EVOLUTION & DEVELOPMENT 13:2, 138–148 (2011)
DOI: 10.1111/j.1525-142X.2011.00464.x

Ontogenic potentialities of the worker caste in two sympatric subterranean termites in France

Laurianne Leniaud,[a,b] Eric Darrouzet,[a] Franck Dedeine,[a] Kiheung Ahn,[c] Zachary Huang,[c] and Anne-Geneviève Bagnères[a]*

[a]I.R.B.I. CNRS UMR 6035, Faculté des Sciences et Techniques, Université François Rabelais, Parc Grandmont, 37200 Tours, France
[b]Evolutionary Biology & Ecology C.P. 160/12, Université Libre de Bruxelles, Avenue F.D. Roosevelt, 50, 1050 Brussels, Belgium
[c]Department of Entomology, Michigan State University, East Lansing, MI 48824, USA
*Author for correspondence (email: bagneres@univ-tours.fr)

SUMMARY In termites, the capacity of workers to differentiate into neotenic reproductives is an important characteristic that deserves particular attention. To gain insight into the differentiation pathway, the potentialities of workers and the endocrinal changes during the formation of neotenics were compared in two sympatric termites, *Reticulitermes flavipes* and *Reticulitermes grassei*. After 1 year of development, 100% of *R. flavipes* worker groups produced neotenics against only 63% of *R. grassei* groups. The average production of female neotenics was significantly higher in *R. flavipes* worker groups compared with *R. grassei* groups and *R. flavipes* produced a greater proportion of female neotenics. Moreover, *R. flavipes* produced more offspring, not only because there were more females, but also because *R. flavipes* females were more productive. Moreover, the offspring produced by *R. flavipes* grew faster than the offspring of *R. grassei*. Both ecdysteroid and juvenile hormone (JH) titers varied significantly during the development of neotenics. The two species showed similar ecdysteroid titer variation patterns. However, the JH titer variation patterns strongly differed: in *R. grassei*, the concentration of JH increased in maturing neotenics then dropped in mature neotenics, whereas in *R. flavipes*, the level of JH was significantly higher than in *R. grassei* and remained constantly high in mature neotenics. Overall, these results suggest that these two species differ strongly in many life-history traits as well as in the physiological control of their caste differentiation system. Possible origins and mechanisms of such interspecific variations are discussed, as well as their evolutionary and ecological consequences.

INTRODUCTION

Polyphenism is the ability of an organism with a given genotype to produce multiple discrete alternative phenotypes by changing from one developmental pathway to another in response to environmental changes (Nijhout 1999, 2003; West-Eberhard 2003). Caste polyphenism in social insects is particularly interesting for studying developmental processes as it involves multiple phenotypes (i.e., castes) that appear and interact within the same generation (Wilson 1971a; Oster and Wilson 1978; Miura 2001; Miyata et al. 2004; Roux et al. 2009; Bourguignon et al. 2009; Korb 2009; Korb et al. 2009a). Switching mechanisms that determine caste phenotype expression result in numerous adaptations not only in response to environmental factors, such as temperature or day length, but also in response to social interactions among nestmates (Noirot 1991; Brent 2009; Rueppell 2009; Nalepa 2010).

Although several recent studies provided supporting evidence for the genetic determination of castes in Isoptera (Hayashi et al. 2007; Matsuura et al. 2009), the classical view that caste systems result from a differential expression of genes between castes still prevails (Miura 2001; Scharf et al. 2005b; Zhou et al. 2006b; Korb et al. 2009b). Research has shown that polyphenism depends on specific mechanisms which are regulated by variations in hormone secretion patterns, altered hormone titers, altered hormone response threshold and changes in the hormone-sensitive period (Nijhout 2003; Brent et al. 2007). Despite recent advances, the mechanisms involved in polyphenism still remain unclear and further research is required.

Caste determination in termites results in a highly plastic social organization that is an example of larval polyphenism (Nijhout 2003; Korb and Katrantzis 2004). This system apparently results from specialized regulation of hemimetabolous

development. Except for primary reproductives, all other individuals in a termite colony are functionally sterile and belong to distinct castes (i.e., workers, soldiers, or nymphs). The developmental pathways are sometimes reversible (Noirot 1989), and individuals, particularly in basal taxa such as subterranean termites (family Rhinotermitidae), can have ontogenic alternatives allowing them to differentiate into several castes.

After being excluded from the winged pathway, immature individuals of both sexes can develop into sterile workers, soldiers, or secondary reproductives (Roisin 2000) (Fig. 1). In the typical development of a rhinotermitid colony, a single pair of primary (winged) reproductives founds a colony with a simple family structure consisting of one queen, one king, and their offspring. In Rhinotermitidae, primary reproductives can be supplemented or replaced by one or more phylopatric secondary reproductives that develop either from nymphs (brachypterous neotenics) or workers (apterous neotenics) (Buchli 1958; Pichon et al. 2007; Fujita and Watanabe 2010).

The development of neotenic reproductives within colonies can have profound consequences on the breeding system and mode of dispersal of colonies and may, therefore, affect both the genetic structure and the dynamics of termite populations. The presence of neotenics increases relatedness and inbreeding within colonies and may reinforce the genetic differences between colonies within populations. By generating numerous offspring, neotenics can also extend the foraging area of the colonies, which are, therefore, more prone to separate into several colonies (i.e., founding colonies by budding) (Thorne et al. 1999; Pichon et al. 2007). Furthermore, the ability of colonies to develop numerous neotenics may increase their capacity to colonize and develop into urban areas. However, the molecular and physiological mechanisms by which neotenics differentiate from workers are not well understood.

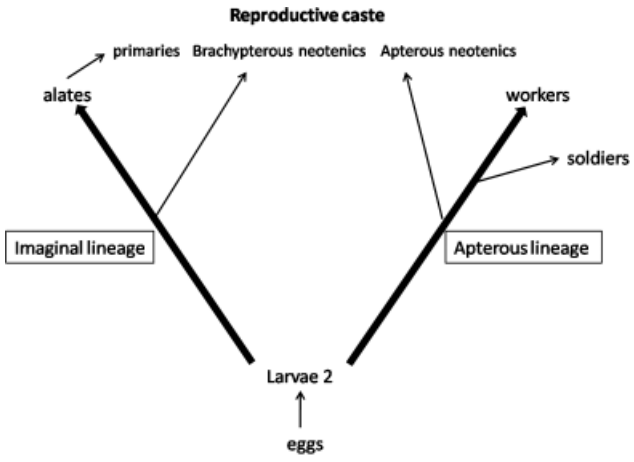

Fig. 1. Caste differentiation and polyphenic development in *Reticulitermes* termites (from Buchli 1958; Scharf et al. 2003).

In termites, studies on hormone titers have focused either on soldier development and/or reproductive physiology (Nijhout 2003; Brent et al. 2005, 2007; Elliott and Stay 2007, 2008; Korb 2009). For example, juvenile hormone (JH) is known to play a key role in caste polyphenism (Park and Raina 2004; Mao et al. 2005), especially in caste differentiation into soldiers (Miura 2001; Scharf et al. 2003; Hartfelder and Emlen 2005) and probably into neotenics (Elliott and Stay 2007, 2008). A small number of studies have focused on ecdysteroids (Brent et al. 2005; Brent et al. 2007; Korb et al. 2009a). However, it is now known that high concentrations of ecdysteroids occur in alate adults (Raina et al. 2003), suggesting that these compounds may play an important role in regulating ovarian development and oogenesis. Despite recent works in endocrinology and gene expression (Miura 2001; Scharf et al. 2003; 2005a, b; Miura 2004; Zhou et al. 2006a, b, c), the knowledge of molecular and physiological processes of caste regulation in termites still remains limited. Zhou et al. (2006b, c, 2007) identified a hexamerin-based mechanism in *Reticulitermes flavipes*, which regulates the development of alternative caste phenotypes. Nevertheless, the exact mechanism controlling termite caste regulation remains to be elucidated.

