Unraveling the molecular determinants of caste development in the honeybee *Apis mellifera*

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In bees, it’s common a few individuals to receive more “attention” during development.
One of the main questions in sociobiology is the comparison between worker and queen bees. Here is a summary of their differences:

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* indicates that the feature is significantly different between worker and queen bees.
The “pre-molecular” saga of caste development

- Higher nutrition
  - Haydak 1943, *J Econ Entomol*

- More hormones
  - Shuel & Dixon 1960, *Insectes Soc*

- More growth
  - Haydak 1943, *J Econ Entomol*

- JH as mediator
  - Shuel & Dixon 1960, *Insectes Soc*

- Exogenous JH -> queens
  - Grading phenotypes, depending on the application’s time
    - Wirtz & Beetsma 1972, *Ent Exp & Appl*
    - Zdárek & Haragsim 1974, *J Insect Physiol*

- Purification of a queen determinator
  - Small hydrophilic molecule (?)
    - Rembold et al 1974, *J Insect Physiol*

- Caste determination as a sequential process
  - Dedej et al 1998, *J Apic Res*
The molecular saga of caste development

CASTE-SPECIFIC TRANSCRIPTION IN THE FEMALE HONEY BEE

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*Department of Entomology, University of Wisconsin, Madison, WI 53706, U.S.A. and †Department of Veterinary Science, University of Wisconsin, Madison, WI 53706, U.S.A.

Differential gene expression between developing queens and workers in the honey bee, Apis mellifera

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Department of Entomology, University of Arizona, Tucson, AZ 85721

DIFFERENTIAL EXPRESSION OF MITOCHONDRIAL GENES BETWEEN QUEENS AND WORKERS DURING CASTE DETERMINATION IN THE HONEYBEE APIS MELLIFERA

MIGUEL CORONA, ENRIQUE ESTRADA AND MARIO ZURITA*

Departamento de Genética y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, APDO-Postal 510-3, Cuernavaca Morelos 62250, México

http://genomewebiology.com/2000/2/1/research/0001/

Research

Expression profiles during honeybee caste determination

Jay D Evans* and Diana E Wheeler†

Addresses: *Bee Research Lab, USDA-ARS, Beltsville, MD 20705, USA. †Department of Entomology, University of Arizona, AZ 85721, USA.

C. Hepperle • K. Hartfelder

Differentially expressed regulatory genes in honey bee caste development
Explaining morphological differentiations: Systemic Approaches
Hormonal modeling of caste-specific gene expression in honeybees

Differential feeding

JH

Ecdysteroids

L1 L2 L3 L4 L5

Queens

Workers

Threshold

Genes activity

Different developmental pathways

Haydak, 1943

Profiles from Hartfelder and Emlen, 2005

Barchuk et al, BMC Dev Biol 2007
General view of caste differentiation in *Apis mellifera*

High JH levels → Physiometabolic genes → Incremental alterations

Low JH levels → Developmental genes → Character state alterations

Queen

Worker
Microarray and regulatory regions data studies on caste differentiation and development

Caste differentiation

Gene expression networks

Barchuk et al, BMC Dev Biol 2007
Regulatory elements found in upstream region of genes over-expressed in L4 Queens

2 putative motifs were found

Motif 1 (similar to DEP4 and AEF-1 binding sites of Drosophila Adh (Ayer and Benyajati, 1992)

7/8 known physiometabolic genes

map = 15; church = 1.7e-10; roc_auc = 0.8

Barchuk et al, BMC Dev Biol 2007
Networks depicting putative gene interaction based on the occurrence of overrepresented motifs in the UCR of DEG between A. mellifera castes.
Working model of caste differentiation in *Apis mellifera*

![Diagram of caste differentiation in Apis mellifera](image)

- **Fat-body**
  - Insulin/IGF
  - Tor

- **Corpora allata**
  - Insulin/IGF
  - Tor

- **Body growth**
  - Ovary development
  - Apoptosis

- **Neurogenesis**
  - Leg structures development

- **Global differential programming of gene expression**

- **Differential feeding**
  - Royal jelly
  - Worker jelly

- **Gut epithelium**
Physiometabolic genes determinant of body size

**Tor**

controls growth in response to nutrients

---

**Target of Rapamycin (TOR) Mediates the Transduction of Nutritional Signals into Juvenile Hormone Production**


José L. Maestro, Juliána Cobo, and Xavier Belles

From the Instituto de Biología Evolutiva (CSIC-UPF), Parque de la Mar, 08028 Barcelona, Spain.

