OP-053 [Bee Biology]
Relative effects of the queen and brood on honey bee worker ovary suppression

Shelley Hoover[1], Leslie Holmes[1], Kelsey Gourlie[2], Alison Mcafee[3], M Marta Guarna[4], Stephen Pernal[5], Ernesto Guzman Novoa[6], Rob Currie[7], Amro Zayed[8], Leonard Foster[3]
[1]Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada
[2]Wildlife Analytics Lab, Lethbridge Polytechnic, Lethbridge, Alberta, Canada
[3]Department of Biochemistry and Molecular Biology, Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada
[4]Project Apis m, Salt Lake City, Utah, USA
[5]Beaverlodge Research Farm, Agriculture and Agri-Food Canada, Beaverlodge, Alberta, Canada
[6]School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada
[7]Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada
[8]Department of Biology, York University, Toronto, Ontario, Canada

Western honey bees (Apis mellifera L.), are a eusocial insect species in which individual members of a colony cooperate to procure food, find and build nests, and reproduce. Typically, the sole reproductive female in a colony is the queen, with daughter workers forgoing personal reproduction in favor of rearing half and full sisters. While workers possess rudimentary ovaries and can lay eggs, they cannot mate, and are thus normally capable of producing only haploid male offspring. Egg-laying workers are uncommon, subject to aggression, and their eggs subject to policing by other workers. Worker reproduction is rare in queenright colonies, but when present it can lead to colony mortality. The presence of a queen, brood, the pheromones produced by both, and nutritional and seasonal factors can suppress worker bee ovary development. To understand the relative importance of queens and brood on worker ovary suppression, we tested their effects in a field experiment with four treatments: 1) queen and brood both present, 2) queen absent, brood present, 3) queen present, brood absent, and 4) queen and brood both absent. Worker bees from each treatment were dissected, and their ovaries were scored according to their level of development. In addition, samples were submitted for proteomics, transcriptomics and microbiome analysis. Results from an ordinal logit regression show a significant interaction between queen and brood status on the probability of observing levels of worker ovary development, whereby the effect of queen status on worker ovary development depends on the presence or absence of brood. Specifically, our results demonstrate that the presence of brood reduces the level of worker ovary development; however the presence of a queen has no impact on ovary development in the absence of brood. In contrast, in the presence of brood, the presence of a mated, laying queen further lowers the probability of observing undeveloped ovaries by 26%. These results suggest that the presence of brood is more influential on ovary development in workers than the presence of a mated queen. Beekeepers should consider adding open brood when requeening queenless colonies.

Relative effects of the queen and brood on worker ovary suppression

Hoover S[1], Gourlie K[1] Holmes L[1], McAfee A[2], Giovenazzo P[3], Pernal S[4], Guarna MM[4,5], Guzman-Novoa E[6], Currie R[7], Zayed A[8], Foster L[2]

1. Department of Biological Sciences, University of Lethbridge, Canada
2. Department of Biochemistry and Molecular Biology, University of British Columbia, Canada
3. Département de Biologie, Université Laval, Canada
4. Beaverlodge Research Farm, Agriculture and Agri-Food Canada, Canada
5. Project Apis m, Salt Lake City, Utah, USA
6. School of Environmental Sciences, University of Guelph, Canada
7. Department of Entomology, University of Manitoba, Canada
8. Department of Biology, York University, Canada

University of Lethbridge

Background

Social Insects

- Individuals coordinate their efforts
- Individuals are specialised for different tasks
- Variation in the complexity of insect societies and degree of caste differentiation
- Differential reproduction

Background

- Primary female reproductive ‘queen’ or queens
- Amount of reproduction by workers varies:
  - In queenright colonies, worker reproduction is often limited
  - Others have frequent production of males by workers
  - Worker male production is common when the primary reproductive(s) are lost (i.e. queenless colonies)

Background

Laying workers:

Background

What determines the identity of egg-layers?