To gain insight into caste polyphenism, the workers' ontogenic potentialities and the endocrinal changes during the formation of neotenics were studied and compared in two *Reticulitermes* species, *Reticulitermes grassei*, and *R. flavipes*. *R. grassei* is found in Spain and along the Atlantic coast in southwest France (Kutnik et al. 2004), whereas *R. flavipes* (previously called *Reticulitermes santonensis* in France) is native to North America and has been introduced into Western Europe (France and Germany) and South America (Chile) (Bagnères et al. 1990; Clément et al. 2001; Vieau 2001; Ye et al. 2004; Austin et al. 2005; Dronnet et al. 2005; Su et al. 2006). These two species live in sympatry along the French Atlantic coast where their colonies exhibit important differences in their social organization, behavior, and breeding system (Clément and Bagnères 1998; DeHeer et al. 2005; Dronnet et al. 2005; Kutnik 2005; Perdereau et al. 2010). Interestingly, the major difference between the two species concerns their capacity to produce secondary neotenic reproductives. Genetic studies revealed that all French colonies of *R. flavipes* produce numerous neotenics whereas these are not always produce in *R. grassei* colonies. Furthermore, genetic analyses showed that the estimated number of neotenics in *R. flavipes* colonies was far greater than the estimated number of neotenics in *R. grassei* colonies. Although the origin of the difference in potential for producing neotenics is unknown, this difference makes comparisons between these species valuable.

This study combined population and physiological experiments to compare *R. grassei* and *R. flavipes,* in order to gain a better understanding of the mechanisms underlying neotenic

differentiation in *Reticulitermes*. The first part of the study was designed to evaluate the development potential of *Reticulitermes* termites using replicated experimental groups of 500 *R. grassei* or *R. flavipes* workers isolated from their respective colonies over a 1-year period. The workers' capacity to differentiate into other castes, especially neotenics, was assessed at both colony and species levels. Based on the findings of a preliminary study using a single colony of each species (Pichon et al. 2007), the type of neotenic (i.e., apterous or brachypterous) that differentiated from workers was also examined and whether regressive molts into the nymphal lineage could occur before differentiation into neotenics as described by Buchli (1958). Finally, the reproductive potential of neotenics was evaluated by measuring the number of offspring produced by each species. The second part of the study focused on possible underlying physiological mechanisms implicated in secondary reproductive differentiation in termites. Radioimmunoassay (RIA) and enzyme immunoassay (EIA) were used to measure JH and ecdysteroid titers in maturing neotenics in order to provide insights into differences in developmental-ontogenic potentiality between the two species.

MATERIALS AND METHODS

Termite collection and rearing

Thirteen colonies of *R. flavipes* from the Ile d'Oléron (Charente Maritime, France) and 17 colonies of *R. grassei* from the forest of La Coubre (Charente Maritime) were collected during the summer of 2006. Species identity was confirmed using cuticular hydrocarbon profiles (Bagnères et al. 1990; Bagnères et al. 1991). Both species are well documented in the respective areas where they were found (Clément et al. 2001). All termites were kept in the piece of wood where they were living at the time of collection.

Orphaning experiments for ontogenic potentialities

For each species, one to three replicate colonies (i.e., subcolonies) were prepared, depending on the size of the original colonies, from six original colonies of *R. flavipes* and 10 original colonies of *R. grassei* (see details in Table 1). Morphological caste phenotypes were identified by visual examination using a binocular lens. For each replicate, 500 fifth to seventh instar workers (i.e., the latest worker stages) were transferred into a LAB3© box (120 mm × 90 × 50 mm, Multiroir3412 Ldt, Perigny s/Yerres, France) containing 150 g of moist Fontainebleau sand and two pieces of pinus (*Pinus pinaster*) wood (4 cm × 4 cm × 1 cm). To ensure an adequate food supply, pieces of poplar wood were added ad libitum. The relative humidity inside each plastic box was maintained at about 90%. Throughout the experiment, replicate colonies were maintained at a temperature of 23 ± 1°C, in constant darkness in a climate-controlled room. Tests began in July 2006 for *R. flavipes* and in August 2006 for *R. grassei*. Each month, the number of newly developed castes and offspring, that is eggs, young larvae, new nymphs and new workers was counted in each subcolony. Workers before the fifth instar were considered newly developed. After 1 year, each new neotenic was weighed and its sex was determined by morphological observation of the seventh sternite (Hayashi et al. 2003). The sex ratio was calculated as well as the proportion of males. The presence or absence of wing buds was used to determine whether the neotenics were brachypterous or apterous. After 1 year, data were available for 9 replicates from the 6 original colonies of *R. flavipes* and 19 replicates from 9 original colonies for *R. grassei*. Survival in each replicate (i.e., number of living workers) was evaluated for both species. Replicate colonies that perished during the experiment were not included in the analysis (see Table 1).

Neotenics recovery for physiological analysis

For each species, 22 subcolonies were prepared from seven original colonies. Morphological castes were identified by visual examination using a binocular lens. For each subcolony, 1,000 fifth to seventh instar workers were transferred to a Lab crystal polystyrene box (120 mm × 90 mm × 50 mm, 3412®, LTD) containing 150 g of moist Fontainebleau sand and two pieces of poplar wood (4 cm × 4 cm × 1 cm). Each week, the formation of neotenics was observed, and in order to have neotenics of known age, new neotenics were marked with an odor-free uni PAINT marker PX 20 (Mitsubishi Pencil Co. Ltd., Tokyo, Japan). Moreover, each week, in order to have a range of neotenics, some neotenics of known age (marked) were collected as required until mature neotenics (formation of eggs) had been collected. Neotenics can be distinguished from workers or nymphs by a longer abdomen, darker pigmentation, slight sclerotization and the presence of eyes and ocelli (Thorne 1996).

Observations and dissections

All neotenics used in this study were observed using a binocular lens and the presence or absence of wing buds was checked. Ovarian development was assessed in neotenic and nymph females by dissecting the individuals under a binocular lens in a drop of saline solution. The number of ovarioles and whether they contained vitellogenic oocytes or not was determined under a dissecting microscope and the lengths of the basal oocytes measured using an ocular micrometer. Oocytes were considered to be vitellogenic and mature if yolk protein could be observed and the volume equaled or exceeded 0.01 mm^3 (Brent and Traniello 2001). Previous ovulation was noted by the presence of *corpora lutea* (small yellow–brown spheres at the base of the ovarioles) (Elliott and Stay 2007). The maturity of neotenics was checked by looking at the oocytes under a dissecting microscope before hormonal analyses. A neotenic was considered mature if there were vitellogenic oocytes or *corpora lutea*.

JH and ecdysteroid analysis

Each neotenic termite was homogenized by sonication in 500-µl chilled methanol with 50 µl NaCl 2%. The methanol extract was washed three times with 500-µl chilled pure pentane. Supernatants (1.5 ml pentane) with JH were stored at −80°C until JH measurement. The methanol extract was kept for EIA to measure ecdysteroids.

Table 1. Caste composition of termite groups after one year of development

Species	Colony	Replicate	Female neotenic	'Visible' male neotenic	Soldiers	Eggs	L1	L2	W3	W4	W5	W6/7	Nymphs	Total offspring	Survival workers
R. f	A	1	3	1	0	0	0	0	0	0	0	8	0	8	40
R. f	B	1	1	2	1	0	1	13	3	16	56	0	16	237	48
R. f	B	2	4	2	2	88	33	33	27	80	10	63	9	428	70
R. f	C	1	2	1	1	48	42	56	82	63	62	61	2	416	128
R. f	C	2	1	1	0	0	0	0	0	0	0	3	0	3	11
R. f	D	1	3	1	0	0	4	4	1	2	8	14	2	33	56
R. f	D	2	1	1	0	0	4	8	10	17	29	20	1	355	18
R. f	E	1	4	1	4	0	1	6	3	0	5	52	5	72	42
R. f	F	1	1	1	1	0	13	10	6	14	98	80	9	230	61
R. g	G	1	1	0	0	7	0	0	0	0	0	0	0	7	239
R. g	G	2	1	1	1	11	0	0	1	0	2	0	0	14	292
R. g	H	1	0	0	4	0	0	0	0	0	0	0	0	0	188
R. g	H	2	0	0	5	0	0	0	0	0	0	0	0	0	195
R. g	I	1	2	0	0	36	17	64	53	28	96	91	5	390	128
R. g	I	2	1	3	2	0	3	12	13	15	45	52	8	148	157
R. g	J	1	1	1	0	0	8	14	11	8	12	0	1	54	111
R. g	J	2	0	0	3	0	0	0	0	0	0	0	0	0	73
R. g	J	3	1	2	1	0	0	0	1	3	5	3	0	12	65
R. g	K	1	0	2	1	0	0	0	0	0	0	0	0	0	121
R. g	K	2	2	0	0	0	0	4	5	9	75	155	19	267	70
R. g	L	1	0	1	4	0	0	0	0	0	0	0	0	0	339
R. g	L	2	1	0	4	0	22	51	27	27	21	6	0	154	267
R. g	M	1	1	1	1	0	0	13	9	12	14	4	0	54	185
R. g	M	2	0	0	1	0	2	0	0	0	0	0	0	0	218
R. g	N	1	0	0	0	0	0	0	0	0	0	0	0	0	166
R. g	O	1	2	0	0	0	0	2	9	0	24	20	2	57	157
R. g	O	2	2	1	0	0	0	0	0	0	0	0	0	0	—
R. g	O	3	0	1	4	0	0	0	0	0	0	0	0	0	268
R. g	P	1													0
R. g	P	2													0
R. g	P	3													0
R. f total	6	9	20	11	9	136	98	130	132	192	268	283	44	1782	474
R. g total	9	22	15	13	31	54	52	160	123	102	294	331	35	1157	3239

