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# On the Origins of Insect Hormone Signaling

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**Abstract**

Hormones are generally thought of as endogenously produced substances, yet many key animal hormones are actually derived intact or as complex precursors from external sources, such as plants. Ecdysteroid hormones in insects are one such example, since insects are incapable of synthesizing steroids endogenously. Therefore, the numerous phenotypic effects of ecdysteroids in insects are actually examples of phenotypic plasticity, where an environmental signal (an ingested sterol) ultimately regulates flexible responses such as molting, oviposition and wing pattern polyphenisms. Here I present the hypothesis that other key insect hormones, notably the juvenile hormones (JH), originated from externally produced substances, initially ingested by herbivorous proto-insects. The plant hormone abscisic acid (ABA) is the most likely candidate: it is ubiquitous among plants, is chemically related to JH, and has juvenilizing effects on some insects. One key argument against this hypothesis is the close similarity of the biosynthetic pathways for JH in insects and a similar hormone in crustaceans, methyl farnesoate (MF). This similarity between JH and MF has led to the commonly-held belief that the aquatic ancestor of insects and crustaceans already had the ability to produce JH, MF or a related substance endogenously. To help distinguish among these possibilities (i.e. an endogenous versus an exogenous origin for JH in insects), I present a sequence comparison of the recently isolated genes encoding crustacean and insect methyltransferase, an enzyme involved in both JH and MF biosynthesis. The lack of orthology between crustacean and insect methyl transferase lends support to my hypothesis of an exogenous origin for JH signaling in insects.

***Abbreviations:*** ABA (abscisic acid), FA (farnesoic acid), FPP (farnesyl pyrophosphate), HGT (horizontal gene transfer), JH (juvenile hormone), Ma (million years ago), MF (methyl farnesoate), MT (methyltransferase).

Hormonal signaling molecules regulate just about every physiological, behavioral and developmental process in insects[1] (reviewed in Nijhout 1994; De Loof 2008). There are several probable explanations for this widespread usage. First, complex organismal functions (such as ecdysis, oviposition and migration) require precise temporal coordination and control. A hormone released into the circulation at a given time can reach all of the cells in the body in a relatively short period, thus allowing for coordination among tissues and cells in different parts of the organism. Second, in addition to this potential for synchrony, morphogenetic hormones—mainly via their binding to nuclear receptors—are well-suited for orchestrating temporally and spatially complex processes such as metamorphosis (Truman et al. 1994). By binding to different receptor isoforms in different cells and tissues at different times, one broad hormonal peak can coordinate a series of events occurring over a period of days or even longer. And third, by placing the release of morphogenetic and other hormones under the control of neurohormones and other neuroregulatory molecules, the timing and amounts of hormones released can be modified by environmental inputs acting on the nervous system[2]. Taken together, this view of hormones suggests the existence of a communication network, in which external and internal signals integrate across a wide range of both spatial and temporal scales. Or, put another way, hormonally-regulated events are notably amenable to phenotypic plasticity (reviewed in Hatle 2003).

Dominant among insect hormones are the juvenile hormones and the ecdysteroids. These two families of insect hormones are unique among chemical messengers in their fundamental importance to insects (reviewed in Nijhout 1994, Flatt et al. 2005). Not only are they required for the successful completion of all insect life cycles, but they function specifically in a wide range of organismal processes in insects, including embryonic development, ecdysis (≈molting) and growth, reproduction, metamorphic transformations, foraging behavior, social caste differentiation, and numerous other polyphenisms (reviewed in Nijhout 1994).

What is the evolutionary history of these functionally diverse yet critical groups of hormones? Ecdysteroids (a class of steroids) and juvenile hormones (JH, a group of sesquiterpenes) are so integral to the biology of the

[1] I use the term "insect" in the broad sense here, including related hexapod groups such as collembolans.

[2] The same logic can apply to plants and fungi, whose plastic responses are not often thought of as being relayed by "nervous systems" *per sé* (but see Wildon et al. 1994; Li et al. 2002).

widest range of living insect groups that their uses are presumably ancient, perhaps dating back to the origin of the Ecdysozoa[3] or even before. As far as we know, all insects and crustaceans utilize ecdysteroids and sesquiterpenes (JH in insects; methyl farnesoate in crustaceans) as regulators of both somatic growth and some aspect of reproduction (Laufer and Biggers 2001, Nijhout 1994). It is thus tempting to consider the internal use of ecdysteroids and sesquiterpenes to be a plesiomorphy (ancestral characteristic) for the arthropods, and possibly for all ecdysozoans (e.g. Tobe and Bendena 1999). I refer to this as the **ancient hormone hypothesis**.

Ecdysteroids are chemically derived from cholesterol or other sterols, and insects are incapable of producing cholesterol or any sterol endogenously (reviewed in Robbins et al. 1971; Behmer and Nes 2003). Thus, it is quite curious that insects rely so heavily on hormones that they cannot produce themselves without first obtaining complex precursors derived from plants or other organisms on which they feed (see Robbins et al. 1971; Svoboda et al. 1975; Pennock 1977; Behmer and Nes 2003). Roundworms (Nematoda) and crustaceans are also apparently incapable of synthesizing sterols endogenously, and must obtain cholesterol or other complex precursors from their diet (Rothstein 1968, Kanazawa 2001). In fact, there is, to my knowledge, no published evidence for endogenous ecdysteroid (or any sterol) production in a single member of the proposed Ecdysozoa clade! This leads to the perhaps surprising conclusion that ecdysteroid signaling in insects is actually an example of phenotypic plasticity, since the myriad cellular, developmental and behavioral processes regulated by ecdysteroids are dependent on an environmental cue: ingested sterols.

