OP-060 [Bee Biology]
Embryogenesis of haploid honey bee (Apis mellifera L.) takes longer than diploid worker

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INTRODUCTION: During honey bee development, the embryonic stage is crucial, as it is when all the fundamental organ rudiments of adult bees are formed. There are notable physiological and social behavioral differences between worker bees and drones, primarily influenced by genetic regulation. The initial molecular basis at the proteomic level for the embryonic development of both workers and drones has been identified, but a comprehensive understanding of the significant events involved in embryonic establishment remains elusive.

OBJECTIVES: To elucidate the molecular regulatory mechanisms underlying tissue differentiation during the embryogenesis of drone and worker bees through a high-resolution investigation.

METHODS: We implemented a state-of-the-art approach that combines precise and extensive sampling with high-throughput proteomics technology and in-hive hatching observations to conduct a comprehensive comparison of male and female bee embryo development.

RESULTS: This work identified a total of 7,591 proteins, marking a 2.5-fold increase in protein coverage compared to previous investigation. Our comparative analysis reveals distinct strategies in embryonic development between worker bees and drones. Notably, proteins involved in fatty acid metabolism and the activation of biological pathways related to organ establishment, such as the hedgehog and wnt signaling pathways were initiated earlier in drones, indicating that drone development begins ahead of that of worker embryos. Furthermore, ribosomal proteins essential for material support were consistently expressed through the later stages in male bees, highlighting that drone embryogenesis lasts longer than that of workers. Specifically, the results of in-hive inspection indicate a developmental gap of approximately four hours between the two embryos. Additionally, the patterns of cytoskeletal proteins and antioxidants reflect distinct strategies employed to support the development of drone embryos.

CONCLUSION: This study offers new insights into the molecular basis of honey bee embryos and establishes a theoretical and practical foundation for further investigation into the mechanisms of embryonic development.

Based on proteomics to investigate the mechanism of embryogenesis between honeybee worker and drone

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Background

1. Honeybee colony (*Apis mellifera* L.)

Queen

Worker

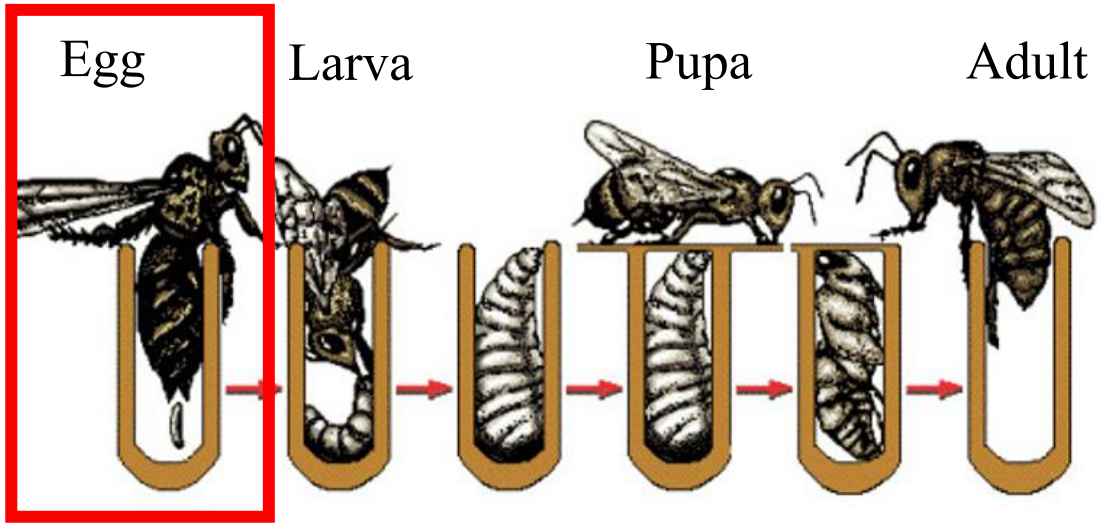
Drone

diploid

haploid

2. Development

Honey bees undergo complete metamorphosis through 4 distinct stages.

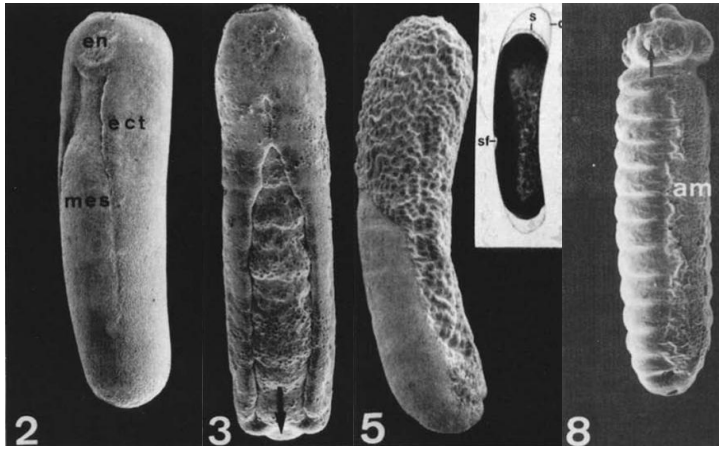


During embryonic development, the formation of various organ rudiments launches (Fleig, 1986), which directly influences the biological characteristics of adult bees.

3. Research on bee embryogenesis

Morphological level

the entire process of worker embryogenesis is characterized by four stages: embryonic disc formation （7 h ~ 33 h） , gastrula formation （33 h ~ 40 h） , embryonic band formation （40 h ~ 55 h), and larval body formation （55 h ~ 72h) (Fleig, 1986).



Genetic level

Genomic methods have been widely applied to study the embryonic development processes of fertilized eggs in diploid worker bees, as well as the coordination and regulation of genome activation in unfertilized eggs of haploid male bees(Wang, 2021).

Haploidy and sex selection significantly influence the evolutionary rates of genes within the honey bee genome (Slater, 2022).

Transcriptional level

The differences between them primarily driven by alternative splicing, which is more diverse than previously understood pathways of sex determination(Netschitailo, 2021).

the transcriptome of diploid mutant embryos provides essential insights into gene expression differences associated with sex differentiation, which is valuable for further exploring sexual dimorphism and developmental processes in Hymenoptera (Wang, 2021).

Protein level

The male embryonic patterns of *Apis cerana* and *Apis mellifera* have developed distinct biological characteristics, which are evident in their respective proteomes (Zhou, 2013).

Day 1 is the preparatory stage for embryonic development in honeybees. Days 2–3 are the critical period for organ formation, during which organ systems gradually develop after 46 hours(Fang, 2014).

The embryonic stage is the optimal period for genetic modification.

The environmental requirement: temperature and humidity;

simple and easily controllable;

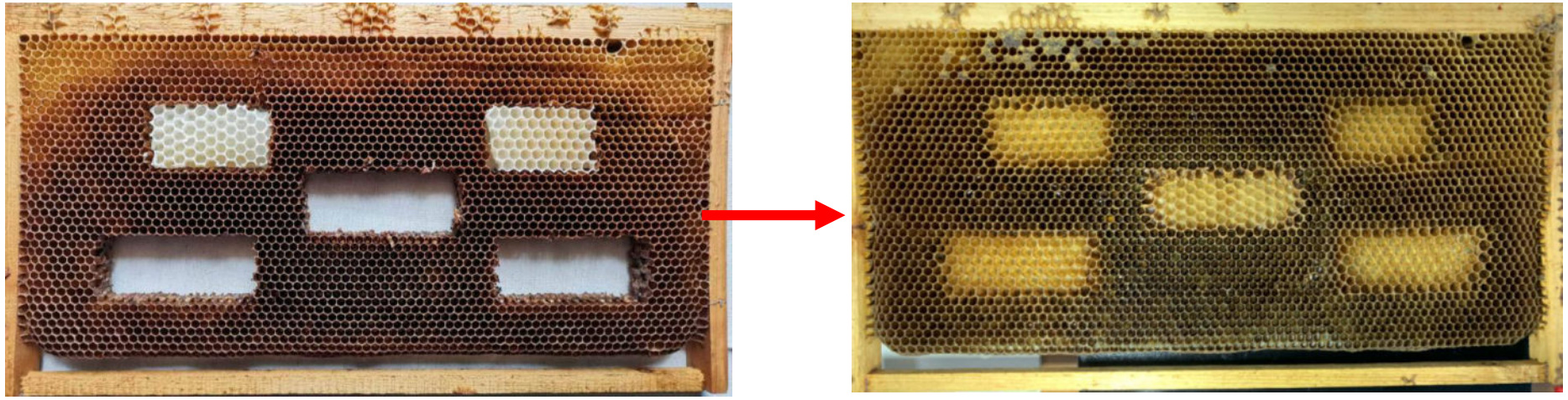
most suitable for genetic manipulation.

We know that drone eggs take longer to hatch than worker (Harbo and Bolten.1981) , but the exact time difference is still unknown.

Furthermore, the molecular basis underlying the differences in embryonic development between drone and worker remains unclear.

