Review

Behavioral Neuroendocrinology of Reproductive Death Processes in Invertebrates

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Behavioral Neuroendocrinology of Reproductive Death Processes in Invertebrates

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Abstract: In much of neurobiological research, senescence and death are regarded as negative outcomes of aging. In the lives of animals, however, death may occur in many different ways, including as a natural result of reproduction, a common mode of death in invertebrates. This review examines the intersection of the nervous system, behavior, and reproductive death in three study systems that span the diversity of neural forms and life history strategies in invertebrates. In *C. elegans*, hermaphrodites experience a shortened lifespan as a result of extreme physiological changes caused by mating. In octopuses, signaling from the neuroendocrine center causes females to deteriorate over the duration of egg-brooding, dying before their offspring hatch. Lastly, in bumblebees, the entire colony perishes due to behavioral changes induced by the interplay of signaling between queens and her workers. Each model offers unique insights into the neuroendocrine control of reproductive death.

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Introduction

Invertebrate animals exhibit an incredible diversity of neural forms and life history strategies that underlie their evolutionary success in nearly every biome on earth. Only three invertebrate lineages have evolved brains, defined as a centralized collection of neural centers with distributed and hierarchical functions: annelids, cephalopod molluscs, and arthropods [1]. Animals in other invertebrate lineages have diffuse nerve nets or, as in the case of the well-studied nematode worm *C. elegans*, cerebral ganglia [1,2]. Reproductive strategies in invertebrates range from semelparity, classically defined as reproducing once before death, to iteroparity, or reproducing multiple times before death [3–5]. Here, we highlight research on three invertebrates that span a range of reproductive strategies and neural structures: the nematode worm, the octopus, and the bumblebee. Each study system provides important mechanistic insights into the neuroendocrine systems that regulate reproductively-triggered senescence and death, revealing shared mechanisms and lineage-specific adaptations.

C. elegans

Short generation times, well-established genomic tools, and visual markers of organismal fitness make *C. elegans* a useful model organism for probing mechanisms of reproduction-triggered senescence and longevity [6]. *C. elegans* also displays a noteworthy survival strategy; under stressful environmental conditions, larvae can develop into dauers, a specialized state that promotes survival and dispersal (Figure 1a) [7]. Formation of this alternate life stage is controlled by the nuclear hormone receptors, such as DAF-12, and their ligands, including the dafachronic acids [8–10]. Most *C. elegans* worms are self-fertilizing hermaphrodites (Figure 1a). Male *C. elegans* are rare, constituting less than 0.1% of the population [11,12]. Reproduction with males is necessary for genetic diversity, yielding larger and healthier clutches compared to self-fertilization, but it comes at a cost (Figure 1b). Exogenous sperm triggers development of severe senescent phenotypes in hermaphrodites, including uterine tumors, shrinking, pools of yolk lipoproteins in the body cavity, intestinal atrophy, and shortened lifespan. This is mediated through perturbations of the insulin/insulin-like growth

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factor (IGF1) signaling pathway (IIS), an evolutionarily-conserved modulator of lifespan [13–19]. IIS in *C. elegans* is mediated through DAF-2, the sole receptor for insulin-like peptides [20]. DAF-2 mutants, which exhibit reduced IIS signaling, experience increased lifespans [21]. Both DAF-2 and IIS play key roles in reproduction and life stage transitions, such as dauer formation.

