Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history

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Summary

Understanding how traits are integrated at the organismal level remains a fundamental problem at the interface of developmental and evolutionary biology. Hormones, regulatory signaling molecules that coordinate multiple developmental and physiological processes, are major determinants underlying phenotypic integration. The probably best example for this is the lipid-like juvenile hormone (JH) in insects. Here we review the manifold effects of JH, the most versatile animal hormone, with an emphasis on the fruit fly *Drosophila melanogaster*, an organism amenable to both genetics and endocrinology. JH affects a remarkable number of processes and traits in *Drosophila* development and life history, including metamorphosis, behavior, reproduction, diapause, stress resistance and aging. While many molecular details underlying JH signaling remain unknown, we argue that studying ''hormonal pleiotropy'' offers intriguing insights into phenotypic integration and the mechanisms underlying life history evolution. In particular, we illustrate the role of JH as a key mediator of life history trade-offs. *BioEssays* **27:999–1010, 2005.**

Abbreviations: CA, corpus allatum, corpora allata; CC, corpus cardiacum, corpora cardiaca; CNS, central nervous system; DAF, dauerformation; DILPs, *Drosophila* insulin-like peptides; ECR, ecdysone receptor; 20E, 20-hydroxy-ecdysone; IGF, insulin-like growth factor; INR, insulin-like receptor; IPCs, insulin producing cells; JH, juvenile hormone; MET, methoprene-tolerant; PTTH, prothoracicotropic hormone; RA, retinoic acid; USP, ultraspiracle; YP, yolk protein.

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Introduction

Many traits cannot evolve independently because they are coupled by genetical, developmental and physiological mechanisms.[1–6] But what exactly are these mechanisms? While connections among traits can be described as genetic correlations and trade-offs caused by pleiotropy or linkage, the detailled physiological and molecular mechanisms mediating such trait correlations have remained elusive.[1–8] Traditionally, evolutionary biologists have focussed on quantifying phenotypic integration rather than on understanding the mechanisms underlying trait architecture and trade-offs.[3–9]

Hormones are regulatory molecules signaling to distant target tissues throughout the organism. They transduce environmental and genetic signals via hormone receptors to affect gene transcription in target tissues, thereby exerting multiple phenotypic effects.[3,4] Consequently, hormones provide a mechanistic link between the environment, genes and whole organism traits, and the manifold regulatory effects of hormones ('hormonal pleiotropy') offer promising insights into phenotypic integration.[3–12] Hormones are now thought to coordinate the integrated expression of multiple traits across environmental conditions (endocrine-mediated phenotypic plasticity) and to constrain their simultaneous evolution.[3–9] Since major parts of development and life history are under endocrine control, hormones and endocrine genes might be major determinants of pleiotropy, life history correlations and trade-offs.[3–8]

The importance of hormones in the insect life cycle has been recognized early. In 1934, Vincent Wigglesworth (1899–1994) showed that ablation of endocrine glands (the corpora allata, CA) in the bug *Rhodnius* causes a precocious molt to the adult, whereas re-implantation of CA ensures a juvenile molt.[17,18] The endocrine factor produced by the CA was named juvenile hormone (JH); its chemical structure was determined by Röller and colleagues in 1967.[19] Since Wigglesworth's classical studies, it has become clear that this lipid-like molecule is a major endocrine regulator in insects, representing the probably most versatile animal hormone.[17,20–26] Yet, our molecular understanding of JH signaling has remained limited and many developmental and physiological functions of JH still await discovery.[21,25,26]

As is the case for the other major insect hormone, 20-hydroxy-ecdysone (20E), progress is likely to come from the fruit fly *Drosophila melanogaster*, a system with extensive genetics, mutant stocks and an array of molecular and physiological tools.[26–28] Here we review the manifold roles of JH in *Drosophila* development and life history.[26,28–30] We illustrate that JH has intriguing effects upon traits and trade-offs of major interest to the evolutionary biologist.[3–16] Although we focus on the pleiotropic effects of JH on *Drosophila* life history, increasing evidence suggests an important role of hormones in the life history evolution of lizards,[4–6] birds,[6–8] insects,[6,9,11,13,16,20,22–24] echinoderms,[13,15] and plants.[13] Comparative endocrinology has made major advances in understanding how endocrine functions evolve and how hormones affect evolutionary processes;[10,11,13,16,23,29] for a comparative evolutionary perspective on how hormones regulate life history transitions see the recent paper by Heyland et al. in this journal.[13] By integrating endocrinology into evolutionary biology, as exemplified by the work of Anthony Zera on crickets,[6,14] the time is ripe for opening the mechanistic black-box of life history, a promising challenge for both developmental and evolutionary biologists.

Life history evolution, trade-offs and hormones

Life history traits are integrated by genetic, physiological and developmental mechanisms, typically measured as genetic correlations.[1–6] Genetic correlations imply that traits cannot evolve independently: selection on a trait causes correlated responses in other traits.[1–6] For example, selection experiments often reveal negative genetic correlations between fitness components, so-called trade-offs, that constrain the simultaneous evolution of two or more traits (see Fig. 1).[1,2]

Life history theory is concerned with explaining the evolutionary diversity of life cycles,[1,2] yet rarely addresses the mechanisms underlying variation and integration of life history traits.[3–9,13,14] While genetic correlations are known to be caused by pleiotropy or linkage,[1–3] the specific genes involved are in most cases unknown. For example, life history theory often assumes the existence of multiple life history effects of a single gene or allele (life history pleiotropy; see Refs. (1–3), yet we have only a handful of examples of loci with pleiotropic effects on fitness components.[1,31] Even if pleiotropy is common, what are the molecular mechanisms by which pleiotropic genes affect multiple traits?[3–6,9,31] Causally understanding genetic correlations requires the identification of specific genetic and physiological mechanisms.[3–6,31]

