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Environmentally responsive reproduction: neuroendocrine signalling and the evolution of eusociality

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Eusociality is a rare but successful life-history strategy that is defined by the reproductive division of labour. In eusocial species, most females forgo their own reproduction to support that of a dominant female or queen. In many eusocial insects, worker reproduction is inhibited via dominance hierarchies or by pheromones produced by the queen and her brood. Here, we consider whether these cues may act as generic 'environmental signals', similar to temperature or nutrition stress, which induce a state of reproductive dormancy in some solitary insects. We review the recent findings regarding the mechanisms of reproductive dormancy in insects and highlight key gaps in our understanding of how environmental cues inhibit reproduction.

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Introduction

Insects, such as most animals, are adapted to cope with adverse environmental conditions, including poor resource availability, decreased day length or falling temperatures. One strategy that insects have evolved to deal with these adverse conditions is dormancy: a state of suppressed development, reproduction or metabolism [1–4].

There are two types of dormancy: quiescence and diapause [1]. Diapause is an adaptive response to an environmental cue (e.g. day length) that is predictive of upcoming unfavourable environmental conditions [1] and involves distinct ecophysiological phases [2]. In contrast, quiescence is considered an immediate or direct response to unfavourable conditions (e.g. deficiency in nutrition) and may, or may not be, adaptive [2]. Throughout this article, we use 'dormancy' as a general term to encompass both quiescence and diapause [1,2]. This general term reflects that some examples of dormancy, particularly in adult *Drosophila melanogaster*, are not easily classified as either quiescence or diapause [1,2,4]. This general term also reflects that there are some similarities in the underlying physiology, mechanisms and phenotypes of both quiescence and diapause [1,2,4,5].

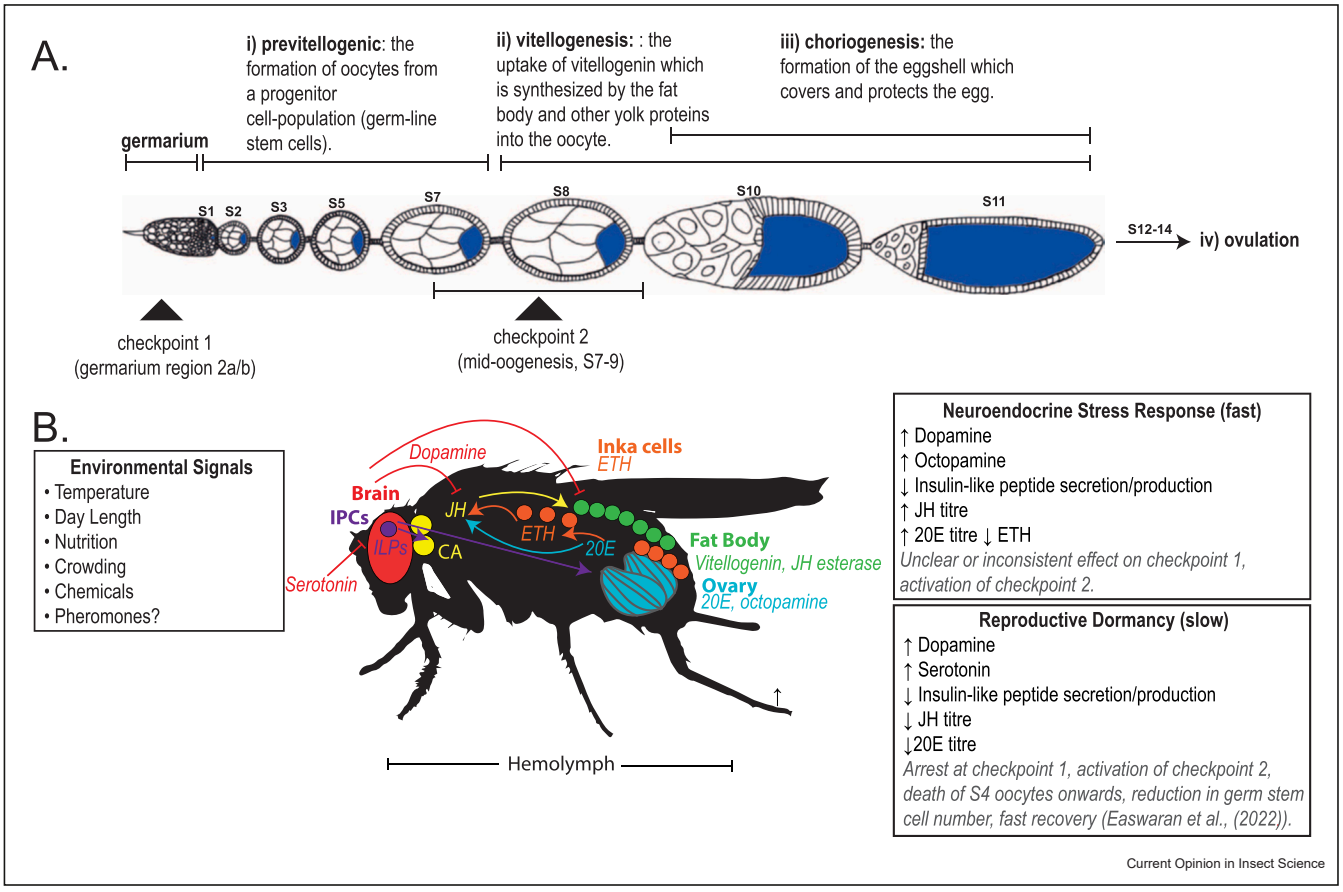
Dormancy can affect different insect-life stages by arresting or slowing the development of the embryo, larvae or pupae [6]. In adults, unfavourable environmental conditions can result in a temporary pause in reproduction, a phenomenon known as reproductive dormancy [3]. Reproductive dormancy allows insects to tailor their reproductive strategy to the prevailing conditions enhancing survivability and long-term fitness [5]. In this article, we focus on reproductive dormancy.

