Flight muscles degenerate by programmed cell death after migration in the wheat aphid, Sitobion avenae

Honglin Feng (hf329@cornell.edu)
https://orcid.org/0000-0001-6803-3413
Xiao Guo
Chongqing Academy of Agricultural Sciences
Hongyan Sun
University of Miami
Shuai Zhang
Chinese Academy of Agriculture Sciences
Jinghui Xi
Jilin University
Jiao Yin
Chinese Academy of Agricultural Sciences
Yazhong Cao
Chinese Academy of Agricultural Sciences
Kebin Li
Chinese Academy of Agricultural Sciences Institute of Plant Protection

Research note

Keywords: aphid, Sitobion avenae, flight muscle, ubiquitin, programmed cell death

Posted Date: August 28th, 2019

DOI: https://doi.org/10.21203/rs.2.13011/v1

Version of Record: A version of this preprint was published on October 21st, 2019. See the published version at https://doi.org/10.1186/s13104-019-4708-z.

Abstract

Objective Previous studies showed that flight muscles were degenerated after migration in some aphid species; however, the underlying molecular mechanism remains virtually unknown. In this study, using the wheat aphid, Sitobion avenae , we aim to investigate aphid flight muscle degeneration and the underlying molecular mechanism.

Results Wheat aphid starts to differentiate winged or wingless lines at the second instar nymph, determined at the third instar, and then fully developed at the fourth instar. After migration, the flight muscles degenerated via programmed cell death, which is evidenced by a Terminal deoxynucleotidyl transferase dUTP-biotin nick end labeling assay. Then, we identified a list of differentially expressed genes before and after tethered flights using differential-display reverse transcription-PCR. One of the differentially expressed gene, ubiquitin-ribosomal S27a, was confirmed using qPCR. Ubiquitin-ribosomal S27a is drastically up regulated following aphids' migration and before the flight muscle degeneration. Our data suggested that aphid flight muscles degenerate after migration, during which endogenous proteins may be degraded to reallocate energy for reproduction.

Introduction

Many aphids develop wing polyphenism and winged aphids can explore new habitats by migration (1). After winged aphids migrated, the indirect flight muscles (IFM) degenerate after settling on new host plants and the onset of larvipostion (2–6).

IFM degeneration is previously depicted as a regulated physiological process (7, 8) in insects including the vetch aphid, *Megoura viciae (*4) and the pea aphid, *Acyrthosiphon pisum (*5). The regulation of IFM degeneration involves multiple factors: (i) juvenile hormone (JH), JH treatment induces IFM degeneration (8–10). (ii) neural factor, denervated muscles in the presence of JH initiated degeneration earlier than the innervated muscles in cricket (11). (iii) specific protein, several specific proteins were induced despite that the overall protein synthesis decreases during *A. pisum* IFM degeneration (8). For example, ubiquitin, a marker for programmed cell death (PCD), accumulates when aphids undergone extensive IFM degeneration (7, 8). Although IFM degeneration has been suggested as an active PCD, limited is the direct evidence and unclear is the underlying molecular mechanism.

The wheat aphid, *Sitobion avenae,* is a major grain crop pest. In China, *S. avenae* migrates from southern to northern China (12) and causes ~10% wheat yield losses every year (13). Here using *S. avenae,* we investigated aphid wing development and PCD during IFM degeneration. Then we identified genes involved in IFM degeneration by comparing gene expression pre- and post-migration. One differentially expressed gene, ubiquitin-ribosomal S27a (*RPS27a)* was analyzed for its expression during IFM degeneration.

Methods

Aphids

We generated a *S. avenae* population with identical genetic background using a single wingless aphid. Aphids were raised on wheat seedlings >30 generations of parthenogenesis at 22°C with a 16h light/8h dark photoperiod.

We obtained the winged aphids by manipulating aphid densities. Under low-density, one wingless aphid was reared on a joint-stage wheat to maintain the wingless morph. Under high-density, 80 wingless adult aphids were reared on a single ripe wheat to induce the winged morph (6).