Variation in endocrine mechanisms has repeatedly been suggested as a major mechanism underlying life history evolution,[3–16,20,24] but hormonal aspects of life history evolution have remained understudied. Remarkably, however, hormones commonly play a conserved role in the coordination of life cycles, both among and within species. Life history transitions, for instance from larva to adult or from a non-reproductive to a reproductive state, are typically controlled by hormones,[10,13] and genetic correlations and trade-offs among life history traits are often hormonally mediated.[3–8] For example, the trade-off between flight capability and reproduction in wing-dimorphic crickets, *Gryllus firmus*, is regulated by JH and JH esterase (JHE);[6,14] the trade-off between egg number and egg size in the side-blotched lizard (*Uta stansburiana*) is mediated by follicle stimulating hormone (FSH);[4–6] and testosterone affects the trade-off between reproduction and immunity in the dark-eyed junco (*Junco hyemalis*).[6–8] In particular, endocrine loci may exhibit life history pleiotropy since they affect hormone signaling, the hormone in turn exerting multiple phenotypic effects ('hormonal pleiotropy').[3–8] Variation in endocrine mechanisms may thus be an important substrate for the evolution of coregulated life history traits and trade-offs.[3–8]

JH is a key mediator of insect development and life history

In insects such as *Drosophila*, JH now emerges as an endocrine regulator of development, life history and fitness trade-offs. JH is a sesquiterpenoid lipid-like hormone secreted by the corpora allata (CA), endocrine glands of the head situated behind the brain (see Fig. 2; Refs. 17,21,23,32). Fig. 2.

Insects produce at least eight forms of JH-like compounds (0, I, II, III, JH3 bisepoxide [JHB3], 4-Methyl-JH, 8′-OH-JH III, 12′OH-JH III), JH III being the most common type.[17,21,23,35,36] In *Drosophila*, the CA produces two JH's, JH III and JHB3.[17,35] While the endocrine function is better understood in the former, the latter appears to be the major product of the mature CA in *Drosophila* (also see Fig. 2; Refs. 17,35). While the molecular details underlying JH action remain poorly understood, JH is known to respond to various internal (e.g. hormonal, genetic) and external (e.g. temperature, nutrition, photoperiod) factors, to regulate and coordinate the expression of entire gene batteries, and to simultaneously affect multiple phenotypes.[22–24] Remarkably, JH affects a vast array of phenotypic traits and physiological or developmental processes, including preadult development, imaginal disc proliferation, organ looping, metamorphosis, ovarian development, sexual maturation, pheromone production, locomotor and courtship behavior, diapause regulation, migration, various morphological polyphenisms, division of labor in social insects, neuronal architecture, memory, learning, immune function, lifespan and many others.[6,14,16,17,20–25,28–30,37] During each particular life cycle stage and in response to internal or environmental signals, JH coregulates the expression of many of these traits or functions. By physiologically mediating pleiotropic gene effects and genotype by environment interactions (phenotypic plasticity), JH might represent a major mechanism underlying life history polymorphisms and

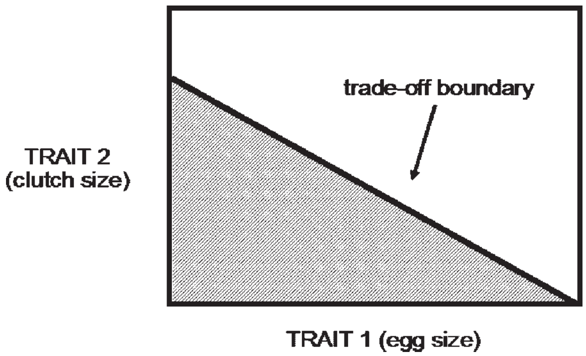

Figure 1. A negative genetic correlation (trade-off) between two fitness components (redrawn from Ref. 2). If two fitness components trade off, selection cannot maximize fitness by simultaneously increasing the trait value of both traits: improving fitness through an increase of one trait decreases fitness through a decrease of the other trait.[1,2] Consider a bird population in which females producing small clutches lay eggs of large size, whereas females that produce large clutches tend to lay small eggs.[1,2] The negative association between clutch size and egg size results in a negative genetic correlation, a trade-off. The trade-off boundary separates the space of realized phenotypes (hatched area) from the space above the trade-off boundary, containing those phenotypes not found in the population.[2] Thus, the trade-off imposes a constraint on phenotypic evolution: only those trait combinations below or on the trade-off boundary can be attained in phenotype space. Selection favors those phenotypes that lie on the trade-off boundary, resulting in a genetic correlation of unity.[1,2]

polyphenisms in insects.[3,6,14,22–24] In particular, genetic variation in JH signaling might be an important determinant of variation in life history strategies.[3,6,24] As we shall sketch below, the functional versatility of JH is also manifest in *Drosophila* development and life history.[28,30] Considering some classical endocrine disorders in *Drosophila* mutants provides a first glimpse of the remarkable pleiotropy mediated by insect hormones such as JH and 20E.[28,30,33,34,38–40]

Endocrine-mediated life history syndromes in *Drosophila* mutants

In the late 1930s, Ernst Hadorn recognized the importance of endocrine effects on *Drosophila* development by showing that the ring gland is partially homologous to the CA and serves a hormonal function.[28,33,34] Hadorn studied a mutant of *lethal (2) giant larvae (l(2)gl)*, now known to be a tumor suppressor gene, and found that the mutant's developmental arrest and lethality is caused by an endocrine deficiency.[28,33,34] In *lgl* mutants, the puparium is formed later or not at all; by transplanting different wild-type tissues into mutant larvae, Hadorn found that implantation of a ring gland provides a partial rescue of puparation. The same effect can be achieved by injection of 20E; yet whether *l(2)gl* mutants have altered JH function remains unknown.[28]