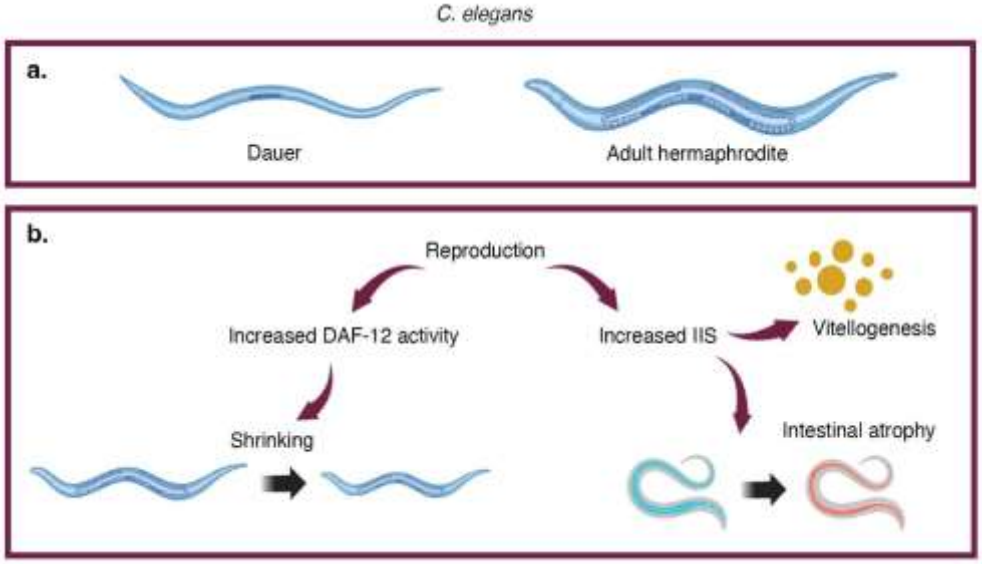

Figure 1. Overview of senescent phenotypes induced by reproduction in *C. elegans*. **a.** Dauers are an alternate developmental stage that promotes survival under stressful conditions. Most *C. elegans* worms are self-fertilizing hermaphrodites. **b.** Reproduction leads to intestinal atrophy, increased vitellogenesis, and shrinking.

Mating increases IIS and leads to downstream physiological changes in hermaphrodite worms. DAF-2 mediated inhibition of the DAF-16 transcription factor causes increased production of yolk protein through autophagy of the gut [13,16]. As eggs develop, the gut fills with pools of yolk protein. This extra yolk is expelled along with the fertilized eggs and is subsequently consumed by developing larvae [13,22]. Larvae that consume this yolk experience increased overall fitness, however, yolk overproduction lead to immediate detrimental outcomes in mated hermaphrodite worms (Figure 1b) [13,22]. Intestinal atrophy can lead to shortened lifespan, and the overproduction of yolk and depletion of self-sperm lead to teratoma-like uterine tumors [23,24]. Worms that lack functional DAF-2 or DAF-16 in the intestines exhibit less intestinal atrophy, reduced accumulation of yolk protein, and lower mortality rates as compared to mated wildtype worms [13]. Taken together, the increase in IIS caused by reproduction leads directly to gut autophagy and senescent phenotypes.

Mating also decreases lifespan through modulation of the DAF-9/DAF-12 and DAF-16 pathways [15]. Sperm decreases activity of DAF-9, a cytochrome P450 oxygenase, leading to a reduction in dafachronic acids [15,25]. Despite the decrease in dafachronic acid production, mated worms show increased activity of DAF-12, a nuclear receptor involved in osmotic regulation and longevity pathways [15,26]. Dysregulation of osmotic adaptability leads to shrinking due to water loss and shortened lifespan (Figure 1b) [15]. DAF-2 signaling leads to the cytoplasmic localization of DAF-16, causing reduction in the activity of the transcription factor and leading to a loss of fat, possibly through yolk overproduction. Loss of fat causes shrinking and is associated with reduced lifespan. The decreased DAF-9 and DAF-16 activity and increased DAF-12 activity after mating lead to fat loss, osmotic stress susceptibility, whole body shrinking, and reduced lifespan [15].

In *C. elegans*, reproduction presents a tradeoff between decreased longevity with increased offspring fitness. The dafachronic acid and IIS pathways that are essential in development also cause senescent phenotypes after reproduction [17,20,27]. Our understanding of how mating in worms recruits these same molecular pathways to accelerate the transition from adulthood to death provides a useful template for studying similar phenomena in other invertebrates.

