

Exploring the Physiological Mechanisms Underlying Sublethal Imidacloprid-Induced Early Foraging Behavior in Honey Bee Workers

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Colony collapse disorder (CCD) of the honey bee, *Apis mellifera*, is an abnormal phenomenon marked by the sudden loss of most worker bees, including hive bees and foragers, in a colony. Previous studies have shown that exposure to environmental stressors, such as the neonicotinoid pesticide imidacloprid, can trigger early behavioral maturation in hive bees and impair the flight and homing abilities of foragers. These symptoms are closely linked to the rapid collapse of honey bee colonies. The transition from hive tasks to foraging behavior is coordinated with the increasing level of juvenile hormone (JH) III, which plays a crucial role in age polyethism. Elevated levels of JH III in young hive bees can cause early behavioral maturation and result in precocious foraging activity. Despite these insights, the molecular and physiological mechanisms underlying stress-induced precocious forager behavior remain largely unknown, but they may be associated with the level of JH III.

To investigate the molecular and physiological mechanisms underlying sublethal imidacloprid-induced precocious foraging, we used next-generation sequencing (NGS) and gas chromatography-mass spectrometry (GC-MS) to monitor trends in transcriptome profiles and JH III titers in workers of different ages between imidacloprid-treated and control groups. Our findings indicate that JH biosynthesis-related genes were significantly upregulated in young workers exposed to sublethal doses of imidacloprid during the larval stage. Additionally, the transcriptomic profiles of imidacloprid-treated young workers closely resembled those of older control workers, suggesting accelerated behavioral maturation at the molecular level. Correspondingly, JH III titers increased earlier in imidacloprid-treated young workers, further supporting the physiological mechanism of JH III in mediating stress-induced precocious foraging behavior.

The outcome of this project provides genetic and physiological evidence that exposure to sublethal imidacloprid during the larval stage may disrupt the age polyethism in the colony; this could potentially impair individual bee development and threaten colony stability. Our results highlight the need for further research into the long-term ecological impacts of neonicotinoid exposure on honey bee populations.



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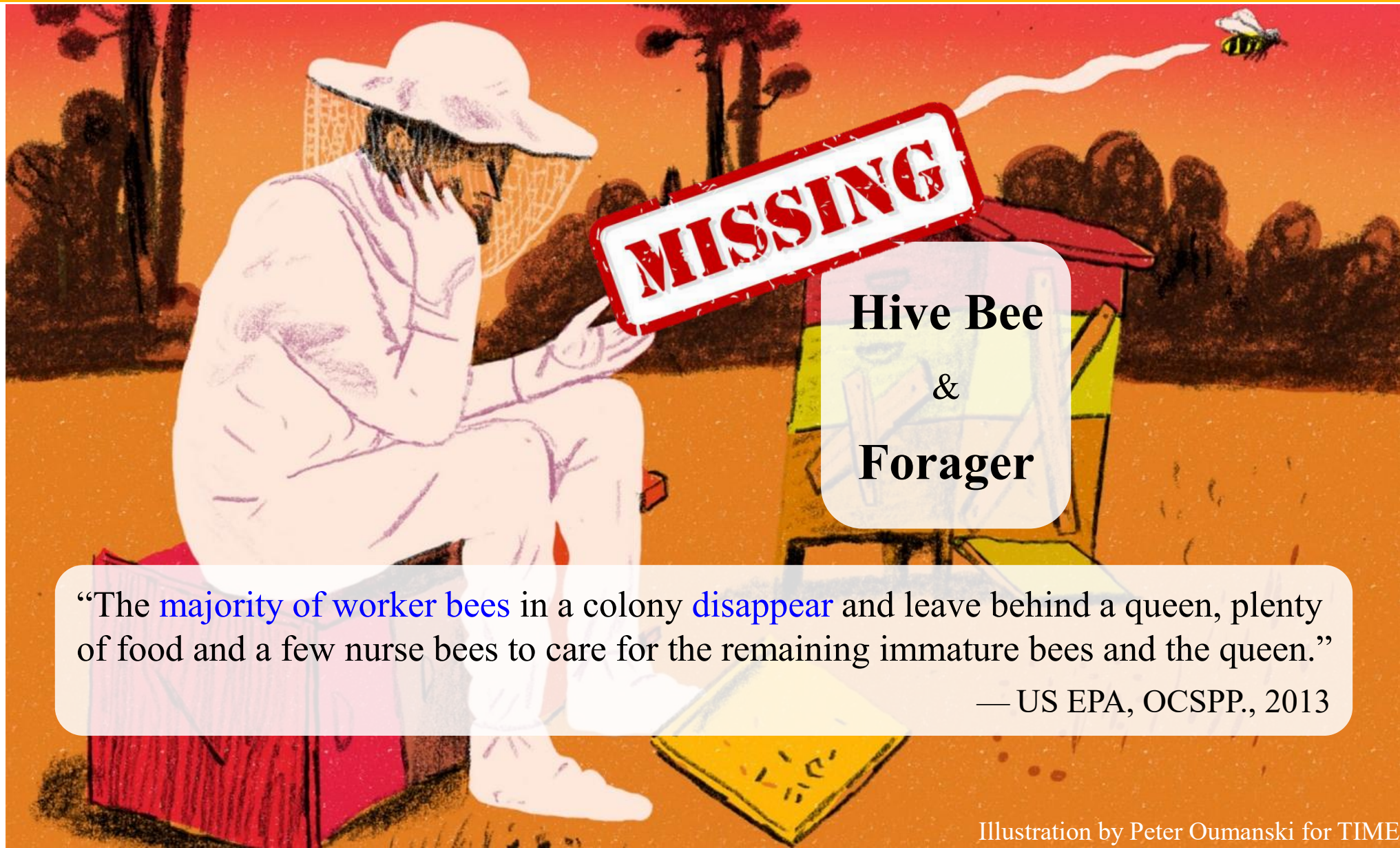
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Speaker : **Wan-Yi Chen**