Another endocrine disorder in *Drosophila* is found in a mutant of *mama* (*maternal metaphase arrest*[1]; also known as *adipose*[female sterile] or *female sterile (2) adipose*), showing developmental defects that suggest an impairment of endocrine function; a similar syndrome occurs in *fs(2)B* (*female sterile (2) Bridges*).[28,38,39] While homozygous mutants are externally normal, they show abnormalities in three inner organs: (1) the fat body, which hypertrophies due to excessive fat accumulation, (2) the ovaries, which have a reduced number of oocytes, and (3) the CA, which hypertrophies to an abnormally large size.[28,38] Hypertrophy of CA and fat body can be rescued by implantation of wild-type ovaries, indicating an endocrine feedback between ovary, fat body and the CA.[28,38] The endocrine details underlying this life history syndrome have yet to be determined.[28,38,39]

The Hawaiian *Drosophila mercatorum* provides more direct evidence for a role of JH in development and life history.[3,40] In this species, the naturally occurring genotype *abnormal abdomen* (*aa*, also called *underreplication*[abnormal abdomen]) exhibits increased developmental time, early sexual maturation, increased fecundity and decreased female longevity (Table 1). Since JH is known to affect sexual maturation and egg production, this life history syndrome is thought to be caused by changes in JH signaling.[3,40] Indeed, *aa* flies have decreased JHE activity, which presumably leads to an unusually elevated JH titer.[3,40] More recent evidence now suggests that JH has major pleiotropic effects on the *Drosophila* phenotype, ranging from development to aging (Table 1, Fig. 3).

The role of JH in *Drosophila* development and metamorphosis

JH probably evolved primarily as a reproductive hormone, but is most well known for its effects on preadult development and metamorphosis.[11,13,17,21–23,29,30,41–43]. Evolutionary modifications of JH signaling have played a key role in the evolution of insect metamorphosis.[11,13,29,43] In embryos of basal, hemimetabolous insects, JH mediates the transformation from pronymphal to larval stages by suppressing morphogenesis and inducing premature differentiation.[29] In more derived groups of holometabolous insects, JH has a markedly weaker effect on embryonic growth, development and differentiation. Growth and differentiation of various tissues, typically suppressed by JH in ancestral insects, are no longer inhibited by JH and early-growing imaginal discs are formed.[29] During evolution, the inhibitory action of JH has thus been shifted from early ontogenetic processes to metamorphosis and, possibly, to the adult stage.[29]

JH is present throughout late embryonic and larval development and serves a 'status quo' function: 20E determines the timing of larval instar molts, while the continued presence of JH ensures that 20E-induced molting leads to another larval stage. JH thus determines whether the insect molts to a larva, pupa or adult. Metamorphosis occurs only

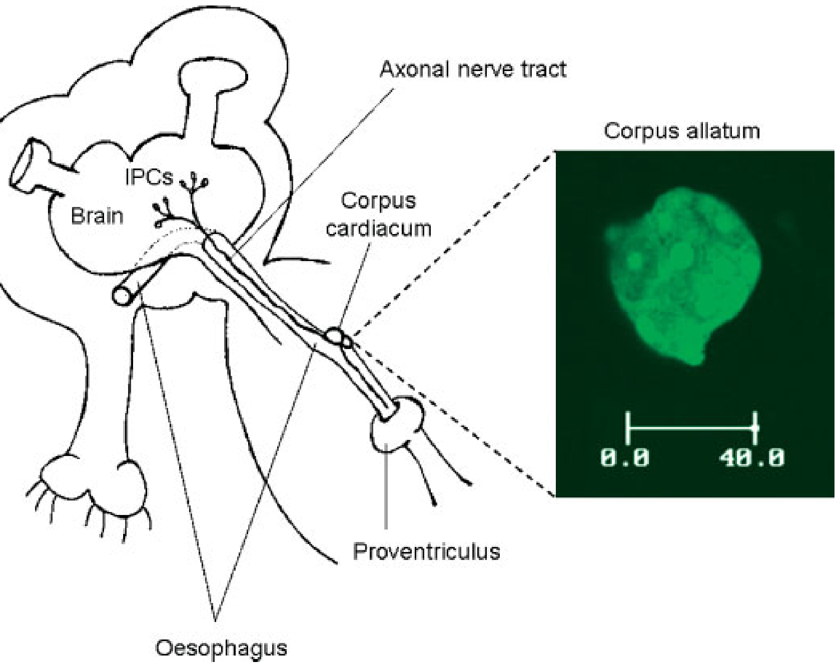

Figure 2. The retrocerebral neuroendocrine complex in *Drosophila*, consisting of the corpus allatum (CA) and the corpus cardiacum (CC), is attached to the fly brain. The CA of higher dipterans is an endocrine gland in the posterior region of the head producing two forms of juvenile hormone (JH), JHIII and JHB3, the latter being the major product of the CA. In the pars intercerebralis of the central nervous system (CNS), median neurosecretory cells (insulin-producing cells, IPCs) produce insulin-like peptides; in direct or indirect response to insulin signaling, the CA produces JH. The secretion of JH is also controlled by neurosecretory cells that release either allatostatins (inhibitory peptides) or allatotropins (stimulatory peptides). These neurosecretory cells in the brain send axonal projections to the CA through the CC, which in fly larvae are situated at the two ends of the horseshoe-shaped ring gland, consisting of the CA, the CC and the larval prothoracic glands.[32,35] While the large cells of the ring gland produce the ecdysteroid hormone 20-hydroxy-ecdysone (20E), a major insect developmental hormone, the smaller medial cells are thought to produce JH.[23,27,32–35] In adult insects, the CA forms a distinct, separate structure.[17,21,23,32] Inset: The corpus allatum (CA) of an adult female *D. melanogaster*. In adult *Drosophila*, the single CA and the CC are close together and remain connected by axonal projections from the brain, while the larval prothoracic gland cells of the ring gland have degenerated.[32–35] The picture shows a confocal microscope image, with the CA in fluorescent green. Expression of GFP (green fluorescent protein) in the CA was achieved by driving a UAS-GFP responder with a CA-specific Gal4 driver. Scale bar: 50μm.