To investigate flight muscle development and degeneration, we collected the winged aphids every 24h from eclosion (0 day) to migration (5^{th} day), to reproduction (8^{th} day) until death observed. For each timepoint, half of the aphids were kept in petri dishes with moist filter paper for morphological, histological, and apoptosis examinations. The other half of aphids were treated following a freeze-drying procedure (14), then dissected for qPCR analyses.

Morphological and histological examination

The external morphology of aphid thorax was observed using a scanning electron microscope (SEM). Aphids were fixed in 3% glutaraldehyde for 24 hrs and transferred to 1% osmic acid. Then aphids were saturated with ethanol, exchanged using isopentyl acetate, and dried in a Hitachi CO_2 Critical Point Dryer system. Aphids were then coated with gold (Au) in a sputter coater (Hitachi, IB–5) and imaged under a Hitachi S–570 SEM.

The inner morphology of aphid thorax using histological staining. Aphids were fixed in 4% paraformaldehyde for 4 hrs. The specimens were dehydrated in a serial of ethanol solution (70%, 80%, 90%, 100%, 10 mins/each), cleared in xylene, and embedded in paraffin. Serial sections (5μm) were cut and stained with hematoxylin and eosin for imaging (Fig S1).

Terminal deoxynucleotidyl transferase dUTP-biotin nick end labeling (TUNEL) assay

To examine apoptosis, we performed a TUNEL assay using an *in situ* apoptosis detection kit (Boster, China). Briefly, paraffin-embedded aphids were sliced into 5μm serial sections, which were rehydrated in xylene 20 mins and a serial of ethanol solutions (100%, 90%, 80%, 70%, 10 mins/each). Then all specimens were permeabilized using Proteinase K (1:200) for 10 mins and quenched using 3% H_2O_2 for 10 mins to inactivate the endogenous peroxidases. Quenched specimen were labeled with TdT Labeling Reaction Mix (TdT:DIG-dUTP:Buffer = 1:1:18) for 2 hrs at 25°C. After a wash with 0.01M TBS buffer, specimens were incubated with anti-DIG antibody (1:100) for 30 mins, followed by an incubation with

SABC (1:100) for 30 mins and Diaminobenzidine for 15 mins, then counterstained with Hematoxylin for 3 mins. Finally, specimens were washed with 100% ethanol, 100% xylene, and mounted for imaging.

Using TUNEL, we also examined the IFM degeneration on fasted aphid. For fasting, newly eclosed winged aphids were transferred to water-soaked sponges. Every 6 hrs, aphids were transferred back to rearing plants for 2 hrs to avoid death. In parallel, aphids reared on plants were collected as a control.

Differential-display reverse transcription-PCR (DDRT-PCR)

To identify genes differentially expressed pre- and post-migration, we performed tethered flight using 3–4 days post-eclosion winged aphids. For pre-migration, aphids were tethered (not flighted) and flash-frozen. For post-migration, aphids were tethered and flighted for 24 hrs. Aphid flight were monitored using a flight-mill program (15) and flighted aphids were collected for analyses.

For DDRT-PCR, we designed three one-base anchored oligo-dT 3’ primers and eight arbitrary 5’ primers (13-mers) designed according to the RNAimage DD Kits (GenHunter) (Table S1). We performed a PCR with each anchor and arbitrary primer pair (3 x 8 pairs) using cDNAs generated from RNAs that extracted from single aphid. The PCR products were visualized using 6% SDS polyacrylamide gel electrophoresis (Fig S2). Differentially expressed genes were excised from the gel and purified for sequencing. Obtained sequences were blast annotated on NCBI.