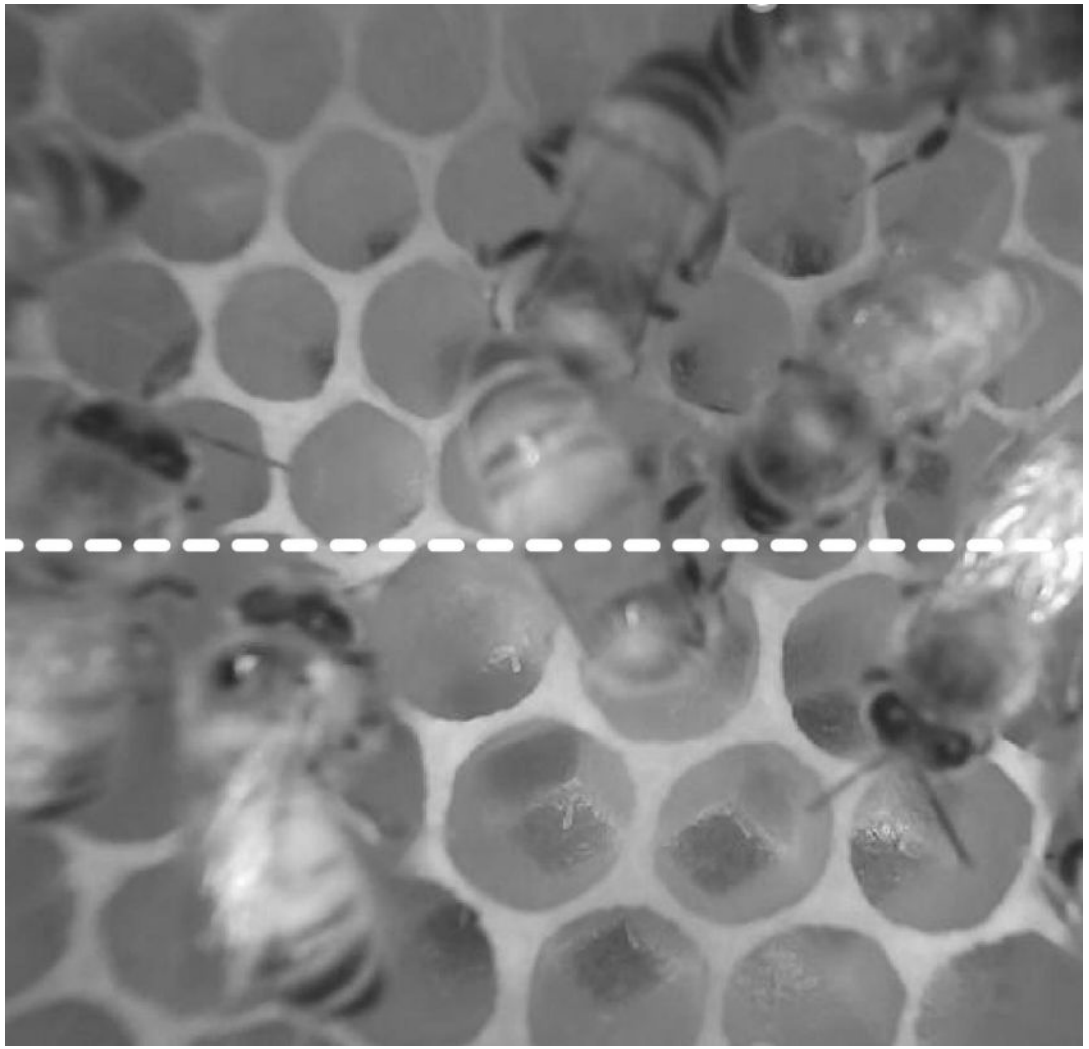
Experiment 1.