More curious still is the fact that the ability to produce sterols internally from relatively simple precursors (e.g. mevalonate or acetate) is apparently widespread among other groups of 'non-ecdysozoan' animals (reviewed in Kanazawa 2001), and the evolutionary pattern among animal phyla seems to indicate multiple gains and/or multiple losses of this trait. In fact, internal sterol production is the rule rather than the exception in all eukaryotes (reviewed in Behmer and Nes 2003). Even ecdysteroids themselves are

[3] The Ecdysozoa (Aguinaldo et al. 1997) are a proposed monophyletic group that would unite a diverse array of molting animals. This super-phylum grouping includes what are arguably the two most dominant animal taxa on the planet at this time: the Arthropoda (insects, spiders, crabs, etc.) and the Nematoda (roundworms). The Ecdysozoa also includes less known groups, such as water bears (Tardigrada), velvet worms (Onycophora), horsehair worms (Nematomorpha), fanghead worms (Priapulida, with probable relatives in the Burgess Shale) and awlhead worms (Kinoryncha or Echinoderida).

synthesized by organisms in a wide array of kingdoms, including fungi, plants and 'non-ecdysozoan' animals (see Dinan 2004). But how is it possible that such globally dominant and ancient groups as arthropods and nematodes are unable make a common hormone that they absolutely need in order to grow? Why have some insect taxa evolved such elaborate mechanisms of utilizing particular sterols to produce ecdysteroids (Behmer and Nes 2003), and none have apparently figured out what so many other living forms have: how to make the hormone themselves? Did the ancestors of arthropods and nematodes originally have this capacity, and then lose it? Or, did they never have it? In any event, lack of ability to synthesize this essential compound from simple precursors apparently has not held back the insects and nematodes in their ascendancy to taxonomic dominance.

The origin of juvenile hormones (JH) in insects is also of interest. These sesquiterpene hormones may exceed even ecdysteroids in the breadth of their involvement in various biological processes (reviewed in Nijhout 1994, Flatt et al. 2005). A similar sesquiterpene, methyl farnesoate (MF), has been isolated from several crustaceans, and has apparent roles in reproduction (reviewed in Laufer and Biggers 2001). The diverse and critical functions for JH across insects, the extremely close chemical similarity of JH and MF, and the fact that both JH and MF are involved in reproduction seems to support the ancient hormone hypothesis.

Still, a closer look at the role of juvenile hormones across insects uncovers some curious patterns. First, a comparative analysis of the precise reproductive functions of JH in insects reveals substantial differences across insect taxa. Even vitellogenesis (the synthesis of egg yolk), the process often described as the most fundamental role of JH in insect reproduction (e.g. Wyatt 1997), does not appear to depend upon JH in several notable insect groups. Indeed, in the lepidopterans (moths and butterflies), the canonical vitellogenic role for JH is only found among a derived group of butterflies, though taxon sampling there is still low (see Chapter 11: Table 4 and Figure 4, this volume). These puzzling patterns are rarely mentioned in print when discussing JH evolution. Furthermore, the precise roles for MF in crustacean reproduction are, relative to JH, very poorly studied. The glands that produce JH in insects (the corpora allata; henceforth CA) and MF in crustaceans (the mandibular organ; henceforth MO) are nearly universally described as homologous. Nevertheless, evidence for this characterization is rarely presented or analyzed (e.g. Stay and Tobe 2007). In those few papers that do carefully consider the evidence for homology, the argument mainly rests upon similar ultrastructural features of their respective glandular cells

and similar embryonic origin (as well as the production of MF by the MO). While similar embryonic origins of the respective glands are indeed found across the majority of insects and crustaceans examined, within-class variations exist, casting possible doubt on the homology argument.[4] In the case of the ultrastructural similarities, the cellular features that are cited to suggest homology (such as the ER ultrastructure and prominence of vacuoles) are, in fact, common characteristics of steroid/lipid secreting cells across animals. Indeed, the two initial papers suggesting an endocrine function of the MO (Hinsch & Hajj 1975; Byard et al. 1975) both noted the strong ultrastructural similarity to vertebrate steroid secreting glands. Surely, such similarities in crustacean and vertebrate glandular ultrastructure are due to homoplasy, so one wonders why CA-MO similarities could not be due to homoplasy as well. In fact, comparative analyses of MO ultrastructures within decapod crustaceans reveal rather dramatic differences between species (Byard et al. 1975). Finally, as Tobe and Bendena (1999) have noted, the innervation patterns of the MF and CA are quite different. In sum, the common presumption of homology for endocrine signaling in insects and crustaceans overlays a more complex story than is often acknowledged. Do the comparative observations cited above regarding ecdysteroid and sesquiterpene signaling cast doubt on the veracity of the ancient hormone hypothesis?

I noted above that all insects obtain their sterols from the environment, and that therefore, ecdysteroid signaling represents a form of phenotypic plasticity. Like steroids, structurally diverse sesquiterpenes have been isolated from a vast array of plants, fungi and algae (see below), suggesting that sesquiterpenes were probably abundant at the time that the first insect ancestors invaded land. Were the earliest insects likely exposed to a consistent source of sesquiterpenes, as was surely the case with steroids? If so, is it possible that the history of both of these hormones in insects began as plastic responses to highly active, externally produced compounds?

