Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Insect Science

The distinct roles of insulin signaling in polyphenic development

H Frederik Nijhout and Kenneth Z McKenna

Many insects have the ability to develop alternative morphologies in response to specific environmental signals such as photoperiod, temperature, nutrition and crowding. These signals are integrated by the brain and result in alternative patterns of secretion of developmental hormones like ecdysone, juvenile hormone and insulin-like growth factors, which, in turn, direct alternative developmental trajectories. Insulin signaling appears to be particularly important when the polyphenism involves differences in the sizes of the body, appendages and other structures, such as wings, mandibles and horns. Here we review recent advances in understanding the role of insulin signaling, and its interaction with other hormones, in the development of polyphenisms.

Address
Department of Biology, Duke University, Durham, NC 27708, USA

Corresponding author: Nijhout, H Frederik (hfn@duke.edu)

Current Opinion in Insect Science 2018, **25**:58–64

This review comes from a themed issue on **Development and regulation**

Edited by **David Angelini** and **Yuichiro Suzuki**

For a complete overview see the Issue and the Editorial

Available online 26th November 2017

https://doi.org/10.1016/j.cois.2017.11.011

Introduction

Polyphenisms are a specialized form of phenotypic plasticity in which insects develop discrete alternative phenotypes in response to specific environmental signals. The seasonal forms of butterflies, the castes of social insects, and the winged/wingless and sexual/parthenogenetic forms of aphids are among the well-known examples. The alternative forms are believed to have adaptations that suit them to alternative environments. Polyphenic development in insects has several well-defined stages. First there is a period during which the environmental signal is received and integrated into the nervous system. This is followed by a period during which the alternative morphology and physiology develop.

The relevant environmental signal that induces the switch to an alternative developmental pathway is species-specific and can include day length, temperature, nutrition and pheromones. Environment-sensitive periods usually occur in the larval stage, often in the last larval instar, and sometimes in earlier instars. The actual developmental switch, by contrast, typically occurs at the time of molting, and usually during the metamorphic molts.

Little is known about how the environmental information is stored, but it must involve some change in the central nervous system, probably the brain. The switch to alternative developmental pathways that follows environmental induction has been shown to be controlled by hormones in all cases that have been studied. Juvenile hormone (JH) and ecdysone are the most common hormones involved in controlling the polyphenic switch.

Both JH and ecdysone control gene expression by binding to receptors that act as transcriptional regulators. Secretion of ecdysone and JH are controlled by the brain's neuroendocrine hormones: the prothoracicotropic hormone (PTTH) controls ecdysone secretion and allatotropins and allatostatins control the secretion of JH [1–5]. It can therefore be said that the brain ultimately controls the alternative developmental pathways that lead to the polyphenism.

Changes in the temporal pattern of secretion of ecdysone and JH lead to changes in the pattern of gene expression, which presumably alters the biochemical, physiological and developmental processes that lead to a particular phenotype. In the seasonal polyphenism of *Junonia coenia* a 24-h shift in ecdysone secretion in the pupal stage is responsible for the alternative color pattern phenotypes. There is a 28-h long critical period for color pattern development during the early pupal stage in *Junonia*, and if ecdysone is secreted during this period the pale *linea* morph develops, whereas if ecdysone is secreted later, the dark *rosa* form develops [6]. Ecdysone is also involved in seasonal color pattern polyphenism in *Bicyclus anynana* [7,8]. JH controls a much broader range of polyphenisms including horn polyphenism in the beetle *Onthophagus taurus* [9], queen caste determination in honeybees [10,11], the soldier caste in the ant *Pheidole bicarinata* [12,13] and lower termites [14], and wing polyphenism in planthoppers [15]. In each of these cases, JH acts during a relatively brief critical period, so that one morph develops if JH is absent (or below a threshold), and a different morph develops if JH is present (or above a threshold) during the critical period.

