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CHAPTER THREE

Physiological and Genomic Mechanisms of Social Organization in Wasps (Family: Vespidae)

Jennifer M. Jandt*, Amy L. Toth*,†,1
*Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa, USA
†Department of Entomology, Iowa State University, Ames, Iowa, USA
1Corresponding author: e-mail address: amytoth@iastate.edu

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Abstract

The family Vespidae provides an excellent group for studying transitional steps associated with the evolution of social behaviour. The family contains species with a wide range of social behaviour, from solitary to highly eusocial, with variation both across and within social subfamilies related to the extent of differentiation of reproductive queen and non-reproductive worker castes. Here, we explore physiological and genomic mechanisms that influence the development of reproductive castes—in both larval and adult stages—and mechanisms that influence non-reproductive division of labour among workers. We synthesize what is known to begin to understand wasp social evolution from a mechanistic perspective. However, since most studies on mechanisms of social organization in wasps have focused on the model genus Polistes, we point out a need for more studies on solitary and advanced eusocial species. We emphasize that by filling these gaps, future comparative studies of these mechanisms will provide key insights into hypotheses underlying the evolution of sociality—such as solitary ground plan, novel genes, and genetic toolkit hypotheses.

1. INTRODUCTION
1.1 Why study physiology and genomics of Vespidae?

Mechanisms that influence social behaviour have been studied most extensively in other hymenopteran families, especially the bees (in the family Apidae) and ants (family Formicidae). However, if the goal is to glean evolutionary insights, the family Vespidae is an excellent study system due to the presence of solitary through highly social species (Fig. 1). By understanding the mechanisms, such as nutritional and reproductive physiology, hormones, and genes, that regulate social behaviour across species with varying degrees of sociality, we can gain valuable insights into how eusociality may have evolved. Mechanistic hypotheses developed in wasps (West-Eberhard, 1996) have been fruitfully used to generate new, empirically testable hypotheses about social evolution (Johnson and Linksvayer, 2010; Linksvayer and Wade, 2005). Studies of wasps, paired with those of other independently evolved social lineages such as bees and ants, provide an informative ‘natural experiment’ to examine whether convergently evolved phenotypes are associated with the same or different physiological and genetic mechanisms.
Within the Vespidae, a complete spectrum of solitary to primitively and advanced eusocial species is represented within a single monophyletic lineage, making it one of the best systems for studying the evolution of sociality (see Table 1 for descriptions of levels of sociality). Some phylogenies of the Vespidae, based on morphological and behavioural traits, indicate a single origin of sociality in the family (reviewed by Carpenter, 1991). A different study, based solely on molecular data, suggests two independent origins (Hines et al., 2007), although this interpretation has been challenged (Pickett and Carpenter, 2010). More data are needed to resolve these relationships, but if the two-origin hypothesis is correct, then the transition to eusociality can be studied in two lineages within the family. In addition, there is evidence that morphological castes have been gained and lost multiple times within the vespids (Noll and Wenzel, 2008; Noll et al., 2004). Thus, the vespids provide unparalleled opportunities to engage in comparative, phylogenetically controlled analyses of the mechanisms underlying transitions in social evolution.

Vespid wasps have been important models for studying the evolution of cooperation and eusociality. There has been much emphasis on studies of the model genus Polistes, which exhibits an intermediate form of social

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**Figure 1** Social characteristics and available genomic information for the six subfamilies (and most commonly studied genera) of vespid wasps. (p), genus in photo image; n/a, not applicable (because solitary); E, some EST sequences; T, transcriptome; G, genome. References for genomic resources: 1 (Hoffman and Goodisman, 2007), 2 (Sumner et al., 2007), 3 (A.L. Toth, unpublished data), 4 (Ferreira et al., 2013), 5 (Toth et al., 2007), 6 (Berens et al., 2015), 7 (A.L Toth, unpublished data).
behaviour referred to as primitive eusociality (Table 1) that involves a unique blend of both cooperation and conflict. This has led to a wealth of studies elucidating both the importance of inclusive fitness (Field et al., 1998; Klahn, 1979; Reeve, 1991; Ross and Gamboa, 1981; Seppä et al., 2002; Strassmann, 1981), as well as direct benefits to the evolution of cooperation (Queller et al., 2000; Reeve, 1991; Zanette and Field, 2008). However, beyond Polistes, there is much more to be learned about the evolution of sociality by studying other vespid wasps, and in particular via a deeper understanding of the physiological and genomic mechanisms of sociality in this group of insects.

Although there have been numerous studies on the physiological and genomic basis of social organization in primitively eusocial species in the genus Polistes (reviewed in Jandt et al., 2014), relatively few of these studies have utilized mechanistic data for an integrative understanding of the

<table>
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<td>‘Nest sharing’</td>
<td>Multiple reproductive females on the same nest, no evidence of sterile workers</td>
<td>Eumeninae</td>
</tr>
<tr>
<td>Facultatively eusocial</td>
<td>Reproductive castes sometimes present (flexible within a species)</td>
<td>Stenogastrinae</td>
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<td>Primitively eusocial</td>
<td>Reproductive castes always present but caste switching can occur, no morphological differences between castes</td>
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</tr>
<tr>
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<td>Reproductive castes always present, morphological differences between castes, nests founded by single female</td>
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<td>Reproductive castes always present, morphological differences between castes, nests founded by swarms</td>
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*Secondary loss in some species.
evolution of sociality. Fewer still have considered the roles of physiological and genomic mechanisms over the course of social evolution by comparing taxa at different levels of sociality (Table 1).

1.2 The purpose of this review

Here, we summarize what is known about the physiological and genomic basis of sociality throughout the family Vespidae. Specifically, we explore the mechanisms that influence reproductive division of labour—in both the larval and adult stages—as well as non-reproductive division of labour among workers. For each, we summarize what is known for each of the major subfamilies. Upon reviewing what is known on these factors, we highlight the imbalance in the amount of information available for certain species relative to others. Finally, we synthesize this information in light of some of the major mechanistic hypotheses for the evolution of sociality and point out gaps in our knowledge, along with exciting opportunities for future research in which we suggest that comparative studies within Vespidae can provide new insights.

1.3 The major groups of vespids and their social behaviour

There are six recognized subfamilies of vespid wasps: Eumeninae, Euparagiinae, Masarinae, Stenogastrinae, Polistinae, and Vespinae (Fig. 1). Because of a nearly complete lack of information on mechanisms of behaviour in two of these (Euparagiinae and Masarinae), our review will focus on the remaining four subfamilies. Throughout the review, we will cover each of these four subfamilies, roughly in order of increasing social complexity (Eumeninae–Stenogastrinae–Vespinae–Polistinae, Table 1). For the purpose of this review, we provide a general overview of the biology of each of these subfamilies. Because this review is not intended to be a complete description of the social biology of wasps, we have necessarily omitted some details about the biology of each family, including references to species for which there are valuable behavioural data. Instead, we narrow our focus to species and genera for which there is available physiological and genetic data.