Advisor : Prof. En-Cheng Yang

Date : 24 Sep 2025

Phenomenon of Colony Collapse Disorder (CCD)

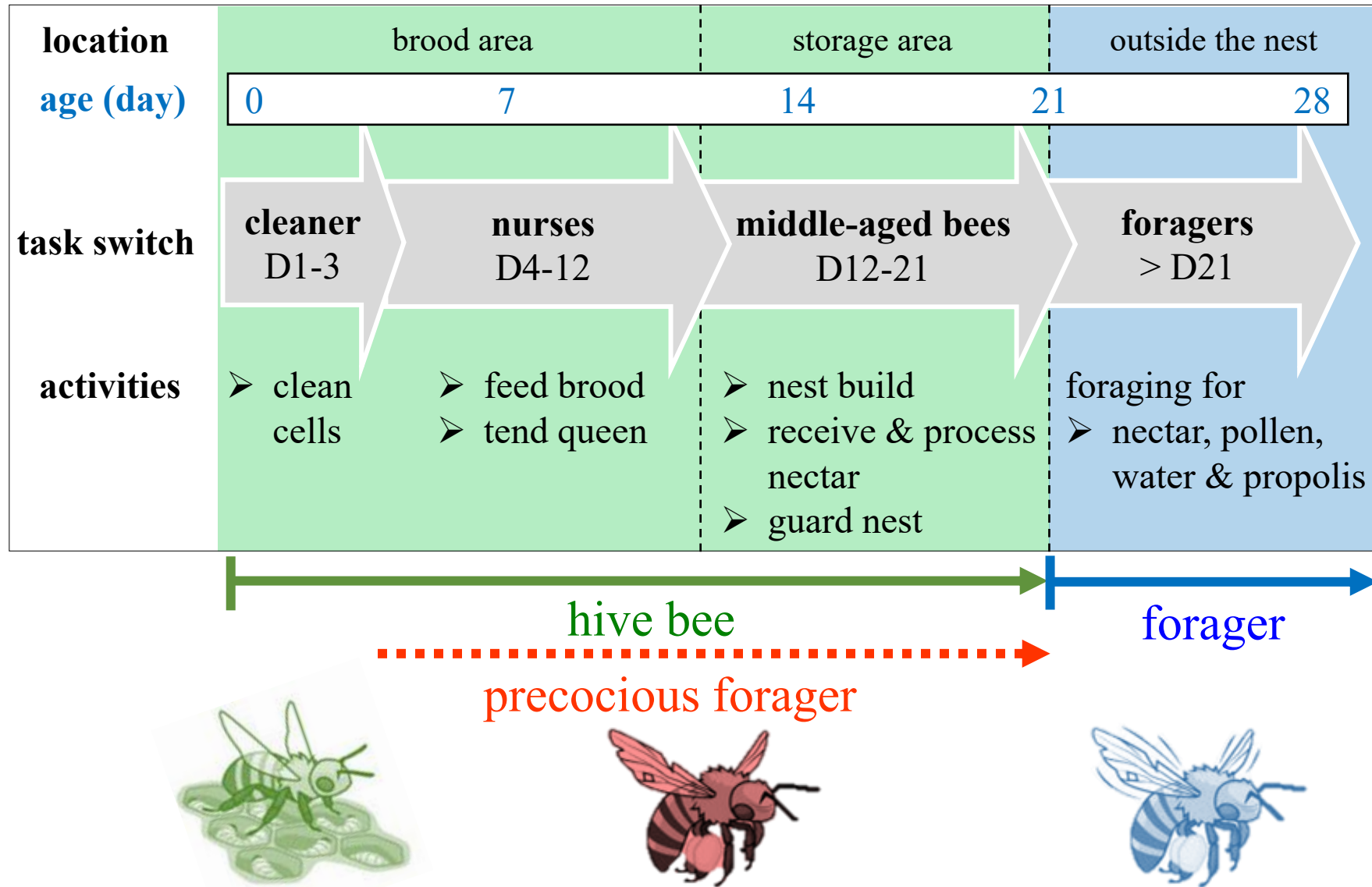


Hive Bee & Forager

“The [majority of worker bees](#) in a colony [disappear](#) and leave behind a queen, plenty of food and a few nurse bees to care for the remaining immature bees and the queen.”

