




RESEARCH ARTICLE

Remodeling of the juvenile hormone pathway through caste-biased gene expression and positive selection along a gradient of termite eusociality

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Funding information

Deutsche Forschungsgemeinschaft,
Grant/Award Numbers: BO2544/11-1,
KO1895/16-1; Spanish Ministry of Economy
and Competitiveness, Grant/Award Numbers:
CGL2012-36251, CGL2015-64727-P; Cata-
lan Government, Grant/Award Number: 2014
SGR 619

Abstract

The evolution of division of labor between sterile and fertile individuals represents one of the major transitions in biological complexity. A fascinating gradient in eusociality evolved among the ancient hemimetabolous insects, ranging from noneusocial cockroaches through the primitively social lower termites—where workers retain the ability to reproduce—to the higher termites, characterized by lifetime commitment to worker sterility. Juvenile hormone (JH) is a prime candidate for the regulation of reproductive division of labor in termites, as it plays a key role in insect postembryonic development and reproduction. We compared the expression of JH pathway genes between workers and queens in two lower termites (*Zootermopsis nevadensis* and *Cryptotermes secundus*) and a higher termite (*Macrotermes natalensis*) to that of analogous nymphs and adult females of the noneusocial cockroach *Blattella germanica*. JH biosynthesis and metabolism genes ranged from reproductive female-biased expression in the cockroach to predominantly worker-biased expression in the lower termites. Remarkably, the expression profile of JH pathway genes sets the higher termite apart from the two lower termites, as well as the cockroach, indicating that JH signaling has undergone major changes in this eusocial termite. These changes go beyond mere shifts in gene expression between the different castes, as we find evidence for positive selection in several termite JH pathway genes. Thus, remodeling of the JH pathway may have played a major role in termite social evolution, representing a striking case of convergent molecular evolution between the termites and the distantly related social hymenoptera.

KEYWORDS

cockroach, gene expression, hemimetabolous eusociality, juvenile hormone, reproductive division of labor, termite eusociality

1 | INTRODUCTION

The evolution of eusociality—where a sterile worker caste forgoes reproduction to help raise the offspring of others—represents one of the most extreme forms of altruism; yet, eusociality has evolved independently in insect orders as distantly related as the holometabolous hymenoptera and the hemimetabolous termites (e.g., Anderson, 1984; Howard, & Thorne, 2010). Similar to most ants and eusocial bees, caste fate in higher termites (i.e., Termitidae) is determined during early development, and can lead to lifetime worker sterility and extreme caste-biases in morphology and lifespan (Okot-Kotber, 1985; Roisin, 2000). In contrast, the reproductive division of labor in lower, wood-dwelling termites (i.e., all Archotermopsidae and Kalotermitidae as well as some Rhinotermitidae) is regulated through

developmental plasticity, where workers retain the ability to become reproductive throughout their lives (Korb, & Hartfelder, 2008; Roisin, 2000; Roisin, & Korb, 2010). Immature termite workers can develop into adult reproductives through a series of progressive or saltatory moults, leading to dispersing, winged sexuals (Korb, & Hartfelder, 2008), similar to the developmental pathway of cockroaches, their phylogenetically closest relatives (Inward, Beccaloni, & Eggleton, 2007; Legendre et al., 2015). Termite workers can also develop into wingless replacement reproductives when the present reproductive is unhealthy or dies. Alternatively, immature workers go through stationary and even regressive moults, or a terminal moult can result in sterile soldiers committed to colony defense. Because such developmental plasticity is the result of endocrine regulation (Brent, 2009; Korb, Hoffmann, & Hartfelder, 2009, 2012), comparing the molecular

mechanisms underlying development and reproduction across a gradient of social complexity can provide insights into termite social evolution.

