参赛队员姓名：Ethan Yihao Li 李亦豪
Sichen Wu 吴思辰
Bernice YX Wang 王誉璇

中学： 华南师范大学附属中学

省份： 广东省

国家/地区： 中国

指导教师姓名：罗宇立 任充华

指导教师单位：华南师范大学附属中学
华南师范大学

论文题目： Bioinformatics modeling and transcriptome analysis of multiple cockroach appendage regeneration

Bioinformatics modeling and transcriptome analysis of multiple cockroach appendage regeneration

Contestants

Ethan Yihao Li, Sichen Wu, Bernice YX Wang

School

The Affiliated High School of South China Normal University

Tutors

Yuli Luo, Chonghua Ren

Location

Guangzhou 510631, China

Abstract

The variation of regenerative capacity in a given multicellular animal represents one of the most complex and intriguing questions in regenerative biology. Cockroaches are ideal models for regeneration research mostly because of their remarkable leg regeneration capacity, but little is known about the regenerative capacity of other appendages. Considering both developmental age and amputation degree, we here performed a holistic analysis of regeneration amongst six appendages in the German cockroach. Bioinformatics modeling reveals that antenna, labial palp, maxillary palp, leg, and cercus have much stronger regenerative capacities than wing pad, and both developmental age and amputation degree significantly affect regeneration capacities of all appendages. When incomplete amputation was performed at early developmental stage, microscope observation shows that the complex sensilla can be fully regenerated on the five appendages rather than wing pad. Moreover, transcriptome analysis, hierarchical clustering, and differentially expressed gene patterning together reveal a significant shift from a function-based profile to a spatially segment-related profile, indicating that a program involved in embryonical development is reactivated during multiple appendages regeneration. This study gives a comprehensive and comparable understanding on cockroach appendages regeneration, and moreover, highlights that multiple appendages are coordinately regenerated to enable the cockroach living as a complex organism.

Key words: appendage regeneration, multiple appendages, bioinformatics modeling, transcriptome analysis, cockroach

Table of Contents

Academic Integrity Statement

We hereby declare that the paper submitted is the research work and research results obtained under the guidance of our supervisors. To the best of our knowledge, the paper does not include research results that have been published or written by others, except for those specifically marked and acknowledged in the article. If there are any inaccuracies, we are willing to assume all relevant responsibilities.

Signature of the contestants: Ethan Yihao Li

Sichen Wu

Bernice YX Wang

Signature of the supervisors: Yuli Luo

Chonghua Ren

Introduction and background

Animal appendage regeneration

Regeneration is an important phenomenon that allows organisms to restore missing body parts by injury. This phenomenon was known to the ancients and described by Aristotle and Pliny, but the first scientific observation of regeneration was reported in crayfish limb by René-Antoine Ferchault de Réaumur at 1712. The regeneration can be classified into five major types, including cellular regeneration, tissue regeneration, organ regeneration, structural (appendage) regeneration, and whole-body regeneration (Figure 1) [1].