An analysis of the substantial fossil record of insects along with comparative biochemistry and genomics can help to provide some answers to these questions. Insects appear in the fossil record during the late Silurian

[4] Although the embryonic origin of the corpora allata in many insects is from ectodermal invaginations in or around the mandibular pouch, it is different in others (reviewed in Kobayashi and Ando 1983), including the rice weevil *Calandra oryzae*, whose corpora allata originate from the antennal mesodermal sacs in the embryo (Tiegs and Murray 1938). Also, in the Collembola (springtails) and other basal insect/hexapod orders, the position of the corpora allata in the adults is suboesophageal and/or lateral, rather than the supraesophageal position seen in most insects (Cassagnau and Juberthie 1983).

to early Devonian, starting at around 410 million years ago ("410 Ma"). And while there is some controversy concerning their original food source (whether live or dead/decaying plant material), the earliest evidence of insect live plant feeding is from spore protoplasts from around 400 Ma, in the late Silurian and early Devonian (Edwards et al. 1995; Habgood 2004; also see Labandeira 1998, 2002a,b). Subsequently, the earliest leaf damage is recorded on seed ferns about 326 Ma, from the late Mississippian (Labandeira, personal communication). Thus, it seems that when live feeding on plants began in earnest, particularly on foliar tissues, gymnospermous plants[5] had largely diversified (Niklas 1997). Indeed, recent evidence suggests that the spectacular radiation of the Coleoptera, which has led them to become by far the most speciose taxon on earth, began on gymnosperms, possibly preadapting[6] them for their multiple, independent moves to angiosperms (Farrell 1998). Therefore, the major events of insect diversification took place when insects were feeding mainly on gymnosperms (Labandeira 1998, 2002b). Phylogenetic comparisons among terpene biosynthetic systems in various plant groups, as well as the near ubiquitous presence of sesquiterpenes in modern land plants (e.g. Asakawa et al. 2001), indicate that these ancient gymnosperms probably had well-developed sesquiterpene biosynthetic capacity (Martin et al. 2004; Bohlmann et al. 1998). Indeed, terpenoids are the largest and most diverse group of plant organic compounds and sesquiterpenes are the largest group of known terpenoids (Ryan 2002). The original function of such sesquiterpenes has been hypothesized as anti-microbial, anti-fungal and insecticidal (reviewed in Bohlmann et al. 2000). Furthermore, algae and even fungi can produce sesquiterpenes (e.g. Anke and Sterner 1991; Smyrniotopoulos et al. 2003), and JH analogs are abundant in a vast array of plants (reviewed in Bowers 1997, Eales 1997). In fact, many plants produce JH intermediates (see below), and sedges (*Cyperus iria*) can actually synthesize *bona fide* JH-III, the active form of JH in most insects (Toong et al. 1988; Bede et al. 2001).

Abscisic acid (ABA) is one noteworthy sesquiterpene found in all groups of land plants, as well as algae and fungi (Jolivet et al. 1991, Oritani and Kiyota 2003, Kroemer et al. 2004). Like JH in insects, ABA is a hormone involved in an extremely wide array of ontogenetic and physiological pro-

[5] The term "gymnosperm" here and throughout refers essentially to the non-flowering seed plants, both living (e.g. cycads, ginkgoes and conifers) and extinct (e.g. seed ferns).
[6] *sensu* Gould (1984): features adapted for one role that are fortuitously suited for another.

cesses in plants, including dessication tolerance, seed dormancy, root growth, root-shoot ratios, cold tolerance, leaf polyphenisms ("heterophylly") and pathogen resistance (reviewed in Davies and Jones 1991, Oritani and Kiyota 2003, Wheeler and Nijhout 2003, Taylor et al. 2004). The similarities between JH and ABA apparently extend to their molecular action: the searches for the JH and ABA receptors have been impeded by a similar series of difficulties, indicating to Wheeler and Nijhout (2003) that each of these sesquiterpenes may act through low affinity interactions with several different endogenous receptor molecules, both nuclear and membrane-based. Furthermore, both JH and ABA are derived from the same sesquiterpenoid precursor: farnesyl pyrophosphate (FPP; see below and Figure 2) (reviewed in Oritani and Kiyota 2003). Given these similarities, the obvious question is: does exposure to ABA have JH-like effects on insects?

Several studies have shown that plants upregulate ABA production in response to insect damage (e.g. Peña-Cortés and Willmitzer 1995), indicating a function for ABA in protection against herbivory (Thaler and Bostock 2004). Still, ABA treatments of adult insects through their food give somewhat complicated results when they are compared across species. In general, leaf-feeding adult insects show a decrease in fecundity when exposed to excess ABA, whereas seed-feeding, carnivorous and detritivorous adult insects tend to show the opposite effect (reviewed[7] in Visscher 1983). Although few studies have been done to determine the effects of ABA exposure on preadult insects, the unpublished results of Carroll Williams suggest that feeding ABA to nymphs of the fire bug *Pyrrhocoris apterus* (Hemiptera: Pyrrhocoridae) can have JH-mimicking effects on cuticle deposition (cited in Visscher 1983), a similar result to that reported by Eidt and Little (1970) with ABA injections into pupae of the mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae).