A role for insulin signaling

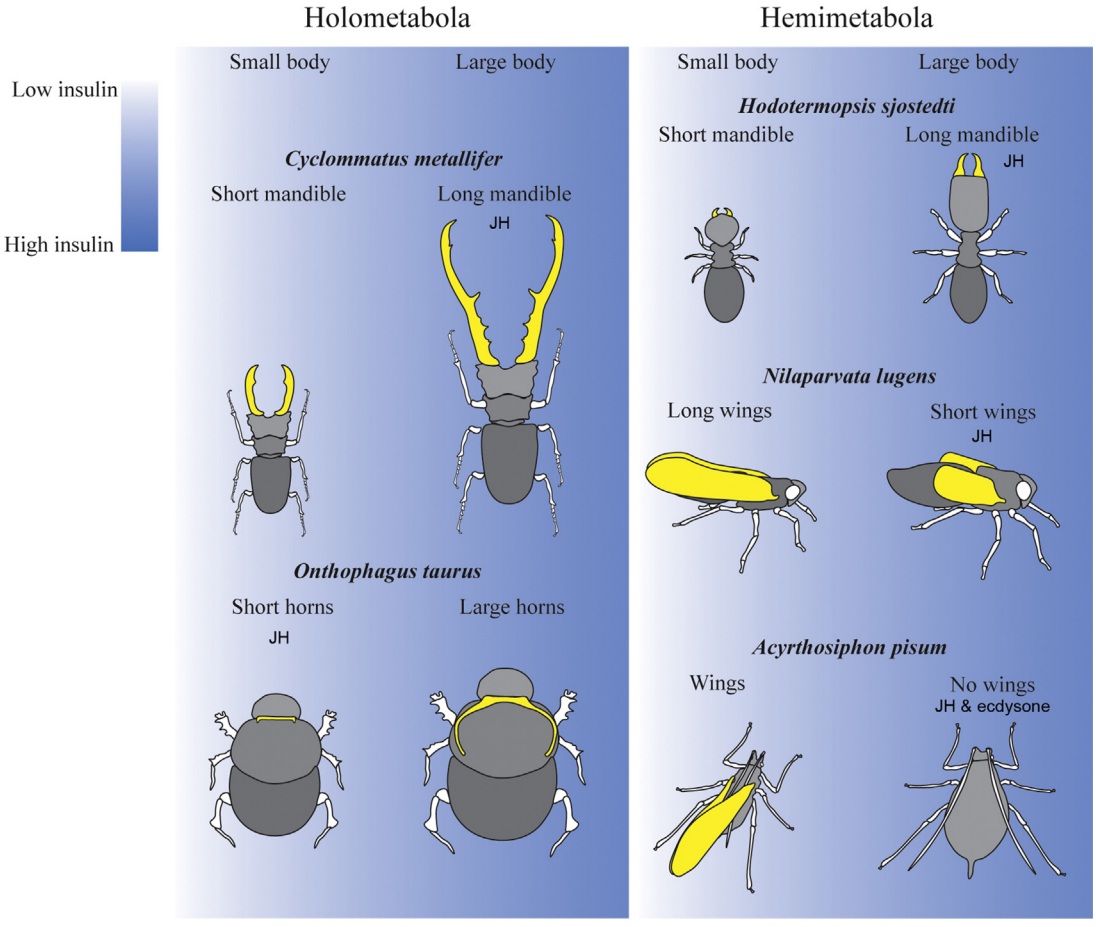
Although polyphenic control by ecdysone and JH has long been known, it is not at all clear exactly what processes are affected by these hormones that eventually lead to the alternative phenotypes. The overall changes in gene expression after hormone treatment are complex, involving hundreds of genes [16–18], and have been relatively uninformative as to mechanism by which morphological differentiation occurs. Targeted studies aimed at specific candidate genes or suspected players in the causal mechanism of polyphenic development, by contrast, have been more informative. For instance the development of polyphenic horns in beetles involves the recruitment of several limb patterning genes that are expressed in the prepupal stage at the locations where the horns will grow out [19].

Other targeted studies in recent years have made it clear that insulin signaling plays an important role in polyphenic development in some systems. Insulin signaling appears to be particularly important when the polyphenism involves differences in the growth and sizes of body, appendages and other structures, such as wings, mandibles and horns (Figure 1). Intuitively, insulin signaling must be correlated with body size, yet how such variation is related to variation in ecdysone and JH signaling to cause the development of alternative morphs is not at all understood. In this article, we review the distinct roles of insulin signaling in hormone biosynthesis and tissue morphogenesis in polyphenic development.

Honeybees

In honeybees queen development is known to be controlled by JH [10,11]. Queen-destined larvae receive higher nutrition in the form of royal jelly, and have higher JH titers during a critical period around 40–48 h after hatching [20–22]. It appears that nutrition raises JH in queen-destined larvae via the insulin signaling pathway. TOR, INR, AKT, and FOXO expression are elevated by 40 h after hatching, which is prior to the period where JH rises [23••]. RNAi knockdown of IRS or TOR lowers JH levels [24], and TOR knockdown inhibits queen development [25]. These findings suggest that enhanced

Figure 1



Examples of polyphenisms, controlled by insulin signaling, that affect the sizes of body and body parts. Phenotypes that also require juvenile hormone (JH) or ecdysone signaling at critical periods are indicated.

nutrition stimulates insulin signaling, which, in turn, enhances JH secretion. JH is known to induce massive changes in gene expression during the worker/queen switch [17,23••,26], and may also be responsible for altered patterns of DNA methylation associated with this switch [27]. Interestingly, later in larval development, at around 88 h, developing queen larvae that are switched to a lower-nutrition worker-inducing diet upregulate expression of components of the IIS/TOR pathway, but developing worker larvae that are switched to the higher nutrition queen diet do not. This suggests that insulin signaling mediates a homeostatic mechanism that buffers any changes in nutrition that might occur once a larva is set on either a queen or a worker developmental pathway [23••].