The dynamic expression of RPS27a

The sequence of *RPS27a* was obtained using a rapid amplification of cDNA ends method (3’-RACE). Partial *RPS27a* sequence containing the start codon was amplified using UbS/UbA primers (Table S2) designed based on *A. pisum RPS27a.* The complete C-terminal of *RPS27a* was obtained using a RACE kit (Takara, China) with gene specific primers (GSP1/GSP2) and the kit-provided outer/inner primers (Table S2). To check the conservation of *RPS27a,* the nucleotide and deduced amino acid sequences were aligned with homologous from other insects using DNAMAN (Fig S3).

We quantified the *RPS27a* expression during flight muscle development and degeneration using qPCR in tissues including head, thorax, and abdomen. To calculate gene expression, a credible standard curve was constructed using a series of 10x dilution of a standard sample. The experiment was repeated three times and each experiment includes 3–4 technical replicates for each sample.

Results

Flight muscle development in S. avenae

The external and internal structure in the winged and wingless aphids at the 1st instar was similar (Fig S1A, a). Consistent to *A. pisum* (6), wing primordia were observed in the internal structures of nymphs in

both morphs (Fig S1a). From the 2nd instar, wind primordia developed and enlarged in the winged morph (Fig S1b'), whereas disappeared in the wingless morph (Fig S1b). At the 3rd instar, swollen structures appeared in the winged morph (Fig S1C, C'), which later developed into wing bud. Flight muscles fibers also differentiated (Fig S1c'); the corresponding area was occupied by fat bodies in the wingless morph hereafter (Fig S1c). To the 4th instar, wing buds enlarged into a plate shape; the wing epithelia were folded in a complicated structure, differentiated between the forewings and hindwings (Fig S1d'). Wing hair sensilla were also seen in the winged morph (Fig S1E, F). To adults, the wings were fully developed (Fig S1E, F).

IFM degeneration after S. avenae migration

Histological examination showed that aphid flight muscle degenerate after migration. The flight muscle of an alatae adult was plump at the 1st day after eclosion till the 5th day (Fig 1A, 1B). From the 9th day, the flight muscle started to degenerate. The myofibrils appear to be thin. The diameter of the myofibrils was reduced, and the volume of the interfibrillar sarcoplasmic region increased (Fig 1C); and the degradation continues through the 11th day (Fig 1D). To the 13th day, the flight muscle was degenerated completely (Fig 1E). The contractile fiber vanished, and the intact flight muscle was not visible.

IFM degeneration is a PCD process

IFM degeneration after migration can be attributed to PCD. In sections of aphid pterothorax stained in the TUNEL experiment, no apoptotic signals were observed in alatae aphid before 6th day after eclosion (Fig 2A, B). The first apoptotic signal appeared in aphids at the 7th day as brownish yellow grains (Fig 2C), which is similar to the apoptosis signals in the positive controls. In parallel, we did not find any apoptotic signals in the muscles of the 1st, 3rd, and 7th day fasted aphids (Fig 2D, E, F).

Differentially expressed genes in aphid thorax pre- and post-migration

We identified 36 differentially expressed genes that were annotated and classified into three groups: 1) genes related to apoptosis, including the apoptosis marker, *RPS27a;* 2) genes related to metabolism, including energy re-allocation genes; and 3) genes with unknown functions (Table S3).

S. avenae *RPS27a* and its dynamic expression

The *S. avenae RPS27a* encodes a 150 aa protein (76 aa ubiquitin monomer + 74 aa ribosomal protein). The *RPS27a* are highly conserved across different insect species (84–93.3%) with the highest identity to *A. pisum* (*i.e.* 93.3%) (Fig S3). The divergence of RPS27a was mainly from the N-terminal ribosomal protein, whereas the ubiquitin monomers were almost identical (Fig S3).

The expression of *S. avenae RPS27a* change dynamically pre- and post- IFM degeneration. Initially after eclosion, *RPS27a* remains a constant low expression in all head, thorax and abdomen tissues (Fig 3). The expression started raising from the 3rd day post-eclosion. *RPS27a* expression significantly increased and is significant higher in abdomen than head and thorax in the 6th day, which is one day post-migration and one day before we detected the apoptotic signals (Fig 2C). Right after migration, a sharp decrease occurred in the 7th day and the apoptosis signals were detected (Fig 3).

