

## RESEARCH ARTICLE

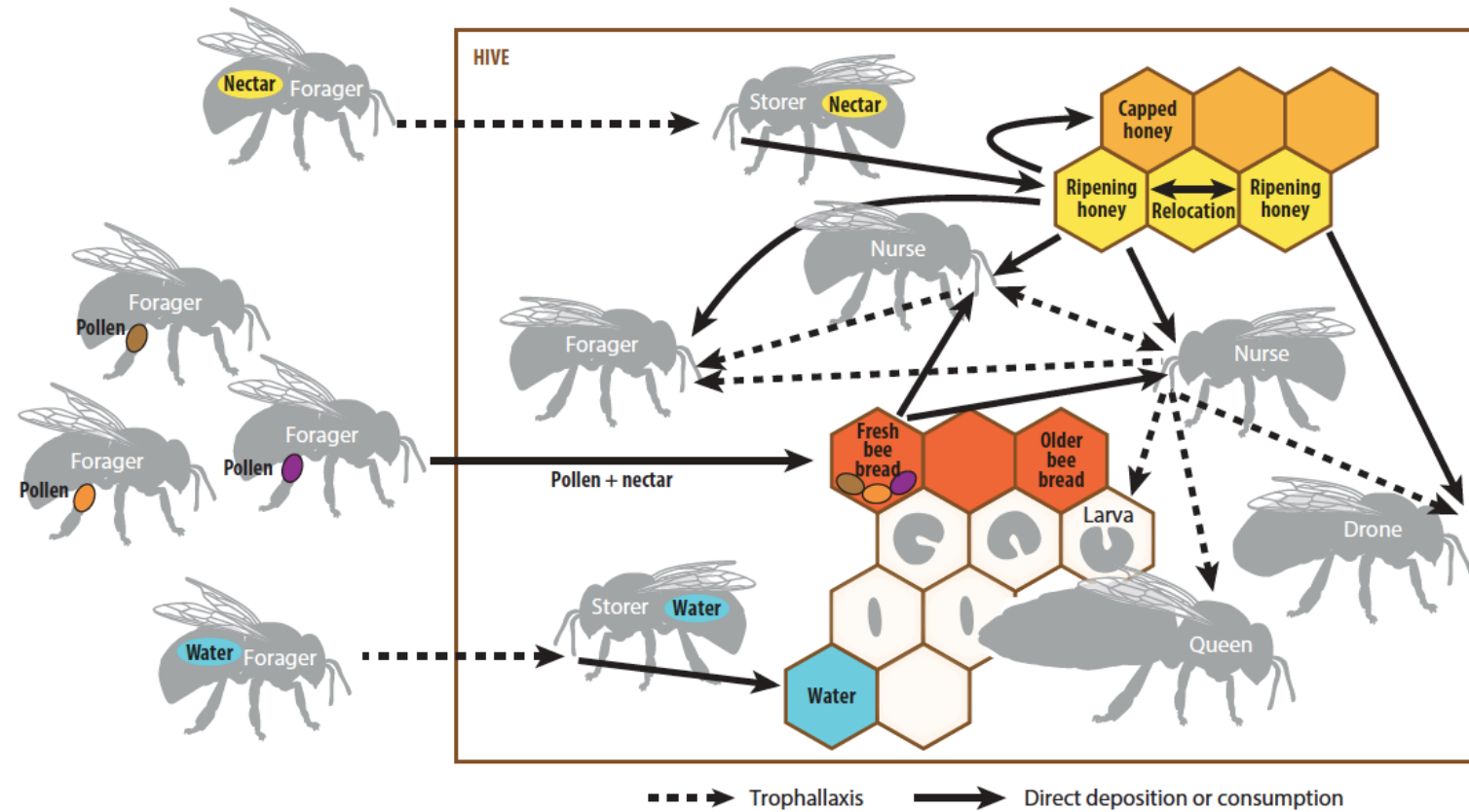
# Differences in the morphology, physiology and gene expression of honey bee queens and workers reared *in vitro* versus *in situ*

**Daiana A. De Souza<sup>1,2,\*</sup>, Osman Kaftanoglu<sup>3</sup>, David De Jong<sup>1</sup>, Robert E. Page, Jr<sup>3,4</sup>, Gro V. Amdam<sup>3,5</sup> and Ying Wang<sup>3</sup>**



Queens and workers reared *in vitro* are the same as queens and workers reared in a natural hive environment?

