in my decisions and their realizations; this support represented the major driving force in the last stages of my studies. I must name my parents and those of my wife who were and still are standing by us. And at the very end: thanks, Lucie, for your unprecedented patience.

Prague, 20. 4. 2008

I hereby declare that this thesis is my own work and effort and that it has not been submitted anywhere with intention to acquire any other academic degree.

View of an incipient colony of *P. simplex,* the royal pair, larvae and the first soldier.

INTRODUCTION

I. SOLDIER CASTE, A KEYSTONE OF TERMITE EUSOCIALITY

Soldier caste is a prominent aspect of the biology of termites. Although they never represent a majority in the termite society and the list of their tasks is notably shorter than that of the workers, the soldiers represent the most conspicuous realization of termite eusociality through absolute devotion to their unique social role: the defence of nestmates. To fulfil this mission, the soldiers underwent an extreme specialization in their anatomy, behaviour, and physiology, and evolved into creatures that are very different from the ancestral life forms of termites, i.e. the winged imagoes, as well as from all other castes. This specialization in defensive tasks is linked with a great simplification of other aspects of life; the soldiers have completely lost the ability to perform many basic biological functions such as individual nutrition. Thus, they represent an excellent example of altruistic sterile individuals ready to sacrifice themselves for the benefit of their colony, while at the same time, fully dependent on nestmates not only in terms of their reproductive potential but also in proximate terms of immediate survival.

Furthermore, soldiers are largely the most diversified of all termite castes both among diverse species and within one given species. An amazing variety of defensive strategies linked with specific features in anatomy, behaviour, and physiology has evolved in soldiers, sometimes represented by two or even three forms in one single species. This richness of forms contrasts with a very conservative appearance of imagoes and workers in most termite species. Paradoxically, the soldiers, a non-reproductive caste that is not directly transmitting its genes to future generations, became a crucial recognition character for termite taxonomy. The formation of the unique and complicated anatomy of soldiers is allowed by a special pattern of ontogenetic development. This two-step mode, with a short-term transitional stage of presoldier, is exceptional among hemimetabolous insects, and is surprisingly conservative in all studied species, despite the diversity of its outcome. The uniformity of the development is one of two major arguments for the largely accepted monophyletic origin of the soldier caste in termites. The other evidence of the ancestral origin of soldiers is their universal presence as permanent helpers in practically all species, with the exception of their secondary loss in two advanced lineages.

While the specific developmental mode is critical in the soldiers' evolution to the present anatomic diversity, the ultimate condition for the defensive specialization of soldiers is their liberation from the multiple tasks performed by working castes. Consequently, due to the eusocial division of labour, the soldier caste could become an ideal "playground" for the evolution. On the other hand, the ability of self-defence, enhanced in termites by the soldier caste, is considered to be a crucial prerequisite for the emergence and maintenance of eusociality in eusocial systems. In other words, the presence of the altruistic sterile caste of soldiers makes the extant termite species eusocial in the strictest sense of the term.

II. OCCURENCE AND PROPORTIONS OF SOLDIERS IN TERMITE COLONIES

Soldierless termite species

The soldiers are present in all termite species with the exception of a few genera of *higher termites* (Termitidae), namely the Paleo- or Neotropical genera from the "*Anoplotermes*-group" (Apicotermitinae), and three other genera from the "*Amitermes*-group" (Termitinae): Oriental *Protohamitermes* and *Orientotermes*, and Australian *Invasitermes*(1-4). The absence of soldiers in these advanced genera is undoubtedly a result of a multiple independent secondary loss of the soldier caste. In these soldierless species, alternative defensive strategies can be seen, performed by workers.

It seems likely that the loss of soldiers is rather a by-product of other selective pressures more so than the result of a low need of defence, normally provided by soldiers. Noirot(5) underlined that the loss of soldiers is linked with the ecological strategy of the soldierless species: the soil-feeding. The developmental pathways in these species reveal a tendency to a reduction towards one monomorphic terminal worker instar, often of one sex(5-7). A hypothetic explanation of this phenomenon was proposed by Roisin(8): the repeated moulting of working stages is retained in species feeding on hard material and enables the renewal of mandibles, whereas in soil-feeding species this expansive regeneration is not needed. An irreversible loss of the soldier caste could appear during this simplification of caste patterns, leading consequently to the evolution of defensive adaptations in workers. On the other hand, Sands(9) postulated that the loss of the soldiers is a consequence of an effective defensive mode, abdominal rupture or autothysis, which was present primarily in soldiers, but adopted by workers. Thereupon, the soldiers would lose their significance and, being too costly defensive strategy, they could subsequently disappear.

But one can also hypothesize other alternative explanations. For instance, the soil-feeders could lose their moulting abilities due to the extreme complexity of their digestive tube or the difficulities in moulting of their cuticular modifications connected with the mechanism of body rupture.

Proportions of soldiers

All other species of termites possess soldiers of extremely diverse anatomy and behaviour, but also of very variable proportion in the colony. The proportion of soldiers in a termite society is subjected to concurrent selective pressures, given that the driving force of this selection is to create the best conditions for reproduction of the society. On one side, there is the need of an adequate defence of the colony, on the other it is the tendency to minimize the expanses for the nutrition of soldiers, individuals fully dependent on care provided by nestmates. This trade-off defines a species-specific optimum in caste proportions, especially the ratio of soldiers to other helpers(10,11). The adaptive value of the caste equilibrium is translated in an optimization of division of labour, the major qualitative step in the evolution of eusocial societies(12,13). This *social homeostasis*(14,15) or *optimal mix*(10) of castes is maintained with a complex feedback mechanism of caste regulations discussed below.

The data about soldier ratio is rather difficult to evaluate because (i) the concerned studies were usually devoted to subjects other than the caste proportions; (ii) the caste ratio usually differs in different parts of the nest and foraging territory; (iii) the caste ratio in laboratory colonies provides only an approximation of the natural situation; (iv) the ratio of soldiers can vary in time with season, colony age, and fitness of the colony. Nevertheless, the available findings suggest that the trend of a species-specific soldier ratio applies at least for the few species scored repeatedly so far. A review of soldier ratios to working castes was compiled by Haverty(16) for 112 species, based on the data from entire colonies, colony peripheries, foraging groups, samples from the nest itself, but also from laboratory colonies, and other samples. The range of soldier proportion is very large; from less than one percent in many species to more than thirty percent in others. The soldier proportions are not clearly correlated with the taxonomy, though few generalizations can be drawn. Kalotermitidae and Termopsidae have a low soldier proportion, ranging from 1 to 5% and rarely above this, as well as termitid subfamilies Termitinae and Apicotermitinae, where soldiers usually represent less than 2%, or they are not present at all(1-4,16-25). Other Termitidae, on the other hand, possess an elevated soldier proportion; this is true for Macrotermitinae, where commonly 5 to 10% of soldiers appear, but first of all for many Nasutitermitinae with usually more than 10%, but sometimes even up to 25% of soldiers, as in *Tenuirostritermes tenuirostris* or 29% in *Longipeditermes longipes*(16,19,26-32). In Rhinotermitidae, the soldier proportions are very variable; from less than 5% in Heterotermitinae to more than 10% in *Coptotermes* and *Prorhinotermes* species, e.g. 25% in *P. inopinatus*(16,25,33-41).

Biology of particular species, namely their nesting habits and type of foraging, can serve in many cases as a better indication to understand the diversity of soldier proportions. One-piece type species(42,43), using wood as a source of food and a shelter at the same time, represented typically by Kalotermitidae and Termopsidae, usually rely on a small number of soldiers guarding the corridors of spatially delimited and compact wooden nest(16,20-24). Many humus and soil-feeders, i.e. numerous Termitinae, Apicotermitinae, and some Nasutitermitinae have few or no soldiers in their societies(1-4,17,19,25). There are alternative hypotheses for the lack of soldiers in these species beside these mentioned above. Deligne et al.(17) draw attention to the fact that these species are often (i) very small and cryptic; (ii) termite inquilines, i.e. they nest in mounds of other termite species, usually considerably bigger in size, which ensure the protection of both populations, or (iii) posses a hard, compact, and complex nest(19,44). On the other hand, true nasute soldiers of arboreal Nasutitermitinae with brittle carton nests are usually very numerous(19,26-31). It is also not surprising to find a high ratio of soldiers in harvesting and open-air foraging Nasutitermitinae, Termitinae, and Macrotermitinae, where up to 90% of soldiers can be present in foraging groups(19,28,29,45-47). The differential pressure of predation between cryptic and harvesting foraging strategies are clearly evidenced by field studies to be the major driving force for the establishment of caste proportions(25).

Other correlation can be found between the number of present soldiers and the defensive adaptations of soldiers themselves. On this basis, two groups with different soldier proportions can be established. Soldiers with dominant mandibular weapons usually represent from 1 to 3%, less frequently up to 10% or more(17). Mandibulate soldiers are often considerably larger than corresponding workers. Their size increase is needed to develop the powerful head musculature and armour, and to face efficiently bigger predators in one-to-one confrontations. In species with predominantly chemical weapons of soldiers, these are very abundant, especially in *Prorhinotermes*, *Coptotermes* (Rhinotermitidae), and Nasutitermitinae(17,19). The high proportion of such soldiers is often compensated by their smaller size, sometimes even smaller than that of the corresponding worker stage, as is common in some true nasute soldiers. The investment in defence in these species is split among many smaller soldiers allowing (i) an efficient synthesis of a big amount of the defensive compound by numerous individuals, and (ii) a very operative mode of group defence with many soldiers emerging suddenly from the nest to attack an intruder or to patrol along foraging columns(19,45,48). This spatial distribution of defensive substance can repel even vertebrate predators(17,49-55).

Variability in soldier proportions

The proportion of soldiers is variable in space. The soldiers logically occur where they are needed; dramatic differences occur in proportions of soldiers between the nest centre (nurseries, royal chamber) and their presence (i) on the periphery of the nest(16,56-59); (ii) along the foraging columns of workers(41,45,47,58,60,61); (iii) by the nest exits during swarming of winged imagoes(62,63), and iv) on specific places, to which soldiers are attracted by immediate chemical, vibrational, or tactile alarm recruitment stimuli(48,64-68).

Proportion of soldiers in termite societies can vary with seasonal cycles, particularly in response to the swarming of winged imagoes. In many species there is the need of a higher soldier ratio during the short period when alates are leaving the nest; these are very vulnerable and become easily a prey of a large variety of predators surrounding the swarming openings. Moreover, the colony itself is in danger while opening the walls of its nest(17,62,69). Therefore, along with seasonal production of nymphs, becoming later winged imagoes, also numerous soldiers develop prior to the swarming to patrol by the swarming openings until workers close them again(6,37,38,62,63,70-72). It should be noted here that the defensive potential of soldiers during swarming was questioned by Grassé(30) who underlined that also other castes leave the nest during the swarming and move chaotically on its surface in a state of an intense agitation, which has little in common with a vigilant defensive bahaviour. Seasonal variations in soldier proportions have also been detected in the composition of foraging columns, e.g. in *Constrictotermes* (Nasutitermitinae)(61).

Other environmental factors can influence soldier proportions, such as temperature, humidity, food availability, and overall colony fitness; the ratio of soldiers decreases when these conditions become unfavourable(73-80). It can be concluded that soldiers, as a dependent caste, are the first to be sacrificed, usually by starvation to death and/or cannibalism by workers, when a colony is subjected to suboptimal conditions(77,79,81,82). We can even go as far as to hypothesize that soldiers may serve as a living emergency food stock allowing the colony to survive stress situations or providing energy to undifferentiated individuals to moult into winged imagoes and disperse when food becomes depleted(77,83).

Positive correlation has been clearly demonstrated between the size of the group and soldier proportions in laboratory conditions. This so-called *group effect* was observed e.g. in rhinotermitids *Reticulitermes* and *Coptotermes*, or in *Kalotermes flavicollis* (Kalotermitidae)(15,33,37,84,85). On the other hand, the ratio of soldiers has been found to decrease with the colony size in one-piece type nesting species *Neotermes koshunensis* (Kalotermitidae); the spatially delimited surface of the nest does not require an increase in soldier number with increasing number of workers(20). However, when considering the dynamics of soldier proportions in relation to the age of the whole colony, the soldier instar origin and body mass must be taken into account; only such approach can describe the dynamics in investment in defence in colonies of different ages(86).

The demographic situation in young, incipient colonies differs fundamentally from that in old, mature colonies, along with requirements for defence, need of a work force, resource availability, disposable undifferentiated larval instars competent to develop into soldiers, and other factors. The establishment of final soldier proportions in mature colonies is preceded by gradual transition from caste proportions specific for incipient colonies. In these incipient societies, the defence of eggs and brood is performed by the pair of reproductives and one or less frequently more *first(s)* soldier(s)(35,36,73,87,88). In lower termites with plastic developmental pathways, this first soldier is derived from an early larval instar, which never develops into soldier in mature colonies; as the colony matures, older instars differentiate in soldiers and the soldier ratio increases (see Chapter VIII. for more details). The first soldiers sometimes develop from the first brood within few weeks after colony establishment, as in *Kalotermes flavicollis* (Kalotermitidae)*, Zootermopsis* (Termopsidae), or *Coptotermes formosanus* (Rhinotermitidae)(73,87,89), but often from later broods and appear as late as during the second year of the colony development, as e.g. in the rhinotermitids *Reticulitermes lucifugus* and *R. hesperus*(34,90). By contrast, high soldier proportions can already be established in an early stage of the colony development in Nasutitermitinae(91). These initial soldier proportions in incipient colonies appear to have an adaptive value rather than to result from any developmental constraints of young individuals and/or societies; this was documented by experiments with removal of first soldiers, which were promptly replaced by new first soldiers in *R. hesperus* and *Zootermopsis angusticollis*(89,90).

Given the complexity of numerous concurrent selective pressures involved in optimization of soldier proportions, and various constraints of particular species due to their phylogenetic position within Isoptera, it is not a real surprise to find quite a few exceptions or paradoxes to the trends mentioned above(19).

III. TERMITE ENEMIES, AGONISM, AND DEFENSIVE STRATEGIES: DEFENCE BY NEST, DEFENSIVE ADAPTATIONS OF WORKERS

Termites as valuable prey

The dominant ecological role of termites does not consist in the turnover of plant material only; due to their amazing abundance in tropics termites became a crucial food source for various predators. The evolutionary trend in nesting of advanced termites, i.e. the centralized nests of very populous colonies, creates a "positive feedback" selective pressure for better defensive capacities; more the defence is efficient, more opportunities it provides for the colony growth. Vice versa, with increasing population sizes of societies, the pressure of predation by specialized predators rises with the concentration of valuable prey on one place. Consequently, also the masses of winged imagoes produced by these colonies can suffer heavy losses due to an enormous predation during swarming and initial phases of colony foundation(92,93).

When considering the "adequate" defence of a society, one must take into account the reproductive and ecological strategy of the given species. The defensive capacities are not based on the performance of soldiers only, but depend also on the nesting and foraging habits, vulnerability of particular individuals, the pressure of predation, cost of soldier production, and cost of other defensive mechanisms, etc. On the basis of summation of these aspects, species can be classified along a spectrum from *tychophile* species, compensating their vulnerability to environmental factors and predation by a high reproductive rate, to *tychophobe* species, reducing this vulnerability by investment in protection and defence(12,44). A large continuum of more or less costly defensive strategies can be found in termites, reflected in defensive adaptations, proportion of particular castes, and in nest structure. Nevertheless, we can state that termites, due to their individual vulnerability and abundance, and high concentration in the nests, are subjected to a high pressure of predation. Consequently, more or less sophisticated defensive mechanisms can be found in every termite species and none of the known species can be classified as entirely tychophile.

TERMITE PREDATORS

Facultative or specialized predators of termites can be found in several classes of arthropods but also among vertebrates. From non-insect arthropods we may cite Onychophora, Arachnida (Chelicerata), namely termite specialists among spiders or the specialized giant velvet mite *Dinothrombium pandorae*, and Chilopoda (Myriapoda)(17,94-97). In Hexapoda, representatives of all entomophagous orders were recorded to attack termite colonies or swarming alates, specialized termite predators occur among Heteroptera: Reduviidae and Coleoptera: Staphilinidae, Carabidae(17,95,98). Among vertebrates, many amphibians (both Anura and Caudata) and reptiles, primarily various lizards (Lacertidae, Iguanidae, Gekkonidae, Chamaeleonidae, Scincidae), but also numerous snakes, represent occasional or exclusive termite-eaters(17,99-105). Most of birds accept without hesitation termites as food, though only few of them are obligatory predators; rarely the birds attack the nest itself, usually they hunt swarming winged imagoes or feed on foraging columns(17,106).

Numerous mammal species are feeding on termites and in most mammalian orders we can find species specialized on termites. The role of termites as dominant food and prerequisite for the radiation of primitive insectivorous mammals at the end of Mesozoic is sometimes discussed and supported by paleontological evidences(107). Although the specialized termitophagy is in many cases considered as an advanced feature, we can find it in numerous primitive representatives of marsupials, monotremes and edentates. In Australian region, echidnas eat on ants and termites, many Australian and American Marsupialia are facultative termite predators, but the numbats are specialists with a dominant termite diet(108-110). Most families of Insectivora and Chiroptera feed occasionally in nests and galleries, or on flying winged imagoes of termites(17,98). Termites are eaten by many Primates, especially by the notorious „fishing“ chimpanzees, and evidences have also been found for a systematic search and consumption of termites by early hominids in southern Africa(111-114). The termite diet still represents a valuable source of essential elements in indigenous tribes in African semi-arid zones(115). Highly specialized termitofagous mammals are among Edentata, i.e. armadillos and anteaters from America, convergently evolved Paleotropical group of Pholidota, i.e. pangolins, and the only species of Tubulidentata, *Oryctoepus afer* or aardvark(116-119). Last but not least, we can find specialized termite-eaters among carnivora, namely the African *Otocyon megalotis* and *Proteles cristatus*, and oriental Ursidae *Helarctos malayanus* and *Melursus ursinus*(17,49,98,120). The specialization on termite diet has been recorded also in one species of Old World pitcher plants (*Nepenthes*), capturing the foraging columns of the nasutitermitine *Hospitalitermes*(121).

TERMITES AND ANTS

An attack of some of the giant predators listed above may undoubtedly represent an abrupt lethal event for the termite colony. Moreover, termites possess poor means to stave off this disaster once their nest is damaged, apart from being unpalatable, as is supposed in some species with high proportion of chemically armed soldiers(17,49,55). Still, the mentioned specialists represent only a minor predation pressure when compared with the major animal predators: the ants. Ants are the second clade, together with termites, which attained the highest level of social organisation and at the same time an equivalent ecological dominance in terms of abundance and biomass, but at different trophic levels; ants being by far the most important predators of arthropods, including termites(122). Wilson described their mutual relationship: "These two superpowers of the insect world have undoubtedly been locked in a struggle of the 100 million years of their coexistence, with ants as the active aggressors and termites the prey and resisters."(123) Therefore, an evolutionary arms race between termites and ants was the designer of weapons and elaborate defensive strategies of termites.

The majority of carnivorous ant species occasionally prey on termites when met in open space, or when the nest is damaged by an intruder or heavy rain and freely accessible. These termitophagous species are distributed in all bigger ant subfamilies, their attack can have a fatal impact on survival of winged imagoes during swarming and subsequent short non-cryptic period in their life. Numerous specialized or obligatory termitophagous species use various strategies: one-to-one attacks in large sized species, group attacks in small ant such as many Myrmicinae, but also the spectacular raids of large groups breaking inside the termite colonies. Termitolestic strategy occurs in small-sized ants, i.e. the nesting near or inside the nest of the host termite, with regular visits of the nest to steal eggs or larvae(17,95,122).

Apart from termitolestic species, we can find among Ponerinae the famous raiding ants attacking almost exclusively termite nests, i.e. the American genus *Pachycondyla* (= *Termitopone*), Asian *Leptogenys*, and African genera *Megaponera* or *Ophtalmopone*. These large ants attack mainly Macrotermitinae and Rhinotermitidae.

Hypoponera eduardi living and hunting in the nests of European *Reticulitermes* (Rhinotermitidae) possess a chemical mimicry on cuticular surface(17,92,95,122,124-130). The nomadic army ants Dorylinae, namely few species of the Old World genus *Dorylus*, attack and occasionally destroy the colonies of various termite species; they have been observed to cause 80% of the mortality in *Macrotermes* (Macrotermitinae) maturing nests(122,124,131,132). Numerous termite predators are known in the family Formicinae, especially in the genera *Camponotus*, *Formica*, and *Lasius*(133). In Dolichoderinae, the genus *Iridomyrmex* is known to attack termites occasionally or systematically as *I. sanguineus*(134). Australian bulldog ants, Myrmeciinae, prey on diverse termite species(122). Myrmicinae are rarely direct hunters of termites due to their small size, though some *Pheidole* and *Decamorium* species are known to attack termites in groups, using their sting to immobilize them(17,95,122,133,135,136). Structural specializations on the head are used by *Eurhopalothrix heliscata* to kill termites in the wood(122). *Myrmicaria opaciventris* has even been proposed as a mean of biological control of termites(137). But above all we can found among myrmicines the termitolestic specialists, e.g. *Solenopsis*, *Carrebara*, or *Paedalgus*(138-141). These nest in vicinity of the nest or in the nest of their host, but separated from termites; the workers are often very small and thus are ideally sized to intrude in the termite nest. It is suspected that some of these species are accepted by termites in the nest just as true termitophiles, e.g. *Stigmacros termitoxenus*(17,95,122,142).

AGONISTIC BEHAVIOUR AMONG TERMITE POPULATIONS AND SPECIES

Termite enemies can be found also among termites themselves; agonistic behaviour often occurs between competing termite populations. The capacity for nestmate recognition is the basic prerequisite for the integrity and cohesion of the society and consequently for the application of kin-selection or colony level of selection. Only genetically related individuals should be the receptors of altruistic behaviour performed by workers and soldiers(13,143,144). When compared with eusocial Hymenoptera, many conspicuous forms of social parasitism lack in termites, as e.g. *dulosis*. On the other hand, termites, such as other social insects, have resources to defend against intra- and interspecific termite intruders, i.e. their foraging territory, and first of all, their nest(11,145). Thus, agonistic interactions often occur in nature between foraging columns (in enclosed arcades or in open space) and underground parts of neighbouring nests, between two colonies, especially in the wooden shelter of one-piece type species, between the hosts and their inquiline species, and between populations competing to occupy a centralized nest(9,43,144,146-154). These interactions may lead to a complete destruction of a colony outcompeted by an other(155-157), but also a non-aggressive mutual avoidance of two competing species(158). Intercolonial and interspecific agonism have also been confirmed by numerous laboratory experiments revealing a high level of aggression (i) between sympatric competing species, but also between allopatric species(98,152,159-163); (ii) between conspecific populations(147,153,158,163-167). These investigations brought rather diversified results concerning the innate vs. acquired character of the capacities for nestmate discrimination, predictability, and stability of agonistic responses among species and populations. Also, the hypothesized correlation between the level of agonism and the genetic relatedness, represented by the level of similarity of recognition cues, i.e. the epicuticular lipids, and the geographical distance between confronted populations, is not always tight, though it is supported by numerous positive evidences(161,165,168-172). Many other studies indicate that the agonism, as well as the cuticular recognition cues, is subjected to large variations among species and population, during seasonal cycles, and changes in environmental conditions(173-176). The resulting behaviour, following the discrimination of a non-nestmate termite, is most often an overt aggression, with means sometimes comparable to those used against other enemies. But often the agonistic patterns are specific for aggression of conspecific termites; sometimes it is primarily the worker caste, which is involved in these interactions(144,151,152,158,164). However, quite a few examples can be listed of passive interactions between conspecific colonies(98,161,176-179).

INTRACOLONIAL AGGRESSION

Lastly, the phenomenon of intracolonial aggression among termites must be mentioned. As in other eusocial societies, the competition over reproduction and nest inheritance frequently occurs also in termites, namely among one-piece nesting Kalotermitidae and Termopsidae. In these families, the unlimited developmental options of the majority of individuals lead to a conflict among nestmates over the development (i) into alate reproductives and (ii) into replacement neotenic reproductives. Violent fights are reported to occur in these societies, which may result in elimination or mutilation of individuals, and a subsequent regressive moulting in mutilated nymphs. In this way, their individual reproduction in alate stage is prevented by other nestmates(180-187). Both inter- and intragroup conflicts in termites have been hypothesized to be an evolutionary context of the origin of the soldier caste as an aggressive phenotype adapted (i) for fights among different familial groups or (ii) for fights among neotenics within the natal colony (discussed in Chapter X.)(153,188,189).

NEST AS A PRINCIPAL DEFENSIVE STRUCTURE

The defensive strategies and mechanisms in termites have attracted deserved attention, and their astonishing spectrum has been reviewed by several authors, namely Deligne et al.(17,190), Prestwich(191-193), Grassé(98), Noirot and Darlington(19). The defence of the termite society must be understood as an integration of *passive* and *active* components; the passive element is represented by the termite nests and galleries, the active one consist of defensive adaptations of workers and soldiers(190).

In eusocial animals, a long-lasting nest providing defence and resource storage represents one of the keystones of social integrity. The occurrence of a nest, along with other defensive strategies, appears to be a major trend in the evolution of eusociality(194,195). The nests of termites are often cited to reach the highest level of organisation in size, structural, and functional complexity among animals(19,98,149). At the same time, their diversity and species-specificity is as pronounced as that of termite soldier caste, and the architecture of nests is also used as an important characteristic in taxonomy. Thus, the nest represents a so-called "frozen behaviour" of its constructors and inhabitants(196); it is an integral constituent of the phenotype of a given species, which co-evolved, subjected to natural selection, together with morphological and behavioural adaptations of particular castes. The benefits provided by integration of these components in the defensive strategy dramatically overreach the scope of solitary individuals. This fact represents an important argument for the conception of multi-level natural selection, acting not only at the level of individuals but also at the level of whole societies, justifying at the same time the use of the controversial term *superorganism*(11,13).