Discussion

IFM degeneration has been mostly studied physiologically in insects such as fruit fly (16), crickets (17–20), bugs (21, 22), and some aphid species (7). Here, we depicted the flight muscle development and degeneration in *S. avenae* and identified some genes that may participate in the degeneration process.

IFM degeneration for energy re-allocation

A trade-off has been proposed between IFM degeneration and reproduction with regard to energy allocation (23). IFM degeneration is regulated throughout development in many insects (21–25), and the products of IFM degeneration are considered to be involved in insect reproduction (26, 27). Here, we identified two groups of genes that differentially expressed pre- and post- aphid migration, including genes relative to cell apoptosis and genes involved in metabolism (Table S3). We hypothesize that aphid IFM degeneration is an active process that first degrades flight muscles by triggering cell apoptosis after migration, which provides energy resources for oogenesis and reproduction by accelerating metabolism (5, 26, 28, 29).

Ubiquitin function as genetic marker for aphid IFM degeneration

Ubiquitin degrades proteins in eukaryotic cells. During eukaryotic cell apoptosis, many short-lived proteins are subjected to ubiquitination and triggers different degenerative processes (30, 31). For example, RPS27a can regulate cell apoptosis by mediating P53 (an apoptosis gene) via inhibiting E3 ubiquitin-protein ligase Mdm2 (32). Kobayashi found that ubiquitin is one of the regulators of flight muscles degeneration in *A. pisum (*8). The increased activity of the ubiquitin indicates a preferential role for apoptosis in turn decreases muscle function.

Here, we found the *S. avenae RPS27a* was significantly differentially expressed pre- and post-migration. *RPS27a* showed a sharp increase before migration and a sudden decrease after migration, which indicated that aphids have been prepared for IFM degeneration. During IFM degeneration, RPS27a may be a trigger or recognized as a signal for other genes in the cell apoptosis pathway to regulate IFM degeneration (30–32). RPS27 may also be used to degenerate the waste proteins to facilitate energy translocation and reproduction (33).

Flight muscle is crucial to migratory insects. The timing of migration and IFM degeneration determines whether these insects can locate preferable plant hosts or better habitats (34). To illustrate the mechanism of IFM degeneration will not only advance our understanding of insects' migratory behavior, but also shed light on the regulation of energy allocation from migration to reproduction.

Limitations

(1) Aphids flighted less distance in the tethered flight. In nature, the timing of aphid migration and IFM degeneration may be different.

(2) The differentially expressed genes pre-/post aphid migration can be further explored using next generation sequencing.

Abbreviations

RPS27a: ubiquitin-ribosomal S27a; IFM: Indirect flight muscles; JH: Juvenile hormone;

TUNEL: Terminal deoxynucleotidyl transferase dUTP-biotin nick end labeling assay;

DDRT-PCR: Differential-display reverse transcription-PCR; SEM: Scanning electron microscope; PCD: Programmed cell death; RACE: Rapid amplification of cDNA ends.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Key R&D Program of China (Grant No. 2017YFD0201700), National Special Fund for Scientific Research on Public Causes (Grant No.200803002) and the Public Welfare Project from Ministry of Agriculture of the People's Republic of China (Grant No.201103022).

Authors' Contributions

HF, XG, KL and YC conceived of the study, HF, XG, HS, JX, and JY and designed the experiments. HF, XG, HS, and SZ performed the experiments and data analysis. HF, XG, and KL wrote the manuscript. All authors contributed to preparation of the final version of the manuscript.

References

1.Kennedy JS. Aphid migration and the spread of plant viruses. Nature. 1950;165(4208):1024–5.

2.Johnson B. Studies on the degeneration of the flight muscles of alate aphids-I:A comparative study of the occurrence of muscle breakdown in relation to reproduction in several species. Journal of Insect Physiology. 1957;1(3):248–50.