— US EPA, OCSPP, 2013

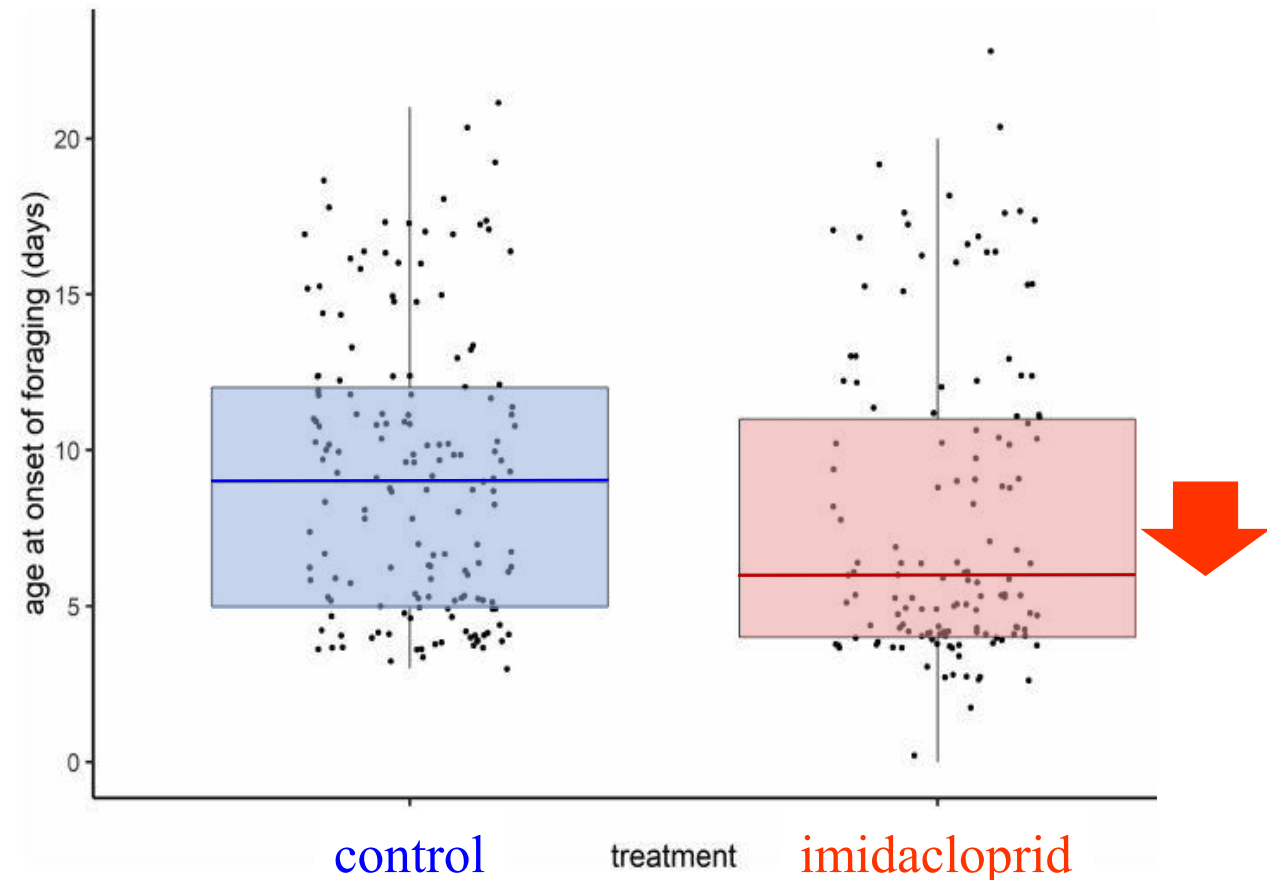
Age Polyethism in *Apis mellifera* L.



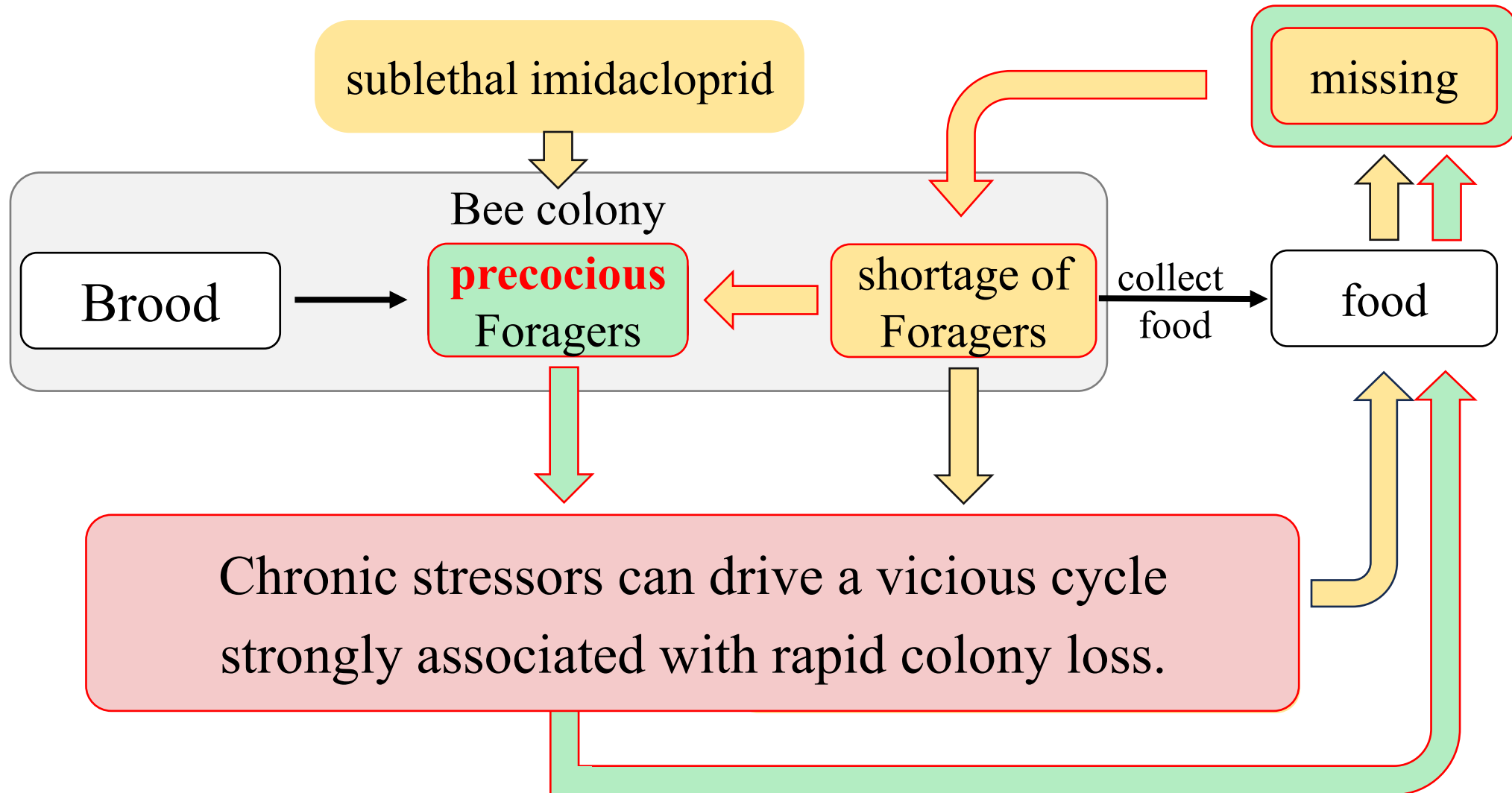
Imidacloprid (IM) Induced Precocious Foraging

