Apidologie (2018) 49:49–70

DOI: 10.1007/s13592-017-0548-9

Review article

The ovary and its genes—developmental processes underlying the establishment and function of a highly divergent reproductive system in the female castes of the honey bee, *Apis mellifera*

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Received 18 May 2017 – Revised 4 September 2017 – Accepted 25 September 2017

Abstract – The strong dimorphism in ovary phenotype seen between honey bee queens and workers represents the anatomical fixation of reproductive division of labor. We review the developmental processes by which the divergent ovary phenotypes become established, mainly focusing on the massive programmed cell death (PCD) that destroys most of the ovariole primordia in the worker ovary during larval development. Ovary-specific transcriptome analyses revealed a set of differentially expressed genes associated with PCD, including two long noncoding RNAs. PCD also plays a major role regulating ovarian activity in adult honey bee workers, and a major effect candidate gene mediating this process is *Anarchy*, previously identified through classical genetics in a rebel worker strain. Finally, we ask how the strong ovary phenotype dimorphism in the genus *Apis* may have evolved, and we discuss this by contrasting honey bees with the equally eusocial stingless bees. Through a comparison of their mating systems (polyandry versus monandry), as well as comparative data on female and male gonad structure across several families of bees, we propose the hypothesis that the exceptional gonad structure in *Apis* queens and drones evolved via shared developmental pathways. Furthermore, we suggest that selection on massive sperm production in *Apis* drones may have been a driving force leading to this exaggerated gonad morphology.

honeybee / gonad development / cell death / differential gene expression / meliponids

Where does the spirit of the hive reside? At least to some extent it is in the ovaries of a crowd of bees working in a dark hive (Robert E. Page Jr.)

1. INTRODUCTION

With this answer, in response to the question posed by Maurice Materlincks' work *The Life of the Bee*, ends the remarkable book *The Spirit of the Hive* (Page 2013). The size of the ovaries, as well as their activity in terms of oogenesis progression, is the main character that distinguishes queens and workers of highly eusocial insects, and a large, active ovary essentially represents

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Manuscript editor: David Tarpy

the fixation of the reproductive primacy of the queen(s) against the thousands of subfertile (non-totipotent) or completely sterile workers living in a colony.

The honey bee, *Apis mellifera*, is a prime example for such structural and functional queen/worker differences in the reproductive system, with very large ovaries, each typically consisting of 120–200 serial units, the ovarioles, in the adult queen and small ovaries made up of 2–12 ovarioles each in the worker (Snodgrass 1956; Linksvayer et al. 2011). Furthermore, the sperm storage organ, the spermatheca, is fully developed in the queen, but only a remnant is present in the worker (Snodgrass 1956). In the adult females, these structural differences generated during preimaginal ovary development are also the basis for differences in ovary function or, in other words, for worker sterility versus queen fertility.

In this review, we will primarily focus on the question of ontogenetic mechanisms that govern female gonad development. We start with the preimaginal stages, giving a brief overview on embryonic gonad development, followed by an in-depth description on processes and mechanisms that generate the divergence in gonadal structure during the critical stages for caste differentiation in the larval stages, when the adult ovariole number becomes determined. The subsequent section is dedicated to the regulation of ovarian activity in adult honey bees, especially the question of worker sterility. Throughout these sections, we will emphasize the importance of tissue or organ-specific molecular studies, since global, whole-body gene expression analyses unavoidably merge the developmental dynamics of different organs, making it difficult to arrive at insightful gene regulatory networks for a specific tissue. This is especially the case for the reproductive system, which contains cellular elements of three different embryonic origins, which, most likely, also exhibit differences in their gene regulatory networks. These elements are the germ cells, the mesoderm-derived (somatic) insect gonads and ovarian ducts, and the ectoderm-derived genital imaginal discs. Furthermore, the transcriptomic contribution of the reproductive system likely represents a very small percentage only in whole-body analyses, especially in larvae, where the fat body is the predominant tissue and of prime importance for larval growth.

Questions concerning evolutionary aspects will be addressed by including data on ovarian activity in stingless bees, the only other group of bees that has reached the same level of a highly eusocial organization as the honey bees, and by drawing attention to parallels with the reproductive system of male bees. Males are the neglected gender, not only among bees (Koeniger 2005) but also among social insects in general. Nevertheless, we believe that understanding gonad development and the reproductive biology of males can shed light on questions that may appear enigmatic, such as the extraordinarily high number of ovarioles in *Apis* queens.

2. DEVELOPMENTAL BIOLOGY OF THE OVARY

2.1. The embryonic gonad

Except for studies investigating the haplodiploid molecular mechanisms of the sex determination pathway acting in early embryonic stages (Beye et al. 2003; Hasselmann et al. 2008), the embryonic development of honey bees has, until recently, not received nearly as much attention as that of *Drosophila*. In fact, for thorough information, one must go back to studies done in the early twentieth century (Nelson 1915; Schnetter 1935), followed later by detailed descriptions of embryonic stages (DuPraw 1967; Fleig and Sander 1986, 1988). Fortunately, this has somewhat changed once the fully sequenced honey bee genome became available, making possible in-depth comparisons with *Drosophila* embryonic development (Cridge et al. 2017). Embryonic development has since came under scrutiny primarily with respect to axial patterning, segmentation and Hox genes (Walldorf et al. 2000; Dearden et al. 2006; Wilson et al. 2010), as well as microRNAs regulating such patterning genes (Freitas et al. 2017). Another line of research addressed zygotic genome activation (Pires et al. 2016). All these studies were driven from the perspective of comparative evolutionary developmental biology. Nonetheless, compared to *Drosophila melanogaster*, very little is actually

known about the development of specific organs in *A. mellifera.*

With respect to gonad development, a major difference to *Drosophila* is apparent already at a very early step, namely the mode of germ cell determination in the blastoderm stage. While the prospective germ cells in *Drosophila* become clearly distinguishable at this stage at the posterior pole of the blastoderm embryo, dependent on pole plasm factors (Mahowald 1962), no evidence was found for such pole plasm determinants in the honey bee (Nelson 1915; DuPraw 1967), even though most of the genes encoding pole cell components involved in *Drosophila* germ cell determination have homologs in the honey bee genome (Dearden et al. 2006). Hence, the mode of germline specification is thought to be quite distinct from that of *Drosophila* and in fact guided by epigenetic factors (Dearden 2006; Cridge et al. 2017). The next developmental step then is the migration towards and arrival and integration of the primordial germline cells in the somatic (mesodermal) gonad. In the view of complete lack of knowledge for honey bees in this respect, we can only assume that this association may be guided by similar mechanisms as in the *Drosophila* embryo (Gilboa and Lehmann 2006; Slaidina and Lehmann 2017).

2.2. Caste-specific ovary differentiation in the larval stages

The still most comprehensive description on the development of the reproductive system in honey bee larvae dates back to the early twentieth century (Zander 1916), presenting analyses from histological sections of drone, queen, and worker larvae, produced by Prof. Dr. Enoch Zander and two of his doctoral students, Fritz Löschel and Konrad Meier. Unfortunately, this study was published in German and thus is not easily fully appreciated nowadays, but it is outstanding for its excellent illustrative tables (6 tables composed of over 100 figures). These figures show the larval gonads positioned bilaterally between the dorsal vessel and parietal fat body in the fifth and anterior part of the sixth abdominal segment. Upon dissection in vivo, they can be localized by the iridescent reflection of their rich tracheal network as elongated banana-shaped structures. In fact, for an embryologist, they do not look too different from the elongated gonads of a day 10–11 mouse embryo. The gonads are apically connected to each other over the dorsal midline, right beneath the dorsal vessel, and at their basal side emerges a string that bilaterally surrounds the midgut and connects the gonads to the ventrally positioned genital imaginal discs (Figure 1**)**. In female larvae, these strings will eventually become the lateral oviducts and, once the larval gut is emptied in preparation for metamorphosis, these strings contract, and the ovaries move ventrally undergoing a 90° rotation. Thereby, the apical side becomes constricted and will become the anterior, distal part of the ovary, where the future terminal filaments are formed. The basal side of the ovary also becomes constricted and forms the region where the basal ends of the ovarioles merge with the lateral oviduct.

