Fyziologie působení farmak a toxických látek

Přednáška č.7
Principy endokrinní regulace a endokrinní disrupce u bezobratlých

Hormonální regulace u bezobratlých:

TABLE I
Examples of Reported Hormones in Different Invertebrate Taxa[a]

Taxon	Reported hormones (example, *controlled process*)
Coelenterata	Neuropeptides (glycine-leucine tryptophan amides = GLWamides, *metamorphosis*); thyroids (thyroxine, *strobilation*); retinoids (9-cis-retinoic acid, *strobilation*)
Nematoda	Ecdysteroids (reported but *functional role questionable*); terpenoids (juvenile hormone (JH) like hormones, *growth*); neuropeptides (FMRFamide, *function unknown*)
Mollusca	Ecdysteroids (reported but *functional role questionable*); steroids (17ß-estradiol, testosterone, progesterone, *sexual differentiation, reproduction in prosobranchs*); terpenoids (JH reported but *functional role questionable*); neuropeptides (APGWamide, dorsal body hormone (DBH), *sexual differentiation, gonad maturation, spawning*; egg-laying hormone (ELH), *spawning*; FMRFamide, *neuromodulation*; molluscan insulin-like peptides (MIPs), *growth, development, energy metabolism*)
Annelida	Ecdysteroids (ecdysone, *functional role unknown*); neuropeptides (FMRFamide, *neuromodulation*)

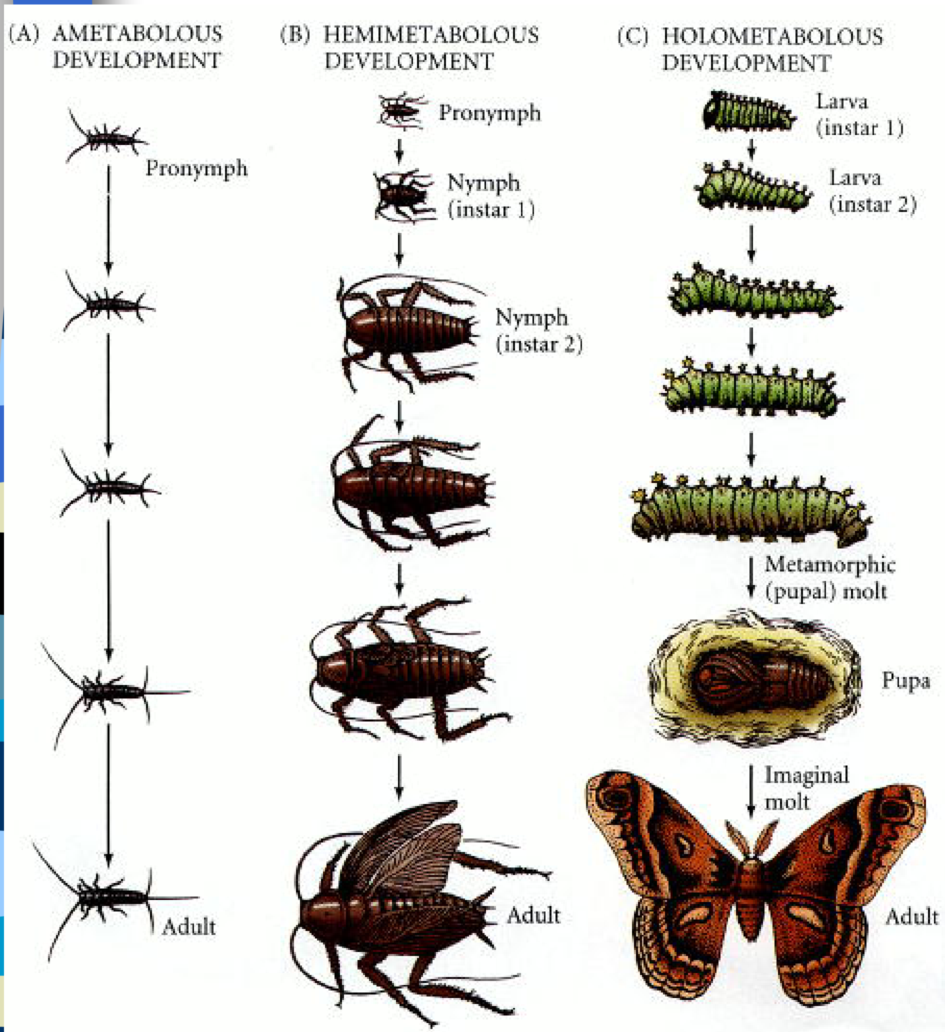
Hormony u členovců:

Crustacea	Ecdysteroids (ecdysone, *molting, vitellogenesis*); steroids (17ß-estradiol, testosterone, progesterone, *functional role under debate*); terpenoids (methyl farnesoate (MF), *metamorphosis, reproduction*); neuropeptides (androgenic hormone, *sexual differentiation, vitellogenesis inhibition*; crustacean hyperglycemic hormone family (CHH), *energy metabolism*; molt-inhibiting hormone (MIH), *ecdysteroid production*; vitellogenesis-inhibiting hormone (VIH), *vitellogenesis*)
Insecta	Ecdysteroids (ecdysone, *molting, egg maturation*); terpenoids (JH, *metamorphosis, reproduction*); neuropeptides (adipokinetic hormone (AKH), *energy metabolism*; allatostatin and allatotropin, *JH production*; bombyxin, *ecdysteroid production, energy metabolism;* bursicon, *cuticle tanning*; diapause hormone, *embryonic diapause*; diuretic hormone (DH), *water homeostasis*; ecdysis-triggering hormone (ETH) and eclosion hormone (EH), *ecdysis behavior*; FMRFamides, *neuromodulation*; prothoraciotrophic hormone (PTTH), *ecdysteroid production*)

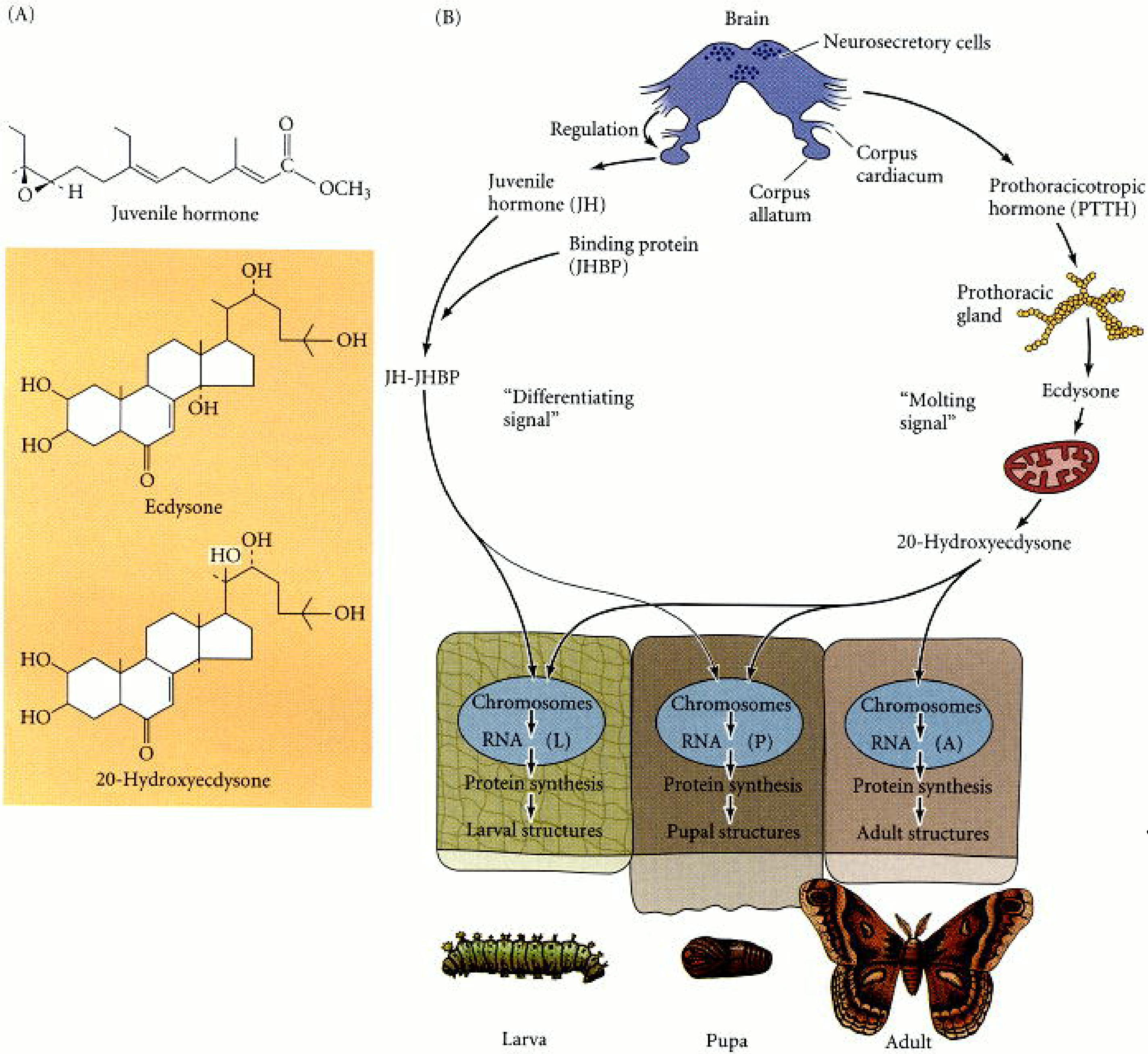
Hormony u ostnokožců a pláštěnců:

Echinodermata	Steroids (progesterone, testosterone, 17ß-estradiol, estrone, *vitellogenesis, oogenesis, spermatogenesis, spawning*); neuropeptides (gonad-stimulating substance = GSS, *spawning*; maturation-promoting factor = MPF, *fertilisation*)
Tunicata	Steroids (testosterone, 17ß-estradiol, *oogenesis, spermatogenesis, spawning*); neuropeptides (gonadotropin releasing hormone analogue, *gonad development*); thyroids (thyroxine, *probably tanning process during tunic formation*)

Metamorfóza hmyzu:



Modes of insect development. Molts are represented as arrows.
(A) Ametabolous (direct) development in a silverfish. After a brief pronymph stage, the insect looks like a small adult.
(B) Hemimetabolous (gradual) metamorphosis in a cockroach. After a very brief pronymph phase, the insect becomes a nymph. After each molt, the next nymphal instar looks more like an adult, gradually growing wings and genital organs.
(C) Holometabolous (complete) metamorphosis in a moth. After hatching as a larva, the insect undergoes successive larval molts until a metamorphic molt causes it to enter the pupal stage. Then an imaginal molt turns it into an adult.



Regulation of insect metamorphosis. (A) Structures of juvenil hormone, ecdysone, and the active moltin hormone 20-hydroxyecdysone. (B General pathway of insect metamorphosis Ecdysone and juvenil hormone together cause molts to keep the status quo and form another larval instar. When there a lower concentratio of juvenile hormone, the ecdysone-induce molt produces a pupa When ecdysone acts in the absence of juvenile hormone, th imaginal discs differentiate, and the molt gives rise t the adult

