

Sociogenomic studies on soldier differentiation in damp-wood termites

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Introduction

- Caste differentiation can be influenced by social interactions among individuals.
- Termite soldiers are a unique caste in terms of their morphology and role (Deligne et al. 1981) (Fig. 1).
- The evolution of the soldier caste is one of the most important events in evolution of termite eusociality (Nalepa 2011).
- Soldier differentiation requires **high juvenile hormone titers in workers** (e.g. Cornette et al. 2008) (Fig. 2).

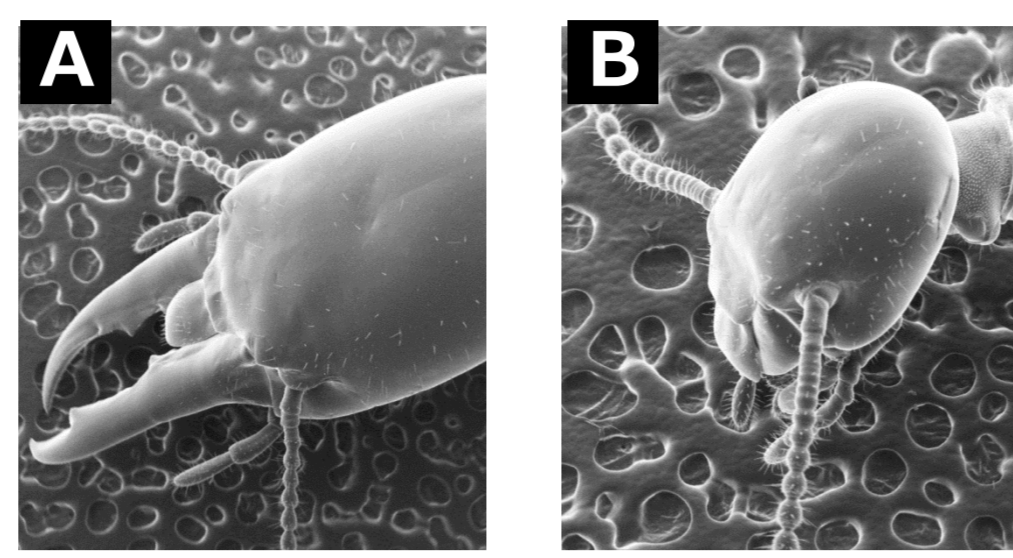
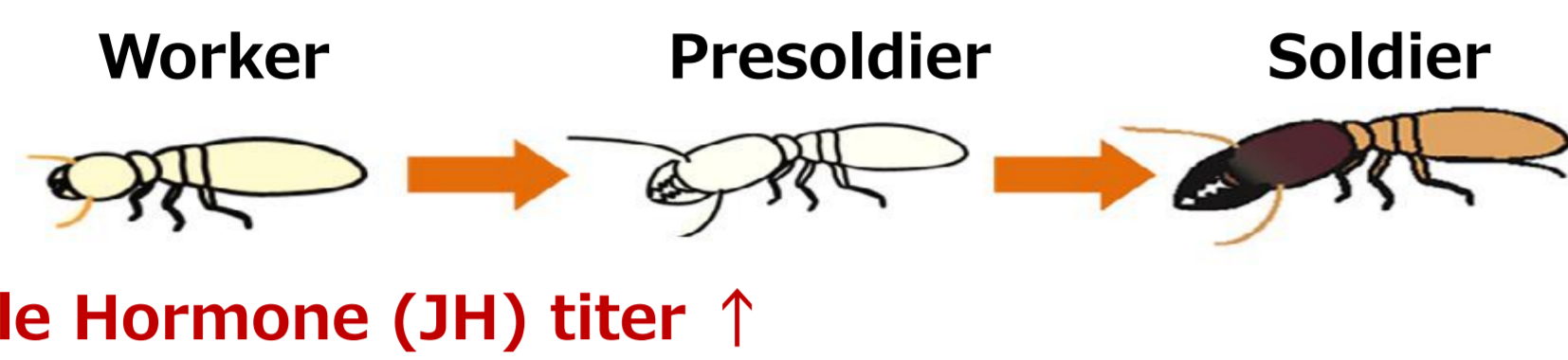
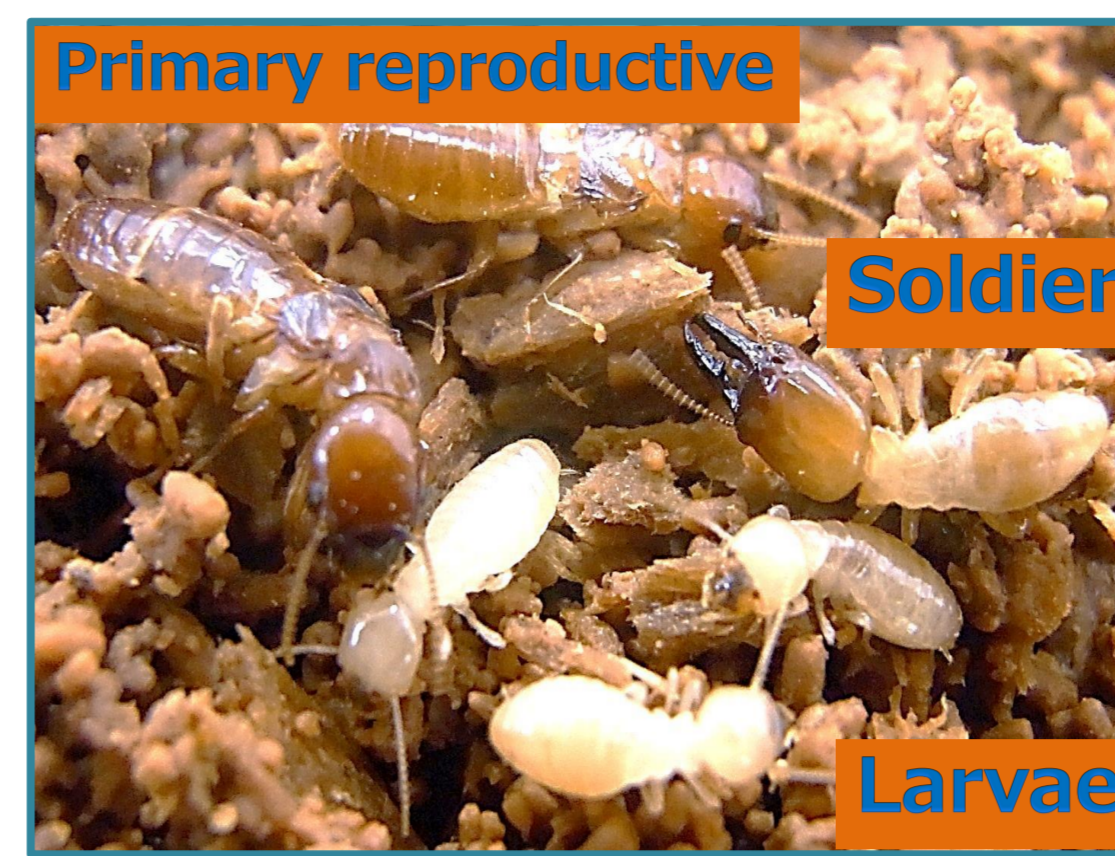
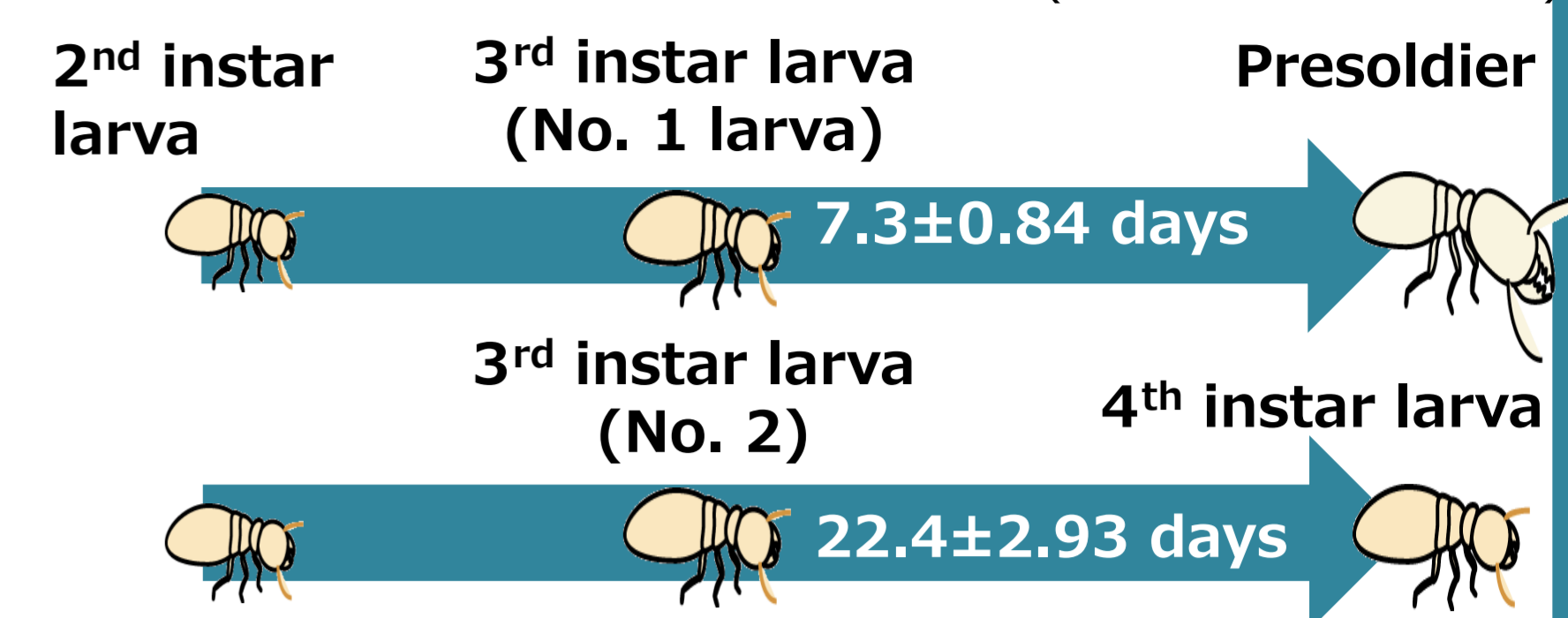
Fig. 1 Heads of soldier (A) and worker (B) of *Zootermopsis nevadensis*.