Among the solitary subfamilies, the majority of studies have been conducted on species in the subfamily Eumeninae (i.e. potter wasps and mason wasps). Among the eumenines, there are some species with well-developed maternal care that includes prolonged brood feeding by mother wasps (also called progressive provisioning)—an important precursor to the evolution of sociality (e.g. Zethus, Eudynerus, and Ancistrocerus, West-Eberhard,
There are also examples of eumenines with even more developed social behaviour, involving nest sharing by multiple reproductive females (e.g. *Zethus* and *Xenorhynchium*, West-Eberhard, 1987a,b) and even overlapping generations (e.g. *Montezumia*, West-Eberhard, 2005). Moreover, although they are solitary, *A. antelope* show a high degree of relatedness among nests within a population (indicative of high levels of inbreeding) (Chapman and Stewart, 1996). These data suggest that high relatedness could have been a pre-condition for the evolution of sociality in vespids. Here, we review the relatively scant literature on physiological factors in these genera that correlate with either reproductive or maternal care behaviour and focus on these factors because of their importance to understanding early stages of social evolution.

The distribution of Stenogastrinae (i.e. hover wasps) is limited to rainforests of the Indo-Pacific region (Turillazzi, 1991). Stenogastrines are considered facultatively eusocial in that female offspring may remain on the nest and care for sisters, and reproductive females may choose to cooperate and found nests together (Turillazzi, 1991). However, all females from a given nest, at any time of the year, can leave the nest, mate, and start a new colony. Colony sizes are very small, on the order of 1–5 individuals in *Parischnogaster nigricans serrei*, for example (Turillazzi, 1991). Among hover wasps, there are species that build nests out of mud or clay (similar to Eumeninae, but see Hermes et al., 2013) and other species that build nests out of plant fibres (similar to Polistinae and Vespinae). In fact, the genus *Liostenogaster* comprises species that do both. However, unlike characteristic polistine and vespine nests, stenogastrines do not construct a petiole from which their cells are built. Instead, the cells are attached directly to the substrate, occasionally scattered or arranged in rows (Turillazzi, 2013). Well-studied genera include *Liostenogaster* and *Parischnogaster* (Turillazzi, 2013).

The subfamily Vespinae (i.e. yellowjackets and hornets) is comprised primarily of social species, most of which are highly eusocial with moderate to large colony sizes (e.g. colonies can produce 50–2000 workers in *V. germanica*, Malham et al., 1991). Colonies generally live in temperate regions with an annual life cycle and are founded by a solitary queen (Matsuura and Yamane, 1990). Notably, there are some exceptions of perennial colonies, multi-queen species, or species with a secondary loss of sociality in which females lay eggs in the nests of other social species (called ‘social parasites’, Greene, 1991; Matsuura and Yamane, 1990). Genetic relatedness within colonies is generally low due to multiple mating by queens; this genetic diversity appears to provide fitness benefits to the colony (Goodisman et al., 2007). Queens and workers have distinct morphologies (called ‘morphological
castes), and there is an age-related worker division of labour. Well-studied genera include: *Vespula*, *Dolichovespula*, and *Vespa* (Greene, 1991; Matsuura and Yamane, 1990).

Finally, the social subfamily Polistineae (i.e. paper wasps) can be further subdivided into two major groups: the independent-founding species (primitively eusocial, including some social parasites) and the swarm-founding species (in the tribe Epiponini, advanced eusocial, see Table 1). Within the primitively eusocial species, queen and worker castes are flexible (i.e. instead of morphological differences, queens and workers are behaviourally/physiologically different), and the primary reproductive is established through a dominance hierarchy (reviewed in Jandt et al., 2014). Colony sizes are small to intermediate (e.g. from 10 to 150 individuals in *Polistes dominula*, Hunt, 2007). Well-studied genera include *Polistes*, *Mischocyttarus*, and *Ropalidia*. In contrast, in the advanced eusocial Epiponini, colonies may be very large (up to 1 million individuals in *Agelaia vicina*, Hunt, 2007; Zucchi et al., 1995), workers specialize on different tasks, and there is a division of nest-building behaviour among workers. Morphological differences between castes (i.e. size dimorphic queens and workers) have been gained and lost within this tribe. Well-studied genera include *Polybia*, *Apoica*, and *Agelaia* (Jeanne, 1991).

2. LARVAL DEVELOPMENT AND ADULT REPRODUCTIVE STATE

Insect larvae undergo large genomic and physiological changes throughout development. Which genes are expressed, and the timing of expression throughout larval development can have significant repercussions on the reproductive physiology of the emerging adult (Pereboom et al., 2005). Here, we explore how physiological and genomic changes throughout larval development, many of which are dependent upon social interactions with adults or differential nutrition, influence reproductive state of adults, with particular attention to queen and worker reproductive caste differences in social species.

2.1 Eumeninae

Although there are no reproductive castes in solitary eumenine wasps, it can be informative to examine the influences of nutrition and hormones during larval development on adult phenotypes, especially in females. This is because comparing the ways in which solitary and social wasps respond to the nutritional and hormonal environment can provide insights into
the ancestral physiological state of female wasps during development. In *Euodynerus foraminatus* and *Ancistrocerus adiabatus*, there appear to be nutrition-dependent effects on fertility in females. Fertilized (female) eggs are provisioned with more food than unfertilized male eggs (Cowan, 1981), which leads to disproportionately larger females (Cowan, 1983), and in *E. foraminatus*, the largest females tend to develop larger ovaries (Tibbetts et al., 2013). In another eumenine species (*Pachodynerus nasidens*), female eggs are larger than male eggs (Cowan, 1983).

Nearly all of the work done on hormones in vespids has focused on juvenile hormone (JH). JH is a terpenoid hormone with many roles in insects, one of the most important being that it is a main gonadotropic hormone in females. Typically, higher JH in larvae and/or adults results in more ovarioles and higher ovary activation (the chapter ‘The Physiology and Genomics of Social Transitions in Aphids’ by Abbot, this volume; the chapter ‘Bumble Bee Sociobiology: The Physiological and Genomic Bases of Bumble Bee Social Behaviour’ by Amsalem et al., this volume; the chapter ‘Old Threads Make New Tapestry—Rewiring of Signalling Pathways Underlies Caste Phenotypic Plasticity in the Honey Bee, *Apis mellifera* L.’ by Hartfelder et al., this volume; the chapter ‘Juvenile Hormone: A Central Regulator of Termites’ Caste Polyphenism’ by Korb, this volume). In eumenine wasps, as is well known in many solitary insects, levels of JH are also correlated with ovary development. When treated with methoprene (a JH analog), *E. foraminatus* females develop larger ovaries (Tibbetts et al., 2013).

On the level of protein expression, in *Monobia quadridens*, storage proteins such as hexamerin increase in expression through female development, with a jump in expression as larvae transition from their final instar to prepupal larvae to pupae (Hunt et al., 2003). Moreover, hexamerin levels vary among newly emerged adults—small females show little to no expression whereas large females show much higher expression. Thus, overall in eumenine females, there is a typical solitary insect relationship between higher nutrition, higher levels of JH, increased storage protein expression, and higher fertility.