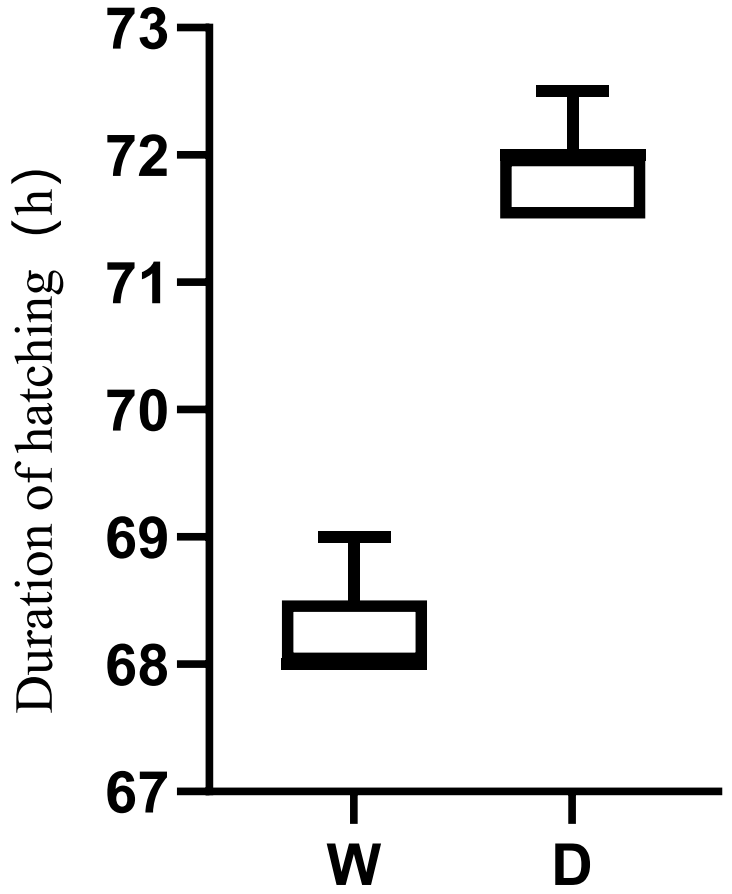
Observation of the development duration



Cage the queen to force it lay eggs for 1h

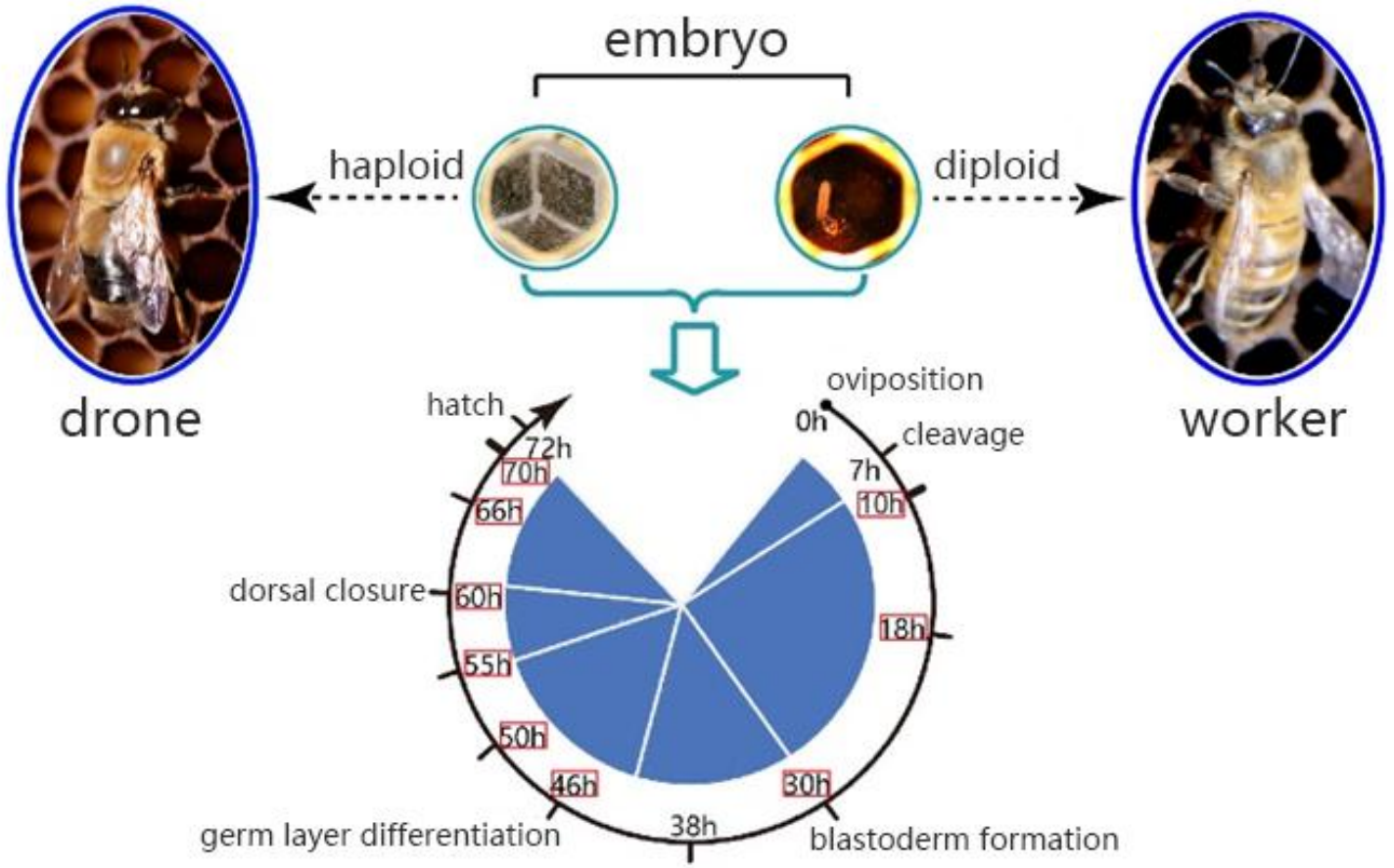
Identical hive temperature and humidity





Drone embryo take another 4 h for hatching

Experiment 2. Proteomic comparison

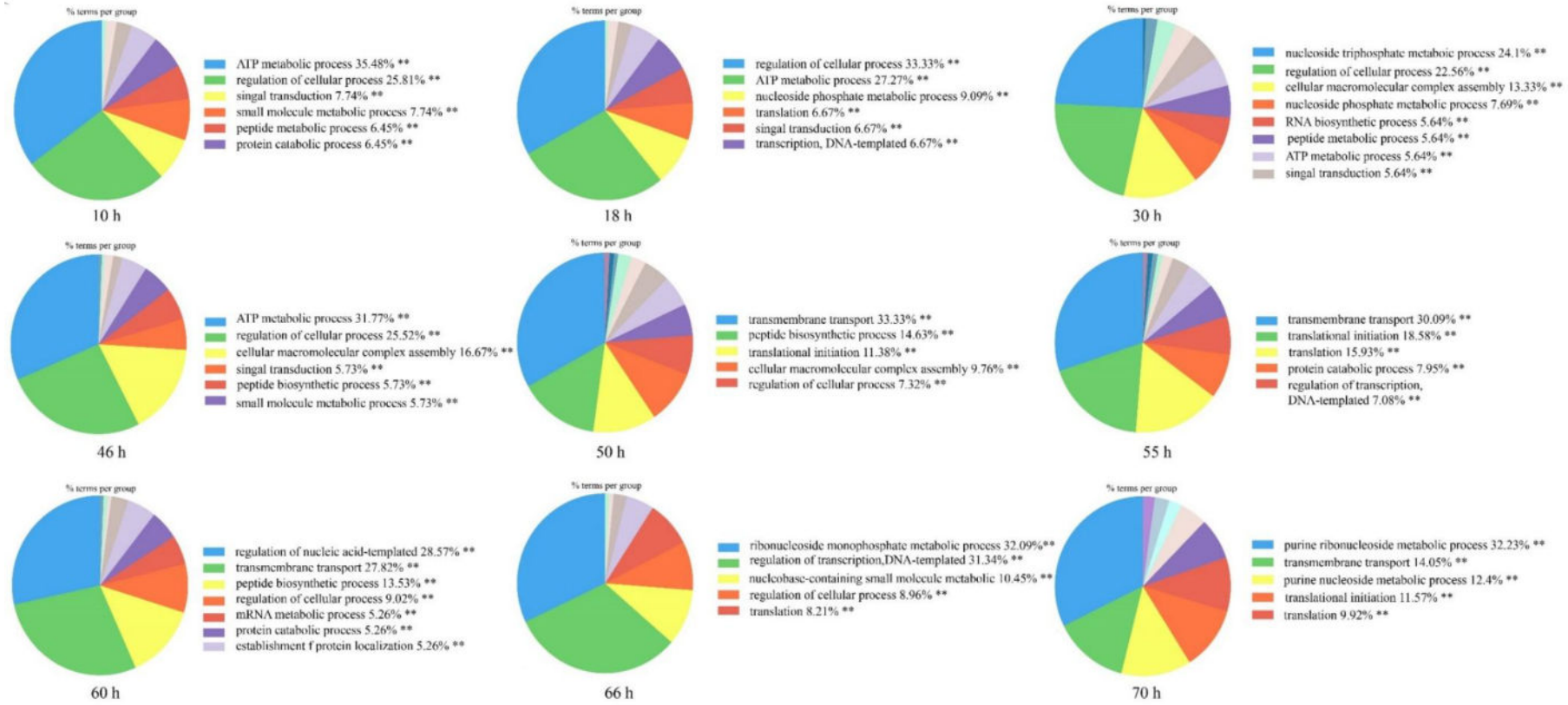


Based on our previous findings (Fang, 2015), selecting 9 key time points for embryonic development.
The sampling points were determined by the corresponding developmental periods, which were not evenly distributed.

Totally, 7,591 proteins were identified, 2,129 proteins exhibited differential expression between drone and worker bees.

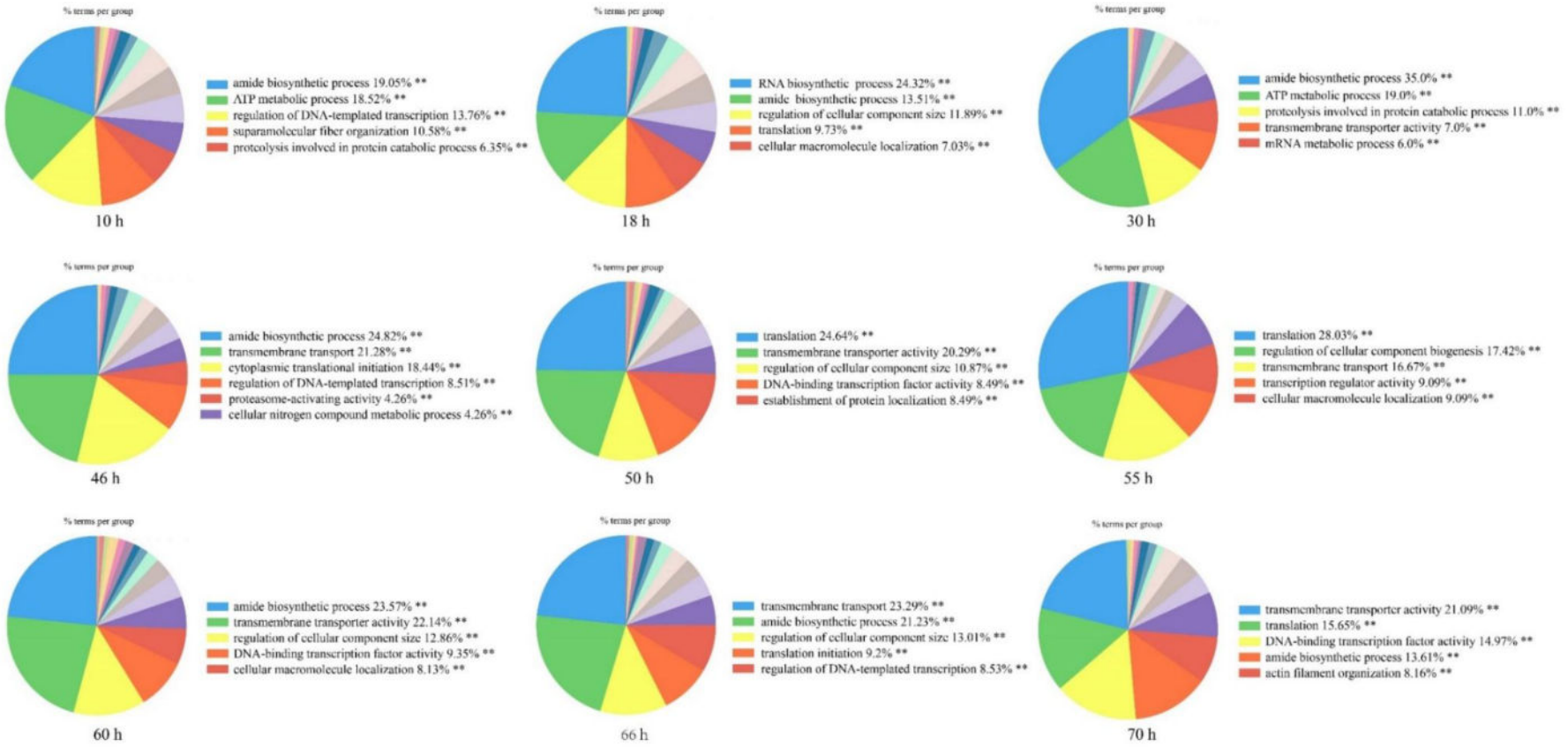
	Qualitative		Quantitative	
	Worker	**Drone**	**Worker**	**Drone**
10h	3525	3613	6	3
18h	3759	3770	138	201
30h	3880	2669	685	233
46h	3594	3462	167	526
50h	4851	4528	272	168
55h	4838	4659	6	6
60h	4623	4961	91	225
66h	4029	4922	237	418
70h	3808	4210	62	270