- Nutrition
- Genetics
- Queen pheromones
- Brood pheromones
- Pheromone response
- Presence of other workers
- Age
- Rearing environment
- Aggression from other workers

Objective

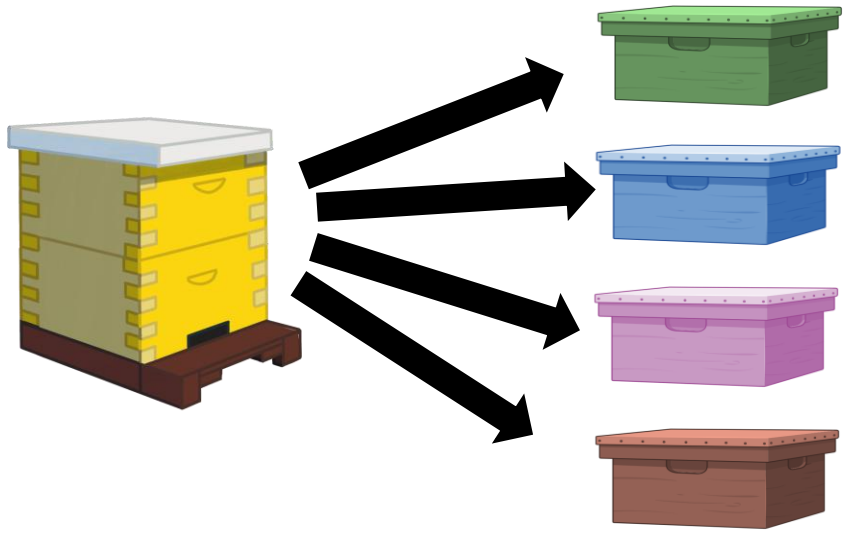
To understand the relative importance of queens and brood on worker ovary suppression

Methods

Four treatments, 6 nucs each:

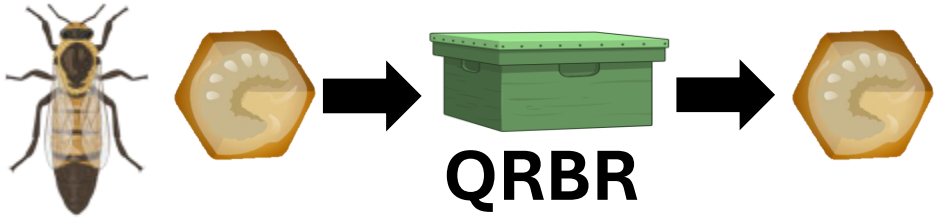
- queenright and broodright **(QRBR)**
- queenless, broodright **(QLBR)**
- queenright, broodless **(QRBL)**
- queenless and broodless **(QLBL)**

Methods

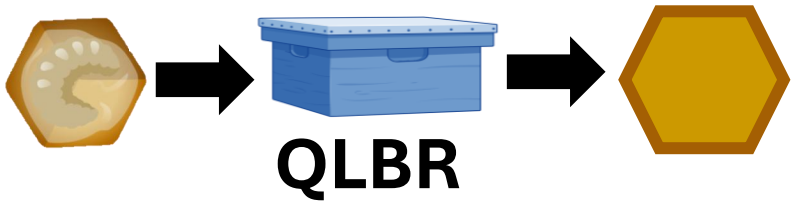


- 6 queenright double brood chamber colonies were each split into 4 queenless nucleus colonies
- Queens introduced to the experimental colonies in the two queenright treatments

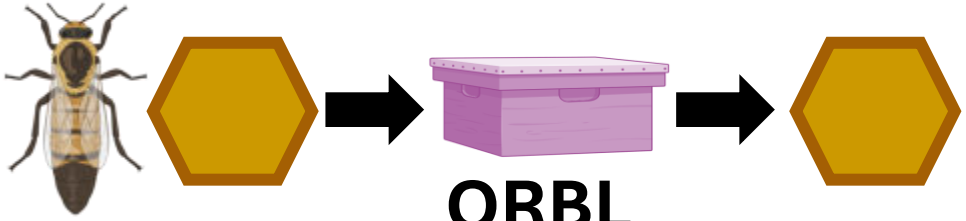
Methods



QRBR – Nucs supplied with new queen, frames containing brood at the time set up, and then open brood frames swapped every 4 days.