### 2.2 Stenogastrinae

Although there have been no direct studies of larval physiology in stenogastrines, the scant evidence available suggests there is no effect of larval nutrition on adult reproductive state and behaviour. Upon eclosion, *Liostenogaster flavolineata* adult females are totipotent regarding their
reproductive potential—all females have the potential to leave the nest, mate, and found a new colony (Field and Foster, 1999). Further, there is no evidence that body size predicts whether females will leave to start their own nest or stay and help at the natal nest (i.e. larger females are not more likely to engage in nest-founding behaviour, Field et al., 1999).

2.3 Vespinae

In *Vespula* yellowjackets, there is a clear relationship between the size of female larvae and their adult morphological caste. Larvae reared in large nest cells, produced at the end of the colony cycle, are fed disproportionately more than larvae reared in ‘worker-cells’ (Schmidt et al., 2012), resulting in larger adult size and individuals that typically become ‘gynes’ or future reproductive queens. Larger larval size may be related to JH as well. Gyne larvae show delayed late instar moults, and it has been hypothesized that an increase in JH at a critical developmental period may be responsible (Greene, 1991; Nijhout and Wheeler, 1982), but we currently lack empirical tests of this hypothesis.

With respect to gene expression, an expressed sequence tag (EST)-based study in *Vespula squamosa* examined expression patterns of several hundred genes throughout larval development (Hoffman and Goodisman, 2007). The results indicate that gene expression patterns are quite similar in young queen and worker larvae, but diverge more as larvae develop. For example, later in development, queen larvae show higher expression of genes related to metabolic processes. Specific hexameric storage proteins show different patterns of expression depending on caste: e.g., VSQ019 and VSQ233 are up-regulated in queen-destined larvae, whereas VSQ232 and VSQ292 (both hexamerin 70b-like ESTs) are up-regulated in worker-destined larvae (Hoffman and Goodisman, 2007). Overall, the available data from Vespinae suggest that, as in Eumeninae, higher larval nourishment is associated with larger size, increased expression of hexameric storage proteins, and a hypothesized connection to higher JH.

2.4 Polistinae

In primitively eusocial polistines, there is also a connection between higher female larval nourishment and increased adult reproduction. In *Ropalidia marginata*, better fed larvae often become reproductively mature earlier (Gadagkar, 2009). In *Polistes* larvae, there is a well-documented connection between larval feeding rate (which is highly correlated with the stage of
colony development, being lower in early development), and the adult caste fate of these larvae. Gyne-destined larvae experience a higher adult:larva ratio, higher feeding rates (Hunt, 2007), and have significantly higher lipid stores than worker-destined larvae (Fig. 2; Jandt et al., in review). Experimental nourishment deprivation of *P. metricus* larvae led to some worker-like changes in ovarian physiology and development time (Judd et al., 2015).

There is less work on the connection between larval nutrition and caste in the advanced eusocial epiponines. Observations of feeding rate suggest that morphological differences between castes in *Polybia sericea* are also the result of differential feeding (Desuó’ et al., 2011). Although more studies are needed on epiponines, including species with more pronounced morphological caste differences than seen in *Polybia*, there is no reason to expect differential larval nutrition is not also, at least partially, responsible for caste differences.

With respect to gene expression, we again see connections between nourishment level and expression of hexameric storage proteins in primitively eusocial polistines. In *P. metricus*, gyne-destined larvae exhibit higher levels of storage proteins, including hexameric storage proteins compared to worker-destined larvae (Hunt et al., 2007, 2010). Like Vespinae, storage

![Figure 2](image-url)  
*Figure 2* A summary of some of the physiological differences (juvenile hormone (JH), ovary, and lipid) in the development of queen and worker caste in vespid wasps (most data based off of studies with *Polistes*). Larvae referred to as ‘gyne-destined’ represent those that are likely to develop into future reproductive queens. *n/a* refers to instances in which data are not available. JH shows a dual function among adults, in a condition-dependent manner: it affects reproductive dominance among better-nourished foundresses and foraging division of labour among more poorly nourished workers.
proteins are synthesized towards the end of larval growth, and gradually diminish throughout pupation (Hunt et al., 2003). However, RNA-interference-based knockdown of expression levels of hexamerin 2 had no influence on the development of worker/gyne phenotype (Hunt et al., 2011). Instead, hexamerin 2 may influence larval development time and ovary development (Hunt et al., 2007, 2011). In P. fuscatus, gyne-destined larvae show higher expression of heat-shock protein 90α, cytochrome P450, inositol oxygenase (an oxidoreductase), and long-wave opsin (Jandt et al., in review). Large-scale genomic analyses of gene expression using RNA-sequencing have shown that many more genes beyond hexamerins are differentially expressed in gyne- and worker-destined larvae, and in response to differential nourishment. Berens et al. (2015) identified nearly 800 transcripts that differed in expression between gyne-destined and worker-destined P. metricus larvae, many of which related to oxidation-reduction processes, and lipid and carbohydrate metabolism pathways. In addition, experimental manipulations of nourishment level resulted in shifts in the expression of some caste-related genes (Berens et al., in review). These data implicate nutritional physiology and nutrition-related pathways as important for larval caste bias in Polistes. However, these data also showed that many caste-related genes are not responsive to nutritional state, suggesting other non-nutritional factors, including social environmental inputs such as vibrational communication (Suryanarayanan et al., 2011) affect caste differentiation in Polistes larvae.

3. ADULT REPRODUCTIVE STATE AND CASTE

As adults, female vespids vary in reproductive success and behaviour. This variation can result from both environmental inputs during larval development, but also from the abiotic and social environment surrounding them as adults. Below, we review what is known about the mechanisms underlying variation in reproductive success (in solitary species and among co-foundresses) or queen–worker caste differences (in eusocial species only). Note that in primitively eusocial vespids, there are also large gradients in female reproductive success related to dominance status within a social hierarchy, both within the queen and worker castes. This subject has received considerable attention in Polistes, and will not be covered in detail in this review; for more information, we refer the reader to a recent review that describes physiological, exocrine, and genomic mechanisms of reproductive dominance in this model genus (Jandt et al., 2014).
3.1 Eumeninae

As mentioned above, wasps in the subfamily Eumeninae do not produce queen and worker castes, but there are differences in reproductive potential among adults. Regardless of body size, adult females of *E. foraminatus* that consume prey produce eggs that are more than twice the size of those produced by females where prey is withheld (Chilcutt and Cowan, 1992). There are also reproduction-related differences among females in terms of their aggressive behaviour. *Symmorphus cristatus* females exhibit aggressive territorial behaviour over nesting sites (Sears et al., 2001). However, there are no studies that explore the physiological or genetic mechanisms of this aggressive behaviour in solitary vespids.