Termite caste differentiation is regulated through varying juvenile hormone (JH) titres (Greenberg, & Tobe, 1985; Korb, 2015; Korb & Hartfelder, 2008; Miura & Scharf, 2010; Watanabe, Gotoh, Miura, & Maekawa, 2014). JHs are major regulators of postembryonic development and reproduction in insects (Goodman, & Granger, 2005; Hartfelder, 2000). High JH titres repress adult morphogenesis in pre-adult stages and JHs regulate developmental plasticity leading to wing polyphenism or castes (Nijhout, & Wheeler, 1982; Simpson, Sword, & Lo, 2011). In the adult stage, their positive influence on fecundity seems to have pleiotropic effects that result in reduced lifespan (Flatt, & Kawecki, 2007). Hence, JHs are considered a central mediator of the longevity/fecundity tradeoff (Rodrigues, & Flatt, 2016). In some eusocial bees and ants, the JH pathway has been co-opted for caste differentiation and division of labor, while either losing or retaining its function as gonadotropic hormone (Amdam & Page, 2010; Cuvillier-Hot, Lenoir, & Peeters, 2004; Formesyn et al., 2014; Guidugli et al., 2005; Penick, Liebig, & Brent, 2011; Ponton et al., 2013; Robinson & Vargo, 1998; Sommer, Hölldobler, & Rembol, 1993). Furthermore, in several primitively social species JH not only regulates gonadotropy but also dominance hierarchies (Barth, Lester, Sroka, Kessler, & Hearn, 1975; Bloch, Borst, Huang, Robinson, & Cnaani, 2000; Giray, Giovanetti, & West-Eberhard, 2005). These pleiotropic effects of JH makes it a prime candidate for the regulation of reproductive division of labor in termite societies.

Like most hemimetabolous insects, the first instars of termites and cockroaches already have the adult body plan and progressive moults mainly lead to an increase in body size and/or wing bud development (Bell, Roth, & Nalepa, 2007; Mullins & Cochran, 1987; Tanaka, 1981). One of the exceptions is the terminal moult into the adult stage, resulting in reproductive maturation through the morphogenesis of external genitalia. JH represses adult morphogenesis in cockroaches by activating the MEKRE93 pathway (Belles, & Santos, 2014): JH binds to its receptor Methoprene-tolerant (Met; Jindra, Belles, & Shinoda, 2015), which belongs to an ancient family of bHLH-PAS transcription factors (e.g., Ashok, Turner, & Wilson, 1998; reviewed in: Jindra et al., 2015). Binding of JH to Met triggers dimerization of Met with another bHLH-PAS protein Taiman (Tai). The resulting JH-Met-Tai complex induces transcription of the target gene Krüppel-homolog 1 (Kr-h1), which inhibits metamorphosis by repressing the “adult specifier gene” E93.

Whether or not the MEKRE93 pathway is activated thus depends on JH titres, which in turn are affected by the interplay among the JH biosynthesis pathway, JH binding proteins, and JH metabolizing enzymes. JHs are sesquiterpenoids produced by the *corpora allata* through the mevalonate pathway, which is a metabolic route based on reductive polymerization of acetyl-CoA that leads to a great diversity of isoprenoid compounds, including cholesterol in vertebrates. In insects, the sterol branch leading to cholesterol was lost during evolution, but, in contrast, insects have the capacity to synthesize JHs with farnesyl diphosphate as the key branch point (Belles, Martín, & Piu-lachs, 2005). Once released, JH is transported through the hemolymph to its target tissue by high affinity JH binding proteins (Goodman, &

Granger, 2005). These binding proteins protect JH from degradation by ubiquitous, nonspecific esterases, explaining why practically all circulating JH is bound to these carriers. Nonetheless, complexed JH is readily degraded by specialized JH esterases and JH epoxide hydrolases, which apparently work in synergy with JH binding proteins to reduce JH titres and promote metamorphosis (Goodman, & Granger, 2005).

Here, we investigate caste-biases in the expression of JH pathway genes along a gradient of eusociality, including two lower termites, *Zootermopsis nevadensis* (Archotermopsidae) and *Cryptotermes secundus* (Kalotermitidae), a higher termite, *Macrotermes natalensis* (Termitidae, Macrotermitinae), as well as the German cockroach, *Blattella germanica*, as a noneusocial outgroup of the termites. In addition, we identify signatures of positive selection in JH biosynthesis genes, genes involved in JH transport, stability, and metabolism as well as downstream JH signaling target genes. Our results suggest that the expression of JH pathway genes has undergone increasing remodeling during termite social evolution, with the most divergent patterns in the higher termite *M. natalensis*.

