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Article in Biological Reviews · September 2008
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Life history and development - a framework for understanding developmental plasticity in lower termites

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(Received 17 September 2007; revised 16 April 2008; accepted 08 May 2008)

ABSTRACT

Termites (Isoptera) are the phylogenetically oldest social insects, but in scientific research they have always stood in the shadow of the social Hymenoptera. Both groups of social insects evolved complex societies independently and hence, their different ancestry provided them with different life-history preadaptations for social evolution. Termites, the ‘social cockroaches’, have a hemimetabolous mode of development and both sexes are diploid, while the social Hymenoptera belong to the holometabolous insects and have a haplodiploid mode of sex determination. Despite this apparent disparity it is interesting to ask whether termites and social Hymenoptera share common principles in their individual and social ontogenies and how these are related to the evolution of their respective social life histories. Such a comparison has, however, been much hampered by the developmental complexity of the termite caste system, as well as by an idiosyncratic terminology, which makes it difficult for non-termitologists to access the literature.

Here, we provide a conceptual guide to termite terminology based on the highly flexible caste system of the “lower termites”. We summarise what is known about ultimate causes and underlying proximate mechanisms in the evolution and maintenance of termite sociality, and we try to embed the results and their discussion into general evolutionary theory and developmental biology. Finally, we speculate about fundamental factors that might have facilitated the unique evolution of complex societies in a diploid hemimetabolous insect taxon. This review also aims at a better integration of termites into general discussions on evolutionary and developmental biology, and it shows that the ecology of termites and their astounding phenotypic plasticity have a large yet still little explored potential to provide insights into elementary evo-devo questions.

Key words: social insect, caste, polyphenism, developmental plasticity, pseudergate, neotenic, wing development, moult, metamorphosis, juvenile hormone.

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I. INTRODUCTION

Caste differentiation in termites is one of the most conspicuous examples of facultative polyphenism in animals, in which individuals show various phenotypes despite the same genetic background. Within a termite colony - with few exceptions where a genetic component might be involved (Hayashi et al., 2007) - the offspring of a single king and queen can develop into workers, soldiers, and two sexual morphs depending on environmental and social stimuli. Each caste generally exhibits a particular behavioural repertoire and often caste-specific morphological characters. As in social Hymenoptera (ants, and some bees and wasps), this caste system of termites is considered to be the basis for the evolutionary and ecological success of these eusocial insects (Oster & Wilson, 1978).

Obviously, such elaborate societies evolved from solitary ancestors. Consequently, a focus of current research is to explain how complex phenotypes can evolve from ancestral solitary forms. Often differences in complex traits among species are not the result of the presence or absence of particular genes, but arise from changes in the mechanisms of gene regulation affecting when and where a gene or an entire regulatory module is expressed. As noted by (Brakefield, 2006, p. 362): ‘There is a limited genetical tool kit and much of the morphological diversity evolution is about old genes performing new tricks. Although existing genetic pathways can be co-opted and subsequently elaborated upon to do something different, and specific genes can take on additional tasks at new times during development and in different tissues via gene duplication and divergence, de novo evolution of new pathways appears to be rare’.

Besides the reproductive polyphenisms in social insects and that of male weaponry in dung beetles, other major types of polyphenisms in insects are sequential (seasonal) polyphenisms in wing length (in its extreme form, winged and wingless morphs) or coloration; these are also often connected to differences in reproductive strategies (for review see Hartfelder & Emlen, 2005). In both termites and ants, the caste syndrome is coupled to a wing dimorphism. This clearly represents convergent evolution since the wingless workers of ants are adults of a holometabolous clade, whereas the wingless workers in termites are either immatures (in the wood-nesting termite families Termitidae and Kalotermitidae, and Prorhinotermes) or have conserved an immature phenotype [the worker caste of the Mastotermitidae, Rhinotermitidae (except Prorhinotermes), Hodotermitidae, Serritermitidae, and Termitidae]. Additional wingless morphs in termites are the neotenic reproductives and soldiers, for which there is no real equivalent in ants. Termites evolved complex societies more than 130 million years ago, probably during the upper Jurassic, (Thorne, Grimaldi & Krishna, 2000) from a cockroach-like ancestor similar to the wood-rove beetles (Nalepa & Bandi, 2000) which form the sister group to present termites (Eggleton, 2001; Inward, Beccaloni & Eggleton, 2007a). Cockroaches as well as termites are characterized by a highly flexible development (Roth, 1981; Nalepa, 1994).

Even though termites are phylogenetically distant from ants, and the two groups have been considered either to be engaged in arms races (Longhurst, Johnson & Wood, 1978; Deligne, Quennedey & Blum, 1981) or in more peaceful co-evolutionary interactions (Dejean, Durand & Bolton, 1996), they share several characters as a result of convergent evolution. Both ants and termites have clearly evolved independently from winged solitary or primitively social ancestors (Wilson, 1971); winglessness is an adaptation to burrowing activities and a nest structure which functions as a fortress against predators. The question, thus, becomes which mechanisms underlie the flexible expression of wing phenotypes in ants and termites, where wings develop in the alate reproductives and where wing development is shut down in wingless reproductives and in the worker and soldier castes.

Pattern formation in wings, including their transformation into very different forewing and hindwing types, is best described in Drosophila melanogaster; not only the individual signaling pathway elements, but even entire gene regulatory networks are found to be highly conserved across species, orders and even phyla (Carroll, Grenier & Weatherbee, 2005). The D. melanogaster wing formation network has been successfully applied to a study on the loss of wings in workers of several ant species (Abouheif & Wray, 2002) which showed that wing disc development in workers is not brought to a halt at a single unique interruption point in the network, but rather can stop at different points among ant genera. Caste polyphenism and its relation to wing bud development has not yet been studied to this extent in termites, but recent progress on endocrine regulation and associated gene expression differences is now gradually shedding light on regulatory mechanisms underlying caste development, especially the fascinating (and notoriously confusing) plasticity of caste fate in the lower termites.

We will summarise here the state of termite research regarding this developmental question. First, we will explain termite caste systems and their distribution among taxa. This is supplemented by summaries of the classification of
Developmental plasticity in termites

Table 1. Classification of termite species

Based on their ecology and particularly nesting and feeding habits, termite species can be grouped into two life types:

<table>
<thead>
<tr>
<th>One-piece nesting termites</th>
<th>Multiple-pieces nesting termites [incl. Abe’s intermediate type; Abe, 1990]</th>
</tr>
</thead>
<tbody>
<tr>
<td>● live inside single piece of dead wood serving both as nest and food source</td>
<td>● well-defined nest separate from foraging grounds</td>
</tr>
<tr>
<td>● with exception of winged sexual’s individuals never leave nest</td>
<td>● workers exploit new food resources outside the nest</td>
</tr>
<tr>
<td>● colony life limited by food availability</td>
<td>● colony life not limited by food availability</td>
</tr>
<tr>
<td>● basal life history</td>
<td>● true, morphologically differentiated worker caste with reduced reproductive potential</td>
</tr>
<tr>
<td>● highly flexible individual development (see false workers)</td>
<td>● Mastotermitidae, Hodotermitidae, Serriotermitidae, Rhinotermitidae (except Prokinotermes), Termitidae</td>
</tr>
<tr>
<td>● Termopsidae, Kalotermitidae, Prokinotermes</td>
<td></td>
</tr>
</tbody>
</table>

Higher termites

An alternative traditional classification of termites is based on their gut symbionts:

<table>
<thead>
<tr>
<th>Lower termites</th>
<th>Higher termites</th>
</tr>
</thead>
<tbody>
<tr>
<td>● bacteria and flagellates in gut</td>
<td>● bacteria only</td>
</tr>
<tr>
<td>● all termites except Termitidae</td>
<td>● Termitidae</td>
</tr>
</tbody>
</table>

Table 2. Developmental terms applied to termites

Lower termites are characterized by a unique flexibility in development which is generated through three molting types:

● **Progressive moult** - a moult characterizing the gradual development from egg via several instars into an adult. Associated with progressive moults is an increase in body size and morphological development. This is the default developmental program in all hemimetabolous and holometabolous insects.