- exposure to 5 ppb imidacloprid causes bees to start foraging ~**15%** earlier

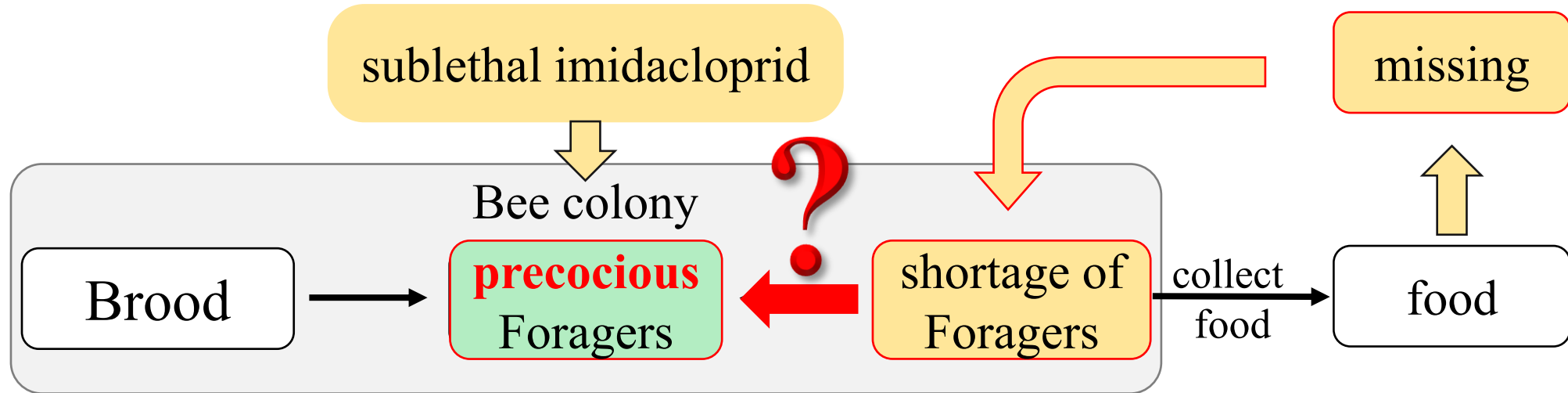
bees exposed to
imidacloprid in the larval
stage only, or **control**



Negative Impacts of Sublethal IM on Bee Colony

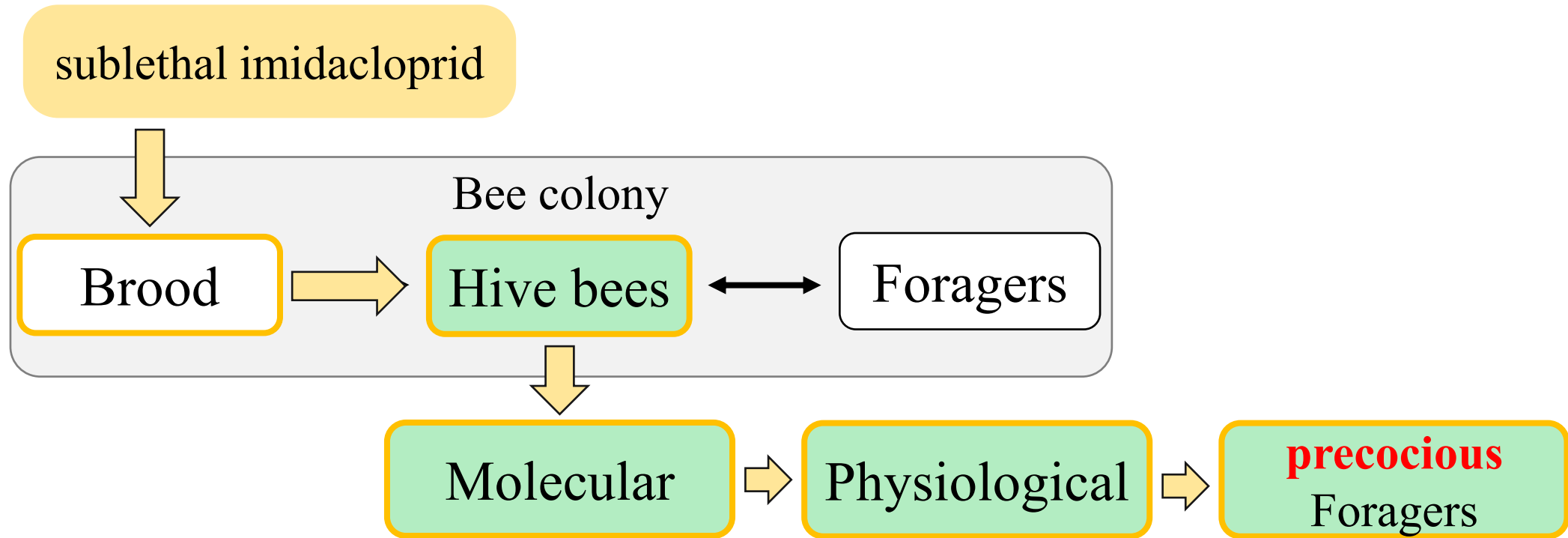


What Causes Precocious Foraging?



Is forager shortage the only trigger of precocious foraging?