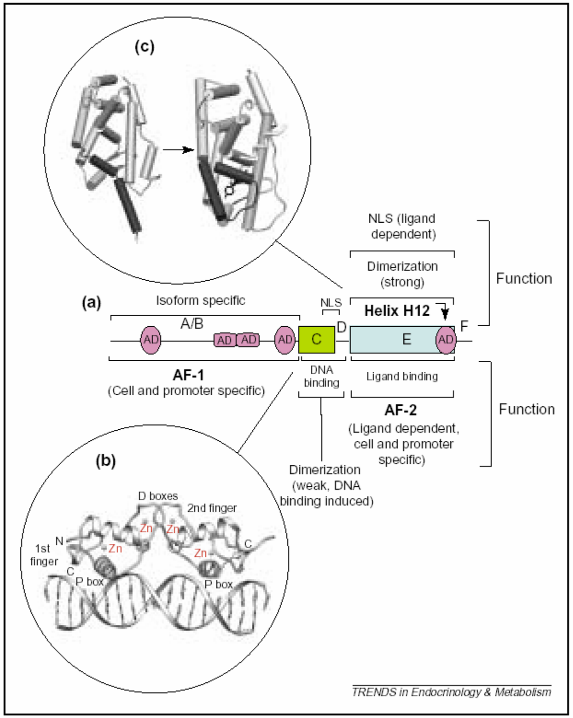
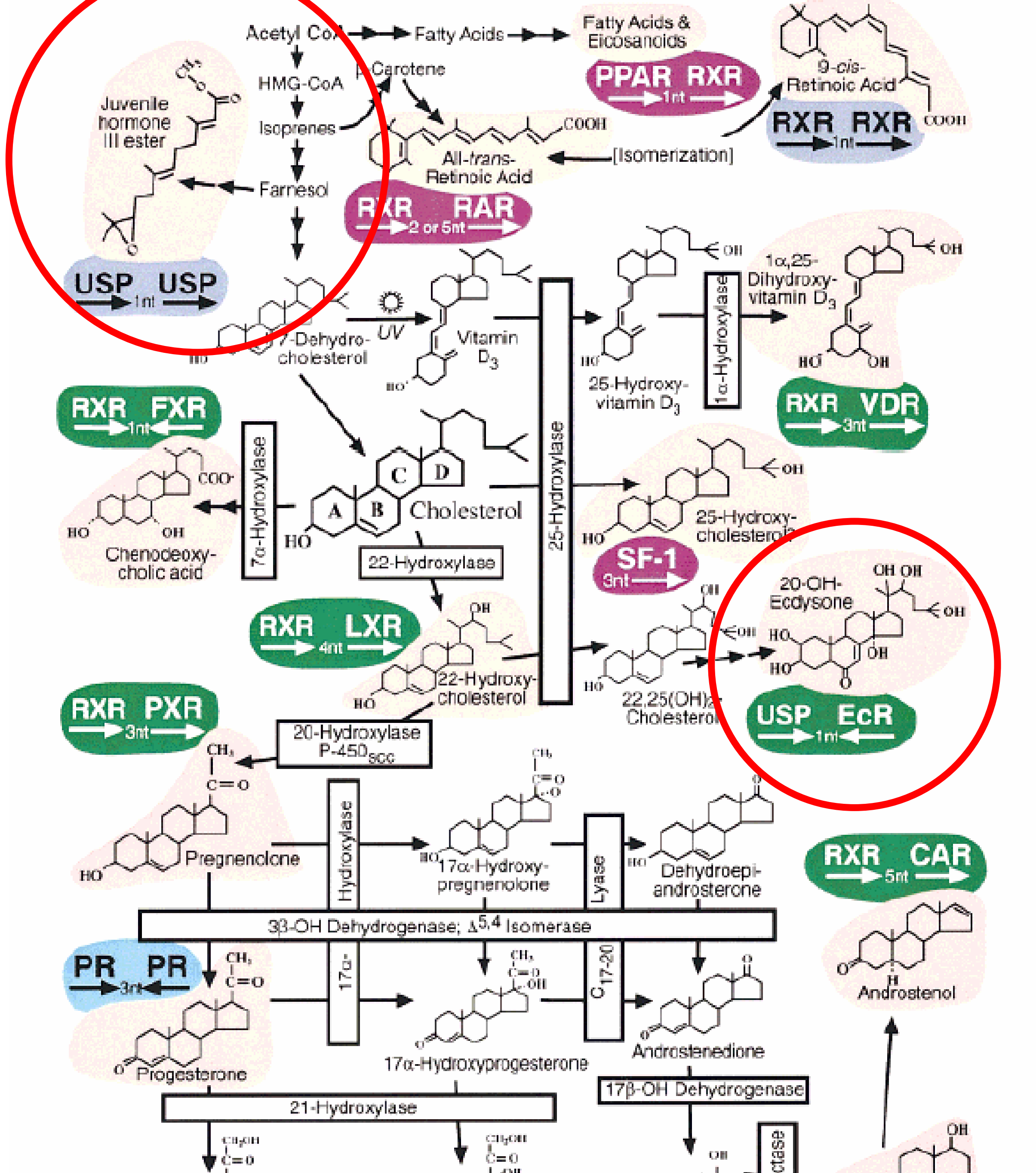
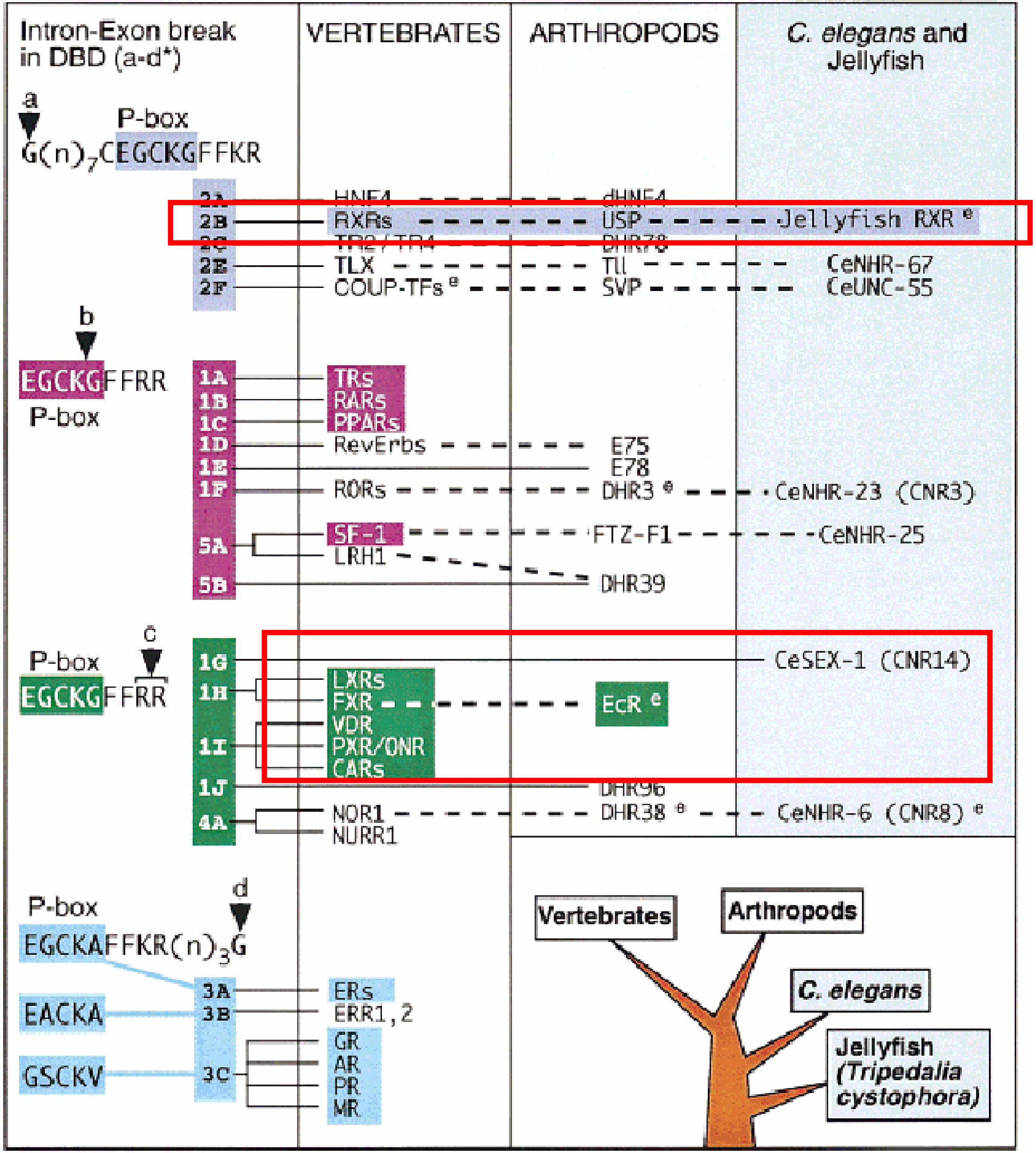


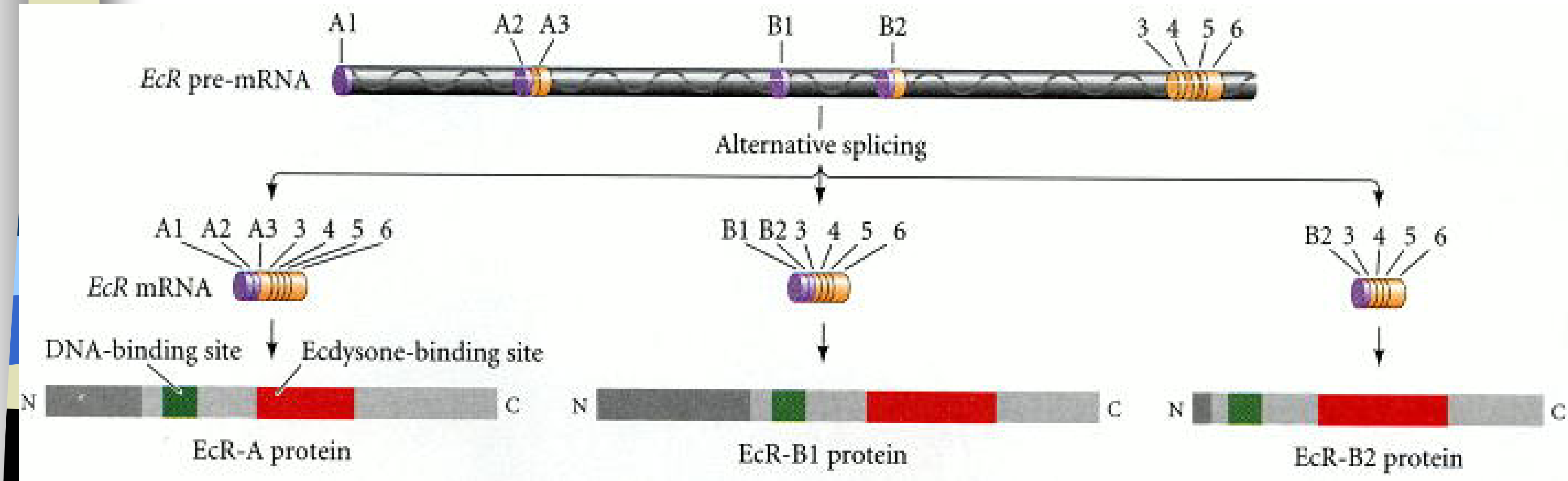
Fig. 1. (a) Schematic of the structural and functional organization of NRs. The evolutionary conserved regions C (DBD) and E (LBD) are indicated as boxes and a black line represents the divergent regions A/B, D and F. Two transcription AFs have been described in several NRs, a constitutively active (if taken out of the context of the receptor) AF-1 in region A/B and a ligand-inducible AF-2 in region E. Within these AFs, ADs have been defined. (b) Estrogen receptor DBD complex on a cognate DNA response element. (c) Agonist-induced changes of the LBD, allowing binding of coactivators (the bound coactivator-binding peptide is shown). Figures 1b,c are three-dimensional views derived from the corresponding crystal structures. Abbreviations: See Glossary.



Another candidate for the JH receptor role is the Methoprene-tolerant (Met) Per-Arnt-Sim (PAS) domain protein, whose loss confers tolerance to JH and its mimic methoprene in the fruit fly *Drosophila melanogaster*. (Konopová et al., 2007


Intron-Exon break in DBD (a-d*)
VERTEBRATES
ARTHROPODS
C. elegans and Jellyfish
a
P-box
G(n)7CEGCKGFFKR
2A
2B
2C
2E
2F
HNF4
RXRs
TR2/TR4
TLX
COUP-TFs
dHNF4
USP
DHR78
Tll
SVP
Jellyfish RXR
CeNHR-67
CeUNC-55
b
EGCKGFFRR
P-box
1A
1B
1C
1D
1E
1F
5A
5B
TRs
RARs
PPARs
RevErbs
RORs
SF-1
LRH1
E75
E78
DHR3
FTZ-F1
DHR39
CeNHR-23 (CNR3)
CeNHR-25
P-box
c
EGCKGFFRR
1G
1H
1I
1J
4A
LXRs
FXR
VDR
PXR/ONR
CARs
NOR1
NURR1
EcR
DHR96
DHR38
CeSEX-1 (CNR14)
CeNHR-6 (CNR8)
P-box
d
EGCKAFFKR(n)3G
EACKA
GSCKV
3A
3B
3C
ERs
ERR1,2
GR
AR
PR
MR
Vertebrates
Arthropods
C. elegans
Jellyfish (Tripedalia cystophora)

Ec receptory:

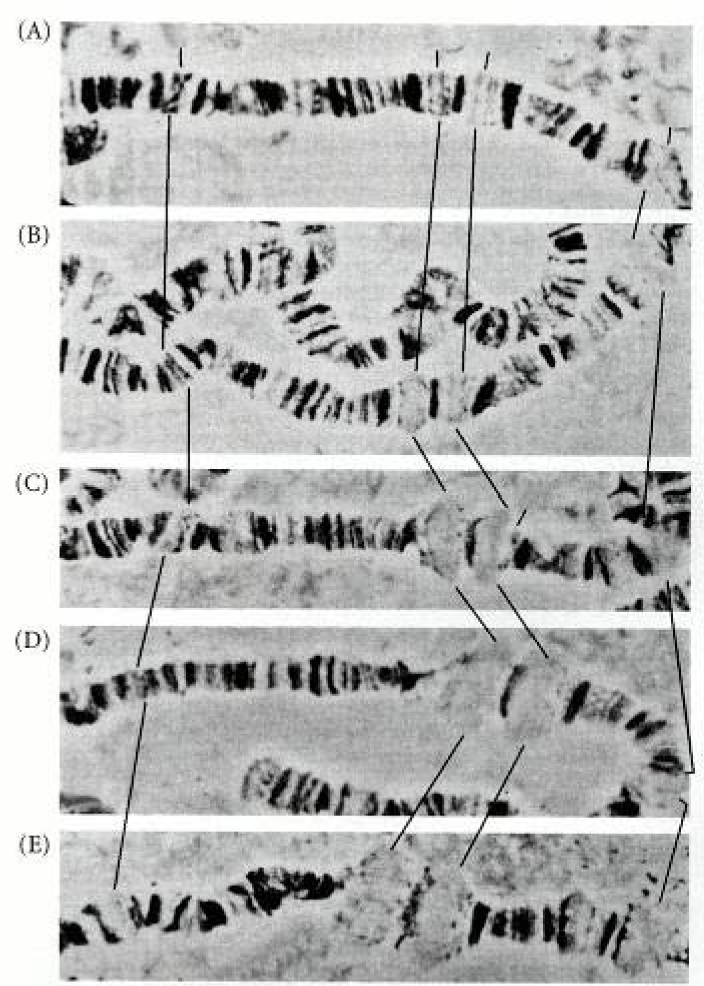


Formation of the ecdysone receptors. Alternative mRNA splicing of the ecdysone receptor (*EcR*) transcript creates three types of *EcR* mRNAs. These generate proteins having the same DNA-binding site (blue) and hydroxyecdysone-binding site (red), but with very different amino termini.