Termite groups of *Reticulitermes flavipes* and *Reticulitermes grassei* are designated by "*R. f*" and "*R. g*," respectively; larvae are designated by L1 and L2, and workers by W1, W2, W3, W4, W5/6.

RIA of JH

Radiolabeled JH (3000 DPM) was added to each sample in 1 ml 60% methanol to monitor recovery. Samples in pentane were dried using a vacuum centrifuge and redissolved in 1 ml 60% methanol. Lipids were removed from the samples as described by Huang et al. (1994). A reverse phase C18 SepPak cartridge (Waters, Milford, MA, USA) was washed with 1 ml 100% methanol and 3 ml 60% methanol. JH extracts (redissolved in 1 ml 60% methanol) were passed through the cartridge, followed by two washes of the sample tube (each with 1 ml 60% methanol). The JH fraction was eluted (with 3 ml 85% methanol), dried to 450 µl with a vacuum centrifuge.

JH was extracted twice by adding 1 ml 0.9% NaCl and 1 ml hexane. The supernatant hexane phase, containing JH, was removed and dried in a vacuum centrifuge. Methanol (100 µl) was added to each tube followed by vortexing. To lower the detection limit of this assay, the following modifications were made to previously published procedures (Huang et al. 1994; Huang and Robinson 1995). Larger aliquots of each sample were used (two aliquots, 30 µl each), which amounted to 40% of the sample in each aliquot, rather than 10–20% as in previous studies. The aliquots were transferred to 10 mm × 75 mm glass tubes and dried, because the large amount of methanol could interfere with binding of JH with the antiserum. A 200-µl aliquot of premixed antiserum (1:14,000) and 4000 DPM of [10-^{3}H (N)]-JH (NEN, 629 Gbq/mmol) were added to each dried tube and then vortexed. These modifications halved the detection limit to about 5 pg per sample. After incubation for 2 h at room temperature, unbound radiolabeled JH was separated from bound JH by incubation with dextran-coated charcoal for 2.5 min. Radioactivity in the supernatant (containing radiolabeled JH bound to antiserum) was quantified by liquid scintillation spectrometry (Packard TriCarb 2100TR, Perkin Elmer, Inc., Boston, MA, USA). The process was calibrated using a standard curve created for each RIA run with known amounts of

racemic JH III (Sigma-Aldrich Ltd., St. Louis, MO, USA). Previous results (Huang et al. 1994) indicate that results from this RIA agree with those obtained with Goodman et al. (1990, 1993) RIAs, both of which have been validated using gas chromatography—mass spectroscopy (Huang and Robinson 1996).

In total, data were available for 50 neotenics of *R. flavipes* and 21 neotenics of *R. grassei*.

EIA of ecdysteroids

After centrifugation (10,000 g, 10 min), the supernatant of the methanol extract was dried in a Speed-Vac and resuspended in 0.1 M phosphate buffer (120 µl, pH 7.4). The ecdysteroids were determined using the EIA method of Porcheron et al. (1989) modified to use a peroxidase labeled 20-hydroxyecdysone (20E) conjugate (De Reggi et al. 1992). The L2 polyclonal antibody used was very sensitive to ecdysone, 2-deoxyecdysone, and 3-dehydroecdysone, but about six times less sensitive to 20-hydroxyecdysone (Bodin et al. 2009).

Each sample was analyzed at least in duplicate. Because ecdysone was used as a standard, ecdysteroid titers are expressed in ecdysone equivalents. Data were available for 43 neotenics of *R. flavipes* and 35 neotenics of *R. grassei*.

Data analysis

To determine the ontogenic potentialities of workers and to study reproductive strategies, all the parameters from the two species were compared: number of soldiers, neotenics, offspring, and number of ovarioles of sexual females, JH, and ecdysone titers. Statistical comparisons were made using Mann–Whitney *U*-tests on ranks, and data are presented as medians ± SIQRs (i.e., semi-interquartile ranges). Rates and proportions were compared using standard Chi-square analyses, with Yates' correction where appropriate. Correlation was calculated according to Spearman's *r* coefficient.

RESULTS

Differentiation of workers into soldiers

After 1 year of development at 23°C, soldiers were produced in five subcolonies (out of nine) of *R. flavipes* and nine subcolonies (out of 19) of *R. grassei* (Table 1). The median (± SIQR) number of soldiers produced by orphan groups of workers was not statistically different between *R. flavipes* and *R. grassei* (1 ± 0.50 vs. 1 ± 0.62, respectively; Mann–Whitney, $U = 71$, $P = 0.49$). There was also no difference in soldier distribution in subcolonies of the two species (Chi square, Yates correction, two-sided, $P = 0.70$).

Differentiation of workers into neotenics

Buchli (1958) reported that workers could revert to the imaginal lineage and could develop into brachypterous neotenics via regressive molts. Unlike Buchli (1958), we found no nymphs earlier than 6 months after egg production and no brachypterous neotenics regardless of species and sex. Consequently, all new neotenics ($n = 59$) that differentiated from workers were apterous (absence of wing pad). The first neotenics were produced 1 month after orphaning in both *R. grassei* and *R. flavipes*. In some experimental colonies of *R. grassei*, female neotenics were found with descendants despite the absence of visible males. In agreement with Pichon et al. (2007), it was assumed that "hidden male neotenics" were present. These males were probably morphologically indistinguishable among workers and so only female neotenics ($N = 15$) could be counted. In *R. flavipes* colonies, in which there was no evidence for the presence of cryptic males, there were a total of 11 males and 20 females (sex ratio = 0.35; Table 1).

Some mature neotenics (1-year-old) exhibited a small dorsal pad that was slightly more developed than in completely apterous neotenics and they may correspond to pseudo-apterous and apterous neotenics described by Buchli in 1958. In the first 2 months, some individuals developed several distinguishing features, that is distinctive body shape (sharply pointed abdomen), much lighter color and a slightly more developed dorsal pad. These individuals were not nymphs and were no longer seen 1 month later among neotenics. Although no definite conclusion can be drawn, these individuals may correspond to pseudo-nymphs (Buchli 1958) or could represent an intermediate stage before the pseudo-apterous neotenic stage.

The production of female neotenics was significantly different between the two species (Mann–Whitney, $U = 34$, $P = 0.0117$). *R. flavipes* produced 2 ± 1 female neotenics in nine replicate colonies while *R. grassei* produced 1 ± 0.5 female neotenics in 19 replicate colonies (Fig. 2). The proportion of replicates with female neotenics was significantly lower in *R. grassei* than in *R. flavipes* (binomial test, $\text{prop}_{R.\ grassei} = 0.63 \pm 0.4$, $P < 0.001$, $N = 19$). All *R. flavipes* colonies produced female neotenics, while only 63% (11 out of 19 colonies) of *R. grassei* colonies generated such females. After 1 full year, no statistical difference was found between the weight of female apterous neotenics in either species (Mann–Whitney, $U = 119.5$, $P = 0.636$). Median weights were 5.2 ± 5 mg for *R. flavipes* and 5.1 ± 5 mg for *R. grassei*.