Despite lacking castes, there are observations from eumeninae that suggest cycles of reproductive and non-reproductive activity. *Zethus miniatus* females, after laying an egg and while the next egg matures, engage in behaviours more typical of ‘workers’—e.g., nest cell building, nest defence (West-Eberhard, 1987a). In the group nesting *Montezumia cortesioides*, females alternate between brood care and aggressive competition for egg-laying opportunities (West-Eberhard, 2005). The possible existence of an ovarian cycle, while not well-documented in eumenines, has formed the basis of some important ideas about the evolution of sociality from maternal behaviour (i.e. the Ovarian Ground Plan, West-Eberhard, 1996, discussed below). Such transitions between a queen-like and a worker-like phase are not unlike those observed in the clonal ant *Cerapachys biroi*. Genomic analyses of *C. biroi* show that shifts between reproductive and foraging phases are accompanied by changes in the expression of genes known to be related to reproduction and foraging in honey bees (e.g. *vitellogenin*, *foraging*, and *malvolio*, Oxley et al., 2014). For eumenines, it will be valuable to focus future studies on possible phasic transitions between queen-like to worker-like behaviour. Specifically, we suggest evolutionary insights can be gained by investigating (a) whether phasic shifts are indeed common in eumenines and whether they are accompanied by physiological changes and (b) whether such phasic shifts are accompanied by changes in the expression of genes related to caste differentiation in social wasps.

3.2 Stenogastrinae

Stenogastrines can divide reproductive behaviour in two ways: between co-foundresses and between queens and daughters that remain at the nest. In *L. flavolineata*, there is no evidence that non-reproductive individuals
are physiologically constrained from developing viable ovaries (Field and Foster, 1999). Among those individuals that remain at the nest, the reproductive dominance hierarchy is linear and age-based (Bridge and Field, 2007). In Parischnogaster nigricans, ovaries develop in females as they age, though some develop faster than others (Turillazzi, 1991). Moreover, in both P. nigricans and L. vechti, mated females tend to have better developed ovaries than unmated females, although it is unknown whether ovarian development increases the odds of mating, or if mating induces ovarian development (Turillazzi, 1985, 1991). Nothing is known about genetic correlates of adult caste; all that is currently known related to stenogastrine genetics is that in Parischnogaster alternata, colonies are comprised of related individuals (Bolton et al., 2006).

### 3.3 Vespinae

Vespine queens are allometrically morphologically different from workers. Before founding a new colony, queens undergo a period of hibernation, during which time the high fat stores accumulated pre-hibernation can be depleted by 30–40% (Matsuura and Yamane, 1990). The single reproductive queen in vespine colonies is the only individual in the colony that can mate and lay diploid (female) eggs. Workers may compete for reproductive opportunities, develop ovaries towards the end of the season, and lay unfertilized (male) eggs, but these are often eaten or ‘policed’ by other workers (Bonckaert et al., 2012).

In Vespula maculifrons, there is no evidence that morphological caste differences between queens and workers are based on hereditary genetic differences (Kovacs et al., 2010). Regarding differential gene expression, there has been a single study that has investigated caste-related expression in adults of three vespine species using an EST-based approach (Hunt and Goodisman, 2010). Across V. maculifrons, V. squamosa, and Dolichovespula maculata, caste-related gene expression levels between species vary widely. Out of seven genes that had caste-related gene expression in honeybees, two had conserved caste-specific patterns across the three vespines (Hunt and Goodisman, 2010). In general, this study found that caste-biased gene expression profiles in vespine wasps seem to have undergone greater rates of evolutionary change than sex-biased profiles. Thus, these data suggest that only a small subset of caste-related genes show conservation across species, providing limited support for the idea that a shared ‘genetic toolkit’ underlies caste differences in social insects (Hunt and Goodisman, 2010). The small
amount of overlap across vespine species is not surprising; recent studies comparing wasps, ants, and bees have also found limited overlap in the expression of specific caste-related genes across species. Instead, there is similarity in which pathways and gene functions are related to caste differences across species (Berens et al., 2015; Toth et al., 2014).

### 3.4 Polistinae

In the tropical, primitively eusocial genera *Ropalidia* and *Mischocyttarus*, females that eclose at any time of the year have a chance of becoming queen. In tropical wasps, where habitats are less seasonal, wasps can initiate colonies at any time of the year, and reproductive physiology can be flexible, changing in response to social conditions. Still, in *R. marginata*, older females tend to have more well-developed ovaries, and wasps fed well are more likely to become egg layers than those that are not (Gadagkar, 2009). JH likely plays a role, as topical application of JH on *R. marginata* adults accelerates ovarian development (Agrahari and Gadagkar, 2003). In *M. mastigophorus*, there is no evidence that body size correlates with reproductive potential, but those individuals with higher fat stores tend to have better developed ovaries (Markiewicz and O’Donnell, 2001). Moreover, there are differences in brain neuroanatomy between queens and workers: the mushroom bodies (the brain region associated with sensory integration and learning) are larger in queens compared to young workers, but there is no difference between the brains of queens and older workers (which also have high reproductive potential, O’Donnell, 2006).

Temperate and tropical species of *Polistes* also possess more subtle, behavioural caste differences, and lack morphologically different queen and worker castes. The ultimate caste fate of females remains flexible throughout much of adult life and is sensitive to changes in the social environment (e.g. dominance hierarchy) as well as individual physiological state. In the temperate-zone *P. metricus*, the queen (mother) has the greatest ovarian development of any colony member. On average, queens have twice as much abdominal lipid compared to their daughter workers, but significantly lower lipids than their daughter gynes (females that will mate, over-winter, and found colonies the following spring). Furthermore, although they are unmated and rarely lay eggs, adult workers actually tend to have better developed ovaries than gynes (which do not activate their ovaries until nest founding the following spring). Instead, gynes have huge lipid stores compared to all other females (Fig. 2; Toth et al., 2009). Among queens and
workers, there is a positive correlation between lipid stores and ovary development—those with greater lipid stores also tend to develop larger and better developed ovaries (Toth et al., 2009).

JH is directly linked to fertility in adult Polistes (Fig. 2; Röseler, 1991; Röseler et al., 1984; Tibbetts et al., 2011a). Moreover, experimentally increasing JH among foundresses leads to an increase in fertility as well (Tibbetts and Izzo, 2009; Tibbetts and Sheehan, 2012). Biogenic amines, such as dopamine and serotonin, have also been shown to be positively correlated with fertility in P. chinensis (Sasaki et al., 2009). Recently, Toth et al. (2014) found a link in the expression of genes with functions related to JH and biogenic amine synthesis and reproductive dominance status in queen and worker castes in P. metricus.

A number of studies have investigated differential gene expression in queen and worker Polistes wasps. In Polistes canadensis, several genes associated with metabolism exhibited twofold higher expression in whole bodies of queens compared to workers, whereas only two genes (those associated with heat shock and imaginal disk development) were up-regulated in workers (Sumner et al., 2006). Several genes that are well known to be associated with reproduction and caste differences in other species (reviewed in Smith et al., 2008) including vitellogenin, major royal jelly protein, and hexamerin 2 also had higher expression in P. canadensis queens compared to workers (Sumner et al., 2006).