While such descriptive morphological data provide insights on the transformation of the larval ovary into its adult form, they lack information on what is actually going on at the cellular level. In fact, for first instar larvae, the only histological data we are aware of are sections prepared by Cruz-Landim 2009). These indicate that germ cells and somatic cells are already structurally organized as individualized ovarioles, separated from one another by prospective peritoneal sheath cells. For second instar larvae, one of these sections (Cruz-Landim 2009) shows clearly individualized short ovarioles, each with an elongated filament at the apical end. Within these primordial ovarioles, large cells with a spherical nucleus can be distinguished, representing a typical germ cell morphology. Such an early appearance of clearly distinct ovariole primordia is quite different from what is seen in the larval ovary of *Drosophila melanogaster*, where ovariole primordia become established and clearly separated from each another only in the third (last) larval instar (King 1970), driven by the formation and organization of terminal filament cell stacks (Godt and Laski 1995; Sarikaya et al. 2012).

Until the fourth instar, the ovaries of queen and worker larvae do not yet show major apparent morphological differences with respect to ovariole structure and number (Hartfelder and Steinbrück

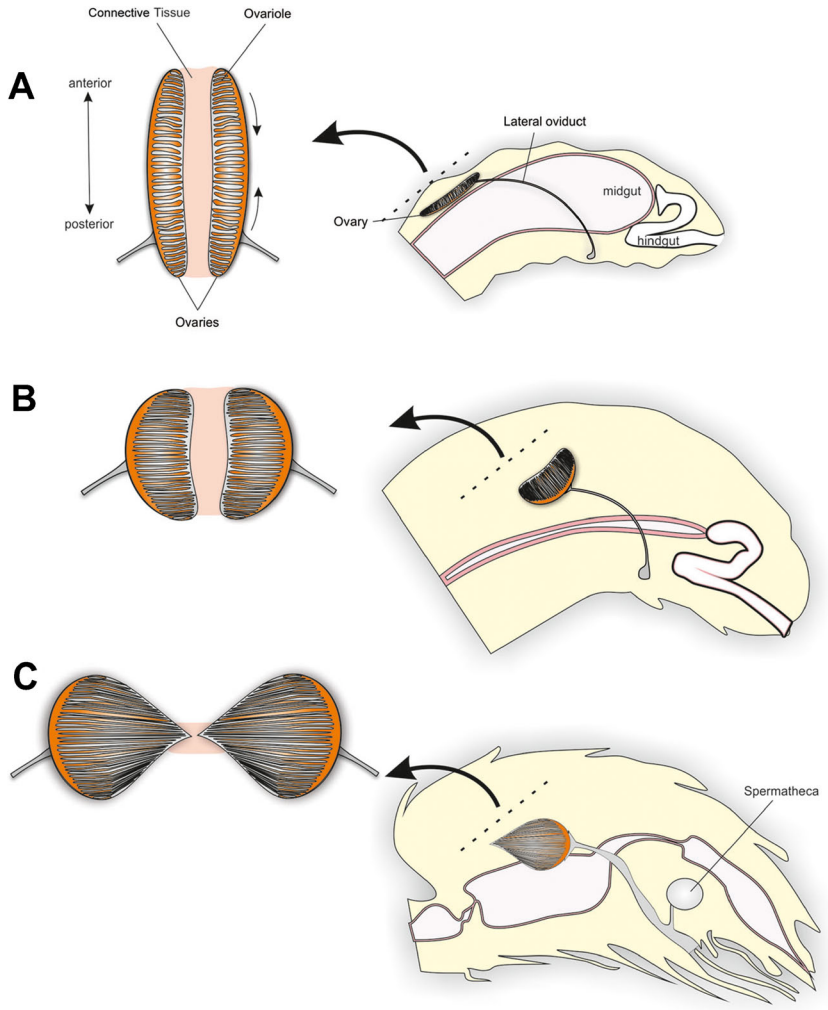

Figure 1. Position and change in format of the honey bee ovaries during postembryonic development. The depicted stages represent **a** an early fifth instar larva, **b** a queen prepupa, and **c** a pharate adult queen. The left column shows the two ovaries as seen when a larva or pupa is dissected dorsally, and the right column shows the relative position of the ovaries in the three developmental stages. Throughout all stages, the two ovaries are connected at their apical sides via a loose band of connective tissue. In these illustrations, we chose to present the situation in queens, but there are no major differences between the two castes, except for ovary and spermatheca size. The schematic drawings of the right column are based on figures from Zander (1916). Artwork was prepared by Douglas Elias Santos.

1997). With the entry into the fifth instar, however, degenerative events gradually become a prominent feature in the ovarioles of worker larvae, as can be inferred already even from their general morphology (Figure 2). This is also the moment when reversal of worker caste fate by transfer of larvae to queen cells becomes increasingly difficult (Dedej et al. 1998).

The degenerative processes in the ovaries of worker larvae are results of programmed cell death (PCD), as detected by TUNEL labeling (Schmidt Capella and Hartfelder 1998), general histological sections (Reginato and Cruz-Landim 2001), and ultrastructure analyses (Hartfelder and Steinbrück 1997). The degenerative process initiates in the germ cell region and is associated with

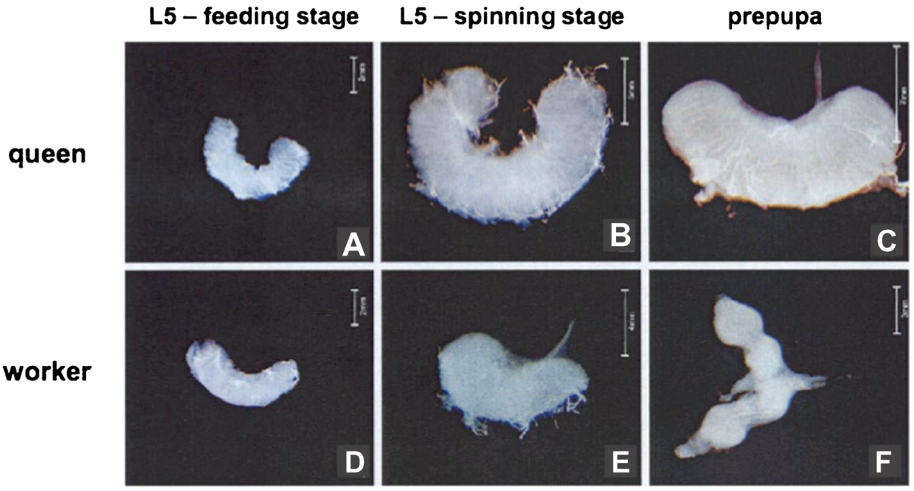

Figure 2. Ovary development in queen (**a**–**c**) and worker (**d**–**f**) larvae of the honey bee. Freshly dissected ovaries from early fifth instar (**a**, **d**), mid fifth instar (**b**, **e**), late fifth instar larvae (**c**), or the entire reproductive system with the two pear-shaped ovaries (**f**). In **a**–**e,** the apical side is up and the basal is down; in **f**, the apical side is already contracted and points to the left.

the degradation of a critical germ cell structure, the polyfusome, a structure typical of the polytrophic meroistic insect ovary (Büning 1994). The polyfusome maintains the mitotically dividing sister germ cells cytoplasmatically connected in the format of a germ cell rosette (Hartfelder and Steinbrück 1997). While polyfusomes are maintained in queen ovarioles, their disruption in worker larval ovarioles is due to the disintegration of the actin/spectrin cytoskeleton, and this is considered a critical step for defining the number of ovarioles that will persist in the adult females (Schmidt Capella and Hartfelder 2002). The onset of PCD is seen in the central region of the ovariole primordia where the germ cells are located, and then extends into the apical and basal ends of the ovarioles. Thus, by the entry into the prepupal phase, over 90% of the ovariole primordia in the worker ovary will become completely degraded (Hartfelder and Steinbrück 1997), and in the pupal stage, the remaining ovariole filaments already permit conclusions regarding adult ovary size.