2 | METHODS

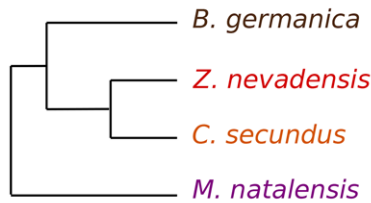
2.1 | Identification of genes involved in JH pathway

To identify JH pathway genes in the three termites and the cockroach, *Drosophila melanogaster* gene IDs were obtained from Cheng et al., 2014 and corresponding protein sequences extracted from FlyBase (Gramates et al., 2017; release dmel_r6.12). Orthologous clusters were obtained using OrthoMCL version 2.0.9 (granularity of 1.5; Li, Stoeckert, & Roos, 2003), including the *D. melanogaster* JH pathway protein sequences and the protein annotations of *B. germanica* (NCBI accession: PYGN000000000.1; NCBI BioProject: PRJNA427252), *Z. nevadensis* (termitegenomes.org; version OGSv2.2), *C. secundus* (NCBI accession: NEVH000000000; NCBI BioProject: PRJNA381866), and *M. natalensis* (GigaDB dataset: 100057; version 1.2). Missing orthologs were manually searched for in the respective proteomes (BLASTP) or genomes (TBLASTN; BLAST version 2.2.30+; e-value cut-off: $1e-3$) with either the *D. melanogaster* ortholog (if missing from all focal species) or an annotated ortholog of one of our other focal species as query. TBLASTN predictions were polished using Exonerate version 2.2.0 (parameter settings: -model protein2genome -percent 50; Slater, & Birney, 2005) and Augustus version 3.2 (parameter settings: -hintsfile < exonerate prediction >; Stanke, Schöffmann, Morgenstern, & Waack, 2006). In total, we found 33 genes from the JH pathway in *B. germanica*, 29 in *Z. nevadensis*, 31 in *C. secundus*, and 27 in *M. natalensis* (Figure 1; Supporting Information Table S2).

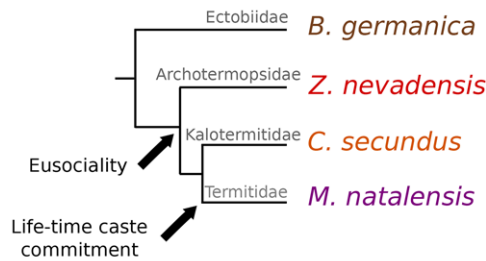
2.2 | Differential gene expression

Developmentally, the final nymphal stages in *B. germanica* are thought to be analogous to termite workers and the adult females to termite queens. The expression of the JH pathway genes was therefore com-