Three isoforms of EcR have been identified in insects, each with a different, stage-specific role in regulation of molting and development. This allows for one steroid hormone to induce a variety of different tissue responses. In general, EcR A is predominant when cells are undergoing a maturation response (from juvenile to adult) and is predominant in imaginal discs, whereas EcR B1 predominates in juvenile cells during proliferation or regression. Little is known about the function of the EcR B2 isoform.

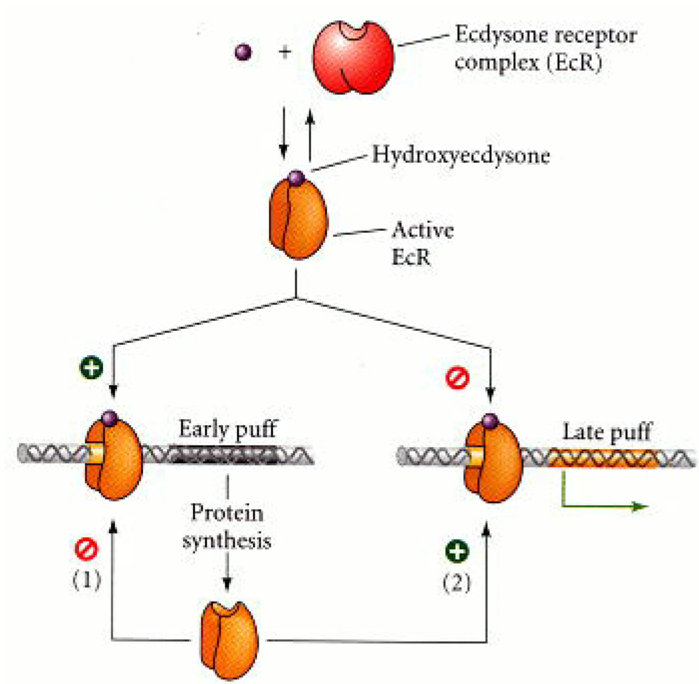
DNA and hormone binding are similar in the three isoforms of EcR. Little is known about the crustacean EcR isoforms and how they change during the molt cycle. However, the EcR that has been cloned from the crab, *Uca pugilator* (U31817, GenBank), shares 85–87% homology with that of *Drosophila* (M74078, GenBank). The differences are primarily in the region of the molecule involved with dimerization. Similar sequence similarities are found between the heterodimeric partner, USP.

There are several ecdysteroids which bind EcR, including 20-hydroxyecdysone, turkesterone, makisterone A, ponasterone A, and muristerone A. Some arthropods may use specific ecdysteroids as their principal molting hormone, but often several ecdysteroids are found within one group. The primary molting hormone for a range of organisms, including some insects and crustacea, is 20-OH ecdysone (20 HE). Among other examples, makisterone A is an important hormone for some crustacea and hemipteran insects.



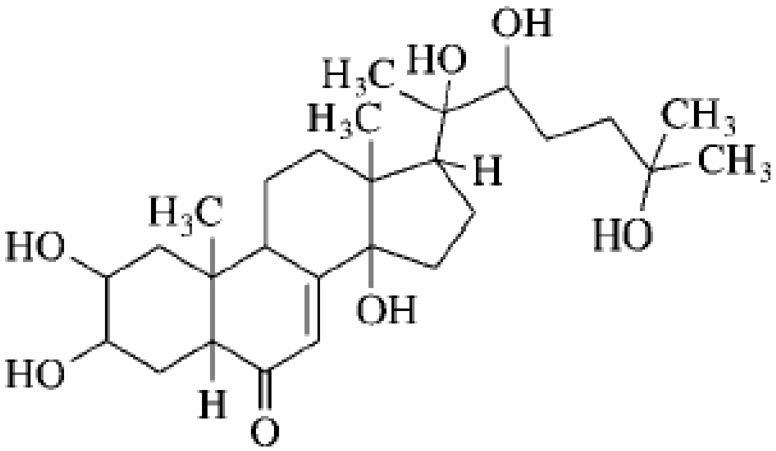
Hydroxyecdysone-induced puffs in cultured salivary gland cells of *D. melanogaster.* (A) Uninduced control. (B-E) Hydroxyecdysone-stimulated chromosomes at (B) 25 minutes, (C) 1 hour, (D) 2 hours, and (E) 4 hours.

The Ashburner model of hydroxyecdysone regulation of transcription. Hydroxyecdysone binds to its receptor, and this compound binds to an early puff gene and a late puff gene. The early puff gene is activated, and its protein product (1) represses the transcription of its own gene and (2) activates the late puff gene, perhaps by displacing the ecdysone receptor. (After Richards 1992.)

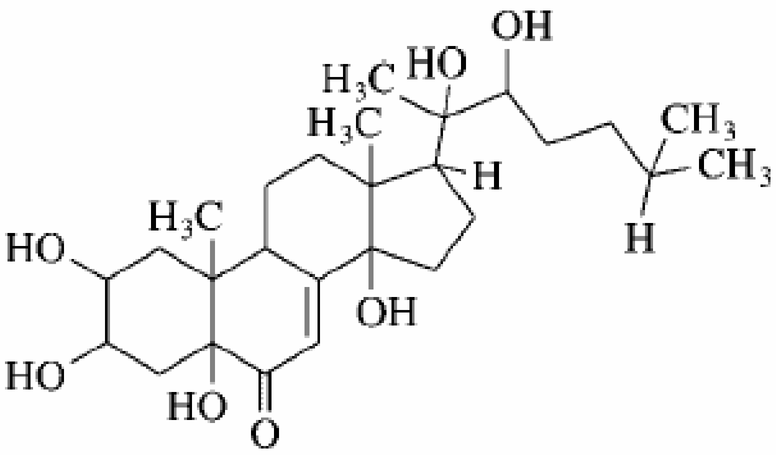


Struktury Ec hormonů:

20-OH Ecdysone
(20 HE)

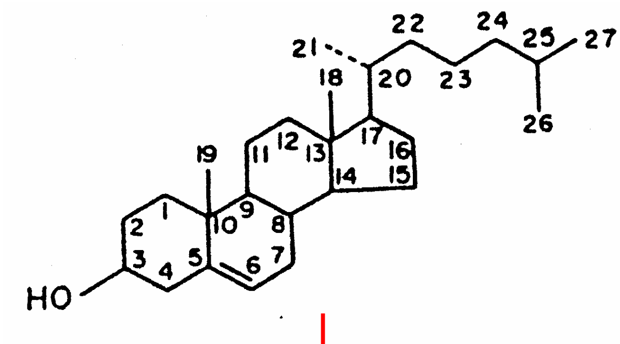


Muristerone A
(MurA)

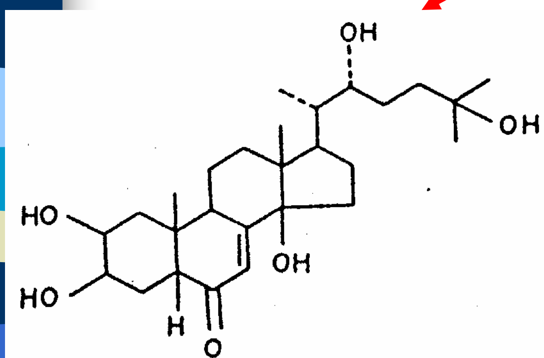


Synthesis of molting hormones

Cholesterol (from diet- a vitamin for insects)



Prothoracic gland

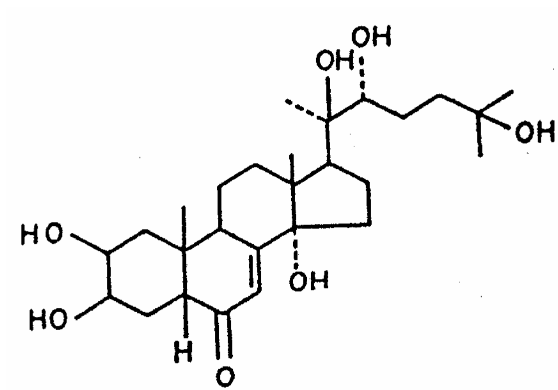


Ecdysone

mono-oxygenase
(fat body, epidermis)

Conjugates
(storage)

Metabolites
(excretion)

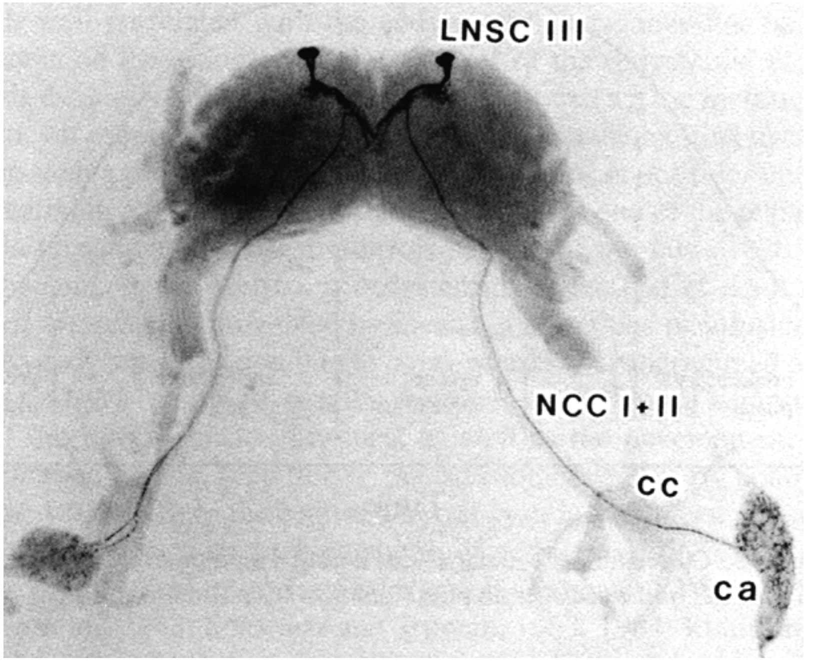


20-Hydroxyecdysone

Prothoracicotropic hormone (PTTH)

- Protein of 30-kDa active as a homodimer linked by a disulfide bond
- Produced by two pairs of lateral neurosecretory cells
- Released from corpora allata (moths) or corpora cardiaca (most insects)

PTTH cells of *Manduca* visualized with anti-PTTH antibody



The role of PTTH

Ecdysone synthesis and secretion are initiated by prothoracicotropic hormone (PTTH), a hormone produced by two pairs of neurosecretory cells in the brain. PTTH was first isolated and characterized from the silkmoth *Bombyx mori*. PTTH has conserved seven cysteine residues, several hydrophobic regions and an *N*-glycosylation site. Only the homodimeric form of *Bombyx* PTTH is biologically active. *Bombyx* PTTH is thought to be a member of the transforming growth factor-β (TGF-β) family.

- **Initiates every molt**
  - **stimulates prothoracic glands to synthesize and release ecdysone**
  - **serves as "mission control" allowing molt if the conditions are right**
    - **factors affecting decision to molt are species-specific**
      - **stretch of the abdomen by a blood meal in *Rhodnius***
      - **completion of the cocoon in some moths**
      - **escape from wet diet in flies**

Fytoekdosteroidy:

Phytoecdysteroids (PEs) are a family of about 200 plant steroids related in structure to the invertebrate steroid hormone 20-hydroxyecdysone. Typically, they are C27, C28 or C29 compounds possessing a 14a-hydroxy-7-en-6-one chromophore and A/B-cis ring fusion (5β-H).