1. Profile of woker embryo proteome



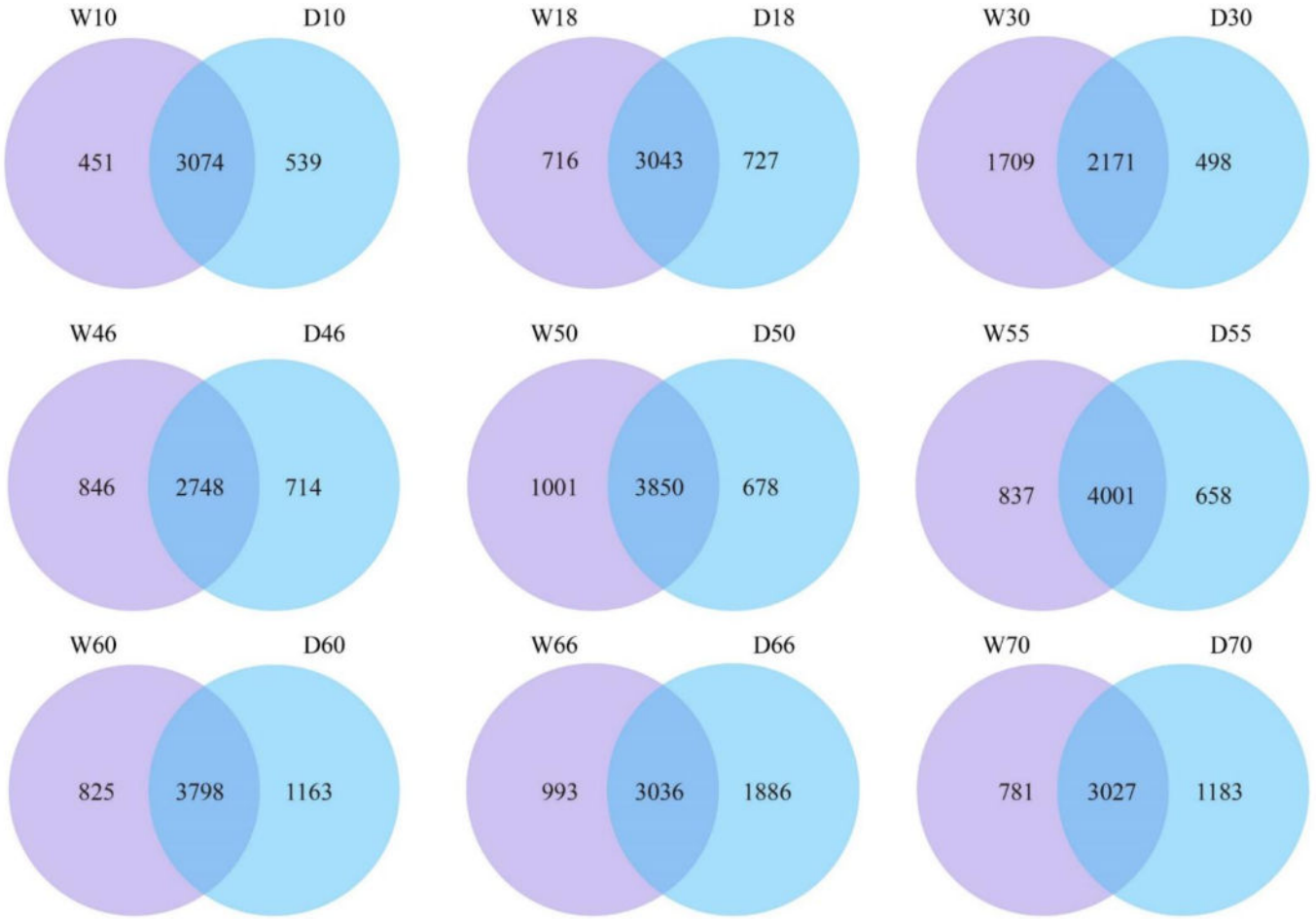
The expressed proteins are mainly related to ATP metabolism, peptide metabolism processes, cellular processes, initiation of translation and regulation of signal transduction, indicating that protein synthesis and energy metabolism, which is consistent with the biological processes of embryonic development.

2. Profile of drone embryo proteome



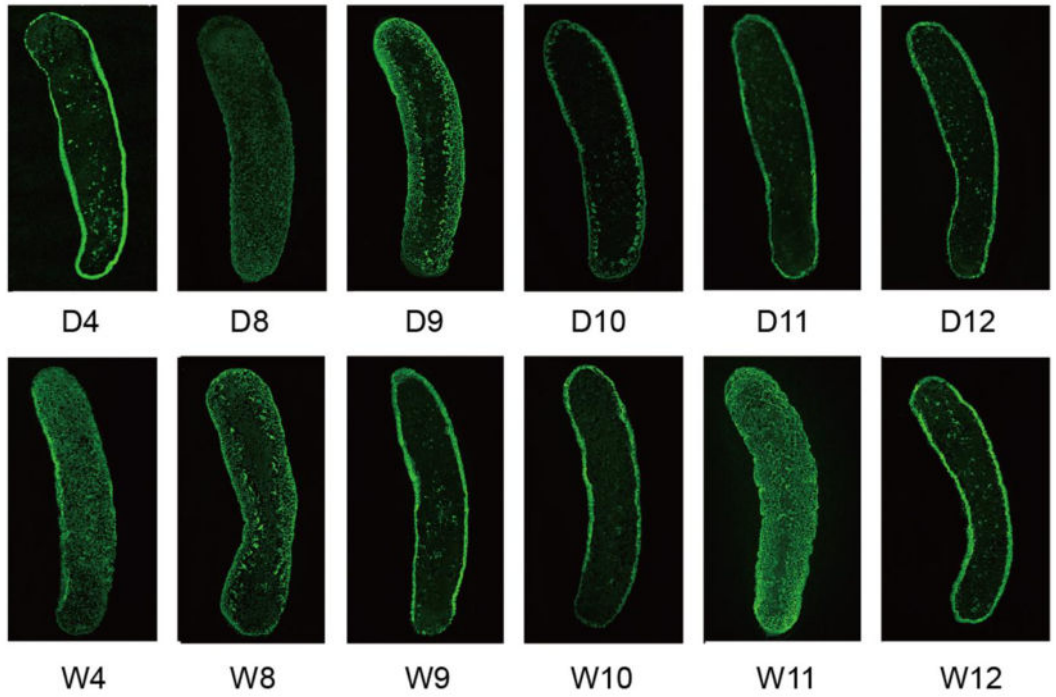
The expressed proteins mainly involved in peptide metabolism, transmembrane transport, translation initiation, assembly of large cellular complexes, and regulation of cellular processes.

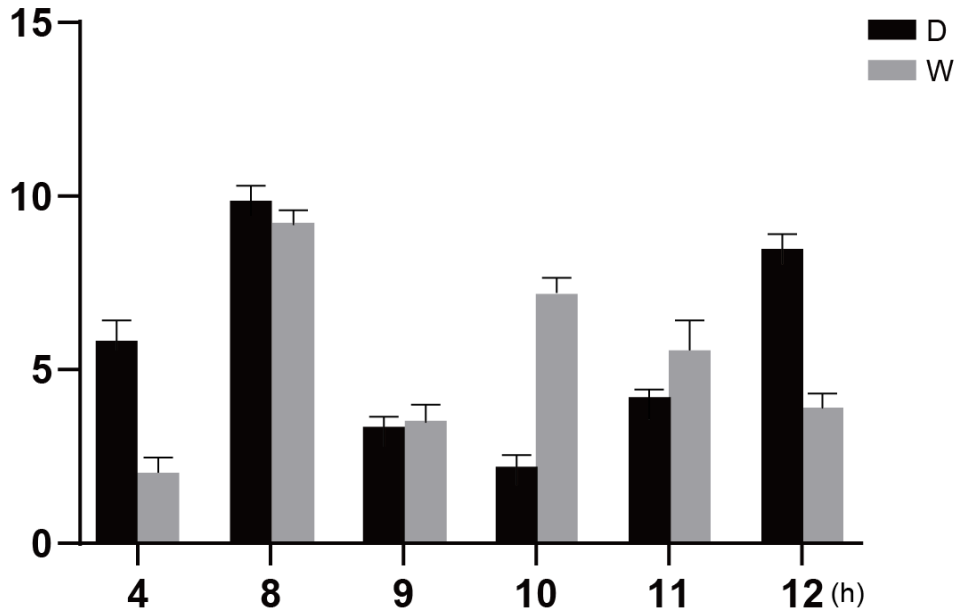
3. Comparison proteomes between worker and drone



4. Quantitative comparison

Early stage of embryogenesis---Tubulin



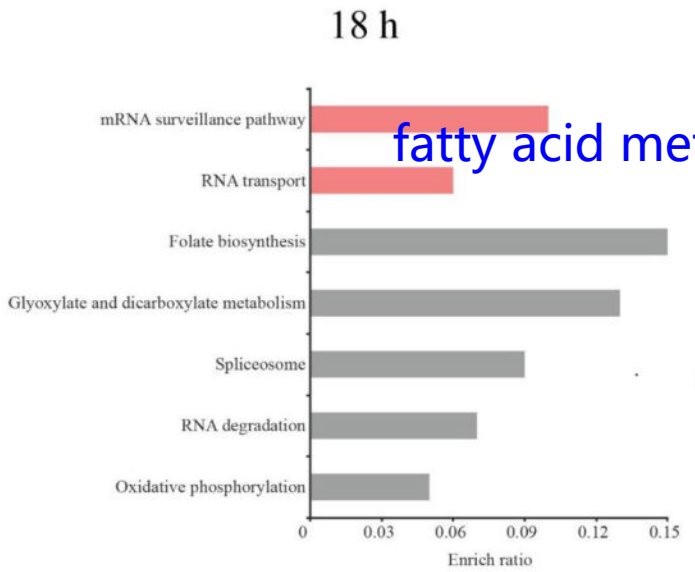


Tubulin constitutes microtubules, involved in the migration of cell morphology, the process of cell transportation, and cell division.

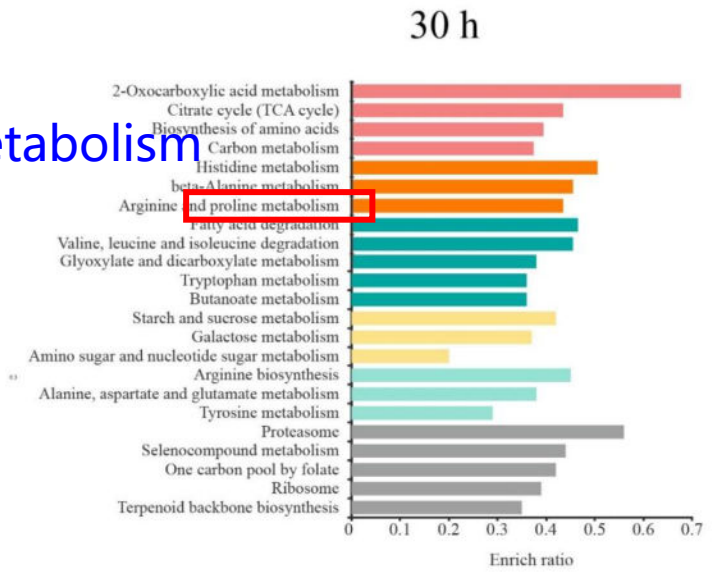
The development of drone embryo may start earlier than that of worker.