Fig. 2 Soldier differentiation requires two molts via a presoldier stage.

- We cannot identify the individual which will differentiate into a soldier in the natural condition.

⇒ **Molecular basis of soldier differentiation remains unclear. (the factor increasing JH titers?, genetic determinant of soldier differentiation?)**

Approach

Fig. 3 The incipient colony of the damp-wood termite *Zootermopsis nevadensis*.Soldier differentiation in the incipient colony of *Zootermopsis nevadensis* (Maekawa et al. 2012)

- The oldest 3rd instar larva (No. 1 larva) always differentiates into a presoldier. The next 3rd instar (No. 2) molts into 4th instar.
- When No. 1 larva is removed from the colony, No. 2 or 3 differentiates into a presoldier.
- The total frequencies of proctodeal trophallaxis from primary reproductive to No. 1 are significantly higher than those of No. 2.

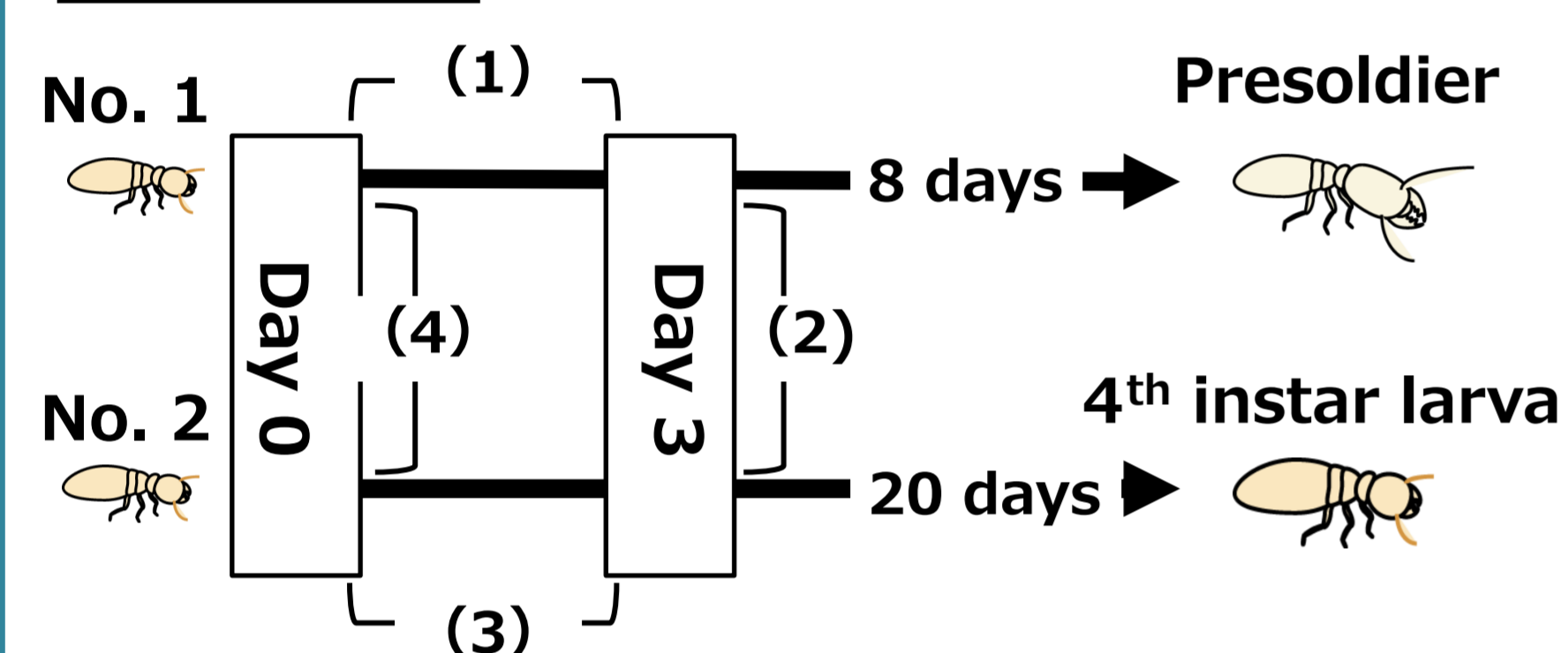
Material Incipient colonies of *Zootermopsis nevadensis*

Objective

To elucidate molecular mechanisms of soldier differentiation

Experiment ① RNA-seq analysis during soldier differentiation

Methods



1. Total RNA was extracted from No. 1 and No. 2 at day 0 and 3 after the molt.
2. cDNA synthesis and purification was performed.
3. Sequence run was performed by the next-generation sequencer (HiSeq 2000).
4. *De novo* assembly and differential expression analysis (1 - 4) were performed.

Results

We could identify 5 highly expressed genes in No. 1 larva at day 3 after the molt. Gene names indicates *Drosophila melanogaster* homologs. Numbers in parentheses show read counts per millions.

* In comparison (3) and (4), *NLaz* is no hit in top 5 genes.

- (1) No. 1 larva at day 0 and 3 after the molt

Head

1. No hit (1.29 vs 476)
2. *CG11905* (3.01 vs 750)
3. *Neural Lazarillo (NLaz)* (3.22 vs 636)
4. *neo* (1.93 vs 159)
5. *CG3108* (1.07 vs 89.0)

Thorax and Abdomen

1. *NLaz* (1.06 vs 775)
2. No hit (1.06 vs 523)
3. *CG11905* (4.87 vs 959)
4. *Sgs1* (169 vs 14082)
5. *CG10625* (2.54 vs 201)

- (2) No. 1 and No. 2 larvae at day 3 after the molt

Head

1. *CG11905* (750 vs 0.40)
2. *NLaz* (636 vs 0.67)
3. No hit (476 vs 0.67)
4. *neo* (159 vs 0.54)
5. *Cuticular protein PxutCPR146* (293 vs 1.88)