Neuroendocrine control of reproduction in female insects

Reproduction in female insects, such as in other animals, is under the exquisite control of the neuroendocrine system. The core neuroendocrine system in insects consists of neuropeptides, juvenile hormones (JH) and 20-hydroxyecdysone (20E) (reviewed in [7]). In *D. melanogaster*, ecdysis-triggering hormone (ETH) is also a key component of the neuroendocrine system regulating oogenesis and female fecundity [8••]. These hormones, together with biogenic amines and insulin signalling [7,9,10], act to regulate oogenesis [11] (Figure 1). However, the exact roles of these components in oogenesis vary widely among insect orders and even between closely related species. For example, JH acts as a gonadotrophin in the bumblebee (*Bombus terrestris*) [12] but not in the honeybee *(Apis mellifera*) [13].

In insects, biogenic amines (predominantly dopamine, serotonin and octopamine) function as neurotransmitters and as neurohormones that are transported within the haemolymph and act on peripheral tissues, including the

Figure 1



Oogenesis and neuroendocrine control of reproduction and reproductive dormancy in *D. melanogaster*. **(a)** Stages of oogenesis in *D. melanogaster* [11] and the ovarian checkpoints that result in cell death in response to environmental cues [30,31]. **(b)** Major neuroendocrine signalling systems that are known to respond to environmental signals and mediate reproduction in *D. melanogaster* [3,8••,32•,39••,40,46].

ovary and fat body [14,15]. These biogenic amines directly regulate oogenesis and oviposition in a range of insects (e.g. [10,16–18]) and also indirectly affect oogenesis by regulating JH titres [19–21].

The species-specific differences in the neuroendocrine signalling network mean it is impossible to come up with a single general model of neuroendocrine regulation of oogenesis in insects [22]. This highlights the need for a detailed molecular understanding of neuroendocrine control of oogenesis and reproduction in a wider range of insects, particularly from phylogenetically diverse taxa.