3.Johnson B. Studies on the degeneration of the flight muscles of alate aphids-II: Histology and control of muscle breakdown. Journal of Insect Physiology. 1959;3(4):367–72.

4.Johnson B. An electron microscopic study of flight muscle breakdown in an aphid *Megoura viciae.* Tissue Cell. 1980;12(3):529–38.

5.Kobayashi M, Ishikawa H. Breakdown of indirect flight muscles of alate aphids (*Acyrthosiphon-Pisum)* in relation to their flight, feeding and reproductive-behavior. Journal of Insect Physiology. 1993;39(7):549–54.

6.Ishikawa A, Miura T. Differential regulations of wing and ovarian development and heterochronic changes of embryogenesis between morphs in wing polyphenism of the vetch aphid. Evolution & Development. 2009;11(6):680–8.

7.Kobayashi M, Ishikawa H. Mechanisms of histolysis in indirect flight muscles of alate aphid (*Acyrthosiphon-Pisum)*.. Journal of Insect Physiology. 1994;40(1):33–8.

8.Kobayashi M, Ishikawa H. Involvement of juvenile-hormone and ubiquitin-dependent proteolysis in-flight muscle breakdown of alate aphid (*Acyrthosiphon-Pisum)*.. Journal of Insect Physiology. 1994;40(2):107–11.

9.Selma-Soriano E, Artero R, Llamusi B. Optical Cross-Sectional Muscle Area Determination of *Drosophila Melanogaster* Adult Indirect Flight Muscles. Journal of Visualized Experiments. 2018(133).

10.Oliver RH, Albury AN, Mousseau TA. Programmed cell death in flight muscle histolysis of the house cricket. Journal of Insect Physiology. 2007;53(1):30–9.

11.Shiga S, Yasuyama K, Okamura N, Yamaguchi T. Neural- and endocrine control of flight muscle degeneration in the adult cricket, *Gryllus bimaculatus.* Journal of Insect Physiology. 2002;48(1):15–24.

12.Zhang X, Zhou G, Shi M, Fang J, Zhao Z, Li S, et al. Studies on the long-distance migration and virus transmission by the aphid *Sitobion avenae* F. Journal of Plant Protection. 1985;12:9–16.

13.Hu XS, Liu YJ, Wang YH, Wang Z, Yu XL, Wang B, et al. Resistance of wheat accessions to the English grain aphid *Sitobion avenae.* PLoS One. 2016;11(6):e0156158.

14.Fujita SC, Inoue H, Yoshioka T, Hotta Y. Quantitative tissue-isolation from *Drosophila* freeze-dried in acetone. Biochemical Journal. 1987;243(1):97–104.

15.Cheng D, Tian Z, Li H, Sun J, Chen J. Influence of temperature and humidity on the flight capacity of *Sitobion avenae.* Acta Entomologica Sinica. 2002;45(1):80–5.

16.Piccirillo R, Demontis F, Perrimon N, Goldberg AL. Mechanisms of muscle growth and atrophy in mammals and *Drosophila.* Developmental Dynamics. 2014;243(2):201–15.

17.Zera AJ, Larsen A. The metabolic basis of life history variation: genetic and phenotypic differences in lipid reserves among life history morphs of the wing-polymorphic cricket, *Gryllus firmus.* Journal of Insect Physiology. 2001;47(10):1147–60.

18.Zhao Z, Zera AJ. A morph-specific daily cycle in the rate of JH biosynthesis underlies a morph-specific daily cycle in the hemolymph JH titer in a wing-polymorphic cricket. Journal of Insect Physiology. 2004;50(10):965–73.

19.Zhao ZW, Zera AJ. The hemolymph JH titer exhibits a large-amplitude, morph-dependent, diurnal cycle in the wing-polymorphic cricket, *Gryllus firmus.* Journal of Insect Physiology. 2004;50(1):93–102.