Offspring production

R. flavipes produced significantly more offspring than *R. grassei* (Mann–Whitney, $U = 32$, $P = 0.0118$, Fig. 3A) not only because there were more females but also because female neotenics of *R. flavipes* had higher fecundity. A female neotenic of *R. flavipes* produced 107 ± 109.5 (median ± SIQR) offspring while a female neotenic of *R. grassei* produced 41.25 ± 63.18 (median ± SIQR) offspring (Mann–Whitney, $U = 40$, $P = 0.0248$, Fig. 3B).

Considering only replicate colonies with female neotenics, the proportion of female neotenics with offspring was significantly lower for *R. grassei* than *R. flavipes* (binomial test,

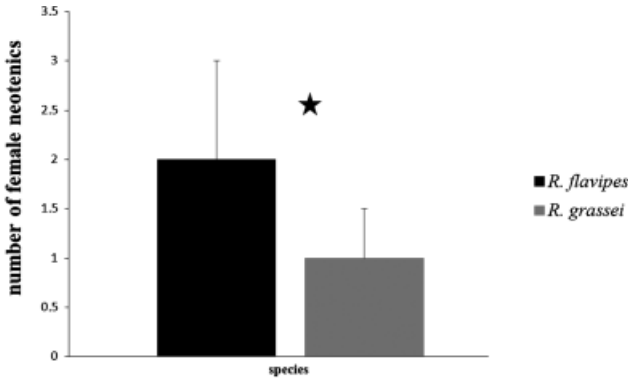

Fig. 2. Number of female neotenics produced per species in 1 year (MW, $U = 34$, $P = 0.011$). Data presented as median values ± semi-interquatile ranges. $^{*}P < 0.05$. *R. grassei*: *Reticulitermes grassei*; *R. flavipes*: *Reticulitermes flavipe*.

$\text{prop}_{R.\ grassei} = 0.83 \pm 0.48$, $P < 0.001$, $N = 12$). All *R. flavipes* replicate colonies with female neotenics produced offspring but only 83% in *R. grassei*. *R. flavipes* produced more nymphs than *R. grassei* (Mann–Whitney, $U = 41$, $P = 0.0288$). On average *R. flavipes* produced 2 ± 4 nymphs (median ± SIQR) whereas *R. grassei* produced 0 ± 0.25 nymphs.

Dissection of female neotenics

The number of ovarioles in mature neotenics differed between species (Mann–Whitney, $U = 39.5$, $P = 0.0009$). The number of ovarioles was 95 ± 7.75 for *R. flavipes* ($N = 30$), 70 ± 14.36 for *R. grassei* ($N = 18$). All ovarioles contained *corpora lutea*. From 0 to 9 mature (vitellogenic) oocytes were found in mature female neotenics of *R. flavipes* (1 ± 4), while 0–14 (0 ± 2.75) were found in *R. grassei*. The number of vitellogenic oocytes did not differ between the species (Mann–Whitney, $U = 195$, $P = 0.74$).

Ecdysone and JH titers

R. grassei

The JH titer was stable during the first weeks of development (week 1 to week 9/10: 2418.90 pg ± 10009.04; median ± SIQR, $n = 17$; Kruskal–Wallis, KW = 0.82, $P = 0.84$) and decreased at maturity even though this decrease was only marginally significant because of the high variance (900.35 pg ± 217.78; $n = 5$; Mann–Whitney, $U = 14$, $P = 0.08$) (Fig. 4A).

Maturing neotenics had a moderate and stable level of ecdysone in their hemolymph (week 1 to week 9/10:155.58 pg ± 36.67, $n = 18$; Kruskal–Wallis, KW = 3.60, $P = 0.30$), which was lower than in mature neotenics (1060.5 pg ± 257.21; $n = 17$; Mann–Whitney, $U = 6$, $P < 0.001$) (Fig. 4A).

In *R. grassei* female neotenics, there was a significant correlation between the level of ecdysone and the number of vitellogenic oocytes (Spearman coefficient $r = 0.91$, $N = 11$, $P = 0.0002$) (Fig. 5).

R. flavipes

The JH titer was stable during the first weeks of development (week 1 to week 10: 4104.74 pg ± 1765.74; median ± SIQR, $n = 25$; Kruskal–Wallis, KW = 5.59, $P = 0.23$) but, after the neotenics were mature and fecund, unlike *R. grassei,* the JH titer did not drop (3500 pg ± 1741.04; $n = 21$) remaining at the same level as that of maturing neotenics (Mann–Whitney, $U = 249$, $P = 0.77$) (Fig. 4B). Moreover, the JH titer in mature neotenics in *R. flavipes* was significantly higher than that in mature neotenics of *R. grassei* (Mann–Whitney, $U = 7$, $P = 0.006$).

However, the titer of circulating ecdysone varied in maturing neotenics. They had a stable and moderate level of ecdysone in their hemolymph (79.75 pg ± 47.42; median ± SIQR of pooled neotenics from week 1 to week 9/10, $n = 21$ Kruskal–Wallis, KW = 2021, $P = 0.69$), which was significantly lower than that of mature neotenics (508.34 pg ± 673.62, $n = 18$; Mann–Whitney, $U = 58$,

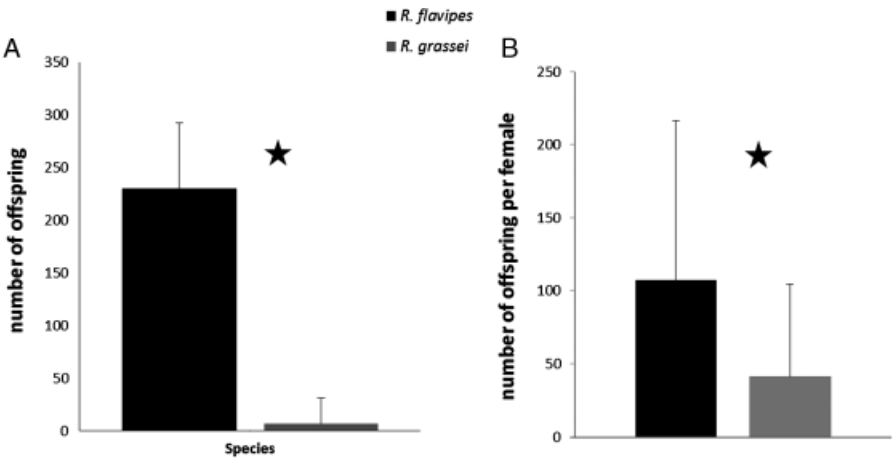

Fig. 3. A. Total number of offspring after 1 year (MW, $U = 32$, $P = 0.0118$). B. Number of offspring per female after 1 year (MW, $U = 40$, $P = 0.0248$.). Data presented as median values ± semi-interquartile ranges. $^{*}P < 0.05$. *R. grassei*: *Reticulitermes grassei*; *R. flavipes*: *Reticulitermes flavipe*.

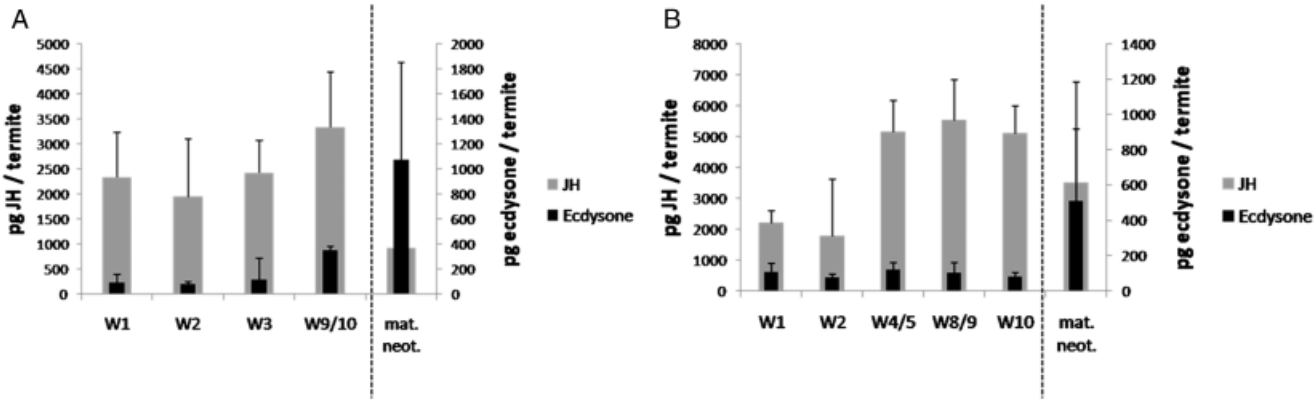

Fig. 4. (A) Amount of juvenile hormone (JH) and ecdysone in pg/termite in *Reticulitermes grassei* neotenic during neotenic development. (B) Level of JH and ecdysone in pg/termite in *Reticulitermes flavipes* neotenic during neotenic development (W, week of development; mat. neot., mature neotenic containing vitellogenic oocytes). Data presented as median values ± semi-interquartile ranges.