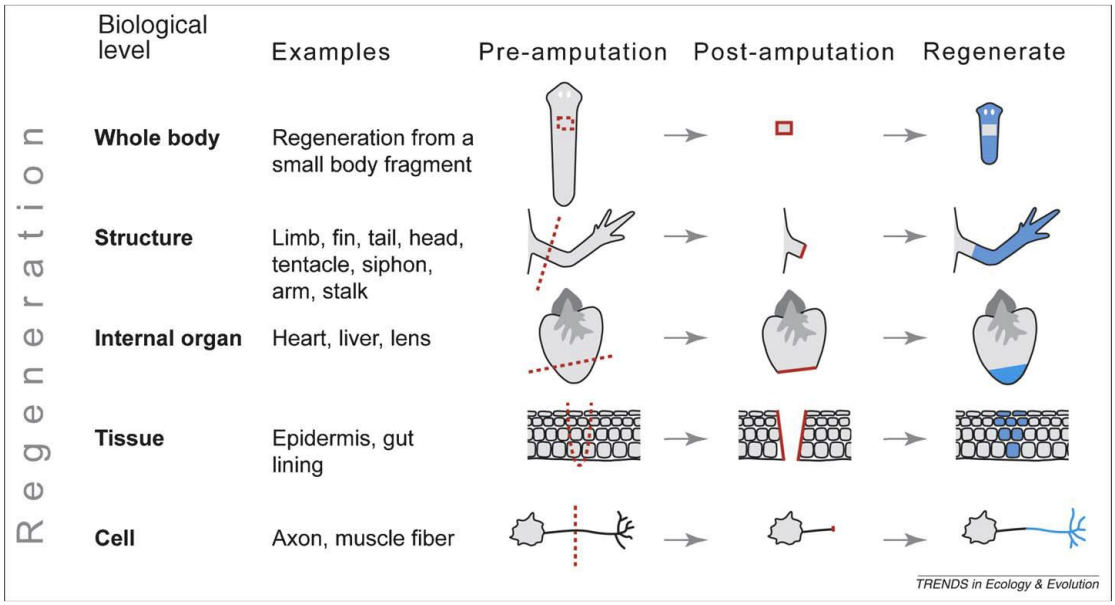

Figure 1: Regeneration at different levels of biological organization [1]

Many animals can regenerate their appendages, including arthropods, amphibians, fish, and deer (Figure 2). However, some mammals such as mice and human species have limited limb regeneration capacity [2]. The variety of regenerative abilities of animal appendages fascinated biologists for centuries [3]. Evolutionary biologists have suggested that the animal appendage regenerative capacity possess very strong phylogenetic trend, namely the regenerative capacity of animals decreases with an increase in anatomical complexity (Figure 2) [1]. Meanwhile, the regeneration capacity can also be affected by multiple factors in a particular species. For example, neonates can regenerate the fingertip but not the full limb, while adults lose all limb regeneration

[4, 5]. Moreover, the complexity of developmental age and damaged site/degree on appendage regeneration brings huge restriction in understanding the capacity of animal appendage regeneration.

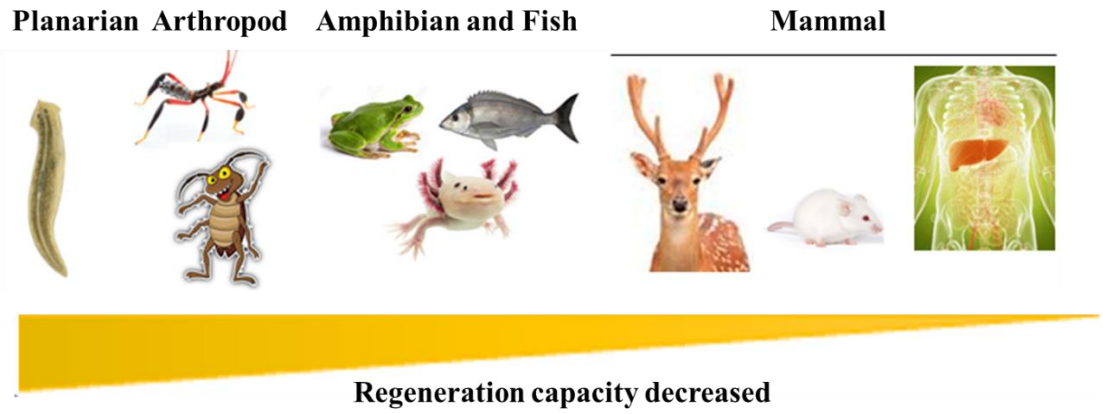

Figure 2: Animals with regeneration capacity at different levels

Multiple appendage development, regeneration and function in insects

Arthropod bodies are formed by a series of appendage-bearing segments, and the evolutionary and ecological success of insects is due in large part to the versatility of their articulated appendages [6, 7]. In general, multiple organs such as cephalic antennae and mouthparts, thoracic wings and legs, and abdominal cerci and analia can be called as appendages (Figure 3) [8]. The variation of regenerative capacity in a given multicellular animal represents one of the most complex and intriguing questions in regenerative biology. Thus, arthropods provide rich resources for comparative analysis of multiple appendage regeneration.