Environmental cues affect reproduction through altering neuroendocrine signalling

Environmental conditions such as temperature and crowding cause alterations to these neuroendocrine signalling networks in insects, particularly biogenic amine, ecdysone and JH signalling (reviewed in [23]). These changes can occur rapidly (within 15 min of an acute environmental cue) and these rapid responses are collectively known as the neuroendocrine stress response (or 'generic stress response'). In *Drosophila virilis*, dopamine, octopamine and tyramine levels are elevated 15 min after exposure to an increased temperature [24]. Fast changes in biogenic amine levels are also seen in response to different environmental cues in diverse species, including mechanical stress in honeybees (*A. mellifera*) [25], mechanical and heat stress in a locust (*Schistocerca gregaria*) and a cockroach (*Periplaneta americana*) [26]. These similarities in the response to different environmental cues and between diverse species indicate that there are some evolutionarily conserved aspects to neuroendocrine stress response. In *D. virilis* and *D. melanogaster*, the increase in biogenic amine levels precedes a rise in 20E [27] and a decrease in JH degradation [19–21,28]. This rise in 20E is thought to pause reproduction by activating the mid-oogenesis checkpoint triggering cell death [29–31] (stage 7–9, Figure 1a). Ecdysone, via ETH regulation of octopamine, also inhibits ovulation [32•] resulting in decreased fertility for

several days following acute exposure to an elevated temperature [28] (Figure 1b). The mid-oogenesis checkpoint is activated in response to diverse acute environmental cues in *D. melanogaster* and *D. virilis* [31], including protein deprivation [33–35], heat stress [29], crowding [36] and exposure to a parasitoid wasp [37].

But how much overlap is there between the neuroendocrine stress response (which governs the response to acute environmental cues) and the neuroendocrine regulation of reproductive dormancy (which occurs due to more sustained or chronic environmental cues)?

We have the best molecular understanding of how reproductive dormancy is regulated in *D. melanogaster*. Reproductive dormancy can be induced in *D. melanogaster* by exposing flies for 7–11 days to low temperatures and short photoperiods and it is rapidly reversible [38]. It is proposed that, similar to the response to acute environmental cues, sustained environmental cues also elevate the levels of biogenic amines, including dopamine [39••]. Enhanced dopamine and serotonin signalling inhibit the production or release of insulin-like peptides from insulin-producing cells [39••]. Insulin signalling is involved in the neuroendocrine stress response [40] and reproductive dormancy in *D. melanogaster* [38,41,42]. Reproduction in insects is sensitive to nutrition (reviewed in [43]), and starvation conditions result in reproductive dormancy in *D. melanogaster* [35]. It has been recently shown that insulin signalling and JH are also key to this process in *D. melanogaster* [40,44•]. Elevated biogenic amines likely act to reduce JH synthesis or release and reduce 20E levels [45,46]. These changes to biogenic amines, JH and 20E, result in reproductive dormancy via activation of the second ovarian checkpoint, similar to that seen in response to acute environmental cues in the neuroendocrine stress response [47]. However, a recent study has indicated that the reproductive repression seen in dormancy is more complete than that seen in response to acute environmental cues. Reproductive dormancy causes arrest of early oogenesis, a decrease in germ stem cell numbers and degeneration of oocytes from stage four of oogenesis [48••] (Figure 1a). This suggests that although many environmental cues activate the mid-oogenesis checkpoint in *D. melanogaster*, there may be additional mechanisms or subtle differences in the response of this checkpoint to specific environmental cues. This highlights a need to better understand how acute environmental cues affect the first checkpoint to determine if the activation of this first checkpoint is similar between acute and chronic environmental cues.

That there are commonalities, both in hormone signalling and the activation of ovarian checkpoints, between the neuroendocrine stress response and reproductive dormancy, suggest that, in *D. melanogaster* at least, reproductive dormancy may have evolved as an extension of the neuroendocrine stress response [47].