when 20E acts in the absence of JH.[23,30,42] Shortly before metamorphosis, the CA stop producing JH, and hemolymph and target tissues exhibit increased JH degradation. Upon attainment of a critical larval weight and a decline of JH to low levels, prothoracicotropic hormone (PTTH) is released, inducing the secretion of 20E. 20E causes cessation of feeding, onset of wandering and pupation; after the pupal molt, 20E is released again and causes the adult molt in the absence of JH.[23,30,42] In many insects, experimentally induced excess of JH during larval development delays metamorphosis, whereas withdrawal leads to precocious metamorphosis.[23] In *Drosophila*, treatment with exogenous JH does not prevent larval–pupal transformation,[30] but prolongs developmental time, disrupts metamorphosis of the nervous and muscular system, disturbs abdominal differentiation and even inhibits eclosion.[41,44] After initiating metamorphosis, JH titers increase again to regulate developmental details of metamorphosis; for example, JH seems to be required for the final stages of histolysis of the larval fat body.[23,30,45]

JH and 20E also affect imaginal disc proliferation and differentiation,[29,30,37,46,47] and recent evidence suggests a role of JH in organ development.[48] In 1943, Bodenstein demonstrated that *Drosophila* imaginal discs continue to grow when transplanted into adult abdomens, but only if larval ring glands are co-transplanted.[46,47] Subsequent experiments with *Drosophila* cell lines and lepidopteran and *Drosophila* imaginal discs indicate that 20E stimulates proliferation and initiates differentiation, whereas high levels of JH combined with low levels of 20E inhibit proliferation and differentiation[29,30,37,46] In the *spin* mutant of *Fasciclin2* (*Fas2*), looping of the genitalia and spermiduct is incomplete and genitalia are under-rotated, suggesting that *Fas2* controls organ

Table 1. Overview of life history effects of JH in *Drosophila*

Genotype	JH phenotype	Development	Sexual maturity	Early fecundity	Lifespan	Effect of JH application
Wild-type *D. melanogaster* (Refs. 30,41,54,59,86; Flatt and Kawecki, unpublished)	Normal JH levels	Decline of JH in the presence of 20E induces metamorphosis	JH promotes sexual maturation	JH promotes vitellogenesis, ovary maturation, and egg production	JH suppresses stress responses, innate immunity, and lifespan	Prolongs developmental time, decreases body size at eclosion, increases fecundity, suppresses stress resistance and lifespan
Wild-type *D. melanogaster* in diapause (Refs. 70–72)	JH downregulated in response to cool temperatures and short days	—	—	Ovarian arrest	Extended	Restores fertility; decreases lifespan to nondiapause levels
Abnormal abdomen (*aa*) in *Drosophila mercatorum* (Ref. 40)	decreased JH esterase; presumably increased JH titer	Prolonged	Precocious	Increased	Decreased	—
Insulin-like Receptor (*InR*) mutants (Refs. 77,83)	JH deficient; gene probably involved in JH synthesis	Prolonged	?	Sterile	Extended	Restores fertility; decreases lifespan to wild-type levels
Chico (insulin receptor substrate) mutants (Refs. 78,83–84)	JH deficient; gene probably involved in JH synthesis	Prolonged	?	Sterile	Extended	?
Methoprene-tolerant (*Met*) mutants (Refs. 51,99,109,112)	Insensitive to JH; gene encodes JH receptor or binding protein	Normal?	Delayed	Decreased	?	Insensitivity to physiological and toxic doses of JH

JH exhibits 'pleiotropic' effects upon fly life history. In particular, JH stimulates reproduction, while decreasing lifespan, suggesting that JH is a key mediator of the trade-off between reproduction and survival. The effects of application of JH refer to treating mutant or wild-type flies with the synthetic JH methoprene. For further information see text, the indicated references, or http://flybase.bio.indiana.edu, a database providing information on the *Drosophila* genome.

looping.[48] In these mutants, the synapses connecting neurosecretory cells to the CA are affected, presumably leading to an abnormally high JH level.[48] This allatotropic effect of the *Fas2^spin^* mutant can be mimicked by application of the synthetic JH pyriproxyfen, phenocopying the *Fas2^spin^* phenotype in a dose-dependent manner.[48] Since JH is related to retinoic acid (RA) and since RA regulates left–right asymmetry in vertebrate organs such as the heart, these results may suggest an evolutionarily conserved role of terpenoid molecules such as JH in organ asymmetry, sidedness and looping.[48,49]

JH regulates multiple aspects of reproduction

JH affects all major aspects of insect reproduction,[16,22,23,30,50–55] and environmental and genetic variation in JH signaling may thus cause variation in reproductive fitness.[3,6,14,16] In male *Drosophila*, JH effects on reproduction are poorly understood.[30,51] JH does probably not affect spermatogenesis, but promotes protein synthesis in the accessory gland.[30,51,52] In female *Drosophila*, JH regulates oocyte maturation and reproductive activity.[30,50,54–60] Upon mating, sex peptide contained in the male seminal fluid stimulates JH synthesis in the female CA and increases, presumably through its effect on JH, egg deposition while simultaneously reducing female receptivity.[53]