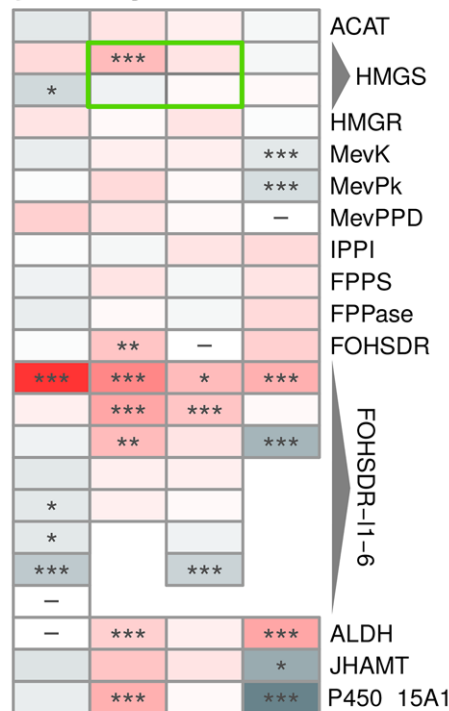
A) Expression clustering



B) Species phylogeny



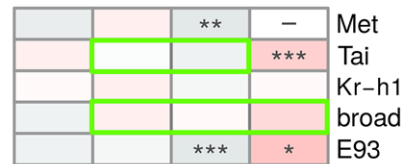
C) JH biosynthesis



D) JH binding proteins



E) JH signalling



F) JH metabolism

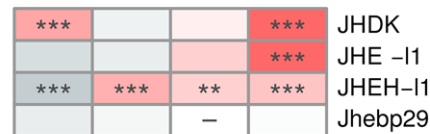


FIGURE 1 Differential expression and positive selection in juvenile hormone (JH) pathway genes. Gene expression comparison between reproductive females and workers/nymphs in two lower termites, *Zootermopsis nevadensis* and *Cryptotermes secundus*, the higher termite *Macrotermes natalensis* and the cockroach *Blattella germanica*, as noneusocial outgroup. (a) Species clustering based on the differential expression profile. Branch lengths represent Euclidean distances. (b) Species phylogeny modified from Harrison et al., 2018. Differential expression of (c) JH biosynthesis genes; (d) JH binding proteins; and (e) JH metabolism genes. Significance levels after FDR correction for multiple testing (all $|\text{Log2FC}| > 1$): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Genes that were not expressed are labeled with a dash. Paralogs are ordered according to differential expression level. Positively selected orthologous clusters are indicated with a green frame. See also Supporting Information Tables S3 and S4 [Color figure can be viewed at wileyonlinelibrary.com]

pared between the adult females (*B. germanica*)/queens (termites) and the final nymphal instars (*B. germanica*)/workers (termites). Specifically, four *B. germanica* RNAseq libraries were used: two replicates of fifth instar nymphs and two replicates of adult, reproductively mature females, in both cases with high JH production (Harrison et al., 2018). For the three termites the following RNAseq libraries were used: *Z. nevadensis*: three worker replicates (pool of third and fourth instar male and female larvae without guts) and three queen replicates (two whole body samples of secondary and one of primary reproductives without

guts; Terrapon et al., 2014), *C. secundus*: three worker (larval instars, without wing buds; whole body samples without guts) and four queen replicates (reproducing primary females; whole body samples without guts; Harrison et al., 2018), and *M. natalensis*: six worker (pool of major and minor workers) and three queen replicates (primary reproductives; whole body samples without guts; Harrison et al., 2018). The age of the termite samples was unknown but caste and age are unrelated due to saltatory and regressive moults in lower termites and life-time caste commitment in higher termites. For further details on the

used samples and data accessions, see Supporting Information Table S1.

RNAseq reads were mapped to the respective genomes using HISAT2 version 2.0.4 (paired-end mode; Pertea, Kim, Pertea, Leek, & Salzberg, 2016). Read counts per gene were obtained using HTSeq version 0.6.0 (htseq-count; Anders, Pyl, & Huber, 2015) and differentially expressed genes were identified using DESeq2 (Love, Huber, & Anders, 2014; BioConductor: Huber et al., 2015; R version 3.2.3: R Core Team, 2015). To account for potentially confounding effects of allometric differences between samples, genes were treated as significantly differentially expressed when $P_{\text{adj}} < 0.05$ and $|\log_2\text{-fold change}| > 1$ (Montgomery, & Mank, 2016). Heatmaps and clustering based on expression profiles were obtained with the R package pheatmap version 1.0.8 (Kolde, 2015).

2.3 | Positive selection

Multiple protein sequence alignments of the OrthoMCL orthologous groups were obtained using MAFFT version 7.123b (parameter settings: -localpair -maxiterate 1000; Katoh, & Standley, 2013), including *B. germanica* and the three termites. The corresponding codon alignments were obtained using PAL2NAL version 14 (parameter settings: -nogap -output paml; Suyama, Torrents, & Bork, 2006). For the one-to-one orthologs, the species tree was used (Figure 1B; from Harrison et al., 2018). For orthologous groups with multiple paralogs per species, we obtained a protein tree using FastTree version 2.1.7 SSE3 (parameter settings: -nosupport; Price, Dehal, & Arkin, 2010). To rule out that the use of a protein, rather than the species tree affected the results, significant paralogous clusters were tested again using the species tree and all possible permutations with only a single protein sequence per species as input. Each permutation yielded qualitatively identical results.