PEs are attracting renewed attention because of their specific effects on invertebrate development (potential in invertebrate pest control) and their varied benign pharmacological actions on mammals (biomedical applications and gene switches). In the past three decades, several thousand species of plants have been surveyed for the presence of PEs and the structures of over 200 PEs have been deduced. The most frequently encountered PE is 20E, the principal physiological inducer of moulting and metamorphosis in arthropods.

Syntéza ekdysteroidů:

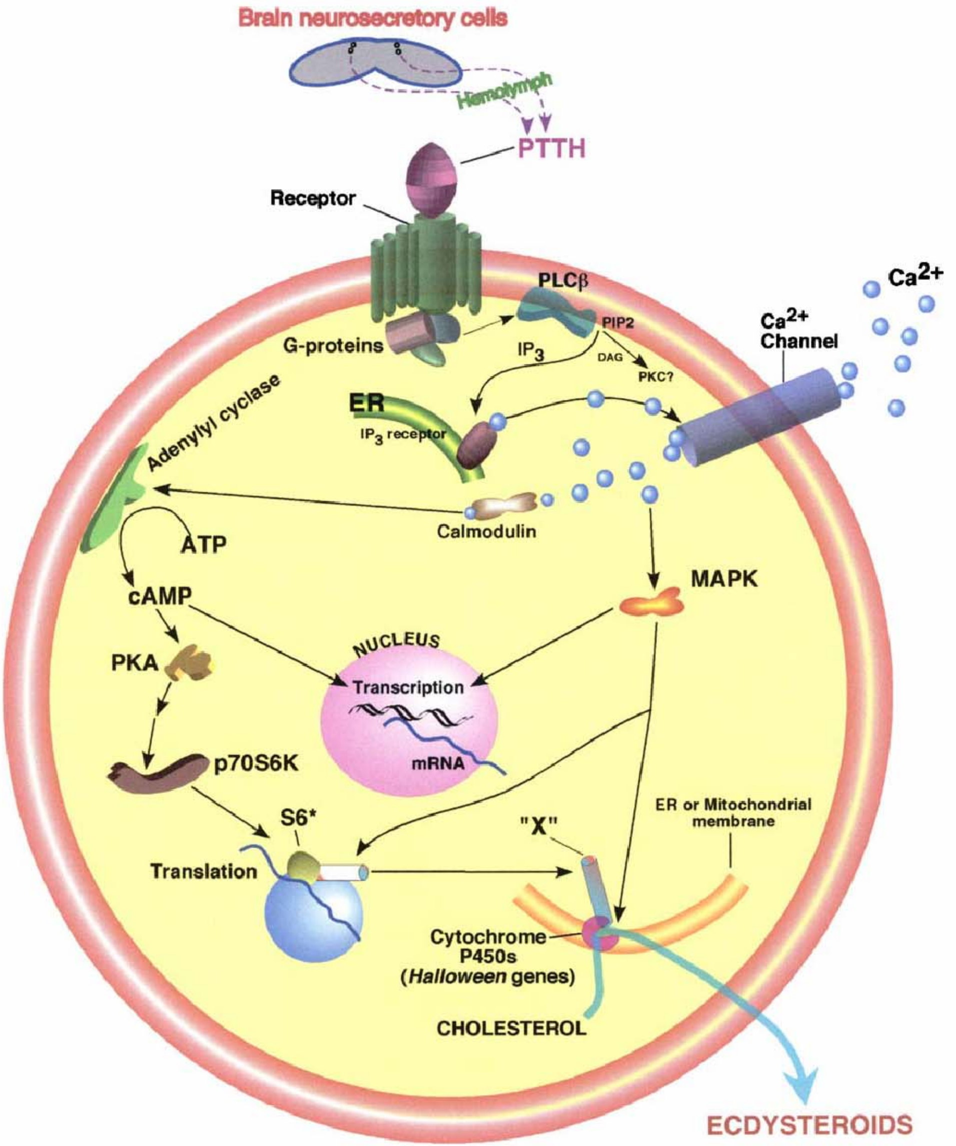
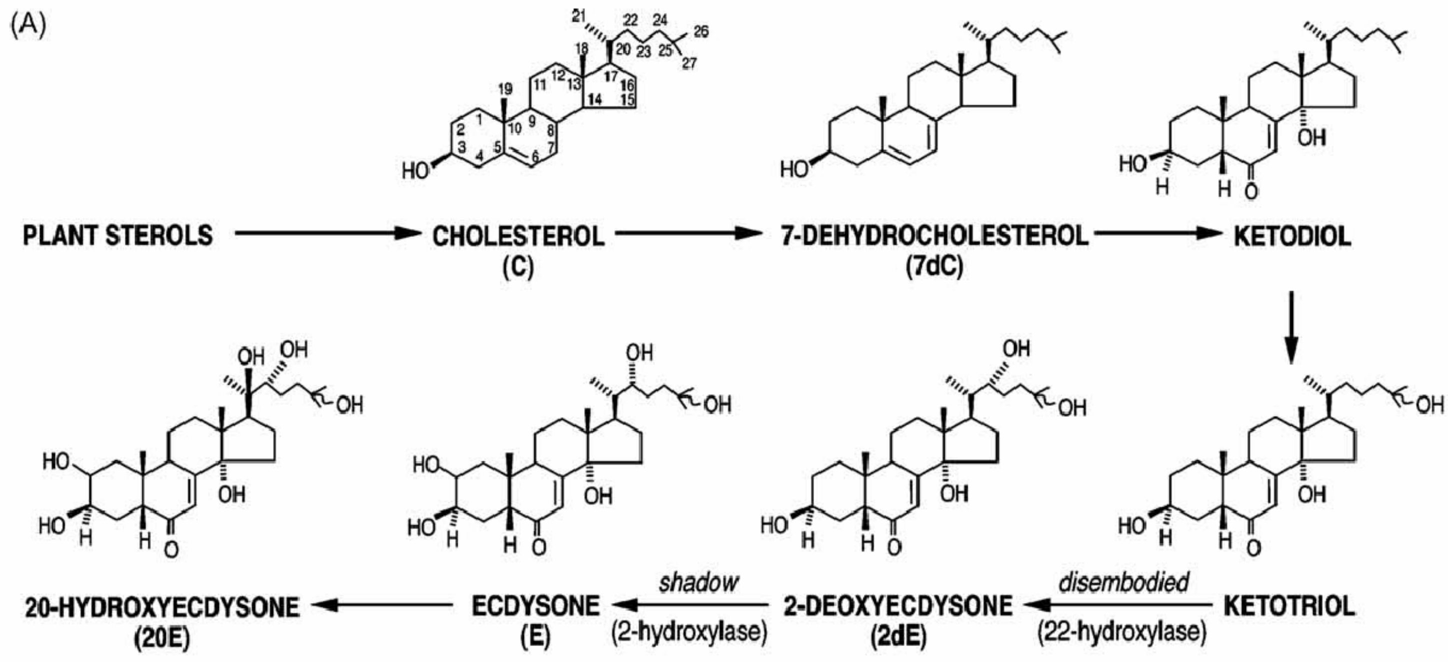
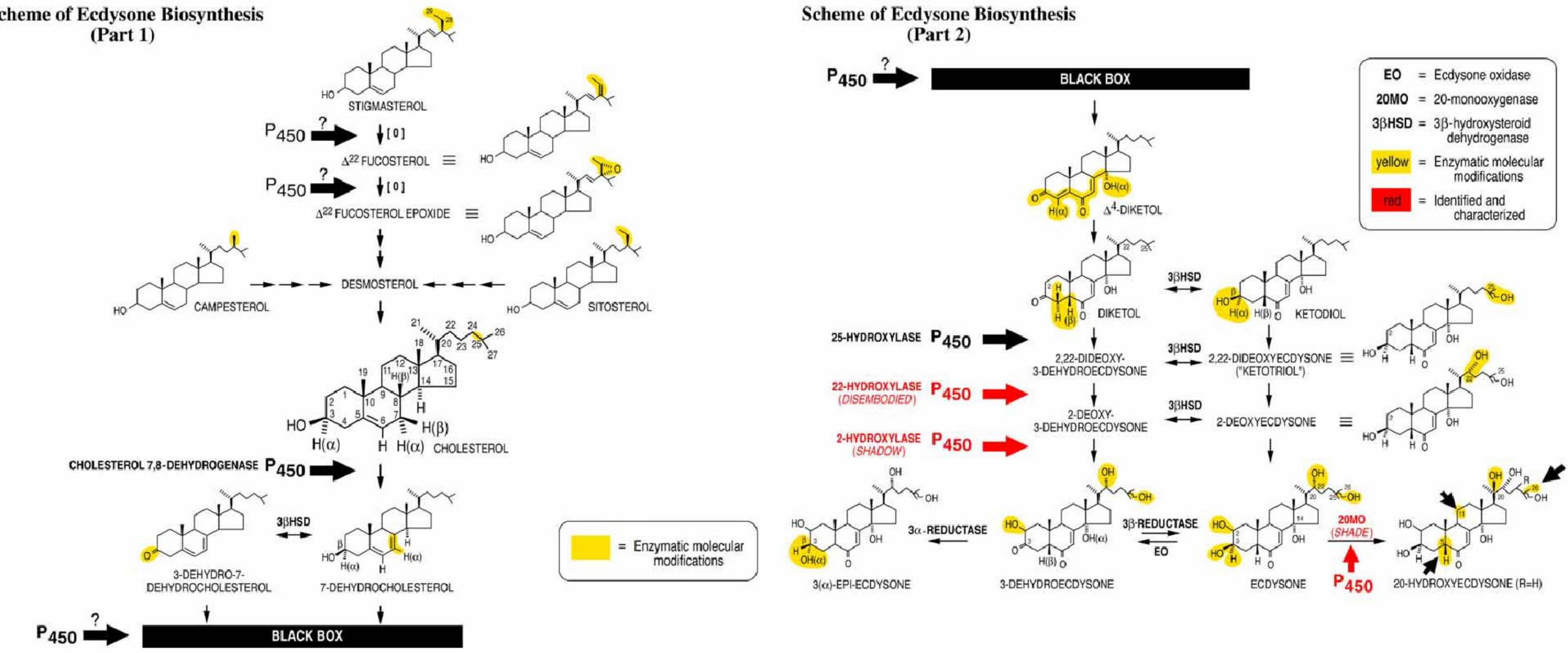


Fig. 2. Transductory cascade of PTTH interaction with a prothoracic gland cell. S6*, multiple phosphorylated form. (Graphics by Robert Rybczynski).

Syntéza ekdysteroidů a úloha cytochromů P450:

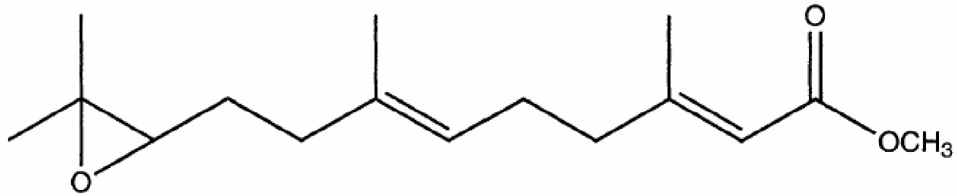


Syntéza ekdysteroidů a úloha cytochromů P450:

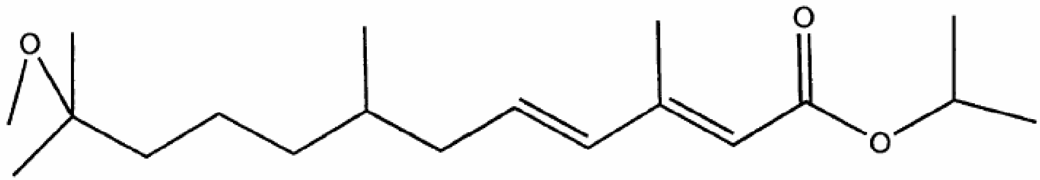


5. (Parts 1 and 2). The biosynthesis of 20-hydroxyecdysone from plant sterols. Question marks denote possible involvement of P450 enzymes. Note specifically where the Halloween gene produ
red). 3-Dehydroecdysone is synthesized in the prothoracic glands of many insects (e.g. *Manduca sexta)* and converted to ecdysone in the hemolymph (left column of part 2). For *Drosophila*, ecdys
nthesized in the prothoracic gland cells of the ring gland (right column of part 2).