Termites

Soldier development in termites is regulated by JH [22,28]. Soldiers in termites have exceptionally large heads and large mandibles. JH levels on the termite *Hodotermopsis sjostedti*, are high prior to a molt to the soldier, and application of a JH mimic during a critical period of the pseudergate larva induces soldier morphology [14]. In *Reticulitermes*, soldiers produce a soldier-inhibiting pheromone that inhibits the transformation of pseudergates into soldiers by suppressing JH levels [29]. Differentiation of some soldier characters require insulin signaling. In *Hodotermopsis* the insulin receptor is highly expressed in mandibular tissue of developing soldiers, and RNAi suppression of the insulin receptor blocks growth and elongation of soldier-specific mandibles [30••].

Aphids

Aphids have a dispersal polyphenism with winged and wingless morphs [31]. Depending on the species, winged morphs are induced by crowding, short day photoperiod, low temperature and diminished food quality, all indicative of a deteriorating environment. The alate/apterous polyphenism of the black bean aphid, *Aphis fabae*, and the pea aphid, *Acyrthosiphon pisum*, is controlled by JH in the sense that exogenous JH can induce the apterous form [32–34].

In the vetch aphid *Megoura crassicauda* presumptive wingless nymphs had significantly higher JH titers than presumptive winged nymphs [35]. And in presumptive winged nymphs exogenous JH partially inhibited wing development resulting in the development of winged/wingless intermediates but also of juvenilized individuals [35]. These findings suggest that JH can suppress development of wings. There is also a role for ecdysone in the pea aphid, *Acyrthosiphon pisum*. Morph determination occurs while the developing larva is still within the oviduct of the mother. Feeding of ecdysone or injection of an ecdysone analog produced fewer winged offspring. Injection of cucurbitacin B (an ecdysone receptor antagonist), and EcR RNAi, produced more winged offspring [36••]. These results indicate that wing development can be induced by maternally supplied ecdysone.

Insulin signaling may play an indirect role in the development of winged and wingless morphs in aphids. Gene products involved in insulin signaling are more highly expressed in the head and thorax of wingless morphs [37]. RNAi of insulin-related peptide 5 (IRP5) in embryos resulted in a smaller body size of wingless nymphs, which is a characteristic feature of winged nymphs [37]. Thus the larger body size of wingless aphids may be regulated in part by insulin signaling.

Beetles

Many scarab and stag beetles have a sex-limited male polyphenism. Males develop large horns or large mandibles, and the size of these structures is non-linearly related to male body size. The nonlinearity of the allometry produces an apparent discontinuity in trait size so that larger males have disproportionally large horns or mandibles and smaller males disproportionally small ones. JH affects horn development in the dung beetle *Onthophagus taurus* in two different ways. During a critical period in the feeding phase of the last instar larva JH acts by reprogramming body growth to stop at a larger body size, so that larger horns develop, and during a second critical period in the prepupal stage JH suppresses horn development [9,38]. In the stag beetle *Cyclommatus metallifer*, JH titers are positively correlated with nutrition, and treatment with exogenous JH increases mandible length in males, but not in females [39].

Sex determination, nutrition, and hormones interact in a complex way. Doublesex (Dsx), a major gene involved in sex differentiation, is differentially upregulated in the head and thorax of larger males of *Onthophagus*, and particularly in the region of horn development [40], and RNAi suppression of Dsx represses horn development in larger males [41]. In *Cyclommatus*, Dsx is also involved in the sex-specific regulation of mandible growth [39]. Dsx is upregulated in the mandibles of larger males and RNAi of Dsx during the prepupal stage yielded males with relatively smaller mandibles [42••]. Gotoh *et al.* [42••] provide a model of how sex determination, nutrition and JH interact in the control of mandible size that may also be applicable to horn size. They suggest that nutrition enhances JH levels as well as insulin signaling in both males and females, and that both hormones potentially stimulate growth of the mandibles. Dsx, in turn, inhibits the effect of JH on mandible growth in females, but stimulates the effect of JH and insulin on mandible growth in males.