The critical factor that influences the level of PCD in the fifth instar honey bee ovary is the hemolymph juvenile hormone (JH) titer. Compared to worker larvae, it is by a factor ten higher in queen larvae at the transition from the fourth to the fifth larval instar (Rembold 1987; Rachinsky et al. 1990). It then drops to basal levels in both castes during the beginning of the larval spinning phase, when the brood cells are closed, and only rises again in prepupae, especially so in queens. Topical application of synthetic JH to fourth instar worker larvae inhibited PCD, as seen by a drastic reduction in TUNEL-labeled cells and conservation of the actin/spectrin cytoskeleton in the respective germ cell rosettes (Schmidt Capella and Hartfelder 1998; Schmidt Capella and Hartfelder 2002).

2.3. Gene expression analyses reveal molecular mechanisms underlying caste-specific ovary development

To understand the molecular underpinnings of how the caste-specific differences in ovariole number are generated, Dallacqua and Bitondi (2014) investigated the expression of two genes known to be important components of the *Drosophila* cell death machinery: the genes encoding an apoptotic peptidase activating factor (Apaf)-related killer gene (*ark*) (Rodriguez et al. 1999), an ortholog of the pro-apoptotic mammalian Apaf-1, and *buffy*, which encodes a member of the B cell lymphoma 2 (Bcl-2) protein family with

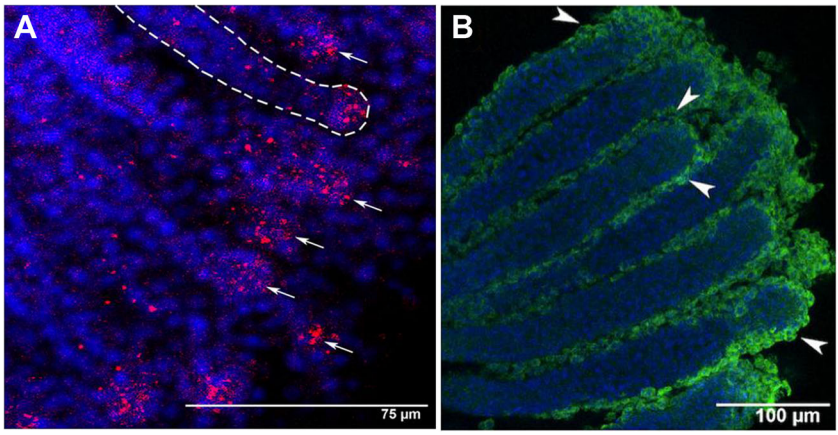

Figure 3. Detection of transcripts of the pro-apoptotic gene *ark* (**a**) and the anti-apoptotic gene *buffy* (**b**) by in situ hybridization on ovaries of mid fifth instar worker (**a**) and queen larvae (**b**); basal ovariole ends are to the left, apical ends to the right. *ark* (red) is predominantly expressed near the apical end of the ovarioles (arrows), whereas *buffy* (green) is strongly expressed in the peritoneal sheaths covering the ovarioles (arrowheads). A white broken line shows the circumference of one of the many ovarioles. Nuclei in blue are marked with DAPI. Modified from Dallacqua and Bitondi (2014).

anti-apoptotic activity (Quinn et al. 2003). The relative quantification of their expression levels in queen and worker larval ovaries showed that pro-apoptotic *ark* becomes highly expressed in workers, but not in queens, at the end of the fifth instar (prepupae), whereas the expression of the anti-apoptotic *buffy* increased earlier, at the spinning phase, in the ovaries of queens, and only increased in the following prepupal phase in workers. Furthermore, by fluorescence in situ hybridization, these authors showed strong labeling for the presence of *ark* RNA in fifth instar worker ovarioles (Figure 3a) in a region where TUNEL labeling had previously indicated the onset of PCD in the germ cell region (Schmidt Capella and Hartfelder 1998). In contrast, *buffy* RNA appeared strongly associated with cells of the peritoneal sheath that surrounds each ovariole in a queen larval ovary (Figure 3b), appearing as if it were shielding the ovarioles from cell death signals (Dallacqua and Bitondi 2014).

Obviously, we use here the term shielding in a symbolic sense, merely inferred from the localization of these transcripts, but not gene function. Nonetheless, while these two genes are clear bona fide candidates acting within the molecular machinery that establishes the divergent ovary size among the female castes of the honey bee, they appear to be relatively late-acting factors, actually associated with PCD execution but not PCD initiation. Since PCD initiating factors cannot be predicted a priori, and are frequently parts of a cell's general gene regulatory networks, including nutrient sensing, we undertook several approaches to identify possible candidate genes involved in caste-specific ovary differentiation.

The earliest of these molecular approaches was a proteomic one, where we compared protein synthesis patterns in fourth and fifth larval instar ovaries (Hartfelder et al. 1995). We could show that general protein synthesis rates become divergent between the queen and worker ovary shortly after the molt to the fifth instar. Furthermore, characteristic expression differences were noted for two low molecular weight proteins that are likely to be heat shock proteins, and which responded to both JH and ecdysteroids. No attempts, however, were made at that time to further characterize and sequence these proteins.

All the subsequent approaches were transcriptome analyses of different levels of complexity and throughput, depending on the methods available at their times. The first followed a differential display RT-PCR (DDRT-PCR) protocol and was done to identify ecdysone-responsive genes in the larval honey

bee ovary (Hepperle and Hartfelder 2001). By that time, the ecdysone response cascade was much better understood than that of JH (Riddiford et al. 2000; King-Jones and Thummel 2010), and measurements of the hemolymph titer in honey bee queen and worker larvae had indicated a significant timing difference in the ecdysteroid titer peak in spinning-phase larvae (Rachinsky and Hartfelder, 1990). From the DDRT-PCR gels, fragments of two genes were cloned and sequenced, one putatively corresponding to a FTZ-F1 homolog and the other to a member of the Cut/CUX1 transcription factor family. Already known by that time as being a component of the ecdysone-mediated transcriptional response, the *βFTZ-F1* gene is now established as one of the major players that integrate the JH and ecdysone responses in insect metamorphosis (King-Jones and Thummel 2010), including honey bees (Mello et al. 2014).