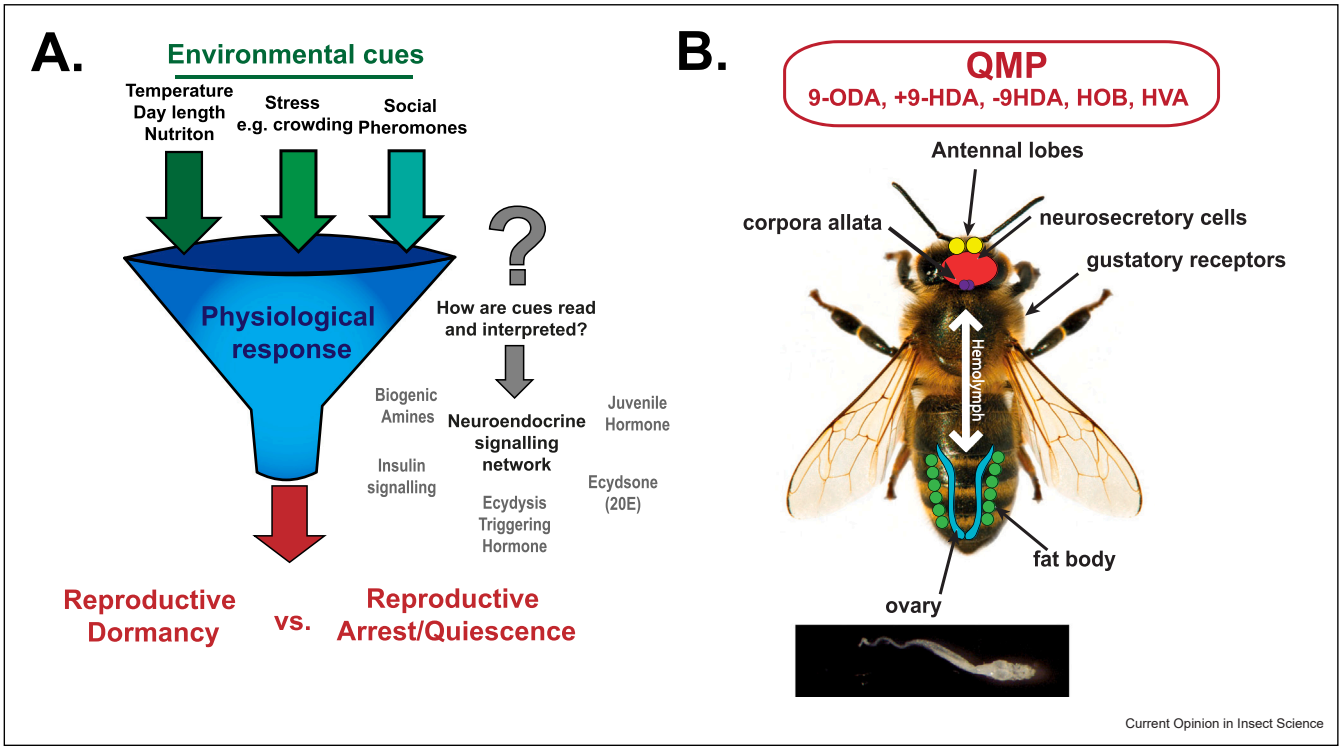
However, in contrast to acute environmental cues which, in *D. melanogaster*, result in increased levels of 20E and JH (Figure 1b), dormancy is associated with low levels of both JH and 20E [49]. On the surface, this suggests that fundamentally different mechanisms underpin the acute neuroendocrine stress response and reproductive dormancy in *D. melanogaster*. However, these differences may, at least in part, be explained by the differential regulation of octopamine. Octopamine is a key regulator of the acute neuroendocrine stress response, but inhibits dormancy [39••]. Octopamine also regulates 20E and JH titres [45,50]. These differences may also arise due to differences in timing between acute and chronic exposure to environmental cues. A possible mediator of this temporal offset may be ETH, which causes the release of JH from the corpora allata [8••]. The decreased levels of ETH associated with reproductive arrest may, with a temporal delay, result in decreased JH levels associated with reproductive dormancy, however, this remains to be tested.

Across a range of species, reproductive dormancy is associated with decreased JH, but the role of 20E is more variable [42]. We, therefore, need a mechanistic understanding of how acute and chronic environmental cues act to induce reproductive dormancy in a wide range of species to determine the extent to which we can make general predictions about how insects will respond to environmental cues. Such predictions may be relevant to assessing the response of insect species to rapidly changing environments, identifying species that may be at risk of decline/extinction or alternatively may have the potential to become invasive. It is also crucial to understand how fundamentally different environmental cues, such as temperature, mechanical stress and chemicals, are detected and interpreted by the neuroendocrine system of different insects to give rise to reproductive dormancy; do diverse environmental cues result in the same alterations to the neuroendocrine network and identical or different effects on reproduction in terms of checkpoint activation (Figure 2a).Also, are these same systems responsive to an even wider range of environmental signals, for example, social signals or pheromones? If so, could neuroendocrine-mediated reproductive dormancy underpin the evolution of different life-history strategies, such as eusociality?