Planthoppers

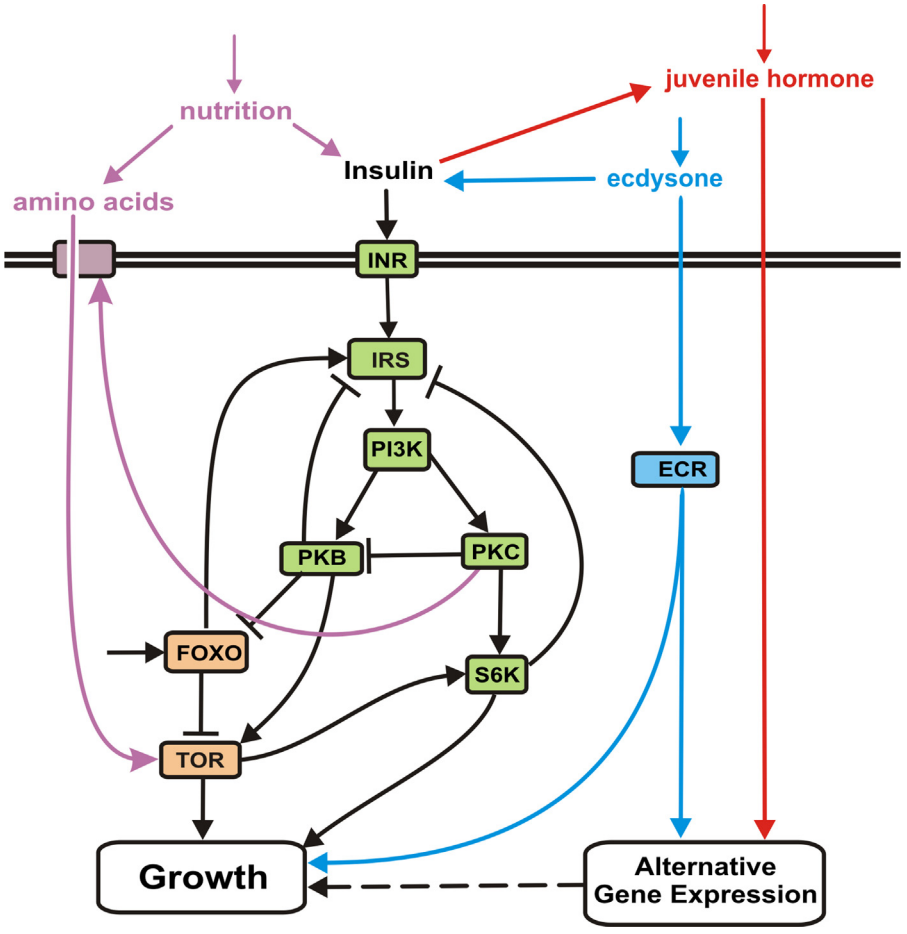
The brown planthopper, *Nilaparvata lugens*, has a long wing/short wing polyphenism that is induced by external

factors like population density, but there is also a strong genetic component with some populations being largely long-winged or short winged [15]. Wing length development is mediated by JH. Treatment of a strain selected to be long-winged with JH results in short-winged adults [43]. Conversely, treatment of a strain selected for having short wings with precocene II (an allatotoxic inhibitor of JH) during a critical period around the molt from the 3rd to 4th nymphal instar results in long-winged adults, and co-treatment with JH prevents this effect [44]. In addition to JH, insulin signaling is also involved in wing-length development in *Nilaparvata*, but in a quite unexpected way. Recently it was discovered that there are two insulin receptor isoforms, InR1 and InR2. Signaling through InR1 leads to the development of the long-winged morph, and RNAi of InR1 produces the short-wing morph. InR2 suppresses the effect of InR1, probably via heterodimerization, and RNAi of InR2 leads to the development of the long-wing morph [45••]. Thus an unusual interaction at the level of the insulin receptor manages the switch between alternative morphs.