The nest in the one-piece type species consists of a piece of wood used as a food source and at the same time as a shelter. The nest is hard, with very limited contact with outside, and suffers relatively low predation. Along with the evolutionary trend to separate the food source from the nest by foraging, a strong pressure of predation shaped the architecture and placement of nests in order to minimize the contact between the nest and the ground. Consequently, two tendencies can be observed: (i) the installation of the nest out of the ground level with a large variety of intermediate situations from underground to arboreal nests, and (ii) the centralization of primitively diffuse nest complexes into one central nest, or several units in case of polycalic nests. In addition, structural defence evolved in the form of (i) subterranean or epigeous galleries lined with more or less resistant building material, and (ii) nest fortification, represented by the royal chamber, outer nest wall, and *paraecie*, an empty space, devoid of termites, surrounding the nest itself. Here again, a variety of structural and material complexity occurs, from rather diffuse nests in soil, with only a hardened royal chamber, to strongly concentrated nests delimited by the outer wall. The thickness varies from simply breakable thin carton in arboreal nests to strongly fortified thick walls of some epigeous and subterranean nests, made of carton or earth. The internal architecture of the nest also participates in defence; often the passages between chambers are as narrow so as to let only one termite pass through at a time. Larger predators are prevented from passing and these openings can be simply sealed by workers or by bodies of living or dead termites(19,98,149). The galleries and cavities in wood may also be filled with building material and prevent the passage of intruders(17).

ACTIVE VERSUS PASSIVE DEFENSIVE ADAPTATIONS

The defence provided by the nest must be considered by far the most important element of termite defence, as it separates the termites from the large majority of predators, i.e. the facultative ones(17). Many *active* anatomic or behavioural defensive adaptations performed by workers and soldiers can be seen as complements of nest structures and co-evolved with the nest architecture. As pregnant examples of this co-evolution, the phragmotic soldiers should be cited; their heavily sclerotized heads fit perfectly to the galleries in the wooden nest and are used as plugs. Their size was found to be subjected to stabilizing selection(17,124). In workers, the renewal and urgent repair of damaged parts of the nest belong among the most efficient defensive activities. Therefore, despite the anthropomorphic tendency to assign the defensive credit mainly to soldiers, their role in many species is rather limited or minute. Grassé(98) stated that only in species defended with numerous chemically and mechanically armed soldiers are these the major defensive force, such as in the rhinotermitid *Coptotermes*, termitids *Macrotermes*, *Syntermes* or *Acanthotermes*, in contrast to some species with massive centralized or one-piece nests, where the role of the few soldiers is rather questionable, as e.g. *Cephalotermes* (Termitinae), Kalotermitidae, and Termopsidae(17,19,98,197,198). We should without doubt add to the list of species well defended by soldiers many Nasutitermitinae(45,48,57,199).

Nevertheless, it is not the aim of the present text to conclude that the existence of the soldier caste in some termites is an aberrant phenomenon, with no real impact on the fitness of the colony. The opposite is the objective, and the few studies devoted to this topic prove that even in groups with low natural proportion of soldiers, their presence may improve proximately the survival or enhance the colony fitness in long-term perspective(124,197).

DEFENSIVE PERFORMANCE AND ADAPTATIONS OF TERMITE WORKERS

In the light of the previous paragraph, workers appear to be crucial defenders of termite colonies, though they are devoid of any conspicuous defensive structures. But their unspecialized anatomy must be seen as a result of their universality in completing a variety of tasks, including the defensive ones, due to a large repertoire of behaviour. Moreover, they are by far the most numerous caste in the colony. First of all, the workers are the builders of nest and repair every breach in the nest caused by environmental impact or intruders. These immediate building activities consist in (i) repair of a breach; (ii) repair and eventual change of the direction of a covered gallery; (iii) sealing of passages between chambers, and (iv) covering of a foreign object, immobile or injured enemy by faecal building material(17,47,58,95,151,200). These everyday building abilities are particularly useful in foraging species and during swarming. The building and repairing behaviour is a complex coordinated activity of all castes, performed by workers and assisted by soldiers, launched by recruitment and/or alarm stimuli; in some species, polyethism may occur among workers during building and repair(47,58,198,200). Some worker instars may even be specialized on repairs, others on nest expansion(201,202). An amazing strategy in building defence has been discovered in several species of Termitinae, where pellets from clay or quartz can be found in nest chambers in order to seal passages when necessary(98,203). Occasionally, it may appear that soldiers, facing an enemy, stay outside the nest while workers prevent them from a return by sealing off the entrances or breaches. Unable to dig an entrance back to the nest, the soldiers fulfil their altruistic role by a self-sacrifice, as is common in Macrotermitinae(19,98).

When faced with an enemy in direct contact, workers can bite with their massive mandibles, while cutting off legs or antennae. This mode of defensive combat is used particularly in termite-termite fights and may result in massive mortality when large numbers of hetero- or conspecific enemies meet. This is true especially in Nasutitermitinae defended by numerous soldiers with purely chemical weapons, that are very effective but not against conspecifics. In such situations, workers are engaged in fights against workers but attack soldiers as well(151,158). The high defensive capacities of workers against heterospecific intruders were demonstrated again in Nasutitermitinae; smaller enemies, typically ants, are often directly crushed by bites, larger ones are slowed down by workers clamped on their body extremities and exposed to attacks from soldiers(48). The open-air foraging hodotermitid workers are not assisted by soldiers and they display high agonistic capacities(95,158,204). Finally, a flight from an endangered area to a safe one is an effective and common mode of defence performed by workers, which can be seen in foraging columns as well as in the nest(17).

The soil-feeding termite species with usually few or no soldiers rely entirely on workers in defensive tasks. Therefore, it is not surprising that these workers are often very active in agonistic behaviour and often very aggressive. A simple defecation in response to endangering stimuli is well known in many termitids, particularly the soil-feeders, such as *Armitermes* or *Curvitermes* (Nasutitermitinae)(205). Workers of *Skatitermes* use their unusually flexible abdomen to deposit with big precision drops of a slimy faecal liquid on enemies; such behaviour is known also in e.g. *Speculitermes*, *Adaiphrotermes*, and *Grigiotermes* (all Apicotermitinae)(1,4,17,205). Another mechanism exists in other soldierless Apicotermitinae as well as in many Termitinae: the intestine and consequently the whole abdomen of workers ruptures as a result of powerful contraction of abdominal muscles(9). More intricate adaptation, an *autothysis* of a hypertrophied labial gland, leading to the rupture of body wall at a specific weak point, was described e.g. in *Grigiotermes*(18,205). It seems probable that the responsibility for the autothysis was wrongly attributed to the labial gland, because in species where a precise study was performed, other glands are involved. This is, for instance, the case of *Ruptitermes*, to which this suicidal behaviour gave its name, where the origin of the secretion was recently attributed to a novel gland, distinct from the salivary glands(206).

Other, less specific defences, often linked with defecation and suicidal behaviour of workers, were described in soldierless species(1,17,193). A hypertrophied mandibular gland has been recently described to participate in defense of workers of *Constrictotermes* (Nasutitermitinae)(207). Finally, the hypothesis that the tarsal glands, found in diverse termite species, serve as a defence against small ants, should be mentioned(208-210).

IV. DEFENSIVE ADAPTATIONS OF SOLDIERS

The soldiers have evolved an amazing and unprecedented variety of mechanical and chemical defensive specializations. This fascinating diversity was probably allowed by the liberation of soldiers from multiple tasks performed by workers and consequent behavioural and anatomic specialization on their single role, defence(211). The richness of forms of termite soldiers is represented mainly by modifications of their heads and internal organs. Except for heavier sclerotization, thorax and abdomen retain usually their larval aspects, or more precisely, reflect in

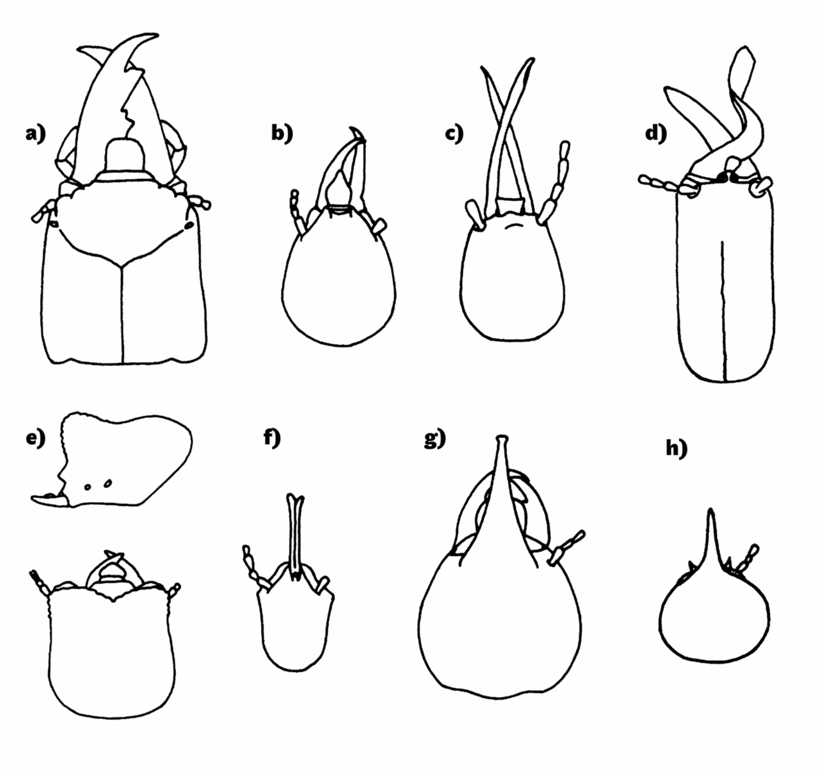

Figure 1.
Drawings of dorsal views on heads of characteristic termite soldier phenotypes.
a – *Archotermopsis* (Termopsidae);
b – *Coptotermes* (Rhinotermitidae);
c – *Promirotermes* (Termitidae: Termitinae);
d – *Pericapritermes* (Termitidae: Termitinae);
e – *Cryptotermes* (Kalotermitidae) (lateral view on top);
f – *Rhinotermes* (Rhinotermitidae: Rhinotermitinae);
g – *Armitermes* (Termitidae: Nasutitermitinae);
h – *Nasutitermes* (Termitidae: Nasutitermitinae).
Modified from 212.

shape and size the original stage and caste from which the soldier developed through the *presoldier* stage. Consequently, the soldiers may bear wing buds or wing rudiments if they have differentiated from a nymph or from a pseudergate, developed by regressive moulting of a nymph.

The thorax and its appendages do not bear any conspicuous novel structures but in many cases they seem to be adaptively shaped to conform to the defensive strategy of the given type of soldier. Therefore we may found a broadly enlarged pronotum protecting the cervix in powerful mandibulate soldiers fighting by biting and crushing, while the agile nasute soldiers have a narrow pronotum allowing a bigger flexibility in motion in their non-contact defensive strategy(6,48). Generally, the pronotum tends to decrease in width from Kalotermitidae through Hodotermitidae and Rhinotermitidae to Termitidae(212). Thoracal tergites may bear projections or spines, e.g. in *Acanthotermes* or *Syntermes* (Termitidae). In Kalotermitidae and Termopsidae, the soldiers derived from nymphs often have well-developed wing buds, typically in the kalotermitid *Pterotermes*. The legs seem to correlate in length and shape with the strategy of defence; they are generally thinner and longer in chemically armed nasute soldiers, whereas in mandibulate soldiers they are stout and shorter(6,212). It is not surprising to find unusually long legs in soldiers of open-air foraging species(46).

The outer anatomy of abdomen does not differ considerably from that of the original caste from which the soldier differentiated. The sexual characters can be more or less distinguished on posterior sternites in lower termites, whereas in Termitidae this distinction is difficult or impossible(212,213). By contrast, the internal anatomy may change substantially with the development of a soldier, especially the digestive tract can be reduced, though it follows the same basic organization as that of workers. The most pronounced reduction of structures, typically hypertrophied in food-provisioning castes and stages, occurs in species where the soldiers are fed with a liquid salivary food. In such species the hindgut, and namely the paunch, is strongly reduced and devoid of symbionts(214,215). After Noirot this situation evolved independently in 20 genera of Rhinotermitidae and Termitidae, particularly in many Termitinae and Amitermitinae(211). On the other hand, the liberation of space in the abdomen of soldiers allowed the development of chemical defensive adaptations; in many cases the abdomen is occupied by the hypertrophied salivary glands (e.g. Macrotermitinae, Mastotermitidae) or reservoirs of the frontal gland (Rhinotermitidae)(211,214). Once again we can see here how the liberation of soldiers from food provisioning allowed an enhancement of defensive adaptations.

The adaptations of the head consist mainly of (i) highly modified mandibles as a mechanical weapon; (ii) a modified labrum, often hyaline on its tip, lanceolate, bifurcated, or trilobed in mandibulate soldiers, vestigial in *nasutes*, and grooved and elongated in *nasutoids*; (iii) the shape of the head capsule, determined by its mechanical function and by the mandibular musculature, often particularly hypertrophied, and finally (iv) often very massive sclerotization of the head capsule. The head also bears specific secretory structures of the defensive frontal gland of various shapes, from a simple opening on the frons, the fontanelle, through labral adaptations in *nasutoids* to a conic nasus in *nasute* soldiers. Another chemical defensive gland, the *labral* gland, is responsible for the hypertrophied labrum in Macrotermitinae, *Syntermes* (Nasutitermitinae), or *Glossotermes* (Serritermitidae)(17,212,216). Other structures of the mouthparts, e.g. maxillae and labium, are rather conservative and do not differ substantially from the imaginal form, though the postmentum correlates in its length with the length of the head, often greatly elongated. The antennae may differ from those of imagoes, especially by the hypertrophy of the third antennal segment in numerous species(212).

MECHANICAL DEFENCE OF SOLDIERS

On the basis of the head and mandibular shape, and mechanical performance of the mandibular weapon, several classifications have been elaborated, of which those by Deligne, Deligne et al. and Prestwich are the most cited ones(17,193,217), and served also for the following overview. The principal features of the head modification in termite soldiers are depicted in the Figure 1. One of the common defensive strategies in termites living in compact nests is a phragmotic behaviour; the soldier serves as a „living plug" and blocks by its head the corridor or gallery. Such an adaptation is known also in ants, e.g. in wood-inhabiting carpenter ants (*Camponotus*). This strategy is typical for primitive drywood termites from the family Kalotermitidae, particularly in the genus *Cryptotermes*; the heads of soldiers are conspicuously adapted to phragmosis, they are heavily sclerotized, short and high in front, labrum is short, antennae protected by ridges, mandibles are powerful and short. Consequently, these soldiers are called phragmotic. With more or less pronounced morphological adaptations, the phragmotic strategy occurs also in other families, regardless of the presence or absence of a chemical defence in these species; e.g. *Reticulitermes* (Rhinotermitidae), *Rostrotermes*, *Cubitermes*, *Armitermes*, *Noditermes*, *Cephalotermes*, *Microcerotermes* (Termitidae)(124,190,218-220). This effective defence is a behavioural complement to the defence performed by the nest itself, and it is usually linked with a low proportion of soldiers.

In majority of termite species, the soldiers use in various ways their mandibles and can be classified as mandibulate soldiers. Among them *biting* or *crushing soldiers* are found in the primitive families of Termopsidae and Kalotermitidae, with mandibles of a general shape similar to that of workers, with a rich marginal dentition, but often strongly hypertrophied. Their mode of operation is biting and crushing enemies with relatively small amplitude. The soldiers themselves are usually robust, and often perform phragmotic behaviour. *Reaping* or *slashing soldiers* are the most common type; they occur in some Kalotermitidae, in most Rhinotermitidae (e.g. *Coptotermes*, *Heterotermes*), in Serritermitidae, and many Termitidae across particular subfamilies (e.g. *Syntermes*, *Cubitermes*, *Macrotermes*). Their mandibles are elongated, usually slightly curved, with sharp points. The marginal dentition is reduced or lost, and the mandibles can easily cross over each other. They have longer angular motion and before they hit their target they are kept open wide by irritated soldiers; they acquire a kinetic energy by rapid closing. A slight modification of this system can be found in *piercing soldiers* whose mandibles are often curved inward and bear prominent marginal teeth, used to pierce the body of an enemy. This mode of fight is common in Rhinotermitinae (e.g. *Rhinotermes*), some Termitinae (*Amitermes*), and Nasutitermitinae with both, mandibles and nasus (*Armitermes*, *Embiratermes*, *Rhynchotermes*). In some Termitinae, namely the cryptic species such as *Termes*, *Cavitermes*, *Tuberculitermes* a peculiar method of mandibular motion has evolved in the *snapping soldiers*. Long and flat mandibles are slightly curved inward and a small flat facet is situated medially near the tip of each of the mandibles. These can be pressed medially one against the other, and the energy exerted by the mandibular muscles is stored in the distortion of the mandibles. Then the mandibles suddenly skip over each other, and the stored energy is released in a violent blow. The adaptiveness of this strategy is considered to apply especially in confined spaces of galleries where the wide opening of mandibles of biting or reaping soldiers is difficult. At the same time such mandibles are usually very long; thereby they allow the soldier to keep a safe distance from the enemy. An ability of both slashing and snapping motion of mandibles has been described in few termitine genera such as *Dentispicotermes* or *Orthognathotermes*. An even more intricate design of the mandibular weapon can be found in soldiers of the genera from the *Capritermes* complex. The mandibles of these *asymmetric snapping soldiers* are asymmetric, with the right one flat and slightly curved and the left one bent and twisted. When locked one into each other, the left mandible can store in its flexion the energy of muscles, and when relaxed, this mandible produces a blow even more powerful then the one of symmetric snapping soldiers. A single blow of *Neocapritermes* is enough to paralyse a large ponerine ant(221).

Deligne et al. underlined the trade-off occurring in mandibulate soldiers between the maximization of the mandibular musculature volume and the necessity of an optimal profile in diameter of the head in order to enter the galleries and tunnels created by workers. These cavities are designed to be as small as possible to allow the passage of termites but to prevent the passage of larger predators(17). Consequently, the head in mandibulate soldiers is often very elongated but comparable in its circular cross-section to that of worker abdomen. The second consequence of the spatial limitation of soldiers by the nest is the development of the peculiar snapping mechanisms of mandible operation(17,193).

But not all soldiers rely on their mandibles to defend their nestmates. The reduction or loss of mandibles occurred independently in two groups. First, the *nasutoid soldiers* of advanced Rhinotermitinae evolved a specific labral tool for distribution of the frontal gland secretion, their mandibles are often vestigial. Second, in the majority of Nasutitermitinae, the typical *nasute soldiers* use the chemical defensive secretion applied with their *nasus* as the only defensive adaptation; the mandibles are reduced. The gut anatomy of Nasutitermitinae, as well as chemotaxonomic studies, suggest that this reduction evolved monophyletically, although alternative diphyletic scenarios were also hypothesized(222,223). The remaining eight Neotropical nasutitermitine genera possess slashing or piercing mandibles in combination with more or less developed nasus. In both non-mandibular types of soldiers new behavioural techniques evolved for the use of their unusual weapons. The behaviour connected with the long-distance discharge of the frontal reservoir in nasutes appears to be particularly complex(45,48).

CHEMICAL DEFENCE OF SOLDIERS, EXOCRINE GLANDS

The chemical defence of termite soldiers is unprecedented in the chemical diversity of defensive substances and their functional properties, as well as in the diversity of glandular sources of these substances and related structures used for the delivery of the secretion(17,193,224,225). Consequently, many studies were devoted to both the chemical diversity and anatomy of the glandular and delivery systems. The reviews by Deligne, Deligne et al., Prestwich, and Noirot served as the basis for the following text(17,193,224-226) if not specified.

The defensive function in soldiers is attributed to several exocrine glands, i.e. the *labral* (or *cibarial*) gland, the *labial* (or *salivary*) gland, and the *frontal* gland. The former two consist of a modification of structures existing in the basic anatomic plan of insects and are associated with the mandibles. The latter represents a novel structure exclusive to termites.

The labral glands occur in soldiers of Macrotermitinae, but probably also in other species; the hyaline tip of the well-developed labrum, covering large part of the mandibles, is known also in Apicotermitinae and in primitive mandibulate Nasutitermitinae. We have recently observed a large labral gland of complicated structure also in *Glossotermes* (Serritermitidae)(216). The glandular epithelium of these glands is located in form of a thickened epidermis under the sclerotized, non-hyaline part of the labrum, but also at the upper surface of hypopharynx. Therefore, the original name of labral gland was reconsidered and cibarial gland is now used. The position of these glands suggests that their secretion impregnates the mandibular edges and can enter the enemy's wound along with the slashing or piercing mandibles of the soldier. However, the chemical composition of the labral glands remains unknown.

A peculiar and unique adaptation of mandibles and glandular defensive structures was observed in soldiers of *Machadotermes* (Apicotermitinae). The mandibles are massive but short and numerous outlets of epidermal glandular cells are located on their hypertrophied basis. The defensive purpose of this glandular set is also hypothetical.

The ancestral function of the salivary or labial glands in workers is the synthesis of enzymes involved in food digestion, nutrition of dependent castes, and the participation on building activities. In most species, these glands are atrophied in soldiers when compared to workers, but in several genera they are hypertrophied. Their paired reservoirs can extend deeply into the abdominal cavity and serve as source of defensive secretion. This adaptation obviously appeared several times in an independent convergent evolution. In *Mastotermes darwiniensis* (Mastotermitidae), the reservoirs penetrate to the depth of the second abdominal segment. Well-developed salivary glands are also present in the subfamily Macrotermitinae, where the reservoirs can extend as far as to one half or two-thirds of the abdomen, or even nine tenths, as in *Pseudacanthotermes spiniger*. The salivary glands communicate with the preoral cavity by ducts opening at the basis of the labium, and the content of the reservoirs can thus be applied on the enemy while the soldier bites. In Macrotermitinae, the drops of saliva of different colours occur between the soldier's mandibles during the combat. Frequently, the soldier pierces the body of the enemy with its reaping mandibles and does not remove them from the wound, but stays locked with the enemy while pumping with its abdominal musculature the content of the salivary reservoirs or cells onto the enemy. The chemical composition of the defensive salivary glands consists mainly of benzoquinones and proteins; such a mixture combines the deterrent and sticky properties due to denaturation of proteins and/or by their reaction with quinones.

According to Mill, an autothysis of salivary glands exists in soldiers of termitine genera *Ortognathotermes*, *Genuotermes*, and *Dentispicotermes*(205). This special type of delivery of defensive chemicals is supposed to occur also in soldiers of a few other species with developed frontal gland. Nevertheless, the glandular origin of the secretion in these exploding termites remains to be confirmed, probably the frontal gland is involved in this system rather than the salivary glands, as has been also the case in *Globitermes*(64,227).

CHEMICAL DEFENCE OF SOLDIERS, FRONTAL GLAND

The frontal weapon represents a unique defensive structure without any equivalent among animals. It is present as a synapomorphy of the most advanced termite families Rhintermitidae, Termitidae, and Serritermitidae(228,229). It consists of the glandular source, the frontal gland, and the delivery structures on labrum, frons and clypeus, whose modified structure ensures the discharge of the frontal secretion. The frontal gland is a thickening or invagination of epithelium subadjacent to the frontal region around the frontal pore (fontanelle). Near the fontanelle, there are attachments of tentorial-fontanellar muscles, which are present in all examined termite species and castes. If the frontal gland is well developed, it forms an unpaired, sac-like structure, which in primitive situations opens onto the frons via the fontanelle.

In workers, pseudergates, and neotenics, only individual glandular cells may appear in the fontanellar region without a formation of prominent glandular epithelium. The frontal gland in imagoes is variable between diverse species. It is either a small sac-like organ located in the posterior part of the head (many Rhinotermitidae, Termitidae: *Odontotermes*, *Bellicositermes*) or occurs as a simple thickening of the epidermis as is common in many Termitidae. The functional significance and chemical composition of the frontal gland in imagoes is largely unknown and deserves a systematic approach(230). The presence of a well-developed frontal gland was demonstrated in presoldiers of few species (Termitinae: *Globitermes sulphureus*, Rhinotermitidae: *Prorhinotermes simplex*, *Reticulitermes santonensis*), some results indicate that it is even functional(64,231,232).

The full development of the frontal gland is attained in soldiers. It varies in different clades and correlates also with the defensive strategies. An enormous development of the frontal gland reservoir is common in Rhinotermitidae and Serritermitidae, where it often extends deep into the abdomen. Consequently, the quantity of the defensive secretion can represent up to almost 40% of the body weight, as in one of the most powerful chemically armed soldiers of *Coptotermes*(228,229,233,234) or up to 50% of dry weight of small soldiers of Rhinotermitinae(235). In Termitidae, the frontal gland is smaller and it is always contained within the head capsule, though it may occupy a very large part of the head, typically in Nasutitermitinae(236-239). A regression of the gland volume and absence of the frontal pore in some genera can be observed in Macrotermitinae, many Apicotermitinae, and the snapping soldier group of Termitinae, relying on other defensive means. A large variety of delivery systems of the frontal gland secretion developed in termite soldiers in close co-evolution with mechanical weapons. They can be classified in the three following categories after Deligne et al.(17): (i) local frontal differentiation; (ii) frontal gutter; (iii) advanced frontal pore. Three behavioural modes can be attributed to these respective categories, listed by Quennedey(240): (I) mandibular biting and simultaneous secretion of the frontal gland content to the wound or onto the enemy; (II) topical application of the poison with a modified brush-like labrum, and (III) ejection on distance of the sticky and poisonous content via a nasus. A fourth category also has to be added: the self-rupture or dehiscence of the frontal gland(228,229).