CodeML version 4.7 (PAML; Yang, 2007) branch-site tests were used to assess signatures of positive selection (parameter settings: -runMode 0 -model 2 -NSsites 2 -fix_omega 1 (null model) or -fix_omega 0 (alternative model) -clade < termites or lower termites or higher termite >). For each orthologous cluster, we performed three analyses specifying the following branch types: (1) Termites: orthologs of all three termites as foreground branches, *B. germanica* ortholog(s) as background branch(es); (2) Lower termites: *Z. nevadensis* and *C. secundus* orthologs as foreground branches, *B. germanica* and *M. natalensis* orthologs as background branches; (3) Higher termite: *M. natalensis* ortholog(s) as foreground branch(es), *B. germanica* and lower termite orthologs as background branches. Significance was evaluated with likelihood ratio (LR) tests and *P*-values were corrected for multiple testing using FDR ($\alpha < 0.05$).

3 | RESULTS

3.1 | JH expression profiles

The expression bias between reproductive females and workers in the JH pathway genes clearly distinguishes the higher termite *M. natalensis* from the two lower termites (Figure 1A): Interestingly, the

expression bias in *M. natalensis*—which, unlike the other two termites, shows lifetime caste commitment and extreme differences in worker and queen morphology, reproduction, and lifespan—sets it apart from all three other species, including the noneusocial outgroup, the cockroach *B. germanica* (Figure 1A). Consequently, the clustering based on the differential expression of workers/nymphs and reproductive females does not reflect the species phylogeny (Figure 1B).

3.2 | JH biosynthesis

Despite the important and conserved function of JH in postembryonic development and gonadotropy, the expression differences of JH biosynthesis genes between workers/nymphs and reproductive females varied substantially between the cockroach and the termites, as well as between the higher and lower termites (Figure 1C; Supporting Information Table S3). In the cockroach, four out of five differentially expressed genes were upregulated in reproductive females, which is in line with the gonadotropic role of JH as well as the higher JH titres found in reproductive females (Korb, & Belles, 2017). Among the two lower termites, most JH biosynthesis genes were biased towards workers, in particular in *Z. nevadensis*, in which not a single one was upregulated in reproductive females (Figure 1C; Supporting Information Table S3). This pattern found in the lower termites strongly contrasts with that in the higher termite, *M. natalensis*, where five out of seven differentially expressed JH biosynthesis genes were upregulated in reproductive females, in particular those genes involved in the last step of JH biosynthesis (P450-15A1; JH epoxidase).

Among the differentially expressed genes, most were upregulated in adult cockroach females but downregulated in reproductive females of the lower termites (Figure 1C). The only JH biosynthesis gene whose expression was nymph-biased in *B. germanica* was one of eight farnesol dehydrogenase-like paralogs (FOHSDR-I1-6). Likewise, the only reproductive female-biased gene in the lower termites was one out of *C. secundus*' seven FOHSDR-like paralogs. Gene duplication and differentiation may have allowed for sub- or neo-functionalization (Dittmar, & Liberles, 2010), which could explain these deviations on the general expression profiles. Indeed, we found that HMGS, which has two paralogs in the cockroach as well as the three termites, was under positive selection in the lower termites (LR-test, $LR = 27.51$, $P_{\text{adj}} < 0.0001$; Supporting Information Table S4).

Intriguingly, the above concepts do not apply to the caste-bias in JH biosynthesis gene expression of the higher termite *M. natalensis*. Among the one-to-one orthologs involved in the final steps in JH biosynthesis, we found both female-biased (JHAMT; JH acid methyltransferase and P450-15A1; JH epoxidase) and worker-biased (ALDH; Aldehyde dehydrogenase) gene expression (Figure 1C; Supporting Information Table S3).