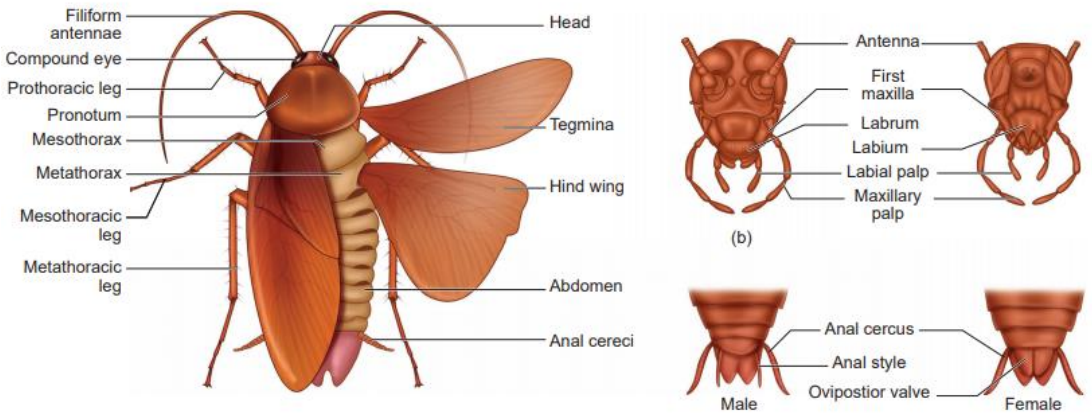

Figure 3: Cockroach appendages [8]

Insecta, as the most diverse metazoan taxon in Arthropoda, hundreds of species have been shown to be capable of appendage regeneration [11]. The hemimetabolous cockroaches have been developed into appendage regeneration model insects due to their strong regeneration ability [12]. By now, the animal appendage regeneration research in vertebrates and arthropods mainly focus on the limbs or legs [13-15], but little is known about the regeneration capacity of other appendage types. Therefore, it's worthy to clarify the key genes for postembryonic homeostatic maintenance and regeneration among different appendage types. Bioinformatics modeling based on the effects of developmental ages and damage sites/degrees on the regeneration ability of multiple cockroach appendages should be able to systematically explain how complex factors coordinate the regeneration of multiple appendages in animals.

In this study, using the German cockroach, *Blattella germanica*, as an insect model of regenerative biology, the regeneration capacity and microanatomy restoration of all six appendages were explored. Bioinformatics modeling suggests that both developmental age and amputation degree play important roles on controlling regeneration capacities of multiple cockroach appendages, showing that all the other five appendages have strong regenerative capacity except the wing pad. Using tissue-specific RNA-seq, the hierarchical relationships among different appendages under homeostatic and regenerative conditions were clarified. We found a significant shift from a function-based profile to a spatially segment-related profile, and the regeneration-associated different expressed genes (DEGs) contribute to this shift.

Materials and Methods

Animal culture

The strain of *B. germanica* used in this study was originally collected from downtown Shanghai in the 1970s and is a well-established laboratory strain bred for 40 years without exposure to insecticides. To maintain the colony, cockroaches were reared in

plastic jars at 28 ℃ and 70% relative humidity in the dark. They were provided with rat chow and water.

Appendage amputation and regeneration ability statistics

Freshly emerged N3-N6 nymphal and adult cockroaches were separated from the colony after moulting. Six kinds of appendages labial palps (l. palps), maxillary palps (m. palps), antenna, wingpad, leg, and cercus were performed for amputation. As bilateral animal, one side of the appendages were amputated and the contralateral ones served as internal controls for analyzing the regeneration ability. The l.palps were amputated at three different regions, named LP1-LP3; the m.palps were amputated at five different regions, named MP1-MP3; the antennas were amputated at four different regions, named A1-A4; either the forewings or hindwings were amputated at two different regions, named Fw1-Fw2 and Hw1-Hw2, respectively; the hindlegs were amputated at five different regions, named L-Co, L-Tr, L-Fe, L-Ti, and L-Ta; the cerci were amputated at two different regions, named C1 and C2.