In P. metricus adult female brains and surrounding fat body, increased expression of vitellogenin and decreased expression of insulin-like peptide 2 are associated with higher reproductive potential (Toth et al., 2009). In fact, queens have twofold higher expression in vitellogenin than workers, and also show higher expression in genes related to oxidation–reduction (Toth et al., 2014). Queens also exhibit higher ovary gene expression for genes associated with for protein folding, mitotic spindle organization, proteolysis, and metabolism (Toth et al., 2014). These data highlight the inter-relationship between genes and pathways related to nutrition, metabolism, and reproduction and their likely roles in caste differences across species (Smith et al., 2008).

Unlike independent-founding Polistes spp., swarm-founding Polybia colonies can have multiple queens. A surprising and unique feature of some epiponine species is that the presence of multiple queens is associated with diminished morphological and physiological differences between queen and worker castes. In Polybia ignobilis, although queens are larger than workers, morphological differences become less apparent as the colony matures or
when there are more queens (Desuó et al., 2011). However, this pattern is the opposite in other species. For example, in Protonectarina sylveirae, queens are smaller in the early colony stages before new workers emerge compared to colonies in later, male-producing stages (Shima et al., 2003). In Po. micans colonies, queens have higher JH titer only when the colony is in the founding phase or if it only has one queen. Otherwise, in established, multi-queen colonies, queens have higher ovarian development and higher ecdysteroid content in the ovaries, but not necessarily differences in JH or ecdysteroids in the haemolymph relative to workers (Kelstrup et al., 2014). Finally, in the temperate-zone Parapolybia indica, young females (though not the youngest) are more likely to replace the lost queens (Suzuki, 2003).

Across 12 genera of polistine wasps, including both independent and swarm-founding species, workers tend to invest more than queens in mushroom body brain tissue responsible for visual processing relative to antennal processing (O’Donnell et al., 2014). Queens, however, tend to have significantly larger mushroom body lip and collar regions than workers (the areas associated with learning and cognition), and this phenomenon is more pronounced among independent-founding species (O’Donnell et al., 2014).

4. ADULT WORKER DIVISION OF LABOUR

In addition to dividing reproductive tasks between queens and workers, in eusocial colonies, workers also divide colony maintenance tasks. One of the major divisions of labour is between foragers and non-foragers, which may spend more time on the nest also engaged in brood-tending or nest-building tasks. In many species, worker division of labour is age-related, called ‘temporal polyethism’. In contrast, as they are solitary, by definition eumenine wasps do not have workers. Therefore, we focus here on the physiological and genomic mechanisms of worker behaviour only in sub-families that divide non-reproductive tasks.

4.1 Stenogastrinae

Among stenogastrines, the probability that an individual forages is age-related and correlates with rank in the dominance hierarchy. For example, in L. flavolineata, the amount of time individuals spend foraging directly correlates with hierarchical rank and ovarian development (Turillazzi, 1991 and references therein). In Parischnogaster gracilipes and P. alternata, middle-aged individuals spend the most time foraging, whereas the oldest are egg layers, and the youngest are less capable of foraging (Turillazzi, 1991).
4.2 Vespinae

*Vespula germanica* workers exhibit an age-based division of labour such that younger individuals are more likely to engage in in-nest tasks, whereas older individuals are more likely to engage in foraging tasks (Hurd et al., 2007). On average, each individual tends to specialize on one type of task each day, but the specific mechanism that influences this specialization or regulates the transitions between tasks is yet unknown (Hurd et al., 2007). In a related species, *V. koreensis*, pulp foraging (a less costly task) is performed by younger foragers, whereas nectar and prey foraging (a task that requires more time and effort for heavier loads) is conducted by older foragers (Kim et al., 2012).

In *Vespula vulgaris*, reduced expression of a cGMP-dependent protein kinase (*Vvforaging*) is correlated with an increase in foraging behaviour among workers (Tobback et al., 2008). This is similar to what is observed in *Pogonomyrmex barbatus* (Ingram et al., 2005), but is the opposite direction of expression observed in *Apis mellifera*, in which expression is higher in foragers (Ben-Shahar et al., 2002). These results suggest a conservation of foraging gene function in division of labour across species, but a difference in the specific patterns of gene activity and brain function that regulate division of labour.

4.3 Polistinae

In *Ropalidia marginata*, there is also evidence of age-based polyethism. Workers transition through on-nest tasks (feeding larvae) to nest building tasks to foraging tasks (collecting nesting material or food, Gadagkar, 2009). However, although JH influences ovarian development, it does not influence the rate at which individuals transition through these tasks—the age that individuals begin to tend brood, build, or forage remains constant regardless of JH application (Agrahari and Gadagkar, 2003).

*Mischocyttarus mastigophorus* workers divide foraging tasks in accordance with dominance rank—socially dominant individuals spend more time foraging for wood pulp, subordinate individuals spent more time foraging for nectar and insect prey (O’Donnell, 1998). Lipid content is also related to foraging rate—those individuals with smaller fat bodies spend more time off the nest engaged in foraging (Markiewicz and O’Donnell, 2001). In addition to nutritional differences, older individuals (typically foragers) have larger mushroom body calyces, as do individuals that are more aggressive and socially dominant (Molina and O’Donnell, 2008).
In *Polistes*, there is also a loose worker temporal polyethism (Shorter and Tibbetts, 2009). In addition, dominance rank and usually also ovarian development are associated with worker task performance. Subordinates may divide non-reproductive tasks based on their relative rank in the hierarchy, the most dominant individuals engaging in the less risky behaviours (Jandt et al., 2014). *P. dominula* alpha subordinates, for example, are more likely to construct the nest, whereas beta subordinates are more likely to forage (Pratte, 1989). *P. metricus* and *P. ferreri* co-foundresses even divide foraging preferences, and these preferences correlate with dominance rank: dominant wasps are more likely to collect vegetable fibre, whereas subordinates will collect nectar or prey (De Souza et al., 2008, Gamboa et al., 1978). Similarly, in *Polistes instabilis*, ovary development and worker reproductive competition correlate negatively with foraging behaviour (Molina and O’Donnell, 2009).

Among co-foundresses, JH is directly linked to reproductive dominance rank (Tibbetts et al., 2011a). Among workers, however, the role of JH may be condition dependent—in larger, fatter workers, JH increases the fertility and dominance of workers, but in smaller workers, JH increases the probability of foraging (Fig. 2; Giray et al., 2005; Shorter and Tibbetts, 2009; Tibbetts and Izzo, 2009; Tibbetts et al., 2011b).

In *P. metricus*, foraging workers tend to have lower fat stores than non-foraging workers (Fig. 2; Toth et al., 2009). Moreover, individuals that are starved as adults not only have lower abdominal lipid, but exhibit an increase in foraging rate (Daugherty et al., 2011). This difference in foraging is also associated with numerous differences in brain gene expression, including several nutrition-related genes in the insulin pathway, and genes coding for oxidoreductases, and fatty acid-binding proteins (Daugherty et al., 2011).