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Octopuses

Of the invertebrates, octopuses have one of the most complex nervous systems, with almost 40 lobes in the central brain and a brain to body mass ratio comparable to that of fish and reptiles [28]. The octopus's many morphological and neural novelties make them advantageous for comparative neurobiological research. The optic glands, small spherical structures located between the optic lobes and central brain mass, are particularly noteworthy. Nicknamed the "self-destruct" system [29], the optic glands are the principal neuroendocrine signaling center of the octopus. Functionally analogous to the vertebrate anterior pituitary gland, the optic glands control reproductive maturation and, ultimately, reproduction-triggered death [29–31].

Almost all species of octopuses are semelparous: females die while brooding their singular clutch of eggs [32]. During the maternal brooding period, the female transitions from feeding to fasting, then undergoes rapid organismal decline [29,30]. Senescent females lose the ability to heal from wounds and exhibit signs of a compromised vestibulo-ocular reflex. In the period preceding death, the female may engage in self-injury behaviors, rubbing her skin off and eating the tips of her arms [29,30]. All physiological changes and maternal behaviors are controlled by secretions from the optic gland. Senescence is abolished if the gland is removed, enabling the octopus to reproduce again and live up to 6 months longer than intact counterparts [29].

Multiomics studies in *Octopus bimaculoides* demonstrate that mating triggers sweeping changes in optic gland neuroendocrinology. Mating upregulates the *daf-36* gene, which remains elevated through the end of life; *daf-36* transcript is the most abundant transcript found in the optic glands of mated females [30,33]. The *daf-36* gene encodes a cholesterol 7-desaturase that converts cholesterol to 7-dehydrocholesterol (7-DHC) (Figure 2) [33–35]. In arthropods and nematodes, this is the first committed step in the synthesis of bioactive steroid hormones from cholesterol [34,35]. Mammals lack a homolog to *daf-36* [35]. In humans, the accumulation of 7-DHC is toxic and can lead to death [36,37]. In octopuses, it is possible that 7-DHC itself, rather than its downstream metabolites, is used as a signaling molecule at the end of life. In addition, high levels of 7-DHC may be lethal, as it is in humans, contributing to the behavioral and physiological changes that lead to death. These findings demonstrate that DAF-36 and 7-DHC share neuroendocrine functions across distantly-related animals.

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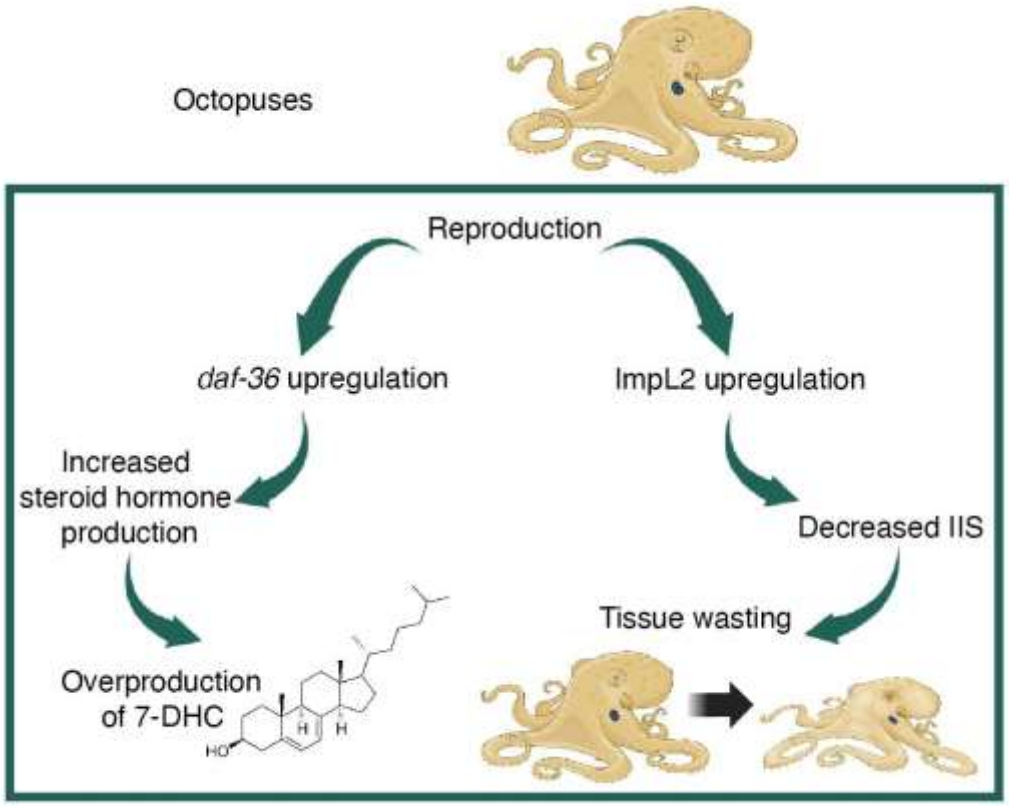