$P = 0.0001$) (Fig. 4B). Moreover, the ecdysone titer in mature neotenics in *R. flavipes* was significantly lower than in mature neotenics of *R. grassei* (Mann–Whitney, $U = 99$, $P = 0.003$). Once again, there was a significant correlation between the level of ecdysone and the number of vitellogenic oocytes (Spearman's coefficient $r = 0.51$, $N = 20$, $P = 0.0081$) (Fig. 5).

DISCUSSION

The reproductive division of labor in social insects is a key feature of their social organization. In subterranean termites, development of neotenic reproductives from workers is a characteristic that has far-reaching consequences on the population biology of these insects and might increase their capacity to spread in urban areas. However, the mechanisms through which neotenics differentiate from workers are still unknown.

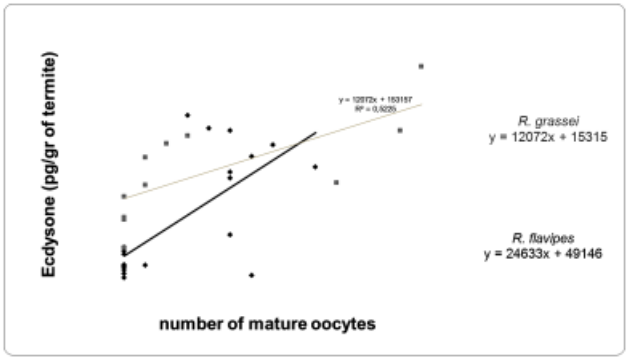

Fig. 5. Positive correlation between the number of mature oocytes and the titer in ecdysone in female neotenics of *Reticulitermes flavipes* (linear regression, $r = 0.91$, $P = 0.00002$) and *Reticulitermes grassei* (linear regression, $r = 0.51$, $P = 0.0081$). Black diamonds represent *R. flavipes* and gray squares represent *R. grassei*.

Our results confirm that isolated workers can differentiate into male and female neotenics at the earliest 1 month after their isolation from their initial colonies. Importantly, all of the 59 obtained neotenics were apterous. In *R. flavipes* replicate colonies, both sexes were observed at the same time, which was not the case in *R. grassei* colonies where no developed males were observed in half of the colonies, as previously reported by Pichon et al. (2007). The presence of hidden males (i.e., cryptic sexually mature males) has now been found in several colonies of *R. grassei* and recently in *Reticulitermes speratus* (Fujita and Watanabe 2010), but never in *R. flavipes*. Pichon et al. (2007) also used genetic analysis to confirm that there was no parthenogenesis in the studied colony of *R. grassei*.

The number of female neotenics observed in the orphaned colonies varied from 1 to 4 in *R. flavipes* colonies and from 0 to 2 per colony of *R. grassei*. These ranges are similar to the number of reproductives observed in orphaned *R. speratus*, *Reticulitermes virginicus* and *R. flavipes* colonies (Watanabe and Noda 1991; Pawson and Gold 1996). The mean production of female neotenics was higher for *R. flavipes* and, interestingly, 100% of the French *R. flavipes* colonies produced female neotenics as opposed to only 63% of *R. grassei* colonies. These empirical results are consistent with the breeding structure inferred in the field in France using microsatellite markers, that is, 100% of extended families for *R. flavipes* and 51.8–100% of extended families for *R. grassei* (DeHeer et al. 2005; Dronnet et al. 2005; Perdereau et al. 2010). As far as we know, this study is the first to provide experimental confirmation of genetic studies of field colonies. These findings might suggest that workers of the two species do not exhibit the same capacity to differentiate into neotenics.

According to the observations of Feytaud (1912, 1966), and the several studies reviewed in Lainé and Wright (2003)

(e.g., Miller 1969; Nutting 1970; Myles 1999), it is usually assumed that secondary reproductives produce collectively more eggs than the primary queen; although other authors claimed the opposite (Buchli 1958; Noirot 1990; Pawson and Gold 1996; Thorne et al. 1999). In this study, female neotenic fecundity was variable but values were of the same order of magnitude as those reported by Pawson and Gold (1996) and Pichon et al. (2007). The colonies of *R. flavipes* produced on average more offspring than the *R. grassei* colonies, not only because more females were produced in *R. flavipes* colonies, but also because each *R. flavipes* female had higher fecundity. Further studies would be useful to determine exactly how many females contribute to reproduction in a colony and in what proportion. Significantly more vitellogenic ovarioles were found in *R. flavipes* neotenics in comparison with those of *R. grassei* neotenics. This result could explain why *R. flavipes* females are very fecund, although it is unknown whether all the ovarioles contain vitellogenic eggs.

Our findings related to hormonal control indicate that both JH and ecdysone titers change dramatically during the development of neotenics. The ecdysteroid titers varied during the development of neotenics and in mature neotenics in both species, and presented the same pattern. The level was moderate during the first 10 weeks of development and reached high levels once the neotenics were fecund. The number of vitellogenic oocytes was correlated with the ecdysone titer in the hemolymph. This result is in accordance with Raikhel et al. (2005) who showed that ecdysteroids promote various aspects of follicle development, especially vitellogenesis. A physiological increase of ecdysteroids can accelerate vitellogenin synthesis, inducing egg maturation and stimulating oviposition (Ogihara et al. 2007).

Interestingly in *R. grassei*, the JH and ecdysone titers follow opposite patterns and the concentration in JH increases in maturing neotenics and then drops in mature neotenics. In *R. flavipes*, the level of JH is significantly higher than in *R. grassei* and remains constantly high in mature neotenics. These increases of JH production in hemolymph are in accordance with previous studies. JH is known to stimulate both the production of vitellogenin by the fat body and its uptake by the ovaries (Engelmann 1983). For example, JH synthesis by corpora allata is correlated with ovarian development in the cockroach *Diploptera punctata* (Stay and Tobe 1977, 1978) and particularly with the number of vitellogenic ovarioles (Elliott and Stay 2007, 2008). Some studies have also investigated this relationship in termites. Greenberg and Tobe (1985) showed that JH production increased in *Zootermopsis angusticollis* neotenics after emergence from larval instars. This increase of JH production is correlated with an increase in the number of vitellogenic oocytes (Brent et al. 2005) while it has been described that ovarian development in termites is regulated by JH (Miura et al. 2003; Scharf et al. 2003). Elliott and Stay (2007) showed that JH reached high levels in female *R. flavipes* neotenics from United States with large numbers of vitellogenic ovarioles, and remained at low levels in females with a small number of vitellogenic ovarioles (as it is the case in the first cycle of neotenics). This study shows the same phenomenon when comparing neotenics from the two termite species. *R. flavipes* neotenics have both a higher level of JH and more vitellogenic ovarioles than *R. grassei* neotenics.

In the absence of alates, some workers can transform into neotenics (Pichon et al. 2007), with abdomens enlarged by fat bodies and ovaries (Thorne 1996). The two reproductive castes (alates and neotenics) are controlled by JH (Wyatt and Davey 1996; Robinson and Vargo 1997). In *R. flavipes* neotenics, the higher level of JH may not only explain the higher number of ovarioles compared with *R. grassei* neotenics, but may also explain the differences between the ontogenic potentialities of these species.