JH also affects vitellogenesis, involving the synthesis and secretion of yolk proteins (YP1, YP2 and YP3) by fat body and ovarian follicle cells and the uptake of YPs by developing oocytes.[30,54–59] Treatment with 20E or the synthetic JH methoprene upregulates YP transcription in the fat body of starved flies or isolated abdomens expressing low levels of YP,[30,58] and treatment with the JH inhibitor precocene reduces the number of vitellogenic oocytes of newly eclosed females.[59] A role of JH in vitellogenesis might also be reflected in mutants of *cricklet* (*clt*).[60] *clt* mutants have reduced YP synthesis, larval fat bodies persisting into adulthood and ovarian arrest at the previtellogenic stage. Methoprene has no effect on fat body synthesis of YP in these mutants and ovarian transplant experiments indicate that females have sufficient JH to promote oogenesis. It has thus been suggested that *clt* encodes a JH receptor or binding protein that is nonfunctional in mutants.[60] JH also regulates uptake of YPs into developing oocytes. Mutations in *apterous* (*ap*) cause sterility due to nonvitellogenic ovaries. The CA of these mutants are JH deficient and methoprene application results in vitellogenic oocytes.[61–64] While *ap^4^* mutants have normal hemolymph levels of YP, YPs are only taken up into oocytes if flies are treated with methoprene.[63]

The vitellogenic effects of JH might be indirect, since vitellogenesis can occur in the absence of JH, but never in the absence of 20E.[65,66] Under this model, JH regulates early YP synthesis and uptake and stimulates ovarian 20E production; in the absence of JH, 20E controls late fat body synthesis of

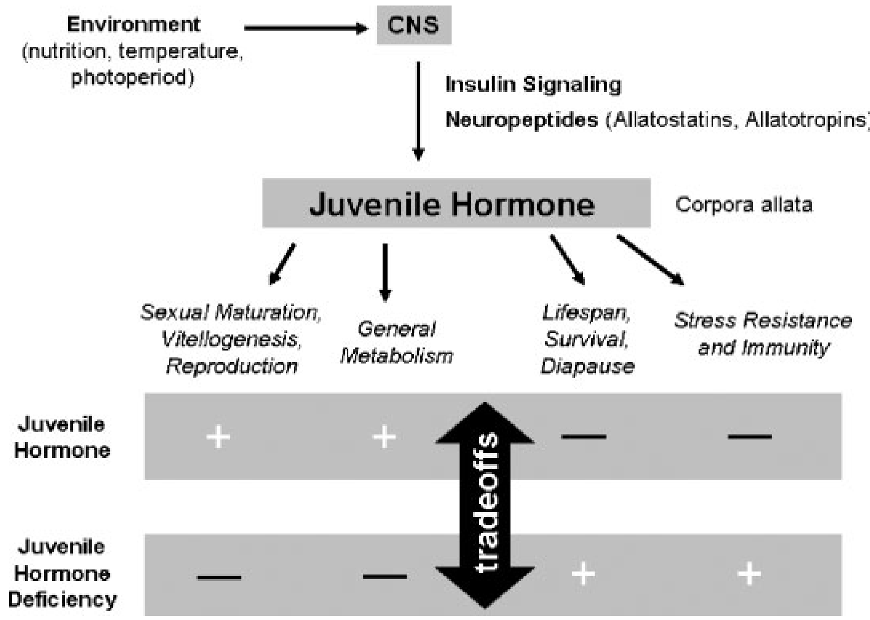

Figure 3. JH regulation of *Drosophila* life history. In response to environmental factors such as nutrition, temperature, and photoperiod, JH synthesis and signaling are modulated by insulin signaling and regulatory neuropeptides such as allatostatins (JH inhibitory neuropeptides) and allatotropins (JH stimulatory neuropeptides). JH in turn coregulates metabolism, reproduction, stress resistance, immune function, and lifespan. In particular, JH signaling promotes overall metabolism and reproductive functions, but suppresses stress resistance, innate immunity, and lifespan, suggesting that trade-offs between these traits are mediated by JH. For example, treatment of wild-type *Drosophila* with the synthetic JH methoprene significantly prolongs developmental time, reduces body size at eclosion, shortens adult lifespan, and yet increases early fecundity (Flatt and Kawecki, unpublished).

YPs.[65,66] This is consistent with the finding that provision of poor food to females at eclosion prevents the increase in vitellogenic-stage follicles and ovarian 20E synthesis, yet methoprene treatment elicits a transient burst of 20E synthesis and restores oocyte production.[67] However, JH and 20E do not always cooperate in promoting egg development. 20E can act as an antagonist of early vitellogenic oocyte development.[68] While 20E application induces nurse cell apoptosis in stage 9 egg chambers, simultaneous methoprene application protects vitellogenic oocytes from the 20E-induced resorption, indicating that JH and 20E act antagonistically to control whether oocytes will progress or undergo apoptosis.[68]

The role of JH in egg development is also manifest in two JH-inducible genes, *JhI-21* and *minidiscs* (*mdn*), whose expression during oogenesis is JH-dependent.[69] Both gene products are expressed in ovarian nurse cells and sequestered into mature eggs; methoprene induces expression of both genes and accumulation of their gene products in the ovaries. In JH-deficient *ap* mutants, levels of *mnd* and *JhI-21* are strongly reduced, but methoprene treatment rescues this defect.[69] *mnd* and *JhI-21* encode amino acid transporters and amino acid availability might be critical for egg development.