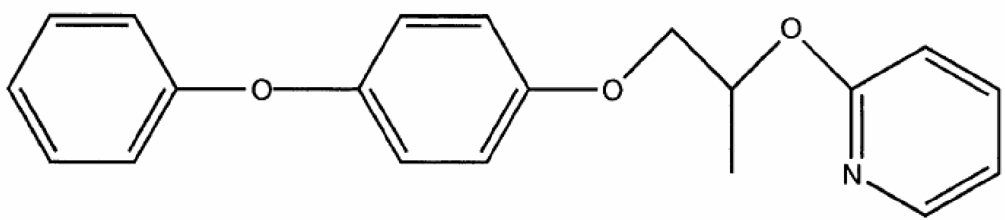
Juvenilní hormon a jeho analogy:



JH III

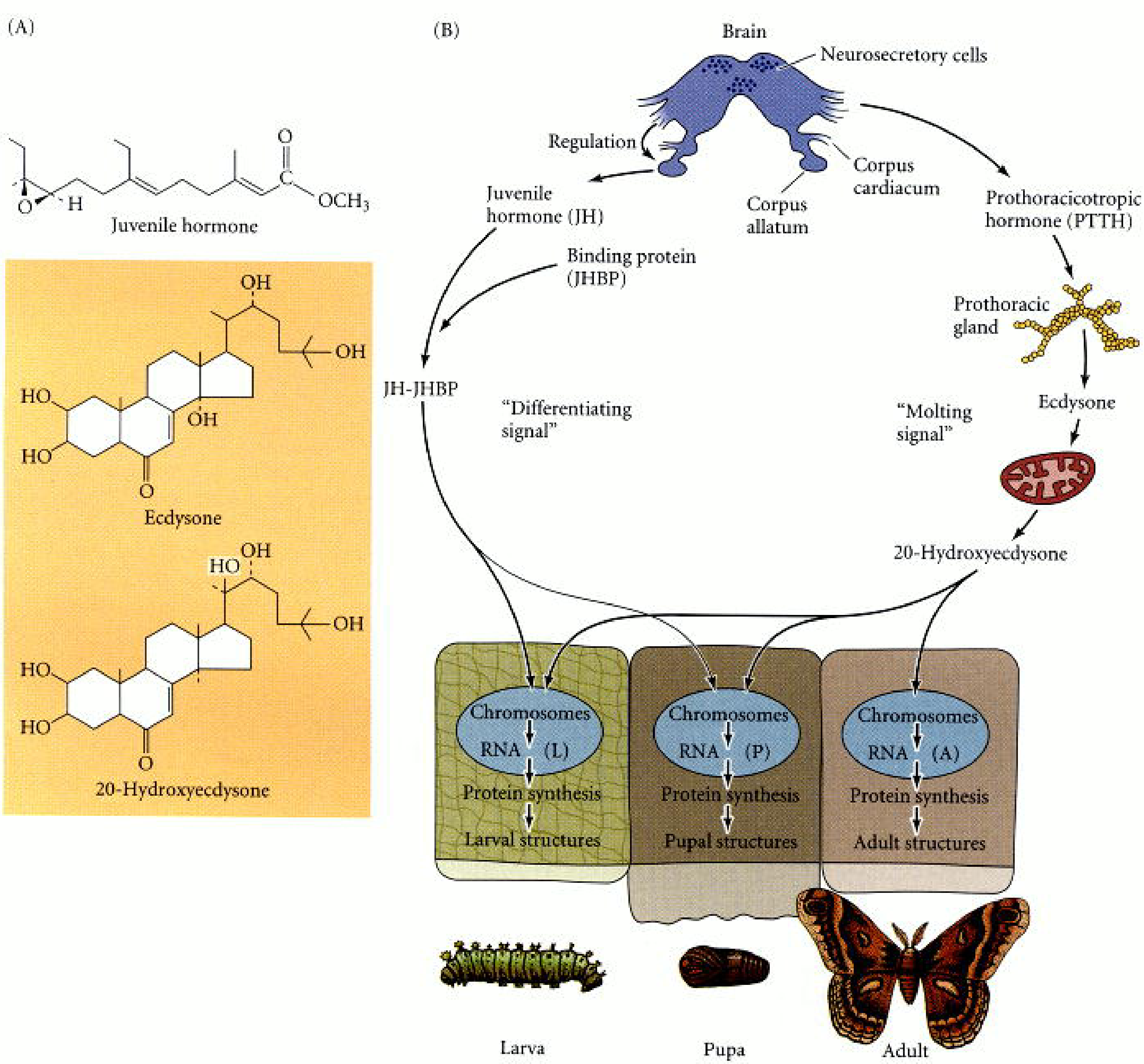


Methoprene



Pyriproxyfen

Fig. 1. Chemical structures of JH III and JHAs.



Regulation of insect metamorphosis. (A) Structures of juvenile hormone, ecdysone, and the active molting hormone 20-hydroxyecdysone. (B) General pathway of insect metamorphosis. Ecdysone and juvenile hormone together cause molt to keep the status quo and form another larval instar. When there is a lower concentration of juvenile hormone the ecdysone-induced molt produces a pupa. When ecdysone acts in the absence of juvenile hormone, the imaginal discs differentiate, and the molt gives rise to the adult

Insect juvenile hormones are critical developmental hormones that have direct effects on both larval development and adult reproductive competence. Most insect orders appear to synthesize a single JH homolog, methyl (2*E*,6*E*)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate (JH III) but Lepidoptera and at least some Diptera synthesize additional homologs.

Although not well documented, regulation of JH production is complicated and involves endogenous neural, neuroendocrine signals and, in some cases, male produced exogenous regulators transferred to the female during mating. Among virgin females, JH is required for vitellogenesis and, thus, females do not become reproductively competent until JH production is stimulated. To date, only two neuropeptides that regulate JH biosynthesis in adult Lepidoptera have been identified. These were identified e.g. from the tobacco hornworm moth (*Manduca sexta*) and are:

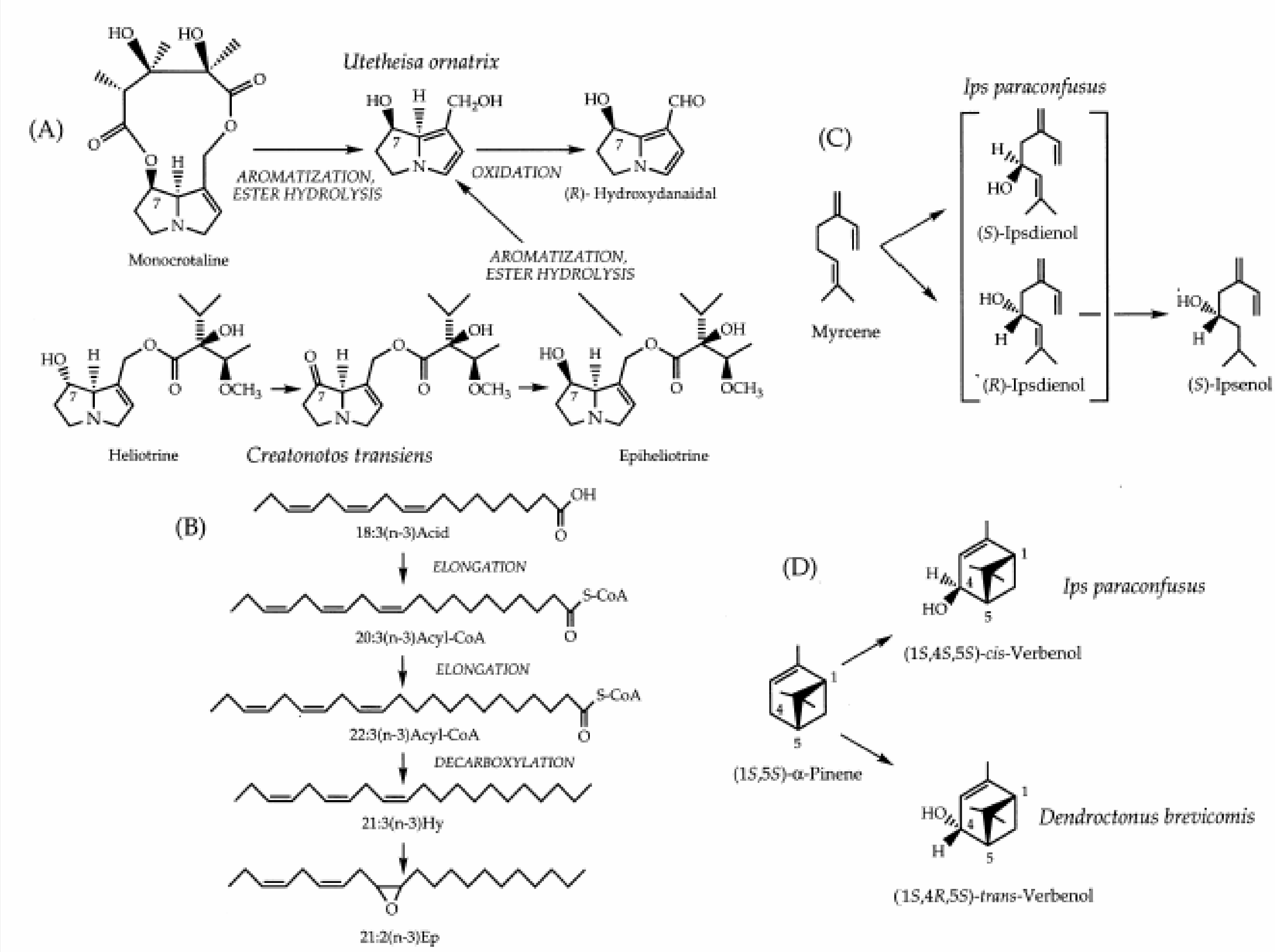
allatotropin

(Gly-Phe-Lys-Asn-Val-Glu-Met-Met-Thr-Ala-Arg-Gly-Phe-NH2)

allatostatin

(*p*Glu-Val-Arg-Phe-Arg-Gln-Cys-Tyr-Phe-Asn-Pro-Ile-Ser-Cys-Phe-COOH).

Hormonální regulace syntézy feromony:



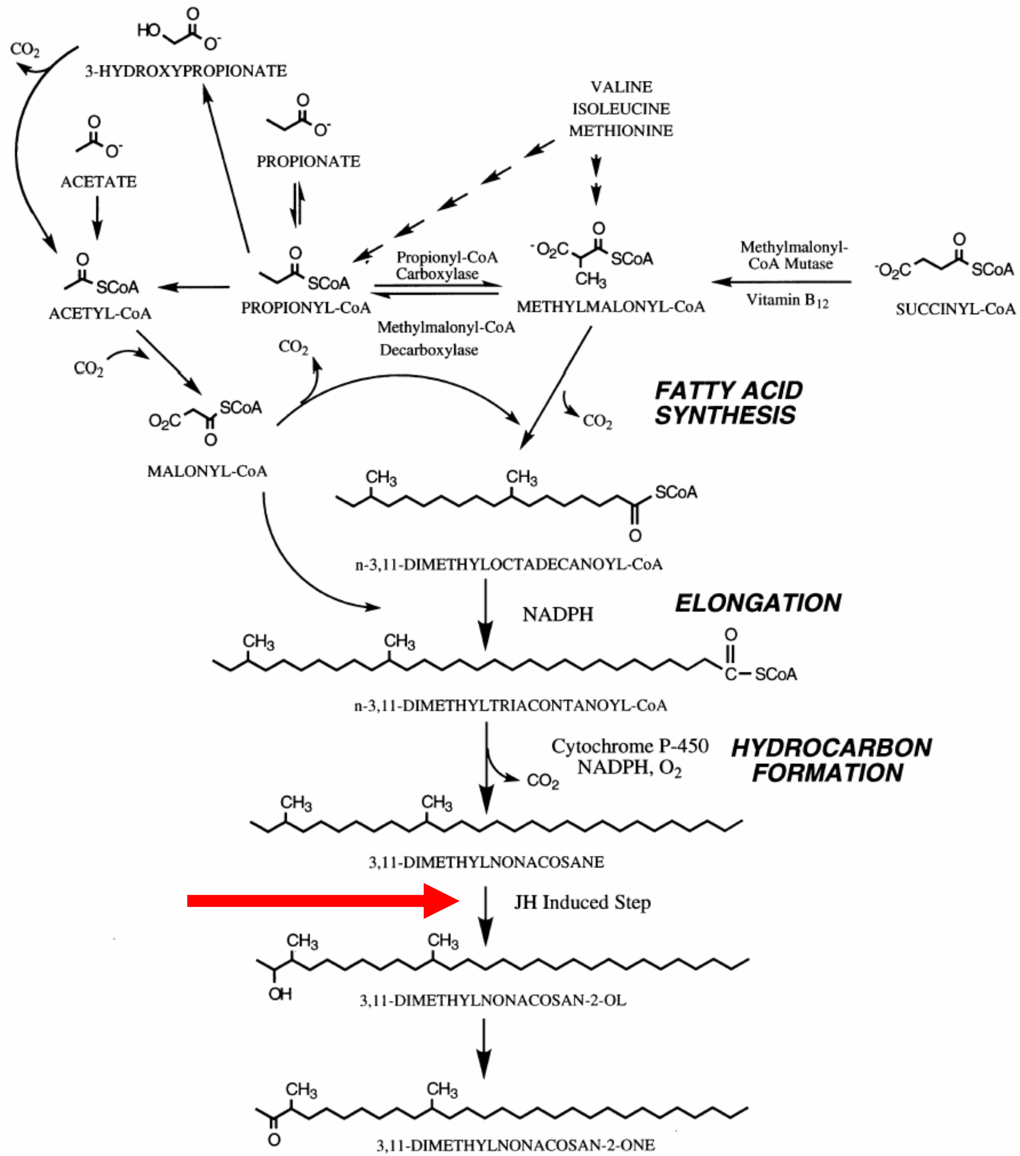
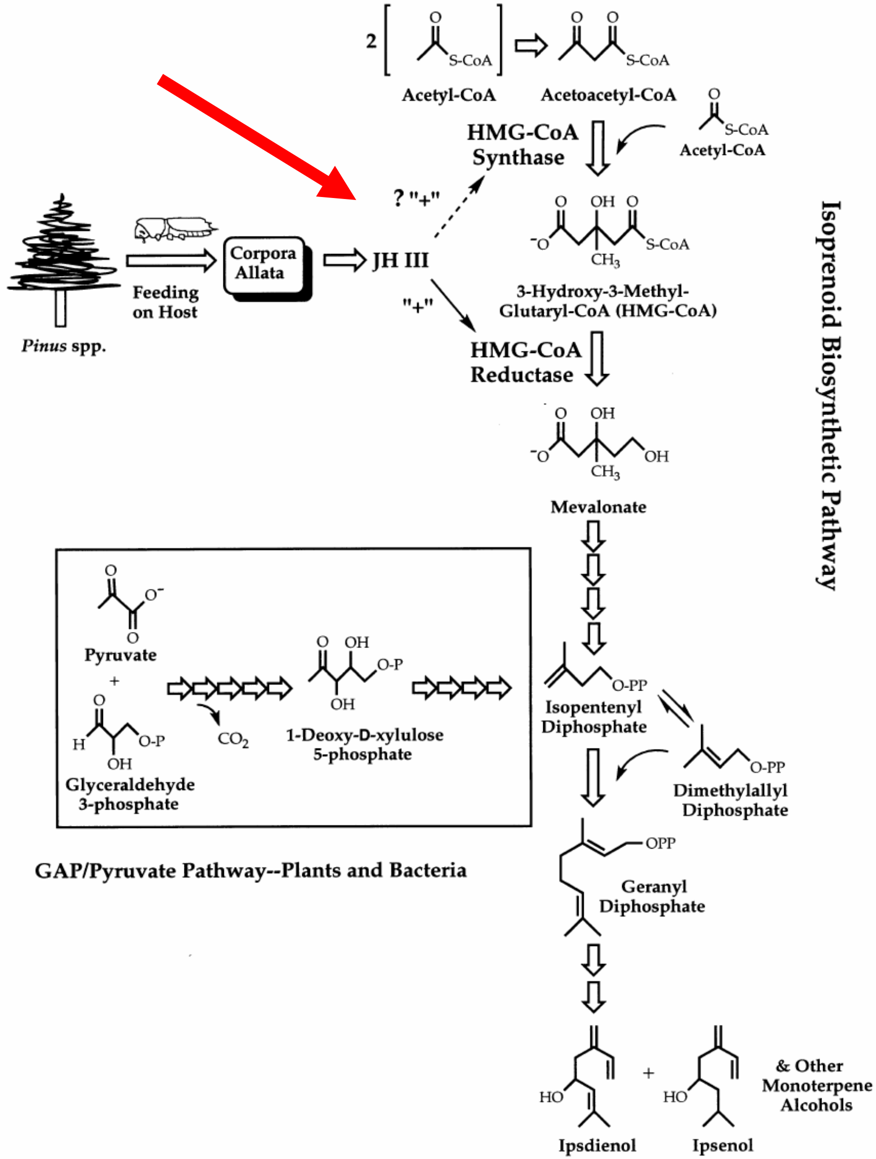


Fig. 2. Blattodean pheromone biosynthetic pathways utilize fatty acid biosynthesis from malonyl–CoA and methylmalonyl–CoA substrates followed by cytochrome P-450-mediated decarboxylation, hydroxylation, and oxidation. The hydroxylation step is regulated by JH III (adapted from Chase et al., 1992 for *Blattella germanica* sex pheromone components).



Coleopteran pheromone biosynthetic pathways as exemplified for *Ips* spp. [e.g. *Ips pini* (Say)] and acyclic monoterpenoid (ipsdienol) pheromone biosynthesis. The classical mevalonate-based isoprenoid pathway is regulated by juvenile hormone III (JH III) at enzymatically catalyzed steps prior to mevalonate. Feeding on host *Pinus* spp. phloem induces synthesis of JH III by the corpora allata.

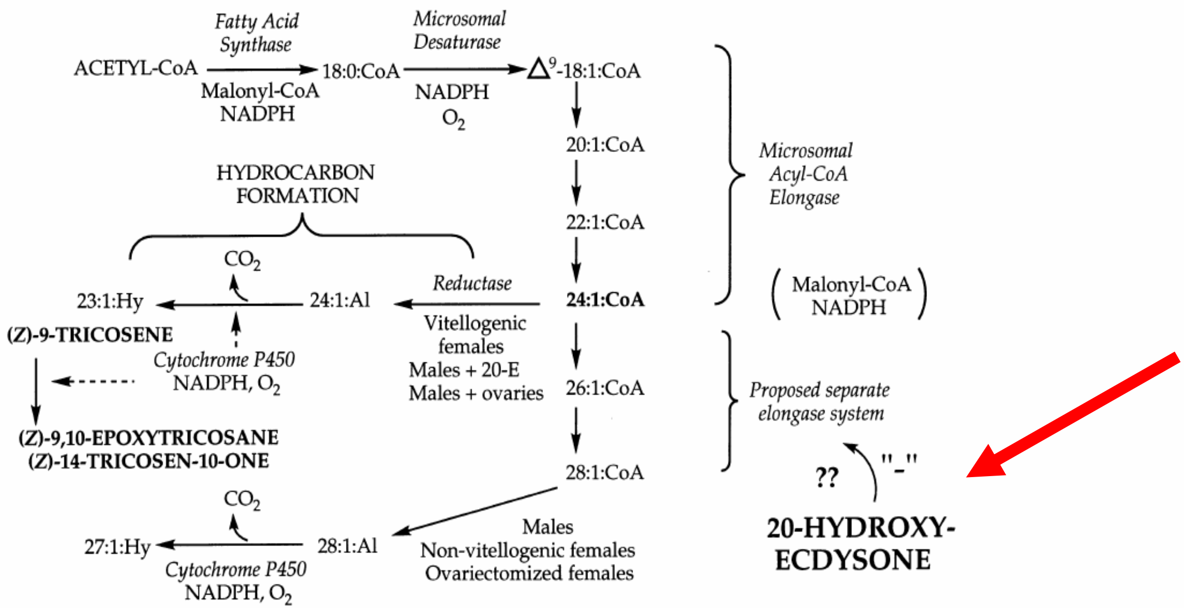
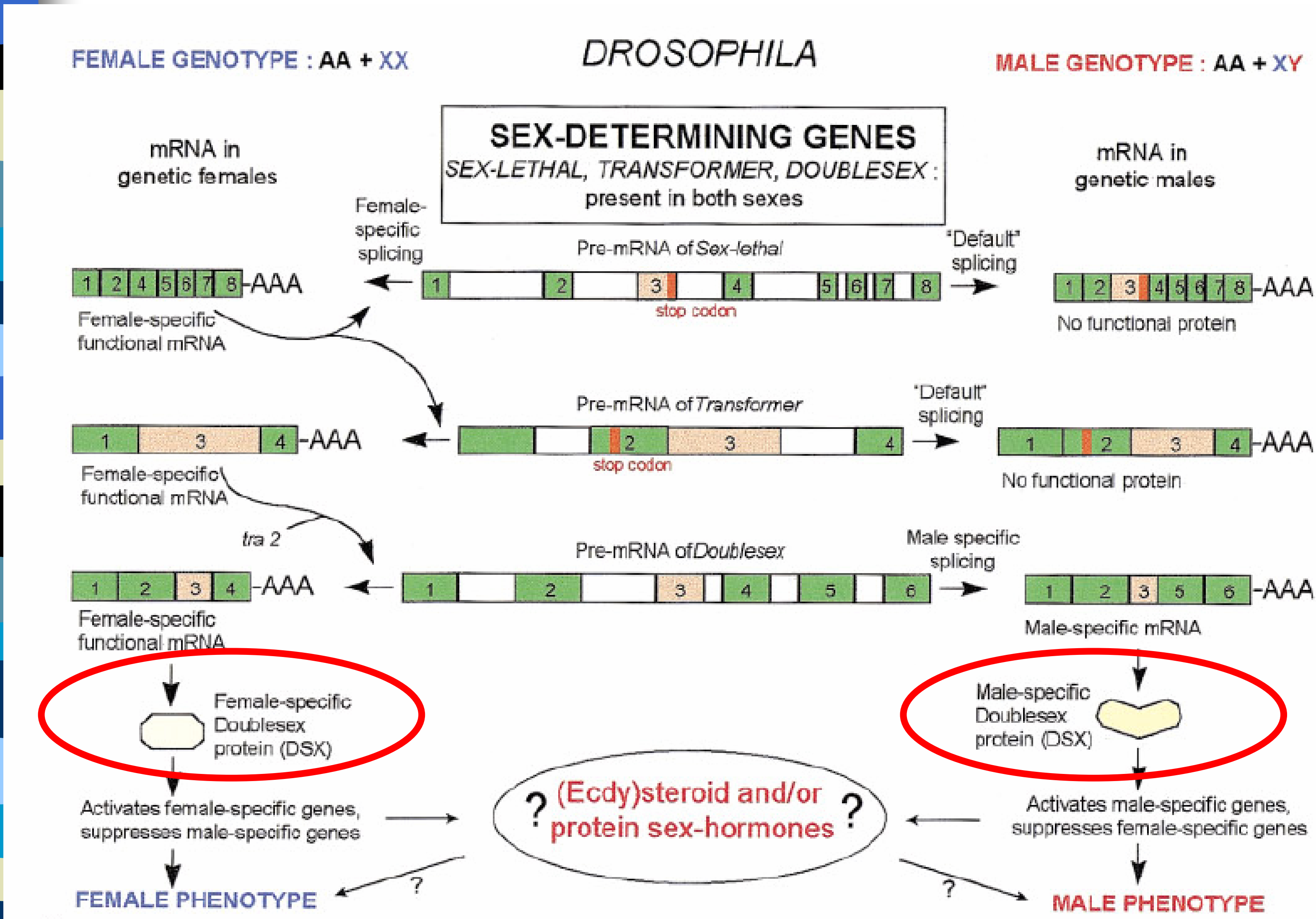


Fig. 4. Dipteran pheromone biosynthetic pathways utilize fatty acid synthesis, desaturation, elongation, and reductive decarboxylation. The proposed regulatory steps for 20-hydroxyecdysone are the secondary elongation system. Unsaturated hydrocarbons can be further modified to the epoxides (adapted from Blomquist et al., 1987a for the common house fly, *Musca domestica* L. sex pheromone components).

Vyskytují se u hmyzu pohlavní hormony?



Endokrinní disrupce u bezobratlých:

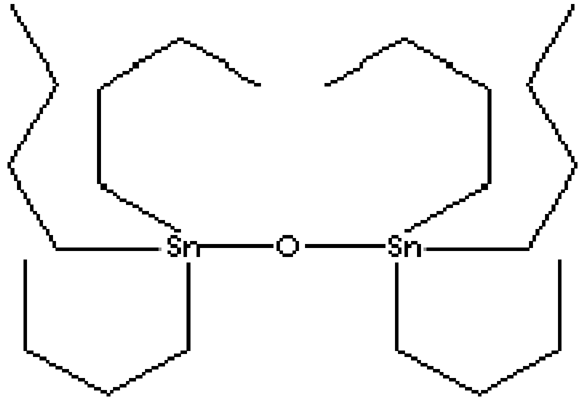
The issue of endocrine disruption (ED) in invertebrates has generated remarkably little interest in the past compared to research with aquatic vertebrates in this area. However, with more than 95% of all known species in the animal kingdom, invertebrates constitute a very important part of the global biodiversity with key species for the structure and function of aquatic and terrestrial ecosystems. Despite the fact that ED in invertebrates has been investigated on a smaller scale than in vertebrates, invertebrates provide some of the best documented examples for deleterious effects in wildlife populations following an exposure to endocrine-active substances. The principal susceptibility of invertebrates to endocrine-active compounds is demonstrated with the case studies of tributyltin effects in mollusks and of insect growth regulators, the latter as purposely synthesized endocrine disrupters.