QLBR - Supplied with frames containing brood at the time set up, and empty comb replaced with unrelated open brood every 4 days. Queen cells cut out.

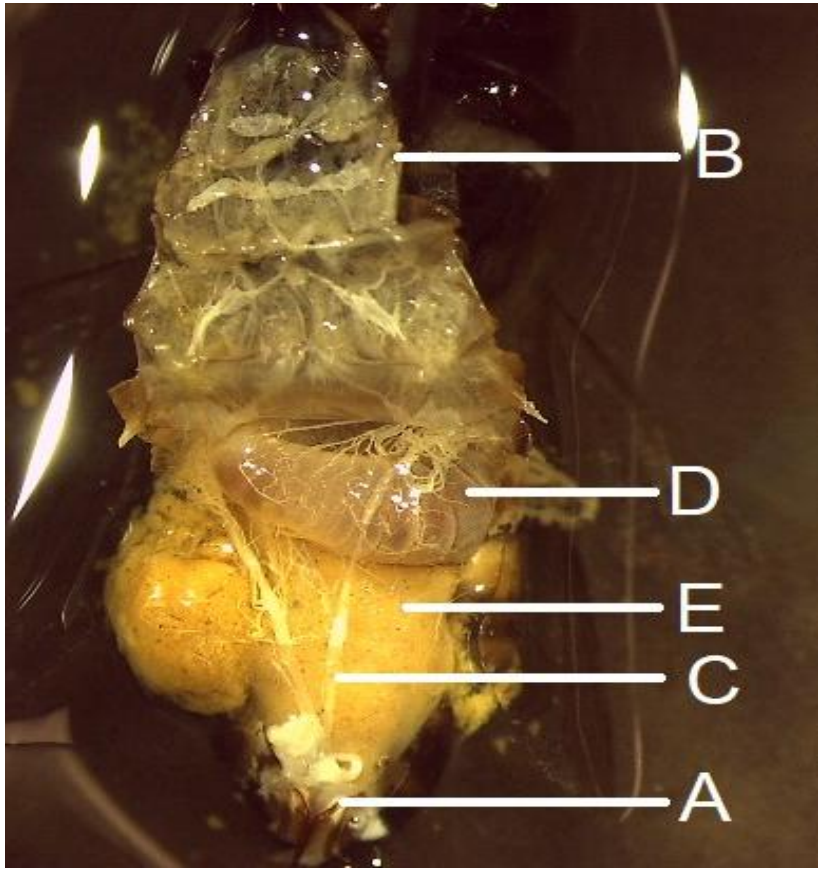


QRBL – Supplied with a new queen confined to a large cage made from queen excluder material that enabled interaction with workers but prevented queen access to comb to lay eggs. Broodless frames swapped every 4 days.

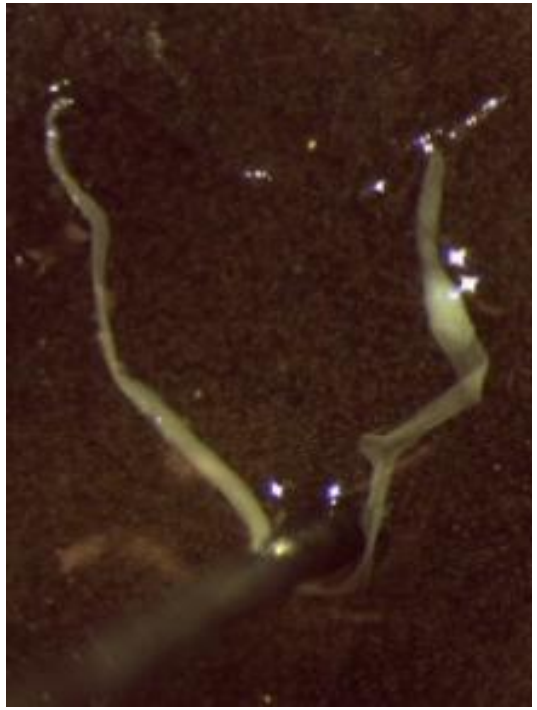
QLBL – Broodless frames swapped every 4 days. Queen cells cut out.