Processes leading to the large number of neotenics in most of the *Reticulitermes* colonies remain unclear. *Reticulitermes* termites have a poorly defined nest organization and primary reproductives do not have a genuine "royal chamber" as in most of the higher termites (Wilson 1971b; Rueppell 2009). For these reasons, reproductives are likely to be more vulnerable to predation and it would be a valuable adaptation for the colony to have multiple reproductives (Howard and Haverty 1979). Another function for the development of multiple reproductives would be to allow rapid colony growth and increase chances of survival (Howard and Haverty 1979; Brent 2009). In this respect, it should be noted that *Reticulitermes* have a propensity for undergoing colony fragmentation or developing satellite nests containing neotenics. The important role of neotenics in colony expansion has been previously reported (Pawson and Gold 1996). A high development potential would ensure the survival of the satellite nests that often break off in *Reticulitermes* colonies (Myles and Nutting 1988; Lenz et al. 2000). The consequences in terms of colony genetic structure are dramatic since the presence of neotenics can increase both the level of inbreeding within colonies and the degree of relatedness among nestmates, and also can reinforce genetic contrast between colonies (Ross 2001).

In conclusion, these comparative analyses indicate that *R. flavipes and R. grassei* differ strongly in several life-history traits. In comparison with *R. grassei*, French colonies of *R. flavipes* produce relatively more neotenics in each colony, neotenic females are more fecund and the offspring generated grow faster. The high fecundity of *R. flavipes* neotenics may be a great advantage in the field, in addition with the absence of intercolonial antagonism (Clément and Bagnères 1998) allowing high dispersion and expansion of the colony in competition with endemic species such as *R. grassei*. As an illustration, field colonies of French *R. flavipes* appear to be

larger than colonies of *R. grassei* (Dronnet et al. 2005; Perdereau et al. 2010). Larger colonies always have an advantage both in survival and in nest competition (Thorne et al. 2003). The ability of the French colonies of *R. flavipes* to produce numerous neotenics contrasts with the finding that most US colonies of *R. flavipes* do not have such a capacity, or do not have productive neotenics, and are often headed only by the founding pair of primary reproductives (Vargo 2003a, b; Vargo and Husseneder 2009). This difference may represent a change in the social organization of this species that occurred after introduction in Europe, and might confer an enhanced longevity of French colonies. Such evolution after introduction may have been crucial to explain the success of *R. flavipes* colonies in invading new environments, especially in urban areas in France but also in Germany, Chile, and Canada. As discussed in a review by Moczek (2007), evolution could be illustrated here by contrasting reproductive potentials between native and nonnative species. *R. flavipes* also shows colony fusion (Perdereau et al. 2010) and is highly competitive with *R. grassei* in various respects (behavior, chemical ecology, genetics) when living in sympatry (Perdereau et al. 2010). Taken overall, these results suggest that *R. flavipes* could have better colony dynamics in comparison with *R. grassei* in their common geographical range and demonstrate that this differential developmental pathway may have resulted in high neoteny, allowing budding as the only viable reproductive strategy in the nonnative populations.

Acknowledgments

We wish to thank The Region Centre for providing L. Leniaud with a PhD grant. We are also grateful to the CNRS and the University François Rabelais de Tours (especially the doctoral school) for financial help. Funding to Z. Y. Huang was provided by Michigan Agricultural Experiment Station. We wish to express our gratitude to Simon Dupont and Charles-Edouard Imbert for their help during collection, Nicole Mandon for her help during ecdysteroid experiments, Colin Brent for his helpful comments, Jean-Paul Monge for the gift of the L2 polyclonal antibody, and Tony Tebby for correcting the English.

REFERENCES

Austin, J. W., Szalanski, A. L., Scheffrahn, R. H., Messenger, M. T., Dronnet, S., and Bagnères, A.-G. 2005. Genetic evidence for the synonymy of two *Reticulitermes* species: *Reticulitermes flavipe*s and *Reticulitermes santonensis*. *Ann. Entomol. Soc. Am.* 98: 395–401.

Bagnères, A.-G., Clément, J.-C., Blum, M. S., Severson, R. F., Joulie, C., and Lange, C. 1990. Cuticular hydrocarbons and defensive compounds of *Reticulitermes flavipes* (Kollar) and *R. santonensis* (Feytaud): polymorphism and chemotaxonomy. *J. Chem. Ecol.* 16: 3213–3244.

Bagnères, A.-G., Killian, A., Clément, J.-L., and Lange, C. 1991. Interspecific recognition among termites of the genus *Reticulitermes*: evidence for a role for the cuticular hydrocarbons. *J. Chem. Ecol.* 17: 2397–2420.

Bodin, A., Jaloux, B., Delbecque, J. P., Vannier, F., Monge, J. P., and Mondy, N. 2009. Reproduction in a variable environment: how does *Eupelmus vuilleti*, a parasitoid wasp, adjust oogenesis to host availability? *J. Insect Physiol.* 55: 643–648.

Bourguignon, T., Šobotnik, J., Hanus, R., and Roisin, Y. 2009. Developmental pathways of *Glossotermes oculatus* (Isoptera, Serritermitidae): at the cross-roads of worker caste evolution in termites. *Evol. Dev.* 11: 659–668.

Brent, C. 2009. Control of termite caste differentiation. In J. Gadau and J. Fewell (eds.). *Organization of Insect Societies. From Genome to Sociocomplexity*. Harvard University Press, Cambridge, MA, pp. 105–127.

Brent, C. S., Schal, C., and Vargo, E. L. 2005. Endocrine changes in maturing primary queens of *Zootermopsis angusticollis*. *J. Insect Physiol.* 51: 1200–1209.

Brent, C. S., Schal, C., and Vargo, E. L. 2007. Endocrine effects of social stimuli on maturing queens of the dampwood termite *Zootermopsis angusticollis*. *Physiol. Entomol.* 32: 26–33.

Brent, C. S., and Traniello, J. F. A. 2001. Social regulation of testicular development in primary and secondary males of the dampwood termite *Zootermopsis angusticollis* Hagen. *Insect. Soc.* 48: 384–391.

Buchli, H. 1958. L'origine des castes et les potentialités ontogéniques des termites européens du genre *Reticulitermes* Holmgren. *Ann. Sci. Nat. Zool.* 11: 263–429.

Clément, J.-L., and Bagnères, A.-G. 1998. Nestmate recognition in termites. In R. K. Vander Meer, M. D. Breed, M. L. Winston, and K. E. Espelie (eds.). *Pheromone Communication in Social Insects*. Westview Press, Boulder, CO, pp. 126–155.

Clément, J.-L., et al. 2001. Biosystematics of *Reticulitermes* termites in Europe: morphological, chemical and molecular data. *Insect. Soc.* 48: 202–215.

DeHeer, C. J., Kutnik, M., Vargo, E. L., and Bagnères, A.-G. 2005. The breeding system and population structure of the termite *Reticulitermes grassei* in southwestern France. *Heredity* 95: 408–415.

De Reggi, M., Pitoizet, N., Gharib, B., and Delbecque, J.-P. 1992. New enzyme immunoassays for ecdysteroids using peroxidase as enzyme and polyclonal or monoclonal antibodies. In Abstracts of the Xth Ecdysone Workshop, Liverpool, p. 91.

Dronnet, S., Chapuisat, M., Vargo, E. L., Lohou, C., and Bagnères, A.-G. 2005. Genetic analysis of the breeding system of an invasive subterranean termite, *Reticulitermes santonensis*, in urban and natural habitats. *Mol. Ecol.* 14: 1311–1320.

Elliott, K. L., and Stay, B. 2007. Juvenile hormone synthesis as related to egg development in neotenic reproductives of the termite *Reticulitermes flavipes*, with observations on urates in the fat body. *Gen. Comp. Endocrinol.* 152: 102–10.

Elliott, K. L., and Stay, B. 2008. Changes in juvenile hormone synthesis in the termite *Reticulitermes flavipes* during development of soldiers and neotenic reproductives from groups of isolated workers. *J. Insect Physiol.* 54: 492–500.

Engelmann, F. 1983. Vitellogenesis controlled by juvenile hormone. In R. G. H. Downer and H. Laufer (eds.). *Endocrinology of Insects*. Vol. 1. Alan R. Liss, New York, pp. 259–270.