3.3 | JH binding proteins

Juvenile hormone binding proteins (JHBP) are important for the transportation of JH to its target tissue and prevent JH degradation

by generalist esterases. In the cockroach, both JHBP paralogs are upregulated in reproductive females compared to nymphs (Figure 1D), which is consistent with the female-biased expression of the majority of differentially expressed JH biosynthesis genes (Figure 1C). Interestingly, the JHBP expression difference between reproductive females and workers in the termites is exactly opposite to that of JH biosynthesis genes. That is, differentially expressed JH biosynthesis genes in the two lower termites *Z. nevadensis* and *C. secundus* are biased toward workers; yet, JHBP expression tends to be female-biased, at least in *C. secundus* ($P_{\text{adj}} = 0.065$; Supporting Information Table S3). Conversely, the higher termite *M. natalensis* shows predominantly female-biased expression of JH biosynthesis genes, but strong upregulation of the JHBP in workers. The aberrant expression of the JHBP in *M. natalensis* might have been driven by a shift in functionality, as we found evidence of positive selection on the JHBP in the higher termite (LR-test, LR = 14.34, $P_{\text{adj}} = 0.0022$; Figure 1D; Supporting Information Table S4), but not in the lower termites (LR < 0.1, $P_{\text{adj}} = 0.5$).

3.4 | JH signaling

In postembryonic development the most general role of JH is repressing metamorphosis through the MEKRE93 pathway (Belles, & Santos, 2014), whereas in the adult of most hemimetabolous insects, including termites (reviewed in Korb, 2015), JH plays a gonadotropic role by inducing the production of vitellogenin (Raikhel, Brown, & Belles, 2004). In the cockroach, neither the JH receptor Met nor its co-receptor Tai was differentially expressed between nymphs and adult females, nor were any of the downstream JH signaling genes differentially expressed (Figure 1E).

In the lower termite, *C. secundus* (but not *Z. nevadensis*), Met, as well as the adult specifier E93 were upregulated in reproductive females (Figure 1E). Reproductive female-biased expression in *C. secundus* contrasts with the worker-biased expression of Tai and E93 in the higher termite, *M. natalensis* (Figure 1E). Of the differentially expressed JH signalling genes in the termites, caste-bias mimicked that of the JH binding proteins (Figure 1D), rather than that of genes involved in JH biosynthesis (Figure 1C).

Interestingly, two out of the five JH signaling genes showed signatures of positive selection in the termites. Specifically, *broad* appears to have evolved at a different rate in the three termites compared to the noneusocial outgroup, *B. germanica* (LR-test, LR = 23.30, $P_{\text{adj}} < 0.0001$; Supporting Information Table S4). The coreceptor Tai was positively selected in the lower termites (LR = 10.55, $P_{\text{adj}} = 0.0084$), but not in the higher termite *M. natalensis* (LR = 0.99, $P_{\text{adj}} = 0.5$). None of the positively selected orthologs were differentially expressed (Figure 1E), which may indicate that they underwent a change in functionality that goes beyond mere shifts in the regulation of caste-specific gene expression.

3.5 | JH metabolism

JH is rapidly degraded by JH esterases and JH epoxide hydrolases. In the cockroach, reproductive female-biased expression of the JH metabolism gene JHEH-I1 (JH epoxide hydrolase-like 1; Figure 1F),

combined with a similar expression pattern in JH biosynthesis genes (Figure 1C) points toward rapid turn-over of JH in adult females. In the termites, the expression of some JH metabolism genes was worker-biased, especially in the higher termite *M. natalensis*, where three out of four JH metabolism genes were upregulated in workers (Figure 1F). Although the fourth JH metabolism gene, *Jhebp29* (JH esterase binding protein 29), was not differentially expressed in any of the termites, the selection analyses showed tentative signs of positive selection in the termites (LR-test, LR = 6.92, $P_{\text{adj}} = 0.062$; Supporting Information Table S4).