# Social role of the bees



Wright, G. A., Nicolson, S. W., & Shafir, S. (2018). Nutritional physiology and ecology of honeybees. *Annual review of entomology*, 63.



### Queen

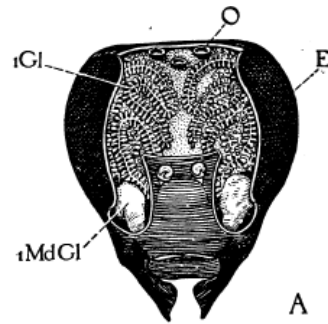
- Larger (150-250 mg)
- Huge abdomen
  - reproductive apparatus
    - ovaries with 200-400 ovarioles
  - Spermatheca

The quality of a queen is highly associated with her reproductive ability

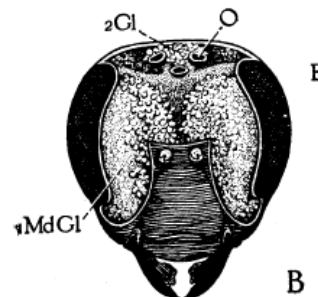
### Worker

- Smaller (80-110mg)
- Specialized morphological traits
  - Hypopharyngeal glands
  - Wax glands
  - Notched mandibles
  - Pollen basket
- do not produce eggs

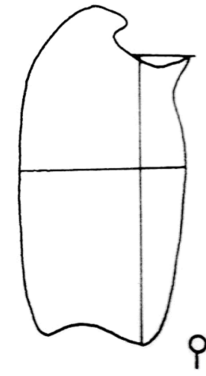
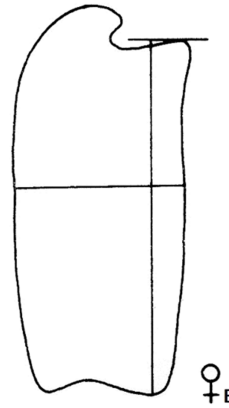
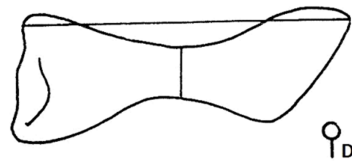
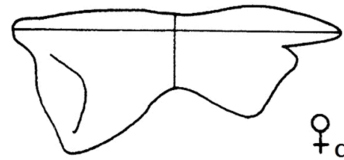
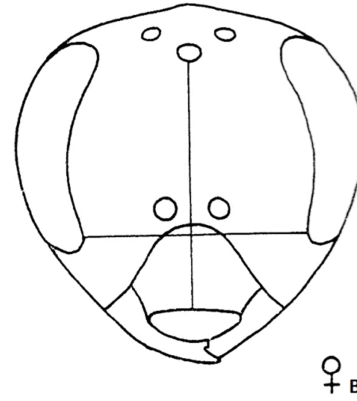
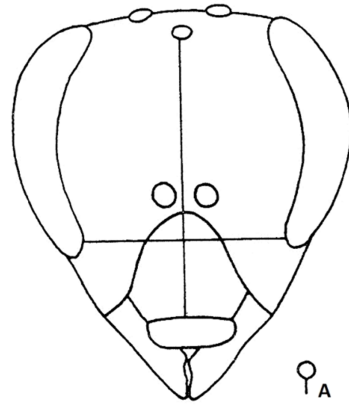
Worker



Queen



Snodgrass, R. E. (1956). *Anatomy of the honey bee*. Cornell University Press.