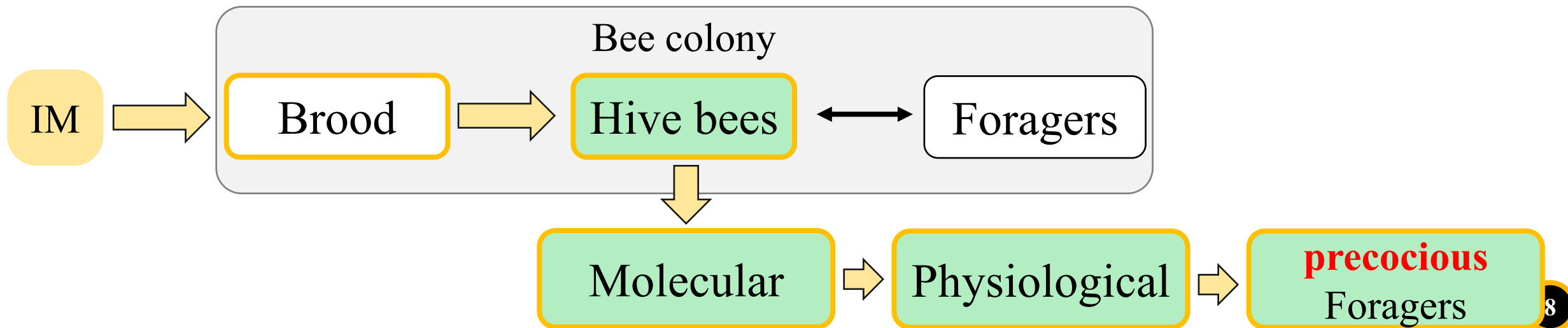
Our Central Hypothesis



Does sublethal imidacloprid alter physiology to induce early maturation?

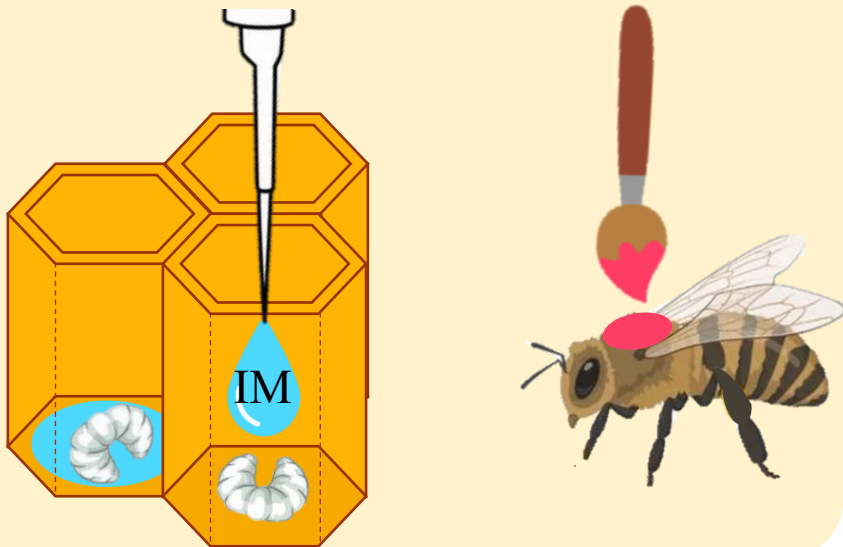
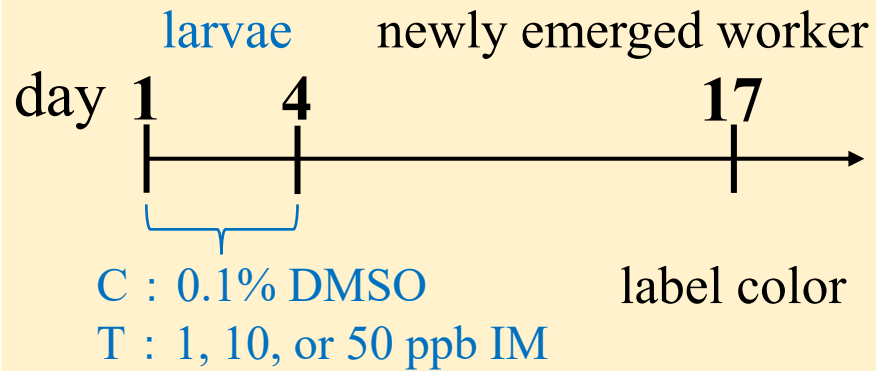
Aims

- **Aim 1**
Determine whether larval sublethal IM exposure shifts adult transcriptome profiles.
- **Aim 2**
Assess whether larval sublethal IM exposure alters worker physiology.
- **Aim 3**
Control for colony context by introducing treated bees into healthy host colonies (removing forager shortage effects).

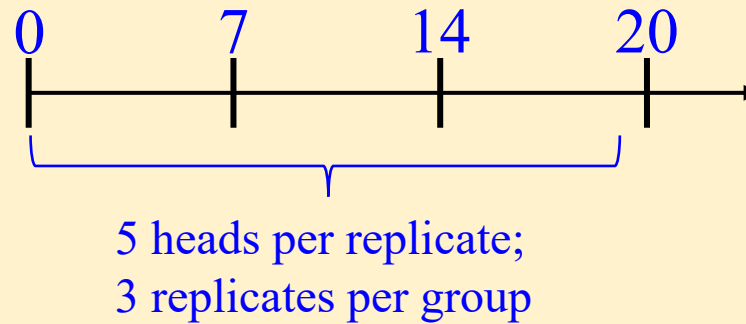


Measuring Gene Expression Changes Following Sublethal IM Exposure

larvae treated (IM) (D1 – 4)



adult sampling (D0 – 20)