Early-middle stage of embrogenesis

worker

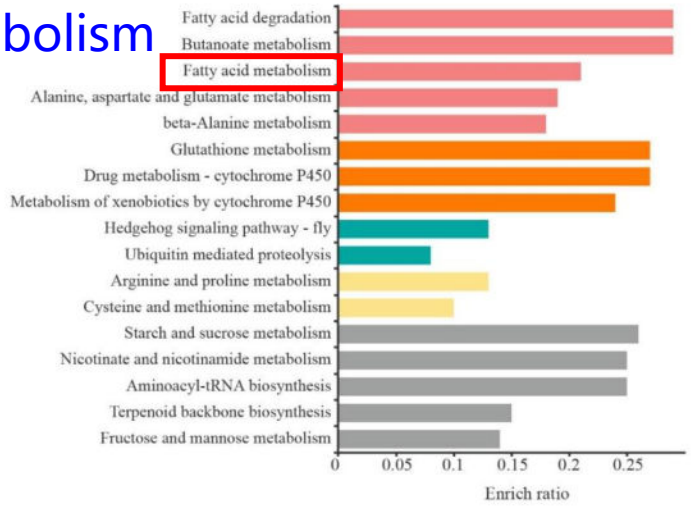


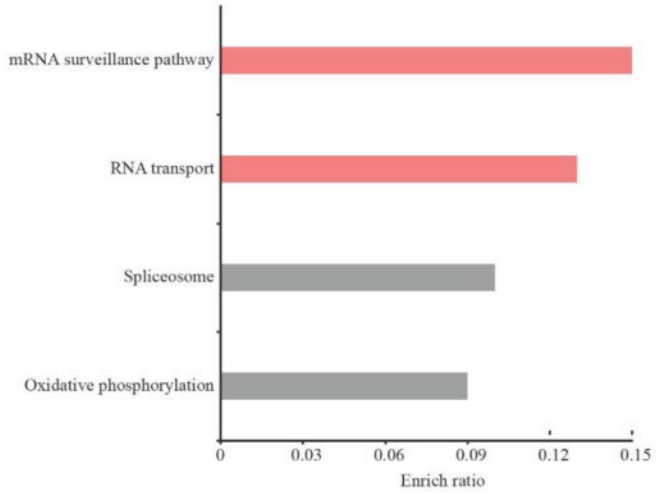
fatty acid metabolism



fatty acid metabolism

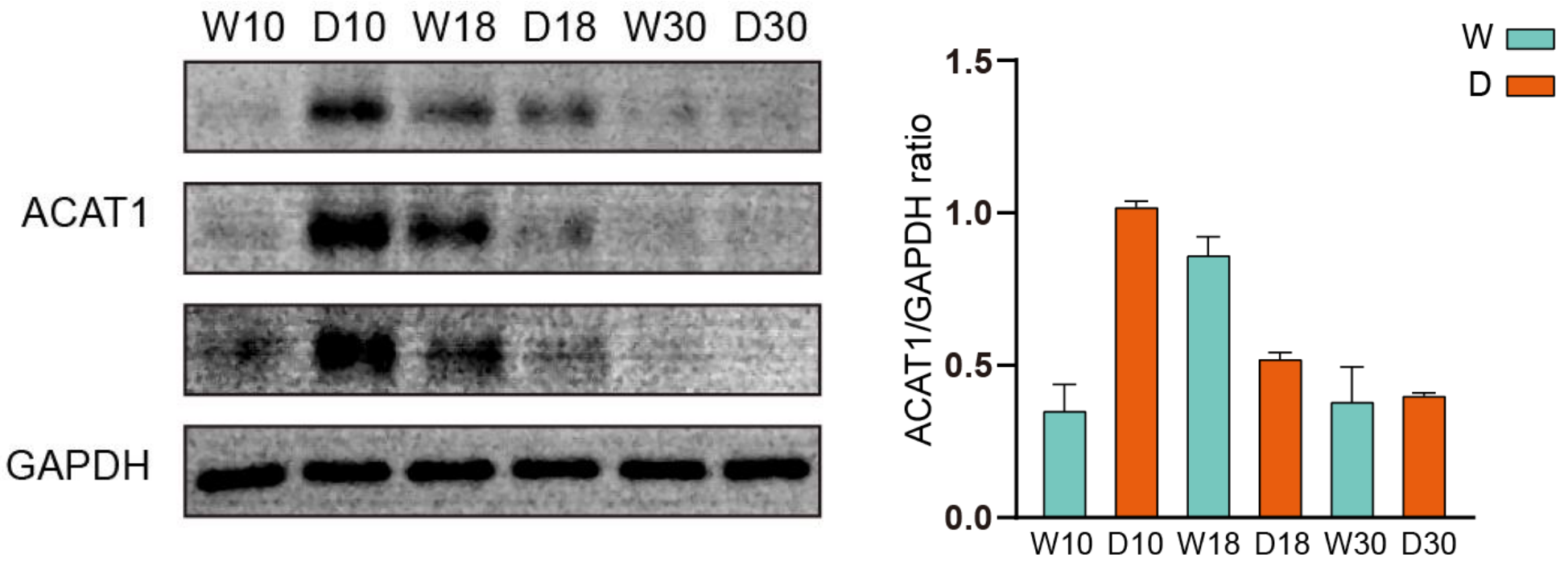
drone





fatty acid metabolism provides energy for cell division.

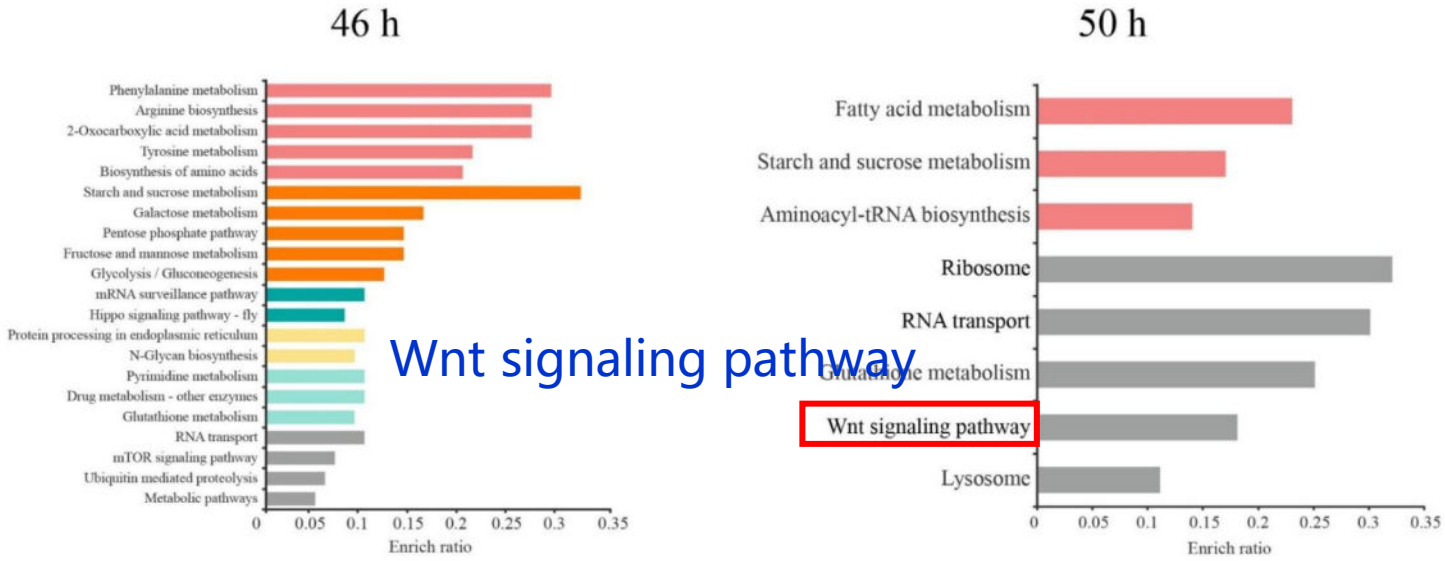
fatty acid metabolism---ACAT1



Events related to fatty acid metabolism, drone may occur earlier than worker.