Feytaud, J. 1912. Contribution à l'étude du termite lucifuge (Anatomie-Fondation de colonies nouvelles). *Arch. Anat. Microsc. T.* XIII: pp. 482–607.

Feytaud de, J. 1966. *Le peuple des termites. 127p. Que sais-je?* PUF, Paris.

Fujita, A., and Watanabe, H. 2010. Inconspicuous matured males of worker form are produced in orphaned colonies of *Reticulitermes speratus* (Isoptera: Rhinotermitidae) and participate in reproduction. *J. Insect Physiol.* 56: 1510–1515.

Goodman, W. G., Coy, D. C., Baker, F. C., Xu, L., and Toong, Y. C. 1990. Development and application of a radioimmunoassay for the juvenile hormones. *Insect Biochem.* 20: 357–364.

Goodman, W. G., Huang, Z.-Y., Robinson, G. E., Strambi, A., and Strambi, C. 1993. A comparison of two juvenile hormone radioimmunoassays. *Arch. Insect Biochem. Physiol.* 23: 147–152.

Greenberg, S., and Tobe, S. S. 1985. Adaptation of a radiochemical assay for juvenile hormone biosynthesis to study caste differentiation in a primitive termite. *J. Insect Physiol.* 3: 347–352.

Hartfelder, K., and Emlen, D. J. 2005. Endocrine control of insect polyphenism. In L. I. Gilbert, K. Iatrou, and S. Gill (eds.). *Comprehensive Insect Molecular Science*. Vol. 3. Elsevier, Oxford, pp. 651–703.

Hayashi, Y., Kitade, O., and Kojima, J. 2003. Parthenogenetic reproduction in neotenics of the subterranean termite *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *Entomol. Sci.* 6: 253–257.

Hayashi, Y., Lo, N., Miyata, H., and Kitade, O. 2007. Sex-linked genetic influence on caste determination in a termite. *Science* 318: 985–987.

Howard, R. W., and Haverty, M. I. 1979. Comparison of feeding substrates for evaluating effects of insect growth-regulators on subterranean termites (Isoptera Rhinotermitidae). *J. Georgia Entomol. Soc.* 14: 3–7.

Huang, Z. Y., and Robinson, G. E. 1995. Seasonal-changes in juvenile-hormone titers and rates of biosynthesis in honey-bees. *J. Comp. Physiol. B* 165: 18–28.

Huang, Z. Y., and Robinson, G. E. 1996. Regulation of honey bee division of labor by colony age demography. *Behav. Ecol. Sociobiol.* 39: 147–158.

Huang, Z. Y., Robinson, G. E., and Borst, D. W. 1994. Physiological correlates of division-of-labor among similarly aged honey-bees. *J. Comp. Physiol. A* 174: 731–739.

Korb, J. 2009. Termites: an alternative road to eusociality and the importance of group benefits in social insects. In J. Gadau and J. Fewell (eds.). *Organization of Insect Societies. From Genome to Sociocomplexity*. Harvard University Press, Cambridge, MA, pp. 128–147.

Korb, J., Hoffmann, K., and Hartfelder, K. 2009a. Endocrine signatures underlying plasticity in postembryonic development of a lower termite, *Cryptotermes secundus* (Kalotermitidae). *Evol. Dev.* 11: 269–277.

Korb, J., and Katrantzis, S. 2004. Influence of environmental conditions on the expression of the sexual dispersal phenotype in a lower termite: implications for the evolution of workers in termites. *Evol. Dev.* 6: 342–352.

Korb, J., Weil, T., Hoffmann, K., Foster, K. R., and Rehli, M. 2009b. A gene necessary for reproductive suppression in termites. *Science* 324: 758–758.

Kutnik, M. 2005. Evolution, génétique et structure coloniale du termite souterrain *Reticulitermes grassei* (Clément). Thèse de Doctorat, Université François Rabelais, Tours, 200pp.

Kutnik, M., Uva, P., Brinkworth, L., and Bagnères, A.-G. 2004. Phylogeography of two European *Reticulitermes* (Isoptera) species: the Iberian refugium. *Mol. Ecol.* 13: 99–113.

Lainé, L. V., and Wright, D. G. 2003. The life cycle of *Reticulitermes* spp. (Isoptera: Rhinotermitidae): what do we know? *Bull. Entomol. Res.* 93: 267–278.

Lenz, M., Kard, B., Mauldin, J. K., Evans, T. A., Etheridge, J. L., and Abbey, H. M. 2000. Size of food resource determines brood placement in *Reticulitermes flavipes*. In 31st Annual Meeting of the International Research Group on wood preservation, Kona, HI, pp. 1–8.

Mao, L. X., Henderson, G., Liu, Y. X., and Laine, R. A. 2005. Formosan subterranean termite (Isoptera: Rhinotermitidae) soldiers regulate juvenile hormone levels and caste differentiation in workers. *Ann. Entomol. Soc. Am.* 98: 340–345.

Matsuura, K., et al. 2009. Queen succession through asexual reproduction in termites. *Science* 323: 1687–1687.

Miller, E. M. 1969. *Caste Differentiation in the Lower Termites. Biology of Termites*. Academic Press, New York, pp. 283–310.

Miura, T. 2001. Morphogenesis and gene expression in the soldier-caste differentiation of termites. *Insect. Soc.* 48: 216–223.

Miura, T. 2004. Proximate mechanisms and evolution of caste polyphenism in social insects: from sociality to genes. *Ecol. Res.* 19: 141–148.

Miura, T., Koshikawa, S., and Matsumoto, T. 2003. Winged presoldiers induced by a juvenile hormone analog in *Zootermopsis nevadensis*: implications for plasticity and evolution of caste differentiation in termites. *J. Morphol.* 257: 22–32.

Miyata, H., Furuichi, H., and Kitade, O. 2004. Patterns of neotenic differentiation in a subterranean termite, *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *Entomol. Sci.* 7: 309–314.

Moczek, A. P. 2007. Developmental capacitance, genetic accommodation, and adaptive evolution. *Evol. Dev.* 9: 299–305.

Myles, T. G. 1999. Review of secondary reproduction in termites (Insecta: Isoptera) with comments on its role in termite ecology and social evolution. *Sociobiology* 33: 1–43.

Myles, T. G., and Nutting, W. L. 1988. Termite eusocial evolution: a re-examination of Bartz's hypothesis and assumptions. *Q Rev. Biol.* 63: 1–23.

Nalepa, C. A. 2010. Altricial development in subsocial cockroach ancestors: foundation for the evolution of phenotypic plasticity in termites. *Evol. Dev.* 12: 95–105.

Nijhout, H. F. 1999. Control mechanisms of polyphenic development in insects—in polyphenic development, environmental factors alter same aspects of development in an orderly and predictable way. *Bioscience* 49: 181–192.

Nijhout, H. F. 2003. Development and evolution of adaptative polyphenisms. *Evol. Dev.* 5: 9–18.

Noirot, C. 1989. Social structure in termite's societies. *Ethol. Ecol. Evol.* 11: 1–17.

Noirot, C. 1990. *Sexual Castes and Reproductives Strategies in Termites. In An Evolutionary Approach to Castes and Reproduction*. Springer-Verlag, Berlin, Germany, pp. 5–35.

Noirot, C. 1991. Caste differentiation in Isoptera—basic features, role of pheromones. *Ethol. Ecol. Evol.* 1: 3–7.

Nutting, W. L. 1970. Composition and size of some termite colonies in Arizona and Mexico. *Ann. Entomol. Soc. Am.* 63: 1105–1110.

Ogihara, K., Horigane, M., Nakajima, Y., Moribayashi, A., and Taylor, D. 2007. Ecdysteroid hormone titer and its relationship to vitellogenesis in the soft tick, *Ornithodoros moubata* (Acari: Argasidae). *Gen. Comp. Endocrinol.* 150: 371–380.

Oster, G. F., and Wilson, E. O. 1978. *Castes and Ecology in the Social Insects*. Princeton University Press, Princeton, NJ.

Park, Y. I., and Raina, A. K. 2004. Juvenile hormone III titers and regulation of soldier caste in *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *J. Ins. Physiol.* 50: 561–566.