● **Stationary moult** - an intermittent moult that is associated with a lack of increase in body size and morphological development. This type of development occurs in several insect species and is frequently associated with periods of food shortage, when a larva or nymph is not capable of passing a critical mass threshold in an instar. In some termites it might also be linked to the wear of mandibles.

● **Regressive moult** - a moult that is characterized by a decrease in body size and/or regression of morphological development, generally a reduction of wing bud size in nymphal instars. This type of development is unique to termites.

In contrast to other hemimetabolous insects where postembryonic development is characterized by a progression of nymphal instars, termites show two distinct types of instars:

● **Larval instar(s)** - instar(s) without externally visible wing buds

● **Nymphal instars** - instars with externally visible wing buds; these instars characterize the gradual, progressive development into winged sexuals.

Larval instars are sometimes further split into:

● **Dependent larvae** - the first up to the second or third larval instar, when larvae supposedly still depend on brood care by ‘workers’ (but see ‘false workers’ in Table 3)

● **Independent larvae** - all other larval instars, when larvae care for themselves. They comprise the false workers and the pseudergates sensu lato (see Table 3).

Termite species (Table 1), developmental terms (Table 2) and classification of castes (Table 3) to provide a comprehensive introduction to termite terminology, essential for wider recognition and comprehension of termite research. We will mainly concentrate on phylogenetically basal termite taxa, but occasional notes on higher termite taxa are given where appropriate. Second, we will summarise what is known about ultimate causes influencing cooperation and altruism in termite societies. Third, we will describe current views about proximate mechanisms underlying social complexity in termites starting with environmental and social triggers that affect different developmental trajectories, then outlining the underlying endocrinology of termite caste development, and presenting the current understanding of differential gene expression during termite caste differentiation. Finally, by embedding our knowledge within a general framework of insect development, we will argue that termite studies can add novel facets to our understanding of the evolution of holometabolous development from hemimetabolous ancestors and we speculate about fundamental facilitators that may help to explain the exceptional position of termites as the only diploid group within the highly social insects.

There is a plethora of literature published on termites and on how best to eradicate them. Undeniably, we have come a long way since the pioneering descriptions on termite life cycles by Eugène Nielen Marais (1937), but there remain enormous gaps in our knowledge on this group of insects; we can still share his observation that ‘The entomologist who made the acquaintance of the termite for the first time, would be justified in thinking it to be an immigrant from a different planet’ (Marais, 1937, chap. 9, par 1).
Table 3. Classification of termite castes

**Reproductives**

Individuals that reproduce within a colony, generally one female (queen) and one male (king). These can be:

- **Primary reproductives** - reproductives that found a new colony after a nuptial dispersal flight. They develop gradually via several nymphal instars into winged sexuals (alates) that shed their wings (dealates) after the nuptial flight. They are characterized by stark sclerotization, the presence of compound eyes and wing marks (remnants of the wings' articulation after they have been shed).
- **Adultoids** - alates that shed their wings and reproduce within the natal nest (they are not neotenics).
- **Neotenic reproductives** - wingless reproductives that develop within the natal colony via a single moult from any instar after the third larval instar. At this neotenic moult, their gonads grow and they develop some imaginal characters while maintaining an otherwise larval appearance; some characters, like wing pads, may regress. Neotenic reproductives are characterized by the absence of wings and usually by the lack of compound eyes. The cuticle is less sclerotized than in primary reproductives. They are subdivided into:
  - **Replacement reproductives** if they develop after the death of the same-sex reproductive of a colony.
  - **Supplementary reproductives** if they develop in addition to other same-sex reproductive(s) already present within a colony.

Depending on the termite life type and the instar from which they develop the neotenics can be further classified into:

- **Neotenis** (sensu stricto): They can be either apterous neotenics developing from a larval instar or brachyterous neotenics developing from a nymphal instar. They are found in lower termites. Neotenics developing from nymphs are sometimes also called secondary reproductives, while those developing from workers are called tertiary reproductives.
- **Ergatoids**: neotenics developing from workers in higher termites
- **Nymphoids**: neotenics developing from nymphs in higher termites

**‘Workers’**

The majority of individuals within a colony belong to the so-called ‘worker’ caste, although they do not necessarily have to work (see ‘false workers’). With a few exceptions among some higher termites, they are not restricted to a specific sex.

A clear separation should be drawn between the ‘workers’ of the one- and multiple-pieces nesting termites (see Table 1) as they are not equivalent in function and development:

- **False workers** - the majority of the individuals within a colony of one-piece nesting termites. They differ from the (true) workers of multiple-pieces nesting termites as they are totipotent larvae that lack morphological differentiations. Correspondingly, they are less involved in truly altruistic working tasks, such as foraging, brood care, or building behaviours. Therefore, they may rather be regarded as large immatures that delay reproductive maturity (‘hopeful reproductives’).
- **True workers** - workers in colonies of the multiple-pieces nesting termites. They can be considered altruistic individuals as they perform most tasks within a colony (e.g., foraging, brood care, and building behaviour) except for reproduction and specialized defence. Although they sometimes, especially in lower termites, still have some reproductive options (for instance as neotenic reproductives), their morphological differentiations (especially their sclerotisation) largely restrict their developmental capability (a notable exception is *Mastotermes darwiniensis*). In functional terms, these true workers, often just called workers, are equivalent to the workers of the social Hymenoptera, even though the latter are imagos, whereas the true workers here are preimaginal stages.

An alternative technical term can be found that distinguishes workers with a flexible development and options for direct reproduction from workers with restricted developmental trajectories:

- **Pseudergates** - ‘workers’ of many lower termites (including one- and multiple-pieces nesting species) that have broad developmental options, generally including progressive, stationary and regressive moults. Current use of this term often lacks the precision of its original definition (Grasse & Noirot, 1947) for individuals that develop regressively from nymphal instars to ‘worker’ instars without wing buds.

We propose here the following definitions which we use consistently throughout our review:

- **Pseudergates sensu stricto** - individuals that develop via regressive moults from instars with wing buds (‘nymphal instars’) to instars without wing buds (‘larval instars’), as defined by Grasse & Noirot (1947).
- **Pseudergates sensu lato** - ‘workers’ of the lower termites that have the potential to undergo progressive, stationary, and regressive moults. They belong to the ‘false workers’ of the one-piece nesting termites and those ‘true workers’ of the multiple-pieces nesting termites that belong to the lower termites and have a flexible development. They comprise larval and nymphal instars.

**Soldiers**

This caste is unique among social insects in function and development. Soldiers are ancestral in termites and evolved prior to a true worker caste. Unlike the soldiers found in other social insects, this caste is monophyletic in termites.

- **Soldiers** - a clearly altruistic caste that is always sterile and that is morphologically and behaviourally specialized for defence of the colony against predators and competitors.
- **Presoldiers** - a single transitional instar during development from ‘worker’ to soldier.

II. CASTE PATTERNS IN TERMITES

In termites two reproductive and two non-reproductive castes can be distinguished: primary and neotenic reproductives on the one hand, and soldiers and true workers on the other, with the latter two forming the majority of the individuals of a colony (Noirot, 1990; Roisin, 2000). The occurrence of the castes differs among families (Roisin, 2000) (Fig. 1). While soldiers are present in all families with exception of a few genera in the Termitidae (higher

Developmental plasticity in termites

![Phylogenetic tree](image)

**Fig. 1.** Phylogenetic tree with life types and the occurrence of different castes in termites. OP- one-piece life type termites which nest in a piece of wood that serves both as shelter and food; MP- multiple-pieces life type termites where nest and food are separated. Unresolved positions are shown in grey or marked ?. Traditionally, termites are classified into lower and higher termites according to the presence or the absence of protozoan gut symbionts. For the monotypic Serritermitidae the caste system is separated. Unresolved positions are shown in grey or marked ?. Traditionally, termites are classified into lower and higher termites according to the presence or the absence of protozoan gut symbionts. For the monotypic Serritermitidae the caste system is separated. Unresolved positions are shown in grey or marked ?. Traditionally, termites are classified into lower and higher termites according to the presence or the absence of protozoan gut symbionts. For the monotypic Serritermitidae the caste system is separated. Unresolved positions are shown in grey or marked ?.