After each molting, the length of regenerated and contralateral l.palps, m.palps, antennas, legs, and cerci were measured; while the area of nymphal wingpads or adult wings were measured. The regeneration abilities were calculated by dividing the length or area of the regenerated appendages by the contralateral ones. At least ten animals were treated in each group, and the statistics were carried out on average values.

After that, bubble chart drawn using the geom_point function of R package ggplot2 were used to describe the regeneration ability of different appendages after limb amputation at different ages and in different regions. The scatterplot is drawn using the geom_point function of R package ggplot2 to visualize the regeneration process of different appendages after amputation at different ages. A geno_smooth function was adopted for fitting remain proportion and regenerative ratio, as well as amputation instars and regenerative ratio using ggplot2 package in R. A scatter3d function of car package in R was applied to draw three-dimensional scatterplot about the remain proportion, amputation instars and regenerative ratio, nonparametric regression is used to fit regression surfaces with different appendages.

Scanning electron microscopy

Appendages were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in cacodylate buffer (0.2 M) for at least 2 h. After rinsing twice with the same buffer, the samples were treated with 1% osmium tetroxide (Ted Pella) at 4 °C for 1 h. The tissues were dehydrated with increasing concentrations of ethanol at 15 min intervals. Finally, the samples were subjected to critical-point drying to complete the dehydration process. The samples were attached to stubs with double-stick tape, coated with gold-palladium in a sputter coating apparatus and then observed via scanning electron microscopy (SEM) at 5 kV (JEOL JSM-6360LV). The types and microstructure of the bristles and other sensilla were observed.

RNA-Seq and data analysis

The N4 instar cockroaches were selected for RNA sequencing (RNA-Seq) experiment. The treatments LP2, MP4, A2, Fw2, L-Fe, and C2 for l.palps, m.palps, antennas, wingpads, legs, and cerci were performed, respectively. To detect the regenerative gene expression, the tissues around the amputation site (AM groups) were harvested for RNA extraction; while the contralateral unamputated wildtype tissues (WT groups) were harvested to detect homeostatic gene expression (Figure 4).

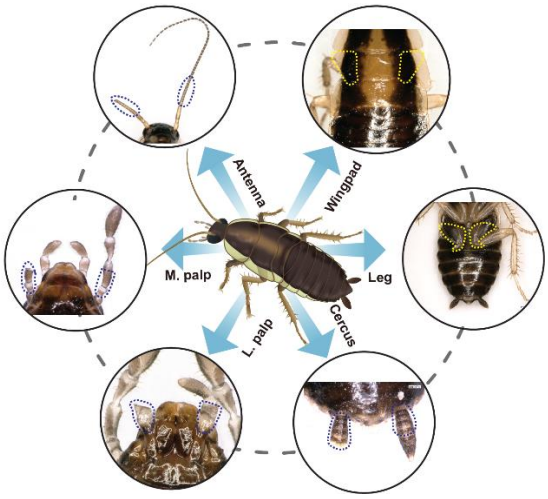

Figure 4: Diagram of appendage amputation sits and tissue collection

A total of 1 μg of RNA per sample was used as input material for RNA preparation. Sequencing libraries were generated using a NEBNext Ultra™ RNA Library Prep Kit for Illumina (NEB, E7770S) following the manufacturer's recommendations. Clustered libraries were sequenced on an Illumina platform and paired-end reads were generated. Raw FASTQ-format data were first processed through in-house Perl scripts. RSEM software was used to map the reads to the reference genome (Bger_1.1). Gene expression levels were estimated as fragments per kilobase of transcript per million fragments mapped (FPKM) values, and differential expression analysis between the AM and WT groups was performed using DESeq2. Genes with adjusted *P*-values < 0.05 and $\log_2$foldchange > |1| in DESeq2 were considered as differentially expressed. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and GO term enrichment analysis were performed for DEGs. Upset plot was performed using the OmicStudio tools at https://www.omicstudio.cn/tool for set analysis of six different appendage DEGs. R package pvclust was used to calculates approximately unbiased P-values for hierarchical clustering through ten resampling scales (0.5-1.4, incrementing by 0.1) and 1,000 bootstrap repetitions per scale; theatmaps of gene expression were drawn using R package pheatmap. Genes within certain clusters displayed similar expression profiles, barring minor variations.