In sum, these various results suggest that ABA can either act as an antagonist or an agonist of JH signaling, depending on the ontogenetic stage and the feeding mode of the insect in question. Why do we see such variability in ABA effects across insects? Perhaps it is unrealistic to expect a uniform effect of such a potent and potentially detrimental plant hormone among insects as a whole. Put simply, 400 million years of co-evolution between insects and plants have undoubtedly resulted in a wide variety of insect responses to ABA, as well as plant counter-responses to these insect

[7] There have been a mere handful of additional studies on this topic since 1983, including Bur 1985; Yesilada and Bozcuk 1995, 1996. The results of these studies are essentially consistent with those reported in and reviewed by Visscher (1983).

responses, etc. (see Fraenkel 1959, Herout 1970 for more general discussions of this idea). Therefore, in order to infer anything reliable about the evolutionary history of ABA-insect interactions, we need more data on the roles of ABA in basal plant taxa, as well as its effects on basal hexapod orders.

In the mean time, it seems reasonable to assume that the earliest insect feeders were exposed to highly bioactive sesquiterpenoid compounds, including ABA and possibly other JH-like chemicals (see Slama et al. 1974, for a review). What if the biological effect of these ancient sesquiterpenes was similar to juvenoids in modern insects: namely, in repressing adult differentiation? Presumably, then, successful ancient gymnosperm-feeding insects eventually developed a degree of resistance (or biochemical accommodation) to such sesquiterpenes, ultimately allowing for adult differentiation to occur on such host plants. At this point, the first juvenile hormonal "control" of development would have been in place, and the source of the hormone would have been exogenous, a situation similar to that of ecdysteroids in insects today[8] . Such a process is not unlikely; indeed, many animal hormones have exogenous sources (reviewed in Heyland et al. 2005). Furthermore, an external source of metamorphically-active JH-like compounds has been proposed for marine polychaetes as well (Biggers and Laufer 1992, 1999).

In fact, this idea that insect hormones may have been evolutionarily derived from plant compounds was proposed as early as 1979 by Karel Sláma (page 683), who hypothesized that "some compounds that are present for various other reasons in plants may accidentally fit certain structural requirements and consequently act as animal hormones. Like some other secondary plant substances, the hormonally active compounds in plants could have been a factor of natural selection that modulated the co-evolutionary relationships between plants and their insect herbivores."[9] Abscisic acid and other JH-mimicking sesquiterpenoids in plants are likely examples.

An exogenous origin for sesquiterpenes in insects implies an alternative to the ancient hormone hypothesis outlined above. If the original source of a

[8] The difference is that ecdysteroids are generally not the ingested form in insects; instead, complex sterols are ingested, which the insects then convert to ecdysteroids.

[9] Such ideas were directly descended from Sláma and Williams' (1966) identification of the JH-mimicking "paper factor." The active compound in the "paper factor" is juvabione from balsam fir (*Abies balsamea*), another FPP-derived sesquiterpene with very close chemical similarity to ABA (Bowers et al. 1966; see Figure 2 and legend).

JH-like compound in stem group insects[10] was in fact exogenous, then how was sesquiterpenoid biosynthesis internalized in the ancestors of modern insects, all of whom synthesize juvenile hormones in specialized structures in the anterior of the animal called the corpora allata (Nijhout 1994)?

There is an evolutionary mechanism that can account for this internalization that should be familiar to all aficionados of phenotypic plasticity: genetic assimilation. This term was introduced by Conrad Waddington (1961 for review), who selected heterogeneous populations of *Drosophila melanogaster* vinegar flies for an induced bithorax phenotype (the conversion of the haltere balancer organs into wing tissue) resulting from environmental stress (ether treatment of embryos). Amazingly, some of the flies in the eighth generation of selection showed the bithorax phenotype in the absence of the ether treatment. In other words, what began as an environmentally induced response quickly became a constitutive response: the induced bithorax condition had somehow "assimilated" into the genome, becoming a genetically fixed condition.

Recent selection experiments using either *D. melanogaster* or the mustard *Arabidopsis thaliana* have revealed one possible cellular mechanism for this process of assimilation of environmental effects: inhibition of the normal functioning of the molecular chaperone HSP90, a protein involved in maintaining protein cellular functions under conditions of stress, uncovers cryptic variation that can subsequently be selected for and ultimately fixed genetically (reviewed in Sangster et al. 2004).

The general principle here is that a response dependent on an environmental stimulus in an ancestral state can become assimilated in a derived state. Such an evolutionary pattern was actually proposed more than a half century before Waddington by James Mark Baldwin (1896, 1902), who wrote that "heredity provid[es] for the modification of its own machinery. Heredity not only leaves the future free for modifications, it also provides a method of life in operation of which modifications are bound to come" (1896, page 552). Baldwin's idea of "future modifications" that are "bound to come," clearly prefigures Waddington's bithorax results. Thus, I adopt the formulation '**Baldwinian assimilation**' throughout this chapter to describe the internalization of environmentally induced phenotypes. In the context of the origin of insect hormones, a Baldwinian assimilation hypothesis could account for the possible internalization of sesquiterpene biosynthetic capacity in stem group insects, as an alternative to the ancient hormone hypothesis presented above.

[10] A "stem group" is an extinct taxonomic group that branched off before the all of the living representatives of a given clade appeared. Thus, a "stem group insect" would hold a phylogenetic position basal to all of the living insect orders.