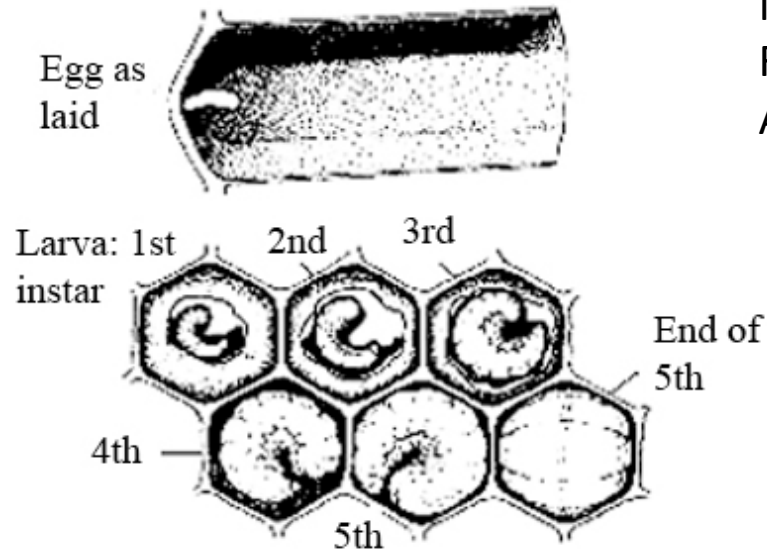


- (A) worker head;
- (B) queen head;
- (C) queen mandible;
- (D) worker mandible;
- (E) queen basitarsus;
- (F) worker basitarsus.

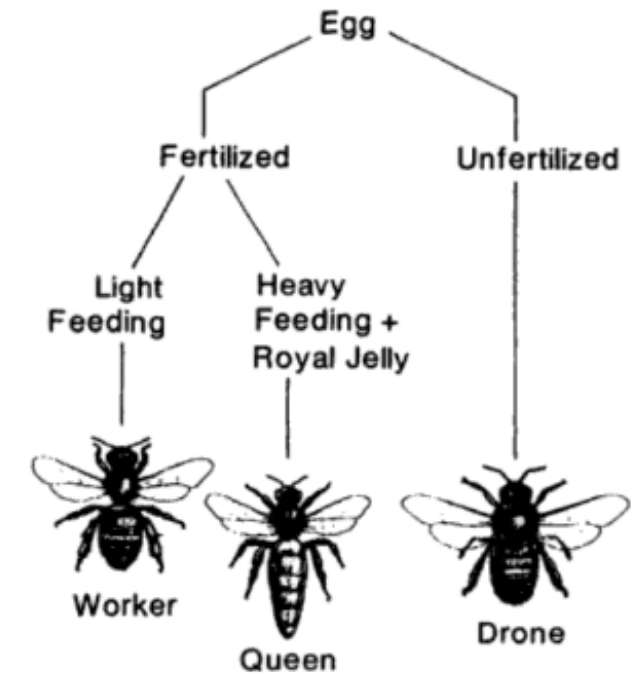
De Souza, D. A., Wang, Y., Kaftanoglu, O., De Jong, D., Amdam, G. V., Gonçalves, L. S., & Franco, T. M. (2015). Morphometric identification of queens, workers and intermediates in in vitro reared honey bees (*Apis mellifera*). *PloS one*, 10(4).

Bee queens and workers develop from the same diploid egg

Nurses bees feed differently the bees.  
First three days: royal jelly  
After 3rd instar different feeding



[bee-health.extension.org/bee-brood-basic-bee-biology-for-beekeepers/](http://bee-health.extension.org/bee-brood-basic-bee-biology-for-beekeepers/)



In vitro: no social control and artificial environment-> impact on morphological traits  
different feeding regimes can produce queen-like, worker-like or intercastes phenotypes

Winston, M. L. (1991). *The biology of the honey bee*. Harvard university press.