Results

Appendage regeneration depends on developmental age and damage degree

Six kinds of body regions, including three cephalic appendages (antennae, l. palps, and m. palps), two thoracic appendage wing pads (forewing pads and hind wing pads) and legs, and one abdominal appendage (cerci), were selected in this study. The amputations were performed at last four nymphal age stage (instars N3, N4, N5, N6), and multiple amputations (the remained proportions were calculated) on each appendage were performed (Fig.5A). Then, the regenerative ratios of l. palps (Fig.5B), m. palps (Fig.5C), antennae (Fig.5D), wing pads (Fig.5E), legs (Fig.5F), and cerci (Fig.5G) were measured at each developmental instars post amputation (N4, N5, N6, A (Adult)). The results show that there are sharp regenerative ratio changes came out for all appendages during

the first molting, while the changes of regenerative ratio became smaller on the following molts. Meanwhile, for all the appendage types, the more molting cycles post amputation they have, the higher regenerative ratio they achieve (Fig.5).

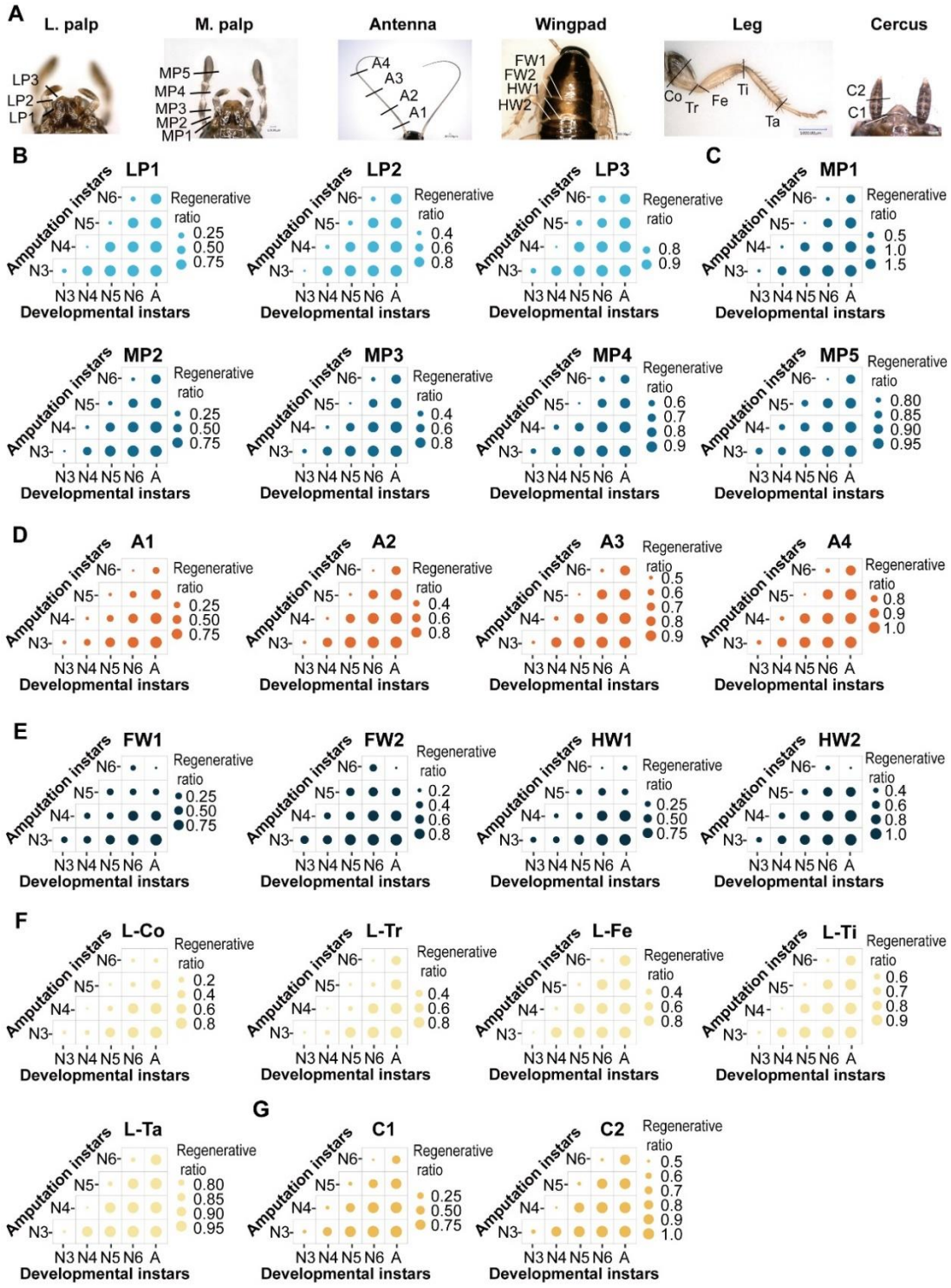

Figure 5: All six kinds of appendages can be regenerated depending on developmental stage and amputation degree. (A). The amputation sites for six kinds of appendages. (B-G). The regenerative ratio of six kinds of appendages at multiple N4, N5, N6, and A stages post amputation were calculated.

Using bioinformatics modeling, we investigated the details how developmental age and damage degree affect regeneration of all appendages. When focusing on the regenerative ratio after eclosion at adult stage, all the six kinds of tissues can be regenerated well (~100%) when amputated at early N3 stage (four molting cycles), while the regenerative ratio decreased sharply when amputated at late N6 stage (one molting left) (Fig. 6A). Importantly, there is a negative correlation between regenerative ratio and developmental age, when amputation was performed, for all six appendages, especially for wing pad (Fig. 6B). Meanwhile, the amputation site (equal to remain proportion) was another key factor which determines regenerative ratio. There is a positive correlation between regenerative ratio and remaining proportion for all six appendages except for L-Fe treatment of leg group (Fig. 6C). L-Fe treatment of leg group harbored the strongest regenerative ability, because trochanter is the critical podomere for leg regeneration (Fig. 6C). The observation in the German cockroach is similar to what we previously found in the American cockroach [12]. Taken account of the amputation instar and remain proportion factors together, the regenerative ratio is negatively correlated with developmental age and positively correlated with damage degree (Fig. 6D).

To summarize the bioinformatics modeling data, we presented a comparative diagram for the regeneration of all the six appendages (Fig. 6E). When amputation degree is more severe, more molting times are needed to make up for regeneration ability; *vice versa*, when amputations were performed at later developmental instars, higher remaining proportions were required to regenerate the whole missing appendages. It is important to note that the regeneration ability of wing pad is weaker than the other five appendages; meanwhile, l. palp and cercus have relatively higher regeneration ability than others, different from the long-standing common idea that leg harbors the strongest regenerative capacity than the other appendages. In short, all six kinds of appendages of German cockroach can be well regenerated when they are amputated at early nymphal developmental stages.