Some recent work on bark beetles in the genus *Ips* (Coleoptera: Scolytidae) might provide a second example where terpene biosynthetic ability evolved *de novo* in insects via Baldwinian assimilation. Conifers produce monoterpene volatiles, such as α-pinene in pines, in response to insect grazing (reviewed in Harborne 1991). Bark beetles, though, are not only resistant to this feeding deterrent, they utilize the hosts α-pinene as a precursor for one of their sex and aggregation pheromones, the monoterpene cis-verbenol (Byers 1981, 1983, 1989). Some *Ips* species (such as *I. paraconfusus*) seem to engage the services of gut symbionts for this production of cis-verbenol, as well as for the synthesis of other monoterpenoid pheromonal components (Brand et al. 1975, Byers and Wood 1981). In addition, this same species and other bark beetles actually have the ability to synthesize their terpenoid pheromones endogenously, and thus independently of pine terpenoid precursors (Byers and Birgersson 1990, Seybold et al. 1995, Hall et al. 2002). In fact, these beetles are the only insects (indeed the only metazoans) known to have an endogenous monoterpene synthase (Martin et al. 2003). Therefore, the most obvious evolutionary scenario is one where an ancestral bark beetle taxon, possibly with the aid of gut symbionts, converted the plant's own monoterpenes into aggregation pheromones. Then, later, some male bark beetles evolved the ability to synthesize the compounds themselves, and could thus initiate aggregation responses and attract mates independent of host-produced volatiles. As in the JH biosynthesis mechanism discussed above, the evolutionary acquisition of this novel terpene biosynthesis mechanism in bark beetles is a perfect candidate for Baldwinian assimilation.

Thus, Baldwinian assimilation might explain the evolution of phenotypic plasticity in response to a wide range of exogenous hormones, pheromones and other chemicals (Figure 1). In the first stage, the animal is exposed to such a chemical, which may induce a harmful, or even a neutral or (less likely) a beneficial response. In the second stage, the insect acquires resistance to any ill effects of the chemical. Not only would this allow the animal to exploit the chemical-producing resource more efficiently, but it would also allow for the environmental signal to be co-opted to induce a particular, selectively favorable (phenotypically plastic) reaction in the animal.[11] During this stage, specific insect receptor molecules, which may

[11] The idea here is that organisms cue their life cycle transitions and other responses to detectable, reliable environmental signals. Such reliable signals (for example: day length, volatile compounds produced by hosts, rainfall, etc.) are precisely what organisms respond to in cases of adaptive phenotypic plasticity. Furthermore, there is substantial precedent in animals for the utilization of potent chemicals derived from food sources as both hormonal regulators of life cycle transitions, and as cues for adaptive plasticity (e.g. Pfennig 1992, Heyland and Hodin 2004; reviewed in Heyland et al. 2005).

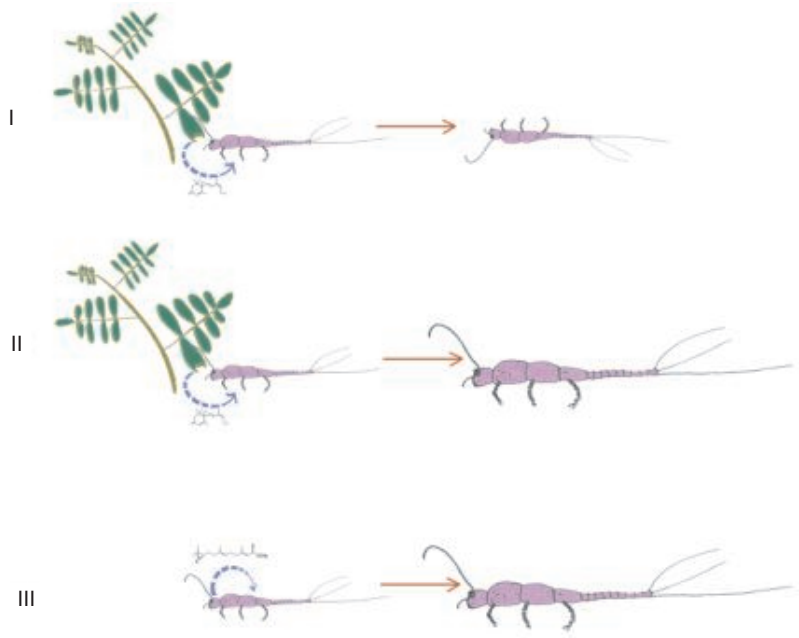


**Fig. 1** Baldwinian assimilation hypothesis for the origin of hormonal signaling in insects. In the first stage (**I**), a potent compound [abscisic acid (ABA) is shown here] produced by a plant induces a detrimental plastic response in the proto-insect feeding on that plant. In the second stage (**II**), the proto-insect acquires resistance to the potent compound. Thus, the proto-insect can now safely feed on this plant. Ultimately, the potent compound is used as a signaling molecule/plasticity cue involved in life stage transitions (red arrow in II) in the proto-insect. At this stage, the insect's genome has adapted so that the external compound produces a beneficial plastic response. In the final stage (**III**), the proto-insect has acquired the ability to synthesize a chemically related compound endogenously [juvenile hormone III (JH-III) is shown here]. Now, the proto-insect can complete the same life stage transitions in the absence of that particular food source. The insect has co-opted and internalized ("assimilated") a formerly environmentally-dependent process.