Aim of  
the  
paper

### Indicators of metabolic states

- Blood sugar (glucose and trehalose) levels
- Abdominal lipids reserves

### Morphological characteristics

- Body weight
- Ovariole number
- Size of the spermatheca

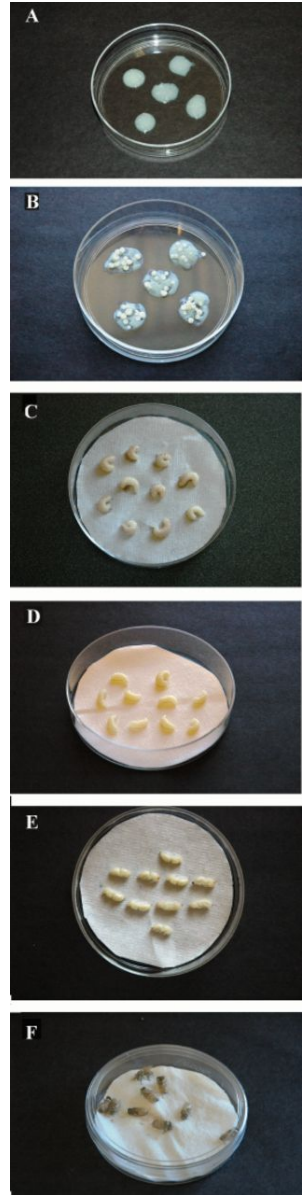
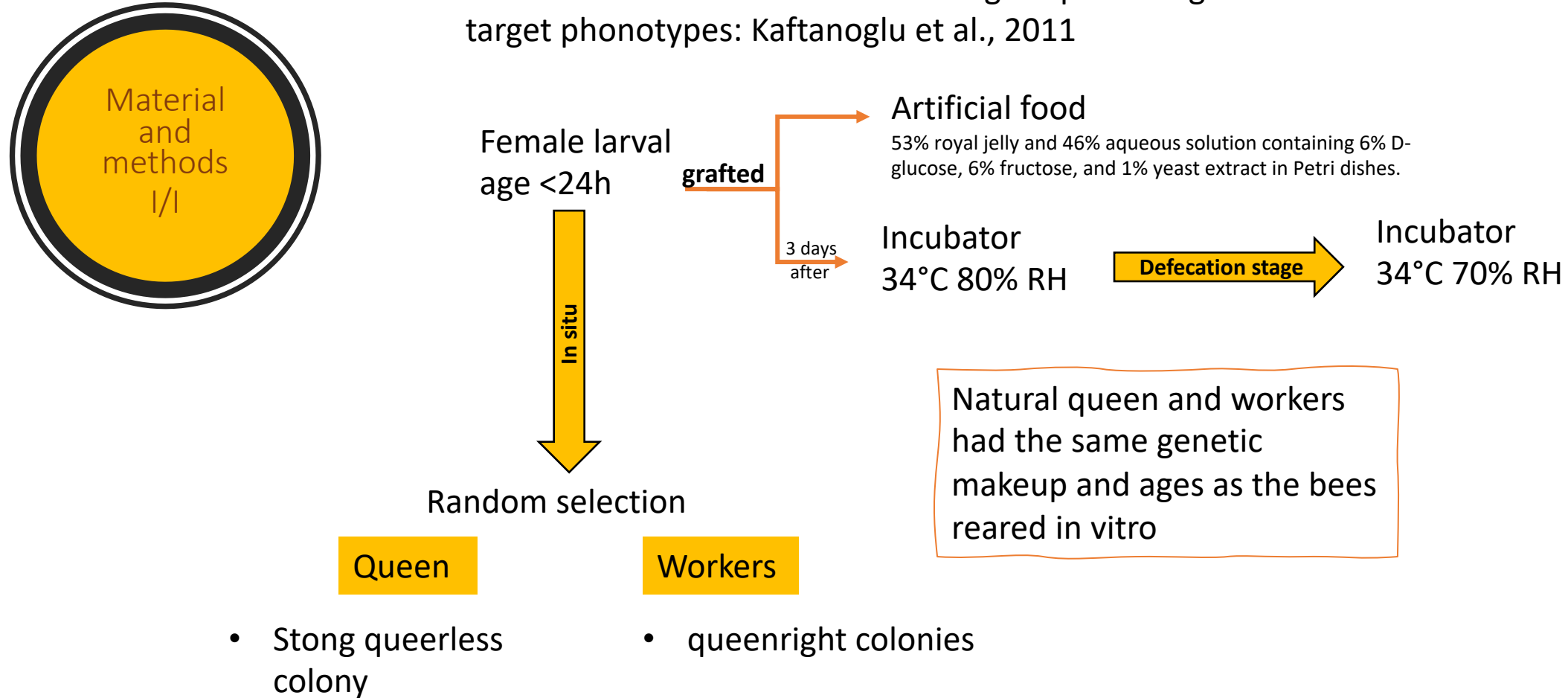
Reproductive and nutritional status gene:

- **vitellogenin (*vg*)**
  - impact on ovarian activation and egg production in queens
  - regulates transition of workers from nurses to foragers
  - component for the larval food produced by workers
- **major royal jelly protein 1 (*mrjp1*)**
  - major components in royal jelly
  - significant influence on honey bee development and ovarian physiology

**Samples:** three 'wild type' colonies maintained at Honey Bee Research Facility School of Life Sciences, Arizona State University, Mesa, Arizona

## Honey bee larvae reared *in vitro* and in the hive

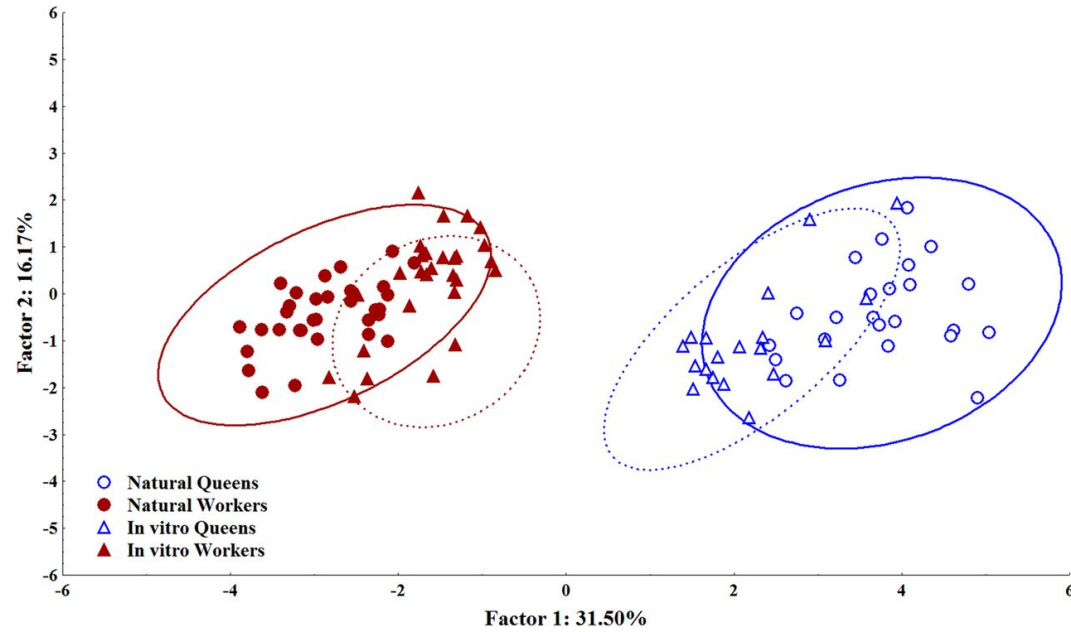
Protocol *in vitro* reared bees with highest percentage of target phenotypes: Kaftanoglu et al., 2011