More recently and with a fully sequenced honey bee genome at hand, a representational difference analysis (RDA) approach was employed to identify differently expressed genes in honey bee ovaries dissected from fifth instar queen and worker larvae (Humann and Hartfelder 2011). From the suppression-subtractive hybridization libraries, expressed sequence tags (ESTs) of 40 and 32 genes, respectively, were identified as overrepresented in queen and worker ovaries, respectively. Strikingly, 60% of the ESTs sequenced from the worker library and 28% of the queen library ESTs represented unpredicted transcripts, i.e., genes that had not been computationally predicted in the honey bee genome, and thus potentially fall into the category of novel genes (Elsik et al. 2014). Validation of the differential expression of 16 of the genes from the two libraries by real-time PCR then confirmed two genes as significantly overexpressed in queen larval ovaries and two in worker ovaries. Among the two queen overexpressed genes, one was *sdr*, a gene encoding a short-chain dehydrogenase/reductase, and the other was an unpredicted gene that we tentatively named Group11.31b based on its genomic scaffold location. Among the worker-overexpressed genes, one was *oat*, which has ornithine-oxo-acid transaminase activity as a predicted molecular function, and the second was another unpredicted gene named Group11.35a, again according to its genomic scaffold localization (Humann and Hartfelder 2011).

The finding that the *sdr* gene was overexpressed in queen ovaries was of interest because in the prior DDRT-PCR screen (Hepperle and Hartfelder 2001), we had already obtained EST hits for this gene, for which we could later show that it is strongly ecdysone-responsive in worker ovaries (Guidugli et al. 2004). The other interesting finding was that the two unpredicted genes with opposite patterns of expression, one being overexpressed in the queen and the other in the worker ovary, mapped genomically to chromosome 11. Once their complete cDNAs were sequenced, computational analysis revealed that they were likely long noncoding RNAs (Humann et al. 2013).

Long noncoding RNAs (lncRNAs) have come to attention in recent years once next-generation sequencing (NGS) methodologies made it possible to obtain high-throughput and high-density transcriptomes at a large scale. As they have no protein-coding potential, their existence could not be computationally predicted in the assembled genomes. The transcriptomic efforts, especially those done on vertebrate species, including humans, however, revealed the existence of thousands of lncRNA genes (Louro et al. 2009; Mattick 2011; Mattick and Rinn 2015), and functional studies soon demonstrated that these have a plethora of functions, especially so in the fine-tuning of transcriptional and translational regulation during developmental processes (Mercer et al. 2009; Mattick 2011).

To our knowledge, in honey bees, only four lncRNAs have been identified so far in terms of their possible functions. These are two lncRNAs identified from studies on brain transcriptomes (Sawata et al. 2002, 2004; Kiya et al. 2012) and the two abovementioned lncRNA genes revealed from the ovarian transcriptomes (Humann and Hartfelder 2011), which were subsequently named *long noncoding ovary 1* (*lncov1*) and *long noncoding ovary 2* (*lncov2*). Both turned out to have an intronic location in the sense strand of protein-coding genes, one (*lncov1*) in a gene of unknown function, only defined by computational prediction, and the other (*lncov2*) in the honey

bee homolog of *fringe*. Fringe proteins are regulators of the Notch signaling pathway, which plays a key role in many cell-cell interaction processes in vertebrate and insect development (Haines and Irvine 2003), including maintenance of the germline stem-cell niche in the *Drosophila* ovary (Yang et al. 2013) and oogenesis in the honey bee (Duncan et al. 2016). Furthermore, the genomic location of the two lncRNAs and the *fringe* gene turned out to be eminently interesting, as they all mapped within a major quantitative trait locus (QTL) for ovariole number variation in honey bee workers, previously identified from crosses of wild-type European and Africanized honey bees (Linksvayer et al. 2009) and also from crosses with bees presenting different preferences in pollen hoarding (Graham et al. 2011).

lncov1 is of particular interest, not only because it was found overexpressed in association with PCD in the ovaries of worker larvae but also because its transcripts were found to localize with cytoplasmatic granules, indicating that it could functionally be involved in translational regulation (Humann et al. 2013). This cytoplasmatic localization prompted us to study potential lncov1 interaction partners. To do so, we performed a pull-down experiment using lncov1 RNA as bait, followed by a proteomic analysis of associated proteins. One of these called our attention, staphylococcal nuclease domain-containing protein 1, also known as Tudor-SN (Tibério, Cardoso Júnior, Lago, Rosa, Hartfelder, unpublished results). In *Drosophila*, *tudor* mutants are sterile (Boswell and Mahowald 1985), and more recently, Tudor proteins were shown to be involved in transcriptional regulation via epigenetic histone modification (Lu and Wang 2013). This shows that we can now gradually build a bridge from the non-hypothesis-driven large-scale analyses to pinpointing genes as bona fide players in the fine-tuning of PCD in the larval worker ovary. Yet, this bridge still lacks a pillar related to the molecular mechanisms of JH signaling.

Though implicated as a crucial factor in honey bee caste development, immediate JH effects on larval ovary development were rarely in focus. Rather, as a general morphogenetic hormone, the look generally was on the JH levels circulating in hemolymph (Rembold 1987; Rachinsky and Hartfelder, 1990), on how differential feeding of the larvae affected JH synthesis (Rachinsky and Hartfelder, 1990; Bomtorin et al. 2012), and on how JH signaling may integrate with nutrient-sensing and metabolic signaling pathways, such as IIS/TOR (Wheeler et al. 2006; Patel et al. 2007; Azevedo and Hartfelder 2008; Wheeler et al. 2014; Hartfelder et al. 2015), EGFR (Kamakura 2011; Hartfelder et al. 2015), and hypoxia (Azevedo et al. 2011; Santos et al. 2016). Furthermore, a mathematical model predicted how the larval feeding regimes and the resultant JH levels would translate into variation in adult ovariole number (Leimar et al. 2012). Other studies already mentioned above had looked at the effects of JH application on PCD in larval ovaries (Schmidt Capella and Hartfelder 1998; Schmidt Capella and Hartfelder 2002), but direct transcriptional JH effects could only be addressed once the paralogous bHLH-PAS domain transcription factors methoprene-tolerant (Met) and germ cell-expressed (GCE) had been definitively identified as the functional JH receptor proteins (Charles et al. 2011; Li et al. 2011). When JH is bound to the dimeric JH receptor complex Met/Taiman (Jindra et al. 2013), the *Krüppel homolog-1* (*Kr-h1*) gene is expressed as an early response gene, and in the case of honey bee larvae, its transcript levels were seen to correlate well with the JH titer (Hartfelder et al. 2015).

With this in mind, the JH response of the set of differentially expressed genes in larval queen and worker ovaries (Humann and Hartfelder 2011) was now assessed by dissecting ovaries of the caste-critical larval stage six hours after the larvae had received a topical application of synthetic JH-III (Lago et al. 2016). The immediate JH response was confirmed by an increase in ovarian *Kr-h1* expression, and a significant increase in transcript levels was noted for *sdr* and *hsp90*, a gene encoding heat shock protein 90.