JH mediates reproductive diapause

In response to stressful environmental conditions, adult insects can enter a state of reproductive diapause (also called quiescence or dormancy), characterized by (1) reduced metabolism, (2) arrested oogenesis, mating and egg production, (3) increased stress resistance, and (4) enhanced survival.[65,70–73] Interestingly, in butterflies (*Danaus plexippus*) and several species of grasshoppers and *Drosophila*, traits specific to reproductive diapause (arrested oogenesis, stress resistance, negligible senescence) are controlled by JH.[65,70–73] For example, diapausing *Drosophila* females have downregulated JH synthesis, exhibit ovarian arrest and show reduced age-specific mortality as compared to synchronously started cohorts of non-diapausing females.[70,71,73] This diapause phenotype can be rescued by methoprene, which terminates ovarian arrest, increases sensitivity to oxidative stress and reduces post-diapause longevity (Table 1).[71] Reproductive dormancy may however not be exclusively regulated by JH. While treating diapausing females with JHIII or JHB3 restores vitellogenesis,[72] warming flies from 11°C to 25°C also terminates diapause and results in an increase of ecdysteroid, but not JH synthesis.[66] More importantly, 20E application can also elicit vitellogenesis in diapausing flies, suggesting that both 20E and JH affect diapause termination.(65,66) Reproductive diapause is an example of adaptive, hormonally mediated life history plasticity. Since JH regulates both fecundity and longevity in a phenotypically plastic and antagonistic way, JH might be an important mediator of senescence plasticity and the trade-off between reproduction and longevity (see Table 1, Fig. 3 and Refs. 70,71,73).

JH is a pro-aging hormone downstream of insulin signaling

Insulin signaling is of major importance for regulating energy metabolism, growth, reproduction and longevity in *Caenorhabditis elegans*, rodents and *Drosophila* (Fig. 4; Refs. 73–82). In response to environmental or internal stimuli, *Drosophila* produces several insulin-like peptides (DILPs, encoded by *dilp*1–7) in median neurosecretory cells (insulin producing cells, IPCs) of the pars intercerebralis of the brain. DILPs are secreted into the protocerebrum, at the corpora cardiaca and into the hemolymph and then bind to the insulin-like receptor (INR) at target tissues, including the gonads and the fat body.(74–76) Female flies mutant for *InR* are dwarf, have immature ovaries, are stress-resistant and are extremely long-lived.(77) Strikingly, diapausing *Drosophila* also show ovarian arrest, increased longevity and improved stress resistance, suggesting that diapausing flies 'phenocopy' *InR* mutant phenotypes.(70,71,73,76,77)

Remarkably, for both diapausing *Drosophila* and reduced insulin signaling mutants, survival and reproduction in the fly is proximately regulated by JH, apparently a secondary hormone downstream of insulin signaling (see Fig. 4 and Table 1).(70,73,76,77,83) Several *InR* mutant genotypes are deficient in JH biosynthesis,(76,77,83) and, while *InR* mutant females are infertile with nonvitellogenic ovaries, egg development can be restored by methoprene application.(77)

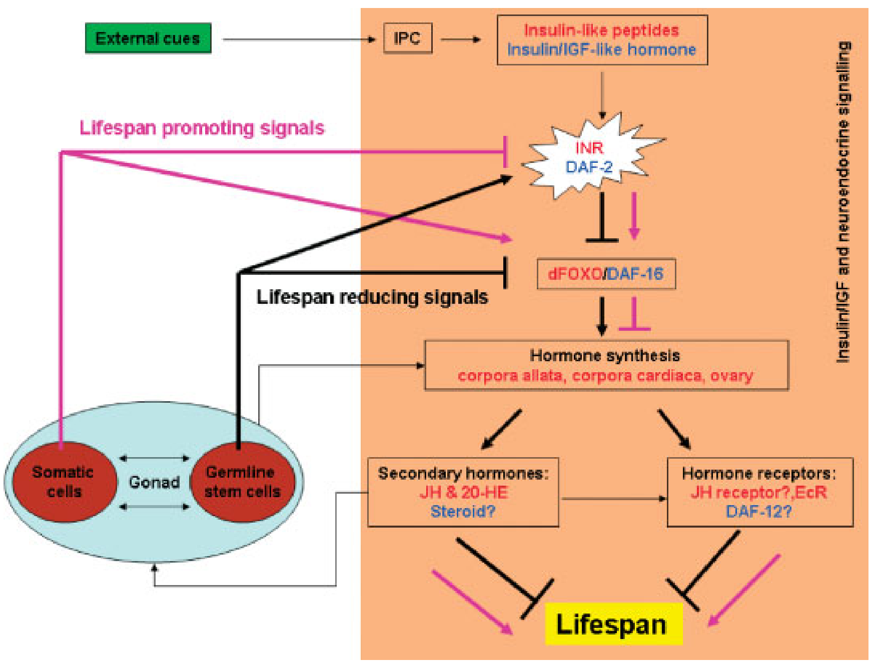

Figure 4. Integrated model for the endocrine regulation of aging, based on similarities between *C. elegans* and *D. melanogaster*.(73,76) External cues like nutrition stimulate insulin producing cells (IPCs) to secrete insulin-like peptides which, in turn, induce insulin/IFG-1 signaling in the CNS by binding to insulin-like receptors (INR in *D. melanogaster* and DAF-2 in *C. elegans*).(73–76) Induction of insulin/IFG-1 signaling suppresses a forkhead transcription factor (dFOXO in *D. melanogaster*, DAF-16 in *C. elegans*).(73,76,79) As in worms, aging in flies is slowed when insulin signaling is reduced: lifespan is extended by mutations in *insulin-like receptor* (*InR*, the *daf-2* homolog) and *chico* (the insulin receptor substrate).(76–78,84) Similarly, activation of dFOXO (the DAF-16 homolog), normally suppressed by insulin signaling, extends lifespan.(73,82) Remarkably, in worms, germline stem cell ablation extends lifespan by 60%, and this lifespan extension requires functional DAF-16.(79–81) Thus, signals from the germline may provide a positive feedback to the CNS and the endocrine system, presumably by suppressing dFOXO/DAF-16 and by activating unknown secondary pro-aging hormones.(79–81) Remarkably, in *Drosophila*, JH is reduced in long-lived fly mutants of *InR* and *chico*, and methoprene treatment restores the extended mutant lifespan to normal levels.(73,76,77,83) Furthermore, long-lived *Drosophila* insulin-signaling mutants recapitulate the phenotype of diapausing flies. Strikingly, diapausing wild-type flies have downregulated JH and extended lifespan,(70,71) and methoprene application reduces lifespan to non-diapause levels. JH might thus be a secondary pro-aging hormone downstream of insulin signaling.(73,76,77,83)