4 | DISCUSSION

JH is a key regulator of termite caste differentiation, while retaining its conserved gonadotropic role in adult females (Korb, & Belles, 2017). By comparing gene expression in workers/nymphs and reproductive females of three termite species and their noneusocial outgroup, the cockroach *B. germanica*, we have demonstrated that: (1) the expression of JH pathway genes varies between the termites and the cockroach. While expression of JH genes in *B. germanica* is consistent with higher JH titres in reproductive females, lower termite workers upregulate JH biosynthesis genes but also JH epoxide hydrolases, suggesting a high JH turn-over rate in this caste; (2) The two lower termites consistently differ in their caste-bias expression of JH pathway genes from the higher termite *M. natalensis*. In the latter, the expression profiles imply a higher JH production and a slower JH metabolism in adult females compared to workers, but also a higher JH stability in workers due to the upregulation of JH binding proteins; (3) the changes in the JH pathway in termites goes beyond mere changes in gene expression patterns as several key components of the JH biosynthesis, JH binding, and JH signaling pathways underwent positive selection in the termites. These findings suggest that both the protein sequences and the gene regulation of the JH pathway underwent substantial changes during termite social evolution.

The gonadotropic role of JH (Maekawa, Ishitani, Gotoh, Cornette, & Miura, 2010) appears to be similar in adult female cockroaches and lower termite queens (Belles, 2005; Korb, & Belles, 2017). In line with this, both reproductive female cockroaches and termite queens are characterized by high JH titres (Korb, & Belles, 2017). Nonetheless, we found striking differences in the expression of JH pathway genes between the cockroach *B. germanica* and the lower termites, *Z. nevadensis* and *C. secundus*. In the cockroach, several JH biosynthesis genes and both JHBP genes were upregulated in adult females compared to nymphs. These findings suggest an overall higher production of JH in adult female cockroaches, combined with an efficient transport to target tissues due to association with binding proteins. Nonetheless, none of the downstream signaling genes were differentially expressed in the cockroach, which may be because of rapid metabolism of complexed JH by the upregulated JH epoxide hydrolases or because JHBP are involved in processes other than the transport of JH. Contrasting to cockroach females, the reproductive females of lower termites downregulated JH biosynthesis genes, as well as JH epoxide hydrolase, compared to workers. This latter finding resembles lower JH degradation

rates in honey bee queens compared to workers, due to the downregulation of JH metabolism genes (Bomtorin et al., 2014).

In the two lower termites, our findings are consistent with higher JH production in workers but lower JH turn-over in reproductive females. This may explain why the lower termite *C. secundus* shows reproductive female-biased expression of the JH receptor and transcription factor Met, although it remains unclear why none of the JH signaling genes were differentially expressed in the other lower termite, *Z. nevadensis*. The upregulation of Met in reproductive *C. secundus* females is consistent with the fact that this factor transduces the vitellogenic signal of JH, for example, in neotenic reproductives of *Reticulitermes speratus*, a lower termite (Elliott, & Stay, 2008; Saiki, Gotoh, Toga, Miura, & Maekawa, 2015). The lack of a similar signal in Kr-h1 is however surprising as Kr-h1 is an early response gene of JH activity. Equally surprising is the upregulation of the adult specifier E93, as the role of this transcription factor in adult insects is unknown. These findings, together with the evidence for positive selection in two key JH signaling genes suggests that termite JH signaling deviates from that in other hemimetabolous insects. Decoupling of JH production and JH stability/degradation may be an important mechanism by which lower termites retain the conserved gonadotropic function of JH while exploiting the JH pathway for flexible caste determination.