Pawson, B. M., and Gold, R. E. 1996. Caste differentiation and reproductive dynamics of three subterranean termites in the genus *Reticulitermes* (Isoptera: Rhinotermitidae). *Sociobiology* 28: 241–251.

Perdereau, E., Bagnères, A.-G., Dupont, S., and Dedeine, F. 2010. High occurrence of colony fusion in a European population of the American termite *Reticulitermes flavipes*. *Insect. Soc.* 57: 393–402.

Perdereau, E., Dedeine, F., Christidès, J.-P., Dupont, S., and Bagnères, A.-G. 2010. Competition between invasive and indigenous species: an insular case study of subterranean termites. *Biol. Invasions* DOI: 10.1007/s10530-010-9906-5.

Pichon, A., et al. 2007. Development of experimentally orphaned termite worker colonies of two *Reticulitermes* species (Isoptera: Rhinotermitidae). *Sociobiology* 50: 1015–1034.

Porcheron, P., Morinière, M., Grassi, J., and Pradelles, P. 1989. Development of an enzyme-immunoassay for ecdysteroids using acetylcholinesterase as label. *Insect Biochem.* 19: 117–122.

Raikhel, A. S., Brown, M. R., and Dellès, X. 2005. Hormonal control of reproductive processes. In L. I. Gilbert, K. Iatrou, and S. Gill (eds.). *Comprehensive Insect Molecular Science*. Vol. 3. Elsevier, Oxford, pp. 433–491.

Raina, A., Park, Y. I., and Florane, C. 2003. Behavior and reproductive biology of the primary reproductives of the formosan subterranean termite (Isoptera: Rhinotermitidae). *Sociobiology* 14: 37–48.

Robinson, G. E., and Vargo, E. L. 1997. Juvenile hormone in adult eusocial hymenoptera: gonadotropin and behavioral pacemaker. *Arch. Insect Biochem. Physiol.* 35: 559–583.

Roisin, Y. 2000. Diversity and evolution of caste patterns. In Abe, T., Bignell, D. E. and Higashi, M. (eds.). *Termites: Evolution, Sociality, Symbioses, Ecology*. Kluwer Academic Press, Dordrecht, the Netherlands, pp. 95–119.

Ross, K. G. 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Mol. Ecol.* 10: 265–284.

Roux, E. A., Roux, M., and Korb, J. 2009. Selection on defensive traits in a sterile caste—caste evolution: a mechanism to overcome life-history trade-offs? *Evol. Dev.* 11: 80–87.

Rueppell, O. 2009. Aging of social insects. In J. Gadau and J. Fewell (eds.). *Organization of Insect Societies. From Genome to Sociocomplexity*. Harvard University Press, Cambridge, MA.

Scharf, M. E., Ratliff, C. R., Hoteling, J. T., Pittendrigh, B. R., and Bennett, G. W. 2003. Caste differentiation responses of two sympatric *Reticulitermes* termite species to juvenile hormone homologs and synthetic juvenoids in two laboratory assays. *Insect. Soc.* 50: 346–354.

Scharf, M. E., Ratliff, C. R., Wu-Scharf, D., Zhou, X., Pittendrigh, B. R., and Bennett, G. W. 2005a. Effects of juvenile hormone III on *Reticulitermes flavipes*: changes in hemolymph protein composition and gene expression. *Insect Biochem. Mol. Biol.* 35: 207–215.

Scharf, M. E., Wu-Scharf, D., Zhou, X., Pittendrigh, B. R., and Bennett, G. W. 2005b. Gene expression profiles among immature and adult reproductive castes of the termite *Reticulitermes flavipes*. *Insect Mol. Biol.* 14: 31–44.

Stay, B., and Tobe, S. S. 1977. Control of juvenile hormone biosynthesis during reproductive-cycle of a viviparous cockroach. 1. Activation and inhibition of corpora allata. *Gen. Comp. Endoc.* 33: 531–540.

Stay, B., and Tobe, S. S. 1978. Control of juvenile hormone biosynthesis during reproductive-cycle of a viviparous cockroach. 2. Effects of unilateral allatectomy, implantation of supernumerary corpora allata, and ovariectomy. *Gen Comp. Endoc.* 34: 276–286.

Su, N. Y., Ye, W. M., Ripa, R., Scheffrahn, R. H., and Giblin-Davis, R. M. 2006. Identification of Chilean *Reticulitermes* (Isoptera: Rhinotermitidae) inferred from three mitochondrial gene DNA sequences and soldier morphology. *Ann. Entomol. Soc. Am.* 99: 352–363.

Thorne, B. L. 1996. Termite terminology. *Sociobiology* 28: 253–263.

Thorne, B. L., Breich, N. L., and Muscedere, M. 2003. Evolution of eusociality and the soldier caste in termite: influence of intraspecific competition and accelerated inheritance. *Proc. Natl., Acad. Sci. USA* 100: 12808–12813.

Thorne, B. L., Traniello, J. F. A., Adams, E. S., and Bulmer, M. 1999. Reproductive dynamics and colony structure of subterranean termites of the genus *Reticulitermes* (Isoptera Rhinotermitidae): a review of the evidence from behavioral, ecological and genetic studies. *Ethol. Ecol. Evol.* 11: 149–169.

Vargo, E. L. 2003a. Genetic structure of *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae) colonies in an urban habitat and tracking of colonies following treatment with hexaflumuron bait. *Environ. Entomol.* 32: 1271–1282.

Vargo, E. L. 2003b. Hierarchical analysis of colony and population genetic structure of the eastern subterranean termite, *Reticulitermes flavipes*, using two classes of molecular markers. *Evolution* 57: 2805–2818.

Vargo, E. L., and Husseneder, C. 2009. Biology of subterranean termites: insights from molecular studies of *Reticulitermes* and *Coptotermes*. *Annu. Rev. Entomol.* 54: 379–403.

Vieau, F. 2001. Comparaison of the spatial distributiion and reproductive cycle of *Reticulitermes flavipes* Feytaud and *Reticulitermes lucifugus grassei* Clément (Isoptera, Rhinotermitidae) suggests that they represent introduced and native species, respectively. *Insect. Soc.* 48: 57–62.

Watanabe, H., and Noda, H. 1991. Small-scale rearing of a subterranean termite, *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *Appl. Entomol. Zool.* 26: 418–420.

West-Eberhard, M. J. 2003. Phenotypic accommodation: adaptive innovation due to developmental plasticity, with or without genetic change. *Integr. Comp. Biol.* 43: 970–970.

Wilson, E. O. 1971a. Social insects. *Science* 172: 406.

Wilson, E. O 1971b. *The Insect Societies*. Harvard University Press, Cambridge, MA.

Wyatt, G. R., and Davey, K. G. 1996. Cellular and molecular actions of juvenile hormone .2. Roles of juvenile hormone in adult insects. *Adv. Insect Physiol.* 26: 1–155.

Ye, W., et al. 2004. Phylogenetic relationships of neartic *Reticulitermes* species (Isoptera: Rhinotermitidae) with particular reference to *Reticulitermes arenincola* Goellner. *Mol. Phylogenet. Evol.* 30: 815–822.

Zhou, X., Song, C., Grzymala, T. L., Oi, F. M., and Scharf, M. E. 2006a. Juvenile hormone and colony conditions differentially influence cytochrome P450 gene expression in the termite *Reticulitermes flavipes*. *Insect Mol. Biol.* 15: 749–761.

Zhou, X., Tarver, M. R., Bennett, G. W., Oi, F. M., and Scharf, M. E. 2006b. Two hexamerin genes from the termite *Reticulitermes flavipes*: sequence, expression, and proposed functions in caste regulation. *Gene* 376: 47–58.

Zhou, X. G., Oi, F. M., and Scharf, M. E. 2006c. Social exploitation of hexamerin: RNAi reveals a major caste-regulatory factor in termites. *Proc. Natl. Acad. Sci. USA* 103: 4499–4504.

Zhou, X. G., Tarver, M. R., and Scharf, M. E. 2007. Hexamerin-based regulation of juvenile hormone-dependent gene expression underlies phenotypic plasticity in a social insect. *Development* 134: 601–610.