Methods

Worker bees from each treatment were dissected



Ventral view of a dissected worker honey bee abdomen including the stinger (A), the created abdominal flap (B), both ovaries (C), the ventriculus (D) and the rectum (E).



0: Undeveloped / resting
Inactive, thread-like, ovarioles not distinguishable

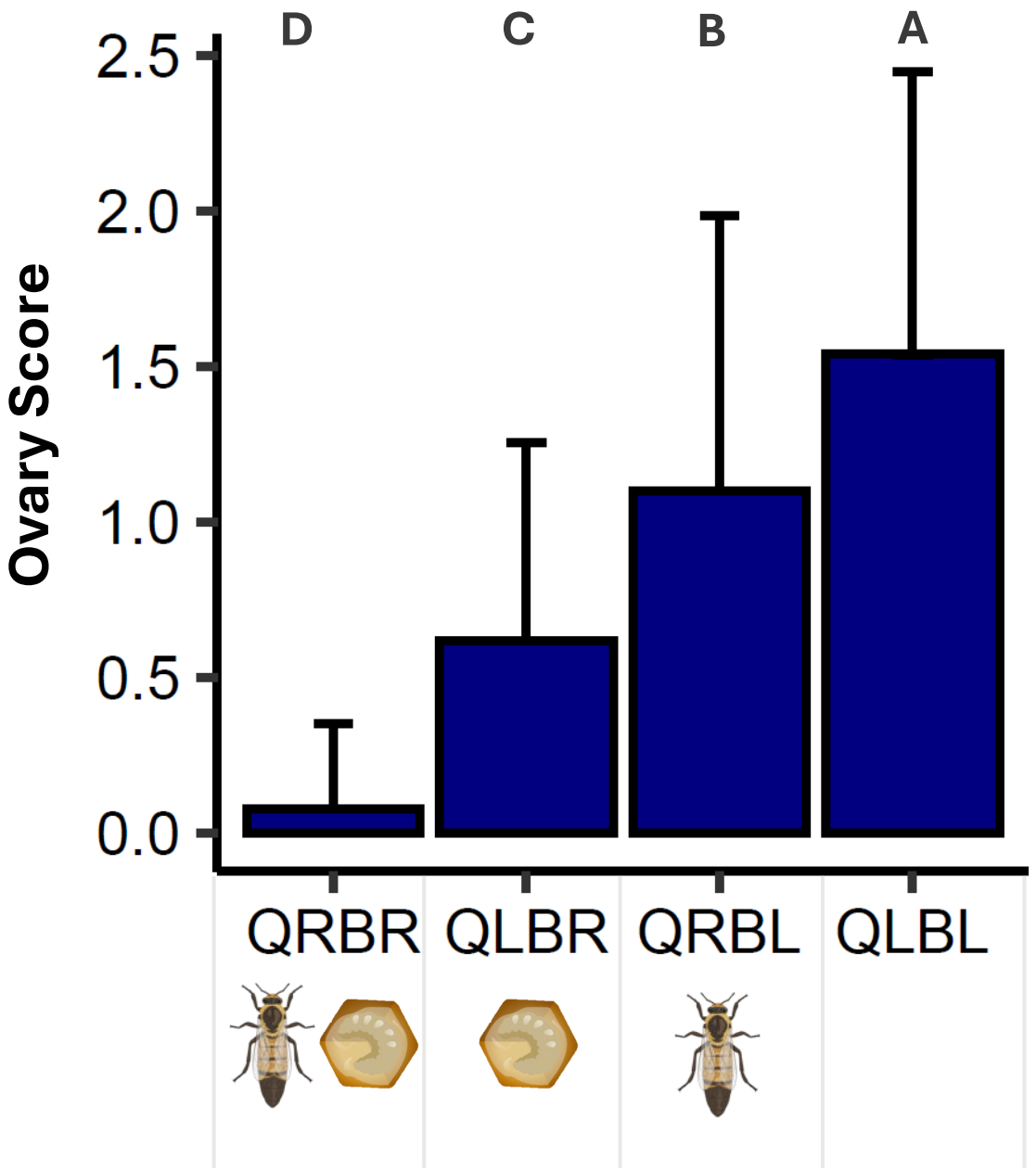
1: Partially developed, no eggs
Ovarioles slightly swollen, no distinguishable eggs