The simplest design of the frontal apparatus consists of a small round fontanelle located in the middle of the frons, which is plane, without specific structures. Such soldiers can be found in e.g. Macrotermitinae (*Macrotermes*), Termitinae (*Cephalotermes*), and Rhinotermitidae (*Termitogeton*). The secretion of these soldiers is generally volatile, and is supposed to play the role of poisons, alarm substances, and deterrents. The frontal gutter appears in Rhinotermitidae and Termitidae, though the most advanced development can be seen first of all in soldiers of Rhinotermitinae subfamily. The most simple situation is that of e.g. *Psammotermes* (Rhinotermitidae: Psammotermitinae) or *Amitermes* (Termitinae), where the frons and/or clypeus form a gutter-like structure facilitating the transport of the secretion from the frontal pore to the mandibles. The Rhinotermitinae soldiers possess a conspicuous gutter crossing frons, clypeus, and long, strongly modified labrum, terminated in an apical labral brush made of hundreds of spines. This brush is used to smear the poisonous secretion onto the enemy. Such soldiers, whose mandibles are small or vestigial, are called *nasutoids*, typically the minor soldiers of *Rhinotermes* or *Dolichorhinotermes*. In addition, in few species within Rhinotermitinae, functional slashing mandibles occur in soldiers, such as in *Parrhinotermes* or the major soldiers of *Rhinotermes*. If the frontal gutter was designed to transfer the secretion from the fontanelle to the place of application, the third type of modification consists in displacement of the frontal pore itself. In many species, the frontal pore migrates towards the anterior to approach the basis of the mandibles, where the secretion is discharged, as in *Cubitermes* (Termitinae) or typically in *Coptotermes* (Rhinotermitidae: Coptotermitinae). Another qualitative step in the application of the frontal gland secretion is the evolution of the nasus in Nasutitermitinae, which can serve in topical application or spraying of the secretion directly onto the enemy, independently on mandibles(48). These are still present in the few primitive genera of Nasutitermitinae, but become vestigial in most of genera with the typical *nasutes*. Finally, the mechanism of a rupture of the body wall, or frontal gland dehiscence, analogous to the autothysis of salivary reservoirs in soldiers and workers, represents a very efficient way to discharge the frontal secretion. This is the case of *Serritermes serrifer* (Serritermitidae), *Globitermes*, and *Apilitermes longiceps* (both Termitinae)(64,227-229,241). The former two examples represent species in which the abdomen is ruptured, whereas in the latter the head breaks during the discharge. In these species the frontal pore is lacking or is present but non functional. Similar type of self-sacrificing behaviour was described for the first time in carpenter ants (*Camponotus*), and named *autothysis*. Such soldiers in termites are called "walking bombs", "exploding termites", or also "kamikaze"(11,205,242), though the discharge of the secretion can be rather a slow outflow(11).

CHEMICAL COMPOSITION OF THE FRONTAL GLAND SECRETION

Due to the systematic work of numerous chemists, many chemical compounds involved in the chemical defence of termite soldiers were identified in the paste decades. The defence by the termite frontal gland proved to be chemically more novel and variable than the chemical defence in any other insect taxon of comparable size; the impressive richness is attained especially in the most advanced chemically armed soldiers of Nasutitermitinae(192,193). Many observations were published on the defensive chemistry of termites, reviewed and classified particularly by Prestwich(191-193,242-244). These references are the basis for the following brief classification. From functional point of view, the frontal gland chemicals can be categorized as follows: (i) Greases, non-volatile and non-polar substances preventing the healing, are present namely in mandibulate Termitidae in form of long-chain alkanes, alkenes, and diterpenes in their oily secretions. Lipophilic compounds were also identified in the frontal gland of mandibulate Nasutitermitinae. (ii) Irritants, fatty acids or terpenoid compounds that deter predation without overt toxic effects, are the most represented compounds in biting soldiers. In Termitinae, there are monoterpene or sesquiterpene hydrocarbons, alcohols, ethers, ketones, and other compounds. Rhinotermitidae possess sesquiterpenes and diterpenes, in advanced Rhinotermitinae ketones or vinyl ketones occur. Nasutitermitinae are also supplied with many irritants in their sticky secretion, such as monoterpenes and sesquiterpenes which acts as agents of disorientation for ants and feeding deterrents even for vertebrates. (iii) Contact poisons, lipophilic and highly reactive compounds, are typical for Rhinotermitidae. In *Prorhinotermes*, a toxic nitroalkene (*E*)-1-nitropentadec-1-ene, a unique nitro compound used in defence(11,245), is present in high amount. In Rhinotermitinae, the fast moving agile small soldiers with labral brushes contain in their huge reservoirs vinyl ketones and ketoaldehydes, known to be highly toxic to ants. In conspecifics of these soldiers, detoxification mechanisms were identified(246,247). (iv) Glues, materials that rapidly become tacky after exposure to air, are found in the immense frontal glands of *Coptotermes* soldiers in form of mucopolysaccharides, but above all represent the most abundant and diversified constituent of the secretion in *nasutes*. The advanced Nasutitermitinae are the richest source of monoterpenes among insects; they are also very rich in di- and sesquiterpenes, many of them are unique for termites(17). These terpene hydrocarbons are constituents of the glue, a defensive tool used to immobilize enemies, predominantly ants, which is undoubtedly responsible for the ecological success of advanced Nasutitermitinae(192,243). (v) Antimicrobial and antifungal action of the frontal gland seretion was observed in *Nasutitermes* species, attributed to the present terpenes(248,249).

EVOLUTION OF DEFENCE IN TERMITE SOLDIERS

The presented variety of defensive mechanisms described in termite soldiers allows us to deduce few general traits in the evolution of defence by termite soldiers. The three main weapons, the mandibular, the salivary, and the frontal one co-evolved, together with related behaviour, as mutually complementary or mutually exclusive mechanisms. The mandibular weapon is apparently primitive, derived from the worker mandibles. The following evolutionary succession of the mandibular types, accompanied by an increase of mandibular efficiency, is proposed by Deligne(217): crushing, slashing, symmetric snapping, asymmetric snapping. The salivary and cibarial glands have independently evolved in several lineages in close relationship with the mandibular mechanism and always represent its complements or upgrade. A clear exclusion exists between the frontal and the salivary glands; the frontal gland and even the frontal pore are greatly reduced or vestigial in Macrotermitinae with developed salivary glands. The frontal gland appears to evolve as an auxiliary weapon to the mandibular mechanism, as is the case of most Rhinotermitidae and Termitidae. In three apparent cases, one of the two became a dominant weapon and the other one was subjected to regressive evolution. First, in numerous snapping soldiers, the frontal gland is reduced. Second, the labral mechanism of delivery of frontal secretion takes over the mandibular weapon in Rhinotermitinae. Third, the mandibles are reduced in the advanced Nasutitermitinae, which evolved once from the ancestor with both, the nasus and efficient mandibles(17,192,222,223).

But a common trend in this co-evolution of particular weapons is the passage (i) from one-to-one contact fights of large soldiers to distance combat of numerous smaller soldiers, and (ii) from piercing and crushing to more efficient physical attacks causing an immediate immobilisation or knock-out of the opponent. These trends are typically realized in I) the snapping soldiers, above all the asymmetric snapping soldiers with violent mandibular strikes; (II) the nasutoid soldiers of Rhinotermitinae, applying rapidly the frontal secretion with their labral tool, and (III) the nasute soldiers of Nasutitermitinae, spraying on distance the sticky secretion from their nasus(17,217).

The species with glue-using active and small nasute soldiers represent almost 25% of all termite species and are diversified in various ecological niches. Most of the freely foraging termite species are found among them (*Constrictotermes, Hospitalitermes, Longipeditermes, Lacessititermes, Rhynchotermes, Trinervitermes* or *Tenuirostritermes*), the soldiers being effective against ants as well as against certain vertebrate predators. Some results indicate that regardless of the discharge mechanism of the frontal secretion, the high concentration itself of irritants in the bodies of soldiers may be the effective deterrent causing general distastefulness of the termite group(48-55,233,250,251).

V. COORDINATION OF DEFENSIVE ACTIVITIES: ALARM, RECRUITMENT, POLYETHISM

The described defensive activities of particular castes are not performed individually; they are subjected to co-ordination by means of information transfer in the society. Here again, the role of soldiers appears to be very specialized. Apart from the direct fighting, the defensive function *sensu lato* of soldiers also comprises initiation and propagation of alarm and recruitment stimuli to nestmates in endangering situations. The modes of alarm and recruitment in termites can be classified as follows: (i) vibratory or mechanical alarm; (ii) chemical alarm by means of alarm pheromones from the soldier frontal gland or/and trail pheromones produced by the sternal gland, and (iii) tactile, physical contact between the alerted individual and the recruited individual. The three mentioned modes are usually combined and are not exclusive for soldiers, though soldiers play the major role in alarm communication.

Alarm communication by vibrations

Body vibration is a widespread activity performed by soldiers, workers, nymphs but also larvae and reproductives in many species. It is often accompanied by drumming produced by the contact of the head or the abdomen with the roof of a gallery or a nest, or with the substrate. Also the ability of termites to perceive vibrations is well known; termites are particularly sensitive to vibratory disturbances of the nest and react by initiation of alarm behaviour, i.e. typically by an outcome of soldiers and by a retreat of workers(19,65,252). Though the vibratory movements produced by termites often represent a sound clearly audible to man, experiments have shown that the alarm in termites is not initiated by airborne sounds but by substrate vibrations, perceived by the subgenual organ on termite legs(252,253).

The communication by vibrations has been first studied in lower termites, namely in the damp-wood species of the genus *Zootermopsis* (Termopsidae) and the dry-wood termite *Kalotermes flavicollis* (Kalotermitidae). Several categories of vibrations were recognised, i.e. (i) *longitudinal oscillatory movements*; (ii) *vertical vibratory movement* or *head-banging* or *drumming*, and (iii) *zigzag horizontal movement* or *complex vibratory movement*, i.e. a combination of horizontal and vertical jerks(254-258). In addition, stridulatory structures have been described on body surface in *Kalotermes flavicollis*, suggesting that high frequency sounds are associated with vibratory movements(255).

The functional significance of these vibratory movements in *Zootermopsis* has been attributed to two different modes of alarm. First, the *general alarm* appears when a strong disturbance affects all members of the group or colony. In such groups, a majority of soldiers and a minority of larvae and pseudergates begin to perform the head-banging or drumming movements, soldiers hitting both the roof and the floor of the gallery, while pseudergates hit the substrate only slightly or not at all. This vibratory alarm is perceived by the nestmates; their speed of motion accelerates and the onset of building activities can be observed. But it does not elicit a positive feedback response by inducing them to vibrate. On the contrary, it is subjected to exponential decrease in time and it cannot be transmitted to groups non-confronted with the disturbing stimuli. The other mode, the *specific alarm*, consists in an initial excitement of one or few individuals (pseudergates). These alerted individuals perform a longitudinal vibratory movement at the encounter with the stimulus, then retreat and run away while laying an odour trail until they meet a nestmate. When in contact with a nestmate, they perform the complex vibratory movement to alert and recruit the other termite. Thereby, the alarm is spread among many termites and the recruited individuals can be observed to follow the odour trail to the spot of disturbance, where they often start building to eliminate this disturbance. The longitudinal and the complex vibratory movements can transform one into another, the longitudinal movement being a sign of a lower excitement(256-258).

The situation in basal lower termites appears to be primitive, especially by the absence of a positive feedback mechanism of the transmission of alarm vibrations. This feedback does not seem necessary in the small colonies inhabiting compact wooden nest. In populous nest of advanced species, foraging for food on a large territory, namely in Termitidae, the vibratory alarm reached a higher level of complexity and caste specialization(256,259). First, the head-banging is performed by soldiers only, as has been documented in *Coptotermes* (Rhinotermitidae) and Termitidae. Second, the general alarm by head-banging elicits an immediate positive feedback reaction of other soldiers that start to drum with their heads as well. The reaction on such drumming alarm is clearly polyethic: soldiers are recruited to drum, workers rapidly retreat(256). Such a fast and effective propagation of alarm is well known to occur in Macrotermitinae, in the nest but also in the foraging groups, and was described in details for *Macrotermes* and *Pseudacanthotermes* species(256,260,261). The synchronized vibrations of macrotermitine soldiers in the foraging territory, such as in *Pseudacanthotermes*, are clearly audible as rhythmical hissing sounds. It has been hypothesized that these sounds may act as a warning aposematic signalization for ants but also vertebrate predators, or a territorial signal preventing heavy losses in conflicts between populations, e.g. in *Macrotermes*(66,260). Apart from the general alarm, the specific alarm recruitment has been described in *Macrotermes*. In basic traits it is similar to that observed in *Zootermopsis*, i.e. the major worker, excited by an encounter with an alien, runs towards its nestmates and alerts them by direct physical contact accompanied by vibratory movements. The alerted nestmates emerge soon after and follow the odour trail laid before by the alarm initiator(257,262).

Finally, also a peculiar alternative explanation of termite-produced vibrations should be mentioned. Evans et al.(263) assigned to vibrations produced by pseudergates of the kalotermitid *Cryptotermes secundus* a role in determination of the size of wooden blocks.

ALARM COMMUNICATION AND RECRUITMENT BY CHEMICAL CUES

Chemical communication is by far the most important mode of information transfer in termites. The alarm communication is not an exception, though alarm pheromones *sensu stricto* are described exclusively in termites possessing the frontal defensive gland. On the other hand, the defensive behaviour cannot be strictly separated from the foraging behaviour in species collecting food from outside, and many aspects of the recruitment in response to endangering stimuli are common with the recruitment initiating the foraging for food(200). Thus, two aspects of soldier defensive behaviour are presented below, i.e. (i) the recruitment performed by soldiers during foraging, mediated by the sternal gland, and (ii) the alarm communication *sensu stricto,* mediated by the frontal gland pheromones.

Food provisioning from outside the nest is a general trend in the evolution of termite ecological strategies. Foraging species can be systematically found in Hodotermitidae, advanced Rhinotermitidae, and above all in many Termitidae. Among Nasutitermitinae, we can also find many species foraging freely without protective tunnels or galleries. Soldiers are present in high numbers during foraging, they are usually the first to leave the nest, proceed on the front of a foraging column, stand and patrol along the foraging trails and around the foraging site. Finally, they are the last to re-enter the nest at the end of foraging(48,60,65,264-268). Hodotermitidae are the rare exception to this rule with workers alone present on foraging sites without soldiers, though these are present in the colony(204). Apart from the obvious defensive role in the vulnerable foraging columns, other tasks are attributed to soldiers of some species during foraging. The soldiers of the nasutitermitines *Nasutitermes costalis* and *N. corniger* were found to initiate the foraging, to search for food, and to recruit other soldiers and workers to a food source. To do this, they use the same communication system as for the defensive recruitment by means of trail pheromone from their sternal gland. Food recruitment proved to be a complex pattern of events, with first only soldiers exploring the territory and recruiting other soldiers by the trail pheromone towards the food source. Only after a reinforcement of the odour trail by numerous soldiers and physical contact of soldiers with workers, the workers leave the nest to attain the food source. Soon after workers start to build a covered gallery(65,268). Analogous situation has been later described in other foraging species, such as *Schedorhinotermes lamanianus* (Rhinotermitidae)(264), though in other species it could not be confirmed, as e.g. in *Nasutitermes lujae*(267). The described behaviour of soldiers could be classified as a participation in food provisioning in a role of scouts, known in social Hymenoptera. But alternatively, this mechanism can be considered as an apparently adaptive modification of the defensive strategy, designed to minimize the exposition of vulnerable workers to predation and to immobilize or deter enemies – ants before the onset of a permanent trail and building of an arcade. Thereby, in this case, soldiers fulfil two tasks at the same time: they are scouts and defenders. This idea was the starting point for a theory proposed by Stuart, that the foraging evolved as a modification of the defensive behaviour. In primitive non-foraging species, the trail produced by the sternal gland of an alerted individual recruits soldiers and workers to a site of disturbance, and workers start to build and repair a breach in the nest, or to bury an intruder or an unknown object. The origin of the recruitment and subsequent building of the foraging galleries could be explained by an adaptation of this defensive behaviour; the foraging would represent a so-called "food alarm"(200).

The alarm communication *sensu stricto* consists in local recruitment by means of short-range attractants. The frontal gland secretion of soldiers mediates this communication, so it is logically restricted to Rhinotermitidae, Serritermitidae, and Termitidae. The alarm substances are included in the blend of defensive compounds in the frontal secretion and released during the discharge of the gland reservoir by excited and/or fighting soldiers. This pattern or alarm initiation has been described in few rhinotermitids, e.g. *Schedorhinotermes*, several *Reticulitermes* species, and numerous termitids, e.g. many *Nasutitermes* species, *Velocitermes*(48,59,68,199,264,269-272). Despite the mentioned observations of the alarm function and the extensive list of chemically identified compounds from frontal secretion in various termites, the confirmations by biotests of particular compounds, responsible for alarm, are rare. The volatiles involved in alarm communication are monoterpenes, e.g. α-pinene and limonene in *Nasutitermes rippertii* and *Velocitermes velox,* carene and limonene in *N. costalis*, and α-pinene in *N. princeps* (all Nasutitermitinae)(68,270,273,274). The participation of these compounds in communication is evidenced also by their high enantiomeric purity, which is frequent in pheromones(273,275-277).

The reaction elicited in nestmates by the release of alarm pheromones is caste-specific. Soldiers are always attracted to the source, while workers usually retreat. Only after a certain delay are they attracted by the pheromone itself, other components of frontal secretion, or by means of recruitment performed by soldiers with use of sternal gland trail pheromone. The workers then participate in defence, particularly in nasute species, either by direct fighting and biting, or by building and depositing faecal material on enemies immobilized by soldiers(48,65,264,268). The alarm recruitment by soldiers can elicit instar-specific responses in workers, as observed in *N. princeps*(68). A curious observation is the initiation of alarm by faeces deposited by workers of *Hodotermes mossambicus* (Hodotermitidae)(278). The singularity of this example may be explained by the lack of our knowledge rather than by its uniqueness.

Polyethism among soldiers

The hemimetabolous post-embryonic development of diploid termites allows a very complex pattern of development of soldiers. These can be derived from different castes, i.e. larvae, workers, nymphs, of various instars and sexes. Consequently, polymorphism in the soldier caste results from differential caste and instar origin of soldiers. This polymorphism can be reflected in size of the soldiers, as is common in lower termites. In advanced Rhinotermitidae and Termitidae, there exists conspicuous polymorphism represented by two or even three forms of soldiers in a given species. Polymorphism and its ontogenetic background are given in more detail in Chapter VII. The polymorphic soldier forms may differ fundamentally in their anatomy and behaviour, and consequently, polyethism within the soldier caste may be observed. Logically, this polymorphism is more pronounced in species with diversified ecology, where also the diversification of tasks performed by soldiers can apply. Therefore, the observations of functional polyethism in soldiers are common in foraging species.

When minor soldiers of Rhinotermitinae, equipped with the frontal brush, e.g. in *Schedorhinotermes* or *Rhinotermes*, are confronted with the major mandibulate soldiers, they seem to belong to another species due to completely different anatomy. The minor soldiers in *Schedorhinotermes* are known to participate in foraging for food; they defend the foraging columns but also function as scouts in recruitment. The major soldiers, by contrast, do not leave the nest and do not stay in its peripheries. The frontal gland of minor soldiers is considerably smaller than that of the major soldiers and apart from defensive substances it contains also alarm pheromones. The composition of the frontal secretion differs between the two types of soldiers(264,279). Similar situation was described in a macrotermitine *Pseudacanthotermes*, where minor soldiers defend the foraging columns(58,266). Also in *Macrotermes subhyalinus* only minor soldiers occur in foraging groups at the extremity of the gallery and they are the first to be recruited to a breach in the nest. In *M. bellicosus,* major soldiers appear the first on the nest surface when it is damaged(19,262,280,281). The small soldiers in the nasutitermitine *Nasutitermes exitiosus* participate in both the defence of the nest and in foraging groups. The large soldiers represent a majority of soldiers in the foraging groups, but they do not fight when disturbed, they retreat and flee instead. However, they posses an active frontal gland and are supposed to transmit alarm excitement to the colony(57,199). A different mode of polyethism occurs between the two soldier morphs in another nasutitermitine *Trinervitermes*. Small soldiers accompany the foraging groups, whereas the large soldiers only occasionally leave the nest. Their hypothetical function is the defence against the attacks of hypogeous doryline ants(30).

Temporal polyethism of particular castes within an instar is not as ubiquitous phenomenon in termites as in social Hymenoptera, or in other words, it is hard to study. Nevertheless, there is evidence for the shift of roles during the individual life of workers and large soldiers of *Macrotermes bellicosus*(282,283). In both cases, younger individuals remain in the nest while the old ones are the only to forage for food.

VI. INTEGRATED DEFENCE

STATIC AND MOBILE WARFARE

Particular elements of defence presented in the above text, i.e. defence by nest, defence by workers, defence by soldiers, alarm, and recruitment are integrated into a complex defensive strategy that is characteristic for a given species. Within the variety of defensive features observed in termites, it is complicated to establish any systematic classification of defensive strategies. Deligne and Pasteels proposed a tool for this classification by distinguishing two general defensive components of nest defence: *mobile warfare* and *static warfare*(190). Mobile warfare consists of an active defence performed by soldiers and/or workers, i.e. by an efflux of defenders towards the site of disturbance, released by endangering stimuli, coordinated and enhanced by alarm pheromones, mechanical and trail pheromone recruitment. The nest usually does not provide efficient security but allows, due to large peripheral chambers, a passage and circulation of numerous termites. Static warfare, on the other hand, represents a passive resistance, phragmotic behaviour of soldiers obstructing the passages in the nest, its entrance or breach, while workers rapidly repair the damages and seal the openings. Such nests are commonly armoured and the cavities on their peripheries are small and narrow to allow blocking.

These two opposite principles are variously combined in particular species and compose the characteristic defence. The best example that represents the mobile strategy can be found in the arboreal *Nasutitermes* species. Their carton nest is fragile and breakable, the peripheral chambers spacious, soldiers and workers very agile and aggressive, soldiers numerous, and apparent alarm recruitment usually occurs when the nest is disturbed(57,58). Among arboreal species an opposite trend towards the static warfare can be seen in *Microcerotermes* (Termitinae), whose nests are also made from carton but they are harder, compact, and compartmentalized. The soldiers are relatively rare and perform phragmotic behaviour without any alarm recruitment. Typical static warfare can also by found in species with massive epigeal nests made of hard carton or earth (e.g. Termitinae: *Cephalotermes*, Macrotermitinae: *Sphaerotermes*). But above all the static warfare is a *modus vivendi* in numerous cryptic soil-feeding species(19,190).

However, the situation is even more complicated in many cases, namely in advanced species with complex nests, diversified, polymorphic, and polyethic castes. The two principles are often combined; e.g. in the macrotermitine *Macrotermes bellicosus*, several lines of defence exist. Primarily the mobile component, the major soldiers, emerges on the surface when the nest is opened; immediately after the major workers repair the breach, often closing irreversibly the soldiers outside the nest. Finally, the internal nest cavities are designed for phragmotic blocking of passages by both soldier types(19,281). The mentioned assistance of soldiers during emergency repair performed by workers is a very common feature, sometimes considered as the primary role of soldiers(47,48,58,198,281).

MODALITY OF DEFENSIVE RESPONSE

The reaction of a particular colony is not uniform in all circumstances. The defensive response in both the individuals and whole groups is modulated by the intensity and type of alerting stimulus. On the individual level, a threshold intensity of excitement necessary to launch defensive behaviour applies, as well as the type of this behaviour performed. For example, a soldier of *Globitermes* (Termitinae) can fight with its mandibles as well as explode by autothysis(64). Thus the qualitative form of defensive activities and the number of individuals involved can reach several levels, reflecting the seriousness of the given situation. It has been mentioned above that the vibrational signalling can include an element of intensity. Different behavioural sequences are launched during general and specific alarm in the nest. The differential response adapted to the intensity of vibrational signalling was observed on the foraging sites in *Pseudacanthotermes* and *Macrotermes* (Macrotermitinae)(260,266). Also the chemically mediated recruitment and alarm shows dynamic modalities reflected in the behaviour and final number of recruited individuals(48,65,268). This hierarchical pattern of behavioural responses, depending on the intensity of alarm stimulus, is noted by Stuart, who also proposes homeostatic feedback control mechanisms eliciting a pacification of individuals after the cessation of responsible stimuli, i.e. the death of intruders or repair of damaged buildings(200). Last but not least, the alarm recruitment is context-dependent so that different stimuli require different type of responses. Depending on the type of intruder, diverse strategies may occur as an optimal reaction; from an overt aggression on one side to a static defence and tightening of the defensive line on the other(164). Specific identity of enemies elicit different involvement of particular castes, as demonstrated by the increased role of workers in intraspecific aggression, particularly in nasute species(151,158,164). The response of termite can be subjected to a trade-off between the risk of predation and the availability of food; when food is scarce, the predation tolerated by termites on foraging sites before they retreat is higher(284).