Methoprene treatment of long-lived and JH-deficient dwarf females also restores life expectancy towards that of wild type, suggesting that JH has lifespan-shortening effects.[77] Similarly, mutants of the insulin receptor substrate *chico* are long-lived, sterile dwarfs[78,84] and exhibit JH synthesis deficiency (Ref. 83 but also see Ref. 85, Table 1). The notion that JH is a pro-aging hormone is also supported by the observation that surgical removal of the CA extends lifespan in grasshoppers and butterflies (discussed in Ref. 70).

While nematodes lack a recognizable JH and the secondary pro-aging hormones in worm insulin signaling are unknown,[76] both diapause and lifespan are controlled by insulin signaling in *C. elegans*. Worms with mutations in *daf-2* (*dauerformation 2*), the *C. elegans* homolog of *InR*, can bypass dauer diapause (a nonfeeding, stress resistant larval state) and exhibit extended adult lifespan.[70,71,73,76,77] The regulation of life history (growth, diapause, reproduction, lifespan) by insulin-like signals and downstream hormones thus seems to be evolutionarily conserved (see Fig. 4; Refs. 70,71,73,76,77).

JH suppresses stress resistance and immunity

In many organisms, aging is accompanied by decreased stress resistance and immune function. Remarkably, JH not only has pro-aging effects, but also suppresses stress resistance and innate immunity,[71,73,86–88] important components of Darwinian fitness. Reproductively dormant *Drosophila* with downregulated JH exhibit greater resistance to heat and oxidative stress than nondiapausing flies,[71] and methoprene treatment of female *D. melanogaster* increases the number of vitellogenic oocytes, while decreasing resistance to oxidative stress and starvation resistance.[86] In the mealworm beetle (*Tenebrio molitor*), the levels of phenoloxidase (PO), an antimicrobial agent, is reduced by mating and application of the JH inhibitor fluvastatin increases immune function.[88] Similarly, the trade-off between immune function (PO levels) and sexual advertisement (pheromone production) is mediated by JH in this species.[87] Whether JH regulates immunity in *Drosophila* requires formal study—preliminary results from our laboratory suggest that JH functions to suppress expression of genes involved in defense and stress response, including several antimicrobial peptides (see Ref. 73 and unpublished observations). By increasing reproduction at the expense of stress resistance, immunity and longevity, JH may thus be an important proximate mechanism underlying evolutionary trade-offs between reproductive and survival functions (Table 1, Figs. 3, 4).

JH modulates neuroanatomy and behavior

Insect hormones such as JH and 20E also affect neuroanatomy, behavior, learning and memory.[22,23,89] In crickets (*Acheta domesticus*), JH promotes neuroblast proliferation in the mushroom bodies, brain structures important for learning and memory.[90,91] In honeybees (*Apis mellifera*), JH treatment has profoundly positive effects on the maturation of short-term olfactory memory.[92] JH-treated bees show very good 1-hour short-term associative memory and perform consistently better than untreated individuals for at least their first week of life.[92]

In *Drosophila*, our knowledge of neuronal and behavioral hormonal effects is much more limited. While 20E in *Drosophila* is known to alter mushroom body and axonal growth, nerve terminal development and the function of the neuromuscular junction,[93] the neurodevelopmental effects of JH have not received much attention. A direct neuronal role of JH comes from experiments applying JH analogs. Methoprene treatment at metamorphosis disrupts a fly's neuroanatomy and pyriproxyfen treatment of prepupae and pupae alters the shape and complexity of the adult dendritic tree of sensory neurons in a time-dependent manner.[44,94]

The behavior of the fly, in particular its courtship and mating behavior, is also affected by JH. For example, Manning in 1966 implanted CA–CC complexes into pharate adult females and found that this causes females to become precociously receptive to courting males, approximately 24 hours earlier than normal.[95] In JH-deficient *apterous* mutants, females exhibit abnormally reduced receptivity to courting males. Receptivity positively correlates with JH production among 17 *ap* genotypes, suggesting that low JH reduces receptivity.[96] Similarly, JH affects the reproductive behavior in the Carribean fruit fly (*Anastrepha suspensa*): mated 7-day-old males produce three times more JH than 7-day-old virgin males; topical JH or methoprene application enhances sexual signaling, pheromone release and mating at an earlier age as compared to control males.[97] Another example comes from sexually dimorphic locomotory behavior in *Drosophila*, controlled by a few neurons in the pars intercerebralis.[98] Male locomotor behavior can be feminized by transplanting a few specific pars intercerebralis neurons from a female into a receiving male. Remarkably, this feminization can be mimicked by feeding males with the JH inhibitor fluvastatin; the effect is reversible by simultaneous application of methoprene, suggesting the control of the behavior by JH.[98]