As previously mentioned, the *sdr* gene has sprung up repeatedly and over decades of research as a differentially expressed gene in the larval honey bee ovary, and as directly regulated by the morphogenetic hormones JH (Lago et al. 2016) and makisterone A (Guidugli et al. 2004) [note that makisterone A is the predominant ecdysteroid moiety in honey bees; Feldlaufer et al. 1985]. This makes the *sdr* gene an interesting candidate for

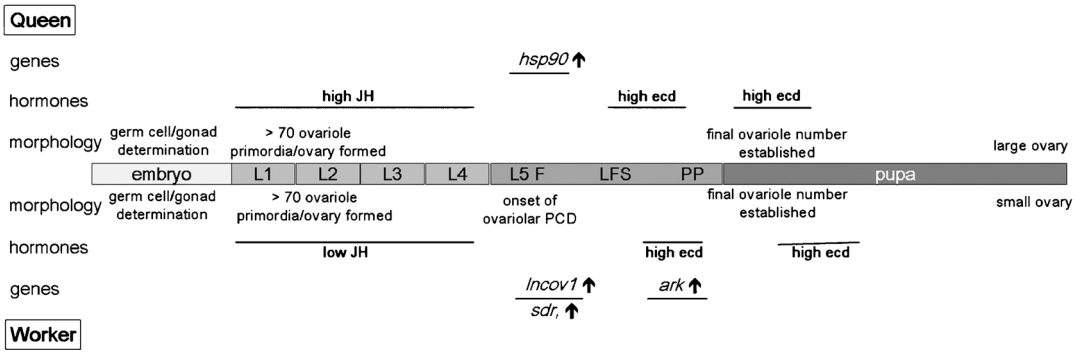

Figure 4. Schematic summary of the developmental steps and events as they occur in the embryo, the larval instars L1 to L5 and the pupal stage, leading up to the morphologically distinct ovary phenotypes of adult queens and workers. L5F, L5S, and PP are the feeding, spinning, and prepupal phases of the L5 instar. Shown are the main morphological characters, periods of major differences in juvenile hormone (JH) and ecdysteroid (ecd) hemolymph titer, as well as the phases where certain genes specifically addressed in this review are differentially expressed.

further in-depth functional studies in honey bee caste development. Among the five predicted genes that we could identify in the honey bee genome as encoding members of the SDR family, the one of primary interest has the GenBank entry AAP45005.1. SDRs are a large and phylogenetically ancient family of NAD(P)(H)-dependent oxidoreductases, and they exert a variety of functions in the cellular metabolism (Kavanagh et al. 2008). A function that could be of specific interest in the context of honey bee caste development is their sensor function of the cellular redox state, because this could provide a direct link between the hormonal regulation of this *sdr* gene and the cellular redox potential seen in the honey bee larval fat body (Santos et al. 2016). Furthermore, it could also provide a link to the evolution of sociality in bees, as an *sdr* homolog was recently identified as differentially expressed in relation to bivoltinism in the non-social bee *Tetrapedia cornuta* (Araujo et al. 2017, this volume).

The sequential steps and events in the preimaginal development of the honey bee ovary are summarized in Figure 4, illustrating that we have made some headway concerning the molecular underpinnings of honey bee caste development at the tissue-specific level, i.e., the larval ovary. Clearly, there will still be much work to do to put together the puzzle of all the developmental signaling pathways and their interactions, but at least for the two morphogenetic hormone systems, JH and ecdysteroids, the methoprene-tolerant-Krüppel homolog 1-E93 (MEKRE93) module (Bellés and Santos 2014) is now a good and solid paradigm for the gene regulatory network underlying insect metamorphosis, and this is the context wherein caste development of highly eusocial Hymenoptera is ontogenetically embedded.

Nonetheless, there is still the question on how the dramatic structural difference in the reproductive system of honey bee females has evolved, and this question has recently been addressed in studies that paved the ground for a mechanistic understanding of reproductive bias and worker sterility in adult honey bee and other social Hymenoptera (Duncan et al. 2016; Ronai et al. 2016a). As this topic has recently been thoroughly reviewed (Ronai et al. 2016b), we will only highlight some of these aspects in the next chapter.

3. OVARIAN ACTIVITY IN ADULT HONEY BEES

3.1. Worker sterility—ovary activation and deactivation in adult *A. mellifera* females

Any researcher working on honey bees has, at least once, included an introductory sentence in a manuscript saying something like *in the honey bee, workers are facultatively sterile, while a*

queen can lay over 1000 eggs per day. Of course this describes quite concisely the reproductive division of labor in the colony of these highly eusocial insects. But why are workers facultatively sterile, or better to say subfertile? And has anyone ever counted the number of eggs a queen actually lays per day?

Though subfertile, workers can activate their ovaries in the absence of a queen, and in some exceptional cases, like the Cape honey bee *Apis mellifera capensis*, the "Clone" in South Africa, and the "anarchistic worker" phenotype originally found in colonies in Australia, they can also do so in the presence of the queen. In Capensis workers and those of the Clone, worker reproduction is associated with a specific switch in developmental modes, i.e., between arrhenotoky and thelytoky (Goudie and Oldroyd 2014; Goudie et al. 2015; Cole-Clark et al. 2017). But examples of arrhenotokous worker reproduction, as in the anarchistic workers, are seen as an exception in the genus *Apis*. Nonetheless, they are actually a rule in the much more diverse stingless bees, the Meliponini.

In stingless bees, young workers involved in brood cell construction and provisioning of the brood cells lay trophic eggs during the so-called provisioning-oviposition process (POP) (Zucchi et al. 1999). Eaten by the queen, these eggs are an important nutritional resource. Alternatively, the workers can also lay reproductive eggs shortly before cell closure. These eggs will give rise to males, and such worker-produced males actually represent a considerable proportion of the males produced in the colonies. Strikingly, the participation and contribution of the workers in colony reproduction are quite variable among stingless bees (Toth et al. 2004; Velthuis et al. 2005; Hartfelder et al. 2006) and can involve chemical inhibition of ovarian activity (Nunes et al. 2014), similar to honey bees, where queen pheromone has an inhibitory effect (Ronai et al. 2016c). A recent multiple-generation genetics study on *Melipona scutellaris* colonies showed that workers were the mothers of 23% of the males, and interestingly, 81% of these were the offspring of workers from superseded queens (Alves et al. 2009). Thus, daughters from a previous queen do not only live longer but also may reproductively parasitize the next-generation workforce (Alves et al. 2009).

So, in distinction to honey bee workers, where oogenesis is blocked at certain steps during follicle development (Tanaka and Hartfelder 2004; Ronai et al. 2015), the ovary of a stingless bee worker goes through the same cycle of activity as that of a queen (Tanaka et al. 2009), the only difference being the number of serial follicles in the ovarioles and the fact that the ovaries of workers undergo cellular degradation as these grow older and become foragers.

Furthermore, stingless bee queens and workers have essentially the same number of ovarioles (Martins and Serrão 2004), but in queens, these are much elongated, and when fully active, the abdomen of the queen is highly physogastric (Engels and Imperatriz-Fonseca 1990). Hence, not the appearance of laying workers in a colony, but rather the subfertile status of honey bee workers, is apparently the exceptional condition among highly eusocial bees. So the question is, what are the underlying molecular and physiological circuitries and what may be the evolutionary causes.

As shown by us (Tanaka and Hartfelder 2004) and Ronai et al. (2015), the occurrence of PCD at certain steps in the oogenesis process creates a mechanism to block reproduction in honey bee workers, and since PCD results from an interplay between pro- and anti-apoptotic factors, this also creates the possibility for reversion of reproductive activity in queenless workers. In their recent comprehensive review, Ronai et al. (2016b) convincingly summarize evidence for evolutionary and mechanistic causes underlying worker sterility in honey bees. They argue that PCD has been incorporated in sequential steps of the ontogenetic program of worker development and reproduction, starting with the inhibition of spermatheca development and degradation of individual ovarioles or germ cells during larval development, followed by selective PCD at four steps during the life cycle of adult workers, including death of germline stem cells and of early germ cell clusters, degradation of early and late follicles, and the loss of entire ovarioles as the workers age and become foragers (Ronai et al. 2016b, 2017). Degradation of late follicles has also been observed in laying workers and in queens of *A. mellifera* (Patrício

and da Cruz-Landim 2008), and the histological characteristics indicate that the degenerated follicle remnants may be equivalent to the yellow bodies frequently observed in ovaries of social insects in connection with aborted or successful oviposition events (Kelstrup et al. 2014).