Figure 2. Effects of reproductive senescence in octopuses. The daf-36 and IIS pathways are involved in reproduction-triggered death in octopuses.

As in *C. elegans* and other animals, the IIS pathway plays an important role in mediating reproduction-triggered death in octopuses (Figure 2). When maternal fasting begins, the optic glands upregulate the production of ImpL2, a homolog to insulin-like growth factor binding protein [30]. In *Drosophila*, increased ImpL2 production occurs under starvation conditions and suppresses IIS activity, ultimately promoting survivorship [38]. However, in octopuses, increased ImpL2 is found only in fasting, senescent females. Non-senescent females under experimentally-induced starvation conditions have comparable ImpL2 expression as females who are fed normally. These findings suggest that different mechanisms control octopus IIS pathways under starvation and senescent conditions [39]. Increased ImpL2 also causes whole body tissue wasting in *Drosophila* cancer models [40,41]. This cachexia-like wasting resembles the physiological markers of senescence in octopuses, strongly suggesting that high ImpL2 promotes tissue loss at the end of life (Figure 2). Remarkably, studies of the optic gland reveal that *daf-36* and insulin signaling pathways are critical to the control of lifespan after reproduction in both octopuses and *C. elegans*.

Although most octopuses are semelparous, *Octopus chierchiae* is iteroparous: males and females reproduce multiple times, and females can brood multiple clutches [42,43]. The maternal behavior of *O. chierchiae* offers insight into the evolution of reproduction-triggered death. *O. chierchiae* mothers feed for most of the brooding period. They stop eating when their eggs begin to hatch, but resume feeding again 2-3 days before hatching is complete. During this time, the female eats any young remaining in the area [43]. The behaviors and unique life history of *O. chierchiae* aligns with Wodinsky's original hypothesis that the inhibition of feeding and maternal death in other octopus species serves to prevent cannibalism of the young by the mother [29].

The *O. chierchiae* optic glands likely rely on alternate signaling systems or compensatory mechanisms to mediate iteroparity. Transcripts that are upregulated in mated *O. bimaculoides*, such as the steroidogenic enzymes, may be more dynamically up- and downregulated through each cycle of mating and brooding in *O. chierchiae*. Additionally, the steady increase in ImpL2 production during

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O. bimaculoides senescence may be entirely absent in *O. chierchiae*. Future comparison of brooding O. *chierchiae* neuroendocrinology to senescent O. *bimaculoides* profiles will be instrumental in clarifying the functional roles of transcriptional changes in senescent octopuses.

Bumblebees

Bumblebees offer the opportunity to understand post-reproductive senescence and death on the scale of the whole colony as well as on an individual basis. In both *Bombus impatiens* and *Bombus terrestris*, colonies are founded in the spring and die by the fall [44]. In the beginning of the colony cycle, the queen only produces diploid female workers, who cooperatively perform nursing and foraging tasks to keep the colony running (Figure 3a). As the colony ages, the queen begins to produce future queens, called gynes, and haploid males. At this stage, previously cooperative workers abruptly become aggressive, competing with each other and the queen to produce males (Figure 3b) [45]. Food provisioning and other nest tasks are neglected in favor of reproduction, and few broods are successfully reared. Instead, workers remove or eat each other's eggs, including the eggs of the queen, and attempt to oviposit. The fierce competition phase of the colony may result in the premature death of the queen [44,46,47]. At the end of the colony cycle, female workers, males, and the queen all die. Gynes are the lone survivors of the colony, undergoing diapause before initiating their own colonies the next year. Ultimately, reproductive competition destabilizes the maintenance and productivity of the colony, leading to its demise. Examining the neuroendocrinology of reproductive competition can elucidate mechanisms by which the behaviors of individuals contribute to the death of the entire social group.