The very last modality of the defensive activity is often an escape of termites from the endangered site and retreat of individuals into the nest centre or deeper into the ground. Even a systematic evacuation of the whole colony was observed when faced to a general attack of ants, including the advanced Macrotermitinae with highly physogastric queen(131).

DEFENCE OF FORAGING COLUMNS

The foraging for food outside the nest above ground, i.e. foraging within covered arcades, under the ground with collection site above ground, and in the open-air, is linked with specific defence requirements. It is in this circumstance where the soldiers appear to play the major role. The general characteristics of the defensive strategies of above-ground foraging observed in different species can be summarized in the following points: (i) the abundance of soldiers in the foraging columns; (ii) efficient chemical defence of soldiers; (iii) specific pattern of temporal and spatial distribution of soldiers and workers; (iv) specific activity of soldiers during foraging; (v) timing of foraging allowing to avoid the exposition to predation; (vi) general unpalatability of the foraging columns.

The proportions of soldiers in termite colonies were already summarized above and their dominant presence in the foraging columns has been underlined; this can attain even up to 90%, as documented in extreme case of *Tenuirostritermes tenuirostris* (Nasutitermitinae)(45). The soldiers are usually the first to leave the nest or the outlet from underground galleries, so only after accumulation of a certain number of soldiers the workers emerge. The soldiers are on the front of the column and represent its leaders. In few cases, the soldiers were found to be directly responsible for the localization and recruitment towards the food, as mentioned above(45,46,60,268). In species foraging inside covered arcades, the soldiers are lining the strong odour trail of the foraging pathway and the collection site in a guarding position, until the arcade is built by workers. The proportion of soldiers decreases as soon as the foraging area and the nest is connected with a complete arcade(65,198,264,266-268,285).

In the open-air foraging species, e.g. *Hospitalitermes*, *Longipeditermes*, and *Lacessititermes* from South Asia, *Rhynchotermes* and *Tenuirostritermes* from Neotropics, African *Trinervitermes* (all Nasutitermitinae), Australian *Drepanotermes* (Termitinae), and *Macrotermes carbonarius* (Macrotermitinae), the soldiers are also distributed along the flanks of the columns in guarding posture, in perpendicular position to the direction of the path. Similarly, on the foraging area, they climb on elevated points, and stay in guarding position with heads and antennae situated outwards. The soldiers are also closing the foraging column, and are the last to come back to the nest(45,46,60,280,286-288). Such pattern of organization is markedly close to that known in army ants (e.g. *Eciton*).

The organisation of open-air foraging columns probably reaches its highest complexity in *Longipeditermes* species (Nasutitermitinae). Its dimorphic soldiers, minor and less numerous major soldiers, flank the columns on both sides; but also inside the column there is a well-organized circulation. Workers on the way to the foraging site are on the periphery of the column and those going back to the nest are in its centre. This pattern, observed also in *Macrotermes carbonarius*, seems to be adaptive because the best protected workers are those loaded with food(46,289).

The timing of the open-air foraging for food is a common mechanism to avoid strong predation. Many open-air foragers have the main active period in the night, such as *Pseudacanthotermes* (Macrotermitinae), *Hospitalitermes*, or *Tenuirostritermes* (both Nasutitermitinae)(45,60,266,286). The harvesting termites of Hodotermitidae, which possess eyes and have diurnal activity, forage in specific daily periods when the pressure of predators on the given locality is minimal(278).

One of the possible explanations of the success of chemically armed soldiers consists in their distastefulness for predators, what makes them unpalatable in big amounts. Regardless of the individual capacities of particular soldiers, their defensive structures, and size, the overall biomass provides protection for the foraging groups as well as for the nest peripheries, most often attacked. The role of such soldiers is not to be good fighters, but rather to be in the right place at the right time in large numbers. The direct distastefulness for vertebrates, including the specialized predators, was recorded in some cases. Of course, the specialists will not avoid termites. But they can be forced to move to another place as they are irritated and/or attacked by rising proportion of recruited soldiers. Such behaviour was described in specialists such as tamandua and giant anteaters, numbats, and aardwolves(49,51,55). The distastefulness or deterrence to ants is a common passive defence in Rhinotermitidae and Nasutitermitinae; it can be combined with other effects, such as chemical disruption of ant behaviour, preventing them from recruiting nestmates or causing hyperactivity. These aspects cannot be separated from other defensive abilities of soldiers, i.e. the immobilization and elimination of enemies through sticky or toxic secretion(48,193,233,251,290). In this context, the evolution of mechanisms of autothysis can be understood as a more efficient mechanism of self-sacrifice and distribution of glandular content then just being eaten.

ASSESSMENT OF EFFICIENCY OF DEFENCE

The quantification of efficiency and cost of particular defensive strategies is rather difficult. However, there are some comparative studies testing the presumptions about the adaptive significance of particular defensive strategies, included in the preceding text. Coles and Howse have proven the importance of the chemical defence in foraging of several sympatric species of a primitive nasutitermitine *Syntermes*. These species combine the mandibular and frontal chemical defence. A continuum can be established from fully mandibulate species with small frontal gland to species with large frontal gland and small mandibles. Along this continuum a clear correlation was observed between the ability of epigeal foraging and the investment in chemical defence. Moreover, the chemically armed soldiers were smaller, their size compensated by a considerably higher number of soldiers in the foraging parties, ensuring the required distribution of chemical defence(51,66). In another comparative analysis, several parameters, such as palatability for anteaters, mechanical resistance of nest, number of soldiers, and latency of the onset of repairing activities, were compared in several Neotropical nasutitermitine species with chemical, combined, and predominantly mechanical defence, and in one soldierless termitine species. Here again, the presumed opposite tendencies between investment in static and mobile warfare were confirmed. More soldiers defended fragile nests than solid armoured nests, and the inhabitants of these fragile nests were less palatable for anteaters than the individuals from solid nests. The species with mechanical defence and the soldierless termitine *Grigiotermes* with workers performing autothysis, reacted by a retreat into the solid compartmentalized nest. There they were fighting and blocking physically the access to its centre. The time necessary for a repair of the damaged nest was considerably lower in the species with static warfare(51,66). According to some authors, the lowest ratio between investment into defence and its efficacy can be found paradoxically among the soldierless species performing one of the chemical defensive strategies, i.e. the body rupture or autothysis. The defensive performance is high and the workers are not a good prey. Given that the valuable nymphs and winged imagoes occur only during a short period, the production of permanently present and dependent soldiers is a costly alternative to the apparently lower investment into defensive structures in workers(9).

When attempting to classify species along a spectrum form tychophobe to tychophile, the reproductive capacities and longevity of the societies should be evaluated as a function of the investment in defence. In some characteristic cases, these two factors display the presumed positive correlation, as demonstrated for instance by Noirot and Darlington on two African sympatric termitine species, *Cephalotermes rectangularis* and *Cubitermes fungifaber*(19). The former one apparently invests a lot in its hard compartmentalized carton nest with thick walls and an armoured royal cell. The cell is occupied by a highly physogastric queen and the lifespan of colonies is estimated to be several decades. *Cubitermes*, on the other hand, has mushroom-shaped nests with thin walls of low-cost faecal material, creating a brittle, primitive structure. This vulnerability is not compensated by a high presence of soldiers. The queen is poorly physogastric, and the longevity of such colonies is estimated to be at the most 5 years. The turnover of such societies, demonstrated by many incipient and young nests in one locality, is high(19).

In conclusion, the following has to be repeated about the defence in termites. The integrated defence of termite nests represents an excellent manifestation of the basic characteristics of social insect societies, i.e. the highly specialized division of labour, not only in terms of reproduction but also among defensive, food-provisioning, and building tasks, elaboration of collective defensive edifice out of reach of solitary individuals, and finally an information transfer and processing allowing an integration of these elements. Therefore, "if the concept of superorganism applies anywhere, it is surely to the termite colony."(19)

VII. ONTOGENETIC DEVELOPMENT, POLYMORPHISM, MORPHOGENESIS, AND SEX RATIOS OF TERMITES SOLDIERS

The complex social division of labour in termites is linked with a very pronounced polyphenism, represented by the existence of several castes defined by their anatomy, behaviour, and ontogeny. The striking resemblance between the caste systems of termites and these known in eusocial Hymenoptera is reflected in the terminology designating the particular castes, such as workers, soldiers, kings, and queens. Nevertheless, the mechanisms underlying the formation of caste systems in these two groups are fundamentally different, and despite the functional convergence of castes, there are many aspects in their biology reminding us that "the termites have climbed the heights of eusociality from a base extremely remote in evolution from the Hymenoptera"(11).

Basic determinants of caste system in termites

There are two major features appropriate to termites determining the formation and richness of their caste system. First, termites are hemimetabolous insects. The basic pathway of post-embryonic development proceeds from an egg through a succession of *larval* (apterous) and *nymphal* (brachypterous) stages to winged imagoes, the ancestral life form of termites, playing a role of *primary reproductives*. The development along this *nymphal* ontogenetic line is marked by a progressive formation of imaginal structures, namely wings and gonads. As in other hemimetabolous insects, the immatures are mobile, active, and able to feed themselves. The formation of other castes must be understood as a modification or complication of the "normal" development through a deviation from the basic developmental pathway(6,291-294).

Through an irreversible deviation from an early larval instar, the developmental line of *true permanent workers* is derived in Mastotermitidae, Hodotermitidae, Rhinotermitidae (with the exception of *Prorhinotermes* and *Termitogeton*), and Termitidae. This *apterous* developmental line consists of one or several larval instars and one or more worker instars. The workers can undergo stationary of progressive moults to higher instars or stop moulting at a specific terminal instar, often the first one. In the rest of termite species, i.e. Kalotermitidae, Termopsidae, *Prorhinotermes*, and *Termitogeton* (Rhinotermitidae) the social tasks are performed by late larvae and nymphs, and especially by *false workers* or *pseudergates*. These temporarily specialized helpers can be derived from late larvae by a *stationary* moult without marked changes in anatomy, or from nymphs by a *regressive* moult, during which the nymphal wing buds disappear or regress. Pseudergates retain future prospect of return to the nymphal line by moulting into nymphs, and subsequently in winged imagoes. The regressive moult is a developmental phenomenon common in lower termites but extremely rare in other insects. One or two, exceptionally even three moults are required for the development of a *neotenic* (*secondary*) *reproductive* from larvae, nymphs, pseudergates, or workers. Soldiers develop always through a short-term transitional stage of a presoldier; they can be derived from larvae, nymphs, workers, or pseudergates. This brief synopsis of existing developmental options illustrates the complexity of the caste systems in termites with many possible developmental "decision points" leading to many alternative developmental directions. This complexity results in a network of developmental pathways characteristic for particular species, genera and/or families. These pathways define the characteristic caste constitution of the given species, sometimes including a polymorphism within a single caste due to differential ontogenetic origin of its members. Moreover, all the mentioned castes, with the exception of the winged imago, are immature and possess their *moult* (*prothoracic*) glands. Consequently, with the exception of the very specialized castes of soldiers and neotenic reproductives, the other castes usually retain the capacity of moulting. Thereby, an individual can during a single life be a larva, nymph, pseudergate, presoldier, soldier, or a neotenic reproductive; but it can also proceed to the alate stage. Alternatively, it can become a true worker and later develop in a neotenic reproductive or soldier. The mentioned stationary and regressive moults are unique features of the termite post-embryonic development, allowing alteration of individual ontogeny. As a result, the terms *stage* and *instar* gain a particular meaning in termites and are far from being synonyms(6,34,292,295-300).

The second determinant of the termite caste system is the diploidy of termites. The diploid males and females can proceed through an identical developmental pathway to the imago, with very slight or no sexual dimorphism. The other castes, derived from the nymphal developmental line, are also primitively bisexual and uniform. In many advanced species, sexual dimorphism and specialization occur within particular castes, i.e. the two sexes differ in size, their ratio is not egalitarian, or one of the sexes is not represented at all. Sexual dimorphism can be the basis for a division of labour leading to sexual polyethism. The involvement of either sex in a particular caste does not follow any universal rule; it varies on specific and generic level. All individuals in the society share the same genetic constitution, an omnipotent diploid genetic pool, which is subjected to differential genetic expression of particular phenotypic programs, controlled by environmental and social regulation often until very late stages of post-embryonic development. The realized phenotypes, characteristic for particular castes, are based on the expression of several more of less independent genotypic sets, i.e. (i) the sexual (germinal) adult traits; (ii) non-sexual (somatic) adult traits; (iii) soldier traits(296,301). Therefore, the termite caste system represents a particular case of polyphenism, the *caste polyphenism*, in which several distinct phenotypic programs can be executed in the course of time, in response to intrinsic and extrinsic stimuli(292,301,302). The fact that an individual can express several phenotypes in a succession is also described by the term *temporal polymorphism*. In other words, "polymorphism in termites is a developmental phenomenon"(292).

Instar and caste origin of soldiers – linear developmental model

It is far beyond the scope of this text to review the diversity of developmental pathways described in various species, therefore the following paragraphs will focus mainly on the developmental origins of presoldiers and soldiers in particular castes and instars; the differentiation of secondary reproductives is omitted. The following text refers to one of the two alternative models of developmental pathways observed in termites; the *linear* model without true workers and the *forked* model with a distinct apterous line leading to true workers.

The linear developmental model is characteristic for Kalotermitidae, Termopsidae, and two rhinotermitids, *Prorhinotermes* and *Termitogeton*. Despite a few alternative theories, it is largely acknowledged as an ancestral mode of caste formation. The social tasks are performed by only temporarily specialized helpers, i.e. larvae, nymphs, and pseudergates(35,184,187,295,296,303-306). Their involvement in the altruistic tasks can be seen as a postponement of the imaginal development. The developmental patterns are characterised by high complexity and individual ontogenetic options are flexible until late stages. This developmental model is linked with the primitive nesting habits of one-piece type species, using a piece of wood as an unstable source of food and shelter at the same time. The individual ontogenetic flexibility and a retained option of imaginal development in pseudergates allow a developmental "decision" between altruistic helping and individual reproduction. The individual can shape its inclusive and exclusive benefits on the basis of resource avalability and prospective fitness of the colony(42,43,306-308). Characteristic developmental options within the linear developmental model are depicted in Figure 5 on page 53.

The developmental pathways in Kalotermitidae were studied above all in *Kalotermes flavicollis*, but the established pattern corresponds well to the development of other species (*Kalotermes banksiae*, *K. aemulus*, *K. rufinotum*, *Neotermes connexus*, *N. koshunensis, N. papua*)(15,87,184,185,309,298,310-312). The imago is preceded by at least seven immature stages, two, eventually three of them are nymphs with wing buds. Late larvae and nymphs can moult into pseudergates by stationary or regressive moults; these can undergo further stationary moults or revert to the nymphal line. Larvae from the fourth instar on can differentiate into neotenic reproductives. The soldiers can originate in larvae from the third (occasionally also from the second) instar on, from nymphs, and pseudergates. The soldiers derived from nymphs bear rudiments of wing buds of various sizes. The origin of soldiers from early instars (2-3) occurs in incipient colonies only; in the advanced colonies the soldiers derive from later instars. The soldiers deriving from different instars are logically of different sizes, but are typically monomorphic and reveal only slight allometric differences in outer anatomy(87,298). As a rare exception, the two soldier morphs reported from *Incisitermes schwarzi*, differing clearly in size and in head shape, are considered(313). The existence of two soldier forms was also noted in *Kalotermes dispar* or *Incisitermes mcgregori*(30). The sex ratio among soldiers is usually egalitarian, though male-biased situations were recorded in *I. schwarzi*, *Neotermes papua*, and *N. connexus*(185,310,314).

The above described linear developmental pattern and origin of soldiers applies in its basis also for Termopsidae in the several species studied in details so far, i.e. *Zootermopsis* spp., *Hodotermopsis japonica*, *Porotermes adamsoni*(315-317). The existence of regressive moults of nymphs was recorded in several cases(315,318). The nymphal stages are two, with the exception of only single nymphal stage reported in *Hodotermopsis sjostedti*(319). This feature is very rare and is shared with the two rhinotermitid cases of linear development. In mature colonies, the apterous or brachypterous soldiers develop from late larvae of the sixth instar, from pseudergates, and nymphs. In incipient colonies the individuals from the third instar on are competent for the development into soldiers(315-316,320). Sexual dimorphism and biased sex ratios are not reported. In six species of Termopsidae from the genera *Zootermopsis*, *Stolotermes*, and *Archotermopsis*, peculiar individuals were recorded sharing the anatomy of neotenics and soldiers(188,321). These individuals have developed gonads, sometimes as well as in the neotenic reproductives, and display underdeveloped soldier characteristics on their heads. Females were observed to lay normal functional eggs. Such individuals are called *fertile soldiers* or *reproductive soldiers*(188,297,321). Due to the simultaneous expression of reproductive and soldier traits these individuals should more appropriately be called *soldier neotenics* or *intercastes neotenic-soldier*(15,19,187). The functional significance of these soldiers is discussed below in Chapter X. The ontogeny and origin of soldiers in the rhinotermitids with linear developmental pattern, *Prorhinotermes* and *Termitogeton*, are described in Chapter XI.

Instar and caste origin of soldiers – forked developmental model

The transition from linear to the forked developmental model must have occurred several times, regardless of which of the alternative phylogenies of Isoptera one accepts(322-326), given that we consider the linear model as a primitive state (see Figure 3 on p. 36). The emergence of this pattern of caste formation is fundamentally driven by changes in ecological strategies of termites, i.e. the foraging for food outside the nest. This ecological driving force revealed to be a more powerful architect of the caste systems in extant termite species than the phylogenetic constraints. The food provisioning of these separate type species ensures a qualitative step to a higher stability, fecundity, and longevity of the societies; from a limited short-term colonies to theoretically immortal populous settlements. Consequently, the prospective inclusive fitness of helpers increases and the altruistic caste of workers resigns irreversibly on individual dispersal(42,43,187,295,296,306).

The forked pathway is characterised by an early decision between the imaginal development and the apterous line, i.e. before the first or second larval moult (see Figure 2). The nymphal line comprises more brachypterous nymphal stages than that of the linear model. The developmental pathways are less complicated and more rigid; the individual options tend to be more limited. Biased sex ratios occur together with sex-based polymorphism within particular castes. Also the caste and instar origin of soldiers is confined to fewer stages, predominantly in the apterous line. On the other hand, the instar origin of soldiers as well as their sex can be reflected in their polymorphism(6,8,293,294,296).

The nymphal and apterous developmental line in *Mastotermes darwiniensis*, the only extant species of Mastotermitidae, separate after the first larval moult; the imago is preceded by as many as eleven instars. The apterous line consists of five larval instars followed by worker instars retaining ability to moult. Only from these workers can the soldiers as well as neotenics be derived in mature colonies. In incipient colonies, the fourth and fifth larval instars differentiate into soldiers. This schema by Watson(88) and Watson et al.(327) is not universally recognised, its weak points are criticized e.g. by Roisin(8).

The developmental pathways in Hodotermitidae are characterised by the occurrence of a marked sexual dimorphism of the apterous line, diverging from the nymphal one after the second moult. The third instar larva is followed by a succession of instars gradually changing from larval to worker characteristics. This apterous line is either represented only by males in *Anacanthotermes* or by larger males and smaller females in *Hodotermes mossambicus*. Size dimorphism in workers was also described in *Microhodotermes viator*. The nymphal line consists of six or seven nymphal stages. Soldiers are always derived from later instars of the male apterous developmental line; brachypterous soldiers were not observed(8,294,328,329).

The developmental patterns in Rhinotermitidae are as variable as other aspects of the biology within this diversified family. With the exception of the mentioned representatives with linear development, all other species studied so far possess the true workers and thereby also the early divergence of the apterous line. Even within these species, the variability in the complexity and specialization of the developmental patterns is striking. On one side, the genus *Reticulitermes* (Heterotermitinae) displays a primitive forked development characteristic by large complexity and individual flexibility. The separation of the apterous line from the nymphal line occurs after the second moult. The apterous line consists of larval and worker instars able to moult all over their life.

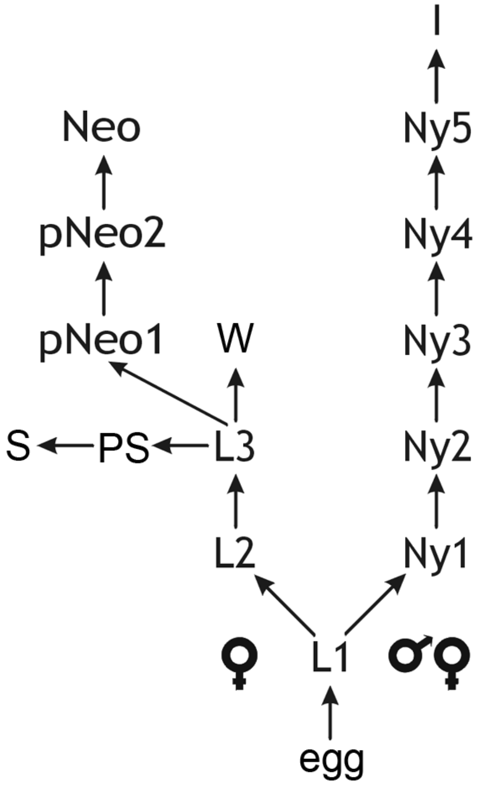

Figure 2.
Schema of the developmental pathways in *Parrhinotermes browni* (Rhinotermitidae: Rhinotermitinae). L – larva; PS – presoldier; S – soldier; Ny – nymph; I – imago; Neo – neotenic reproductive; pNeo – transitional instars in the neotenic development.
Redrawn after 299.

The nymphal line comprises six nymphal stages and imago. The majority of soldiers is derived from workers of the fifth or sixth instar, but in principle soldiers can develop from every instar of the apterous line; in incipient colonies primarily from early instars, i.e. the third instar workers. Also, brachypterous soldiers may derive from the last four nymphal stages(34,330,331). According to Buchli, early nymphal stages can undergo regressive moults and become pseudergates, able to moult and occasionally revert to the nymphal line. These pseudergates can also develop into soldiers(34). The sexual dimorphism or sexual specialization is absent except for the prevalence of females among the soldiers derived from nymphs(34,332). The pattern of caste development in *Reticulitermes* is sometimes considered as an example of an intermediate situation between the linear and the forked developmental model, undergoing gradual transition from facultative and reversible regression of nymphs to pseudergates to an irreversible separation of workers at an early stage(34,296).

Developmental pathways of *Coptotermes* (Coptotermitinae) are basically similar to that described above for *Reticulitermes*, but display a few advanced features: six nymphal stages follow the separation of two developmental lines after the second moult, five progressively growing worker stages are followed by further instars without significant growth. Regressive moults of nymphs were not observed. In mature colonies, the monomorphic soldiers develop from workers from the seventh instar on; soldiers are exclusively females in *C. lacteus*, whereas male soldiers are three time more numerous than female in *C. formosanus*(333,334). In incipient colonies, the soldiers probably develops from earlier instars, i.e. from the worker of the third instar(335).

The development of other rhinotermitids was described in details only in the subfamily Rhinotermitinae. It can be characterised as an advanced, forked development. It shares its principal traits with many Termitidae, especially the sexual specialization and sexual and instar-based polymorphism of neutral castes. These aspects can be even more pronounced than in many termitids. The caste system and developmental pattern in *Schedorhinotermes lamanianus* is typified by a bifurcation of the apterous and nymphal lines after the first moult. Five nymphal stages lead to the imago. The apterous line consists almost exclusively of females, and consequently, all soldiers, which derive only from the apterous line, are of the female sex. Thus, extreme sexual caste polymorphism occurs with males destined to become winged imagoes, rarely neotenics, and exceptionally workers or soldiers. Moreover, histological studies have shown that also the determination of females to follow either the imaginal or the apterous line, occurs at the embryonic level. Therefore, the two developmental lines diverge even before the larvae hatch from eggs(300). The apterous line consists of three larval stages followed by worker stages. Second and third larval stages are dimorphic; the morphs with smaller heads represent first instars of an independent developmental line leading to small and medium soldiers. The third soldier form, the large soldier, derives from workers. The soldier polymorphism in *Schedorhinotermes lamanianus* is extremely pronounced, as well as in other *Schedorhinotermes* species. Two or three soldier forms occur, the small soldiers differ dramatically from the large soldiers in both anatomy and behaviour, as is described in Chapter V. The medium soldiers are also supposed to be behaviourally specialized(336). The pattern of soldier polymorphism in *Schedorhinotermes* is similar to that of a few other Rhinotermitinae, e.g. *Rhinotermes* and *Dolichorhinotermes*. In all these cases, dimorphism occurs with small soldiers being nasutoids and large soldiers with more or less developed mandibles(190,337).

Another species of Rhinotermitinae with precisely described developmental pathways is *Parrhinotermes browni* (Figure 2). Here again, the two developmental lines split after the first moult, also the nymphal line is comprised of five nymphal stages. However, the developmental options within the apterous line are greatly reduced. It consists of two larval instars, the second of them moults either in a single terminal worker stage or in a monomorphic soldier, analogous to large soldiers of polymorphic Rhinotermitinae(299). All members of the apterous line are females. The exclusive presence of females among workers and soldiers was noted also in *Dolichorhinotermes*(8).

In another genus of Rhinotermitinae, *Acorhinotermes*, the soldiers are monomorphic, but these are typical nasutoids analogous to small soldiers(17,337).

In other subfamilies of Rhinotermitidae, the data is rather scarce. Genus *Psammotermes* displays a pronounced polymorphism in the soldier caste with two or even three types of soldiers recognised; these types are mandibulate but possess also the frontal gutter. As in other Rhinotermitinae, the frontal gutter is more pronounced in small soldier forms, but the differences are not as striking(30,337,338).