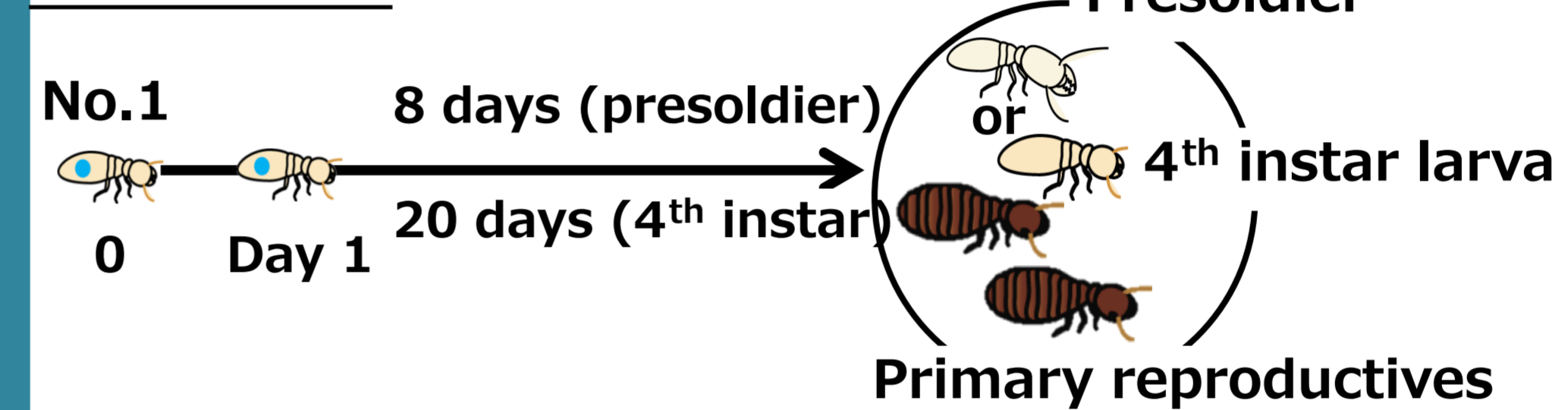
Thorax and Abdomen

1. *NLaz* (775 vs 0.75)
2. *CG11905* (959 vs 1.32)
3. No hit (523 vs 0.58)
4. *Obst-A* (831 vs 3.49)
5. *neo* (227 vs 1.00)

- *NLaz* homolog was highly and specifically expressed in No. 1 larvae.

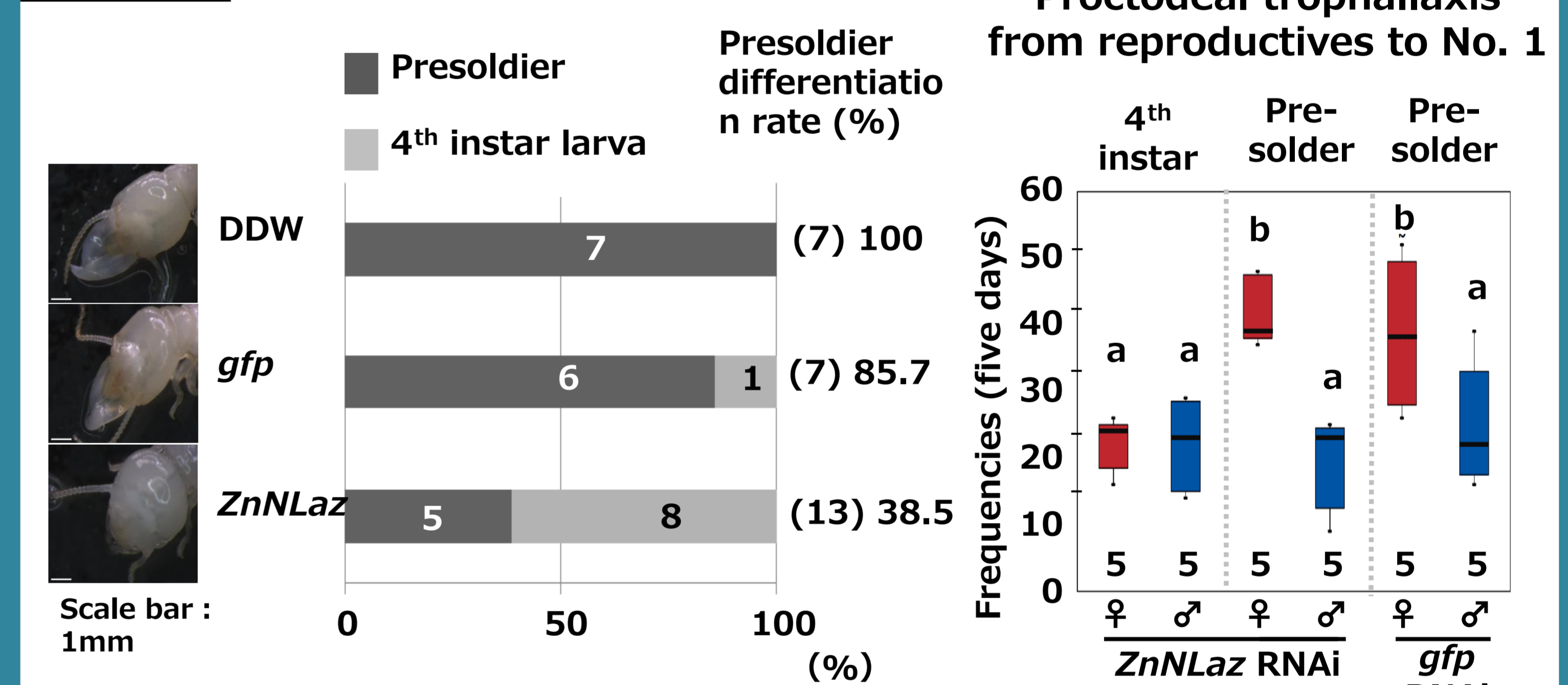
Experiment ③ Roles of a candidate gene *ZnNLaz*