The elusive nature of the JH receptor

While the search for a JH receptor has yielded very limited results, as recently discussed by Wheeler and Nijhout in this journal,[25] we have two interesting candidate genes for the receptor.[25,26,29,99] The *Drosophila* nuclear hormone receptor gene *ultraspiracle* (*usp*)[100–104] encodes a retinoid X receptor,[100,101] and JH is closely related to retinoic acid.[49] Interestingly, USP indeed binds JH[100,103] and forms a heterodimer with the ecdysone receptor (ECR).[26,27,101] USP is an essential component of ECR since it is required for ECR activity in vivo;[101] for instance, both ECR and USP are required for regulating the timing and progression of ovarian differentiation during metamorphosis.[102] Interestingly, JH has now been reported to act as a USP ligand suppressing 20E-dependent ECR transactivation.[104] However, USP does not fulfill the criterion of high-affinity hormone binding typically required for a hormone receptor.[26,29]

Another candidate for the JH receptor is the X-linked gene *Methoprene-tolerant* (*Met*; also called *Resistance to Juvenile Hormone*, *Rst(1)JH*), a member of the basic helix–loop–helix (bHLH-PAS) family of transcriptional regulators.[26,44,99,105–112] Mutations at *Met* confer resistance to JH- or methoprene-induced lethality and external morphogenetic abnormalities and alter JH reception during late larval development.[105,106,109] The fat body cytosol of mutants has a 10-fold lower binding affinity for JH III than wild type,[106] and a new study reports that MET directly and specifically binds JH III (as well as other JHs and methoprene) with high binding affinity.[111] Although MET does not belong to the nuclear hormone receptor family, it is thought to transduce JH signals.[26,105–109] MET may function as a JH-dependent transcription factor since JH can activate the expression of a reporter gene fused to MET in a transient transfection assay using *Drosophila* S2 cells.[111] In addition, *Met* affects a number of life history traits, perhaps supporting the notion that MET is a JH receptor (Table 1; Refs. 51,99,109,112). However, some tissues showing MET expression are not known JH targets and *Met* null mutants show no apparent defects in embryogenesis or larval development, as one would expect if MET is a JH receptor.[29,108]

Since both USP and MET are somewhat problematic candidates for the JH receptor, it remains possible that the JH receptor does not fit classical models of hormone receptor function. Indeed, several lines of evidence indicate that JH may function through membrane (rather than nuclear) receptors and protein kinase C (PKC) signaling.[25] Clearly, the elusive nature of the JH receptor limits our current understanding of JH signaling and its role in life history evolution.[25,26,29,99]

Evolutionary modifications of hormone signaling

How might evolution alter developmental outcomes and life history through evolutionary changes in endocrine mechanisms? And how can the remarkable life history variation among insects be reconciled with the employment of the same major hormone? During evolutionary time, the same hormones have often been functionally co-opted multiple times.[11,13,16,37,43] For example, while JH has many evolutionarily conserved functions, the details of JH function can be remarkably variable among species, as is the case for reproduction.[16] Evolutionary modifications of the hormone response may be facilitated by its modular structure.[11,13,16] Not all tissues or cells are hormone-responsive, for example when they do not express the appropriate hormone receptor,[11,13,16,37] and different responsive tissues or cells may have different hormone sensitivities.[11,13,16,37] In particular, hormone responses may be both spatially and temporally heterogeneous, for instance due to variation in tissue- and cell-specific patterns of hormone receptor expression.[11,37] Evidence for evolutionary variation in hormone receptor expression is now available for the case of the EcR/Usp complex in the ovaries of gall midges (discussed in Refs. 13 and 16) and is suggested by a study of *Met* in *Drosophila*.[99]

Conclusions

Here we have illustrated that JH is a remarkably versatile molecule with major effects on various aspect of development and life history in *Drosophila* and other insects, including many components of Darwinian fitness. In particular, we have reviewed evidence suggesting that JH is an important mediator of life history trade-offs, not only in *Drosophila*, but also in grasshoppers, butterflies and beetles. Although the effects of JH signaling can vary greatly among species (e.g. Refs. 16,22, 23,29) JH typically has negative effects on stress resistance, immune function and lifespan, yet exerts positive effects on reproduction. This suggests that the life history effects of JH may be evolutionarily conserved among insect species.

Life history variation caused by genetic polymorphisms (e.g. as seen in insulin signaling mutants) or phenotypic plasticity (e.g. as seen in diapausing insects) may involve the same endocrine mechanisms. In the case of reproductive diapause and senescence plasticity, insects use JH to coordinate and trade off, the expression of ‘reproductive functions’ versus ‘survival functions’ in response to environmental cues. Remarkably, this phenotypically plastic diapause syndrome is constitutively recapitulated in JH-deficient insulin signaling mutants and JH is now known to be a downstream effector of insulin signaling affecting both reproduction and lifespan. Since hormones such as JH affect multiple traits (hormonal pleiotropy), endocrine loci (e.g. *InR, Met*) may thus exhibit a remarkable degree of genetic pleiotropy. This functional versatility may be caused by alleles with different effects on endocrine signaling (e.g. as seen for different alleles of *InR* or *Met*), with a given hormone affecting multiple physiological and developmental aspects of the phenotype. Hormonal loci are thus promising candidate genes underlying life history and its phenotypic integration, and environmental or genetic variation in endocrine signaling might be an important substrate for life history evolution.

As advocated by Heyland et al. in a recent issue of BioEssays, hormones in development and evolution deserve increased attention from evolutionary biologists.[13] While there has not yet been sufficient interdisciplinary crosstalk, the combined study of endocrinology, developmental genetics, and evolutionary biology promises to yield fascinating insights into the development, functional architecture, and evolution of complex phenotypes. In particular, we are now able, using the tools of genetics and endocrinology, to open the black-box of life history evolution and fill it with mechanism.

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