20.Zera AJ. Intermediary metabolism and life history trade-offs: lipid metabolism in lines of the wing-polymorphic cricket, *Gryllus firmus,* selected for flight capability vs. early age reproduction. Integrative and Comparative Biology. 2005;45(3):511–24.

21.Socha R, Sula J. Flight muscles polymorphism in a flightless bug, *Pyrrhocoris apterus* (L.): Developmental pattern, biochemical profile and endocrine control. Journal of Insect Physiology. 2006;52(3):231–9.

22.Socha R, Sula J. Differential allocation of protein resources to flight muscles and reproductive organs in the flightless wing-polymorphic bug, *Pyrrhocoris apterus (*L.) (Heteroptera). Journal of Comparative Physiology B. 2008;178(2):179–88.

23.Lorenz MW. Oogenesis-flight syndrome in crickets: age-dependent egg production, flight performance, and biochemical composition of the flight muscles in adult female *Gryllus bimaculatus.* Journal of Insect Physiology. 2007;53(8):819–32.

24.Tanaka S. Effects of wing-pad removal and corpus allatum implantation on development of wings, flight muscles, and related structures in the striped ground cricket, *Allonemobius-Fasciatus.* Physiological Entomology. 1985;10(4):453–62.

25.Tanaka S. De-alation, flight-muscle histolysis, and oocyte development in the striped ground cricket, *Allonemobius-Fasciatus.* Physiological Entomology. 1986;11(4):453–8.

26.Nair CRM, Prabhu VKK. Entry of proteins from degenerating flight muscles into oocytes in *Dysdercus-Cingulatus* (Heteroptera, Pyrrhocoridae). Journal of Insect Physiology. 1985;31(5):383–8.

27.Stjernholm F, Karlsson B. Flight muscle breakdown in the green-veined white butterfly, *Pieris napi* (Lepidoptera: Pieridae). European Journal of Entomology 2008;105(1):87–91.

28.Bhakthan NM, Borden JH, Nair KK. Fine structure of degenerating and regenerating flight muscles in a bark Beetle, *Ips-Confusus.* I. Degeneration. Journal of Cell Science. 1970;6(3):807–19.

29.Bhakthan NM, Nair KK, Borden JH. Fine structure of degenerating and regenerating flight muscles in a bark beetle, *Ips-Confusus.* II. Regeneration. Canadian Journal of Zoology. 1971;49(1):85–9.

30.Lee JC, Peter ME. Regulation of apoptosis by ubiquitination. Immunological Reviews. 2003;193(1):39–47.

31.Gupta I, Singh K, Varshney NK, Khan S. Delineating crosstalk mechanisms of the ubiquitin proteasome system that regulate apoptosis. Frontiers in Cell and Developmental Biology 2018;6.

32.Sun XX, DeVine T, Challagundla KB, Dai MS. Interplay between ribosomal protein S27a and MDM2 protein in p53 activation in response to ribosomal stress. The Journal of Biological Chemistry. 2011;286(26):22730–41.

33.Kano R, Kubota A, Nakamura Y, Watanabe S, Hasegawa A. Feline ubiquitin fusion protein genes. Veterinary Research Communications. 2001;25(8):615–22.

34.Feng H, Sun H, Yin J, Li K, Xi J, Cao Y. Progress of molecular biology researches in apoptosis of insect flight muscles. Chinese Journal of Applied Entomology. 2011;48(3):701–9.