Methods



1. siRNA (*ZnNLaz*, *gfp*) 5ng or DDW 32.2nL were injected into No. 1 larva at day 1 after the molt.
2. No. 1 was reared with primary reproductives in a petri dish until the molt into a presoldier or 4th instar.
3. Behavioral observation was performed (5 days, 120 min./day).

Results



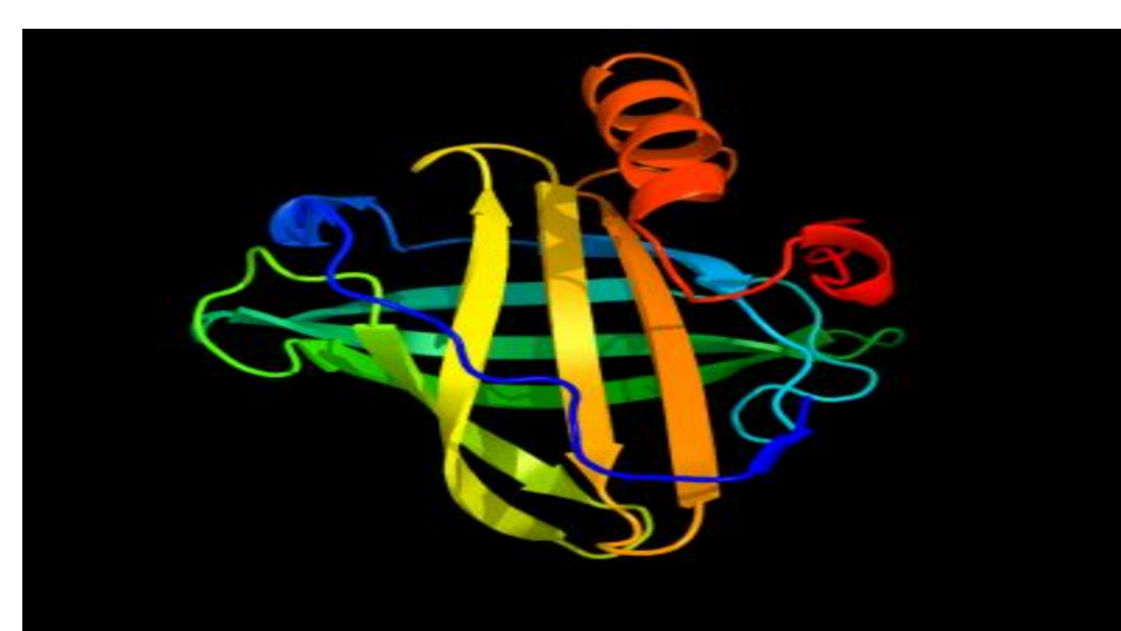
(two-way ANOVA followed by Tukey's test, $p < 0.05$)

- *ZnNLaz* RNAi in No. 1 resulted in strongly decreased presoldier differentiation rates.
- The total frequencies of proctodeal trophallaxis from female reproductive were significantly low in the case of the molt into 4th instar.