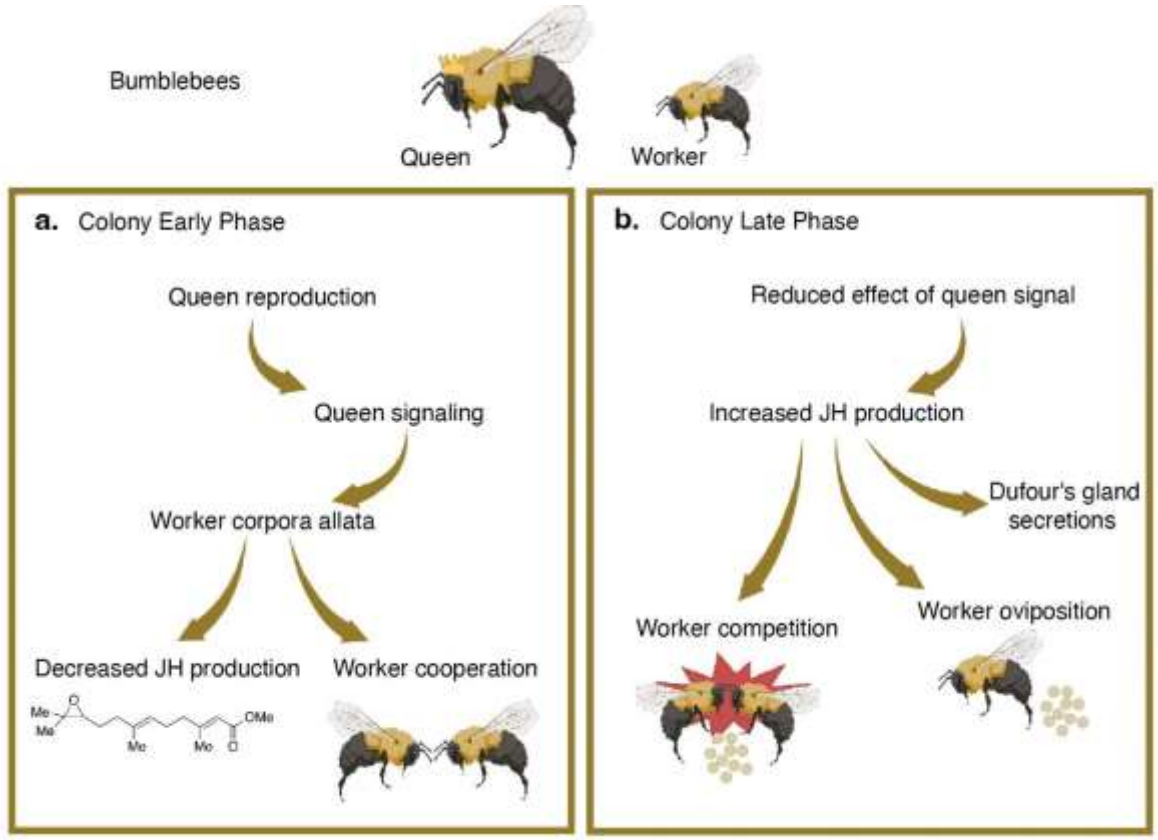

Figure 3. Colony death in bumblebees. **a.** In the early phase of the colony, workers are cooperative and non-reproductive. **b.** In the late phase of the colony, changes in queen signaling and worker JH production cause workers to exhibit overt antagonism with each other and the queen as they compete to lay eggs.