Synthesis and interpretation of the role of insulin signaling in polyphenisms

The effects of JH, ecdysone and insulin, and the interactions among these hormones are complex. The mechanism by which JH differentially activates tissue specific response pathways is unclear. All tissues must be more or less sensitive to JH for the regulation of metamorphosis, and this is probably why JH acts during restricted critical periods that separate its role in tissue specific switching to an alternative morph from its role in the maintenance of larval characters. Exactly what determines a period of

Figure 2



Hormonal pathways that control insect polyphenisms. Juvenile hormone (JH) and ecdysone are stimulated by the brain in response to environmental signals. Both hormones can alter patterns of gene expression and directly or indirectly affect growth of specific tissues. In feeding insects, nutrition stimulates secretion of insulin, and in non-feeding stages insulin secretion is stimulated by ecdysone [56]. Insulin can stimulate the secretion of JH and also stimulates tissue-specific growth by stimulating the uptake of amino acids into cells and by activating the cell's protein synthesis machinery via PKB, S6K, FOXO and TOR. (Abbreviations: INR, insulin receptor; IRS, insulin receptor substrate; PI3K, phosphoinositol kinase; PKB, protein kinase B; PKC, protein kinase C: S6K, ribosomal protein S6 kinase; FOXO, forkhead transcription factor type O; TOR, target of rapamycin; ECR, ecdysone receptor).

JH-sensitivity is still unknown, but it is likely to involve a changing temporal and spatial pattern of expression of receptors and proteins that interact with JH. This has been poorly studied.

Insulin appears to have two roles in the pathways that control polyphenic development. First, insulin signaling is involved in the nutrition-dependent secretion of hormones like JH, which are the immediate triggers for the alternative developmental pathways. Second, insulin signaling is involved in the response pathway of hormone-induced growth in cases where the morphs differ in the sizes of traits like wings, mandibles or horns. Both these features of insulin signaling, its response to nutrition and its stimulation of growth are well-established [46–50]. Extracellular amino acids stimulate insulin secretion, and one of the responses to insulin signaling is an increase in the rate of amino acid import into the cell, at least in vertebrates [51–55], but this has not yet been thoroughly studied in insects. Amino acids that enter the cell activate TOR, which, in turn activates protein synthesis required for growth. TOR is also regulated by the insulin signaling cascade via Akt and FOXO (Figure 2).

Much remains to be understood about how insulin and other hormones interact to control polyphenisms. We see many interesting targets for future research and pose these in the form of questions to be answered, in no particular order. (1) What determines a critical period for hormone action? (2) Why are there so many insulins but only one (or two) receptors? (3) Is insulin signaling systemic or autocrine/paracrine (or both)? (4) Do amino acids stimulate insulin secretion in insects? (5) What is the mechanism by which nutrition regulates the JH titer? (6) Is insulin signaling sufficient to explain growth? (7) Does any insulin-like peptide acting on the same receptor in the same cell produce the same effect? (8) Do different cells or tissues respond differently to the same insulin, and why? (9) Is it possible develop an *in vitro* system to study polyphenism? (10) How is environmental information stored? (11) What controls the neuroendocrine secretion of insulin? (12) How is JH secretion controlled? (13) How do the hormonal mechanisms that induce polyphenisms facilitate morphological divergence and speciation? These diverse questions highlight the great biological scope of interest in polyphenisms that is not restricted to just its hormonal control, but poses many questions that are of general interest in development and evolution.

Acknowledgements

Supported by grants IOS-0744952 and IOS-1557341 from the National Science Foundation. We thank Laura Grunert Sachiko Kobayashi and Anna Kudla for critical comments on the manuscript.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Kataoka H, Toschi A, Li JP, Carney RL, Schooley DA, Kramer SJ: **Identification of an allatotropin from adult *Manduca sexta***. *Science (New York, NY)* 1989, **243**:1481-1483 PubMed: https://www.ncbi.nlm.nih.gov/pubmed/17839751.

2. Kramer SJ, Toschi A, Miller CA, Kataoka H, Quistad GB, Li JP, Carney RL, Schooley DA: **Identification of an allatostatin from the tobacco hornworm *Manduca sexta***. *Proc Natl Acad Sci* 1991, **88**:9458-9462 ISSN: 0027-8424, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/1946359.

3. Li S, Ouyang YC, Ostrowski E, Borst DW: **Allatotropin regulation of juvenile hormone synthesis by the corpora allata from the lubber grasshopper, *Romalea microptera***. *Peptides* 2005, **26**:63-72 ISSN: 0196-9781, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/15626505.

4. Nijhout HF: *Insect Hormones*. Princeton, NJ: Princeton University Press; 1994.

5. Nijhout HF: **Development and evolution of adaptive polyphenisms**. *Evol Dev* 2003, **5**:9-18 ISSN: 1520-541X, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/12492404.

6. Rountree DB, Nijhout HF: **Hormonal control of a seasonal polyphenism in Precis coenia (Lepidoptera: Nymphalidae)**. *J Insect Physiol* 1995, **41**:987-992 ISSN: 0022-1910.

7. Koch P, Brakefield P, Kesbeke F: **Ecdysteroids control eyespot size and wing color pattern in the polyphenic butterfly *Bicyclus anynana* (Lepidoptera: Satyridae)**. *J Insect Physiol* 1996, **43**:223-230 ISSN: 0022-1910.