Phylogenetic tree: Mastotermitidae (MP): p, n, w, s
- Termopsidae (OP): p, n, s
- Kalotermitidae (OP): p, n, s
- Hodotermitidae (MP): p, n, w, s
- Serritermitidae (MP): p, n, w, s
- Rhinotermitidae (OP): p, n, s
- + Termitidae (MP): p, (n), w, (s)

Table 3: Elements of the Life Cycle of Termites

<table>
<thead>
<tr>
<th>Termite family (life type): castes</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower termites</td>
<td>Mastotermitidae (MP): p, n, w, s</td>
</tr>
<tr>
<td>Higher termites</td>
<td>Termopsidae (OP): p, n, s</td>
</tr>
<tr>
<td></td>
<td>Kalotermitidae (OP): p, n, s</td>
</tr>
<tr>
<td></td>
<td>Hodotermitidae (MP): p, n, w, s</td>
</tr>
<tr>
<td></td>
<td>Serritermitidae (MP): p, n, w, s</td>
</tr>
<tr>
<td></td>
<td>Rhinotermitidae (OP): p, n, s</td>
</tr>
<tr>
<td></td>
<td>+ Termitidae (MP): p, (n), w, (s)</td>
</tr>
</tbody>
</table>

**Table 3:** The lower termites are 'true workers' (see Table 3). True workers with flexible development including regressive moults together with false workers have often been called 'pseudergates'. We propose here to use the term 'false workers' and compound eyes and a less sclerotized cuticle than in alates. They may originate via a single moult from any instar after the third. At this neotenic moult, their gonads grow and they develop some imaginal characters while maintaining an otherwise larval appearance; some characters, like wing pads, may even regress. This stands in contrast to the development of winged sexuals which is gradual and generally occurs via several nymphal instars with increasing wing pad development.

Recently reported for a Reticulitermes species (Hayashi et al., 2007), caste development in these taxa is not genetically determined. In the pathway leading to reproductive's false workers can either develop into winged sexuals that found a new nest (primary reproductives) or they can become neotenic replacement reproductives in the natal nest if the same-sex reproductive of the colony is unhealthy or dies. Neotenic reproductives in Termopsidae, Kalotermitidae and Proshinotermes are characterized by the absence of wings and compound eyes and a less sclerotized cuticle than in alates. They may originate via a single moult from any instar after the third. At this neotenic moult, their gonads grow and they develop some imaginal characters while maintaining an otherwise larval appearance; some characters, like wing pads, may even regress. This stands in contrast to the development of winged sexuals which is gradual and generally occurs via several nymphal instars with increasing wing pad development.

Associated with the lack of a true worker caste in Termopsidae, Kalotermitidae and Proshinotermes is a characteristic life type. These termites live inside a piece of wood that serves both as food and nest and which they, except occasionally in a few species, never leave to exploit new resources (one-piece nesting termites; Abe, 1987, 1990). Consequently, when the food is depleted, the colony dies. In contrast to the typical drywood and dampwood termites, however, it has recently been shown for some Proshinotermes species that they can move to a new nest site when food supplies are low (Roisin & Parmentier, 2006). Interestingly, neotenic development can be triggered by adding new potential nest sites (i.e. pieces of wood) at a distance of a few centimetres, and some data indicate that these neontics...
become the kings and queens of the new nest in a budding process (Roisin, 2006). Thus, although these neotenics do not reproduce in the natal nest, as occurs in the Termopsidae and Kalotermitidae, immatures of Pronotermes species can become reproductives without a costly winged dispersal process.

Strikingly, this option to become an unwinged reproductive that avoids a costly nuptial flight also exists in the most basal termite family, the monotypic Mastotermitidae; Mastotermes darwiniensis lacks totipotent large immatures and has true workers (Fig. 2B). Recent results show that similar to derived termite taxa, such as the Termitidae, there is an early separation into two developmental pathways, the apterous and nymphal lines, in M. darwiniensis (Watson & Abbey, 1985; Watson & Sewell, 1985; Parmeuter, 2006). These have been called neuter and alate lines respectively, but the former terms seem to be more appropriate (see Roisin, 2000). Individuals can either develop via nymphal instars into winged sexuals (nymphal line) or they can become workers (apterous line) (Fig 2B). Interestingly, these true workers have the option to develop into wingless neotenics reproductives (ergatoids; neotenic derived from workers) which have been shown to head most of the field colonies that originate primarily via colony budding (Goodisman & Crozier, 2002; M. Lenz, personal communication). Although alates do occur, they are apparently less successful in colony foundation; field colonies with primary reproductives were not found in recent studies (Goodisman & Crozier, 2002; M. Lenz, personal communication). Thus, M. darwiniensis displays mosaic evolution of ancestral and highly derived traits. Convergently to derived taxa, like some Rhinotermitidae and the Termitidae (Fig. 2C), it evolved a separate apterous worker line, although from a developmental point of view, this worker line is not equivalent to those in the derived taxa; both the nymphal and apterous lines in M. darwiniensis have direct reproductive options, the nymphal line in the form of winged sexuals and the apterous worker line in the form of unwinged neotenics reproductives. In M. darwiniensis the neotenics, on which colony reproduction largely relies, originate from the workers, whereas in most other termite species nymph-derived neotenics are found (M. Lenz, personal communication). Clearly, in all basal termite clades neotenic reproduction is common (Fig. 1) and presents an alternative to winged dispersing sexuals.

Neotenic reproduction, which only has been lost secondarily in some derived clades, thus, can be considered a synapomorphy that characterizes termites. Its ancestral origin goes hand in hand with the transition to eusociality and has long been claimed to be fundamental for the
evolution of eusociality in termites (Myles, 1988; Thorne, 1997; but see also Roisin, 1999). Reproduction as wingless neotens might be regarded as an alternative breeding tactic that avoids the cost of winged dispersal. Early separation during development into a nymphal and apterous line in species with true workers (Rhinotermitidae (except Proxorhinetes), Serritermitidae, Hodotermitopsidae, Termitidae) which forage outside the nest (multiple-pieces nesting termites, sensu Abe, 1987) (Fig. 2C) might further imply that the termite caste system, including true workers, arose from a wing-polyphenism reflecting alternative breeding tactics (sensu Gross, 1996).

III. ULTIMATE CAUSES INFLUENCING CASTE DEVELOPMENT IN LOWER TERMITES

Their developmental flexibility combined with a basal phylogenetic position make one-piece nesting termites ideal subjects to study the ultimate causes for the development of immatures into soldiers and two types of reproducitives (wingless neotens and dispersing winged sexuals). In solitary insects, wing polyphenisms reflect alternative breeding options: winged individuals disperse to breed elsewhere, while wingless adults reproduce at the natal nest (philopatric breeding) or close to it. The ‘developmental decision’ whether to stay or leave in these solitary insects generally depends on density and food availability at the natal nest, which will both influence reproductive opportunities and the direct fitness of philopatric breeding (Roth, 1981; Müller, Williams & Hardie, 2001; Braendle et al., 2006). The default option in solitary insects is to become a winged sexual, as dispersal in all organisms is generally selected for to avoid competition with relatives and inbreeding (Hamilton & May, 1977). Abundant food resources at the nest, opportunities to meet unrelated mating partners close to the nest, or a lack of incest avoidance (e.g. through secondary mechanisms that protect against inbreeding depression) combined with high dispersal costs may, however, select for philopatric breeding and the evolution of wingless morphs (e.g. Alexander, 1974; Braendle et al., 2006).