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In bumblebees, signaling systems in both the queen and the workers act simultaneously to modulate reproduction, aggression, and ultimately, colony longevity. Exocrine organs, including the Dufour's gland and the mandibular glands, produce compounds that are essential for chemical communication and social identification [48–50]. The queen's chemical secretions reduce reproductive output of the workers. In *Bombus terrestris*, this occurs through the suppression of the workers' corpora allata, an endocrine gland that produces juvenile hormone (Figure 3a) [51,52]. Juvenile hormone controls development and metamorphosis in arthropods and serves as a major gonadotropic hormone in social insects [53,54]. High levels of juvenile hormone induce oogenesis and impacts social dominance in bumblebees. By suppressing juvenile hormone synthesis, the queen can monopolize reproduction: workers that lack juvenile hormone have underdeveloped ovaries, do not lay eggs, are less aggressive, and hold a less dominant position in worker groups (Figure 3a) [48,53].

As the colony develops, the queen to worker ratio diminishes. Faced with a high worker population, the effectiveness of the queen's chemical secretions may also decline [46,55]. This enables individual workers to overcome reproductive suppression and take advantage of the opportunity to oviposit [56]. Reproductive workers exhibit elevated juvenile hormone levels and increased social dominance, effectively inhibiting the reproduction of other workers (Figure 3b) [57,58]. Additionally, Dufour's gland secretions of reproductively active workers become similar to that of bumblebee queens; high juvenile hormone levels cause the removal of esters that signal reproductive sterility in nonreproductive workers [48,59,60]. This difference in chemical identity may function to protect sterile workers during the competition phase, signaling to reproductive workers that they are not a threat, or act as an ancillary chemical cue for inhibiting reproduction of other workers.

The death of the bumblebee colony as a result of reproductive competition arises from dynamic actions of hormone and pheromone signaling. The queen's chemical secretions and reproductive behaviors are key drivers in controlling competition amongst her workers, but modulation of chemical cues in the workers themselves may also contribute to the transition from cooperativity to antagonism. For example, juvenile hormone exerts gonadotropic effects which have downstream consequences on the social milieu [48,53]. In bumblebees, rapid and socially-dynamic chemical signaling causes a stark shift from cooperativity to reproductive aggression that ultimately destroys the colony.

Conclusions

Research conducted in these three systems emphasize the highly intertwined roles that developmental and metabolism-related signaling pathways play in the control of reproduction and lifespan. In *C. elegans*, reproduction triggers IIS and dafachronic acid based senescent phenotypes in hermaphrodites [13]. Octopuses brood their young until dying, undergoing a dramatic decline that culminates in starvation, self-cannibalism, and self-injury [29,30]. Bumblebee colonies undergo a JH-mediated rapid decline during the production of fertile offspring [44,48].

Although the phenotypes, behaviors, or life histories discussed here may seem extreme, they only begin to represent the full spectrum of reproductive death found in invertebrates. For example, aphagous mayflies lack a fully formed gut or mouth as reproductive adults. Because mayfly adults have no opportunity to replenish energy reserves, regulation of metabolism likely plays a key role in the regulation of their reproduction and death. They mate and die rapidly, living only days to weeks as adults [5,61]. Future experimental studies might test whether administration of nutrients during the adult phase could extend lifespan, or if adults that do not reproduce tend to live longer than those who do. Reproductive cannibalism is prevalent in several species of spiders and mantids: females kill and eat their male mates [62–66]. In a particularly noteworthy case, the male dies without female intervention immediately upon insertion of the second pedipalp [64]. It is possible this behavior promotes the fitness of the female during reproduction, and thus increases the chances of reproductive success for both the male and female. Future research into the mechanistic basis of this phenomenon should not only examine the offspring fitness of mothers who consumed their mates, but also how the death of the male changes the reproductive success of other males. Deepening our

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understanding of the three animals we discuss in depth here and broadening the neuroendocrinology of reproductive death to other non-model animals will greatly increase our appreciation for the evolutionary richness and diversity of life history strategies present amongst all animals.

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