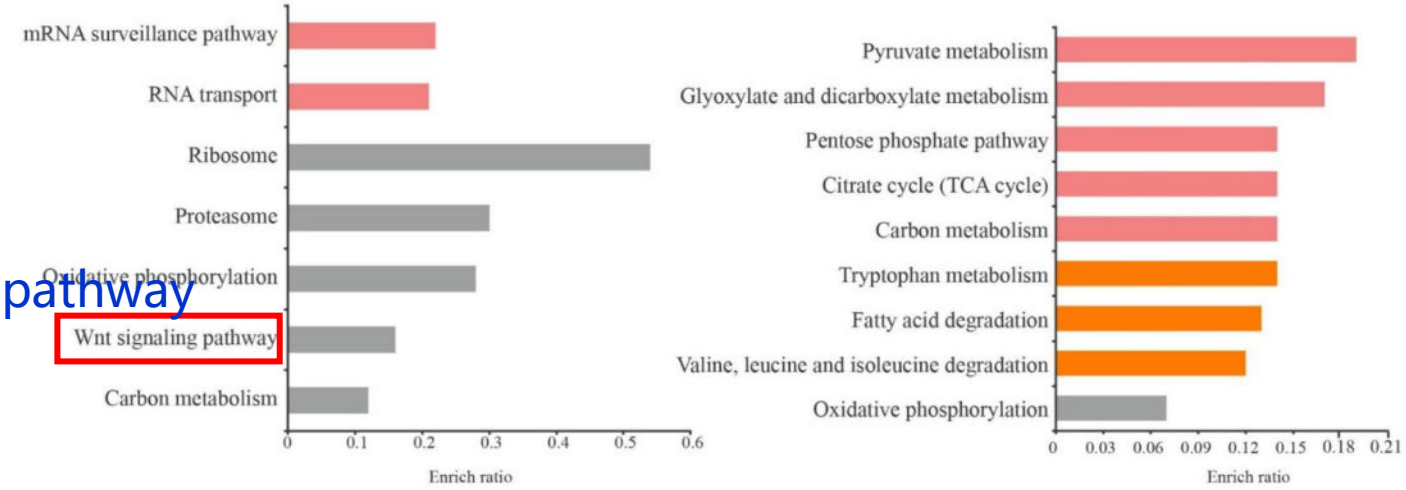
Middle stage of embryogenesis

worker



Wnt signaling pathway

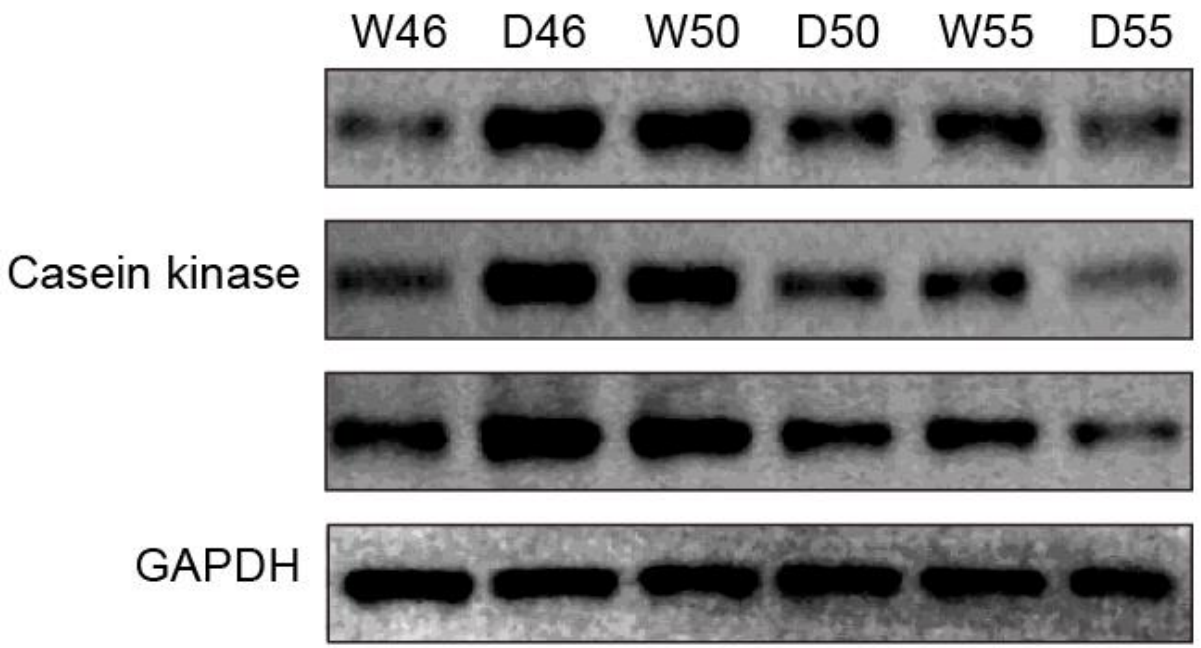
drone

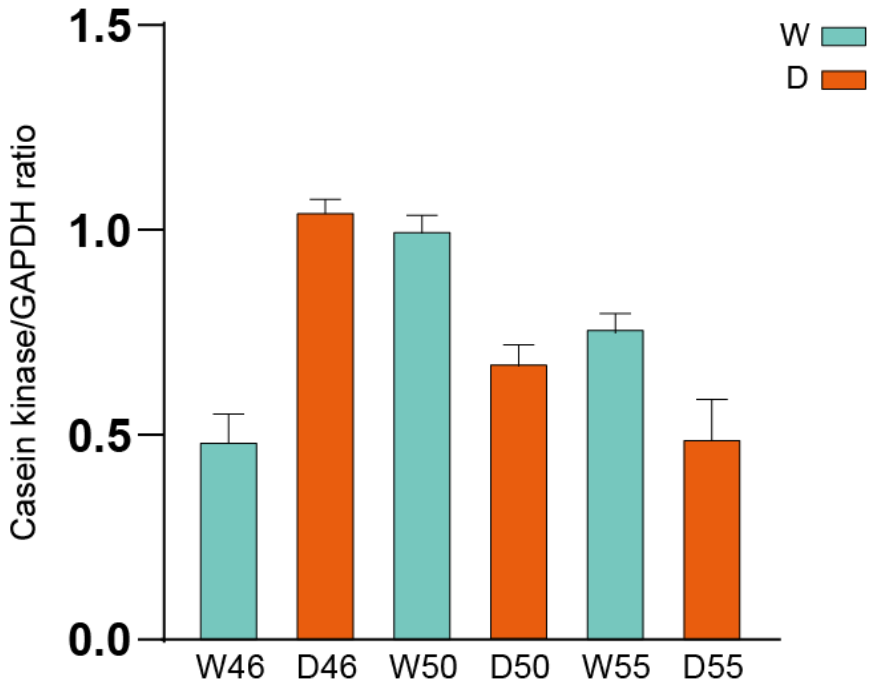


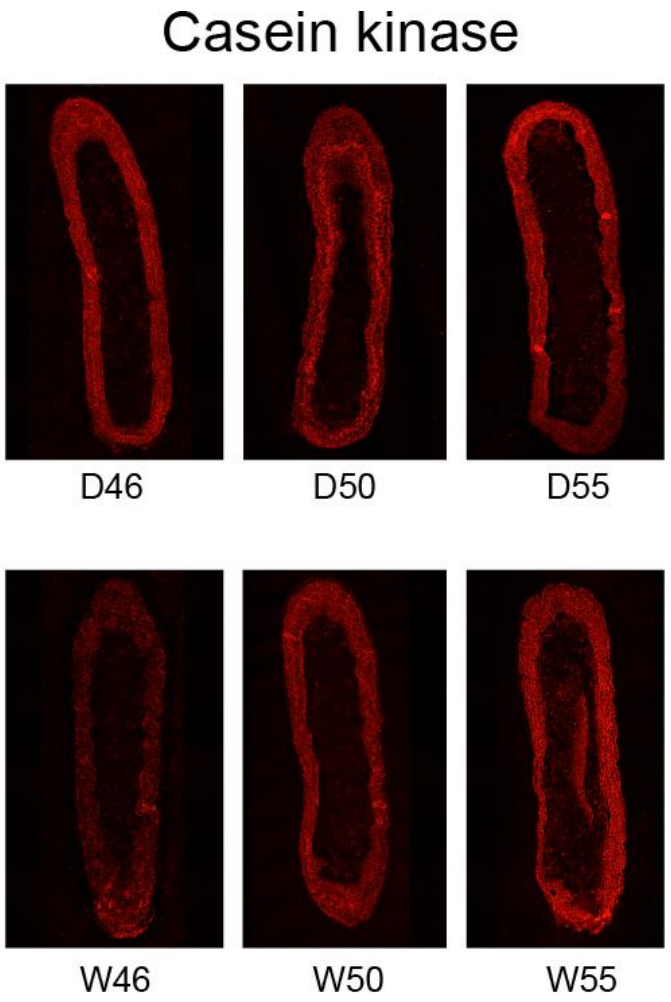
Wnt signaling pathway

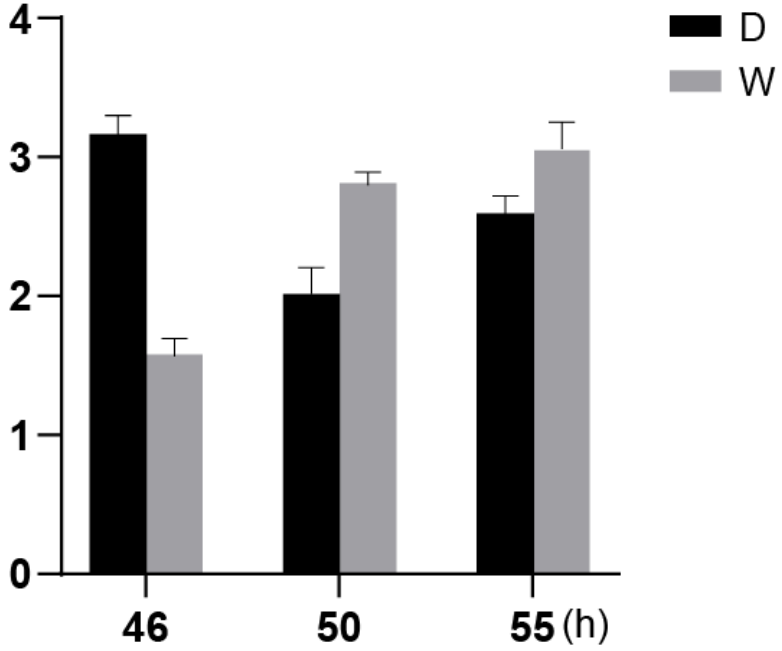
Wnt signaling pathway, involved in organ rudiment formation and tissue differentiation .