PCD in the ovary is not unique to social insects. Rather, it is an adaptive response to unfavorable environmental conditions, allowing insect females to reduce their egg laying rate once there is a shortage of nutrients. Mechanistically, it allows *Drosophila* females to adjust their reproductive output via insulin/TOR signaling (Pritchett and McCall 2012). Hence, the conclusion is that ovarian PCD in social bees is actually built on an ancestral program allowing reproductive fine-tuning. This program has become co-opted or, better to say, selectively driven to an extreme in honey bee workers that do not reproduce in the presence of the queen (Ronai et al. 2016b).

An interesting connection between PCD and oogenesis regulation through an evolutionarily conserved developmental signaling pathway, Notch signaling, has recently been evidenced (Duncan et al. 2016). Notch signaling has a repressive effect on the early steps of oogenesis in the worker ovary when a queen is present, and blocking Notch function activates the worker ovary, suggesting that Notch signaling has been co-opted into the social circuitry of female reproduction/worker sterility in honey bees. Interestingly, this may actually represent a link also with ovary development in the larval stage, since one of the long noncoding RNAs, *lncov2*, genomically maps within the intron of the honey bee homolog of *fringe/lunatic fringe*, which is an activator of Notch signaling and related to FOXO/insulin signaling in the *Drosophila* ovary (Yang et al. 2013).

In more general terms, high-throughput analyses designed to distinguish between activated and inactivated ovaries revealed characteristic proteomic differences in workers, indicating regulation via endocrine and neuroendocrine factors (Cardoen et al. 2012). Transcriptomic analyses on ovary activation states in both queens and workers subsequently revealed a highly complex set of differentially expressed genes, including 824 genes shared among the two castes with respect to equivalent ovary activation states (Niu et al. 2014). In an attempt to gain further insights into regulatory pathways, a transcriptomic analysis of microRNAs then confirmed that queens and workers share a set of 19 differentially expressed microRNAs (Macedo et al. 2016), which could be mapped into a regulatory framework that integrated this data with the prior proteomics and transcriptomic analyses.

Interestingly, there is always a certain number of rebel workers present in queenright *A. mellifera* colonies that defy the queen's inhibitory signals and activate their ovaries. Naturally, their presence has been observed in the context of swarming (Woyciechowski and Kuszewska 2012) and may be related to instability in the interpretation of the queen pheromone signal and also to the absence of brood pheromone (Mohammedi et al. 1998; Maisonnasse et al. 2009). An elevated occurrence of such rebel workers has, however, been noticed in colonies that could be genetically selected as the "anarchistic worker strain," and this has led to the identification of a gene, named *Anarchy*, associated with this trait (Oldroyd et al. 1994; Oldroyd and Osborne 1999). The *Drosophila* homolog of Anarchy is a peroxisomal membrane protein (PMP34). *Anarchy* expression was shown to be a good predictor of the workers' ovary status. Elevated *Anarchy* expression was shown to be associated with a non-activated ovary state in workers and with the presence of a queen. Furthermore, by in situ hybridization, *Anarchy* transcripts were found associated with degenerating oocytes, and the RNAi-mediated knockdown of *Anarchy* function resulted in the upregulation of the anti-apoptotic factor *buffy* (Ronai et al. 2015), thus putting in evidence a striking and direct connection of this gene with PCD regulation in the ovaries of larval honey bees (see above, Dallacqua and Bitondi 2014).

With a focus on understanding the genomic underpinnings of worker sterility, transcriptome analyses done in a comparison of wild-type workers and workers from the anarchistic strain revealed a set of genes as overexpressed in abdomen of anarchistic workers, including vitellogenin and a gene encoding an enzyme of the AdoHycase superfamily (Thompson et al. 2008). As the latter is considered a candidate

for modulating the activity of DNA methyltransferases, this finding would again, as already seen in postembryonic development (Kucharski et al. 2008), present a link to epigenetic factors that mediate ovary activity in honey bees (Thompson et al. 2008). Subsequently, by integrating data from such brain and abdomen transcriptomes in a meta-analysis, Sobotka et al. (2016) now present a view on a complex transcriptional regulatory network that they interpret as depicting a social transcriptome underlying worker sterility in honey bees. Such meta-analysis perspectives can provide information on hub genes that interconnect gene regulatory modules and should be useful in heuristically guiding functional studies on this central aspect in honey bee social biology.

3.2. Vitellogenin and JH in honey bee reproduction and division of labor

Notwithstanding the importance of such large-scale approaches to the understanding of ovarian function in honey bees, a prime factor for oogenesis undoubtedly is the synthesis of vitellogenin by the fat body and its release into the hemolymph, from where it is then sequestered by the growing oocytes. Vitellogenin uptake occurs by receptor-mediated endocytosis, either via spaces that broaden between follicle epithelial cells (Engels 1973) in a process termed patency or by transepithelial transport (Fleig et al. 1991). The receptor mediating the incorporation of vitellogenin into the oocyte and its subsequent processing into vitellin is the honey bee homolog of the *Drosophila* yolkless protein. The honey bee vitellogenin receptor (VgR) has been cloned and sequenced, and its expression in ovaries was found to be directly related to the state of ovary activation in both queens and workers. Furthermore, in situ hybridization analysis revealed that the *vgr* gene is highly expressed in nurse cells composing the trophic chamber, from where the respective mRNA is transported to the growing oocyte (Guidugli-Lazzarini et al. 2008). Since the oocyte nucleus is in the meiotic prophase and, thus, transcriptionally inactive, with the exception of a few genes, this shows that the nurse cells play an important role, not only in previtellogenic but also in vitellogenic growth of the oocyte. While this is, to our knowledge, the only study directed towards the understanding of the molecular underpinnings of vitellogenin uptake, vitellogenin itself is certainly one of the best-studied proteins in the honey bee.

Vitellogenin is a 180-kDa protein (Wheeler and Kawooya 1990) encoded by a single gene (Piulachs et al. 2003), and its functional 3D structure has been predicted by a modeling approach (Havukainen et al. 2011). The levels of vitellogenin in the hemolymph of honey bee queens have been shown to increase during the first days of their adult life cycle and to stay high during the rest of their life, representing up to 60–80% of the total protein content in hemolymph (Engels 1974; Hartfelder and Engels 1998). Even in queenright non-reproductive workers, the vitellogenin hemolymph levels are elevated while they perform brood rearing tasks within the colony, but then drop as they become foragers. As this transition to foraging behavior is associated with an increase in JH levels (Huang et al. 1994; Huang and Robinson 1996), a mutual repressor circuitry has been proposed and experimentally confirmed as governing this age-related behavioral transition (Amdam and Omholt 2002; Guidugli et al. 2005; Nelson et al. 2007; Marco Antonio et al. 2008).

Two aspects are intriguing in this respect: the fact that (1) JH triggers the initial increase in vitellogenin levels in both queens and workers as they are about to emerge from their brood cells (Barchuk et al. 2002) and that (2) JH has no further function in the maintenance of the reproductive status in queens (Hartfelder and Engels 1998), but assumes a repressor function on vitellogenin expression in the context of behavioral maturation of workers (Amdam and Omholt 2002). JH is the major gonadotropic hormone in the reproductive cycles of female insects (Wyatt and Davey 1996; Raikhel et al. 2005). Since honey bee queens have no cyclic ovarian activity, but reproduce continuously at high rates, the loss of this JH function should not be seen as a surprise, as JH would only be required to initially trigger vitellogenin synthesis in the late pharate adult stage, before they emerge from the brood cell. Furthermore, since *A. mellifera* workers are suppressed in their reproductive activity by the presence of the queen and larval brood pheromones, a

long-proposed hypothesis is that JH, once freed from its adult gonadotropic function, may have been evolutionarily co-opted for controlling division of labor among the honey bee workers (Robinson and Vargo 1997).