Figures

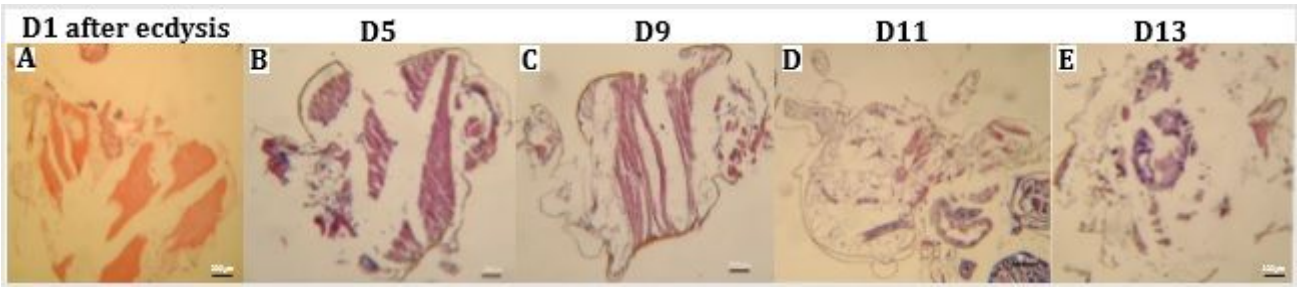

Figure 1

Breakdown of flight muscles of alatae aphids. Mesothoraces of alatae aphids at various stages were fixed, sectioned at 5 μm thickness and stained on the (A) 1st day, (B) 5th day, 9th day, 11th day, and (C) 13th day after the final ecdysis.

