Hormones in control of growth and form - developmental plasticity and alternative phenotypes in social insects

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polyphenism in social insects

diana wheeler, iussi, sapporo 2002

explain this
are these just puzzling Nature’s games?

ecological success suggests otherwise
Premises

- alternative phenotypes are flexible response systems to specific environmental or genetic cues
- they are molded by natural selection on a groundplan of development and reproduction
- hormones are the primary mediators that generate phenotypes distinct in size, form and function
juvenile hormones

produced by the corpora allata in the retrocerebral endocrine complex
ecdysteroids

produced by the prothoracic gland (larva/pupa) or gonads (adults)

biosynthesis dependent on dietary steroids
JH and ecdysteroids control molting and metamorphosis

larva of helometabolans is equivalent to pronymph of hemimetabolans
(Truman and Riddiford, 1999)
multiple functions of JH in insect reproduction

- migration
- mating
- oviposition

sex peptide
mag
male

vitellogenin
patency
female
multiple functions of ecdysteroids in reproduction
castes in *Apis mellifera*
180-200 ovarioles per ovary

2000 and more eggs per day

2-12 ovarioles per ovary

functionally sterile
differential feeding of young larvae is the environmental inducer for the expression of different caste morphologies by acting on the larval endocrine system
differences in juvenile hormone and ecdysteroid titers

coordinate divergent developmental pathways
ovary of fourth instar queen and worker larvae

- both castes with over 150 ovariole primoridea per ovary
- few visible differentiation markers

ovary structure: fifth instar queen larvae

- cystocyte rosettes form by incomplete cell division
- fusomal material connects cystocytes until ring canal formation
ovary structure: fifth instar worker larvae

- few cystocyte rosettes
- signs of cell death noticeable in early fifth instar larvae

- complete degeneration of most ovarioles by prepupal stage
organization of the cytoskeleton in ovaries of fifth instar larvae

queen

maximally ramified polyfusomes

dissociation of actin from spectrin initiates programmed cell death

worker

Schmidt Capella & Hartfelder (2002)
a high HJ titer in L4 prevents cell death

intact ovarioles of worker prepupa treated with JH in L4

JH acts on actin/spectrin association

Schmidt Capella & Hartfelder (2002)
Conclusions
the larval JH titer is a key player in a switch mechanism of honey bee caste development (and probably also in all other social insects)

JH directly affects cellular differentiation processes

the question is, how, especially at the level of differential gene expression
approaches connecting hormonal regulation to complex networks of gene expression in social insect castes

A) analysis of phylogenetically conserved network modules – patterning genes

morphogenesis of wing imaginal discs in ants: *queens have wings, workers do not*

Abouheif & Wray (2002): expression patterns of genes controlling wing development in *Pheidole morrisi*
queens:
high JH levels in embryonic (and late larval) stages

soldiers:
low JH levels in embryonic, but high levels in last larval instar

workers:
JH levels low in embryonic and last larval instar
genetic network underlying wing development in flies

A: mid-embryo

B: late embryo

C: late larva

D: metamorphosis

Vein positioning, Cell growth and identity, Intervein cell differentiation, Bristle differentiation, Notum / blade differentiation
ants are monophyletic, but wing development can be blocked at different levels in the patterning network (en, sal, etc.)
approaches connecting hormonal regulation to complex networks of gene expression in social insect castes

B) by discovery of specific (novel) genes

analysis of differential gene expression during honey bee caste development

(Evans & Wheeler, 1999 and 2000; Corona et al., 1999)

(Hepperle & Hartfelder, 2001; Guidugli et al., 2004)
250 ESTs of differentially expressed genes in larval stages cluster into: early vs. late larval and queens vs. workers

Evans & Wheeler, 2000
components of ecdysone response cascade identified by DDRT-PCR on fifth instar ovaries

makisterone A

EcR/USP

DHR3
E74
E75
BR-C

βFTZ-F1

transcription factors (e.g., cut-like)
cell adhesion molecules
metabolic enzymes

early genes
early-late genes
late genes

SDR mRNA
mak A
rRNA

(Hepperle & Hartfelder, 2001; Guidugli et al., 2004)
the Beenome – a big step forward in bee genomics

http://www.hgsc.bcm.tmc.edu/projects/honeybee/

Honey Bee Genome Project

the near complete and assembled sequence information of the honey bee genome:

already provides high power tools for functional genomic studies
ApisEST project

produced 5,000 open reading frame ESTs from 87 mini-libraries covering the entire life cycle of honey bee workers

the majority of orthologs corresponds to ESTs from the dipterans (Drosophila and Anopheles)