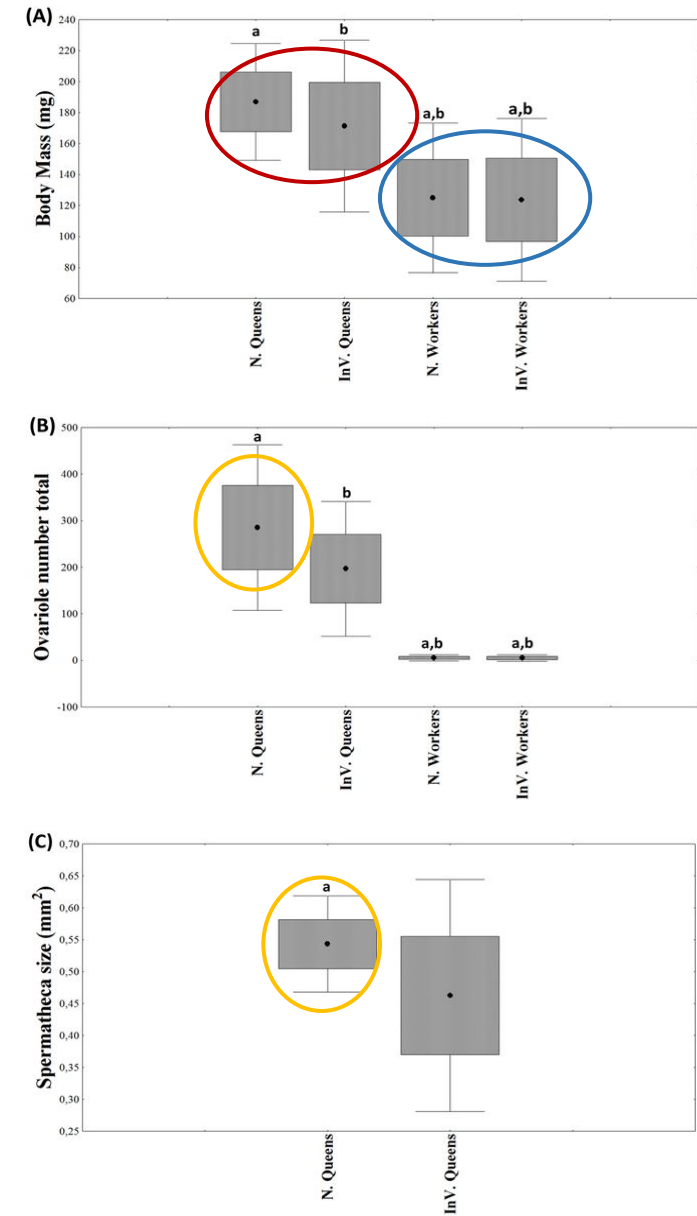
Kaftanoglu et al., 2011



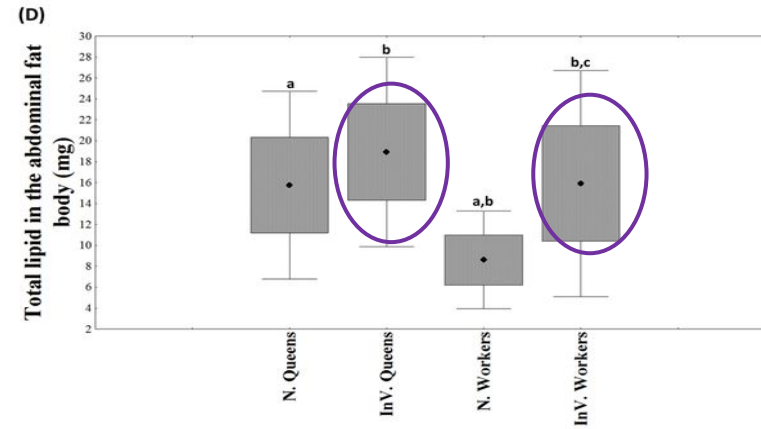
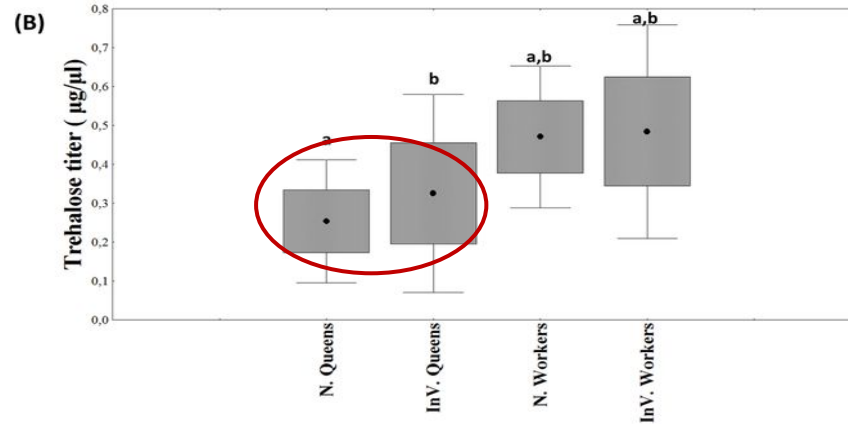