Wnt---Casein kinase





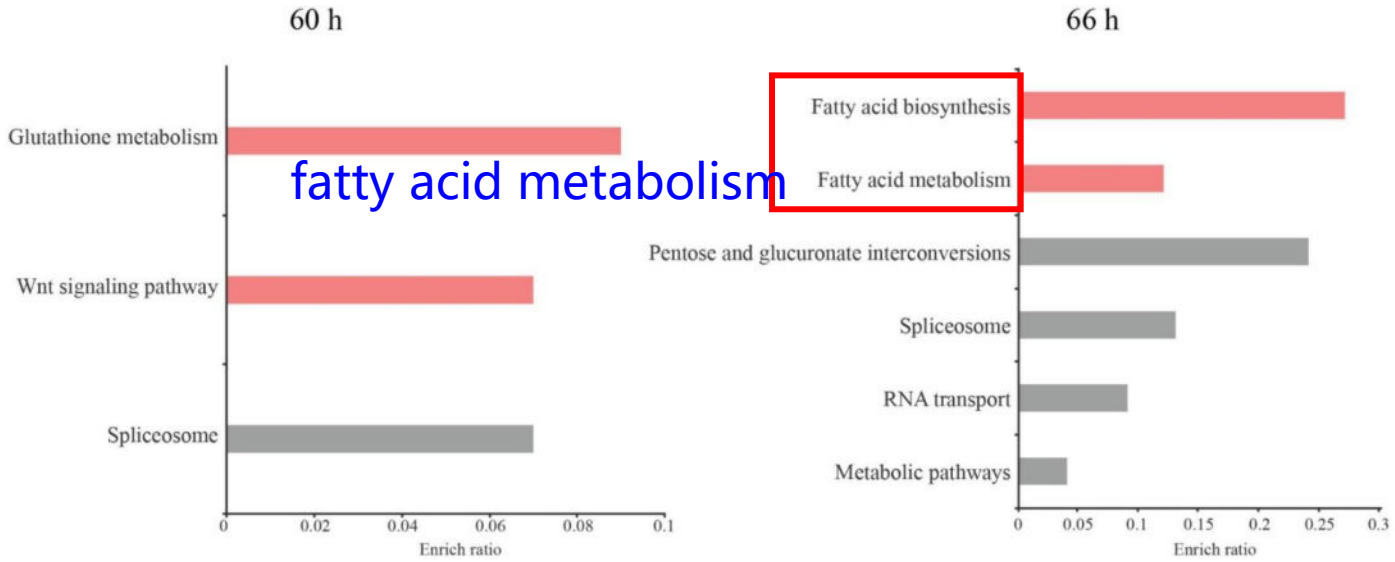




wnt signal pathway in drone embryo launch earlier than worker, means rudimentary organs of drone may form earlier than worker.