Is reproductive constraint in eusocial insects an example of reproductive dormancy?

Eusocial insects are defined by their reproductive division of labour [51], with subordinates or 'workers' remaining reproductively inactive to support a reproductive dominant or 'queen'. The reduction of

Figure 2



Environmental cues and reproductive dormancy in insects. (a) A diverse range of environmental cues impact neuroendocrine signalling in insects. However, there are key gaps in our knowledge regarding a) how different species are responding to environmental cues and stressors and b) how these diverse stressors are read and interpreted to result in reproductive dormancy. **(b)** In the honeybee, reproduction in workers is repressed, at least in part, by QMP. This is arguably the best-studied example of reproductive constraint in a eusocial inset, yet there are key gaps in our understanding of how QMP is detected and how this signal is transmitted to the ovary to repress oogenesis [86,87].

reproductive capacity can be absolute, for instance, some ant species lack ovaries altogether [52], or can be partial; where workers retain some capacity to reproduce, as is seen in the honeybee (*A. mellifera*). The reduced reproductive output of workers relative to queens arises from behavioural and developmental mechanisms termed 'reproductive constraints' [53]. The ability of workers to produce offspring creates conflict within the colony [54] and, as a result, the ability of workers to reproduce is often conditional on external cues [53].

Although reproductive constraints could have evolved via novel molecular pathways, it is thought that they are more likely to have arisen through the co-option of pathways that controlled reproductive and foraging processes in solitary ancestors (ovarian ground-plan hypothesis) or a conserved genetic 'toolkit' (reviewed in [55]). From this perspective, can reproductive constraint, which is crucial for the evolution of eusociality, be considered analogous to reproductive dormancy in solitary insects?

In primitively eusocial species, such as the bumblebee (*Bombus impatiens)* [56], *Polistes* spp. [57] and *Ropalidia marginata* [58] paper wasps, colonies are often small. Reproductive rights are therefore determined behaviourally through aggression and dominance hierarchies, particularly in the early stages of colony development. Dominance hierarchies are also important in some ponerine ants (e.g. *Diacamma* spp. and *Dinoponera quadriceps*), which have evolved to be queenless [59].

Biogenic amines mediate aggressive behaviour in various insects, including *D. melanogaster* (e.g. [60–62]) and are important in generating dominance hierarchies in primitively eusocial species. In bumblebees and the queenless ant *Streblognathus peetersi*, brain octopamine levels positively correlate with rank [63,64]. While in *Polistes* wasps and ponerine ants, dopamine levels, rather than octopamine levels, were associated with dominance status [65–67]. However, elevated biogenic amines may be a consequence rather than a cause of aggression in some species. For example, in *Diacamma* sp., topical

application of dopamine did not increase aggression [65], and in *Harpegnathos saltator*, individual brain dopamine levels were reduced by conspecific aggression [66].

Both JH and ecdysteroids are implicated in establishing dominance hierarchies in some, but not all, primitively eusocial insects (e.g. [68]). But, given the ovary is the primary site for ecdysteroid biosynthesis in adult insects [69,70], disentangling reproductive effects from aggression and dominance is difficult. In the ponerine ant *D. quadriceps*, JH was found to regulate reproduction but not aggression, suggesting that, in some species, JH and reproduction are decoupled from dominance [71]. Although there are species-specific differences in how dominance hierarchies are determined, there is clear involvement of the neuroendocrine signalling network and more research needs to be done to determine what commonalities, if any, exist between species.

Queen pheromones, neuroendocrine signalling and reproductive dormancy

In large eusocial colonies, worker reproduction is curtailed by pheromones produced by the queen or her brood (reviewed in [72]). In Hymenoptera, where eusociality has arisen independently at least 10 times, the majority of queen pheromones are nonvolatile saturated hydrocarbons [73] that may have evolved from ancestral fertility signals [72,73]. The mechanism of action of these pheromones and how they inhibit reproduction in workers is, as yet, largely unknown. However, neuroendocrine signalling may play a key role, for example, JH is associated with reproduction in the wasp *Vespula vulgaris* [74] and dopamine has been found to correlate positively with reproduction in queenless worker bumblebees, paper wasps and some ants (reviewed in [75]). However, more studies are required to link pheromone exposure with the underlying molecular mechanisms that repress reproduction to determine how these pheromones function and how they might have evolved. Importantly, pheromone activity may be context-dependent [76•] and this should be considered when testing the molecular function of pheromones.