A number of studies have explored the basis of worker division of labour in *Polybia* wasps. Workers can engage in one of three task groups: in-nest work (nest construction, brood care, etc.), on-nest work (receive materials from returning foragers), and foraging (Jeanne et al., 1988). Like other polistines, workers transition from in-nest to foraging tasks as they age, and this transition is correlated with a number of physiological traits: lipid content gradually decreases (O’Donnell and Jeanne, 1995b) and ovarian status decreases (O’Donnell, 2001). In *P. occidentalis*, smaller individuals transition from in-nest to out-of nest tasks more slowly, and are more likely to achieve dominant social status (O’Donnell and Jeanne, 1995a). Topical application of a JH analog (methoprene) on day-old *P. occidentalis* workers
leads to accelerated temporal polyethism, suggesting that JH facilitates this transition (O’Donnell and Jeanne, 1993). In Polybia wasps, foragers also exhibit a division of labour for a preference of foraged materials: water, pulp, honey, and prey (O’Donnell and Jeanne, 1990). There is evidence that the probability to forage on a specific material may be genetically linked. In Polybia aequatorialis, forager specialization on pulp, water, nectar, or insect prey is associated with specific segments of DNA (based on an analysis of anonymous markers, O’Donnell, 1996).

5. TOWARDS A SYNTHESIS: UNDERSTANDING WASP SOCIAL EVOLUTION FROM A MECHANISTIC PERSPECTIVE

In the data reviewed above, we have simultaneously presented information on physiology and genomics. This was intentional—physiology is shaped by its underlying genomics, and in turn gene expression is responsive to the internal physiological environment. In addition, both physiology and genes can influence group dynamics, and at the same time the nest environment itself can potentially have important influences on organismal physiology and behaviour (Fig. 3). We wish to highlight the importance of considering physiology and genomics, rather than considering these as separate systems. In fact, this type of integrative perspective has a strong tradition of leading to novel ideas and important insights into social evolution. Below, we review some of these ideas, emphasizing the role that Vespidae have played, and as we project, will continue to play, in developing heuristic hypotheses about social evolution.

Paper wasps, especially primitively eusocial species in the genus Polistes, have provided excellent fodder for the development and empirical testing of hypotheses about the proximate and ultimate factors contributing to the evolution of eusociality (Table 2). Here, we highlight several emerging ideas relating to the evolution of eusociality that have been addressed using physiological and genomic data from vespid wasps (Table 2). The first, on the level of hormones, relates to the changing role of JH from gonadotropin to regulator of division of labour. The next three represent mechanistic hypotheses for how wasp castes evolved from solitary physiological and behavioural ‘ground plans’ (reviewed in Toth and Robinson, 2007). The final two ideas deal with the relative contributions of deeply conserved genes or recently evolved (novel) genes.
5.1 Split-function hypothesis

The role of JH is known to have changed radically during insect social evolution. In a wide variety of non-eusocial insects, JH is well known to be a powerful regulator of female reproductive state, and high JH during both pre-adult development and during adulthood are associated with increased ovary size, ovary activation, and egg-laying behaviour (Chapman, 2012). In striking contrast, JH is the major regulator of worker division of labour in adult honey bees and has no apparent association with reproduction (Hartfelder, 2000). Instead, in honey bees, high JH is associated with foraging (the chapter ‘Old Threads Make New Tapestry—Rewiring of Signalling Pathways Underlies Caste Phenotypic Plasticity in the Honey

Figure 3 The dynamic interplay between multiple levels of biological organization, from genes to hormones to physiological systems, can affect social organization in paper wasps. Wasp drawings adapted from Hunt et al. (2011).
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<th>Description</th>
<th>Prediction(s)</th>
<th>Supporting studies</th>
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| Split-function hypothesis         | The ancestral reproductive function of JH became split into a dual function, regulating reproduction in queens and behavioural division of labour among workers | 1: JH regulates reproduction in solitary species  
2: JH regulates both reproduction and worker division of labour in eusocial species  | Giray et al. (2005), Shorter and Tibbetts (2009), and Tibbetts et al. (2013)                                                                |
| Ovarian ground plan hypothesis    | The ancestral phasic shift between egg-laying/egg-development and brood care/foraging evolved into reproductive and non-reproductive castes, respectively | 1: Gene expression patterns related to worker behaviour will be similar to those of maternal individuals  
2: Maternal care related gene expression patterns from solitary species will resemble patterns of workers in eusocial species | Toth et al. (2007)                                                                                                                                |
| Diapause ground plan hypothesis   | The ancestral diapausing phenotype evolved into the gyne phenotype           | 1: Genes related to diapause in solitary species will be related to gyne phenotype in eusocial species                                            | Hunt et al. (2007) and Hunt et al. (2010)                                                                                                  |
| Genetic toolkit hypothesis        | Deeply conserved genes related to solitary behaviour (including maternal behaviour, reproduction, food-searching behaviour, aggression) are involved in the evolution of eusocial behaviour | 1: Similar sets of genes will be differentially expressed in queens and workers from species with different origins of eusociality  
2: Genes associated with behaviour such as maternal behaviour, feeding, aggression in solitary species will be related to worker behaviour, social foraging, and social aggression in eusocial species | Berens et al. (2015), Toth et al. (2014), but see: Hunt and Goodisman (2010), Toth et al. (2010)                                     |
| Novel genes hypothesis            | Novel social phenotypes (e.g. worker caste in social insects) are the result of new genes, most likely the result of rapid protein evolution | 1: Genes that are differentially expressed between castes are likely to be taxonomically restricted genes, or genes with no homology to sequences from other taxa  
2: Genes with caste-biased expression are more likely to show rapid rates of protein evolution | Ferreira et al. (2013)                                                                                                                        |
Bee, Apis mellifera L.’ by Hartfelder et al., this volume; Robinson, 1987) and experimental manipulations of JH levels can cause worker bees to accelerate the age-related shift to foraging behaviour (Sullivan et al., 2000). These observations led to the suggestion that during social evolution, the function of JH shifted from a gonadotropin to a regulator of worker foraging, and that we should be able to find intermediate species in which JH regulates both reproduction and worker behaviour in a condition-dependent manner. Studies on Polistes wasps have provided tests of this hypothesis. JH is well known to affect ovary activation and dominance status in Polistes (Rösel, 1991; Rösel et al., 1984; Tibbetts et al., 2011a), and subsequent work demonstrated that JH can also affect worker division of labour such that JH-treated workers are more aggressive (Giray et al., 2005) and more likely to initiate foraging at a young age (Fig. 2; Shorter and Tibbetts, 2009). In addition, the effects of JH appear to be condition-dependent; JH can apparently activate ovaries in wasps that have high nutritional state, but it is more likely to lead to changes in worker foraging behaviour in wasps that have low nutritional state (Shorter and Tibbetts, 2009). Overall, studies of Polistes support the hypothesis that JH, once freed from constraints on regulating reproduction in the physiological context of non-reproducing workers, was selected to take on new functions in regulating colony-level traits such as foraging division of labour. Subsequently, in some advanced eusocial species such as honey bees, the reproductive function of JH can be completely lost; whether the same is true in vespid wasps remains to be seen, but such evidence would represent a compelling example of convergent evolution of hormonal mechanisms across social insect lineages (Toth and Robinson, 2007; the chapter ‘Old Threads Make New Tapestry—Rewiring of Signalling Pathways Underlies Caste Phenotypic Plasticity in the Honey Bee, Apis mellifera L.’ by Hartfelder et al., this volume).