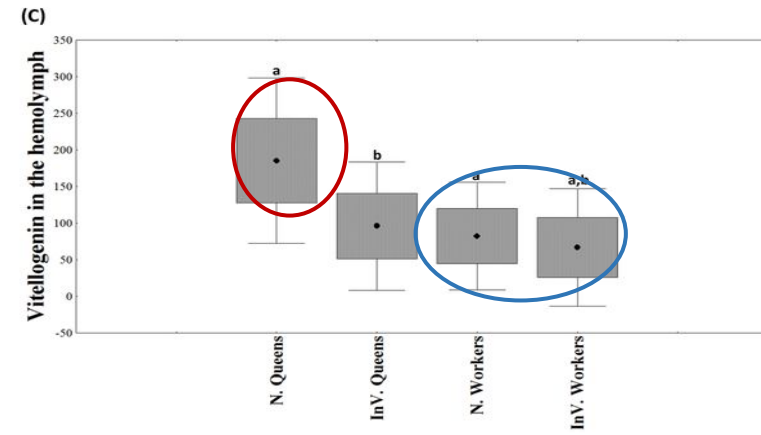
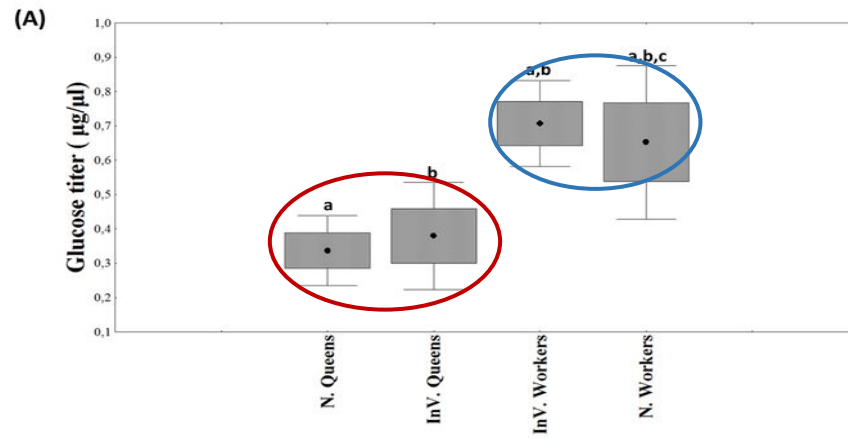
# Identification of in vitro queen-like and worker-like bees