2: Partially developed, eggs developing
Small but distinguishable eggs

3: Developed, with eggs
Contains at least one fully developed appearing “sausage-shaped” egg

Each pair of ovaries was assigned a score

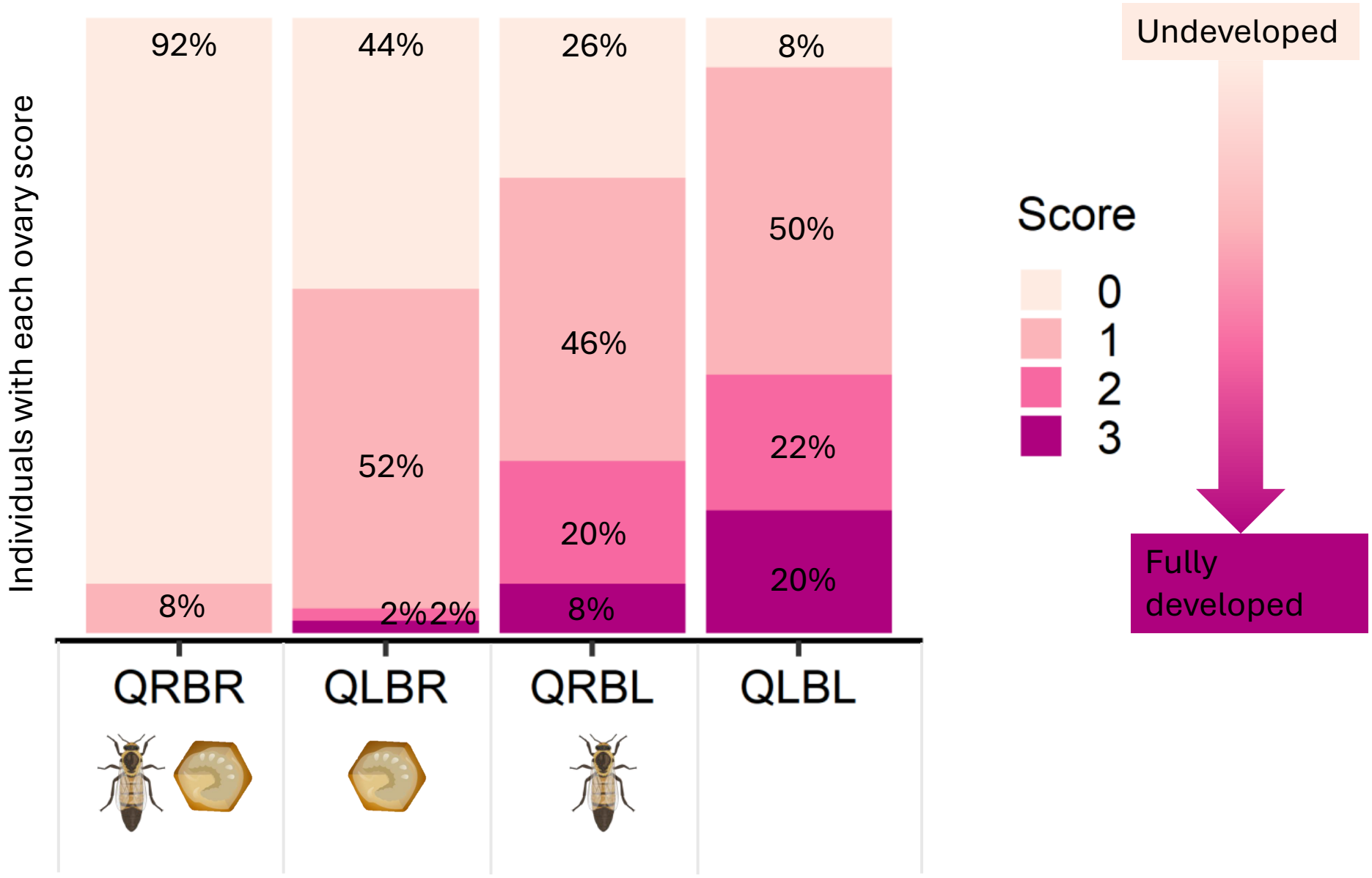
Results



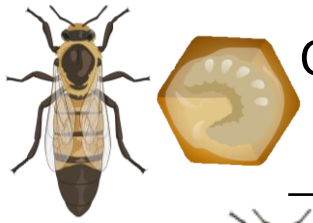
Absence of queen or brood causes increased ovary activation, especially broodless conditions

F(4, 195) = 29.03, P < 0.001; P<2.2e-16)

Results

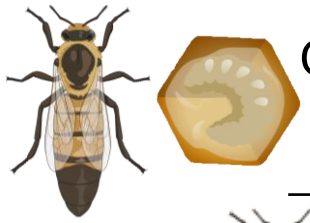

Individuals with each ovary score
92%
8%
44%
52%
2%
2%
26%
46%
20%
8%
8%
50%
22%
20%
QRBR
QLBR
QRBL
QLBL
Undeveloped
Score
0
1
2
3
Fully developed

Next Step – ‘omics & Pheromones



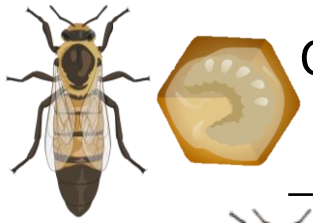
Comparison with QRBR	Tissue	Number of differentially expressed proteins		Number of differentially expressed genes	
		Down	Up	Down	Up
QRBL	Abdomen	145	227	497	649
	Gut	0	0	1048	1120
	Head	272	164	2538	2422
QLBR	Abdomen	0	0	1	4
	Gut	0	0	73	10
	Head	0	0	0	0
QLBL	Abdomen	0	19	126	357
	Gut	0	0	257	284
	Head	171	75	2140	2048

Next Step – ‘omics & Pheromones



Comparison with QRBR	Tissue	Number of differentially expressed proteins		Number of differentially expressed genes	