8. Kooi RE, Brakefield PM: **The critical period for wing pattern induction in the polyphenic tropical butterfly *Bicyclus anynana* (Satyrinae)**. *J Insect Physiol* 1999, **45**:201-212 ISSN: 0022-1910, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/12770367.

9. Emlen DJ, Nijhout HF: **Hormonal control of male horn length dimorphism in *Onthophagus taurus* (Coleoptera: Scarabaeidae): a second critical period of sensitivity to juvenile hormone**. *J Insect Physiol* 2001, **47**:1045-1054 ISSN: 0022-1910, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/11472767.

10. Dietz A, Hermann HR, Blum MS: **The role of exogenous JH I, JH III and anti-JH (precocene II) on queen induction of 4.5-day-old worker honey bee larvae**. *J Insect Physiol* 1979, **25**:503-512 ISSN: 0022-1910.

11. Wirtz P, Beetsma J: **Induction of caste differentiation in the honeybee (*Apis mellifera*) by juvenile hormone**. *Entomol Exp Appl* 1972, **15**:517-520 ISSN: 0013-8703.

12. Wheeler DE: **The developmental basis of worker caste polymorphism in ants**. *Am Nat* 1991, **138**:1218-1238 ISSN: 0003-0147.

13. Wheeler DE, Nijhout HF: **Soldier determination in ants: new role for juvenile hormone**. *Science* 1981, **213**:361-363 ISSN: 0036-8075, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/17819911.

14. Cornette R, Gotoh H, Koshikawa S, Miura T: **Juvenile hormone titers and caste differentiation in the damp-wood termite *Hodotermopsis sjostedti* (Isoptera, Termopsidae)**. *J Insect Physiol* 2008, **54**:922-930 ISSN: 0022-1910, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/18541259.

15. Iwanaga K, Tojo S: **Effects of juvenile hormone and rearing density on wing dimorphism and oöcyte development in the brown planthopper, *Nilaparvata lugens***. *J Insect Physiol* 1986, **32**:585-590 ISSN: 0022-1910.

16. Scharf ME, Wu-Scharf D, Pittendrigh BR, Bennett GW: **Caste- and development-associated gene expression in a lower termite**. *Genome Biol* 2003, **4**:R62 ISSN: 1465-6906, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/14519197.

17. Evans J, Wheeler D: **Differential gene expression between developing queens and workers in the honey bee, *Apis mellifera***. *Proc Natl Acad Sci* 1999, **96**:5575-5580 ISSN: 0027-

8424, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/10318926.

18. Evans J, Wheeler D: **Expression profiles during honeybee caste determination**. *Genome Biol* 2000, **2** research0001.0001-0006.

19. Moczek AP, Rose DJ: **Differential recruitment of limb patterning genes during development and diversification of beetle horns**. *Proc Natl Acad Sci U S A* 2009, **106**:8992-8997 ISSN: 0027-8424, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/19451631.

20. Rachinsky A, Strambi C, Strambi A, Hartfelder K: **Caste and metamorphosis: hemolymph titers of juvenile hormone and ecdysteroids in last instar honeybee larvae**. *Gen Comp Endocrinol* 1990, **79**:31-38 ISSN: 0016-6480, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/2354779.

21. Rachinsky A, Hartfelder K: **Corpora allata activity, a prime regulating element for caste-specific juvenile hormone titre in honey bee larvae (*Apis mellifera* carnica)**. *J Insect Physiol* 1990, **36**:189-194 ISSN: 0022-1910.

22. Nijhout HF, Wheeler DE: **Juvenile hormone and the physiological basis of insect polymorphisms**. *Q Rev Biol* 1982, **57**:109-133 ISSN: 0033-5770.

23. •• Wheeler DE, Buck NA, Evans JD: **Expression of insulin/insulin-like signalling and TOR pathway genes in honey bee caste determination**. *Insect Mol Biol* 2014, **23**:113-121.
Along with [24], this paper demonstrates that insulin signaling regulates JH biosynthesis. Further, these two papers establish that elevated insulin signaling facilitates queen development.

24. Mutti NS, Dolezal AG, Wolschin F, Mutti JS, Gill KS, Amdam GV: **IRS and TOR nutrient-signaling pathways act via juvenile hormone to influence honey bee caste fate**. *J Exp Biol* 2011, **214**:3977-3984 ISSN: 0022-0949, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/22071189.