The developmental patterns in the family Termitidae are globally diverse, but at the same time, the pathways are stable, rigid, and more specialized within particular species. Nevertheless, there are a few basic features common to all species. Most prominent is the strict separation of the nymphal and apterous developmental line after the second moult. There are also proofs of even much earlier determination of future development in some species, i.e. at the embryonic level(6,300,339-342). This separation also restricts the behavioural and ontogenetic potentialities. There are five nymphal stages in all higher termites, they can differentiate neither in soldiers nor in brachypterous neotenics, and they are, with few exceptions, fully dependent on care provided by helpers. The developmental patterns of the apterous line in the family Termitidae reach the highest level of sexual dimorphism and sexual specialization within the apterous line. One can note examples of marked simplification of the development, with a single developmental line leading to monomorphic workers and soldiers. But in other cases, two types of workers and even three types of soldiers can occur; in the most common situations females are the larger caste. Workers differentiate from larvae of the second instar or from larvae of the third instar in Macrotermitinae. The moult from the larva is not accompanied by a marked change in anatomy but with a dramatic change in behaviour from a dependent larva to an active worker. The future sexual dimorphism of workers occurs already in larvae of the second instar. The worker caste can be represented by a single terminal stage or by a succession of instars with stationary of rarely progressive moults (e.g. in Nasutitermitinae: *Trinervitermes*)(5,6).

Soldiers originate in either a few or a single stage of the apterous line, i.e. in the second instar larva or in the worker. In few cases (e.g. Macrotermitinae: *Acanthotermes*; Nasutitermitinae: *Trinervitermes*, *Tenuirostritermes, Velocitermes*) they develop from a specialized larval line. All soldiers are often derived from one sex, e.g. male in many Nasutitermitinae or female in many Termitinae and Macrotermitinae. The soldiers can be of the opposite sex than the waste majority of workers, e.g. in Nasutitermitinae. The polymorphism of soldiers is reflected mainly in the size and often pronounced allometric differences; the particular morphs do not develop specific structures(6,8). The following text will briefly summarize the development of the apterous line in Termitidae with emphasis on the origin of soldiers. It is based mainly on the review by Noirot(6) if not specified.

The developmental pathways in Termitinae are often very simple; workers are not sexually dimorphic, they can moult several times; monomorphic soldiers are derived from both sexes (e.g. *Amitermes*, *Termes*, *Drepanotermes*)(343,344). In *Microcerotermes,* a sexual dimorphism in workers appears; soldiers derive mainly from large workers, i.e. females. Dimorphic soldiers were noted in several species of *Basidentitermes*(30). In other genera, such as *Noditermes*, *Cubitermes*, *Pericapritermes*, *Orthotermes*, the workers appear to be only a single monomorphic stage; soldiers derive from these workers and are of one sex, usually female. A very similar pattern probably exists in Apicotermitinae, i.e. single worker stage, female soldiers only.

Three groups with characteristic developmental pattern can be distinguished among Nasutitermitinae. First, the *Syntermes* group displays an unusual sexual dimorphism of workers with bigger males; the workers sometimes moult again. Soldiers are usually, but not always, derived from male workers. In *Cornitermes*, all workers and soldiers are male and monomorphic. In *Embiratermes*, slightly dimorphic workers are either males or females; soldiers develop from female workers. In *Rhynchotermes*, the sexual dimorphism is conspicuous, male workers do not moult; there is no worker stage in females; the third larval instar leads to female soldiers(345).

The group represented by *Nasutitermes* is characterised by repeated moulting of female workers. Soldiers are predominantly males that usually develop from the single male worker stage, or from several worker stages, if these exist (e.g. *Nasutitermes*, *Hospitalitermes*)(346-349). Sometimes they are derived from a special larval line leading to the single soldier morph in *Tenuirostritermes*, to small soldiers in *Trinervitermes*, *Velocitermes*, and probably *Longipeditermes*. In these species, large soldiers develop from the single male worker stage(16,91,350). Soldier dimorphism may also be based on sexual dimorphism of workers; e.g. in *Nasutitermes exitiosus*, the non-combative large workers are females, the small soldiers are males. Sexually dimorphic soldiers occur also in *Leptomyxotermes*(8,57,199). And finally, the third group, with typical genera *Subulitermes* and *Coatitermes*, has a weak sexual dimorphism with a single worker stage or several stages; soldiers develop from both sexes, more often from male workers.

Three larval stages precede a single worker stage in Macrotermitinae. Sexual dimorphism is marked and inverse compared to majority of other Termitidae, i.e. males are larger. Soldiers are of the female sex with the exception of *Sphaerotermes*. They can be (i) monomorphic, as in *Sphaerotermes*, *Protermes*, *Microtermes*, and *Odontotermes*, where they derive from the larval stage preceding the worker, though according to Grassé(30) several species with dimorphic soldiers can be listed among these genera; (ii) dimorphic, such as in *Macrotermes*, *Bellicositermes*, *Ancistrotermes*, and *Pseudacanthotermes*, where large soldiers develop from female workers; or (iii) trimorphic in *Acanthotermes acanthothorax,* due to origin of the third form in a specialized larval line(351-353).

General trends in the evolution of caste systems and soldier polymorphism

Ecological and phylogenetic determinants can be applied when attempting to explain the occurrence and maintenance of diverse developmental patterns and resulting polymorphism in workers and soldiers. The ecologically primitive and basal lineages of Kalotermitidae and Termopsidae, together with more advanced rhinotermitid genera *Prorhinotermes* and *Termitogeton*, share primitive nutritional and nesting habits, with low stability of resources and limited diversity of inhabited environment. The great flexibility of individual developmental options, lack of irreversible task specialization in form of permanent worker caste, and the absence of sexual specialization and dimorphism, correspond well to these determinants. The soldiers developing from both sexes of larvae, pseudergates, and nymphs, reveal only a size polymorphism accompanied by slight allometric differences based on the size of the original instar. Anatomic, behavioural and/or spatial specialization among soldiers is lacking. This situation is largely acknowledged as primitive in the evolution of termite caste patterns(8,42,43,187,295,296,306,354,355).

The separation of nesting and food resources results in an increased stability of societies and diversification of environment linked with foraging for food and building activities. These circumstances accompany the occurrence of the distinct true worker caste in termites and the polymorphism in workers and soldiers, characterised by (i) sexual dimorphism and specialization; (ii) instar-based polymorphism, and (iii) polymorphism within an instar. All these phenomena have very probably evolved independently and polyphyletically in the lineages with true workers (see Figure 3). The polymorphism and sex specialization is ubiquitous among these taxa, and it is rare to find examples with egalitarian sex ratio in monomorphic castes of workers and soldiers, e.g. *Reticulitermes* (Rhinotermitidae) or *Amitermes* (Termitinae). None of the two sexes seems to be predestined to develop in large or small workers and/or soldiers. Correlation can be drawn between sexual dimorphism of workers, often combined with instar-based polymorphism, and sound-wood feeding or harvesting strategy. Within Macrotermitinae, harvesting species display a higher rate of polymorphism; the same rule applies for many Nasutitermitinae. Moreover, also the intra-instar variability appears to be greater in the species with diversified environment(5,8). The wood-feeding versus humus-feeding strategy can be considered as another trend-setting factor. In humus-feeding species an obvious trend to monomorphic workers appears due to the absence of sexual dimorphism or absence of one sex among workers. Also, the workers do not increase in size during moults to higher instars or do not moult at all(5,8,356).

Polymorphism of the soldier caste is subjected to analogous selective forces when facing the diversified environment of harvesters and centralized-nest builders. Their polymorphism reflects all types of polymorphism of larvae and workers from which they develop. In addition, specialized developmental lines occur, leading to additional soldier morphs. On the other hand, as already mentioned, the simplification of caste patterns in humus feeders might be responsible for the loss of the soldier caste(5,8,356).

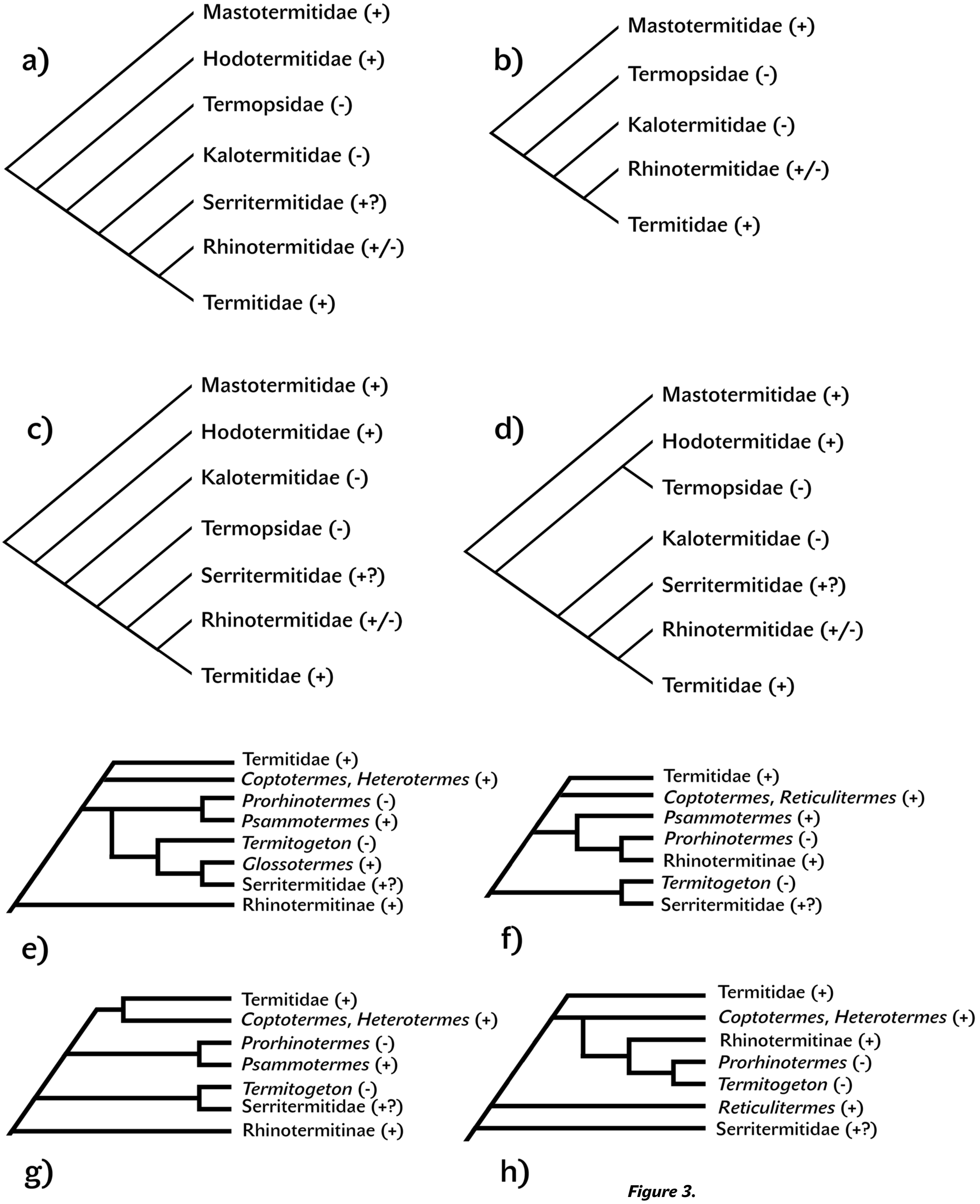

Figure 3.
Major phylogenetic hypotheses concerning the relationships among termite families (a-d) and within the family Rhinotermitidae (e-h). Redrawn and modified from a) 323; b) 322; c) 514; d) 324; e) 528; f) 529; g) 325; h) 530. Presence or absence of true worker caste is indicated in brackets.

It should be noted here that alternative scenarios of the evolution of the worker caste and the resulting polymorphism exist, even in very recent studies. Watson and Sewell(303,304) assigned in early 1980's a derived status to the linear developmental model with temporarily specialized pseudergates. This caste pattern was described as being an efficient adaptation to unstable conditions in one-piece type termite species. This approach was disclaimed in several subsequent papers and the derived origin of the true workers is widely recognised, although it implies a multiple origin of the worker caste(295,296). Nevertheless, this assumption was recently challenged by molecular phylogenetic analysis suggesting the most parsimonious resolution to be a single origin of the worker caste and its multiple loss(324). Also the ecological significance of the linear developmental model and related caste system was infirmed. The implications of this hypothesis are subjected to present-day discussions(324,325,357-359). Only recently, another extensive reconstruction of termite phylogeny has been published by Inward et al.(325), suggesting that the multiple origin of the true worker caste is as likely event in the evolution of present caste patterns as the multiple loss of workers. Moreover, the authors have confirmed the perfect correlation between the nesting types and presence or absence of the worker caste. They concluded, just as Shellman-Reeve(43) a few years earlier, that the ecological constraints reveal to be a more powerful determinant of termite caste patterns than the phylogenetic relationships among termite groups. Consequently, mapping the trait by parsimony may be misleading (see Figure 3).

DEVELOPMENT OF SOLDIERS IN INCIPIENT COLONIES

The production of soldiers differs fundamentally between incipient and mature colonies, both in the proportion of the soldier caste and in their instar origin. In species where multiple instars are competent to differentiate into soldiers, earlier instars develop in first(s) soldier(s) in incipient societies. As the colony matures, it goes through a transition from the production of soldiers from early instars to late instars. Despite of the scarcity of precise evidence from incipient colonies, this rule seems to apply universally for the species with linear development, but also for numerous species with forked development. In lower termites, the first soldiers most commonly develop from the larvae or workers of the third instar, as e.g. in several Rhinotermitidae (*Reticulitermes*, *Schedorhinotermes*, *Parrhinotermes*)(34,299,300,336), Kalotermitidae (*Kalotermes*, *Neotermes*)(87,185,298), Termopsidae (*Porotermes*)(316). In *Mastotermes* the fourth instar was recorded to differentiate into soldiers(88). Rarely, the development of minute first soldiers from as early as the second instar was recorded, as in the kalotermitid *Kalotermes* and probably in the rhinotermitid *Prorhinotermes*(35,87). The anatomy of the first soldiers in lower termites does not differ substantially from that of the soldiers derived from late instars. Nevertheless, they are considerably smaller and some allometric differences can be observed in the head shape, which increases relatively in its width in higher instars due to the need to harbour powerful muscles(88).

The development of incipient colonies was also studied in several species of higher termites. The individuals in these young societies are constantly smaller than those in the mature colonies. Nevertheless, the workers and soldiers develop along identical developmental pathways as in the adult colonies, as has been proved in *Amitermes*, *Cubitermes* (Termitinae)(7,360), *Tenuirostritermes*, *Trinervitermes* (Nasutitermitinae)(91,361), *Macrotermes*, and *Ancistrotermes* (Macrotermitinae)(351,362,363). The correlation between individual weight and the size of the colony is well established; the size differences can be explained by a considerably shortened duration of the development of particular instars in incipient colonies(293,364,365). However, in rare cases, the soldiers in incipient colonies are produced from an earlier instar that in the mature ones, as e.g. in *Pericapritermes urgens* (Termitinae)(7,293) or *Nasutitermes ephratae* (Nasutitermitinae)(366). The first soldiers in these species develop from the larva of the second instar normally preceding a worker. The development of small soldiers from the second instar of a specialized larval line is normal in macrotermitine *Acanthotermes acanthothorax*; these soldiers are the most abundant soldier category from the trimorphic set regardless of the age of the colony(7,293). In species with polymorphic soldiers, the smallest morph usually differentiates in first soldiers; the bigger morphs develop in soldiers only after the colony reaches a certain size(293,351,363).

The first soldiers often inhibit the development of further soldiers for a long time, and remain, together with the royal pair, the only defenders of the colony during its the early stage(89,90). When watching these so-called *nanitic* soldiers, developing even in the presence of older instars that could become soldiers, two questions necessarily arise: 1) „Why this dynamics in instar origin of soldiers appear in numerous species, often very different in many aspects of their biology?“, and 2) „What is the mechanism controlling the colony-age dependent differentiation of soldiers from different instars?" The first question remains unanswered, though few interpretations can be hypothesised. Proximate physical constraints may explain the need of small soldiers when the colony is young and bigger soldiers as the colony grows older, for instance to fit better to the galleries made by the workers or pseudergates of increasing instar age and size. The influence of the selective forces on the optimization of soldier head size was proposed by Matsuura for *Reticulitermes speratus* (Rhinotermitidae), where the author documented a stabilisation of the head width, compared to other body parameters, in soldiers performing phragmotic behaviour(124). The author later extended this approach and tried to explain also the biased sex proportions of soldiers by the selection for the bigger sex, i.e. females, driven be the need of an optimal head size of soldiers(367).

In this context, one could accept the need of optimal head size as a driving force of the colony-age dependent origin of soldiers. But it does not explain at all the situation in the non-phragmotic majority of species. Few attempts have been made to explain the adaptive value of the soldier instar origin in terms of inclusive fitness gained as a result of alternative developmental decisions. According to Shellman-Reeve, a pseudergate or worker should maximize its inclusive fitness in flourishing colonies by helping as long as possible as a worker (pseudergate), and only after proceed into the soldier role(43). In consequence, the soldiers should derive in preference from older instars. In reality, the soldiers usually really are among the oldest individuals of the colony, but still this hypothesis does not help to explain the soldier instar origin in incipient colonies(368).

Horiuchi et al.(369) elaborated a mathematical model integrating the demographic characteristics of incipient versus mature colonies in lower and higher termites, and asked the question: „Who should become soldier, young or old instar?" The (future) inclusive fitness of colony members in starting colonies is in the model defined by the rate of population growth allowing a colony to mature within short time. In this fashion, the colony can avoid an accidental death and start earlier the second period of its life: the production of winged imagoes. Based on the predictions of the model, the production of soldiers from young instars should be favoured when (i) the ratio of young undifferentiated instars is high and the workload of helpers is high; (ii) there is a need of numerous soldiers for the defence of colony members, and (iii) the increase in size due to moulting is more useful for a helper than for a soldier in the tasks they perform. Indeed, these predictions seem at the first view very plausible. First, in one-piece type species, the fertility of the queen(s) is limited as well as the capacity of the nest to harbour an extremely growing population. Thus in time, the ratio of young undifferentiated instars decreases. Second, the proportion of soldiers needed in one-piece type species decreases with the growth of colony population due to the limited surface to be guarded. In contrast, in species foraging for food a constant ratio of soldiers to workers seems necessary(20). Consequently, in one-piece type termites the production of soldiers should switch gradually from early to late instars, whereas the separate type species with a constantly very high brood production and high need of defence of foraging columns should produce soldiers predominantly from young instars. The principal deficiency of the presented model is the third prediction, which is crucial and at the same time very hard to quantify and test. Moreover, the colony-age dependent strategy of soldier production is known also in separate type species as noted above.

The theory by Horiuchi et al.(369) was criticized by Crosland et al.(86), and a simpler one was proposed. The authors infirm several major assumptions of the model due to their poor support, i.e. (i) the assumption that the soldier ratio is decreasing in one-piece type termites is an oversimplification, because the fewer soldiers derived from later instars are bigger; therefore the investment in defence should be measured in biomass; (ii) the need of work force provided by helpers is always high, because maturing one-piece type colonies start to produce winged imagoes; (iii) in species where the fertility of the queen would be considerably limited, the strong pressure toward physogastry, and/or the reproduction by multiple supplementary queens, would undoubtedly compensate the low fertility of one queen. Crosland et al.(86) recall the functional explanation of the gradual change in the soldier instar origins, i.e. the need of optimal body proportions, namely the head width and height, to fit accurately to the galleries of the nest. This allows the soldiers to (i) move freely across their nest and (ii) perform the phragmotic defensive behaviour. Given the current state of our knowledge, none of the presented hypotheses can satisfactorily explain this curious phenomenon, which does not seem to be strictly linked with one-piece type nesting. Moreover, the defensive efficiency of the nanitic soldiers appears ridiculous when compared with the manifold bigger soldiers derived from older instars.

The second question, concerning the proximate mechanisms of the control of colony-age dependent soldier production, is even more difficult to answer because we lack any information about the specificity of hormonal and pheromonal stimulation in incipient colonies. We can therefore only hypothesize a specific pheromonal influence of the reproductives on young larvae in incipient colonies and/or a specific sensitivity of these larvae to morphogenetic hormones. These topics are discussed below in Chapter IX.

MORPHOGENESIS OF SOLDIERS

The soldier is always a terminal instar, though formally it has to be classified as a juvenile stage, because it possesses developed moult glands. Nevertheless, the extreme anatomic specialization definitely prevents it from further moulting to higher instars(292). The complicated morphogenesis of a soldier, resulting in an abrupt change of internal and external anatomy, occurs always in two successive steps, in a very consistent pattern, common to all termites. The first moult leads to an intermediate developmental stage of presoldier (*white soldier* or *soldier-nymph*). The presoldier is unpigmented, unsclerotized, and fully dependent on the care provided by helpers in form of liquid salivary food. Moreover, the presoldiers are inactive and cannot perform any defensive behaviour though they are able to walk and move from one place to another within the colony, as is common in immatures of hemimetabolous insects.

It is obvious that the stage of presoldier has no functional significance for the colony, and it is only a transitional stage required to accomplish the intricate rebuilding of body structures, particularly the cephalic part and the internal organs. In two successive moults, a dramatic change in anatomy occurs, unprecedented in Hemimetabola. During this stage, the constant care of helpers allows an accumulation of nutrients in the fat body; the body mass may increase by 60 or more percent(30).

The duration of the presoldier stage is very short and surprisingly consistent in all termites. It ranges most often from 10 to 15 days(7,15,34,352,370,371), rarely it has been reported to be shorter, as in incipient colonies of *Nasutitermes ephratae* (Nasutitermitinae)(366), or longer, e.g. 20 days in mature colony of *Macrotermes michaelseni* (Macrotermitinae)(372) or 22 – 25 days in *Cubitermes heghi* (Termitinae)(7,373). Prior to the moult to the soldier stage, the presoldier stops all activities and remains in a static posture in a so-called „hypnosis“. This phase may represent more than a half of the presoldier stage duration, and is apparently accompanied by important internal morphogenetic events(30). Due to the two-step mode of development via an inactive stage, and because of a development of fundamentally new structures during this process, the soldier development was accurately compared to the metamorphosis through the pupal stage of holometabolous insects(373).

Few studies were devoted to the dynamics of the morphogenesis of soldiers with regard to the two successive moults(231,374-378). During the whole process, completely new structures appear, such as the frontal gland reservoir or the nasus in Nasutitermitinae; other structures are greatly modified or subjected to a growth, which is unprecedented in successive instars in Hemimetabola. For instance, the head can grow in length by 80% and mandibles by 180% in *Kalotermes flavicollis* (Kalotermitidae), and undoubtedly more in species such as *Promirotermes* (Termitinae); the nasus of nasutitermitine soldiers can easily exceed the length of the whole head in numerous species, not only in the accurately named *Cyranotermes*(231,376,378,379). The overall growth of the body may be very important in mandibulate soldiers, in many cases they are clearly the biggest non-reproductive individuals of the colony; e.g. the body mass of *Cubitermes* (Macrotermitinae) soldiers exceeds by 55% that of workers from which they developed. It is already mentioned above that the nasute soldiers usually do not grow dramatically, and are sometimes even smaller than the previous instars. During the two moults the antennal segments are usually added, most often one or two(212,34,300). But opposite trend was recorded in rare cases toward a constant number of segments, or even a reduction of their number, as e.g. in *Tenuirostritermes* (Nasutitermitinae)(26,34,212,352,375).

The morphogenesis of the external structures occurs mainly during the first moult from an undifferentiated individual to a presoldier(231,375-378). The growth of the mandibles in future mandibulate soldiers is the *primum movens* of the whole morphogenetic process, including a necessary hypertrophy of mandibular musculature and consequent growth of the head. This growth is highly allometric, resulting in dramatically different shape of the head. By the first moult, the head as well as the mandibles greatly elongate, the growth of the head in width is less pronounced. By the second moult, the mandibles grow in length again, but less than during the previous moult. Also the head length and width grow considerably, mainly in the posterior part of the head. The mandibles elongate allometrically, the growth in the apical part is more pronounced than that in the basal part. The shape of the labrum is modified in each of the moults, from the worker-like labrum with round anterior margin to the most common lanceolate shape through an intermediate appearance in presoldiers(231,375-378).