		Down	Up	Down	Up
QRBL	Abdomen	145	227	497	649
	Gut	0	0	1048	1120
	Head	272	164	2538	2422
QLBR	Abdomen	0	0	1	4
	Gut	0	0	73	10
	Head	0	0	0	0
QLBL	Abdomen	0	19	126	357
	Gut	0	0	257	284
	Head	171	75	2140	2048

Next Step – ‘omics & Pheromones



Comparison with QRBR	Tissue	Number of differentially expressed proteins		Number of differentially expressed genes	
		Down	Up	Down	Up
QRBL	Abdomen	145	227	497	649
	Gut	0	0	1048	1120
	Head	272	164	2538	2422
QLBR	Abdomen	0	0	1	4
	Gut	0	0	73	10
	Head	0	0	0	0
QLBL	Abdomen	0	19	126	357
	Gut	0	0	257	284
	Head	171	75	2140	2048

Discussion

- Lack of either queen or brood, and especially both, subtly increases the reproductive activity of workers.
- The lack of brood could more broadly disruptive to ovary development and changes in gene / protein expression in workers than the lack of a mated queen.
- Beekeepers should consider adding open brood when requeening queenless colonies.

Thank you

Jeff Kearns
Fairo Dzekashu
Jemma Todoschuk
Jessenia Buzunis-Delagneau
Lan Tran
Rodrigo Ortego Polo
Lance Lansing
Yuming Shi
Huan Zhong
Aidan Jamieson