transcriptome analysis by NGS

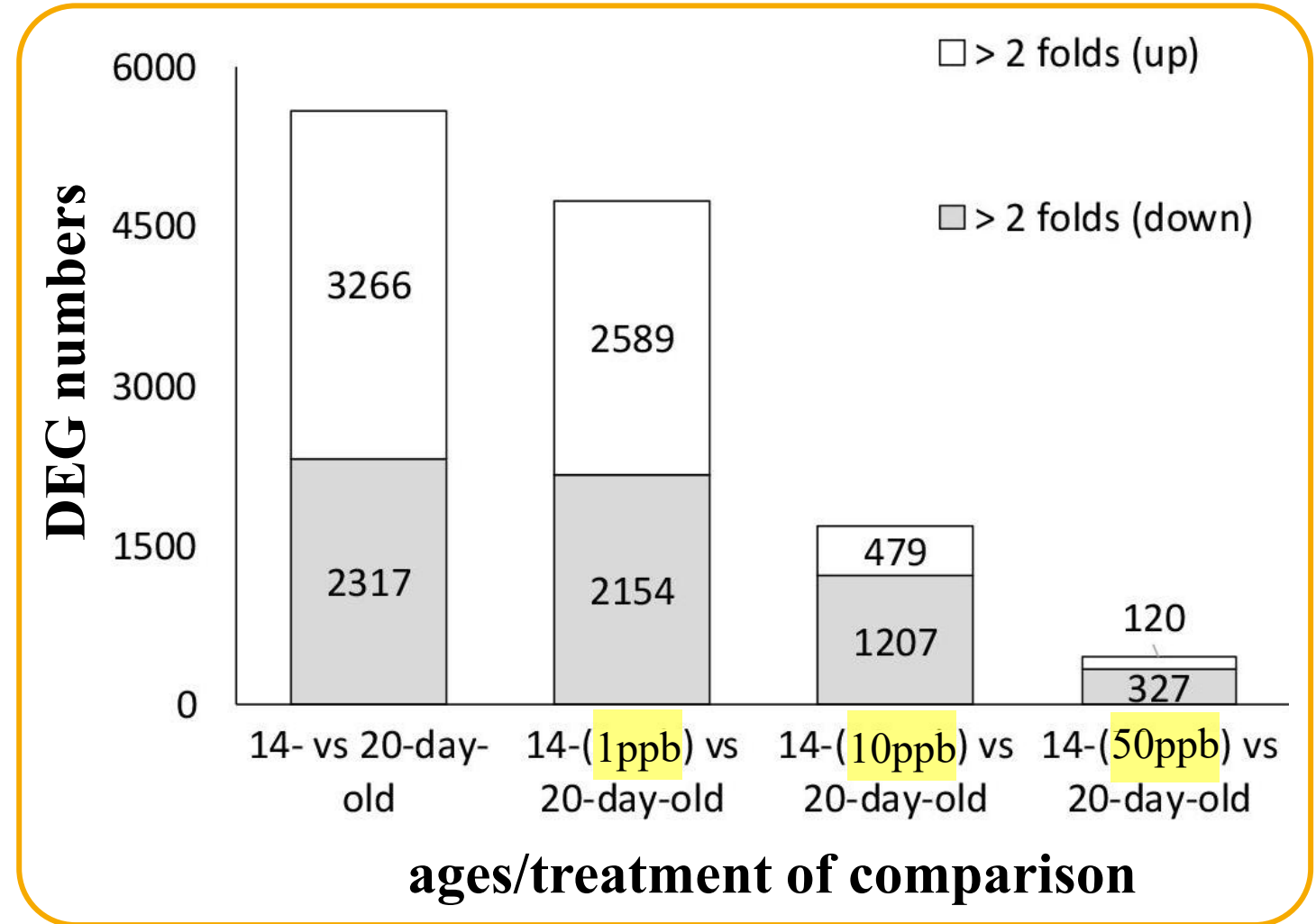
RNA extraction

library preparation

data analysis by
**Next Generation
Sequencing (NGS)**

Transcriptomes of Treated Young Workers Resemble Older Controls

- fewer DEGs (differentially expressed genes)
= more similar transcriptomes
- 14-day treated $\approx$ 20-day control (esp. 50 ppb)
- dose-dependent effect



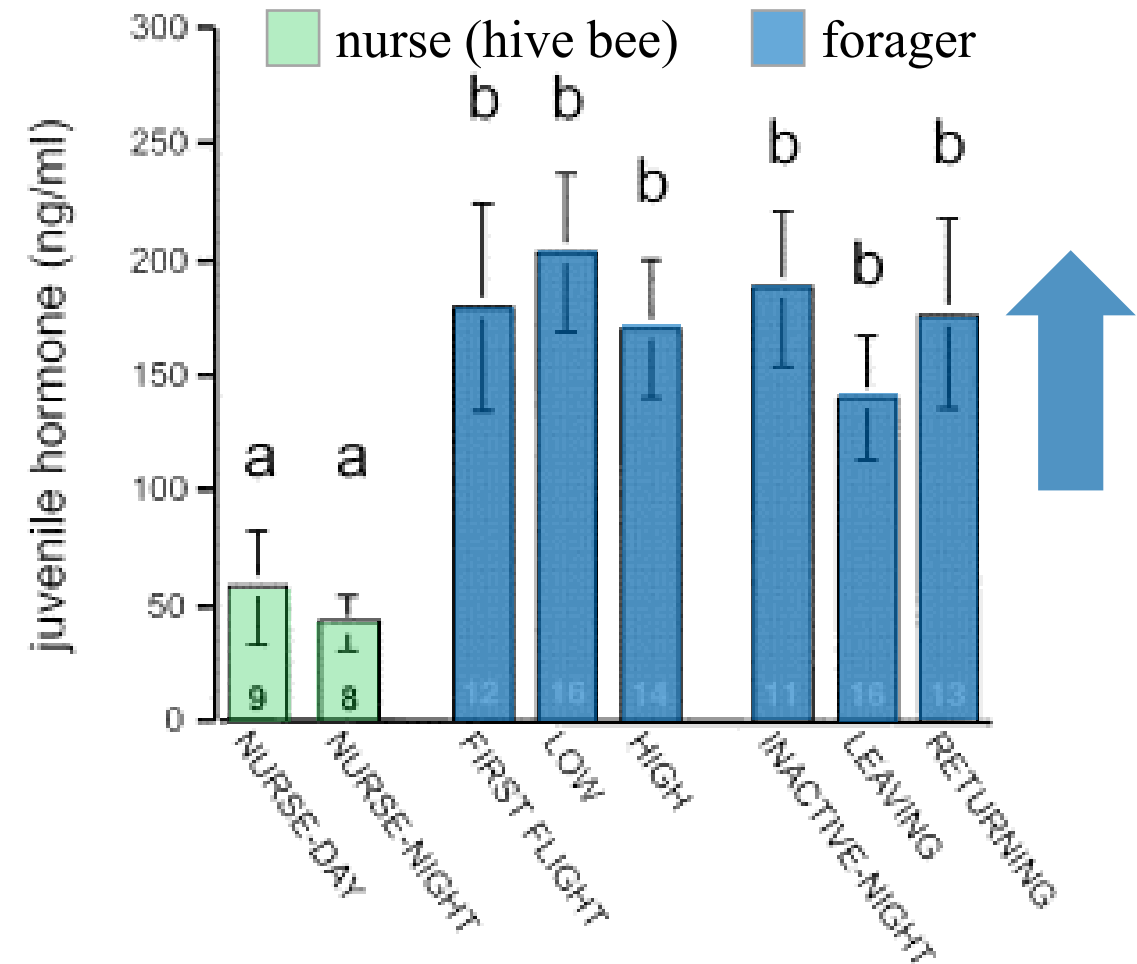
Up-regulation of Foraging Regulators After Sublethal IM Treatment