5.2 Ovarian ground plan hypothesis

Originally proposed by Evans and West-Eberhard (1973), the ovarian ground plan hypothesis (OGPH) posits that different elements or modules of solitary behaviour were uncoupled during evolution to produce distinct castes (West-Eberhard, 1996). For paper wasps, this idea was based on a proposed cycle of ovarian activation (egg laying) alternating with a period of ovarian inhibition, accompanied by foraging for larval provisions and
maternal care of developing larvae. Specifically, the ovarian ground plan idea proposes that these two modules of solitary behaviour, specifically foraging/provisioning and egg laying/egg-development, were separated during evolution and manifest as two distinct groups of females—egg-laying ‘queens’ and non-egg-laying, foraging ‘workers’. Along with these different behavioural modules, the OGPH suggests selection on correlated suites of behavioural, physiological, and gene expression traits produced two distinct female groups, and in this way the first rudimentary castes were born. This idea was supported by observations on solitary wasps such as *Zethus* (West-Eberhard, 1987a), but the objection has been raised that there may not be a clearly defined ovarian/foraging cycle that can be separated into distinct modules (Hunt, 2007). Nonetheless, even without a strict ovarian cycle, the general idea of the OGPH, specifically, that maternal/offspring care and egg-laying elements of solitary behaviour can be separated into distinct castes, has been one of the most influential ideas about how castes evolved in the Hymenoptera and has led to several related hypotheses.

A related idea, termed the maternal heterochrony hypothesis (MHH) focuses on the idea that worker behaviour arose due to a change in the timing of the expression of genes related to maternal care, to occur in females that had not yet mated nor reproduced (Linksvayer and Wade, 2005). Empirical evidence supporting the OGPH and MHH has come from studies on primitively eusocial *P. metricus*, which showed similar patterns of brain gene expression in maternal females (foundresses) and workers, compared to queens and gynes (Toth et al., 2007). A related hypothesis termed the reproductive ground plan hypothesis (also called the ‘forager reproductive ground plan hypothesis’, Oldroyd and Beekman, 2008) posits that regulation of foraging division of labour among workers arose from a further split of solitary gene networks related to reproduction (egg laying and maternal behaviour, Amdam et al., 2004, 2006). Evidence for this idea comes from data on the egg yolk protein *vitellogenin* from honey bees (Amdam et al., 2006), as well as data on the insect gonadotropic hormone JH from both honey bees and ants (Amdam et al., 2004; Dolezal et al., 2012). To date, there have been no direct tests of the forager reproductive ground plan hypothesis in paper wasps.

### 5.3 Diapause ground plan hypothesis

The diapause ground plan hypothesis (DGPH), proposed by Hunt and Amdam (2005), posits that the reproductive caste in paper wasps is related
to the expression of diapause-related behaviour, physiology, and gene expression. In temperate paper wasps, female offspring reared later in the colony cycle show many characteristic traits of insect diapause: they are typically larger, with extremely high fat stores, and inactivated ovaries (Hunt and Amdam, 2005). These represent the gynes (future reproductive queens) that will over-winter in hibernaculae (sheltered areas with clusters of both related and unrelated wasps) and found colonies the following spring. Just as food availability during larval development determines whether individuals of some solitary insect species will develop into adults or enter diapause and develop into adults the following season, a similar process might regulate whether a social wasp larva develops into a non-reproductive or a reproductive adult. For example, *P. metricus* gynes are more well-nourished than workers, and they do enter a quiescent period prior to founding colonies and egg-laying the following season (Toth et al., 2009). According to this idea, the molecular machinery underlying diapause in solitary insects is involved in caste determination in social wasps (Hunt and Amdam, 2005; Hunt et al., 2007). Molecular level support for this notion comes from measurements of storage proteins known to mediate diapause, including hexameric storage proteins (Hunt et al., 2007, 2010). Gyne-destined larvae have higher levels of *hexamerin* mRNAs and protein expression than worker-destined larvae. In addition, development time in gyne-destined larvae is extended as predicted for a diapause phenotype (Hunt et al., 2007). Additional studies are needed, especially on tropical *Polistes* and solitary vespids, to better understand the relevance of the diapause phenotype to social evolution in a phylogenetic context.

Are the OGPH and DGPH mutually exclusive hypotheses? Not necessarily. There can potentially be multiple ground plans selected in concert, simultaneously acting to pull caste phenotypes apart by influencing multiple gene networks and physio-behavioural systems. New data on a genomic scale are beginning to provide some first hints at the complex and multifactorial nature of the genomic regulation of caste differences in wasps.

### 5.4 Genetic toolkit hypothesis

Solitary ground plan hypotheses generally focus on the principle of evolutionary co-option—specifically, that ‘old’ or deeply conserved genes involved in solitary phenotypes are retooled or simply shifted in their expression to regulate social phenotypes. A more general version of this idea has been put forward as the genetic toolkit hypothesis, based on principles from evolutionary developmental biology or ‘evo-devo’ (Toth and Robinson,
This idea suggests that a small set of deeply conserved genes involved in solitary phenotypes were co-opted for the evolution of social traits such as caste-specific physiology, behavioural specialization, and social organization. These genes may have functions including reproduction and diapause, but are not limited to them; other fundamental solitary phenotypes include aggression and food-searching behaviour. This hypothesis predicts that similar genes and/or pathways will be associated with social behaviour across multiple, independently evolved social insect lineages. A series of studies that used *Polistes* wasps as a focal point compared gene expression patterns associated with maternal behaviour (Toth et al., 2007), foraging (Daugherty et al., 2011), reproduction (Toth et al., 2010), aggression (Toth et al., 2014), and caste differences (Berens et al., 2015) across social species. Overall, these studies found evidence for a relatively small, but statistically significant overlap in gene expression patterns associated with similar types of social behaviour across wasps, bees, and ants. In some cases, the overlap across species on the gene level was very small and not significant (Berens et al., 2015; Ferreira et al., 2013; Toth et al., 2014). However, an analysis on the pathway level showed that indeed there was significant overlap across lineages on the level of conserved enzymatic pathways (e.g. glycolysis) and functional types of genes (e.g. genes related to oxidation–reduction activity) associated with caste development across bees, ants, and wasps (Berens et al., 2015). These data provide some supporting evidence for the genetic toolkit hypothesis, but suggest that the toolkit itself is rather loose. Unlike the classic evo–devo cases of *Hox* genes being repeatedly involved in the generation of morphological novelty in development (Carroll, 2008), there are no specific ‘major player genes’ that are always associated with caste evolution in multiple lineages. Instead, data suggest that largely different, lineage-specific genes affect caste development, but that there are certain pathways and gene networks that are repeatedly recruited for caste differences across multiple origins of sociality.