Experiment ② Amino acids sequence of *ZnNLaz* and protein structure

Results

Signal peptide
 MDWLRLLILLGSAITVTAQVPSFWGCPEKNIPMPDFDLSK
 FLGVWYEVLRVPTLMEAGASCVKNSYQTSDGKIH
 VENELKPLGQPKRVVPGELKVSFGKAGEGKITIKYKV
 PILPSWETTYHILNTDYKSYAVLWNCASYGIMNIQS
 AWLLARERKPPGTTLQRAYGVLDAYGLPRNYLVETN
 HDDCDIASEAPEVPSNPV PANATRQSSVLAITTPAKEIPSEVSDNT
 STKPTNEKLAEDKAPLESASPSTMEDVPKAPSETTQDNNAASETNVT
 SEKIKIPSDTMADIKNGKV
 Red : amino acids sequence with beta barrel structure
 Blue : alpha helix
 Underline : lipocalin 2 domain

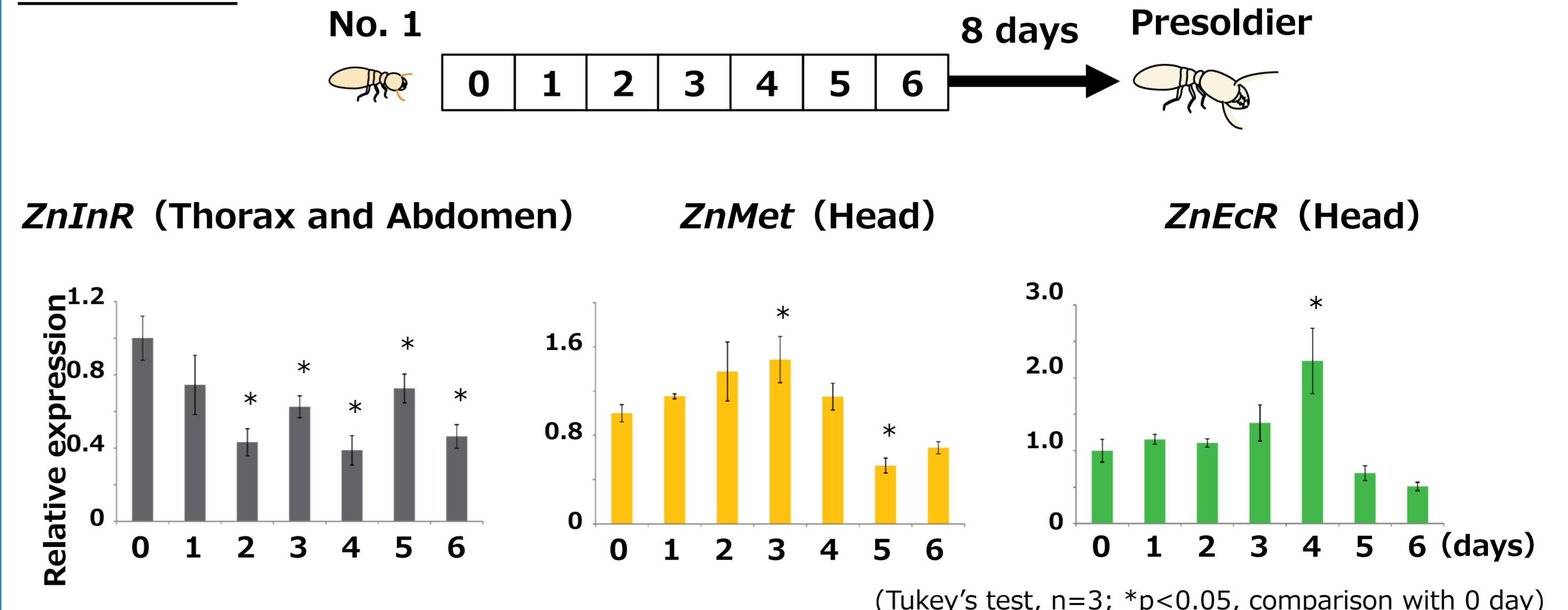


Protein structure inferred by the Phyre2 software. Completely similarity with the lipocalin family was shown.

- *ZnNLaz* protein had a conserved lipocalin 2 domain with a specific beta barrel structure.

Experiment ④ Expression analyses of hormone receptor genes

Results



(Tukey's test, $n=3$; $*p < 0.05$, comparison with 0 day)

Insulin signaling pathway :
Low level at 2-6 days.

- Insulin signal pathway could be activated.

JH · Ecdysone signaling pathway :
High level at 3, 4 day low level at 5, 6 day

- Each hormone activity could be rapidly changed.

Conclusion We identified that *ZnNLaz* was a candidate genetic determinant related to developmental fate for presoldier differentiation.

Discussion These results suggest *ZnNLaz* ① regulates presoldier differentiation.

② involves in social interactions and hormone signal pathway.