In the ancestors of termites, it can be hypothesised that the first individuals to remain at the nests ‘choose’ philopatric breeding to avoid costly dispersal, while helping evolved only secondarily. This is illustrated by an extant drywood termite species, Cryptotermes secundus. We will concentrate on this species here because it not only exhibits an ancestral one-piece nesting life type (Korb, 2007a) but also is one of the most thoroughly studied. References to other lower termites will be given where appropriate. In Cryptotermes secundus, like in all one-piece nesting termites, totipotent individuals have the option to develop into winged sexuals or to stay in the nest with a chance of becoming a wingless neotenic replacement sexual when the same-sex reproductive of the colony dies. There is no local resource competition, as the nest constitutes a bonanza food resource that generally outlasts the lifetime of the founding primary reproducitives (Korb, 2008). If the nest quality declines, individuals are predicted to show an increased tendency to develop into winged sexuals. Experiments have confirmed this, showing that reduced food availability (Korb & Lenz, 2004; Korb & Schmidinger, 2004) or a high parasite load (Korb & Fuchs, 2006) at the nest together with large group sizes lead to increased development of dispersing sexuals. Furthermore, the number of individuals developing into winged sexuals that leave the nest can be explained by the relative probability of inheriting the nest versus successfully founding a new colony (Korb, 2008). As has been suggested for termites in general (Nutting, 1969), the probability of successfully founding a nest is extremely low in C. secundus (< 1 %). The chances of inheriting the colony are on the same order of magnitude and depend on colony size, age of the present reproducitives, and the potential longevity of the nest (Korb & Schneider, 2007). These three variables explain the variation in the number of individuals developing into dispersing sexuals in field colonies. This suggests that individuals remaining at the nest as neotens gain direct fitness benefits as has also been proposed by Myles (1988): dispersal is risky, while the nest presents a safe haven (sensu Kokko & Ekman, 2002). Similarly, in Zootermopsis nevadensis inheritance of the natal breeding position after intercolonial encounters seems to favour the development of soldier-like neotens (i.e. neotens with soldier-like traits, misleadingly also called reproductive soldiers) which are a peculiarity of the Termopsidae (Thorne, Breisch & Muscedere, 2003). At least in C. secundus, indirect fitness benefits gained through raising siblings seem to be less important (Korb, 2007b): the ‘decision’ to develop into a winged sexual is independent of the number of young present in the nest. If individuals were staying in order to raise young, one would predict a negative correlation between these variables: individuals would be less likely to leave the colony when there are more offspring to raise. The lack of a correlation was explained by subsequent observations of an absence of brood care in this species (Korb, 2007b).

Although C. secundus is the only species for which we have such detailed results derived from field as well as laboratory experiments, these results might apply to one-piece nesting termites in general (Korb, 2008) for several reasons: (i) all one-piece nesting termites have totipotent false workers that can develop into winged sexuals and neotenic reproducitives; (ii) reports exist for many one-piece nesting termites that a reduction in food availability triggers the development of winged sexuals (Buchli, 1958; Lenz, 1976, 1994; La Fage & Nutting, 1978; Korb & Lenz, 2004); (iii) they live within a bonanza-type food resource, removing the value of food provisioning for nestmates as all individuals have easy access to food, and a lack of specialized brood care has been recorded for at least four other species [Zootermopsis nevadensis (Howse, 1968); Z. angusticollis (Rosengaude & Traniello, 1993); Cryptotermes domesticus and Cryptotermes cynocephalus (J. Korb, personal observations)]; (iv) they live in a wooden nest that is well protected against predators, while dispersal is relatively risky (Nutting, 1969; Myles, 1988). Taken together, it appears that breeding opportunities as neotenic reproducitives offer high incentives for staying at the nest in one-piece nesting termites. Hence, as in solitary

insects with wing polymorphism, there seem to be two alternative tactics: stay and breed as wingless sexuals, or leave as winged sexuals to reproduce elsewhere.

It can therefore be suggested that the safety of the nest, together with the chance of inheriting the natal breeding position, led to delayed dispersal and the formation of family groups. In C. secundus, as well as in other termites (Haverty, 1977, 1979; Haverty & Howard, 1981; Shellman-Reeve, 1997), the first soldier only develops after such a group has become established. In C. secundus this commonly is at the end of the first year after colony foundation, when about 20 false workers are present. Thereafter, the number of soldiers within a colony is adjusted to colony size, so that a rather constant and species-specific proportion of soldiers will be present within a nest (Haverty, 1977, 1979; Haverty & Howard, 1981). These sterile soldiers defend their family against predators and competitors, hence gaining indirect fitness benefits. This was shown in a field experiment with C. secundus in which soldiers were removed and their re-development hormonally suppressed: soldier-less colonies had a lower fitness than similar-sized control colonies (Roux & Korb, 2004).

IV. PROXIMATE MECHANISMS UNDERLYING CASTE DEVELOPMENT

(1) Social and environmental triggers of polyphenisms in termites and other hemimetabolous insects

For most organisms displaying alternative phenotypes, neither phenotype exhibits higher fitness overall. Rather, there is a trade-off, with the relative fitness of the different phenotypes being contingent upon environmental conditions. The evolution and maintenance of polyphenisms, therefore, requires and is a consequence of variation in the environment. For the evolution of polyphenisms several conditions must be met. First, environmental conditions must influence development to generate different phenotypes. Second, the resulting phenotypes must exhibit higher than average fitness in their respective environments. The factors acting as triggering cues may be the same as the selective agent, or they may be different. As the developmental environment of a phenotype often precedes the selective environment for the adult organism, an environmental cue must at least be correlated with future selective factors (West-Eberhard, 2003). Environmental control of alternative phenotypes can, therefore, evolve in organisms living in spatially or temporally variable environments in which cues can be used to predict reliably the future selective environment (Moran, 1992).

In termites, the cues triggering wing development are identical with the selective agents that favour dispersal of individuals. Reduced food availability or increased parasite load (both causing reduced fitness benefits for individuals staying at the nest) immediately induce a change in behaviour of C. secundus linked to development into winged sexuals (Korb & Schmidinger, 2004): false workers increase food acquisition behaviours and spend less time moving. More strikingly, while proctodeal trophallaxis (anal feeding) is reduced at the colony level, those individuals which are the most active feeders of other individuals develop progressively into nymphal instars. These results support a long-standing, but so far unproven suggestion that inhibitory substances are transmitted within the colony via proctodeal trophallaxis (Lüscher, 1974). Similarly, large group sizes which decrease an individual’s chance of inheriting the nest, function as triggers of winged sexual development.

Assuming that termite societies evolved as a consequence of immatures (false workers) following conditionally two alternative reproductive tactics (dispersing or staying at the nest), and that such alternative tactics (“should I lay or should I go?”) are common in solitary hemimetabolous insects and are frequently associated with wing length polyphenisms, one would expect that the mechanisms underlying the development of alternative phenotypes are evolutionarily conserved. Indeed, similar to one-piece nesting termites, food quality or quantity and population density (i.e. group sizes) are factors known to affect wing development and reproductive physiology in a large number of hemimetabolous insects, including aphids, locusts and crickets (reviewed by Zera, 2003; Hartfelder & Emlen, 2005; Braendle et al., 2006). Thus, the plethora of results on reproductive physiology in cockroaches (for review see Raikhel, Brown & Bellés, 2005) may heuristically guide future in-depth studies on this major aspect of sociality in termites.