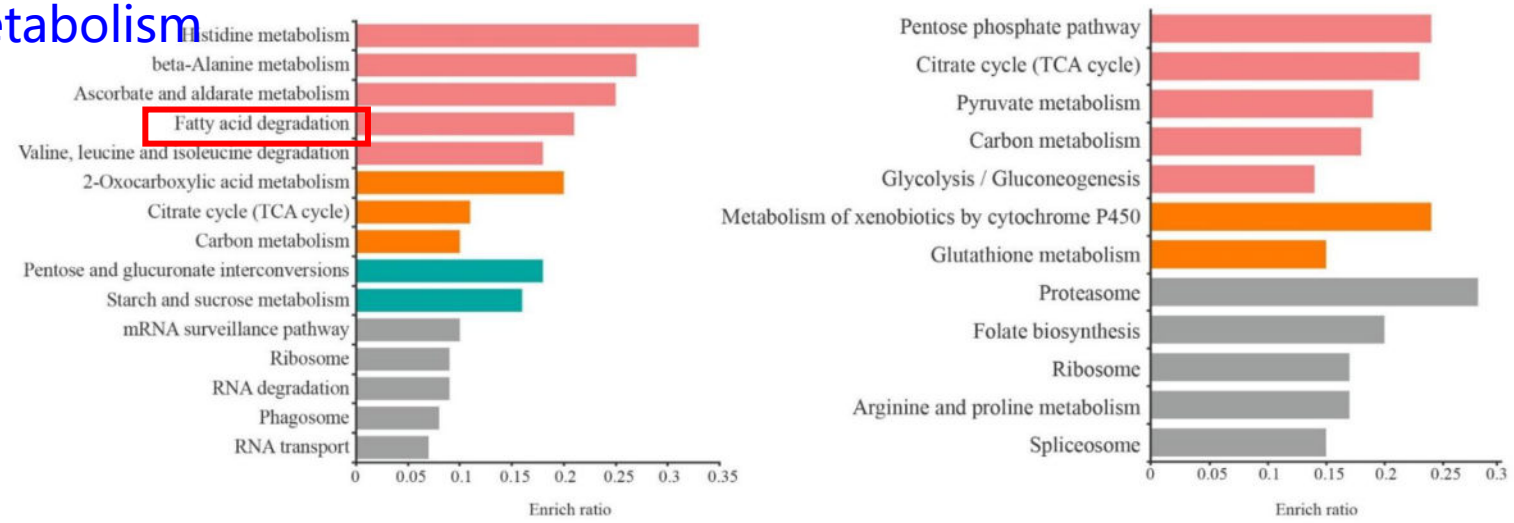
Middle-late stage of embryogenesis

worker



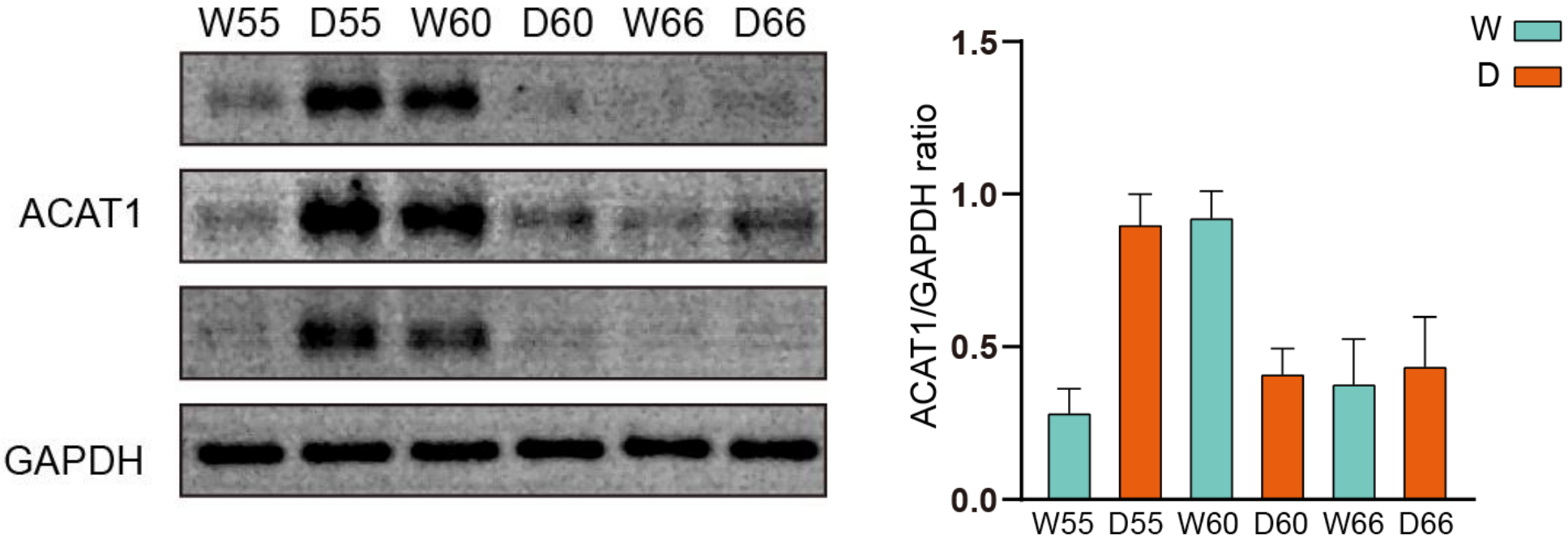
fatty acid metabolism

drone



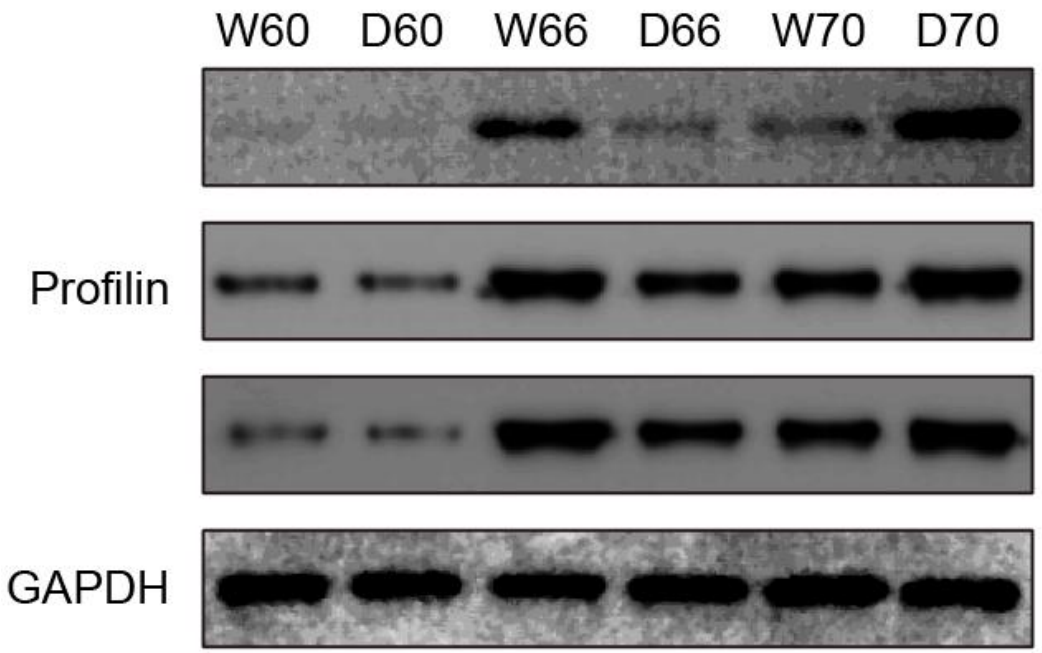
fatty acid degradation, forming a cavity structure, marking the formation of the midgut organ.

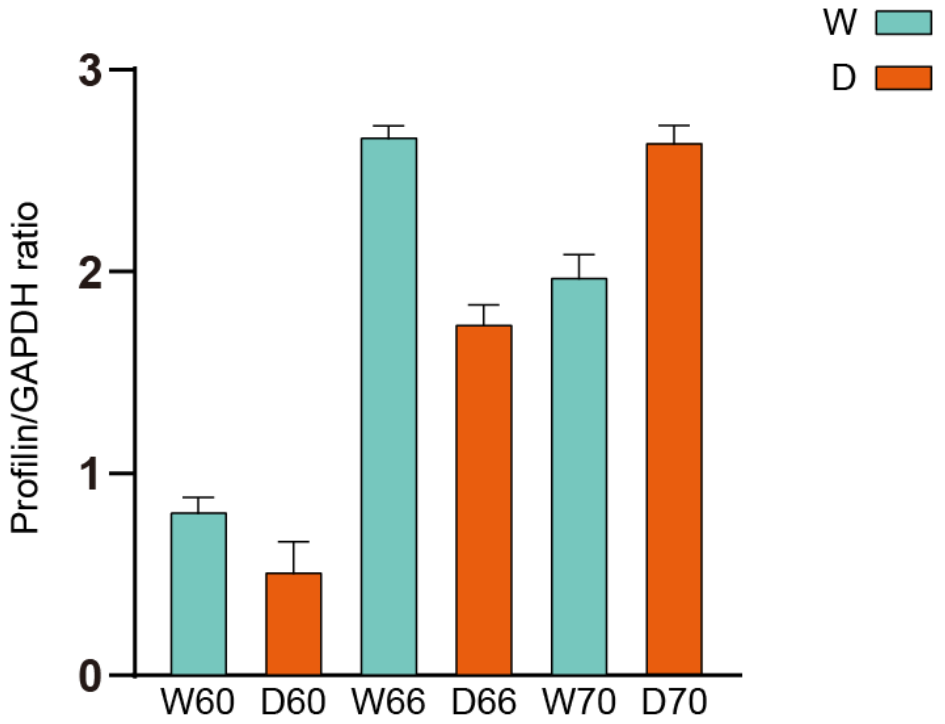
fatty acid degradation---ACAT1



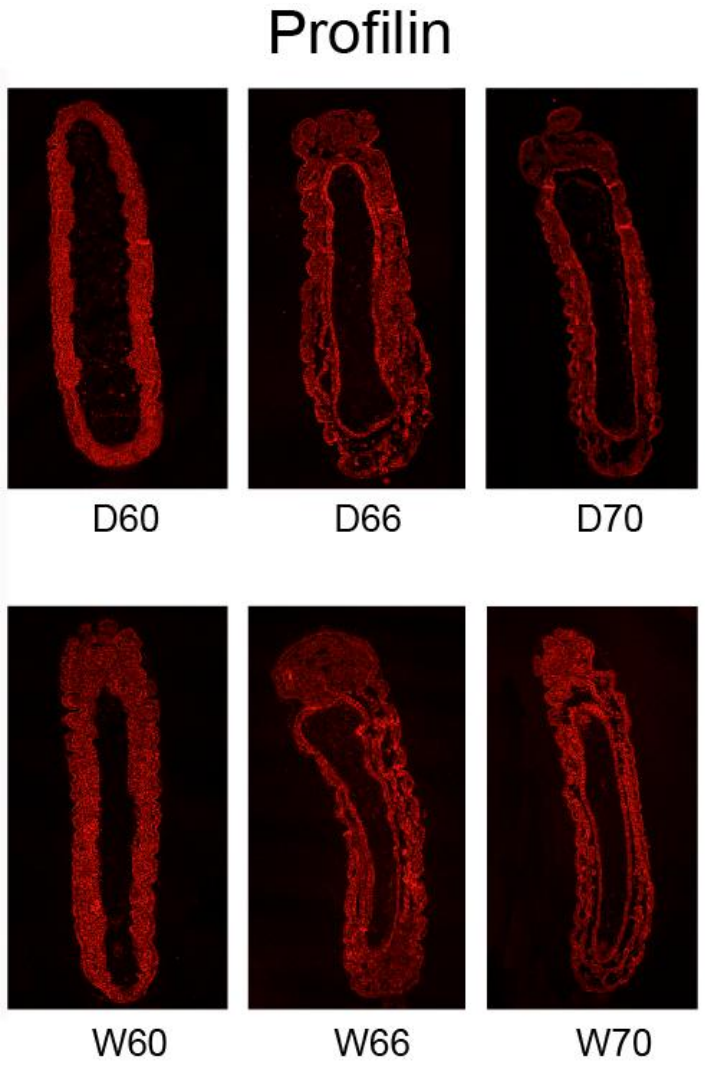
fatty acid degradation, take place earlier in drone embryo.

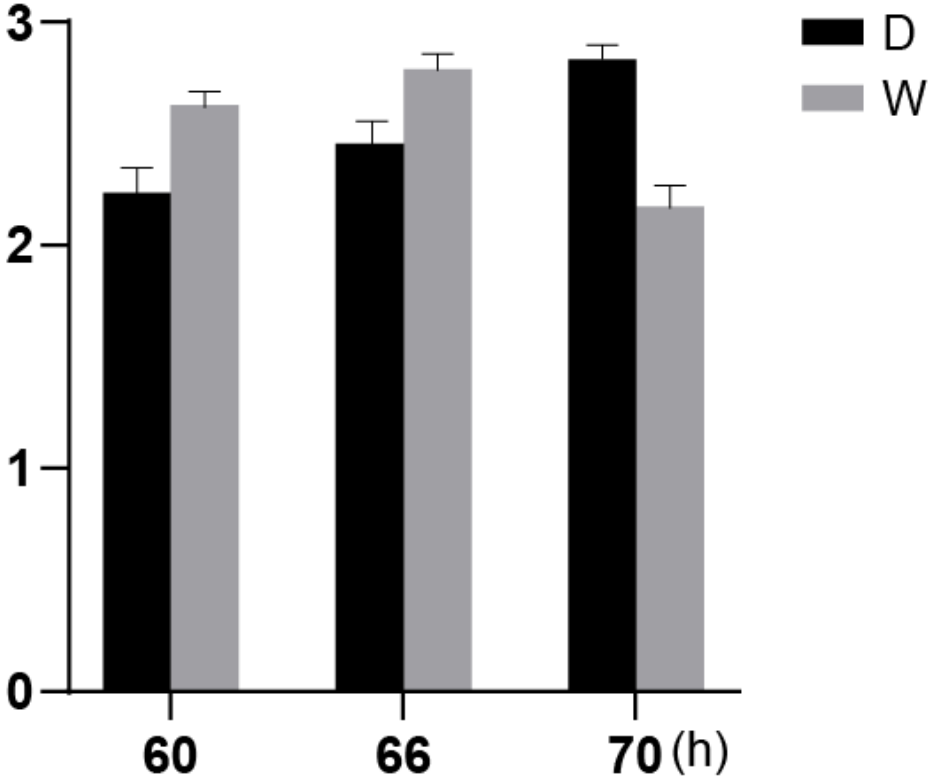
Late stage---Profilin





regulates the polymerization and depolymerization processes of actin, and is related to dorsal closure.





dorsal closure in worker embryo happen earlier than drone, indicates drone embryogenesis ended relatively late.

Conclusion

- Worker and drone embryos adopt similar strategy in organic development.
- The tissue differentiation of drone occurs earlier, and last 4-hour longer than worker.

Thank you!