Imposex:

The first adverse effects of TBT on mollusks were observed in *Crassostrea gigas* at the Bay of Arcachon, one of the centers of oyster aquaculture in Europe with ball-shaped shell deformations in adults, and a dramatically decline of annual spatfall. These effects led to a break-down of local oyster production in the bay with marked economic consequences. Laboratory and field analyses revealed that TBT from antifouling paints was the causative agent with trace concentrations as low as 10 to 20 ng TBT/L in ambient water being already effective. Another TBT effect in mollusks was initially described in a number of regions worldwide in the early 1970s without identifying the organotin compound as the causative agent at that time: A virilization of female prosobranchs, which has been termed as **imposex**. Imposex is characterized by the formation of a penis and/or vas deferens on females of gonochoristic prosobranch species and is induced at lower concentrations than all other described TBT effects. Furthermore, it is a specific response of organotin compounds under field conditions. Today, imposex is known to occur in more than 150 prosobranch species.

Imposex:

Tributyltin (TBT)



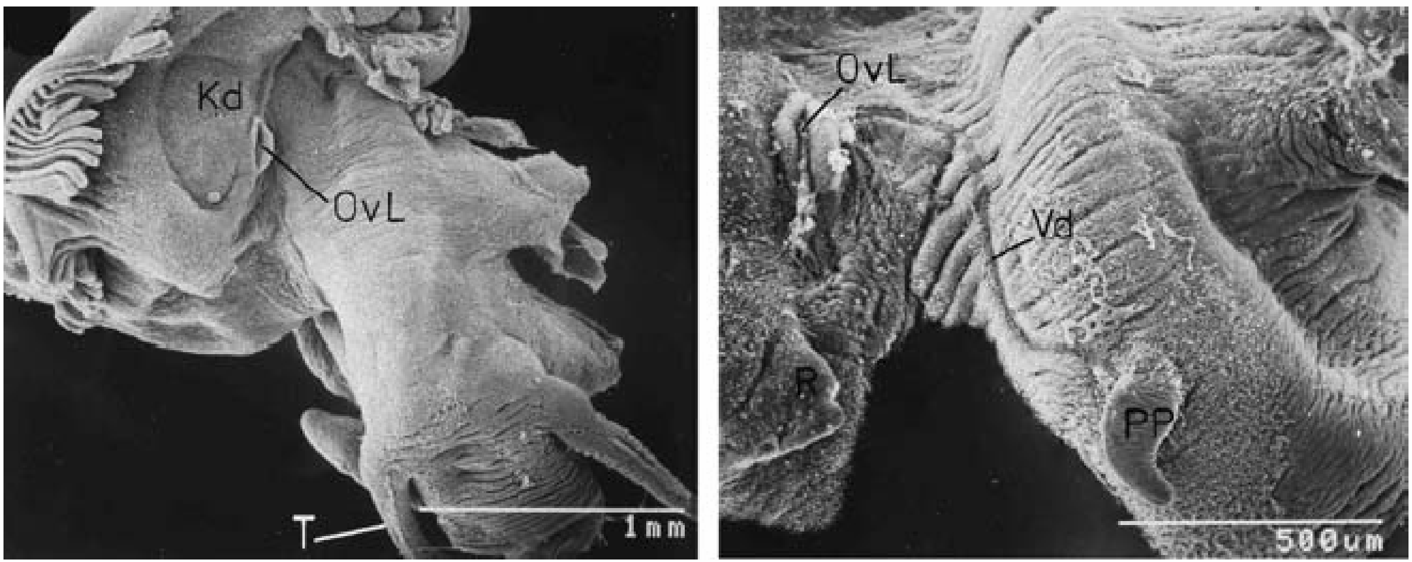


Fig. 1 *Hydrobia ulvae*. Scanning electron micrographs of females with their mantle cavity opened. Left: normal female without imposex; right: sterilized female in the final stage of imposex with blocked oviduct. Abbreviations: Kd, capsule gland; OvL, Ooparous opening of oviduct (open left; blocked right); PP, Penis; T, tentacle; Vd, vas deferens.

The periwinkle *Littorina littorea* develops a closely related virilization phenomenon as a response to TBT exposure, termed as **intersex**. Intersex females are either characterized by male features on female pallial organs, specifically by an inhibition of the ontogenetic closure of the pallial oviduct or female sex organs are supplanted by the corresponding male formations particularly by a prostate gland. Comparably to imposex, the intersex response is a gradual transformation of the female pallial tract, which can be described by an evolutive scheme with four stages.

Intersex development causes restrictions of the reproductive capability of females. In stage 1, a loss of sperm during copulation is possible and consequently the reproductive success is reduced. Females in stages 2-4 are definitively sterile because the capsular material is spilled into the mantle cavity (stage 2) or the glands responsible for the formation of egg capsules are missing (stages 3 and 4). Due to female sterility, periwinkle populations can be in decline but are not likely to become extinct because of the planktonic veliger larvae produced by the species, as long as aqueous TBT levels are not beyond mortality threshold concentrations for the larvae (Matthiessen *et al.* 1995).

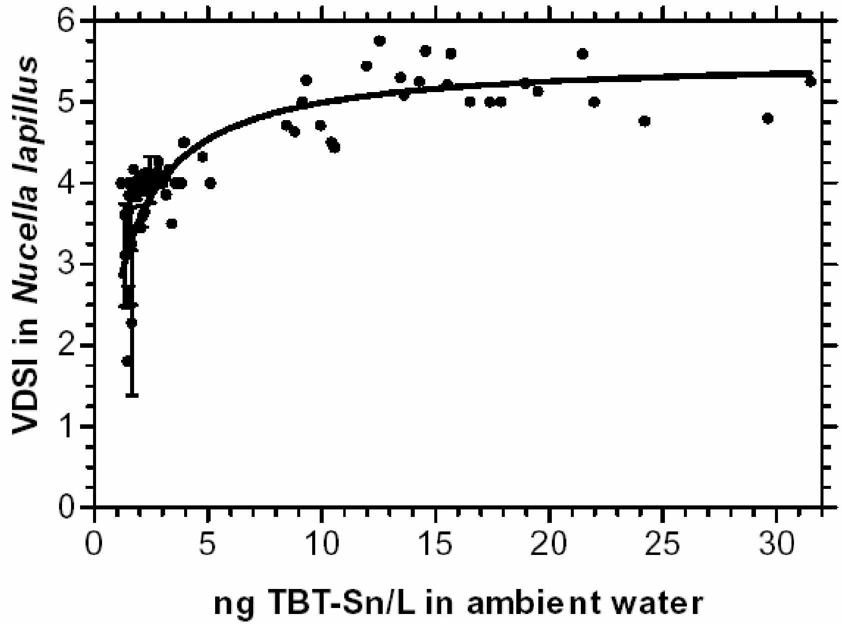


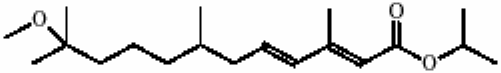
Fig. 2 *Nucella lapillus*. Relationship between aqueous TBT concentrations and imposex intensities: $y = (5.54\ x) / (1.12 + x)$; $n = 151$ population samples from 81 stations; $r = 0.688$; $p < 0.0005$.

One of the most important lessons to be learned from the "TBT story" and its effects in mollusks is that EDCs may impact different levels of biological integrations from molecules to communities affecting also the survival of populations in the field. Furthermore, the case history of TBT provides evidence for vertebrate-type steroids playing an important functional role in a number of invertebrate groups, including prosobranchs.

Alternative Insecticides: Insect Growth Regulators

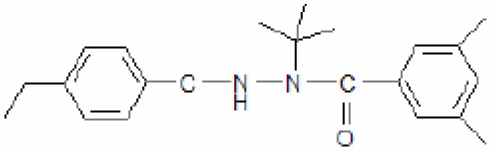
- "Nature and the pesticide industry apparently have decided that the best way to poison an animal is through its nervous system"
- most insecticides are nerve poisons, so mammalian selectivity must arise from differences in pharmacokinetics or metabolism
- a better way to go: find chemicals that attack biological processes *unique to insects* = biorational design
    - insects must shed their skin periodically to grow = molting
    - insects undergo metamorphosis between life stages
    - both processes are under strict endocrine control
    - 1. *juvenile hormone* (JH) titer in blood determines the next step in development; 2. *Ecdysone* (molting hormone) stimulates the molting process
- insecticides have been developed that mimic JH and ecdysone
- practical problem: agent must be present at the critical period to influence development (narrow window of susceptibility)

- Methoprene (Precor, Altosid) – first commercial *JH mimic* (Zoecon, 1978). LD_{50} = 34,600, MSR = 1.7 x 10^6
- used as a mosquito larvicide (approved by WHO in drinking water for mosquito control); feed to livestock to control flies in manure; home control of fleas; control of stored product pests, mushroom pests. Used in Japan on silkworms to increase silk production.
- about 10 additional JH mimics have been commercialized



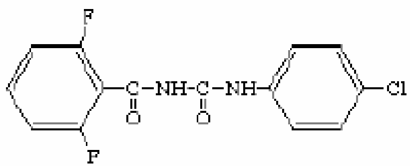
methoprene

- Tebufenozide (Mimic, Confirm) – first commercial *ecdysone agonist* (Rohm & Haas, 1991). LD_{50} > 5000 mg/kg
- very effective against Lepidoptera and Colorado potato beetle – induces lethal premature molting
- first insecticide jointly registered by the EPA (USA) and the PMRA (Canada), 1996

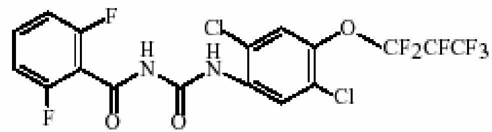


tebufenozide

- Diflubenzuron (Dimilin) – first commercial *chitin synthesis inhibitor* (Phillips, 1972). LD_{50} = 4640 mg/kg.
- prevents insects from completing a molt by interfering with the synthesis of chitin, the main constituent of the integument. Not a direct action on chitin synthetase, but prevents the final step in activation of the enzyme. Slow acting.
- major uses: boll weevil on cotton, gypsy moth and other forest pests – used in Kitsilano in 1979 against gypsy moth



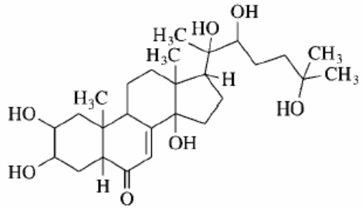
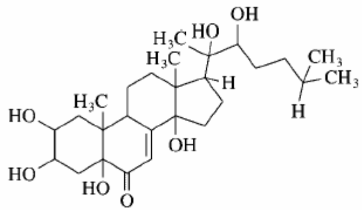
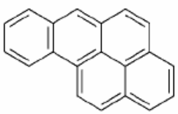
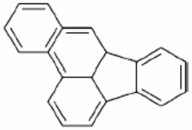
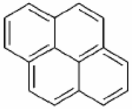
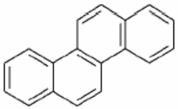
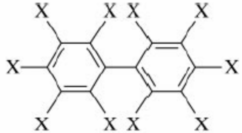
diflubenauron



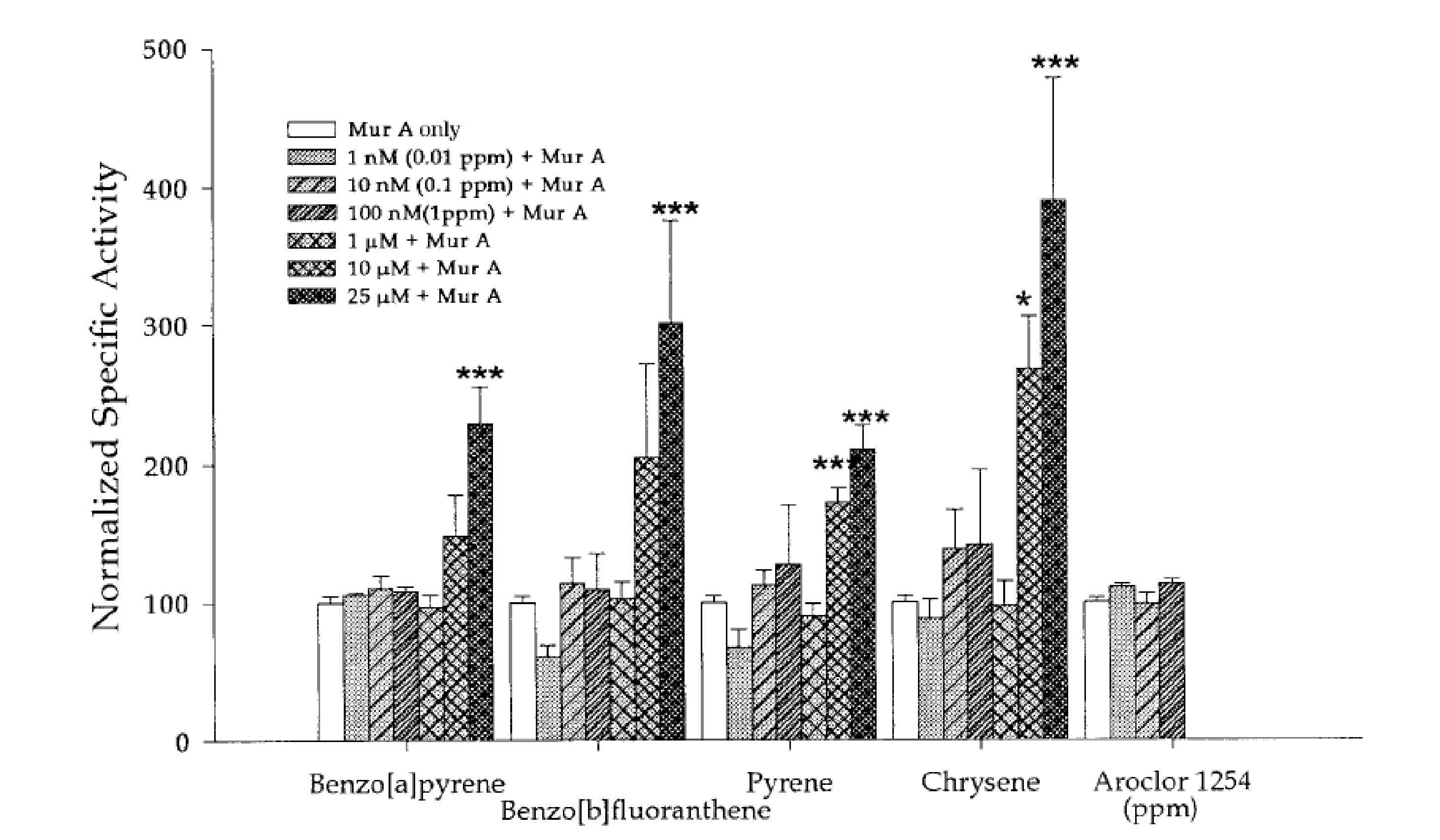
lufenuron

- Lufenuron (Program) – developed by Novartis in 1994. Oral IGR for flea control in dogs and cats – prevents egg development.
- LD_{50} = 2000 mg/kg; pet dose ~ 20 mg/kg