	fly ortholog	bee gene	7-day-old adults			14-day-old adults		
Imidacloprid (ppb)			1	10	50	1	10	50
Nursing regulators	<i>usf</i>	GB40634	up					
	<i>cfp1</i>	GB43820						
	<i>nautilus</i>	GB55306						
Foraging regulators	<i>usp</i>	GB42692				up	up	up
	<i>Creb-B17A</i>	GB46492					up	up
	<i>slbo</i>	GB44204						
	<i>retained</i>	GB55596				up		up
	<i>atf3</i>	GB53401						
	<i>labial</i>	GB51303						
	<i>deformed</i>	GB51299				up	up	
	<i>dorsal</i>	GB42472						
	<i>hairy</i>	GB47799				up	up	up
	<i>eyeless</i>	GB50342					up	
	<i>stripe</i>	GB50091				up	up	up
	<i>cwo</i>	GB52039					up	up

Gene list is referenced
from Khamis *et al.*, 2015.
Sci. Rep. 5:11136.
doi:10.1038/srep11136
Chen *et al.*, 2021. *Front.*
Genet. 12, 665927.

Juvenile Hormone (JH III): A Key Regulator of Task Switching

- nurse to forager transition is accompanied by an **increase in JH III** titers
- foragers have significantly higher JH III titers than hive bees



Up-regulation of JH Synthesis Genes After Treated to Sub-IM

- DEGs of JH-III biosynthesis & degradation pathway

Symbol		14-day-old adults		
Imidacloprid (ppb)		1	10	50
Mevalonate pathway	<i>HMGR</i>	up	up	up
	<i>MK</i>	up	up	up
JH synthesis specific steps	<i>LOC408559</i>	-	up	up
	<i>FaMeT</i>	up	up	up
	<i>MFE</i>	down	down	up
Methyl transfer portion	<i>JHAMT</i>	up	up *	up *
	<i>AHC</i>	up	up	up
	<i>AK</i>	up	up	up
JH degradation	<i>JHEH</i>	-	up *	up *
	<i>JHE</i>	down *	down *	down *

up-regulation of JH synthesis genes

+

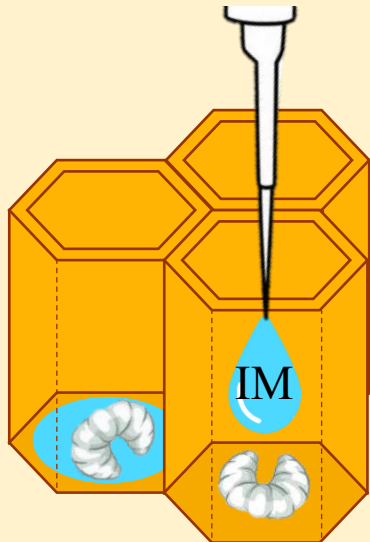
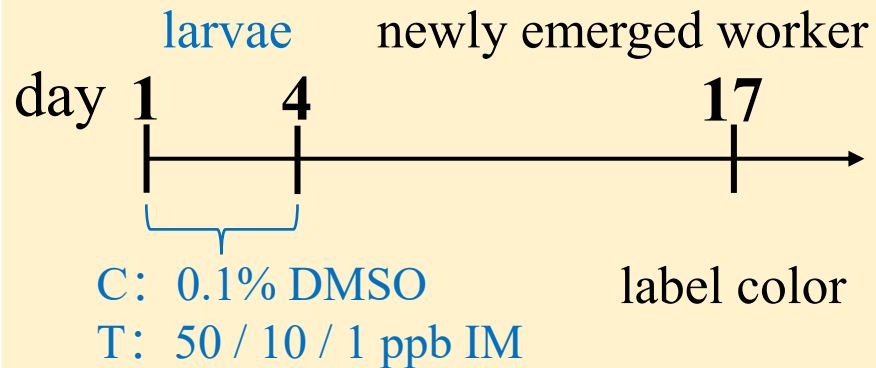
down-regulation of JH hydrolysis genes

↓

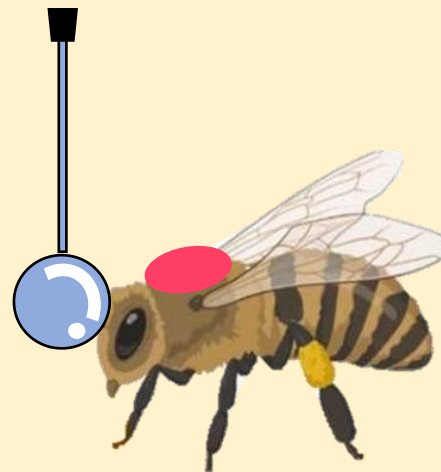
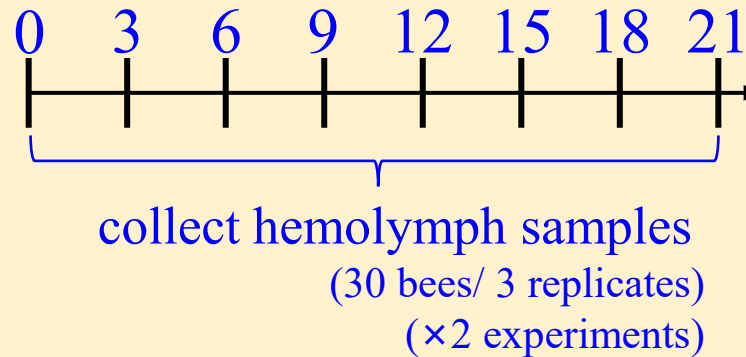
predicts higher JH levels