have started out at low affinity for the external signal, are gradually modified through natural selection to attain higher and higher affinities (see for example Tallamy et al. 1999). Such a receptor molecule could either be something akin to a detoxification enzyme, or it could be a member of a pre-existing signaling cascade that induces a particular physiological or neurophysiological reaction to the presence of the novel plant signal (see Baker 2005). In the third stage, the animal is primed for internal synthesis of the chemical (or a similar substance), as the "favorable reaction" could then

occur in a wider diversity of environmental contexts, where and when the specific exogenous chemical is absent. Stage three, then, represents a fertile condition for the Baldwinian "internalization" of a synthetic mechanism for the exogenous chemical by genetic assimilation.[12]

There is a second (albeit not entirely mutually exclusive) evolutionary mechanism that could account for the acquisition of plant chemical synthetic enzymes by herbivorous insects: **horizontal gene transfer** (HGT). For example, there is quite strong evidence that several bacterial genes involved in manipulating plant chemistry and physiology, including cellulases, have been acquired by plant parasitic nematodes via HGT (reviewed in Bird and Koltai 2000). And despite widespread understanding that pieces of DNA have the ability to cross species boundaries (as in yearly flu virus outbreaks as well as HIV evolution), the possibility remains underappreciated that modern organismal genomes may be, in reality, patchwork mosaics of DNA derived both horizontally and vertically. Indeed, Palmer et al. (2004) reached the shocking conclusion that the "true" tree of all living forms may never be knowable, due to the exceedingly high rates of HGT among bacteria and cyanobacteria.

These findings have at least two implications for the Baldwinian assimilation hypothesis delineated above. First, the insect receptor molecules that I postulate to have some fortuitous affinity for the plant chemical ("stage two" above) could have been acquired by HGT from a bacterium (or other microbe), one that, perhaps, had a longer term association with the plant than the insect in question. In this way, in a literal evolutionary instant, the ability to bind the plant chemical could have been acquired. Second, the internalization of the chemical synthetic mechanism ("stage three" above) could have also been a HGT event, either from the plant itself, or from some plant-associated microbe.

Thus we have three working hypotheses to explain the acquisition of plant signaling systems by insects: the Baldwinian assimilation hypothesis, the horizontal gene transfer (HGT) hypothesis, and the ancient hormone

[12] The scenario I present here can be seen as broadly consistent with Waddington's (1960, Figure 9) evolutionary outline for assimilation, where genetic change involves four different but overlapping "subsystems" operating somewhat sequentially. The first two subsystems ('exploitive' and 'epigenetic') involve environmental variation and behavioral responses to them, and can be called "phenotypic plasticity" in modern parlance. The third ('natural selective') subsystem involves novel combinations of alleles yielding new phenotypes from standing variation. In the final ('genetic') subsystem, changes result from mutation.

hypothesis.[13] The main prediction of the Baldwinian assimilation hypothesis is that different lineages exposed to the same chemical would evolve internal synthesis by quite different mechanistic (convergent) routes (see Hodin 2000). Thus, for example, the mechanisms of methyl farnesoate (MF) synthesis in crustaceans would be substantially different from the mechanisms of JH biosynthesis in insects. In other words, the biosynthetic enzymes would have been independently co-opted in crustaceans and insects, and would not necessarily be orthologs. The HGT hypothesis predicts that the enzymatic proteins involved in these hormone synthesis pathways would be more similar to specific plant or microbe proteins than they are to any proteins in other ecdysozoans or other animals. The null hypothesis here is the ancient hormone hypothesis: namely, that the JH and MF biosynthetic pathways are homologous, in that sesquiterpene biosynthesis was present in the last common ancestor of insects and crustaceans. In this case these enzymes would be expected to be orthologous in crustaceans and insects, and would also be found in other animals.

Distinguishing among these hypotheses requires detailed comparative biochemistry and genomics. In insects, MF is a JH precursor (Figure 2). Do insects and crustaceans use similar or different biosynthetic enzymes to produce MF? The key steps in the insect JH and crustacean MF biosynthetic pathways involve conversion of farnesol to MF via oxidase, dehydrogenase and methyltransferase activities (Figure 2). Do crustaceans use orthologous enzymes here? What about those plants and other organisms that produce JH or related compounds? Are these independently-evolved biosynthetic pathways at all similar to the pathways in arthropods?

The starting point for JH biosynthesis in insects is the same as for cholesterol synthesis in other eukaryotes, as well as for abscisic acid (ABA) synthesis in plants and fungi: farnesyl pyrophosphate (FPP; see Figure 2).[14] The enzyme responsible for catalyzing the formation of FPP is a FPP synthase (not shown in Figure 2), and, not surprisingly, FPP synthase orthologs are found in plants and animals (Poulter and Rilling 1981; Bellés et al. 2005). Therefore, with FPP we have an example where the same

[13] The former two (assimilation and HGT) together might be examples of "phylogenetic espionage" hypotheses *sensu* Schultz (2002). The scenario outlined above for the evolutionary acquisition of monoterpene production in bark beetles as sex and aggregation pheromones is another plausible example. Shultz (2002) and Schultz and Apel (2004) provide several additional and striking examples of parallel uses of hormones and other chemical signaling molecules in plants and herbivorous animals.