Polycyklické aromatické uhlovodíky mohou aktivovat EcR:

Compound	Chemical structure	Steroid interactions	References
20-OH Ecdysone (20 HE)		Ecdysone agonist	Cottam and Milner (1997) Yao *et al.* (1993)
Muristerone A (MurA)		Ecdysone agonist	Cottam and Milner (1997) Yao *et al.* (1993)
Benzo[*a*]pyrene (BaP)		AhR agonist Anti-estrogen Anti-androgen Not estrogenic in fish	Hankinson (1995) Tran *et al.* (1996) Chang and Liao (1987) Thomas and Smith (1993)
Benzo[*b*]fluoranthene (BbF)		AhR agonist	Hankinson (1995)
Pyrene		No AhR activation	Poland and Knutson (1982)
Chrysene		AhR agonist No estrogen receptor (ER) interaction	Hankinson (1995) Tran *et al.* (1996)
Aroclor 1254	 X = H or Cl, 54% Cl	AhR agonist Thyroid hormone Not antiestrogen Estrogenic (?) in fish	Hankinson (1995) Jacobson and Jacobson (1996) Goldey *et al.* (1995) Krishnan and Safe (1993) Flouriot *et al.* (1995) Thomas and Smith (1993)

Polycyklické aromatické uhlovodíky mohou aktivovat EcR:



Polycyklické aromatické uhlovodíky mohou aktivovat EcR:

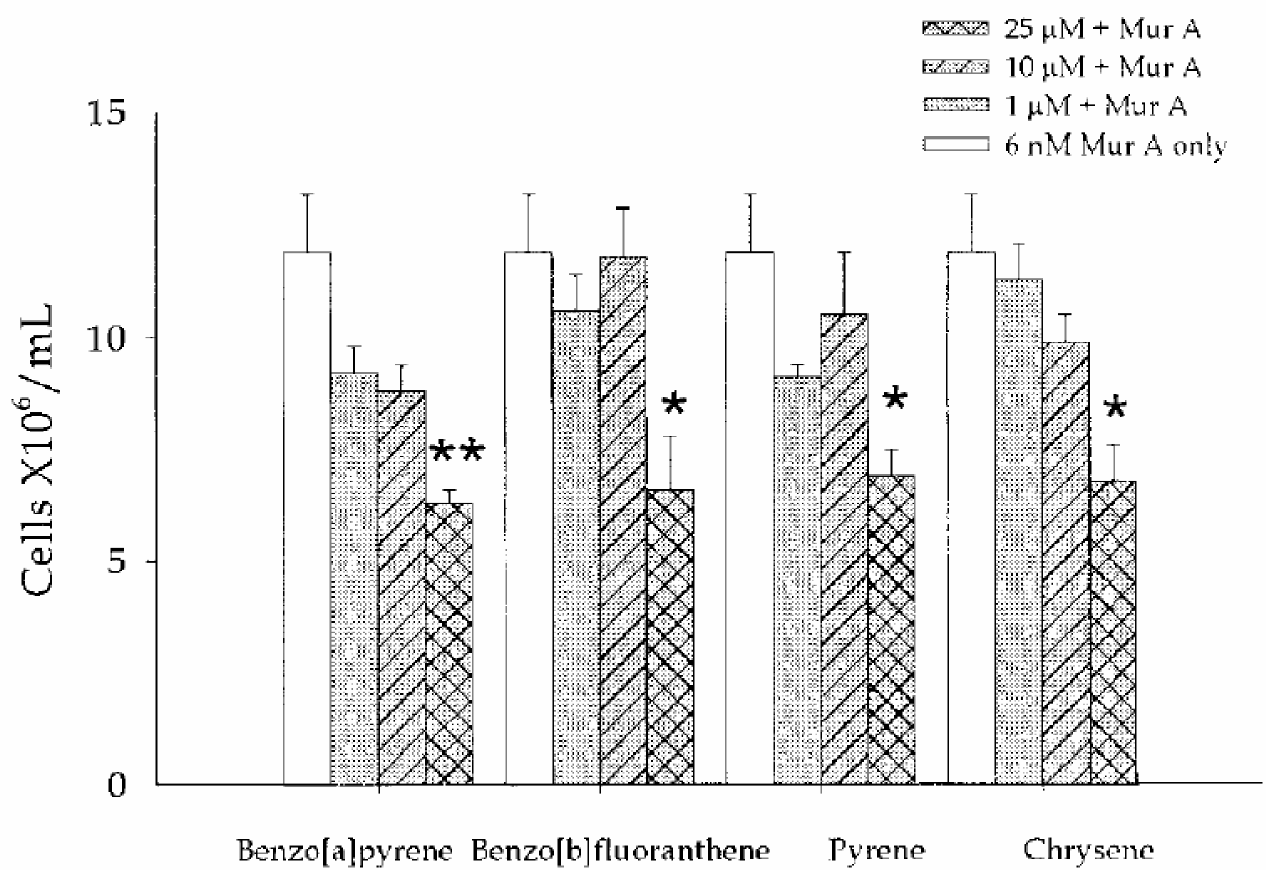


FIG. 4. Interaction of PAHs and Muristerone A in Cl.8+ cells. The four PAHs each enhance the differentiation response in Cl.8+ cells. This represents the average of three assays, each done in triplicate. Bars, SEM. *$p < 0.05$; **$p < 0.01$ different from 6 nM Muristerone A alone.

TABLE V

Representative Laboratory and Field Studies in Which Endocrine Disruption May Be Occurring in Insects[a].

Species	Contaminant	Concentration (range), effects	Lab/field	Reference
Chironomus tentans (all life stages)	4-Nonylphenol	12.5-200 µg/L; Reduced survival at high concentrations	Lab	Kahl *et al.* 1997
Macromia cingulata (larvae)	Tannery and paper pulp mill effluent	5, 10, 15, 20, 20%; Shortened time to first molt (tannery), arrested larval molting (paper pulp)	Lab	Subramanian and Varadaraj 1993
Chironomus riparius (larvae-adult)	Phathalate esters	100, 1000, 10000 mg/kg dw; No effects on survival, development or emergence	Lab	Brown *et al.* 1996
Chironomus spp.	Industrial effluent containing metals	Ambient at site; Higher level of mentum deformities in exposed larvae in both field and lab conditions	Field and Lab	Dickman and Rygiel 1996
Chironomus thummi (larvae)	Organic and inorganic pollutants	Ambient at site; Prevalence of morphological deformities related to pollutants	Field	De Bisthoven *et al.* 1995
Chironomus spp.	Possible exposure to pollutants	Ambient at site; Deformed mouth parts, heavily pigmented head capsules, unusually thick head capsule and body wall	Field	Hamilton and Saether 1971

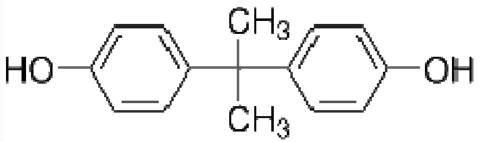
[a]Modified from deFur *et al.* (1999).

TABLE III
Representative Laboratory Studies in Which Endocrine Disruption May Be Occurring in Mollusks[a].

Species	Contaminant	Concentration (range), Effects	Lab/field	Reference
Lymnaea stagnalis (adult)	DDT, MCPA	50, 500 μg/L (DDT); 10, 100 mg/L (MCPA); Fecundity alterations	Lab	Woin and Bronmark 1992
Mytilus edulis (adult)	Cd	100 μg/L; Spawning stimulation, inhibition of gonadial development	Lab	Kluytmans *et al.* 1988

[a]Modified from deFur *et al.* (1999).

Bisphenol A



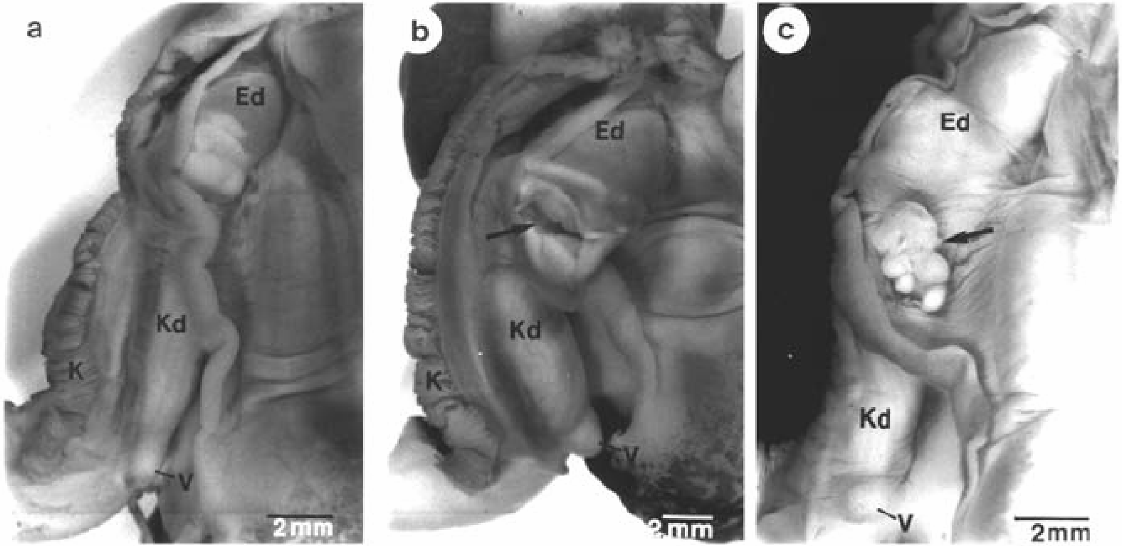


FIG. 3 *Marisa cornuarietis*. Photographs of a control female (a) and of BPA treated "superfemales" (b-c) with opened mantle cavities. In (b) and (c) a rupture in the wall of the pallial oviduct occurs (arrow) with an additional protrusion of the spawning mass in (c). Abbreviations: Ed, albumen gland; K, gill; Kd, capsule gland; V, vagina.

- Chemical signaling systems and their basic mechanisms in the animal kingdom exhibit a considerable degree of conservatism (McLachlan, 2001). Consequently, invertebrate endocrine function should be affected by identical or similar compounds as vertebrates (deFur *et al.*, 1999; Pinder and Pottinger, 1999).
- Highly effective EDCs have been intentionally developed for the purpose of pest control to interfere with hormonal systems of insects. Such endocrine-mediating properties can be assumed as not being unique for the IGRs or this group of arthropods but rather reflect the fact that much less research has been undertaken for other invertebrate groups than insects.
- ED in invertebrates found far less attention than in vertebrates in the past, probably because their hormonal systems are poorly understood favoring investigations with vertebrates and especially fish as systematic groups for ecotoxicological research and routine analyses many scientists feel familiar with.
- Little work has been done on endocrine disruption in invertebrates from the field (with the exception of the investigation of the imposex phenomenon in marine gastropods). The overwhelming majority of laboratory-based studies (56 reports) focuses on mollusks (17 publications), crustaceans (15 cases) and insects (12 reports), thus continuing main tendencies in the pre-1999 literature (Fig. 4a, b).