Measuring JH III Titers Following Sublethal IM Exposure

larvae treated (IM) (D1 – 4)



sample collection (D0 – 21)



JH III measured by GC-MS

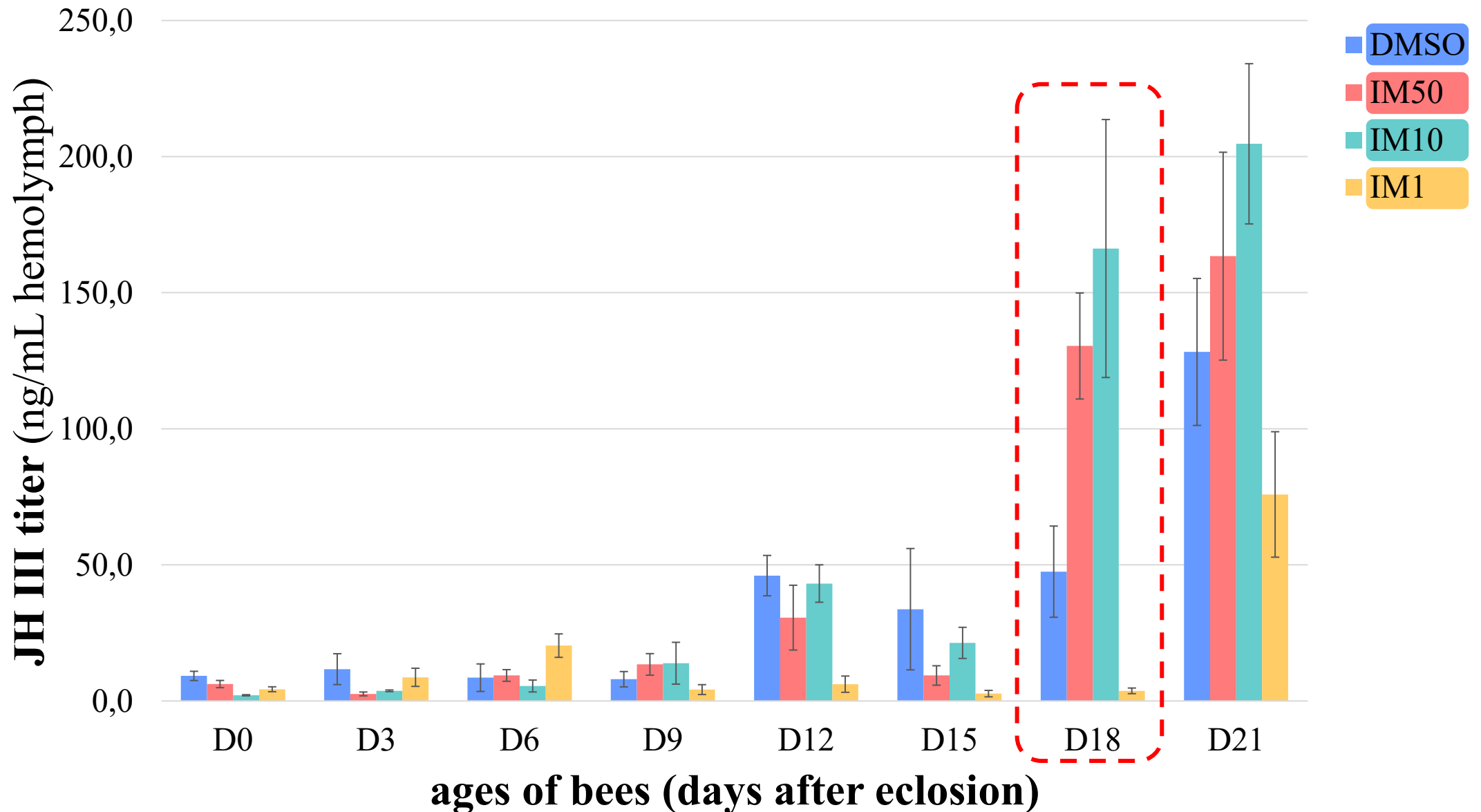
JH III purification

GC-MS analysis



Gas chromatography–mass spectrometry (Agilent 7890/5975)

JH III Titers Rise Earlier After Sublethal IM Treatment



Conclusion

Larval exposure to sublethal IM leads to:

Transcriptomes:

- ✓ Young bees resemble older controls
- ✓ **Up**-regulation of JH biosynthesis & foraging regulators

Physiology:

- ✓ JH titers rise earlier → precocious foraging
(↑ at D18 in 10 & 50 ppb groups)

The outcome of this project provides physiological evidence that exposure to sublethal imidacloprid during the larval stage may disturb the age polyethism within a colony.

Acknowledgements



Advisor : Prof. En-Cheng Yang

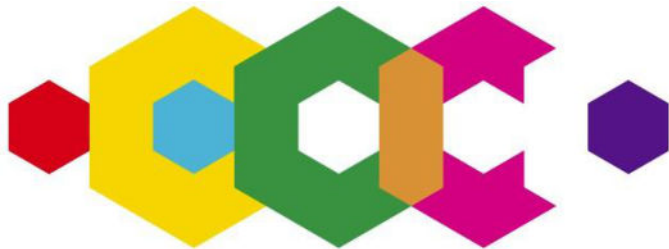
Lab members: Der-Wei Lee

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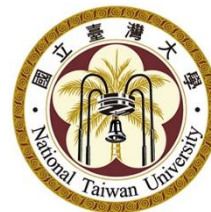
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Thank you for your attention!