The second non-reproductive caste in termites, the soldiers, which are the only individuals in one-piece nesting termites that lose the ability to reproduce, also result from an environmentally induced polyphenism (Lüscher, 1958; Lenz, 1976; Korb, Roux & Lenz, 2003). Their development is triggered by the presence of reproductives, food availability and the size of a colony, while the presence of soldiers inhibits further soldier differentiation (Miller, 1942; Lüscher, 1969; Springhetti, 1969; Haverty & Howard, 1961; Bordereau & Han, 1986; Liu et al., 2005a). This results in colonies having a more or less constant proportion of soldiers in relation to colony size (Haverty, 1977; Noirot & Darlington, 2000). Whether predation pressure also may influence soldier numbers is still controversially debated (Noirot & Darlington, 2000).

(2) Endocrine control of caste development in lower termites

The coordination of growth and tissue differentiation within modular systems, such as the segmented body plan of insects, requires both long-range and short-range signaling by hormones. Ecdysteroids and juvenile hormones are the key factors that drive an insect through larval and nymphal moults and metamorphosis, and their chemistry, haemolymph titres and mode of action in target tissues have been investigated in great detail in a wide variety of hemimetabolous and holometabolous species (for reviews see Nijhout, 1994; Riddiford, 1996; Hartfelder, 2000; Goodman 2008 Cambridge Philosophical Society

& Granger, 2005; Henrich, 2005; Lafont et al., 2005). Furthermore, models have been developed from hormone titre analyses and hormone application experiments that explain the transition from hemimetabolous to holometabolous development through the exploitation of the pronymphal stage and successive recruitment of epidermal cells into imaginal discs (Truman & Riddiford, 1999, 2002). This already highly successful life-history transition has been further extended by the introduction of alternative phenotypes (polyphenisms), which represent adaptive responses to environmental changes without disrupting successful genotypic combinations.

As detailed above, caste development in the lower termites is highly plastic (with certain restrictions in multiple-pieces nesting termites) (Fig 2), making them a challenge to any model on endocrine regulation in hemimetabolous development, because moults are not only progressive or stationary, but can even be regressive. Below we review the literature on morphogenetic hormones and their actions in termite caste development and try to integrate these still fragmentary findings with the much better studied cockroaches, within which the termites are nested (Inward et al., 2007a; see also Lo et al., 2007), and with general ideas on the evolution of insect metamorphosis. The focus will be on the lower termites because caste fate in the higher termites (Termitidae) is generally determined rather early in development and may even involve embryonic predisposition via maternally deposited hormones (Lanzrein, Gentinetta & Fehr, 1985b). Furthermore, moults in higher termites are generally progressive and there are no records of regressive moults in Termitidae.

(3) Juvenile hormone in progressive mouts of lower termites

As illustrated in Fig 2, progressive mouts of particular importance to caste fate in lower termites are (i) the late instar larvae to presoldier/soldier transition, (ii) the transition from late instar larvae to nymph (first nymphal moult, to the nymphal line), and (iii) from late instar larvae/ nymphs to a neotenic replacement reproductive. Of these, the presoldier-soldier transition is at present the best understood.

Although the induction of soldier differentiation by juvenile hormone (JH) and juvenile hormone analogues (JHAs) was one of the earliest findings in JH research (Lüscher, 1969; Howard & Haverty, 1979; for a recent summary see Hrdy et al., 2006), the regulation of JH titre and its mode of action is only now becoming clear, one of the main problems being the role of the social environment.

When monitoring JH and ecdysteroid titres in isolated larvae of the rhinotermid Reticulitermes flavipes, Okot-Kothber et al. (1993) noted a gradual increase in levels of ecdysteroids followed by a steeper increase in JH titre, both peaking at day 9 after isolation, shortly before a presoldier moult normally initiates in isolated pseudergates sensu lato of this species. A radiochemical assay for measuring JH biosynthesis rates was employed to monitor corpora allata activity in pseudergates sensu lato of Reticulitermes flavipes that were kept isolated from their nest in groups of 12–50 individuals (Elliott & Stay, 2008). The results showed an increase in JH synthesis around the time point when some of the pseudergates sensu lato were expected to develop into neotenic reproductive or presoldiers. Also, while JH synthesis rates were generally higher in presoldiers than in pseudergates sensu lato or soldiers, corpora allata activity was considerably lower in presoldiers than in pseudergates sensu lato or neotenes in the pharate stage, that is during the subsequent moulting phase. The authors interpreted this as similar to the terminal stage of development in cockroaches.

A caveat in the interpretation of these results is that the developmental profiles of JH are strongly modulated by season, food availability and colony composition. In the rhinotermid species, Coptotermes formosanus, meticulous studies revealed a strong seasonal cycling of JH titres in soldiers and pseudergates sensu lato (Liu et al., 2005b), similar to earlier observations on corpora allata (CA) volumes in the kalotermitid Kalotermes flavicollis (Lüscher, 1972), JH titre levels in pseudergates sensu lato were either negatively affected by increasing the percentage of soldiers in experimental groups (Mao et al., 2005; Park & Raina, 2005), or were positively affected by improved food or temperature conditions (Liu et al., 2005a). Furthermore, the time course of JH synthesis rates for Reticulitermes flavipes pseudergates sensu lato kept isolated from their nest was shown to be dependent on group size (Elliott & Stay, 2008).

Such social influences on factors controlling caste development are widespread and not limited to soldier development in termites, but rather are a facet of the pleiotropic functions of JH in insect development and reproduction. For example, JH both prevents wing shedding and precocious ovarian activity in immature alates of the termopsid Zootermopsis angusticollis, yet stimulates oogenesis in mated queens in this species (Brent, Schal & Vargo, 2005). This pleiotropic role of JH also became apparent in a study on corpora allata activity in apterous and brachypterous neotenic Reticulitermes flavipes females, where an increase in the number of vitellogenic ovarioles was accompanied by an increase in corpora allata activity (Elliott & Stay, 2007). The critical questions, therefore, are (a) how is the hormone titre regulated, and (b) which are the molecular targets for JH action in termites?

The first inhibitors of JH biosynthesis were discovered in cockroaches and were termed allatostatins (Woodhead et al., 1989; Stay et al., 1991). Members of this large family of neuropeptides have now been identified in all major orders of insects (for review see Stay & Tobe, 2007). Allatostatin immunoreactivity has recently been described in the rhinotermid, Reticulitermes flavipes, showing that lateral and medial neurosecretory cells in the brain innervate the corpus allatum (Yagi et al., 2005). Furthermore, in vitro incubation of termite corpora allata (CA) in the presence of two cockroach allatostatins significantly inhibited JH production (Yagi et al., 2005). Allatostatins, however, do not only regulate JH synthesis but, due to their widespread occurrence and origin as gut-brain peptides, can affect a large suite of body functions, including food intake by modulating gut contraction (Aguilar et al., 2003; Aguilar, Maestro & Belles, 2006). The effects of food quality on
soldier induction (Liu et al., 2005a) may partially be mediated through these routes. In conjunction with their allatostatic effect, these peptides would then link reduced individual food intake due to deteriorating colony conditions to a reduction in the percentage of larvae that enter the presoldier-soldier pathway. The pleiotropic functions of such endogenous regulatory peptides could, thus, provide a switch mechanism that links the social with the internal environment. Links between the social and the internal environment are still little understood, not only in termites but also in the caste development of other social insects. Even though inhibitory pheromones have long been thought to be involved in the development of reproducitve and in the adjustment of caste ratios in termites (Lüscher, 1964; Lefèvre & Bordereau, 1984; Noirot, 1991), the neural substrates for their perception and transmission to the endocrine system are unknown, except for the architecture of the allatostatin-expressing neurons (Yagi et al., 2005).