It is prior to the moult to presoldier when the new cuticular structures develop and must fit into the old cuticle of undifferentiated individual. Consequently, the typical multiple folding of the new cuticle and epidermis within the old cuticle can be seen. The presoldier's mandibles are folded in much smaller and shorter gnawing mandibles of a worker, pseudergate, larva, or nymph. This folded epidermis and cuticle form conspicuous large concavities between the marginal teeth, allowing a future elongation and straightening of the new mandible in presoldier. The presence of multiple folding and compression of epidermal cells, and their subsequent expansion

and unfolding of the whole cuticle after the ecdysis, were compared by Koshikawa et al. to the differentiation of imaginal discs in Holometabola. Once again, a parallel with the holometabolan metamorphosis was drawn(377). The same conclusion has been made about the development of the nasus in Nasutitermitinae. The time period shortly prior to the moult from worker to nasute presoldier is characterised by a formation of *soldier-nasus disc*, a disc structure constituted by multiple folds of the new epidermis of the future presoldier. This structure and its formation has been compared with the formation of imaginal discs in prepupal stage in Holometabola(348,374).
The development of internal organs in presoldiers is rather unknown; the available information concerns the frontal gland. Its reservoir is partially developed in presoldiers of e.g. *Globitermes sulphureus* (Termitinae), *Prorhinotermes simplex*, and *Reticulitermes santonensis* (both Rhinotermitidae)(64,231,232), and one may suppose that to a certain extent it is developed also in presoldiers of other species with frontal gland. Logically, the gland should be non-functional in the presoldier stage, nevertheless, in *R. santonensis,* some of the compounds typical for the soldier frontal gland secretion were identified in lower amounts in heads of presoldiers(231). Among other internal organs, the variations in size and activity were observed in the endocrine glands involved in the regulation of moulting cycle, caste differentiation, and morphogenesis, i.e. moult glands and corpora allata. These dynamics are described in Chapter IX.
It is only after the second moult to the terminal soldier stage, that the cuticle gets its sclerotization and pigmentation, and the typical mandibular musculature develops to operate mandibles or participate in the emptying of the frontal gland reservoir. Subsequently, the frontal gland, if present, starts the biosynthesis of the definite defensive and alarm substances. Also the soldier-specific defensive behaviour appears, subjected to eventual dynamics in time as is described in Chapter V. Despite of the specialization to risky tasks, soldiers often have a long life span, especially in one-piece type termites, where they can be the oldest individuals in a colony. In colonies of *Incisitermes schwarzi* (Kalotermitidae), in which all other members have been already replaced by offspring of the secondary reproductives, the present soldiers were the offspring of the primary reproductives. Their life span could have been estimated to be over five years(368). The soldiers of *Zootermopsis* (Termopsidae) are also supposed to live up to four or five years(315,320), the same life span was found in soldiers of *Reticulitermes* (Rhinotermitidae), reared under laboratory conditions(34). In numerous other species, the life span of soldiers is estimated to be less than four years, and in Termitidae usually less than two years(15,372,380,381).

The genetic background of the caste differentiation of soldiers

The caste differentiation in termites is considered as a differential gene expression of a common genetic pool, allowing the expression of particular caste phenotypes from the polyphenic set of castes(292,302,382). Therefore, thanks to the recent progress in the field of molecular biology, the attention of numerous scientists is attracted by eventual caste-specific genes, characteristic for particular castes, which could document this theoretic frame. In the last few years, several caste-specific genes and/or proteins have been identified, i.e. soldier-specific genes in mandibular glands and developing mandibles in *Hodotermopsis japonica* (Hodotermitidae)(383,384), soldier-specific proteins and genes in a nasute *Nasutitermes takasagoensis*(385-387), and caste specific cytochrome-coding gene expressed in pseudergates and soldiers in *Hodotermopsis sjostedti* (Termopsidae)(388). The caste-specific genetic sets were characterised also for reproductives in *Cryptotermes secundus* (Kalotermitidae)(389). A large set of studies has been recently published, evidencing numerous structural, regulatory, and enzyme-coding genes in association with particular castes, including presoldiers and soldiers in *Reticulitermes flavipes* (Rhinotermitidae)(390-396).

VIII. SOCIAL REGULATION OF SOLDIER DEVELOPMENT

Social regulation mediated by primer pheromones

It has been already shown above how environmental factors such as temperature, food availability, or season affect the social homeostasis reflected in proportions of castes in the society, including the ratio of soldiers. Another regulatory level of individual ontogeny is represented by the social regulation performed by the presence or absence of individuals belonging to particular castes. The gene expression of particular caste phenotypes in undifferentiated individuals is subjected to a multitude of social influences, mediated by a network of putative pheromonal stimuli. The observations of social regulation in termites created a direct inspiration for the introduction of the term *pheromone*, broadly used nowadays for all forms of intraspecific chemical communication(397). The hypothetic substances, responsible for social regulation in termites, were labelled with various names, such as *caste pheromones* or *social pheromones*. They correspond perfectly to the definition of *primer pheromones* used for chemical stimuli affecting physiology, morphogenesis, and ontogeny(11).

Social stimulation and inhibition

Among social regulatory mechanisms both stimulation and inhibition of caste differentiation by the presence of particular caste members may occur. A well-documented phenomenon is the stimulation of the differentiation of soldiers by the presence of reproductives. Such an enhancement of soldier production and proportions was observed in *Kalotermes flavicollis* (Kalotermitidae), when groups of pseudergates were reared in presence of functional reproductives. The moults into presoldiers increased more than five times. The influence of the queen is twice as high as that of the king(398). A similar situation was described in two *Zootermopsis* species (Termopsidae)(399) as well as in *Prorhinotermes simplex* (Rhinotermitidae), in which the addition of neotenic reproductives stimulated the differentiation of nymphs into soldiers, and the frequency of moults increased approximately three times(36). In higher termites, the stimulation by the presence of reproductives has been proved in species with fundamentally different patterns of soldier production and proportions: soldier production increased two times in *Nasutitermes lujae* (Nasutitermitinae) and three times in *Cubitermes fungifaber* (Termitinae) when groups of workers were held with a royal pair or one of its members(400). The stimulation of the production of soldiers by the presence of late nymphs was observed in *Reticulitermes lucifugus* (Rhinotermitidae)(79).

The fundamental principle of the social regulation is without a doubt the inhibition of the formation of castes. This inhibition allows the establishment of a feedback regulatory mechanism complementary to the caste stimulation by reproductives, creating a balanced homeostatic system. It has been demonstrated in many cases that the presence of soldiers inhibits the formation of further soldiers. Already in incipient colonies, the first soldier(s) perform(s) an efficient inhibition of the development of soldiers, often as long as one or two years, as e.g. in *Kalotermes* (Kalotermitidae)(87), *Zootermopsis* (Termopsidae)(89), or *Reticulitermes* (Rhinotermitidae)(90). If this first soldier is removed, it is repeatedly replaced within few days. The same rule applies in larger groups reared without soldiers or with a suboptimal number of soldiers: new soldiers appear until the optimum is reached. The present soldiers, on the other hand, inhibit the differentiation of soldiers as has been documented for instance in kalotermitids *Kalotermes flavicollis* and *Neotermes jouteli* (Kalotermitidae)(181,398), rhinotermitids *Reticulitermes flavipes*, *Coptotermes formosanus*, *Prorhinotermes simplex*, and *Schedorhinotermes lamanianus*(33,36,37,80,300,401,402), but also in higher termites, such as *Nasutitermes lujae*, *N. nigriceps* (Nasutitermitinae), or *Macrotermes michaelseni*(79,403,404,353). The regulatory function of soldiers on the occurrence of other soldiers was observed also in the field, for instance in *Macrotemes bellicosus* (Macrotermitinae). Newly moulted soldiers appeared in high numbers within 26 days after an important loss of the soldier force by the colony(283). In the case of soldier inhibition, a direct contact between undifferentiated individuals and the soldiers is required(353,398).

Source and chemical identity of primer pheromones

The above presented mechanisms of social regulation of soldiers are quite comprehensive and are based on numerous predictable empirical experiences with a large set of species. But the situation becomes complicated when we ask for the functional aspects of its mediation. In other words, the results identifying the particular compounds, responsible for soldier inhibition, are very scarce compared to the experimental evidence of this phenomenon. Logically, the eventual primer pheromones are the first candidates for such a function in the blind societies of termites. Various models of social regulation based on pheromones were elaborated, namely by Lüscher(312). Only a few cases have been studied so far, with results that are rather difficult to generalize and interpret. A hypothesized source of the inhibitory primer pheromone was searched for in the frontal gland of chemically defended species. In *Schedorhinotermes lamanianus* (Rhinotermitidae), Renoux(300) has shown that the body washes of soldiers prevent larvae from developing into soldiers. Further research indicated tetradecenone, present in the frontal gland, to be responsible for this effect. The inhibitory activity, however, was accompanied by increased mortality; therefore the decrease in soldier production can hardly be separated from the toxic effect of the compound. In another chemically defended species, the nasute *Nasutitermes lujae*, Lefeuve and Bordereau clearly demonstrated an inhibitory activity of soldier body extracts and frontal gland extracts on the differentiation of further soldiers. Moreover, the authors proved that the inhibitory effect requires a direct contact of the undifferentiated individual with the chemical stimulus(404). This implies, in agreement with a few other studies, a low volatility of the putative primer pheromone(80). They also hypothesized that this primer pheromone should act (i) via ingestion and direct inhibition of the endocrine glands involved in morphogenesis and caste differentiation, or (ii) via the sensory system, i.e. contact chemoreception, on the neurosecretory system of the recipient's body(404).

The promising results of the mentioned study however imply one major difficulty. They cannot be generalized for all termites because only the advanced lineages possess the frontal gland. Given the uniformity of the formation of the soldier caste, two fundamentally different ways of mediation of the caste inhibition should have evolved in termites. Moreover, subsequent research brought even more ambiguous results. Okot-Kotber et al.(401) reported the inhibition of soldiers in *Reticulitermes flavipes* (Rhinotermitidae) effectuated by the frontal gland extract of its soldiers but also of soldiers from three species of higher termites. The authors concluded that the inhibitory substance could be one compound, used in a broad range of species and acting in an unknown way. The results of this study are hard to accept as a general rule given the obvious toxicity of soldier frontal gland secretion to non-nestmates. This can, once again, be demonstrated by an increased mortality in the experimental groups when compared with inhibition performed by living conspecific soldiers. Korb et al.(405) described recently the inhibitory effect of the head extracts on the differentiation of soldiers in the kalotermitid *Cryptotermes secundus*, which is, of course, devoid of the frontal gland. Though the numeric values of the inhibition were not extremely convincing, probably due to a long duration of the experiment, it has been shown that the source of the eventual pheromone is located on the head or inside the head(405). This was suggested already by Grassé, who pointed out the presence of unicellular glands on the head surface of soldiers, and assigned to them the probable function as the source of pheromonal substances(98).

I cannot omit mentioning the existence of dramatically different scenarios of the pheromonal regulation of caste differentiation. These were supposed by Lüscher, who proposed that juvenile hormone, the key morphogenetic hormone involved in the caste differentiation, plays the role of the primer pheromone mediating the social regulation(406). Though Lüscher himself later rejected this hypothesis and it is not broadly accepted, it was repeatedly invoked by several other authors(185,407). In conclusion, it should by recalled that despite the rich evidence of the social regulation of soldiers, its pheromonal background remains enigmatic, as well as the chemical identity of the responsible compounds and their source.

IX. ENDOCRINE ASPECTS OF THE DIFFERENTIATION OF SOLDIERS

The fundamental role of the juvenile hormone

The proximate level of the control of caste polyphenism in termites, subjected to both environmental and social stimuli, is represented by the endocrine system of individuals. It is responsible for the onset of moults and the differential gene expression of particular phenotypes in response to extrinsic and physiological factors. The endocrine regulation operates on several functional levels, such as the titer of morphogenetic hormones, threshold of sensitivity of tissues, the timing of hormone secretion, the timing of hormone-sensitive period, and finally the cellular response in form of gene expression(301). The crucial role in the control of caste polyphenism at the endocrine level is without a doubt played by the juvenile hormone (JH), the sesquiterpenoid secreted by the endocrine glands *corpora allata*.

JH is a key morphogenetic hormone of insects involved in (i) orchestration of metamorphosis in concert with the moult-inducing ecdysteroids; (ii) regulation of female fertility by stimulating vitellogenin synthesis in the fat body and its uptake by the growing oocytes; (iii) regulation of polyphenism, including the sophisticated caste polyphenism in social insects. Many attributes were given to this omnipresent hormone, such as *status quo* and *juvenile* hormone due to its activity in preventing an insect from precociously turning into an adult, or *pleiotropic* hormone because of the multitude of its roles(408).

The specific role of the JH has been demonstrated in the control of different types of insect polyphenism, e.g. the wing polymorphism in crickets, the phase polyphenism in locusts and aphids, and finally in the caste polyphenism of both dominant groups of eusocial insects: hymenopteran eusocial representatives and termites. The current state of our knowledge indicates that JH is deeply entrenched in practically all aspects of social life in social insects, affecting the generation not only of different phenotypes, but also of neural and resulting behavioural plasticity. In Hymenoptera, for instance, JH is responsible for the caste determination between workers and queens in honey bees and ants, and between the particular morphs in ant workers. Moreover, it is known to regulate the temporal polyethism during the life of honey bee workers(301,302,382,408-412).

The fundamental role of the JH in the triggering of particular alternative phenotypes during the post-embryonic development in Isoptera is well established since several decades. Especially the object of my interest in this text, i.e. the differentiation of soldiers, was the first to be linked with the action of JH. Lüscher in his pioneering transplantation of corpora allata from functional reproductives into pseudergates of *Kalotermes flavicollis* (Kalotermitidae), observed within several days their moulting into presoldiers(413,414). This approach, extended later by Lüscher and Springhetti(415) and Lebrun(370,416) by transplantations of the glands from different castes and also from *Periplaneta americana*, revealed a relationship between the activity of the corpora allata of the donor and the outcome of the subsequent moult of the recipient. The enlarged corpora allata of reproductives, preimaginal nymphs, and adult cockroach females in vitellogenesis stimulate the differentiation of soldiers, whereas the corpora allata from pseudergates and nymphs far from imaginal moulting do not. At the same time, the implantations of active glands prevent individuals from developing into neotenic reproductives(370). Thus, the first coincidence between the production of JH, and the development of soldiers was established. This concept was subsequently confirmed by the first experiments with application of exogenous JH to *K. flavicollis*, revealing an intense capacity of this hormone to trigger the development of various individuals toward soldiers(417).

JH AND ITS ANALOGUES

Extensive research of JH and its functional analogues (JHA) has followed since the early 1970's, proving the capacity of various, often structurally diverse compounds, to stimulate soldier production. The studies, reviewed by Howard and Haverty in 1979(418) and Su and Scheffrahn in 1990(419), covered a large spectrum of compounds and termite species, primarily of lower but also a few higher termites, e.g. *Nasutitermes* (Nasutitermitinae) and *Macrotermes* (Macrotermitinae)(79,420-422). From queens of the later genus, more precisely of *M. michaelseni*, the JH of termites was isolated and identified as JH III in 1976(423). The common motivation of these studies was the quest for an ideal compound for practical use in termite pest control, based on the idea of stimulation of development of superfluous soldiers in the society, and subsequent disruption and collapse of the whole colony due to the high food requirement of soldiers and lack of work force(424).

Besides this primary goal, these investigations brought more precise insight into the endocrine regulation of caste differentiation in termites. Apart from other consequences, the following important phenomena were repeatedly observed in isolated, i.e. orphaned and soldierless, groups of workers or pseudergates treated with JH or JHA: (i) precocious development of superfluous soldiers in the groups, often even from stages that normally do not develop into soldiers; (ii) general increase of moulting events compared to control groups; (iii) decrease in the production of neotenic replacement reproductives; (iv) formation of intercastes sharing the traits of the original caste and the soldier caste; (v) direct mortality through toxicity and indirect mortality due to failure during moulting, loss of intestinal symbionts, starvation, or cannibalism(79,185,331,378,401,420-422,424-440). Complementary information was also provided by experiments documenting the ability of soldiers, initially present in experimental groups, to reduce the effect of the applied JH or JHA(79,401,441,442). Last but not least, by blocking the synthesis or action of endogenous JH or exogenous JH and JHA with precocenes, called *anti-JH* or *JH antagonists*, the decrease of the rate of soldier differentiation was observed(405,439).

CONCEPT OF JH-DEPENDENT CASTE DIFFERENTIATION CONTROLLED BY SOCIAL REGULATION

The raising evidence of the role of JH resulted in establishment of a theoretical concept of JH-titer dependent pattern of caste differentiation in termites, presented by Yin and Gillot(428), Stuart(443), and Lebrun(444). This concept applies above all to lower termites, which possess undifferentiated instars with large developmental options. A high titer of JH, exceeding a certain threshold, is required for soldier differentiation. For the development of sexual and adult traits, a decrease in JH titer is necessary, including a dramatic decrease preceding the imaginal moult of nymphs. This aspect is in agreement with the general pattern of post-embryonic development in insects, accompanied by a gradual decrease of JH titer. A substantial decrease in JH titer induces the expression of sexual characters and results in the development of neotenic reproductives, whereas an increase can lead to regressive moulting of nymphs. Medium titer of JH precedes a stationary moult.

But the mechanism of caste differentiation is not as simple as to depend on the activity of one hormone from one endocrine gland. In other words, the impact of the JH titer on morphogenesis can manifest only during a moult, and the process of moulting is also subjected to endocrine control. As in other insects, the onset of the moult is triggered by the ecdysteroids secreted from the moult glands. The stimuli triggering the development into a presoldier appear to stimulate the moult glands, as can be inferred from (i) the shorter duration of the instar preceding the moult into presoldier when compared to moults to other castes; (ii) the increase in the number of moults in soldierless or JH (JHA) treated groups. This prothoracicotropic effect is most often explained by the action of the JH itself. The corpora allata, stimulated by intrinsic and extrinsic factors, produce JH, which is responsible for (i) the switch of the gene expression toward the soldier phenotype, and (ii) the onset of the moult by activation of the moult glands(231,292,301,429). The predictions concerning the endocrine regulation of caste differentiation of soldiers are documented by the dynamics in the size of the two glands and titers of JH and ecdysteroids, as described below.

The concept of JH-titer dependent caste differentiation could have been consequently incorporated to the framework of the known social regulation by primer pheromones. The current scenario could be summarized as follows: the presence of soldiers in a group acts against the activation of corpora allata in undifferentiated individuals,

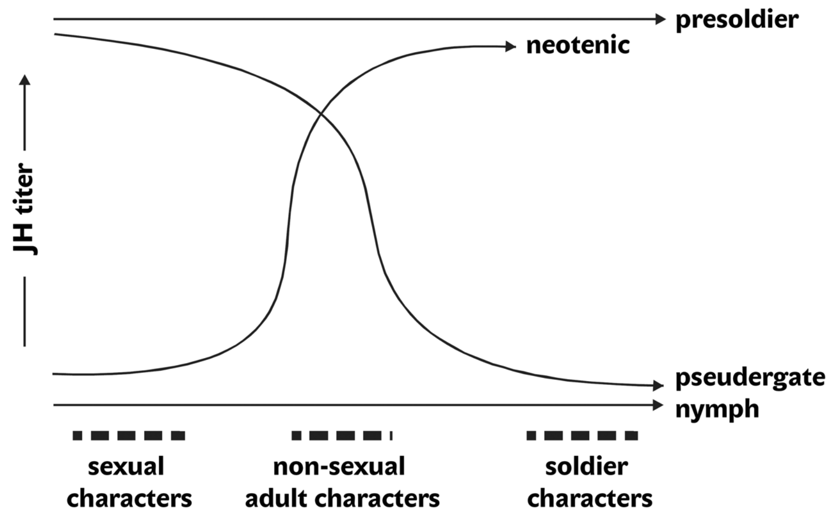

Figure 4.
Model for the endocrine control of caste determination, based on the lower termite *Kalotermes flavicollis* (Kalotermitidae).
Each curve represents a hypothetical JH-titer profile during the course of a pseudergate instar prior to moulting to the caste indicated by the arrow. Bars show the timing of the JH-sensitive periods for various characters.
Redrawn from 301 and modified.

whereas the presence of reproductives stimulates their activity. By the same mechanism, the functional reproductives prevent other nestmates from the development into neotenic reproductives(291,292,444-446). The extrinsic social regulation could hypothetically act not only on the corpora allata, but also directly on the moult glands. The inhibition of soldier development, for instance, could be achieved by an inhibition of the moult glands, which could prevent the individual from moulting into presoldier. This hypothesis can be demonstrated by examples from higher termites, where soldiers derive exclusively from certain stages; the production of soldiers is regulated by the activation or inhibition of moult events in these stages. Such a slowing down of moults in response to inhibitory stimuli has been observed e.g. in small workers of *Nasutitermes lujae* (Nasutitermitinae)(292,404). Surprising results were recently published by Mao and Henderson(371). These concern the delay between isolation of workers in homogenous groups and the occurrence of the first presoldiers in *Coptotermes formosanus* (Rhinotermitidae). This delay displayed marked seasonal variations from 9 to 30 days with a clear trend to be the shortest in the period before and during the regular swarming. The authors hypothesize the adaptiveness of this seasonal pattern but do not propose the endocrine background for these variations. Undoubtedly, evaluation of relationship between the hormonal titers and this variation in developmental time would contribute to better understanding of the phenomenon.

COMPETENCE PERIODS FOR DIFFERENTIATION INTO SOLDIER, FORMATION OF INTERCASTES

Numerous studies on caste differentiation under various conditions suggest that the response of undifferentiated individuals to the extrinsic and intrinsic factors varies during the intermoult period. Another dimension had to be added in the puzzle of termite caste differentiation. It was well established by Lüscher's and Lebrun's experiments that the competence for differentiation into a neotenic is at the very beginning of the pseudergates instar in *K. flavicollis*. In later period of the instar, the pseudergate does not respond to orphaning by moulting into neotenic(312,370,447). Also the competence for soldier differentiation differs during the intermoult. Surprisingly, the data about the *competence period* for soldier differentiation is not consistent in all studied species, despite the general similarity of soldier development. In pseudergates of *K. flavicollis* and *Zootermopsis*, the highest competence is situated in the second half of the instar(429,448), whereas in *Reticulitermes flavipes* (Rhinotermitidae) and *Macrotermes michaelseni* (Macrotermitinae) it is at the beginning of the intermoult(422,426). On the other hand, the excessive doses of JHA applied to termites can induce the moult in individuals out of the competence period. This is apparent from sometimes nearly 100% differentiation of presoldiers or presoldier-like individuals in JHA treated groups(440,449).

The concept of competence periods, known also in other insects, has been incorporated in a dynamic model of caste differentiation in lower termites by Nijhout and Wheeler(301). This model, depicted in Figure 4, postulated three types of morphogenetic programs with three distinct competence periods, e.g. the sexual characters, the non-sexual adult characters, and the soldier characters. In each of these competence periods, the titer of JH decides about the expression of particular set of characters. Consequently, for each type of morphogenesis a characteristic dynamic profile of JH is required. Though the described model could be criticized for an over-simplification of the complex problem, it represents a good synthesis of the findings based on *K. flavicollis* and *Zootermopsis*.

This model also allows an understanding of the occurrence of *intercastes*, individuals sharing the phenotype characteristics of several castes. This phenomenon can be observed occasionally in nature and very often during laboratory hormonal manipulations. In abnormal hormonal circumstances due to an unusual timing and/or presence of an unusual titer of the JH, one of the morphogenetic programs can only partially be induced in the individual or two morphogenetic programs can be released simultaneously. In nature, the intercastes often occur in response to abrupt changes in the social structure of the society, such as the orphaning, or as a result of a parasitic manipulation. Most commonly, these intercastes appear after only a partial expression of (pre)soldier traits following a moult from a larva, nymph, pseudergate, or worker. The second very common category of intercastes is the so-called *pseudoimago*, individual derived from late nymphal instar with imperfect expression of imaginal adult traits(15,450-459).

The experimental intercastes are usually induced by the application of high doses of JH, JHA, or corpora allata transplantation into various castes of various intra-instar ages. For instance, the implantation of active corpora allata to orphaned pseudergates early during the intermoult resulted in simultaneous expression of reproductive and soldier traits. The intercastes reported in most experiments with JH and JHA are of the type nymph-soldier, worker-soldier, or pseudergate-soldier. Their anatomy reveals the presence of a mixture of traits characteristic for the two castes, most often of an intermediary appearance. The development of such intermediary phenotype is most probably due to different sensitivity of particular tissues, organs, and cells to JH stimulation in time. On the cellular level, the response to JH follows the *all-or-none rule*, but the final shape and size of structures or organs is a result of summation of many of these responses(460). The inconsistence in response of particular cells out of the competence period results in a phenotypic continuum between the original caste and the (pre)soldier. This fact is documented e.g. by a biometric study by Lelis and Everaerts(231), who pointed out that with increased doses of JHA offered to groups of *Reticulitermes santonensis* (Rhinotermitidae) an increase in number of moults into presoldiers (intercastes) occurs, probably due to an activation of moult glands. But the morphogenetic process, and its outcome in the form of outer anatomy of intercastes, does not show any dose-dependent features. Regardless of the dose applied the intercastes revealed to be a mosaic of a worker and presoldier. Thus, the final phenotype is driven by the readiness of particular cells to respond to JH (JHA) and not by the dose applied(231,301).

The hormonal background of termite caste differentiation allows us to consider the fertile soldiers in Termopsidae as neotenic-soldier intercastes(19). It should be noted in this respect, that from a certain point of view, all neotenics, appearing as prematurely fertile individuals of various caste and instar origin, could be considered as intercastes, i.e. individuals with precocious partial expression of sexual traits in immatures(296). However, the stable presence of neotenics in many species, as well as their significance in the life history of these species, allows us to classify the neotenics as a regular caste.

DYNAMICS IN THE TITER OF MORPHOGENETIC HORMONES AND ACTIVITY OF ENDOCRINE GLANDS

An alternative approach to the application of JH or JHA to termites when studying the caste differentiation is the histological and physiological analysis of the activity of the glands involved and evaluation of the titres of their hormonal products. These studies have confirmed the general concept of the role of the JH. There is abundant evidence of a dramatic increase in the volume of the corpora allata in individuals destined to become presoldiers and during the presoldier stage in both lower and higher termites(80,226,370,413,416,428,461-463). The correlation between the gland volume and its synthetic activity was demonstrated by the comparison of nuclear vs. cytoplasmic area in the cells of corpora allata of individuals destined to become presoldiers in *Macrotermes michaelseni* (Termitidae)(463).