With its unrivaled status as a model system for social insect biology, a frequently asked question is whether such a vitellogenin/JH regulatory module may also exist and coordinate division of labor in other social insects, and especially so, in other social bees. While JH application experiments provided evidence for such a regulatory module in social wasps (O'Donnell and Jeanne 1993), there is unfortunately little data on actual JH levels in wasp hemolymph, and even less so on vitellogenin synthesis or titers. In fact, the evidence from studies on *Polistes* paper wasps (Röseler et al. 1984) and tropical, swarm-founding social wasps (Kelstrup et al. 2014) points more towards an association of JH with reproduction and dominance than with division of labor. JH levels and ovarian status are strongly correlated in bumblebees (Bloch et al. 2000: Shpigler et al. 2014), and recent data for the stingless bee *Melipona scutellaris* point in a similar direction (Cardoso-Júnior et al. 2017). Bombini and Meliponini comprise a branch of eusocial bees within the Corbiculata that is separated from the Apini (Hedtke et al. 2013). Different from honey bees, the workers of bumblebees and stingless bees are reproductively active at some point in their life or in the colony cycle and, thus, the gonadotropic function of JH should have remained conserved in this branch. This also indicates that the JH/vitellogenin repressor circuitry, which is so clearly evidenced in the honey bee, may actually be more of an idiosyncrasy of this species and possibly also for the other species of the genus *Apis*, than representing a general condition in social Hymenoptera.

Actually, this apparent rewiring of the JH/vitellogenin circuitry in female honey bees may well be related to the queen's polyandrous mating strategy and her exaggerated ovary morphology, with each ovary consisting of well over 100 ovarioles. In this respect, the genus *Apis* strongly differs from the other bee species, as for most of the Andrenidae, Halictidae, Colletidae, and Melittidae, the number of ovarioles per ovary was found to be three (Iwata 1955; Rozen 1986; Martins and Serrão 2004). Within the corbiculate bees, ovariole numbers are apparently somewhat more variable, but also in these, the Meliponini, Bombini, and Euglossini apparently have only between 4 and 18 ovarioles, with the most variability seen in the queens of stingless bees (Cruz-Landim et al. 1998; Lisboa et al. 2005). Yet none of these queens comes anywhere close to the honey bee queens, so the question is, what may actually have been the evolutionarily driving force that led to the highly elevated ovariole numbers in the genus *Apis*? Obviously, one may think that this may be due to the high egg laying rates of honey bee queens compared to those of other social bees, and indeed, for species of stingless bees for which data on daily egg laying rates of queens are available, these do not exceed 30–180 eggs (van Benthem and Velthuis 1995; Martins and Serrão 2004). But these data are for *Melipona* and *Plebeia* species, and these do not have really large colonies. Unfortunately, no such data are available for *Trigona* species, which clearly rival honey bees in terms of colony size. Among the Hymenoptera, similarly high ovariole numbers are only reported for army ants, with over 250 ovarioles per ovary in *Eciton schmittii* queens (Wheeler 1910), and the champions are driver ants of the genus *Dorylus*, where over 15,000 ovarioles have been reported for queens (Hölldobler and Wilson 1990).

Strikingly, what these ant species have in common with honey bees is an exceptionally high frequency of multiple matings of their queens (Kronauer et al. 2007), leading to the conclusion that there is at least a correlation between drastically enlarged ovaries and polyandry and that this could reflect trait co-evolution between the female and male sexes in these taxonomically distinct groups.

3.3. Mating frequency and sperm number—a male sex perspective on female ovary structure in social bees

The evolution of polyandry, which is a rare trait among social insects in comparison to monandry, has direct implications on intracolony relatedness and kin selection theory, and hence is a long-

debated issue (Page 1980; Crozier and Page 1985; Boomsma and Ratnieks 1996; Strassmann 2001; Brown and Schmid-Hempel 2003; Kronauer et al. 2007). Furthermore, an ancestral character state reconstruction based on 267 bee, ant, and wasp species provided a strong argument for monandry being ancestral in all of these groups, and that high levels of polyandry are derived, apparently, in one corbiculate bee genus (*Apis*), one vespine genus (*Vespula*), and seven distinct lineages of ants (Hughes et al. 2008).

The most frequent explanations of why multiple mating could have evolved in the genus *Apis* are avoidance of colony losses due to diploid male production, genetic bias of worker specialization, and immune system response variation against disease threats (Page 2013). Nonetheless, the monandrous stingless bees have to cope with the same challenges and there is no evidence that they are worse off. So, a closer look into the mating biology of these two groups of highly eusocial bees may shed some light on the problem.

The mating biology of queens and drones has been in the focus of bee research over decades (Page 1980, Koeniger and Koeniger 1991) and has recently been reviewed in a comprehensive manner by Koeniger et al. (2014). Also, a more general overview on male mating behavior and mating systems of bees has been provided by Paxton (2005), so the reader is referred to these publications for further information. As stingless bees are not only taxonomically diverse, but also vary a lot in terms of their reproductive biology (Vollet-Neto et al. n.d.), a few points of interest shall be addressed here. First, in most stingless bee species, with exception of the genus *Melipona*, male aggregations and probably also mating occur close to nests where virgin queens are emerging (Michener 1946; Engels and Engels 1984; Engels 1987; Roubik 1990; Cameron et al. 2004). Such aggregations of hundreds to thousands of males form day after day over a certain period and are composed of males from very different locations (Paxton 2000; Kraus et al. 2008), thus guaranteeing genetic diversity. Species of the genus *Melipona* differ from this general stingless bee pattern, and in this respect, they are more similar to honey bees by using non-resource-based *rendezvous* sites further distant from the nest (Sommeijer and De Bruijn 1995; van Veen and Sommeijer 2000).

Besides the mating site location, that is, at none-resource-based drone congregation areas in the air (*Apis*) versus male aggregations close to the colony (stingless bees), the two taxonomic groups differ in another important aspect, i.e., multiple mating (*Apis*) versus single mating (stingless bees). The mating sign left in the queen's vaginal chamber by a honey bee drone can easily be removed by the subsequent drone (Koeniger and Koeniger 1991; Koeniger et al. 2014), thus permitting a series of sequential copulations during the queen's nuptial flight. This is not the case in stingless bees, where the male leaves its endophallus together with the external genitalia trapped in the queen's vaginal chamber. After returning to the nest, the workers typically remove this mating plug only after a few days, and its persistence in the queen's vaginal chamber for at least one day is in fact important to stimulate egg laying (Melo et al. 2001).

While this difference in mating sign/mating plug function is a mechanistic underpinning of the high mating frequency in the genus *Apis*, it does not explain the evolution of multiple mating. Rather, from an evolutionary perspective, one would expect considerable differences in selection pressures acting on the males with regard to sperm production, especially when taking into account that both *Apis* and stingless bee males can mate only once and that in both cases, spermatogenesis ceases during the pupal stage. During adult maturation, all the spermatozoa are or have already migrated to the seminal vesicles and the testes degenerate (Camargo 1984; Koeniger et al. 2014). So, once these males are mature, they are sperm limited, or in other terms, they would have to adjust their sperm production/ejaculate volume during preimaginal development, taking into account the number of the female's expected copulations and the total sperm volume that ends up and remains stored in her spermatheca (Boomsma et al. 2005).