25. Patel A, Fondrk MK, Kaftanoglu O, Emore C, Hunt G, Frederick K, Amdam GV: **The making of a queen: TOR pathway is a key player in diphenic caste development**. *PLoS ONE* 2007, **2**:e509 ISSN: 1932-6203, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/17551589.

26. Wheeler DE, Buck N, Evans JD: **Expression of insulin pathway genes during the period of caste determination in the honey bee, *Apis mellifera***. *Insect Mol Biol* 2006, **15**:597-602 ISSN: 0962-1075, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/17069635.

27. Kucharski R, Maleszka J, Foret S, Maleszka R: **Nutritional control of reproductive status in honeybees via DNA methylation**. *Science* 2008, **319**:1827-1830 ISSN: 0036-8075, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/18339900.

28. Luscher M: **Environmental control of juvenile hormone (JH) secretion and caste differentiation in termites**. *Gen Comp Endocrinol* 1972, **3(Supplement)**:509-514 ISSN: 0016-6480.

29. Watanabe D, Gotoh H, Miura T, Maekawa K: **Soldier presence suppresses presoldier differentiation through a rapid decrease of JH in the termite *Reticulitermes speratus***. *J Insect Physiol* 2011, **57**:791-795 ISSN: 0022-1910, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/21414320.

30. •• Hattori A, Sugime Y, Sasa C, Miyakawa H, Ishikawa Y, Miyazaki S, Okada Y, Cornette R, Lavine LC, Emlen DJ *et al.*: **Soldier morphogenesis in the damp-wood termite is regulated by the insulin signaling pathway**. *J Exp Zool Part B Mol Dev Evol* 2013, **320**:295-306.
This paper is the first to demonstrate a role for tissue specific insulin signaling in caste differentiation in termites. Along with [29], these papers suggest a correlation between soldier development, increased JH titers and insulin signaling.

31. Hardie J: **Photoperiodism in insects: aphid polyphenism**. In *Photoperiodism. The Biological Calendar.* Edited by Nelson R, Denlinger D, Somers D. Oxford: Oxford University Press; 2010: 342-363.

32. Hardie J: **The corpus allatum, neurosecretion and photoperiodically controlled polymorphism in an aphid**. *J Insect Physiol* 1987, **33**:201-205 ISSN: 0022-1910.

33. Hardie J: **Juvenile hormone mimics the photoperiodic apterization of the alate gynopara of aphid, *Aphis fabae***. *Nature* 1980, **286**:602-604 ISSN: 0028-0836.

34. Corbitt TS, Hardie J: **Juvenile hormone effects on polymorphism in the pea aphid, *Acyrthosiphon pisum***. *Entomol Exp Appl* 1985, **38**:131-135 ISSN: 0013-8703.

35. Ishikawa A, Gotoh H, Abe T, Miura T: **Juvenile hormone titer and wing-morph differentiation in the vetch aphid *Megoura crassicauda***. *J Insect Physiol* 2013, **59**:444-449 ISSN: 0022-1910, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/23434762.

36. •• Vellichirammal NN, Gupta P, Hall TA, Brisson JA: **Ecdysone signaling underlies the pea aphid transgenerational wing polyphenism**. *Proc Natl Acad Sci* 2017, **114**:1419-1423.
This paper establishes the role of ecdysone signaling in morph determination in the pea aphid. High ecdysone titers in the mother causes larvae to develop into wingless adults. Elevated insulin signaling in the mother may be a cause for higher ecdysone titers, considering RNAi of insulin-related peptide 5 in the embryo causes smaller body size in wingless nymphs [37].

37. Guo S-S, Zhang M, Liu T-X: **Insulin-related peptide 5 is involved in regulating embryo development and biochemical composition in pea aphid with wing polyphenism**. *Front Physiol* 2016, **7**:31 PubMed: https://www.ncbi.nlm.nih.gov/pubmed/26903881.

38. Emlen DJ, Nijhout HF: **Hormonal control of male horn length dimorphism in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae)**. *J Insect Physiol* 1999, **45**:45-53 ISSN: 0022-1910, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/12770395.

39. Gotoh H, Cornette R, Koshikawa S, Okada Y, Lavine LC, Emlen DJ, Miura T: **Juvenile hormone regulates extreme mandible growth in male stag beetles**. *PLoS ONE* 2011, **6**:e21139 ISSN: 1932-6203, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/21731659.

40. Snell-Rood EC, Cash A, Han MV, Kijimoto T, Andrews J, Moczek AP: **Developmental decoupling of alternative phenotypes: insights from the transcriptomes of horn-polyphenic beetles**. *Evolution* 2011, **65**:231-245 ISSN: 0014-3820, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/20731717.