These findings are in agreement with the values of JH titers in the hemolymph, which are increasing in individuals isolated in groups with few or no soldiers and destined consequently to become presoldiers. This has been documented e.g. in *M. michaelseni*(462,463) and in the rhinotermitids *Reticulitermes flavipes* and *Coptotermes formosanus*(76,402,464-466). Recent studies have demonstrated the correlation between the JH titer in *C. formosanus* and *R. flavipes* and (i) the soldier inhibition by other soldiers; (ii) the size of the group; (iii) the delay from the isolation of workers; (iv) the (un)availability of valuable food and (sub)optimal temperatures, and (v) seasonal changes. The JH titer in workers reveals a negative correlation with the number of soldiers in the group, but a positive correlation with the size of the group, the time after isolation, the availability of valuable nutrients, and the temperature within the range of optimal temperatures(76,402,465,466). The seasonal dynamics are characterised by minimal JH level in autumn and winter, followed by an increase culminating in summer(75). These studies also revealed that by far the highest titer of JH appears in presoldiers, and described the time course of the JH titer during the presoldier and soldier stages(465,466).

The dynamics in the size of moult glands and titers of ecdysteroids were studied by Okot-Kotber(353,462,463,467) in the third instar female larvae of *M. michaelseni,* developing normally either in workers or in presoldiers, and by Okot-Kotber et al.(464) in isolated workers of *R. flavipes*. In agreement with the postulates presented above, a clear correlation between the time course of JH and ecdysteroids has been documented in both species; the titer of both hormones increases in a similar pattern during the first few days of the instar duration in isolated larva or worker, and preceding its moult into soldier.

The detailed study in *M. michaelseni* confirmed that the pattern of ecdysteroid production was correlated with multiple enlargement of moult glands when compared to the situation in larvae determined to become workers. The time course of the ecdysteroid titers revealed two subsequent peaks, a smaller one on the day 4, followed by a dramatically higher peak a few days later on day 10. The first peak coincides with an increase of corpora allata and JH titer, but also with the first detectable onset of the presoldier structures, i.e. the start of apolysis, epidermal retraction, and initial morphological differentiation of the larval mandibles into those of a future presoldier. At the same time, the occurrence of this peak represents the end date of the competence period. The powerful second peak is followed by the ecdysis. All the events in the hormonal cycle appeared earlier than in correspondent larvae determined to moult into workers. Consequently, also the instar preceding presoldier was shorter. This could be considered as another verification of the prothoracicotropic effect of the increase of JH titer in the hemolymph(353,462,463,467). The development of soldiers, so often compared to the imaginal metamorphosis via a pupal stage, is once again showing a feature characteristic rather for the prepupal stage of Holometabola, i.e. the dynamics of ecdysteroids with a first minor peak (the one responsible for imaginal disc stabilization) and a final dominant peak preceding ecdysis.

Alternative scenarios

The described concept of pheromonal and endocrine background of termite caste differentiation, especially the differentiation of soldiers, is largely acknowledged. Nevertheless, alternative hypotheses and scenarios occur, and should be mentioned in this text. Among them, the curious and profoundly speculative theory of Henderson(407) postulates the existence of one single substance playing the role of the primer pheromone and at the same time the role of the morphogenetic hormone. This substance is supposed to be the JH itself. The soldier caste is characterised as an antagonist of the reproductives, absorbing the juvenile hormone produced by them in order to inhibit the development of reproductives from undifferentiated individuals. The presoldiers and soldiers would function as a storage capacity of the JH, their corpora allata enlarge not due to the high synthetic activity but due to the storage of absorbed JH. In this way, the soldier caste would modulate and resolve the conflict over reproduction between reproductives and their progeny, and ensure the equilibrium between the maintenance of a work force and the production of winged imagoes. This curious theory reassumes the old suggestion that the social regulation could be mediated by the juvenile hormone and anti-juvenile substances acting directly at the endocrine level of the recipient(185,399,406).

Recent findings and perspectives in endocrinology of caste differentiation

Current research in the field of JH and JHA is far from being as extensive as in 1970's and 1980's, undoubtedly because of the fact that despite the spectrum of species and compounds tested, the JHA are not practically used in termite pest control. Nevertheless, the perspective of designing an ideal juvenilizing compound still represents for some teams a driving force for laboratory tests of compounds(440,468-470). But the current use of JHA as a tool for hormonal manipulation of termites represents an important methodical approach. It contributed recently to many important findings in endocrinology of the caste differentiation, its regulation, and caste-specific gene expression(377,391,434,471).

Many particular aspects of the JH-titer dependent concept of caste differentiation, proposed on the basis of histological studies and experimental manipulations, were only recently supported by modern methods of hormonal titer evaluation and in vitro measurements of corpora allata activity(402,465,466,472-475). Besides the caste-specific structural genes, proteins and genes involved in regulatory mechanisms of the JH synthesis, transport, and metabolism were recently identified, and provided first evidences of a caste regulating mechanism on the molecular level. Among them the JH-binding proteins from *Zootermopsis* (Termopsidae), responsible for the transport of the JH in hemolymph, are known for a relatively long time(476,477). Very recently a set of hexamerin proteins was identified in the rhinotermitid *Reticulitermes flavipes*, involved probably in the sequestration and storage of the JH from the hemolymph. In this way they slow down or inhibit the action of the JH on the tissues(392,393). The expression of the genes responsible for these hexamerins is prompted by high JH level, their silencing is followed by an expression of a large set of JH-dependent genes(391,395). The hexamerins may be a part of the regulatory mechanism of the caste-specific differential gene expression, underlying the caste differentiation in termites(395). Another recent discovery in the field of endocrine regulation of castes is the description of an allatostatin identified in *R. flavipes*, similar to that of the cockroach *Diploptera punctata* (Blattodea). The neuropeptide is located in the neurosecretory neurons of the brain, corpora cardiaca, and corpora allata. It ensures an efficient inhibition of JH synthesis(478,479).

The above described findings concern the dynamics in hormonal titers, synthesis of JH by corpora allata, its neurohormonal regulation, and molecular regulation of the JH transport on the extra-cellular level. The intracellular and nuclear mode of action of the JH on the gene expression is a phenomenon extensively studied and discussed in various model insect species. The recent findings brought several candidate scenarios, which are not mutually exclusive, and suggest that a multitude of action modes may coexist for the multitude of various roles of JH(480,481).

EVIDENCES FOR GENETIC FACTORS INVOLVED IN CASTE REGULATION

We should keep in mind that the presented mechanisms of pheromone and JH-mediated caste regulation describe well the situation in lower termites and can also be applied for the differentiation of soldiers in higher termites. On the other hand, the developmental decision of individuals between the nymphal and apterous developmental line in advanced species with forked development may probably occur earlier than during the instar preceding the anatomic divergence. This has been suggested by several authors, and also endocrinological and histological studies indicate the existence of a determination at the embryonic level(300,339-341), depending on the amount of JH deposited in eggs by the queen(342,461).

In this respect, we should not omit the fact that in advanced rhinotermitids and termitids, the developmental decision between the nymphal and apterous line is often based on the sex of the individual. Caste differentiation therefore can be considered as genetically based, though we cannot exclude that the sex specialization evolved as a differential ability or sensitivity of particular sex genotypes to epigenetic stimuli. A curious and reminiscent phenomenon was recently observed even in *Mastotermes darwiniensis* (Mastotermitidae)(482). In some of studied colonies, the frequencies of particular genotypes were caste-specific for workers and soldiers. This suggests that the genotype may occasionally be associated with caste. The genetic differentiation of castes within these colonies could reflect differences in the propensities of termites with distinct genotypes to develop into particular castes.

Very recently, the evidence of a strong influence of genotype on the future caste identity of individuals were published by Hayashi et al.(483). In *Reticulitermes speratus* (Rhinotermitidae) the authors discovered strongly differentiated sex and caste ratios in offspring of different crosses of neotenics. Particular genotypes of particular sexes could have been assigned to particular castes, i.e. future nymphs or future workers. The workers appeared to be completely genetically determined whereas the nymphal genotypes could have been manipulated by environmental condition toward the development into workers. The authors hypothesize the evolutionary step from linear pattern with full developmental potential, controlled by pheromones, to a genetic determination of the apterous development towards permanent worker caste. These results contradict the concept of environmentally based caste determination. They should be studied in more detail and extended to other species. In the light of these findings, the whole theory of caste regulation and determination could be subjected to substantial modifications in the future.

X. ORIGIN OF THE SOLDIER CASTE IN TERMITES

The search for evolutionary origins of termite soldiers can hardly be separated from the search for the factors underlying the origin and maintenance of eusociality itself. It is generally accepted that the occurrence of the soldier caste is an early and ancestral event in the evolutionary history of termites. This can be inferred from the common mechanisms of soldier formation across the whole order Isoptera and the ubiquitous presence of soldiers in (practically) all extant species(484). This permanently sterile caste, highly specialized by its anatomy, physiology, and behaviour to perform defensive tasks *sensu lato*, must have evolved only once in the common ancestor of extant termites, as the first permanent altruistic caste. The majority of work tasks in this putative "prototermite" were performed by temporary helpers, i.e. larvae, nymphs and/or pseudergates, only postponing temporarily their individual reproduction. This situation is supposed to persist in extant primitive species lacking the divergent developmental line of true workers. Consequently, based on available phylogenetic studies, altruism in form of permanent sterile helpers must have evolved at least four times; once as the soldier caste and three times as the true worker caste in Mastotermitidae, Hodotermitidae and the Rhinotermitidae + Termitidae clade(187,295,296,325,485). The controversy concerning the multiple origin of the worker caste is described above in Chapter VII. As pointed out by Thorne(187), this multiple origin of sterile helpers does not imply that eusociality itself evolved several times. The soldiers and true workers in the distinct lineages probably originated in the ancestral helper caste, which apparently complied with requirements for the eusocial reproductive altruism by the postponing of its individual reproduction. The passage to a complete sterility is just one more qualitative step towards higher levels of eusociality(486,487).

Since the evolutionary background of the origin of soldiers and workers differs in certain aspects but is common in others, the most pregnant hypotheses concerning the origin of termite eusociality in general will be briefly reviewed, and consequently the specific situation of soldiers will be discussed.

ORIGINS AND MAINTENANCE OF TERMITE EUSOCIALITY

The postulates of the kin selection theory by Hamilton(143) represent the basic framework for understanding the apparent paradoxes of eusocial animal societies, such as the reproductive altruism or even a complete sterility in majority of its members. The concept of costs in terms of the loss of individual reproduction, compensated by benefits in the form of *inclusive fitness* realized by the enhanced production of siblings, provided a plausible explanation of reproductive altruism. This applies particularly in eusocial systems with a high relatedness asymmetry, as is the case in eusocial Hymenoptera and Thysanoptera, with haplodiploid genetic constitution, or asexually reproducing parthenogenetic aphids and polyembryonic parasitic wasps. The high relatedness among siblings offers high gains of inclusive fitness and promotes the evolution of helping. The validity of this explanation is supported by the disproportional frequency of these genetic systems among all eusocial animals and the multitude of eusociality origins in these taxa(11,187,488). But termites, as well as a few other eusocial animals, such as the eusocial shrimps or naked mole-rats, are diploid and consequently do not display any obvious constitutional relatedness asymmetry(489-491). Therefore in these taxa, a plethora of mutually non-exclusive theories took the place of the universal principle of haplodiploid relatedness asymmetry, applied in hymenopterans and thrips.

The hypotheses concerning the evolution of eusociality in termites attempt to identify various factors in the life history and ecology that might have shifted the cost-benefit ratio in a way to favour the origin and maintenance of eusociality in the putative prototermite. These factors presumed to underlay termite eusociality can be classified as follows: (i) relatedness asymmetry created by the reproductive cycle or specific genetic constitution of termites; (ii) conflict or manipulation of altruists by nestmates; (iii) ecological determinants specific for termite societies; (iv) general ecological determinants of eusociality shared by termites with other eusocial animals.

HYPOTHESES BASED ON RELATEDNESS ASYMMETRY

The lack of an apparent relatedness asymmetry promoted a search for more intricate mechanisms that could generate genetic asymmetries in diploid termites and favour helping. The first of the related hypotheses was elaborated by Bartz(492). He underlined the presence of the neotenic reproductives and the inbreeding, resulting from the mating among these neotenics, which are widespread in termites and present in all basic termite lineages(493). If each individual from an outbred pair of primary reproductives is produced incestuously by neotenics in its natal colony, than the offspring of such a pair would be relatively genetically homogenous. This increased relatedness among siblings is higher than that between them and their hypothetical outbred offspring. This bias in relatedness is consequently the prime mover of the occurrence of helping by the offspring to produce more siblings. This relatedness asymmetry is subsequently maintained in the colony by inbred reproduction of neotenics. In the course of a few generations, the repeated inbreeding results in a decrease of the relatedness asymmetry between the siblings and their hypothetical offspring. Thus a conflict over neotenic reproduction should arise among siblings. Consequently, if the inbred reproduction of the colony continues, the selective force for helping gradually decreases. Therefore, the individuals would more likely develop into winged imagoes and attempt their individual outbred reproduction resulting again in offspring relatively genetically homogenous, favoured to help their parents to produce more siblings. The mentioned model predicts cycles of inbred and outbred reproduction, regular occurrence of neotenic reproductives, conflict among siblings over reproduction as neotenics, and the production of winged imagoes by incestuous mating between neotenics. These predictions concur well with many life history traits in primitive extant species of one-piece type nesters. Bartz also pointed out that the presumable termite ancestor was confined to small spatially delimited underbark or rotting wood habitats; such circumstances are predispositions for the occurrence of inbred reproduction. However, the criticism of this theory was based on the fact that the major presumptions of the model were not confirmed in many species, i.e. (i) the high occurrence of inbreeding; (ii) systematic production of primary reproductives by inbreeding of neotenics; (iii) outbreeding of primary reproductives(187,305,494).

Another hypothesis is based on the occurrence of chromosomal translocations that link a substantial part of the genome to the sexual chromosome in a male. This mechanism has been extensively studied in *Incisitermes schwarzi* and other kalotermitids in a series of cytogenetic studies by Luykx and Syren(495-497), but it has been also observed in other termites(498). As a consequence of these translocation complexes, a large part, even a half of the genome, is transmitted from males to males only, its homologous part from males to females. As a result, relatedness asymmetry occurs between sexes, sometimes compared to that observed in haplodiploid hymenopterans. These findings led to the hypothesis, formulated by Lacy(499,500), of the increase of inclusive fitness by mutual altruism among siblings of the same sex, which would in consequence favour the origin of eusociality. This concept was subjected to discussion and criticism, raising the two following major arguments to disclaim its predictions: (i) in a large set of species, such chromosomal translocations were not observed; they seem to occur independently and randomly as an advanced feature, which is not universally shared by the primitive lineages; (ii) in many aspects of the social behaviour, the sex-biased approach is not possible, such as in defence by soldiers and nest building, which serve to all colony members at the same time; (iii) no evidence of sex-biased behaviour was observed within termite colonies(501-504).

INTRAGROUP CONFLICT AND SIBLING/PARENTAL MANIPULATION

The origin and maintenance of helping and reproductive division of labour in social Hymenoptera is often linked with dominance hierarchy, and more or less overt conflict over reproduction within the female societies(11,195). Roisin(186) extended this principle to termites and proposed a hypothesis based on an intragroup conflict among individuals over the development into the alate stage. This theory presumes the existence of a subsocial termite ancestor living in familial groups of parents and their offspring, where the older offspring helped to raise young siblings before proceeding into the alate stage. From this starting point, the hypothesis attempts to explain the origin of permanent helping and sterility as observed in extant termites. In the ancestral familial groups a conflict must have appeared over the decision which of the temporary helpers will remain and help and which of them will become winged imagoes and reproduce, given that the resources are limited.

In extant species, there is a rich evidence of such conflicts among nymphs in the basal termite lineages of Kalotermitidae(182-184,310), Termopsidae(186,315), and few Rhinotermitidae(35,505). They are documented by the presence of wing mutilations preventing the nymphs from becoming alates and resulting in regressive moults or wing pad abscissions in these mutilated individuals. After the regressive moult, the individual may either retry a delayed development into winged imago or remain in the nest to continue in helping as a pseudergate. Thereby it adopts an alternative option that is the best in terms of inclusive fitness in its actual situation. The selection should, in the course of time, favour the evolution of effective helper adaptations to increase the fitness of the losers in these conflicts. Consequently, as the gain from remaining a helper is increasing with effectiveness of these adaptations, also the developmental decision between alate dispersal and helping is not as dramatic. Thus a less spectacular switch mechanism between the two developmental options should have evolved, when compared to overt fights among nestmates. In this way, finally, the permanent helpers, i.e. workers, could have evolved in termites, determined by epigenetic pheromonal stimuli. By small steps of gradual evolution, also the specialized soldier caste could have occurred in the same way by anatomical and behavioural specialization to the non-dispersal role.

An alternative hypothesis based on the same life history traits of extant termites, i.e. on the presence of wing-bud mutilations, has been proposed by Zimmermann(182) and Myles(183). According to these authors, the mutilations are caused by other colony members, nymphs, reproductives, or helpers, and represent a case of sibling or parental manipulation, occurring to prevent nestmates from individual reproduction and to remain in the nest while increasing its fitness by helping. A deficiency of this hypothesis was pointed out by Roisin(186): to be adaptive, the parental or sibling manipulation must have occurred after the evolution of efficient helper phenotype. Therefore, this concept does not explain the origin itself of the permanent helper.

The Roisin's hypothesis of intragroup conflict is supported by large evidence of wing mutilation from the termite lineages, considered to approximate the best the situation in ancestral termite societies. Critical comments were raised by Thorne(187), who underlined these three points: (i) the theory fails to interpret the choice of the helping option as a choice for a hopeful reproduction as neotenic; (ii) it is unclear how the helping behaviour occurs in early instars of the developmental line, which are preceding this alate/helper decision point; (iii) it appears that the wing-bud abscissions in Termopsidae are self-induced by the nymphs before they develop into a neotenic.

The intragroup-conflict hypothesis has been recently reassessed by Korb(506), who considers it to be an overestimation of the significance of wing-bud mutilations, which are in many cases supposed to be the result of stress and disturbance induced by the handling condition in captivity. Her statement is supported by the experimental evidence of wing mutilations and fights in the kalotermitid *Cryptotermes secundus* in response to stressful conditions, whereas in natural or undisturbed colonies such observations were rare. The author also emphasized that more subtle means are used for the regulation of decision between the alate or helper development, i.e. the putative primer pheromonal regulation by reproductives, distributed via proctodeal trophallaxis or body-shaking as signal performed within the group. An extensive study of the developmental decision between alate and pseudergate phenotypes in *C. secundus* revealed that (i) the decision to rest in the nest as helper occurs when the season does not favour the dispersal; (ii) when the food availability is high and the nest displays a long future perspective. When the season is favourable or the food and nest resources depleted, the individual will more likely develop into an winged imago. Thus, rather than losers or winners in conflicts or manipulations, the individual termites should be considered as decision-making units choosing the best option in terms of inclusive fitness on the basis of environmental and social stimuli, and the signals distributed within the colony could be viewed as honest signals rather than pheromonal manipulation(83,506-508). The origin of the helper phenotype, and consequently of the true worker caste, would be an adaptive option to benefit from the inclusive fitness provided by the colony, combined with a hopeful philopatric reproduction as neotenic within the natal nest. This concept is underlying the following theories based on specific and unspecific ecological correlates of the termite life history, favouring the occurrence of eusociality.

Specific ecological determinants in primitive termite societies

The following hypotheses attempt to identify particular ecological conditions and life history characteristics and constraints, proper to the putative termite ancestor, often compared to *Cryptocercus*-like cockroach society, which would favour the origin of eusocial behaviour as adaptive option without a manipulation or relatedness asymmetry. A classical symbiont transfer hypothesis was formulated already by Cleveland et al.(509) in 1934. It is based on the need of transfer of symbiotic protozoa to every nutritionally active instar after its moult, which is accompanied by a complete emptying of the gut and a loss of these symbionts. The xylophagy predisposes termites to parental care and a social life. On the other hand, there is no proof of how these circumstances could contribute to the evolution of other basic traits of eusociality, i.e. the reproductive division of labour and helping(187).

The concept of social evolution in the *Cryptocercus*-like termite ancestors by Nalepa(510,511) postulates several ecological determinants which again act in favour of the evolution of familial life and brood care, and might have been the circumstances of the origin of reproductive division of labour and helping. Among these determinants, the following are crucial: the low quality of food, the one-piece type nest habitat confined to small area, and the necessity of symbiont transfer. In these conditions, a slow development, subsocial families, and monogamy are the starting point for gradual shift in the behaviour of old offspring towards the adoption of brood care and liberation of the founding pair from this task, which would allow it to produce more offspring. In consequence, a helping would arise, as well as a bias in reproductive potential between the founding pair and the helpers, postponing their individual reproduction.

Though these two concepts did not discover any specific selective forces directly explaining the passage to eusociality, they represent a framework enabling such passage in termite ancestors.

General ecological determinants promoting eusociality

It is very probable that the origin of the termite eusociality was not a result of one selective driving force. Rather, it evolved in response to a multitude of particular elements generating a multitude of selective pressures, more or less specifically promoting eusociality. To support this presumption one should compare the ecological and life history traits of extant eusocial animals with those displayed by termite societies. Surprisingly, many of these traits are shared among the phylogenetically unrelated eusocial animal groups. Such a comparison was elaborated by Thorne(187), Alexander et al.(491), and Crespi(194), who pointed out several traits proper to the eusociality, e.g. (i) living in protected nest confined to a small area, often serving as food, resulting in the overlap of generations and parental care, and allowing the onset of helping by older offspring to raise siblings; (ii) relatively slow development, long life span of reproductives, helping as immature stage; (iii) high risk of individual dispersal and reproduction out of the natal nest; (iv) possibility of inheritance of resources in the natal nest during philopatric reproduction; (v) high pressure of predation, cooperative collective defence of resources and nest, specialized defensive morphs. Namely the option of philopatric reproduction, accompanied by inheritance of the nest resources and helpers, was sometimes considered to be the prime mover of the social evolution in termites, responsible for the stay of individuals in the natal nest and for the evolution of helping by these hopeful reproductives, waiting for their reproductive opportunity(43,189,490). On the other hand, these assumptions were disclaimed by Roisin(486), who proposed a more parsimonious justification of helping to be the benefit of inclusive fitness gained by helpers, independently on the prospect of future neotenic reproduction.

The mentioned characteristics shared by termites with other eusocial animals suggest that some or all of them in summation could generate a sufficient gain in terms of inclusive fitness of altruistic castes to explain at least the maintenance of termite eusociality if not its origins. When evaluating this cost-benefit ratio in extant termite species, we must keep in mind also following points: (i) in the highly integrated societies of extant species, the selection acts not only on the individual level but also on the colony level in some aspects of collective behaviour of the society; (ii) the lack of intermediary stages of the social evolution among extant termites, together with the absence of fossil evidence, make difficult the assessment of the circumstances of termite social evolution; (iii) the life-history traits in extant species can be relicts of past evolution, which are maintained not due to their adaptiveness, but due to their irreversible character(13,187,486).

THE CASE OF SOLDIERS

The eusocial societies are apparently linked with an increased selection for defence. The high concentration of brood and/or food resources creates a high pressure of predation and parasitism; the investment in brood care and resources must be somehow defended. The defensive adaptations appear to be among prerequisites of sociality in some animals, such as the sting in Hymenoptera, or they occurred at early stages of social evolution, such as the soldier morphs in termites, eusocial aphids, shrimps, and thrips(195,412,490,512,513). The aspect of defence is underlying most of the ecological determinants common to eusocial systems listed above, which can be reformulated here by citing Crespi(194): "...a combination of three conditions, (1) food-shelter coincidence, (2) strong selection for defence, and (3) ability to physically defend against parasites, predators and usurpers, is sufficient-although not necessary-to explain most of the taxonomic distribution of eusociality. These three conditions appear sufficient for the evolution of eusociality because all known group-living species that satisfy them are eusocial. "

Even in diploid eusocial societies of termites, despite the lack of relatedness asymmetries within the colony, the indirect fitness benefit generated by the defensive activities performed by permanently sterile soldiers must largely compensate their own prospective reproduction and the investment of other helpers in their nutrition. As is already discussed in the chapters concerning the defensive adaptations and efficacy of soldiers, this contribution is obvious in some of the extant species, whereas it may appear questionable in others. Nevertheless, the mechanisms of caste regulation allow a fine tuning of the cost-benefit ratios by altering the soldier proportions.

However, some of the hypotheses, proposed to account for the origin of the soldier caste in termites, search for more powerful driving forces of this evolution than a simple altruistic helping motivated by indirect fitness benefit of the soldiers. The hypothesis by Myles(188,189) as well as that by Thorne et al.(153) attempts to draw a comparison between the circumstances of the life history of the termite ancestors and these observed in the termopsid species, for instance in *Zootermopsis* spp., with the occasional occurrence of the soldier neotenics. Both hypotheses conclude that the combination of fertility with the soldier traits evolved as adaptation for intraspecific aggression motivated by nest inheritance. According to Myles, this aggression occurs among nestmates from the same colony, and the soldier neotenic phenotype is designed to win in the fights over the reproduction. The criticism of Roisin(186,486) can be resumed by the following questions: "If the expression of soldier traits in neotenics is an adaptive advantage for nest inheritance, why are there the non-soldier neotenics in *Zootermopsis* present, usually in majority? And why would this combined phenotype disappear in all other extant species?" Moreover, the use of weapons by the soldier neotenics in within-colony siblicidal fights in *Zootermopsis* is not adequately supported. The re-examination of this theory by Thorne et al.(153) consists in the observation of increased formation of the soldier neotenics in colonies faced to high intraspecific competition among young colonies in a limited nest resource. The occurrence of these morphs enhanced the chances of the natal colony to win in the intercolonial fights and to invade the losing colony. The neotenic soldiers have consequently boosted their own chance for *Accelerated Inheritance* by becoming replacement kings and queens in newly established merged colonies. Nevertheless, the two questions raised by Roisin apply for this hypothesis too, i.e. why this advantageous phenotype is not a universal feature in neotenics of all Termopsidae, but also in all other termites with similar nesting habits? An alternative approach to explain the occurrence of these soldier neotenics is to consider them as intercastes between neotenics and soldiers(486). Though the developmental programs for sexual traits appear to be triggered by opposite stimulation in terms of JH titers, the abrupt changes in this titer occurring during dramatic changes or anomalies in the social structures, such as the orphaning, are known to result in the formation of various intercastes, including morphs with sexual and soldier traits(455,454). Simultaneous expression of the two developmental programs is also possible by hormonal manipulation(415,447)(see also page 117). Moreover, from the anatomic point of view, the soldier adaptations observed in soldier neotenics appear to be an imperfect realisation of soldier phenotype, due to their smaller size and variable anatomy when compared to regular soldiers(188).