[14] FPP is also well-known for its involvement in protein modifications in plants and animals (prenylation), as shown in Figure 2 (see also Poulter and Rilling 1981).

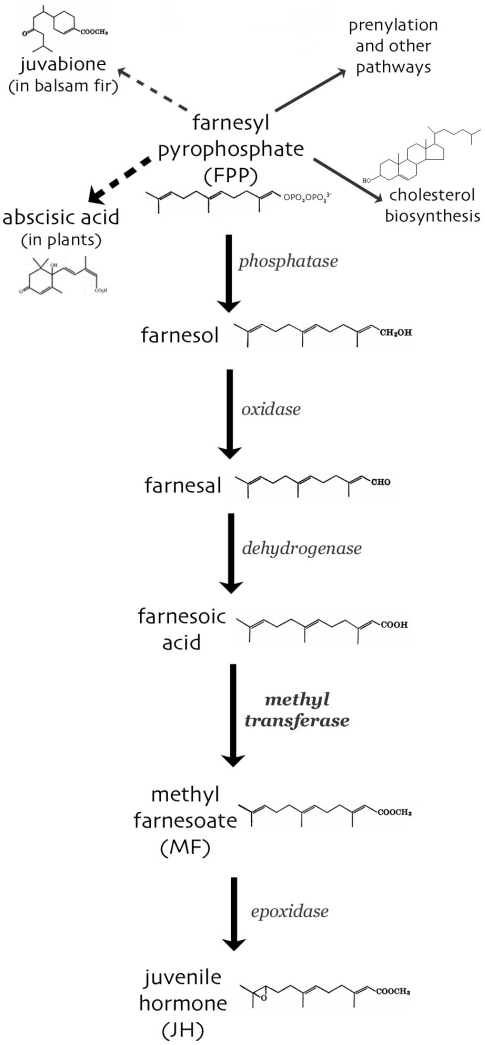


**Fig. 2** The MF/JH biosynthetic pathway in arthropods, and related pathways in plants. Juvenile hormone III (JH-III), the active form in most insects, is shown here. In italics are enzymes that catalyze the different steps in the MF/JH pathway. Each of these enzymes has been identified by their activity, but the genes have, to this date, only been isolated for the crustacean and insect methyltransferases (see the text and Figure 3) as well as the insect epoxidase (cytochrome P450). Crustaceans are not known to make JH. FPP is involved in multiple pathways in various organisms, as indicated (see also the text). Juvabione (the "paper factor") from balsam fir (*Abies balsamea*) is a potent JH-mimic in hemipteran bugs in the family Pyrrhocoridae (see the text). Cholesterol biosynthesis from FPP is conspicuously absent in all arthropods and nematodes.

molecule is produced by an apparently conserved biosynthetic pathway across a wide array of organisms.

Now what about JH/MF biosynthesis in arthropods, a pathway unknown from any other animal group (Figure 2)? Unfortunately, the oxidase and dehydrogenase genes responsible for the conversion of farnesol to farnesoic acid have not yet been identified (Bellés et al. 2005). Recent work, though, has identified methyltransferases (MTs) from a variety of insects and crustaceans. To begin to distinguish among the above hypotheses, I performed sequence alignment comparisons of the different insect and crustacean MTs thought to be responsible for methyl farnesoate biosynthesis: JH acid MT in insects (e.g. Shinoda and Itoyama 2003; Bellés et al. 2005) and farnesoic acid O-MT in crustaceans (e.g. Silva Gunawardene et al. 2002; Ruddell et al. 2003; Bellés et al. 2005). Despite their description by Bellés et al. (2005, page 186) as "orthologs," these insect and crustacean MTs show no significant similarity (Figure 3).

Furthermore, my preliminary BLAST searches of gene and protein databases (not shown) revealed a curious pattern for the JH acid MT: the only sequences that showed significant similarity to the *Drosophila melanogaster* sequence (see Figure 3) were other insect JH MTs and microbe sequences; no other animal sequences showed substantial similarity. Most of these microbe sequences were uncharacterized, but several were bacterial ubiquinone/methyltransferases!

Farnesoic acid O-methyltransferases (FA O-MTs) have been identified in several crustaceans (*e.g.* Figure 3), as well as in insects, including the honeybee *Apis mellifera* and the mosquito *Aedes aegypti*. These FA O-MT sequences are quite highly conserved among the arthropods (e.g. 44.8% amino acid similarity between *Scylla* mud crabs and *Aedes* mosquitos over the entire sequence; data not shown), but show comparatively low similarity with any insect JH Acid methyltransferases (JH Acid MTs; see above and Figure 3). Such levels of similarity are indicative of the fact that both are S-adenosyl-L-methionine (SAM)-dependent methyltransferases, a diverse family of enzymes found throughout prokaryotes and eukaryotes. Equally low levels of similarity are seen in comparisons within insect species between their JH Acid MT and FA O-MT genes. For example, *A. aegypti* JH Acid MT is 35% similar to *A. aegypti* FA O-MT over a 100 amino acid N-terminal stretch (data not shown), about the same similarity in the crustacean-insect comparison shown in Figure 3. A recent study (Burtenshaw et al. 2008) has demonstrated that the *D. melanogaster* ortholog of crustacean farnesoic acid O-methyltransferase is expressed in the ring gland, but that *in vitro* assays and genetic analyses show no evidence that

```
S.serrata FA O-MT          6  HGKTLRFQVKAAHDCHVAFTTGAEETDPMVEVFIG---------------  40
                              ||..||::                .|..|.::::..|
A.aegypti JH Acid MT      25  HGHLLRWK---------------EENEDSLLDIGCGSGDVLIDFVIPMVPP  60

S.serrata FA O-MT         41  ------GWEGAASAIRF--KKADDLVKV--DTPDIVTEAEYREF---WIA  77
                                  |.:.:...:||  |...|:..:  :|.||  |.:...|   |..
A.aegypti JH Acid MT      61  KRARVLGTDVSEQMVRFARKVHSDVENLFFETLDI--EGDISSFLNKWGC  108

S.serrata FA O-MT         78  VDH
                              .||
A.aegypti JH Acid MT     109  FDH
```