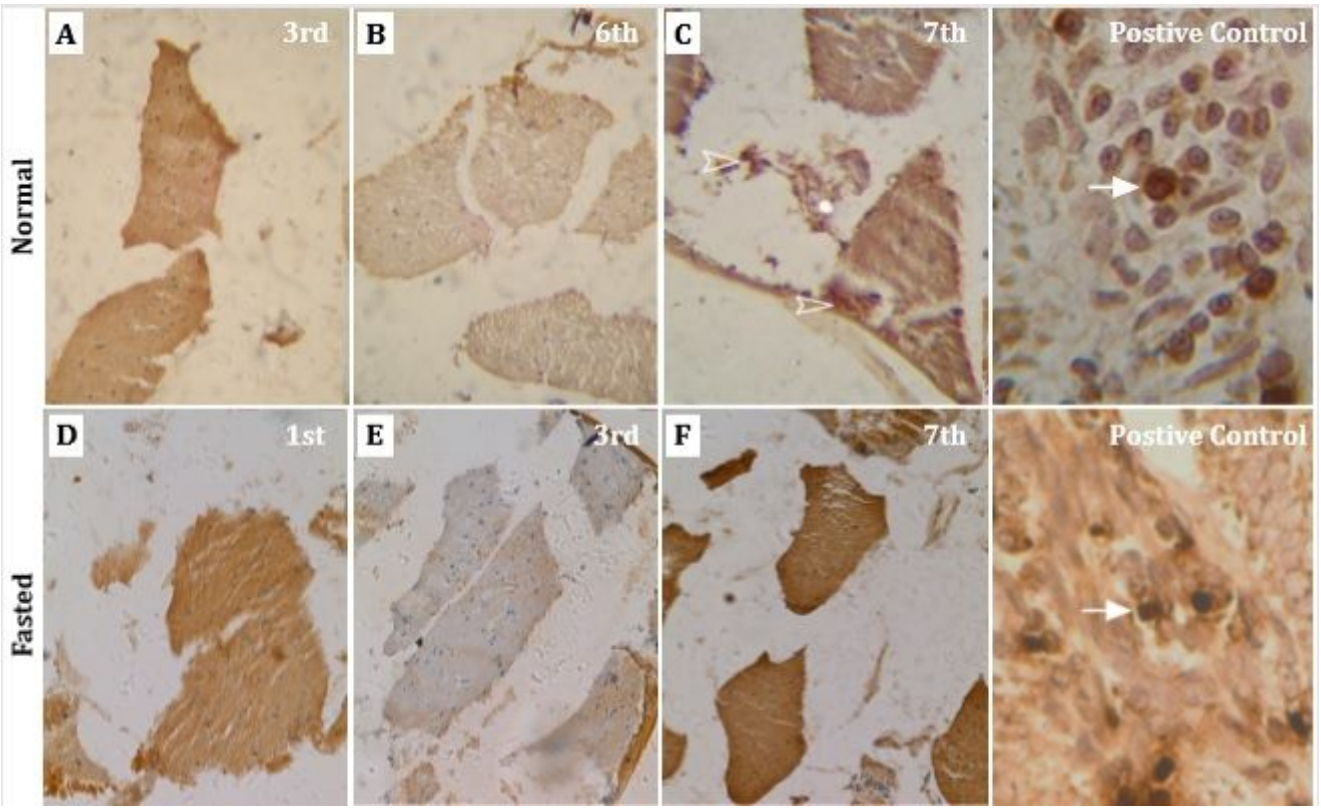

Figure 2

Cross-sections of the mesothoracic flight muscle at various stages. The apoptotic wing degeneration occurs at 7th day after the final ecdysis. All the mesothoraces were sectioned at 5 μm thickness and TUNEL stained on the (A) 3rd day, (B) 6th day, and (C) 7th day after the final ecdysis. The arrowheads indicate apoptotic cells. (D) fasted alatae 1st day, (E) fasted alatae 3rd day, (F) fasted alatae 7th day after the final ecdysis.

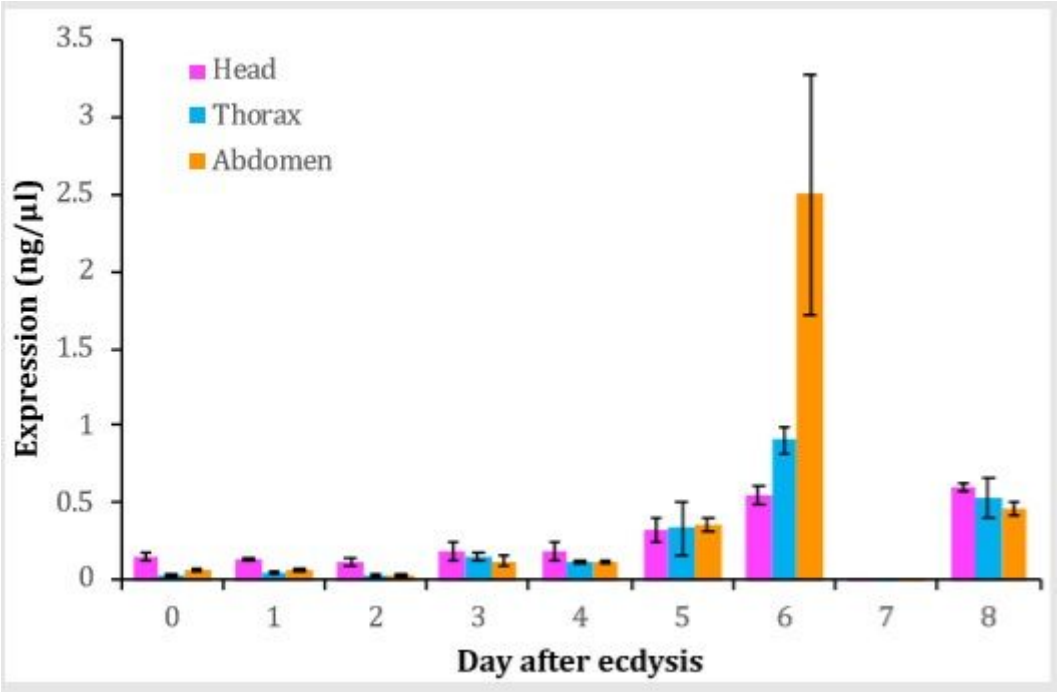

Figure 3

The dynamic expression of S. avenae ubiquitin-ribosomal S27a gene. The aphids we collected are from eclosion (0 day) to breeding finished (8th day). The expression in all three tissues always show stable and relatively lower, except a sharp increase in 5th day, with higher in abdomen than in head and thorax.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.jpg
- supplement2.jpg
- supplement3.jpg
- supplement4.pdf
- supplement5.pdf
- supplement6.pdf
- supplement7.pdf