Nunes et al. (2004) BMC Genomics
approaches connecting hormonal regulation to complex networks of gene expression in social insect castes

C) this genome/transcriptome information sets the stage for a new strategy to identify and sequence key components of conserved patterning modules and investigate the temporal and spatial dynamics of their expression in relation to nutritional and hormonal modulation
such as ... the insulin receptor pathway

in connection with the TOR network, this signaling pathway

controls organ and body size in insects and mammals

provides a link between nutritional status, regional growth trajectories and...

the endocrine system (small PTTHs (bombyxins) are insulin-like peptides)
a putative honey bee insulin receptor

tblastn search with Drosophila IR as query revealed highly significant match (E-score < $e^{-39}$) with contig in honey bee genome

and allowed designing specific primers against the conserved insulin receptor-specific tyrosin kinase domain
AmIR predicted:
7 exons (but C-terminal gap)
cds 3501: 1170 aa

Drosophila IR:
10 exons (novel C-terminus)
2148 aa

Bombyx IR
1472 aa

human IR
1297 aa
IR expression in honey bee caste development

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worker
queen

JH
ecd
approaches connecting hormonal regulation to complex networks of gene expression in social insect castes

D) by comparative phylogeny-based analysis of mechanisms underlying caste differentiation

according to: Engel 1999 and 2000
expression of morphological caste differences requires strong differences in hormone titers in the last larval instar

Cnaani & Hartfelder (2000)
Stingless bees (Meliponini)

- highly eusocial bees
- over 300 species
- strong queen/worker dimorphism
- mass provisioning of brood cells before oviposition by queen or workers
- JH and ecdysteroid titer differences in last larval instar and pupa
- quantity of larval food determines caste in most genera
Meliponini and Trigonini queens and workers reared in the same cell type:

- Genetic factors in caste determination?

Meliponini queens and workers reared in the same cell type:

- Genetic factors in caste determination

Trigonini queens and workers reared in different cell types:

- Only trophic factors in caste determination?

Photos: Freitas
from environmental to genetic caste determination

the *Melipona* classic

the zygotic genotype predisposes females to develop into queens (heterozygosity at two loci)

but the final outcome depends on nutritional status in the last larval instar – acting on the endocrine system

*this looks surprisingly similar to an ESD – GSD transition*
... is this putative ESD - GSD transiton is reflected in the caste-specific gene expression pattern?

RDA of queen vs. worker transcripts
1278 ESTs clustered into 337 Unique Sequences

Judice et al. - submitted
higher diversity (GO-classes) of caste-specific transcripts in workers
validated by quantitative RT-PCR
reveals worker-like expression pattern in JH-induced queens
hormonal dissociation of genotype and phenotype?

Judice et al. - submitted
Polyphenism is a common adaptive life history strategy

- Wing length in planthoppers, soapberry bugs, crickets - dispersal
- Wing length in aphids - dispersal and mode of reproduction
- Color in butterfly pupae - camouflage/temperature control
- Wing pattern in butterflies - camouflage/temperature control
- Color in locusts - dispersal and reproduction
- Caste in termites, social Hymenoptera - reproductive bias/sociality
- Caste in polyembryonic wasps - clonal competition
- Horns in male dung beetles - reproductive strategies

Compiled in Hartfelder & Emlen (2005)
wing length polyphenism in *Gryllus rubens*

triggering factor: population density experienced by nymphs

JH titer controlled by JH esterase (degradation)

lower investment into wings is associated with higher investment into egg production

Zera and Tiebel, 1988; Zera and Denno, 1997
complex phase polyphenism in aphids

overlap of wing polymorphism (dispersal) with reproductive strategies (sexual vs. clonal) and host plant switching

only some switches controlled by JH
phase transition requires several factors and generations:
- crowding
- egg pod foam (maternal factor)
- [His$^7$]-corazonin (foreground color)
- JH (green background coloration)

Tawfik et al., 1997; Tanaka, 2000; Hägele et al., 2000
butterflies: wing color and wing pattern polyphenism

a) Short days, Cool temperatures

b) Long days, Warm temperatures

a) Dry Season, Cool temperatures

b) Wet Season, Warm temperatures

Seasonal phenotypes: interaction of genotype, pupal ecdysteroid titer, patterning genes (dll) and melanization

Precis coenia

Bicyclus anynana

Rountree & Nijhout, 1995; Koch et al., 2003

Brakefield & Larsen, 1984; Brakefield et al., 1998; Beldade et al., 2002
dung beetle horns - polyphenism in male weaponry

a) Large males

b) Small males

hormonally controlled horn size relates to male reproductive strategy