41. Kijimoto T, Moczek AP, Andrews J: **Diversification of doublesex function underlies morph-, sex-, and species-specific development of beetle horns**. *Proc Natl Acad Sci U S A* 2012, **109**:20526-20531 ISSN: 0027-8424, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/23184999.

42. •• Gotoh H, Miyakawa H, Ishikawa A, Ishikawa Y, Sugime Y, Emlen DJ, Lavine LC, Miura T: **Developmental link between sex and nutrition; doublesex regulates sex-specific mandible growth via juvenile hormone signaling in stag beetles**. *PLoS Genet* 2014, **10**:e1004098.
This paper demonstrates the causal link between nutrition, juvenile hormone and double sex in the regulation of male mandible size in stag beetles. Along with [41], these two papers establish a functional role for doublesex in determining tissue sensitivity to insulin and JH in male condition-dependent polyphenisms.

43. Ayoade O, Morooka S, Tojo S: **Enhancement of short wing formation and ovarian growth in the genetically defined macropterous strain of the brown planthopper, *Nilaparvata lugens***. *J Insect Physiol* 1999, **45**:93-100 ISSN: 0022-1910, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/12770400.

44. Bertuso AG, Morooka S, Tojo S: **Sensitive periods for wing development and precocious metamorphosis after precocene treatment of the brown planthopper, *Nilaparvata lugens***. *J Insect Physiol* 2002, **48**:221-229 ISSN: 0022-1910, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/12770122.

45. •• Xu H-J, Xue J, Lu B, Zhang X-C, Zhuo J-C, He S-F, Ma X-F, Jiang Y-Q, Fan H-W, Xu J-Y, Pan P-L, Li Q, Bao Y-Y, Nijhout HF, Zhang C-X: **Two insulin receptors determine alternative wing morphs in planthoppers**. *Nature* 2015, **519**:464-467.
This paper is the first to demonstrate the existence of two functional insulin receptor isoforms. Using RNAi, Xu *et al.*, demonstrate that InR1 causes development of the long-wing morph while InR2 causes development of the short-wing morph.

46. Leopold P, Layalle S: **Linking nutrition and tissue growth**. *Science* 2006, **312**:1317-1318 ISSN: 0036-8075, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16741099.

47. Oldham S, Stocker H, Laffargue M, Wittwer F, Wymann M, Hafen E: **The Drosophila insulin/IGF receptor controls growth and size by modulating PtdInsP(3) levels**. *Development* 2002, **129**:4103-4109 ISSN: 1011-6370, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/12163412.

48. Ikeya T, Galic M, Belawat P, Nairz K, Hafen E: **Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in Drosophila**. *Curr Biol* 2002, **12**:1293-1300 ISSN: 0960-9822, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/12176357.

49. Hafen E: **Interplay between growth factor and nutrient signaling: lessons from Drosophila TOR**. *Curr Top Microbiol Immunol* 2003, **279**:153-167 ISSN: 0070-217X, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/14560957.

50. Brogiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, Hafen E: **An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control**. *Curr Biol* 2001, **11**:213-221 ISSN: 0960-9822, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/11250149.

51. Jones HN, Ashworth CJ, Page KR, McArdle HJ: **Expression and adaptive regulation of amino acid transport system A in a placental cell line under amino acid restriction**. *Reproduction* 2006, **131**:951-960 ISSN: 1470-1626, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16672359.

52. Kilberg M: **Amino acid transport in isolated rat hepatocytes**. *J Membr Biol* 1982, **69**:1-12 ISSN: 0022-2631, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/6811749.

53. McDowell HE, Eyers PA, Hundal HS: **Regulation of system A amino acid transport in L6 rat skeletal muscle cells by insulin, chemical and hyperthermic stress**. *FEBS Lett* 1998, **441**:15-19 ISSN: 0014-5793, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/9877156.

54. Munoz M, Sweiry JH, Mann GE: **Insulin stimulates cationic amino acid transport activity in the isolated perfused rat pancreas**. *Exp Physiol* 1995, **80**:745-753 ISSN: 0958-0670, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/854686.

55. Somwar R, Sweeney G, Ramlal T, Klip A: **Stimulation of glucose and amino acid transport and activation of the insulin signaling pathways by insulin lispro in L6 skeletal muscle cells**. *Clin Ther* 1998, **20**:125-140 ISSN: 0149-2918, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/9522110.

56. Nijhout HF, Callier V: **Developmental mechanisms of body size and wing-body scaling in insects**. *Annu Rev Entomol* 2015, **60**:141-156 ISSN: 0066-4170, PubMed: https://www.ncbi.nlm.nih.gov/pubmed/25341104.