Yamamura(488) attempted to characterize evolutionary circumstances of the occurrence of soldiers as the first (or only) altruistic caste in some eusocial insects and of workers in others, based on genetic systems and rates of relatedness among various individuals in these societies. Using a presumption of a high rate of inbreeding in termite societies, adopted from Bartz(492), he elaborated a mathematical model predicting the presence of altruistic soldiers in termites rather than workers. However, the deficiencies of Bartz's predictions, presented above, infirm the credibility of the hypothesis by Yamamura.

Finally, a few other authors consider the inclusive fitness benefit of soldiers to be a sufficient selective factor for the evolution of the soldier(186,187,197,211,486,510). A question remains to be answered: "Which individuals from the totipotent pool of undifferentiated individuals should become soldiers?" It could be Roisin's "losers" of the intragroup conflicts over reproduction with the lowest chance of individual reproduction as winged imagoes. These individuals could have been prompted to enhance their helping capacities to such an extent as to evolve in the highly specialized sterile caste ready for a self-sacrifice in the name of its natal colony(186). As has already been mentioned, the contribution of the soldier caste to the defence of the colony, and consequently to the inclusive fitness of its members, remains controversial in some cases. Such situations occur for instance in the one-piece type termites living in a compact wooden nest, such as Kalotermitidae. Roux and Korb(197) decided to investigate the impact of soldiers on colony development and fitness, reflected in the number of helpers and winged imagoes produced within one year, in the kalotermitid *Cryptotermes secundus*. Comparison between soldierless colonies, in which formation of new soldiers was prevented by soldier head extracts(405), and control colonies in natural conditions, revealed a non-significant impact of soldiers on the survival of colonies and helper growth. But surprisingly, the soldierless colonies had significantly fewer late nymphs and/or winged imagoes. In other words, at the place of a crucial involvement in colony defence, the impact of soldiers on the production of reproductives has been proved, though the mechanism is unknown. The authors speculate about the possible functional aspects and significance of the soldier impact on the development of alate reproductives, as is for instance a pheromonal signalling of the colony status, providing information about the chance of nest inheritance. Thus, an alternative role could hypothetically be assigned to soldiers, however it has yet to be confirmed.

XI. BIOLOGY OF THE GENUS *PRORHINOTERMES*

PRORHINOTERMES – AN OUTSTANDING GENUS WITH NUMEROUS PECULIARITIES

The eighteen species of the monogeneric subfamily Prorhinotermitinae(337,514) represent a very outstanding group within the family Rhinotermitidae, with several unique biological peculiarities even among termites and insects. Species of the genus *Prorhinotermes* Silvestri, 1909, lack the distinct true worker line; the work tasks are performed by pseudergates, larvae, and nymphs(35,36). Consistently, on the basis of ecological characteristics it is usually classified among one-piece type termites, without large foraging territory and well-defined centralized nest(42,43). The linear pattern of post-embryonic development of *Prorhinotermes* is shared with another monogeneric subfamily Termitogetoninae (genus *Termitogeton*)(515). It contrasts with the forked development present in all other rhinotermitids, sometimes in a very advanced form, as is the case of the subfamily Rhinotermitinae, overreaching in the developmental complexity many higher termites. Within the family Rhinotermitidae, one can find the whole spectrum of existing caste patterns and related ecological strategies, this family is therefore considered as the key taxon for understanding the evolution of true and temporary workers(34,295,296,325,516,517). Moreover, the linear development in the two genera displays one more singularity, i.e. the existence of a single wing-padded nymphal instar preceding the imaginal stage. This accelerated wing development, observed in

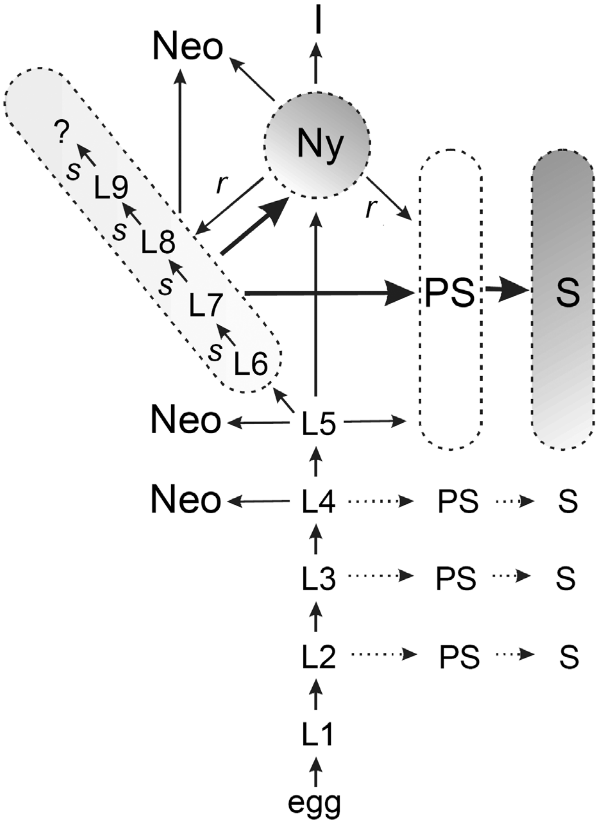

Figure 5.
Schema of the developmental pathways of the genus *Prorhinotermes*. L – larva; PS – presoldier; S – soldier; Ny – nymph; I – imago; Neo – neotenic reproductive; s – stationary moult; r – regressive moult. Dotted lines apply for incipient colonies. Larvae >L5 can be classified as pseudergates. Compiled from 35,36,521.

a single other termite *Hodotermopsis sjostedti* (Termopsidae)(319), is unique among hemimetabolous insects and was denominated as *proneometaboly,* due to its reminiscence to the wing formation characteristic for neometaboly in Acercaria(518). The single nymphal stage in *Prorhinotermes* is also characterised by a unique shape of wing pads; these represent a shield-like structure on meso- and metanotum, originated in an apical fusion of each pair of wing pads(36,519).

In larvae, strange finger-shaped appendages of unknown origin and significance, extending backwards from the pronotum, were found in *P. inopinatus*, *P. flavus*, and *P. canalifrons*. These appendages, resembling wing pads, usually persist until the second or third instar(35,520,521). Tarsal glands are lacking in *Prorhinotermes*(522), whereas they are present in all other rhinotermitids studied(208). Few other, plesiomorphic features of anatomy, separate the genus *Prorhinotermes* from other rhinotermitids, such as the presence of tergal glands in imagoes of both sexes(523,524).

Genus *Prorhinotermes* also displays some unusual traits in its chemical ecology. The main component of the defensive frontal gland secretion of soldiers is a toxic (*E*)-1-nitropentadec-1-ene, one of very rare nitro- compounds present in insects(245,525,526). Neocembrene A was identified as trail pheromone in pseudergates, a compound absent in other rhinotermitids studied so far, but used as trail pheromone in two species of Nasutitermitinae(527).

All these peculiarities justify the classification of the genus *Prorhinotermes* in a discrete subfamily Prorhinotermitinae, though originally the exclusion of the genus *Prorhinotermes* from the subfamily Rhinotermitinae was based on anatomic features of soldiers(337). Often it has been considered as a sister group of all other Rhinotermitidae(334,517,523), but alternative hypotheses were formulated in the recent phylogenetic studies(325,528,529). Therefore, given the unclear phylogenetic position of Prorhinotermitinae within Isoptera, the presence of a unique mixture of primitive and advanced traits, as well as few singular biological features, justify the intense motivation to study the biology of this interesting genus.

GEOGRAPHY AND DISTRIBUTION

The distribution of the genus *Prorhinotermes* is cosmotropical and probably the most insular of all termites. It is present in Malagasy (1 species), Indo-Malayan (11 species), Papuan (3 species), and Neotropical regions

(3 species), with by far the highest diversity in Indo-Malayan region, with 11 recorded species(354,514,519,531,532). This is also where the origins of the genus were very probably located, followed by an overseas dispersal during the Tertiary(531). *Prorhinotermes* usually lives on the coasts or by the waterways on numerous islands, only rarely on the mainland or in rainforest canopy(354). Its records from inland are rather rare, which could indicate lowered competitive abilities(531). On the other hand, *Prorhinotermes* is famous for its viability and resistance to abiotic factors: only a few individuals are enough to establish a vital colony in newly colonized territories. Its cosmotropical distribution and species diversity is the consequence of its ability to cross the open ocean in floating logs or coconuts, and speciate through island isolation. Such a mode of transport is unavailable to practically all other termites(531).

ONTOGENY AND CASTE SYSTEM

The ontogeny of the genus *Prorhinotermes* was studied in details by Miller(36) in *P. simplex* from Florida and by Roisin(35) in *P. inopinatus* from New Guinea. Contrary to his expectations, Miller did not find an early divergence of apterous developmental line. Using a biometric study he distinguished six or seven apterous larval instars (though he called them nymphs) characterised by head widths and the number of antennal segments; with each moult one more article was added. The metrical characteristics in individuals with 15, 16, and 17 antennal articles were broadly overlapping, suggesting the onset of stationary moults from the fifth instar on. He identified the single nymphal stage characteristic for *Prorhinotermes*, and suggested the origin of all nymphs in one larval instar on the basis of the head width.

In extensive experiments Miller discovered the large developmental potentialities of nymphs. Homogenous groups of nymphs survive well; the nymphs are active and self-sufficient. In more than 500 nymphs held in homogenous groups, he observed their capacity to regressive moults into individuals similar to late larvae with reduced wing buds, to differentiate by one moult into apterous neotenics, or into presoldiers with reduced or absent wing buds. The first observation of moult from a nymph to a presoldier occurred on day 24 after their isolation, the regressive moult of nymphs was often preceded by aggressive mutilation of their wing buds. Through another series of pioneering experiments, with more than 1600 nymphs, he proved the capacity of soldiers to inhibit soldier differentiation, as well as the capacity of neotenics to enhance this differentiation. The results suggested a complex functional mechanism of the inhibitory and stimulatory influences since the presence of neotenics in the groups of nymphs did not compensate the inhibitory influence of present soldiers. At the same time, the presence of neotenics proved to prevent nymphs from the development in neotenics. These observations led Miller to the establishment of a linear model of development with six or seven larval instars preceding a single nymphal stage, which has unlimited developmental potentialities. The orphaned and soldierless groups of nymphs can within several weeks develop in functional society with an appropriate ratio of particular castes and produce viable eggs(521).

This concept was re-examined by Roisin with very similar results for *P. inopinatus*. A census of two whole mature *P. inopinatus* colonies revealed a total number of more than 2 000 individuals in one and 8 000 in the other. No systematic sex ratio difference was found between reproductives and soldiers; both sexes were represented in an equal ratio approximately 1:1. The most important modification, made by Roisin in the developmental scheme by Miller, was the discovery of multiple possible origins of nymphs in several successive instars, and introduction of the notion of pseudergates as defined by Grassé and Noirot(298) for *Kalotermes flavicollis* (Kalotermitidae) a few years after the publication of Miller's results. Thus, the fifth instar larva can proceed to the nymphal stage or by a stationary moult to one or several omnipotent pseudergate instars. The nymphs can originate in the fifth instar larva or in pseudergates or various instars. Roisin also observed mutilations of thoracic margins in larvae, nymphs, and pseudergates originated in regressive moults of nymphs. Such mutilated individuals never moulted into nymphs or winged imagoes, though other developmental options were open to them. Based on the mandible sclerotization, the larvae are active by the third instar in incipient colonies, and by the fourth instar in mature colonies. The early larvae from incipient colonies are slightly larger than that from mature colonies. The resulting scheme of developmental pathways is very similar to those described in Kalotermitidae(312) and Termopsidae(315-317), including the occurrence of regressive and stationary moults (Chapter VII). The developmental pathways of the genus *Prorhinotermes* are depicted in Figure 5. According to Miller(36), the neotenics can develop from nymphs and larvae from the second instar on. Unfortunately, he did not mention the method of evaluation of the instar age of neotenics, since the development of functional reproductives from such early stages seems improbable and has no equivalent in termites. Other observations suggest that the larva of the fourth instar is the earliest competent instar for neotenic development in *P. simplex*(521). Roisin(35) did not study the origin of neotenics. The neotenics in *Prorhinotermes* are common, they may coexist within one colony as several or even many replacement and supplementary reproductives. The physogastry in females is marked but not extremely pronounced(36,493,519,521). Siblicidal fights over reproduction in orphaned groups are common and may result in dramatic mortality of isolated groups(521).

The soldiers in *P. inopinatus* were found to originate predominantly in larvae and pseudergates of instars 5 – 7 in mature colonies, but Roisin supposed that any larval instar beyond the first is potentially able to provide presoldiers, depending on the age of the colony. Miller estimated that larvae from the third instar on can develop into soldiers. Under experimental conditions nymphs of both studied species can also develop in presoldiers and soldiers, having variously reduced wing buds(35,36,521).

SOLDIERS

Genus *Prorhinotermes* is characterised by a high ratio of soldiers to other castes; Miller approximated the ratio established after several months in laboratory colonies as 2:7 (22%) in *P. simplex*, Banks and Snyder(519) recorded 11% of soldiers in Floridan field colonies. Roisin(35) observed a ratio of 1:5 and 1:3 (17,5% and 25%) in two field colonies of *P. inopinatus*.

Prorhinotermes soldiers (see Fig. 1 on page 72) are of a slashing type with falcate mandibles and a powerful frontal weapon. The reservoir of the frontal gland, visible through the body wall, reaches deep into the abdomen. The defensive secretion in *Prorhinotermes* consists of the toxic (*E*)-1-nitropentadec-1-ene and a minor constituent, identified as a sesquiterpene (*Z,E*)-α-farnesene(220,245,525). The soldiers do not reveal any obvious functional polymorphism and seem to differ in size only, depending on their instar origin(521).

The numerous soldiers, unusual for one-piece type termites, are involved in guarding inside the nest and in high concentration on its peripheries. They also act as defenders during the outside foraging and moving of the nest to richer food sources, as has been recently observed in *P. inopinatus*(516,517).

ECOLOGY, LIFE HISTORY, AND PHYLOGENETIC IMPLICATIONS

The genus *Prorhinotermes* was traditionally characterised as a one-piece type(42,355,515) termite or single-site nesting termite(43); the colonies can be found on the tropical forest floor, in coastal areas, mangroves, along waterways, or in other swampy and moist localities. The whole colonies are most often located in single branches and logs, in drift wood kept moist by tides, periodic inundations, ocean spray, and coastal weather(35,36,493). The classification among one-piece type termites is in congruence with a typical linear developmental model with pseudergates and nymphs retaining the whole spectrum of ontogenetic potentialities, low physogastry of queens, absence of sexual dimorphism, egalitarian sex ratio, and working larvae and nymphs. These characteristics class *Prorhinotermes* beside kalotermitids and termopsids, lineages believed to represent the primitive stages in the social evolution of termites. On the other hand, the life history and ecology of the genus *Prorhinotermes* displays several features that can be considered as advanced, as for instance a relatively high colony population of several thousands individuals and an unusually high proportion of soldiers, not only among one-piece type termites, but among termites in general. The soldier ratio is comparable to that in species with a powerful chemical weapon used for defence of complex nests and foraging areas, such as the rhinotermitids *Coptotermes* and *Schedorhinotermes*, or diverse Nasutitermitinae(17), producing the highest numbers of soldiers.

This paradox prompted the interest in the ecological strategies of *Prorhinotermes*. Very important findings concerning *P. inopinatus* were only recently published by Rupf and Roisin(517). The authors describe the ability of *P. inopinatus* to forage outside the nest, and construct covered galleries between the nest and a newly found food source. This surprising ability, indicated already in earlier contributions of the authors(35,516), is accompanied by another, even more extraordinary feature, i.e. the nest moving from the depleted nest to the newly found source of wood. The discovery of a food source is followed by the establishment of a long-lasting trail, used by a large fraction of the colony, including neotenics, to migrate to the new nest. Moreover, the foraging appears to be initiated by soldiers, only later followed by pseudergates spreading all over the foraging area. After the establishment of the trail, the soldiers adopt another role, i.e. they stand guard along the sides of the track used by all castes until the pseudergates proceed to shelter the track with a carton arcade.

The presented findings shed new light on the role, abilities, and significance of soldiers in *Prorhinotermes*. The soldiers display complex behavioural patterns during foraging, including initiation of foraging activity and trail guarding, observed only in advanced species such as *Schedorhinotermes lamanianus* (Rhinotermitidae) or some Nasutitermitinae(65,264,268). As a result, their high proportions in the colonies suddenly appear justifiable by the elevated need of soldier presence during foraging activities.

These results demonstrate that external foraging occurs in *P. inopinatus* regardless of the absence of true workers. The authors suggest that caste system with true workers, typical for other Rhinotermitidae, might have evolved from the pattern observed in *Prorhinotermes* given the existence of a selective pressure to increase the efficiency of the foraging. Thus the situation in *P. inopinatus* can be characterised as a transition from one-piece type nesting to a foraging strategy characteristic for intermediate type species, though *P. inopinatus* uses quite unusual type of foraging. A permanent moving of a large part of colony members, including reproductives, to new food sources was only rarely observed in termites(533), but it is based on similar behavioural mechanisms as the foraging for food. It represents its modification or probably its primitive form.

These facts invoke once again the question of the origin and evolution of the true worker caste and the phylogenetic relationships within Rhinotermitidae. It seems obvious that the cases of *Prorhinotermes* and *Termitogeton* are the keys for understanding the evolution of caste patterns in Rhinotermitidae and consequently in Isoptera in general. However, the concurrent hypotheses regarding the origin of true workers(295,296,324,325,357-359) are constrained by the unresolved relationships between several subfamilies within Rhinotermitidae(325,528,529). The classical hypothesis of an early branching of *Prorhinotermes* from Rhinotermitidae, based on morphological peculiarities and plesiomorphies, classified *Prorhinotermes* as a sister group of all other rhinotermitids(334,523). In such a situation, the evolution of the true workers from *Prorhinotermes*-like ancestor would represent a plausible hypothesis in the light of the presented results(517). But the recent molecular phylogenetic studies do not do so, and place *Prorhinotermes* deeper inside Rhinotermitidae(325,528,529). Thus, the evolutionary pattern suggested by Rupf and Roisin, i.e. the passage from pseudergate foraging to true workers, does not have a parsimonious support in the phylogenetic context. On the other hand, the alternative hypothesis by Inward et al.(325), presuming a single origin of true workers for the whole clade Rhinotermitidae + Termitidae and its subsequent loss in *Prorhinotermes,* includes important weak points: it fails to deal with workerless rhinotermitid *Termitogeton*(515) and omits some distinct morphological features and peculiarities, such as the presence or absence of tergal and tarsal glands in *Prorhinotermes*(517).

Only few days before the submission of this thesis, a new extensive molecular phylogenetic study by Legendre et al.(530) appeared in the on-line version of *Molecular Phylogenetics and Evolution*. It brings a strong support to the idea of a secondary loss of true workers in *Prorhinotermes* and *Termitogeton*. The two genera are placed as sister clades inside Rhinotermitidae, as a sister group to the monophylum Rhinotermitinae (see Fig. 3 on page 36). In the light of this new study, the concept of a multiple origin of workers in Isoptera but only one origin in Rhinotermitidae + Termitidae appears parsimonious(325,530). The workerless caste pattern observed in *Prorhinotermes* and *Termitogeton* thus would be a derived situation, suggesting only one secondary loss of true worker caste in the common ancestor of the two genera. However, this study suffers three deficiencies, i.e. (i) no other study so far suggested such a close relationship between *Termitogeton* and *Prorhinotermes*; (ii) the study do not include the genus *Psammotermes* which was assigned to be a sister genus to *Prorhinotermes* by other studies(528,529,325); (iii) it is hard to believe the dramatic separation of *Reticulitermes* and *Heterotermes*, these two genera are generally viewed as very related.

Apparently, it is too early to decide unambiguously which of the concurrent phylogenetic hypotheses describes the best the evolutionary history of *Prorhinotermes* caste pattern. In other words, at the current state of knowledge, the presence of soldier-mediated foraging followed by nest moving in *Prorhinotermes* must be considered as one more unique feature of this curious genus, and together with other singularities, it remains to be explained from evolutionary and phylogenetic points of view.

View of a colony of *P. simplex*.

AIMS

The principle aim of my thesis is the description of various aspects of the biology of the soldier caste in the termite genus *Prorhinotermes* Silvestri, 1909 (Isoptera: Rhinotermitidae). My interest in this genus is motivated by its special life history and phylogenetic position, as it is described in the previous chapter. Understanding the biology of the genus *Prorhinotermes* is a step towards the answer to a few crucial questions concerning the biology of termites in general, such as: "Is the occurrence of true worker caste really a derived trait as has been many times hypothesized? And did it evolve in the family Rhinotermitidae from *Prorhinotermes*-like ancestor? Or the true worker caste existed in the ancestor of all rhinotermitids and it was consequently lost in *Prorhinotermes*? And which were the influences that promoted this loss?" The quest for the answers to these questions, together with the curiosity caused by several other peculiarities of *Prorhinotermes* biology, is prompting the research concerning this genus.

The other source of my motivation was the passion and interest in the termite soldier caste, a phenomenon which is unique from numerous points of view and which is overviewed in the previous chapters. Under the influence of these two inspirations, I decided to focus on the soldiers, a caste which appears to play a vital role in the societies of *Prorhinotermes* species. Therefore, I tried to address the role of the soldier caste in the life history of this genus, especially the ontogeny and morphogenesis of soldiers, population dynamics in incipient colonies, the defensive abilities of soldiers, their involvement in physical defence, in the initiation and mediation of alarm signalisation, and other behavioural patterns of soldiers, as well as the chemistry and ultrastructure of the dominant defensive weapon, the frontal gland. The presented results were obtained by studying three *Prorhinotermes* species from three mutually distant zoogeographical regions, i.e. *P. simplex* from Neotropical region, *P. canalifrons* from Malagasy region, and *P. inopinatus* from Papuan region. Where it was desirable and possible, I tried to compare and/or confirm the results on two or three species with different evolutionary history. In close collaboration with a small team of experts in various branches of natural sciences, I addressed the particular aspects of the biology of the soldier caste in the genus *Prorhinotermes*:

1) The ontogeny of soldiers, i.e. instar origin of soldiers, morphogenetic events during the development of soldiers and its duration, dynamics of soldier production in incipient colonies, presence or absence of poly-

morphism in the soldier caste, defensive chemistry of soldiers, and comparison of the external anatomy and chemistry among soldiers of various instars. This study is presented in the article "ONTOGENY OF SOLDIERS IN *PRORHINOTERMES SIMPLEX*", published in *Insectes Sociaux* in 2006.

2) The behaviour of soldiers, i.e. the behavioural repertoire of the soldiers when facing an enemy, the behavioural pattern during a disturbance of the nest, and the propagation of alarm signalling during these activities. These studies are presented in three articles, i.e. "EGG CARE BY TERMITE SOLDIERS", published in *Insectes Sociaux* in 2005, "(*E*,*E*)-α-FARNESENE, AN ALARM PHEROMONE OF *PRORHINOTERMES CANALIFRONS*", published in *Journal of Chemical Ecology* in 2008, and "AGONISTIC BEHAVIOUR OF *PRORHINOTERMES* (ISOPTERA: RHINOTERMITIDAE)", accepted for publication in *Journal of Insect Behaviour* in March 2008.

3) The morphology and ultrastructure of the frontal gland of soldiers and the chemical composition of its secretion presented in two articles, i.e. "ULTRASTRUCTURE OF THE FRONTAL GLAND IN *PRORHINOTERMES SIMPLEX* AND QUANTITY OF THE DEFENSIVE SUBSTANCE", published in *European Journal of Entomology* in 2004 and "NITROALKENES AND SESQUITERPENE HYDROCARBONS FROM THE FRONTAL GLAND SECRETION OF THREE *PRORHINOTERMES* TERMITE SPECIES", published in *Journal of Chemical Ecology* in 2007.

4) The expression of particular caste-specific genetic programs, and its impact on external anatomy and ultrastructure of organs and tissues during the caste differentiation manipulated by the analogues of juvenile hormone. The results of this study are included in the poster "EXPERIMENTAL INTERCASTES NEOTENIC-SOLDIER IN *PRORHINOTERMES SIMPLEX* (ISOPTERA: RHINOTERMITIDAE)", presented at the *IUSSI French Section Congress, Avignon, 2006*.

The results are presented in the next section as five reprints of original articles, one accepted manuscript, and one poster miniature.