The Journal of Biological Chemistry, 2008, 283, 27082-27089

Queens Workers

Barchuk et al, BMC Dev Biol 2007
Tor pathway and body growth

Testing gene function by microinjecting or feeding (RNAi)

A non-invasive method for silencing gene transcription in honeybees maintained under natural conditions

Francis Morais Franco Nunes a,b,*, Zilá Luz Paulino Simões c

a Departamento de Biologia Aplicada a Agropecuária, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brazil
b Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
c Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

*Tor knock-down* in prospective queens leads to the development of small individuals possessing appendicular structures similar to those of workers.
Royalactin induces queen differentiation in honeybees

Kamakura, 2011, Nature
Working model of caste differentiation in *Apis mellifera*

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**Global differential programming of gene expression**

- **Differential feeding**
  - Royal jelly
  - Worker jelly

**JH**

Barchuk et al, BMC Dev Biol 2007
Explaining morphological differentiation: Developmental Fields Approaches

The initial organ development takes place through the establishment of specific morphogenetic fields.
Some of the most conspicuous morphological differences

Nest-mate recognition

Forraging

Queens

Reproduction

Nursing

Workers

Fahrbach, Annu Rev Entomol 2006

Snodgrass & Erickson, 2000
JH and differential development of the ovary

Schmidt-Capella and Hartfelder, J Insect Physiol 1998

Low JH levels

High JH levels

Workers

Queens
Pollen Collecting Apparatus

http://www.youtube.com/watch?v=l8j-gw3tIPM
*Ub* expression impairs hair development in workers’ tibia

Queens

Workers

Workers’ duties require a “boosted” mini-brain
One of the main questions in sociobiology

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Michener 1974
Differential brain morphogenesis during larval development in honeybee castes
Queen larvae brains develop more and faster than those of workers.

Moda et al. 2013, PLoS One
Cell Quantification in Brains

Developing Brains

Proliferating cells in L4

Moda et al. 2013, *PLoS* One
Schematic diagram of the time of first appearance of the mushroom body neuropil regions in workers and queens

Moda et al. 2013, PLoS One
Systemic microarray hybridization experiments showed a set of neurogenic genes differentially expressed between castes.
By RT-qPCR several of these genes had their profiles validated.

Moda et al. 2013, PLoS One
In situ hybridization showed a higher transcription of shot in queens’ developing brains.

Moda et al. 2013, PLoS One
Immunohistochemical procedures using anti-Shot confirmed transcription findings

Localization of Shot in larvae (L4) brains

Moda et al. 2013, *PLoS One*
Queen larvae brains develop more and faster than those of workers.

Moda et al. 2013, *PLoS One*
Schematic diagram of the time of first appearance of the mushroom body neuropil regions in workers and queens

Moda et al. 2013, *PloS One*
There not seems to be morphological differences between L3 brains. Are there molecular differences?

Moda et al. 2013, *PLoS One*
A typical RNA-seq experiment

Wang et al. 2009, Nature Reviews
Differentially expressed genes between developing (L3) queens and workers brain

- Queens
- Workers

Vieira et al., unpublished
Royal Jelly’s microRNAs might target L3 brains mRNA

Queens

Vieira et al., unpublished
Acknowledgments

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Joseana Vieira – UNIFAL-MG, Minas Gerais
Heloisa Gianelli – UNIFAL-MG, Minas Gerais
Paulo E Alvarenga – UNIFAL-MG, Minas Gerais