Caste differentiation in lower termites is achieved through an unprecedented diversity of moulting types (see Introduction; Korb & Hartfelder, 2008; Korb, & Belles, 2017; Roisin & Korb, 2010). While JH represses adult morphogenesis in cockroaches, the role of JH in successive termite moults is thought to reflect a complex interaction between JH titres, moulting type, developmental phase, and social environment (Cornette, Gotoh, Koshikawa, & Miura, 2008; Korb, 2015; Korb, & Belles, 2017; Korb, Hoffmann, & Hartfelder, 2012; Watanabe et al., 2014; Zhou, Song, Grzymala, Oi, & Scharf, 2006). This complexity and phase sensitivity makes it difficult to obtain standardized gene expression data, especially because the future caste fate of experimental individuals cannot be known for sure. Given these challenges, it is not surprising that we find expression variation between the two lower termites on a gene-by-gene basis. Nonetheless, the clustering of both lower termites based on the complete JH pathway expression profile (Figure 1A), and the consistent worker-biased expression of differentially expressed JH biosynthesis and JH metabolism genes, suggests that these signals are conserved across the two lower termites. The upregulation of JH biosynthesis genes in workers of lower termites is in line with the high JH titres in the larval instar workers of *C. secundus* and in another lower termite, *Hodotermopsis sjostedti* (Cornette et al., 2008; Korb et al., 2009, 2012), supporting the role of JH in repressing worker maturation (Korb, 2015; Korb et al., 2009; Korb, & Belles, 2017). Thus, our results point toward high JH production and rapid turnover in workers, which may facilitate the remarkable developmental plasticity underlying social life style in lower termites.

Unlike lower termites, higher termites like *M. natalensis* show a bifurcated development and caste fate is determined at the stage of the egg or first instar larva (Korb, & Belles, 2017). In theory, such lifetime caste commitment would allow permanent decoupling of the gonadotropic role of JH in reproductive females and its function in repressing adult morphogenesis in workers. While we currently lack

in-depth studies on how JH regulates these two functions in higher termites, our findings do show substantial differences between the higher termite *M. natalensis*, compared to the lower termites and the noneusocial cockroach. First, the caste-biased expression of JH biosynthesis and JH binding protein genes in *M. natalensis* does not resemble that in the other studied species, which may point to a change in functionality, at least in the positively selected JH binding protein. Second, the aberrant worker-biased patterns of JH signaling factors Tai and E93 observed in *M. natalensis* (Figure 1F), points to a more dynamic JH signaling in this caste. This is possibly related to the morphogenetic plasticity required to form pre-soldiers and soldiers from workers. It would not be surprising that Tai, which is a promiscuous transcription factor involved in many morphogenetic and nonmorphogenetic pathways (Lozano, Kayukawa, Shinoda, & Belles, 2014), and E93, which is a typical morphogenetic factor (Belles, & Santos, 2014) would be involved in regulating the worker-soldier caste transition. Third, the differences in JH pathway expression in *M. natalensis* not only sets it apart from the closest relative included in our study, *C. secundus*, but distinguishes it even more from the lower termites than the noneusocial outgroup, *B. germanica* (Figures 1A,B). Collectively, these results suggest that the JH pathway in *M. natalensis* has been subjected to substantial remodeling and highlights the importance of further functional studies on JH synthesis and signaling in the higher termites.

Remarkable parallels in the remodeling of the JH pathway can be found in termites and eusocial hymenoptera, where the JH pathway has likewise been co-opted as a key regulator of caste differentiation and division of labor (Amdam & Page, 2010; Cuvillier-Hot et al., 2004; Formesyn et al., 2014; Guidugli et al., 2005; Penick et al., 2011; Ponton et al., 2013; Robinson & Vargo, 1998; Sommer et al., 1993). Despite 350 million years of independent evolution of termites and social hymenoptera, it appears that natural selection convergently exploited the myriad pleiotropic biological properties of JH, driving the formation of complex societies where reproduction is monopolized by few highly fecund individuals with the help of many sterile workers.

ACKNOWLEDGMENTS

We thank the Deutsche Forschungsgemeinschaft (grants BO2544/11-1 to EBB; DFG grant KO1895/16-1 to J.K.), the Spanish Ministry of Economy and Competitiveness (grants CGL2012-36251 and CGL2015-64727-P to X.B.), and the Catalan Government (2014 SGR 619 to X.B., including FEDER funds) for providing funding for this research.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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How to cite this article: Jongepier E, Kemena C, Lopez-Ezquerria A, Belles X, Bornberg-Bauer E, Korb J. Remodeling of the juvenile hormone pathway through caste-biased gene expression and positive selection along a gradient of termite eusociality. *J Exp Zool (Mol Dev Evol)*. 2018;1–9. <https://doi.org/10.1002/jez.b.22805>