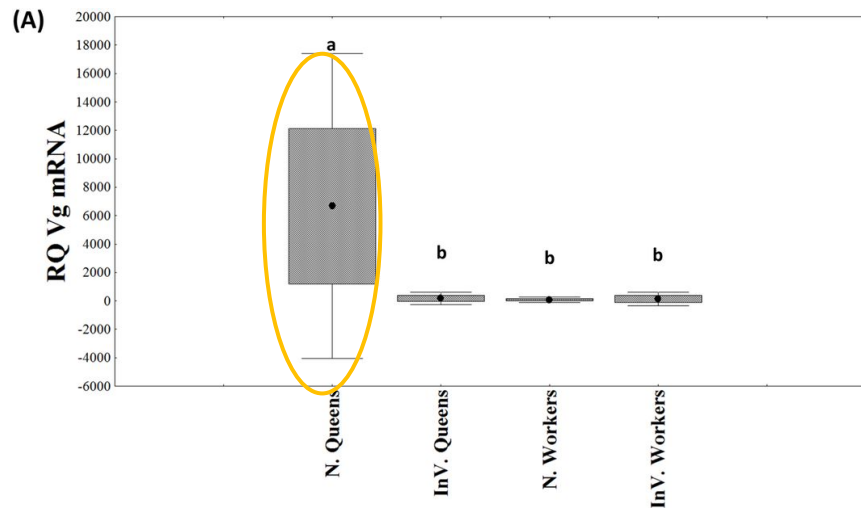
Inter-caste samples were discarded



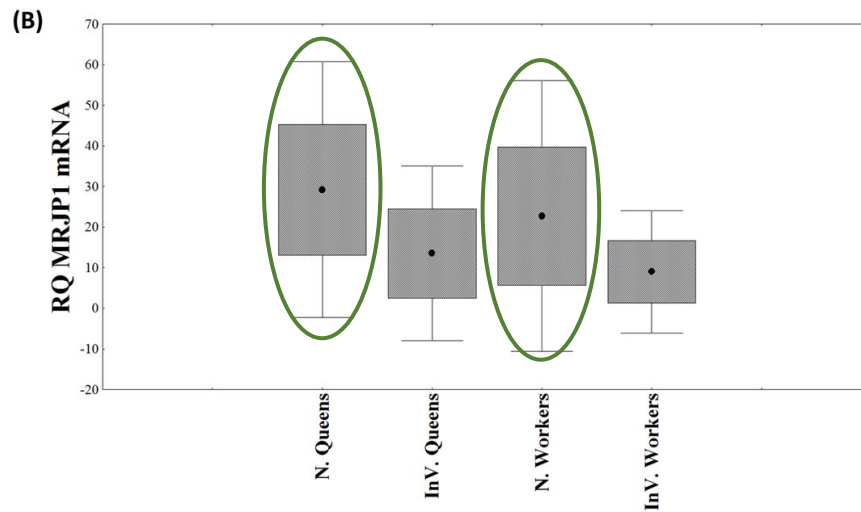
# Metabolic physiology



## Gene expression



A. *vg* gene expression in hive-reared queens was 19 times higher than in *in vitro* queens and 35 times higher than in workers from both rearing systems



B. *mrjp1* gene expression did not differ between castes (both in hive and *in vitro*) . the hive-reared bees had greater expression of this gene than *in vitro* reared bees.



### Comparison of the external morphological characters:

- In vitro queen-like and worker-like differ from natural queen and worker



Are these morphological traits

- a full representation of the differences between queen and worker?
- a good evidence of the quality of adult bee female?

### Indicators of metabolic states (I/II)

glucose and trehalose:

- Queens have lower sugar titers than workers both in naturally and in-vitro bees



The first time that caste-associated metabolic differences in adult bees have been identified.

Queen and worker larvae receive different quantities of sugar they differentially express insulin peptides and insulin receptors, which are responsible for carbohydrate metabolism. Insulin pathway role is not clear.

## Reproductive and nutritional status gene

Vitellogenin (*vg*):

- highest in hive-reared queens

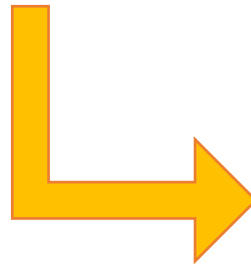


Function of *vg*: immune response, reproduction, and social behaviour, honey bee health and lifespan-> higher fertility and lifespan-> higher in queens

- Low levels in *in vitro* reared queens but they have same morphological traits as natural queens

major royal jelly protein 1 (*mrjp1*):

- Highest in hive-reared queens



Royal jelly contain equal amounts of protein carbohydrate  
*In vitro* artificial diet contains 53% commercial royal jelly and more sugar-> less protein for the larvae  
Protein in the storage royal jelly after a period gradually degrade



## Metabolic state II/II

Lipid level are not effected negatively by artificial food.



high sugar diet -> phagostimulant-> increasing food consumption and growth rate -> queen phenotypic differentiation  
Artificial diet has high sugar concentration-> increasing lipid synthesis



## Conclusion

- By using artificial rearing methods, morphological traits cannot be fully translated into bee quality
- Protein levels, especially Vg in newly emerged bees, are more sensitive to larval nutrition.
- Physiological and gene expression data suggest protein, lipid and glucose metabolisms are regulated independently during the bee larval stage.
- The quality of the in vitro queen-like and worker-like bee is lower than hive-reared bees