Fortunately, there is good data on actual sperm numbers produced by males and those stored in the spermathecae of females over a wide spectrum of solitary and social bees (Garófalo 1980). In this extraordinary study, sperm counts were made for

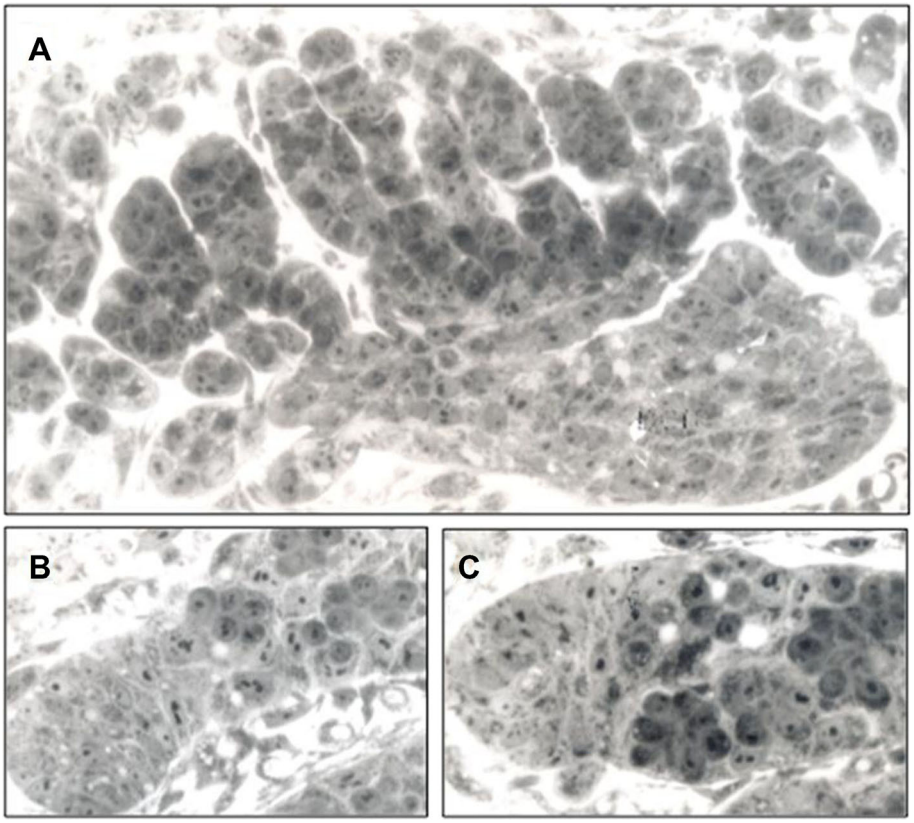

Figure 5. Histological sections of drone larval testes showing testiolar tubules. **a** Short finger-like testiolar tubules are clearly separated from one another and project from a basal cell mass in testes of third instar larvae. Apical is towards the top. **b** Upper end of a testiolar tubule of a fourth instar larvae showing a distinct apical cap region (to the left) followed by small germ cell clusters. **c** Upper end of a testiolar tubule of a fourth instar larvae with already larger germ cell clusters below the apical cap (to the left).

44 species of bees, comprising Colletidae, Andrenidae, Megachilidae, Anthophoridae, Halictidae, and Apidae, including paired sperm count datasets for both male testes and female spermathecae of 12 species. Furthermore, data from another 22 species from the literature were added (including six paired datasets). This dataset clearly shows that sperm number in the testes/seminal vesicles of a stingless bee male is practically identical to the sperm number found in the spermatheca of a mated queen. In contrast, in honey bees, these numbers are highly disparate. Sperm counts in drone ejaculates vary from 430,000 for the dwarf honey bee *A. florea* to over 10 million in *A. mellifera*, while spermathecal sperm counts range from 1 million (*A. florea*) to 5 million (*A. mellifera*) (Baer 2005). Considering a mean mating frequency of 12 in *A. mellifera*, this means that after mating the queen will discard over 90% of the sperm she has received, and only a small percentage actually ends up in the spermatheca. These numbers already indicate that there should be an enormous selection pressure on sperm production in *Apis* males, and this is even further increased by the fact that only about 20–30% of the spermatozoa in a queen's spermatheca will actually be used to fertilize her eggs (Baer 2005). This would mean that once polyandry had evolved, honey bee drones were likely to come under enormous selection pressure to produce a high number of spermatozoa during a very short time of their

life, while this should not have been the case for the males of stingless bees.

Evolution of polyandry in the female sex would thus likely be a driving force not only for sperm competition but possibly also for the generation of a high number of tubules that form as testiolar primordia in the larval testes of honey bee drones (Figure 5). At this stage, the testiolar primordia actually look strikingly similar to the ovariole primordia of females (Hartfelder and Steinbrück 1997).

Our hypothesis of a male-driven origin for the exaggerated morphology of the queen ovary in honey bees is, thus, based on (i) the similarity in gonadal structure shared by the two sexes, not only in honey bees but in insects in general (Büning 1994) and (ii) the fact that the differentiation of the insect testis and ovary is apparently guided by the same basic developmental mechanisms (Godt and Tepass 2003; Green and Extavour 2012). A third argument in favor of the hypothesis is the marked congruence in ovariole and testiole number across bee species, as shown in the comparative morphology studies on ovariole (Iwata 1955; Rozen 1986; Cruz-Landim et al. 1998; Martins and Serrão 2004; Lisboa et al. 2005) and testiole numbers (Ferreira et al. 2004).

4. QUESTIONS FOR THE FUTURE

Could the selection pressure on high sperm production during a short preimaginal time window, as a consequence of polyandry, have driven developmental mechanisms increasing the number of the drones' testiolar tubules? And is it possible that the same developmental mechanisms that the females share with the drones could have then driven or facilitated evolution towards the high (exaggerated) ovariole number seen in honey bee queens? Asking such questions may at first sight seem awkward but certainly would be in line with the not uncommon cross-sexual transfer of traits due to a shared developmental mechanism (West-Eberhard 2003). While this is as yet a hypothetical conjecture, we believe that it should be now possible to use comparative genomics to look at genes associated with gonad formation in the two sexes of bees and across different taxonomic and social levels. This may then shed light on the question as to why the two honey bee castes differ so drastically in ovary structure and why the genus *Apis* is so distinct from the other bees in terms of gonad (ovaries and testes) morphology.

ACKNOWLEDGMENTS

We thank Douglas Elias Santos for producing artwork for this review.

Funding information Financial support was obtained from the Brazilian funding agencies FAPESP (11/03175-5, 2014/28147-3; 2015/05757-5; 17-09128-0) and CNPq (303401/2014-1).

AUTHORS' CONTRIBUTIONS

KH wrote the review. GJT, DCL, RDP, and MMGB provided original data and discussed subsequent drafts of the manuscript. All authors read and approved the final manuscript.

L'ovaire et ses gènes – les processus de développement sous-jacents à l'établissement et la fonction d'un système reproductif hautement divergent dans les castes de femelles de l'abeille, *Apis mellifera*

Abeille / développement des gonades / mort cellulaire / expression génique différentielle / Meliponini

Das Ovar und seine Gene - entwicklungssteuernde Prozesse in der Etablierung und Funktion des hochdivergenten Reproduktionssystems der Kasten im weiblichen Geschlecht der Honigbiene, *Apis mellifera*

Honigbiene / Gonadenentwicklung / Zelltod / differentielle Genexpression / stachellose Bienen

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