### 5.5 Novel genes hypothesis

As an alternative to the focus on ‘old genes’ with the genetic toolkit hypothesis, an emerging idea focuses instead on ‘new genes’, that is, novel or taxonomically restricted genes that show no homology to known sequences from other species. Instead of novel phenotypes being the result of recycling old genes, the novel genes hypothesis suggests that novel social phenotypes are the result of new genes, most likely the result of rapid protein evolution.
Recent studies on honey bees have suggested that novel, rapidly evolving genes are more likely to be over-expressed in workers in advanced eusocial honey bees (Harpur et al., 2014; Johnson and Tsutsui, 2011). One study on *P. canadensis* also found novel genes to be more likely to be differentially expressed between adult queens and workers (Ferreira et al., 2013). However, a subsequent study on a different species of *Polistes* (*metricus*), did not find any association between novel genes and caste differences in larvae (Berens et al., 2015). A comparative study on ants (Simola et al., 2013) suggests that novel genes abound in every single examined lineage of ants. The ant comparisons suggest that, rather than being related to fundamental caste differences, these novel genes may be more likely to be involved in highly derived, lineage-specific social traits. At this time, it is still not completely clear whether conserved genes or novel genes contribute more to social evolution. Additional studies are needed, especially utilizing solitary and social species in direct comparisons within a monophyletic group, rather than relying on cross-family comparisons.

### 6. GAPS IN OUR UNDERSTANDING AND FUTURE DIRECTIONS

The Vespidae are an excellent, arguably one of the best, monophyletic groups for studying evolutionary transitions associated with eusocial evolution. This family contains representatives that are solitary, subsocial, facultatively social, primitively social, and advanced eusocial. Morphological castes have been gained and lost multiple times (Noll and Wenzel, 2008; Noll et al., 2004). Many vespid species are highly ecologically successful, and even invasive, and can be reared in semi-natural or laboratory settings (Jandt et al., in review) making them attractive model organisms (Jandt et al., 2014). Considerable progress has been made in recent years on understanding the physiological and genomic mechanisms of sociality in vesps. However, we suggest that the biggest and most informative insights are still awaiting us. This is because nearly all research to date has focused on a relatively small number of species, most of which are primitively eusocial, especially *Polistes*. While this genus is extremely informative for understanding some aspects of social evolution, we are in dire need of more studies on additional genera, especially solitary and swarm-founding advanced eusocial species (Table 3). While these species are often more difficult to locate (solitary), work with, and study (including some highly aggressive advanced eusocial species), a deeper knowledge of the mechanisms regulating pre-social and
<table>
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<tr>
<th>Genus</th>
<th>Larval development and adult reproductive state</th>
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n/a, not applicable (solitary).
social behaviour in these groups will be very illuminating. Below, we detail several specific areas that we believe to represent some of the most promising future directions for vespid wasp research.

6.1 Ovarian ground plan hypothesis

The ovarian ground plan and maternal heterochrony hypotheses are based on underlying assumptions about ancestral/conserved roles of genes and hormones related to maternal care and reproduction in solitary species. However, to date, there are no studies that actually address the roles of important reproductive hormones (such as JH) or genes (such as genes with possible roles in maternal behaviour) in a comparative context across both solitary and social species. We suggest that studies on solitary vespids, especially species such as *Zethus* with well-defined maternal care in the form of progressive provisioning, will be critical to a more definitive test of the OGPH and MHH. Under these hypotheses, we would predict that genes with roles in maternal provisioning behaviour in solitary species will have roles in sibling care behaviour in social species.

6.2 Genetic toolkit hypothesis

Thus far, the idea of deeply conserved genes affecting the evolution of novel social traits has been tested using cross-lineage comparisons of, for example, one bee species, one ant species, and one wasp species (Berens et al., 2015). However, a more comprehensive test of the toolkit idea will also encompass multiple species within a lineage. For example, by comparing gene expression patterns associated with maternal behaviour, worker behaviour in primitively social species, and worker behaviour in advanced eusocial species, we can ask to what extent are ancestral mechanisms regulating maternal/sibling care behaviour retained throughout social evolution? Are there more similarities between solitary and primitively eusocial species, with more departure in advanced eusocial species?

6.3 Novel genes hypothesis

Thus far, it has been difficult to assess the importance of novel genes in social evolution, especially in wasps, because of a lack of genomic information. This situation is changing rapidly with the advent of next-generation genomic technologies, and many new insect genomes and transcriptomes, including those of vespids (A.L. Toth, unpublished data), are on the horizon. We suggest that a comprehensive comparison of novel genes, based on full
transcriptome and/or genome sequencing, could be especially informative when including representatives of the major transitional states in social evolution: solitary, incipiently social, primitively eusocial, and advanced eusocial. Using such an approach, it will be possible to identify vespid-specific genes, as well as genus and/or species-specific genes, and assess whether these genes have associations with fundamental eusocial traits (such as caste differences), or whether they are related more to lineage-specific and derived social traits (such as swarm founding).

7. CONCLUSIONS

In summary, a deeper understanding of vespid physiology and genomics will provide not only a template for testing hypotheses on the origins of eusociality, but also on its subsequent elaboration into advanced eusocial traits, including morphological castes, swarm founding, and multiple queens. There is a rich ethological tradition with numerous species of social wasps not discussed in detail in this review, including multiple origins of complex traits such as swarm founding, morphological castes, and the loss of eusociality in social parasites (Jeanne and Hunt, 1992; Matsuura and Yamane, 1990; Noll and Wenzel, 2008; Noll et al., 2004; Smith et al., 2002). Studies of such species could be highly informative for understanding the mechanisms underlying the gain and loss of social traits. Recent technological advances have now made it possible to study non-model species on a genomic scale, and we hope that more attention will be paid on using these techniques to explore new vespid genomes and associated physiological mechanisms. In addition, although we have stressed the importance of nutritional physiology and nutrition-related genes in their influence on both reproductive caste and worker caste differences, it is important to think beyond nutrition—there is still a great deal to be learned about the environmental determinants of individual differences in social insects. We suggest that it will be fruitful in the future to more fully investigate feedback between the social environment (such as chemical and vibrational communication), individual development and physiology, epigenetics, and gene expression (Bengston and Jandt, 2014; Jeanne and Suryanarayanan, 2011; Weiner and Toth, 2012). Finally, although there are numerous mechanistic hypotheses that have been put forward to explain the evolution of sociality in vespid and other social insects, there is a need for a more synthetic approach (Johnson and Linksvayer, 2010) to tie these ideas together across multiple levels of biological organization. We suggest that phylogenetically
controlled comparative studies within the Vespidae can provide the needed empirical and conceptual framework to move towards such a synthesis in the future.

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