After its release into the haemolymph, the extremely lipophilic JH molecule must be bound to transport proteins in order to reach target organs and to remain protected against degradation by JH esterases and/or JH epoxide hydrolases (reviewed by Goodman & Granger, 2005). JH binding proteins have been identified by photoaffinity labeling in haemolymph of the rhinotermitids Reticulitermes flavipes, Coptotermes formosanus and the termopsid Zootermopsis nevadensis (Okot-Kotber & Prestwich, 1991a, b). While these proteins bind JH with high affinity, two general transport and storage proteins, hexamerin 1 (Hex-1) and hexamerin 2 (Hex-2) have recently emerged as major candidates for regulators of soldier development in the rhinotermitid Reticulitermes flavipes. As in other insects, fat body expression levels and haemolymph titres of these proteins are strongly modulated during development and seem to be affected by JH, especially in the case of Hex-2 (Scharf et al., 2005a; Zhou et al., 2006b; Zhou, Faith & Scharf, 2006a; Zhou, Traver & Scharf, 2007b). In turn, JH regulation of hexamerin expression has a marked feedback effect on JH availability to target tissues. Whereas hexamerins of other insects are known to bind JH with low affinity (Tawfik et al., 2006), they apparently do this in a peculiar way in R. flavipes. Based on RNA interference (RNAi) results and Western blotting with a JH-specific antiserum, Zhou et al. (2006b) conclude that Hex-1 might covalently bind JH and that these two proteins interact and form a sink for JH that, at the colony level, would allow the fine-tuning of worker to soldier caste ratios. This termite therefore seems to have exploited an abundant haemolymph protein with pleiotropic functions and co-opted it into a regulatory network of social organization (Zhou et al., 2006a). A similar feedback network involving a hormone of pleiotropic functions (JH) and a phylogenetically equally old transport/storage protein (vitellinogen) has recently been demonstrated to regulate age polyethism in honey bee workers (Amdam et al., 2003, 2006).

While these examples illustrate the co-option of ground plan components of insect developmental and reproductive physiology, there are still a number of questions to be answered. Firstly, the proposed covalent binding of JH is peculiar and raises the following questions: (a) is Hex-1-bound JH accessible to degradation by JH esterase or JH epoxide hydrolase in haemolymph, (b) is it sequestered in Hex-1-bound form into the fat body, and if so, what happens there, (c) can JH-sensitive target tissues also sequester Hex-1-bound JH, and what are the subsequent effects and, most importantly, (d) is this mechanism of JH sequestration a general property of lower termites or is it restricted to Reticulitermes flavipes?

Additional questions have been raised by Hrdy et al. (2006) who showed that the racemic JH-III used in the above experiments on hexamerin function is not a particularly strong inducer of soldier development in Reticulitermes species, when compared to other JHAs. A feasible explanation for the low activity of JH-III would be its well-known lower metabolic stability in insect haemolymph, where it is efficiently degraded by insect JH-esterases (Oakeshott et al., 2005). In addition, the fraction of JH-III that is covalently bound to Hex-1 in Reticulitermes flavipes haemolymph would essentially be unavailable for physiological functions. An important question to answer would, thus, be whether JHAs modulate hexamerin expression similar to JH, that is, do they transcriptionally mimic JH-III and do they also bind to Hex-1?

Regarding intracellular JH clearing, the finding of a JHA-induced expression of a cytochrome P450 transcript in the fat body of the termopsid Hodotermopsis sjoestedti (Cornette et al., 2006) is interesting. This enzyme could be a candidate for intracellular JH degradation, since cytochrome P450s are not only general detoxifying enzymes, but also have been specifically implicated in the metabolism of methyl farnesoate to JH-III (Helvig et al., 2004). Similarly, Zhou et al. (2007a) showed for the rhinotermitid Reticulitermes flavipes that several fat-body-related P450s (CYP4) were differentially expressed after JH treatments, and we found that a cytochrome P450 enzyme was overexpressed in female Cryptotermes secundus neotenics compared to false workers (Weil, Rehli & Korb, 2007; see below).

(4) Molecular underpinnings of caste in lower termites

Attempts to unravel the molecular basis of caste development have also focused on the soldier differentiation pathway, because of its relative ease of induction by JH and JHA applications, and also because of the marked morphological differences in soldiers. The first differential gene expression screens performed on the termopsid Hodotermopsis sjoestedti (formerly H. japonica) (Miura et al., 1999; Miura, 2001) led to the identification of a gene with soldier-specific expression (SOL1) in the mandibular gland of this dampwood termite. It encodes a putative member of the lipocalin family that may be a soldier-specific secretory product of this gland. In the termitid Hospitalitermes mediusflavus, this gland develops from a disc-like structure once a presoldier-differentiating moult has been induced by a high JH titre (Miura & Matsumoto, 2000).

In a follow-up study performed as a differential display reverse-transcription polymerase chain reaction (DDRT-PCR) screen on RNA extracted from mandibles of
Hodotermopsis sjostedti. Koshikawa et al. (2005) investigated tissue-specific differentiation processes induced by the application of a JHA. They confirmed the expected overexpression of cuticle proteins in the mandibles of developing soldiers and also revealed a set of putative transcription and translation regulators (including a staufen orthologue), an actin-binding protein possibly involved in cellular morphogenesis and also a member of the aldehyde dehydrogenase (Adh) family.

Macroarray screens on whole-body pseudergate sensu lato, presoldier, soldier and nymphal RNA of the rhinotermitid Reticulitermes flavipes (Scharf et al., 2003) detected 25 differentially expressed sequence tags (ESTs), 16 of which represented orthologues (E-values < e^{-6}). Pseudergates sensu lato showed a strong overexpression of endosymbiont cellulase genes and soldiers overexpressed cytoskeletal proteins, especially ones related to skeletal muscle, and a cytochrome oxidase I encoding gene. While these were not surprising findings considering the functions of these two castes, the overexpression of vitellogenin in presoldiers is perplexing. Another remarkable point is that most of the unknown genes (no BLAST matches) were overexpressed in pseudergates sensu lato or in soldiers and, thus, may represent novel genes typical for either of these castes, like the SOL1 transcript of the termopsid H. sjostedti.

The molecular basis of development from pseudergates sensu lato to reproductives (especially into neotenic replacement reproductives, since this requires only a single moult) is now under investigation in the kalotermitid Cryptotermes secundus (Weil et al., 2007). Using a highly sensitive suppression subtractive hybridization strategy (Representational Difference Analysis), this study led to the complete or partial cloning of five differentially expressed genes, as validated by quantitative real-time PCR. These genes (a member of the esterase-lipase family, a putative beta-glycosidase, a cytochrome P450 gene, vitellogenin, and an unknown gene) were markedly overexpressed in female neotenics. Surprising findings for vitellogenin transcripts were the lack of sex-specificity (highly expressed both in neotenic females and males) and lack of compartmentalization (expressed in the head, thorax, and abdomen). Vitellogenin has long been considered a sex-specific protein exclusively required for oogenesis. This paradigm has lately undergone considerable change, especially in social insects.

In the honey bee, vitellogenin has been shown to be involved in the regulation of task performance, via repression of the JH titre (Guidugli et al., 2005a), and in longevity (Amadon et al., 2005; Corona et al., 2007), and vitellogenin expression has also been detected in larval stages (Guidugli et al., 2005b). In cockroaches, the induction of vitellogenin by JH is also not restricted to adult females, but has been reported to occur in preadult stages (Lanzrein, 1974; Cruz et al., 2003) and in males (Mundall, Tobe & Stay, 1979).

Such differential expression of several metabolism-related genes in termite caste development as well as the overexpression of a cytochrome C oxidase subunit III in nymphs and pseudergates sensu lato of the rhinotermitid Reticulitermes santonensis (Lienard et al., 2006), may shed light on a largely overlooked aspect of caste development, the importance of metabolic regulation. In this respect, termites parallel the honey bee, where similar observations have emerged from several expression screens that can now be explored in depth on the basis of genomic information (Cristino et al., 2006).

Whereas these studies are informative on metabolic regulation and on the differential expression of structural genes they tell us relatively little about patterning mechanisms involved in the shaping of caste-specific structures. In particular, the shutting down of wing development in termite castes (true workers, neotenics and soldiers) has not yet been investigated at the molecular level, although studies have been initiated to reveal underlying cellular mechanisms (Miura et al., 2004).