The most well-characterised queen pheromone to date is that from the honeybee *A. mellifera*. Honeybee queen mandibular pheromone (QMP) acts to repress reproduction in workers [77] as well as inducing young workers to feed and groom the queen and to perform colony-related tasks [78]. Honeybee QMP differs considerably in terms of chemical composition [78] (Figure 2b) from the queen pheromones of other hymenopterans, which are primarily nonvolatile saturated hydrocarbons [73]. However, despite decades of research, it is not yet clear how QMP is detected by worker honeybees (Figure 2b), although it is seemingly independent of olfaction [79]. QMP exposure affects brain dopamine levels, possibly through one of the specific components, homovanillyl alcohol [80] and high levels of dopamine are associated with reproduction in workers (reviewed in [72,81,82]). Workers exposed to QMP also have low levels of 20E [83] and high levels of JH [84]. This is seemingly inconsistent with the model presented for reproductive dormancy in *D. melanogaster* (Figure 1b). However, JH has a more limited role in reproduction in the honeybee, and there has been extensive rewiring of the neuroendocrine network in honeybees [85]. However, like the reproductive dormancy response in *D. melanogaster* [48••] (Figure 1b), QMP causes an arrest of oogenesis in the germarium [86], and there is evidence of apoptosis occurring in mid–late oogenesis [87]. To fully determine if the honeybee response to QMP is analogous to reproductive dormancy, it is critical to determine if other components of the neuroendocrine network that control reproduction in solitary species (e.g. ecdysis-triggering hormone) are also responsive to QMP, and also to functionally link the changes in brain dopamine levels, the arrest of oogenesis in the ovary (Figure 2b).

Understanding how QMP represses reproduction will allow us to determine how this complex pheromonal blend evolved. Some hints may be gleaned from the fact that QMP, unlike other hymenopteran queen pheromones [88], represses oogenesis in virgin *D. melanogaster* females [88,89]. This is perhaps surprising given that honeybees and *D. melanogaster* last shared a common ancestor ~340 million years ago [90] and QMP has only evolved over the last 55 million years [91]. It has been suggested that QMP has evolved to target an evolutionarily conserved mechanism, possibly derived from an environmental signal linked to nutrition sensing [92], for repressing reproduction. Nutrient sensing pathways have been implicated in establishing and maintaining reproductive skew in eusocial insects [93,94•] and are known to repress reproduction via neuroendocrine signalling in solitary insects (Figure 1b).

Concluding remarks and future perspectives

Reproduction in insects is exquisitely regulated by environmental cues (Figure 2a) via neuroendocrine signalling (Figure 1b). It is important to understand how different environmental cues (e.g. temperature day length, stress and social pheromones) are detected and 'interpreted' by the neuroendocrine system to give rise to reproductive dormancy or arrest in a range of species (Figure 2a). As discussed above, in *D. melanogaster*, there are subtle differences in the way the neuroendocrine system responds to acute and long-term environmental cues (Figure 1b). If this is true for other insects, it will have implications for understanding species adaptation to changing environments, particularly in the context of invasive species and insect declines.

The evolution of eusociality represents one of the major and most successful life-history transitions in animal evolution. The key to this life-history strategy is the reproductive division of labour. The evolution of queen pheromones is key to the evolutionary success of highly eusocial species such as the honeybee. Ancestral queen pheromones are nonvolatile substances made up of cuticular hydrocarbons. QMP is however unusual as it is chemically distinct from the majority of queen pheromones. It has been hypothesised that this complexity has arisen as a result of an evolutionary arms race between queens and workers over reproduction. Determining how these pheromones are synthesised, how they are detected and how they interact with neuroendocrine signalling, is critical to understanding how, at a mechanistic level, eusociality has evolved.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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