**Fig. 3** Crustacean farnesoic acid O-methyltransferase (FA O-MT) is not orthologous with insect JH Acid methyltransferase (JH Acid MT). Alignment (using EMBOSS Align 2006-7 at the European Bioinformatics Institute; http://www.ebi.ac.uk/emboss/align/) of a mosquito, *Aedes aegypti*, JH acid methyltransferase (accession number DQ409061), and a mud crab, *Scylla serrata*, farnesoic acid O-methyltransferase (accession number DQ187991). Numbers (6-80; 25-111) refer to the amino acid positions out of 278 *(A. aegypti*) and 235 (*S. serrata*), respectively. Vertical lines define identical amino acid positions; pairs of dots identify chemically similar amino acids; single dots denote weak similarity; dashes indicate gaps inserted in the two sequences to preserve optimal alignment. These N-terminal portions of the two sequences are the only parts showing substantial similarity (32% with 19.4% identity). The remaining C-terminal regions (not shown) are only 12.2% similar (6.8% identical). This and all alignments referred to in the text were performed using the default settings in the "water" pairwise alignment method in EMBOSS.

this gene actually functions in JH biosynthesis in *D. melanogaster*. Although not discussed in this context by Burtenshaw and colleagues, their data seems more consistent with a scenario of independent evolution of crustacean MF and insect JH functions, as we hypothesize in this chapter.

These comparative genomic and functional data point away from the ancient hormone hypothesis, which would predict orthology between the enzymes catalyzing the farnesoic acid to MF conversion in crustaceans and insects. Instead, the crustacean enzyme known to catalyze the conversion of farnesoic acid to MF (FA O-MT) and the insect enzyme (JH Acid MT) are clearly not orthologous. While these results are at odds with the ancient hormone hypothesis[15], this pattern of non-orthology is exactly what the Baldwinian Assimilation and the HGT hypotheses would predict. The close similarity of JH acid MTs to microbe rather than other animal sequences

[15] It is formally possible, though, that insects and crustaceans shared a common (ancient) JH/MF biosynthetic pathway, but that one or the other taxa replaced their methyltransferase after the insect/crustacean divergence. This would, therefore, be an example of "non-orthologous gene displacement," an evolutionary phenomenon not uncommon among bacteria (Koonin et al. 1996). Comparative analyses of the wide variety of genomes being currently studied should help us determine how common this process is in eukaryotes as well.

provides an intriguing indication that this enzyme may have been acquired in insects by horizontal gene transfer from a plant-associated microbe.

It may be objected that the overall architectures of the insect JH and crustacean MF biosynthetic pathways (see Figure 2) are too similar to have evolved independently in these two related arthropod groups. How can we evaluate this argument? Perhaps we can look at the production of JH-III (the active form in most insects) in sedge plants in the genus *Cyperus*. Is *Cyperus* JH-III produced by a similar biosynthetic pathway as JH in insects? Yes! First, some of the same exact intermediates (farnesol, MF) are found in sedges, and farnesol (e.g. in Rutaceae; Brophy and Goldsack 2005), farnesal [e.g. *Arabidopsis* (Brassicaceae); Crowell et al. 2007), farnesoic acid [e.g. *Xanthostemon* (Myrtaceae); Brophy et al. 2006], and MF [e.g. *Polyalthia viridis* (Annonaceae); Kijjoa et al. 1990] are found in taxonomically disparate plants. In fact, *Arabidopsis*, which produces both farnesol and farnesal (Crowell et al. 2007) is now known to have a *bona fide*, functional farnesoic acid methyltransferase, though it is non-orthologous to the two arthropod genes discussed here (Yang et al. 2006). Second, some of the aforementioned JH intermediates in plants are known to have negative impacts on insects that feed on them (reviewed in Hick et al. 1999), and are in some cases induced by insect feeding (e.g. Schnee et al. 2002). Furthermore, the final step in JH synthesis in sedges is catalyzed by a cytochrome p450 epoxidase (Bede et al. 2001), the same class of enzyme used in insect JH biosynthesis. Given this remarkable parallel evolution of JH biosynthesis in sedges and insects, and the presence of JH/MF intermediates in disparate plants, perhaps it is not so unlikely that similar enzymatic pathways could have evolved independently in crustaceans and insects as well.

Still, to fully evaluate the issues surrounding the origin of insect hormonal signaling, we await further detailed comparative biochemistry, endocrinology and genomics, as well as additional paleontological data. The results of such studies might substantially influence our thinking regarding the mechanisms of evolution, from the evolution of phenotypic plasticity and life histories to macroevolutionary questions concerning the origins of novelty.

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