V. PERSPECTIVES ON HORMONAL REGULATION, CASTE DEVELOPMENT AND TERMITE EVOLUTION

(1) Ecdysteroids and insulin signaling

Whereas the role of JH in termite caste development has received much attention due to its potential in pest control strategies, ecdysteroids have only rarely been studied, despite their importance in moulting cycles and metamorphosis. JH and ecdysteroid titres rise concomitantly in isolated pseudergates sensu lato of the rhinotermitid Reticulitermes flavipes (Okot-Kotber et al., 1993), and the deposition and sclerotization of cuticle in the mandible of soldiers is dependent on an interaction between these two hormones (Okot-Kotber, 1983).

For molecular studies, the ecdysteroid signaling pathway may be more productive since, in distinction to JH signaling, the ecdysone receptor(s) (EcRs) and their dimerization partners, the orphan nuclear receptors of the rexinoid/ultraspiracle (RXR/USP) family have been identified in a large number of species (Thummel, 1996; Henrich, 2005). An EcR-A isoform has recently been identified in the cockroach Blattella germanica, and RNAi experiments have revealed that it is involved in adult-specific developmental processes, including wing development (Cruz et al., 2006). Similar functional RNAi analyses have also been performed for the RXR/USP orthologue of this cockroach showing that BgRXR knockdown arrests the nymphal to adult moult (Martin et al., 2006). The identification of Drosophila EcR and RXR/USP orthologues in cockroaches and the results of the functional assays demonstrate that this nuclear receptor pathway is highly conserved in hemimetabolous and holometabolous insects. Furthermore, besides being a dimerization partner for EcR, the RXR/USP protein has also been suggested as a possible JH receptor in Drosophila melanogaster (Jones & Sharp, 1997; Xu et al., 2002; Jones et al., 2006) and also in the honey bee (Barchuk, Maleszka & Simoes, 2004). Alternatively, with their low-affinity binding characteristics for specific ligands, the RXR/USP nuclear receptors could function as lipid sensors, and thus provide a direct link between nutritional status and moulting or metamorphosis induction (Chawla et al., 2001).
This link between the classic morphogenetic hormones, JH and ecdysteroids, and nutritional status could also involve another phylogenetically old signaling pathway which functions as a local growth regulator, the insulin-/insulin-like signaling (IIS) and the associated target of rapamycin (TOR) pathway (Brogiolo et al., 2001; Edgar, 2006). These flexible response systems to the endogenous nutrient milieu regulate cell growth and cell division, and thus affect organ and body size. Furthermore, they have been shown to interact directly with the function of the prothoracic gland (Colombani et al., 2005; Mirth, Truman & Riddiford, 2005). Through this gateway, an important aspect of metamorphosis, the critical size threshold, becomes directly amenable to natural selection.

In the honey bee, the availability of the complete genome sequence has facilitated the annotation of insulin/insulin-like peptides, insulin receptors, TOR, as well as most of the downstream elements of these signaling pathways (The Honey Bee Genome Sequencing Consortium, 2006). The expression of some of the IIS components has been shown to be strongly affected by the diet provided to honey bee larvae (Wheeler, Buck & Evans, 2006), in correspondence with the caste-specific differential expression profiles found for an insulin receptor (Azevedo & Hartfelder, 2008); interference with TOR signaling by rapamycin administration or RNAi has been shown to affect queen/worker differentiation directly (Patel et al., 2007). The high conservation of IIS components should make this pathway an interesting target for studies on termite caste differentiation. This group of social insects is of singular interest because of its dietary specialization, especially in those lower termite species which continuously reside in and feed on a single piece of dry or damp wood and for which food availability has been shown to trigger different caste developmental trajectories (see above). The question of critical size in the context of nutrition and caste divergence and its consequences for direct and indirect fitness should, thus, be of singular importance to the highly flexible systems of caste determination in the lower termites.

(2) What is the status of the larval stages?

A major problem in termite developmental biology is actually a terminological one. While they are unquestionably hemimetabolous insects, their early postembryonic instars are nevertheless denominated as larvae and not as nymphs, the typical terminology for growth stages of solitary hemimetabolous. In termites, the term nymph is reserved for later developmental stages that exhibit progressively growing wing pads and, thus, are gradually committed to winged dispersal (Noiro, 1990). In some termites only the early stages (first to third instars) are termed larvae, but de facto, the false workers of the one-piece nesting lower termites can also be considered larvae, as long as they have an unsclerotized cuticle, simple mandibles and do not show external wing pads or compound eyes. The traditional terminology of termite developmental stages has been called into question by the discovery of an early larval stage with wing buds in the genus Termitogeton (Rhinotermidae). Their wing buds regress in successive moults until they reappear in the single nymphal stage (Parmentier & Roisin, 2003). A second major problem is how to describe stationary and regressive moults in the lower termites.

From their morphological characters and possibly also their developmental status, the early postembryonic stages of termites (Fig. 3) are more similar to the grub-like larvae of lepidopterans or coleopterans than to the more adult-like cockroach or locust nymphs. They are holometabolan-like with respect to the developmental status of their wing and eye primordia and the cuticle structure, and they are hemimetabolan-like with respect to the body tagmata (more pronounced thorax-abdomen division). In terms of evolutionary trajectories, the postembryonic stages of termites (probably including parts of the false worker/pseudergates sensu lato stage, which is not a single developmental stage but a composite resulting from progressive, stationary and regressive moulting events) could actually be considered as equivalent to the pronymphal stage of Orthoptera and Blattoidea.

This hypothesis would be consistent with the scenario proposed by Truman & Riddiford (1999, 2002) on the transition from hemimetabolous to holometabolous development (but see also Heming, 2003; Minelli et al., 2006; for a wider discussion and alternative viewpoints). In termites external wing buds only start to grow during the nymphal moults (but are present in Termitogeton; Parmentier & Roisin, 2003), and larval termites do not have compound eyes but only primordia (Miura, 2005). In holometabolous insects, wings develop from imaginal discs and the eye primordia of termites resemble the eye imaginal discs of Manduca sexta (Champlin & Truman, 1998; MacWhinnie et al., 2005). The pseudergates sensu lato, which can comprise several instars,

![Fig. 3. Developmental stages of the drywood termite Cryptotermes secundus. (A) ‘Dependent larva’, (B) false workers (larval and nymphal instars).](image-url)
could, thus, be conceived as a platform for flexibility in developmental decisions, leading to soldiers, alates, or neotenic reproductives.

So far, this hypothesis remains to be tested. Some data hint at this scenario, such as a change in mandible shape and an increased degree of sclerotization observed in rhinotermitids following JHA application (Lenz, 1976; Lelis & Everaerts, 1993), similar to results obtained in locust pronymphs (Truman & Riddiford, 1999), and the formation of soldier-alate intermediates (Lelis & Everaerts, 1993; Koshikawa, Matsumoto & Miura, 2002; Miura, 2005) in Heterotermes sjostedi (Termopsidae), which can be interpreted as a trade-off between wing/eye development and that of defence structures on the head.

A revision of termite embryogenesis is clearly required. There are excellent descriptions on the embryonic development of Kalotermes flavicollis and Zootermopsis nevadensis (Striebel, 1960) and Cryptotermes brevis (Kawanishi, 1975), but these do not address the question of whether termites undergo embryonic mouls. In locusts, such mouls are important for the appearance of a pronymphal stage, before eclosion of the larva from the egg. Identification of a pronymphal stage would require detailed JH and ecdysteroid titre measurements to determine whether there are differences in the titre profiles between the larval and nymphal/pronymph stages and whether and how these titre patterns differ from those established for a well-studied basal hemimetabolan insect, the cockroach Nauphoeta cinerea (Lanzrein et al., 1985a). An alternative would be to investigate candidate genes for gene regulatory networks underlying molting and metamorphosis, in an approach similar to that taken by Abouheif & Wray (2002) for understanding the prevention of wing development in ant workers. Both approaches should enable us to clarify the position of the stationary and especially the peculiar regressive mouls in lower termites.

(3) Regressive mouls in lower termites, a major enigma

The regulatory network for the stationary mouls of pseudergates sensu lato may be equivalent to that of supernumerary mouls of solitary hemimetabolous insects, which are associated with little growth and no differentiation. The driving force for stationary mouls in the lower termite pseudergates sensu lato may actually be the wear of their mandibles (Roisin & Lenz, 1999). Renewing this structure in a moult would allow large immatures (i.e. pseudergates sensu lato) to remain for an extended period in the nest with a chance of eventually inheriting the natal breeding position. Regressive mouls are observed in the nymphal-alate transition, where nymphs that did reach the alate moult in one year regress to the false worker stage, accompanied by wing pad reduction (pseudergates sensu stricto) (Korb & Katrantzis, 2004). So why do these individuals not remain as nymphs in the nest and develop into alates early in the next swarming season? This is especially interesting as there seem to be developmental deadlines for each nymphal instar; individuals that fail to reach these deadlines cannot become alates.

There might be two explanations. First, a regressive moult in the nymphal-alate transition could be a consequence of wing pad mutilation. Mutilated wing buds were found in several lower termite species and it was hypothesized that they were the result of manipulations by siblings or parents (Zimmermann, 1983; Myles, 1988; Roisin, 1994, 2006; Miura et al., 2004) similar to the mutilation of gemmae in some queenless ponerine ants (Peeters & Higashi, 1989; Ramaswamy et al., 2004). However, as nobody has actually observed the process of mutilation in nature, its causes are unknown. Results for the drywood termite Cryptotermes secundus suggest that such damage is an artefact of handling conditions (Korb, 2005).

From a theoretical point of view there should be no selective advantage in monogamous colonies to sibling or parental manipulation (Korb, 2003), especially when false workers do not provide much help in raising siblings, as recent data for C. secundus suggest (Korb, 2007b). Why then should parents or siblings manipulate their nestmates to stay in the nest, when these nestmates do not provide costly brood care and when breeding opportunities outside the nest are not limited by intraspecific competition? Roisin (2006) suggested that intracolonal competition might exist for some high-quality resources or brood care. But why then can individuals, which do not disperse under abundant food conditions, become winged sexuals when food availability is reduced? If individuals are capable to develop into alates under reduced food conditions they should be even more capable to become alates under abundant food conditions. These considerations suggest that individuals are staying voluntarily under abundant food conditions.

Conflicts among totipotent individuals over dispersal might occur in fused termite colonies with a within-colony relatedness below 0.5 and if dispersal is a better option then staying at the nest. Further research must show whether these latter conditions are met in any lower termite species under natural conditions. So far, although scant, the available field data suggest that staying in the nest with a chance to inherit the colony is not an inferior reproductive tactic compared to leaving the colony as winged sexual (Korb, 2007b, 2008).

An alternative explanation is that regression might be part of an 'honest signal' in a test for alate competence. All individuals have to start from the same stage as apterous individuals. From this stage, only those individuals that reach the developmental deadlines are the most competent for alate development. Individuals that are less competent would stay for another year and try to gain enough resources for the subsequent nuptial flight.

At the proximate level, the general paradigm for hormonal regulation in metamorphic mouls provides a series of predictions for regressive mouls in the nymphal-alate transition, since the nature of the subsequent moult is always determined during the preceding intermoult period (Riddiford, 1994). A critical factor in the last preimaginal stage is the JH level at the rise of the moult-inducing ecdysteroid peak. We predict that only nymphs with a sufficiently suppressed level of JH will become alates, while an above-threshold JH level during the ecdysteroid
VI. CONCLUSIONS

(1) “Pseudergates sensu lato” are a fascinating example of ample developmental plasticity with far-reaching consequences in terms of the ecological and evolutionary success of termites. We compile here current knowledge on the ecology and life history of lower termites, and set this into a framework of developmental biology, especially the endocrine regulation underlying caste differentiation.

(2) The so-called one-piece nesting termites with their wood-nesting life style are considered a basal group (Roisin, 2000; Thorne & Traniello, 2003; Korb, 2007a). From phylogenetic data on termite families it is not possible to reconstruct the evolution of true workers unambiguously (Thompson et al., 2000, 2004; Grandcolas & D’Haese, 2004; Inward, Vogler & Eggleton, 2007b). Other results leave less doubt about the basal position of false workers, and thus of the one-piece nesting life style (reviewed in Korb, 2007a): (i) a recently published comprehensive phylogenetic analysis on Dictyopterans places the termites within the cockroaches, Blattodea, as a sister group to the woodroaches, Cryptocercidae (Inward et al., 2007a; see also Lo et al., 2007) which have a similar life style to the one-piece nesting termites; (ii) the presence of true workers in the basal group Mastotermes danciniensis, which are not equivalent to the true workers of other multiple-pieces nesting termites (Parmentier, 2006), suggests at least two independent origins of true workers, implying a basal position for one-piece nesting termites.

(3) The one-piece nesting termites show the greatest flexibility in developmental decisions related to caste. Developmental plasticity is, thus, a basal character in termites. Termite caste allometries (as e.g. shown by Koshikawa et al., 2002) are results of phenotypic plasticity that does not involve genetic change. The demonstration of major epigenetic effects on honey bee caste fate through differential genome methylation (Kucharski et al., 2008) furthermore shows that social insect polyphenisms can be interpreted as split developmental reaction norms in terms of Schlichting & Pigliucci’s (1998) approach to phenotypic evolution.

(4) We propose that a pronymphal stage similar to that of the holometabolous clade (Truman & Riddiford, 1999, 2002) may have been translocated from within the egg to a prolonged postembryonic stage at the beginning of termite evolution, in association with the suppression of wing development due to a burrowing feeding habit in a woodroach-like ancestor. Under this scenario, the prolonged postembryonic stage, exemplified by the false worker/pseudergates sensu lato stage, provides a platform for developmental trajectories into wingless and winged reproductive, which present alternative breeding tactics, and, probably as a subsequent evolutionary step, also development into soldiers. The extension of the pronymphal stage of basal hemimetabolans into a sequence of postembryonic instars in such a termite ancestor would then be considered a pre-adaptation for developmental plasticity contingent on the nutritional and social environment. A next step could then have been the assimilation of this contingency into a morphogenetic program that directs the development of alternative phenotypes via endocrine control.

(5) The role of JH has long been explored in proposals for termite pest control strategies and based on such data and hormone titre measurements we propose a scenario of endocrine regulation of caste development that incorporates critical periods and response thresholds (Nijhout, 1994; Hartfelder & Enlen, 2003). Recent results on the differential expression of hexamerin genes in the genus Reticulitermes can be interpreted as a co-option of a general storage protein that belongs to an ancient family of arthropod proteins (Burmester, 2002).

(6) Even though the focus of the current review is on lower termites, the castes of higher termites can be easily integrated into this framework. The restricted plasticity in the apterous line and their much earlier commitment to reproductive castes can be conceived as a change in the timing of hormone-dependent determination steps. The higher JH levels observed in Macrotermes michaelseni eggs dedicated to become reproductives (Lanzrein et al., 1985b) indicate that the lines split at at least two stages, an early one in the embryonic phase, for the apterous/nymphal decision, and a later one in the early nymphal stages, for the worker/soldier decision (Fig 2).

VII. ACKNOWLEDGMENTS

We would like to thank Michael Lenz, Paul Eggleton and two anonymous referees for their helpful comments on this manuscript. We also acknowledge financial support from the Deutsche Forschungsgemeinschaft (DFG, KO 1895/6) and from a Brazilian/German (Capes/DAAD) cooperation program (PROBRAL, 261/07).

VIII. REFERENCES


Developmental plasticity in termites


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