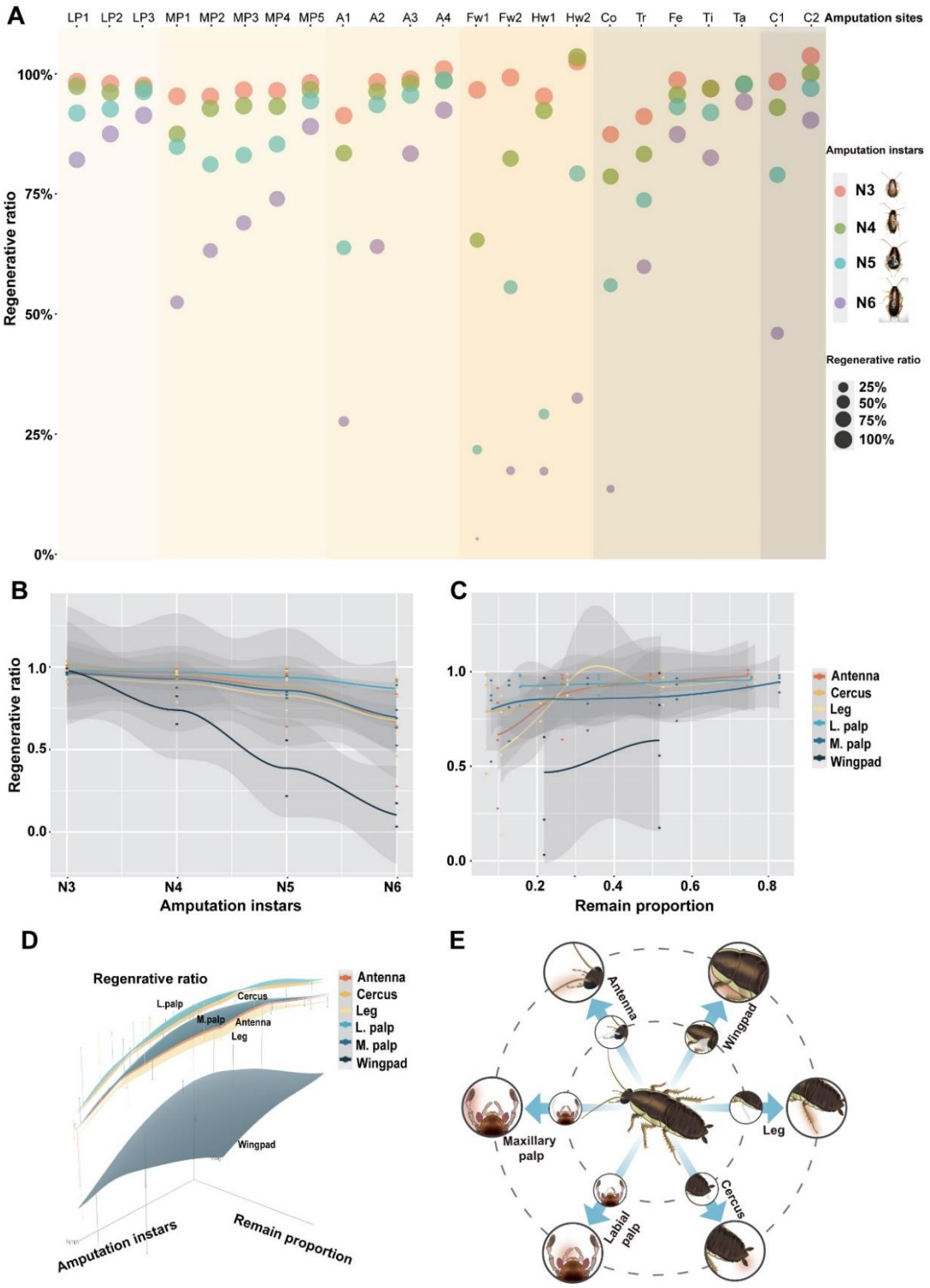

Figure 6: All six kinds of appendages can be regenerated and closely depend on developmental stage and amputation degree. (A). Six kinds of appendages were amputated in different sites, and four instars (N3 (red), N4 (green), N5 (blue), and N6 (purple)) of animals were selected. The regenerative ratio at adult stage were calculated. (B-D). The non-linear curve correlation between regenerative ratio and amputation instars (B), and between regenerative ratio and remain proportion (C); and the correlation between regenerative ratio and amputation instars and remain proportion was shown in tridimensional axis (D). (E). The model for the strong regenerative capacity of all six kinds of appendages post amputations.

The complex sensilla of multiple appendages can be regenerated

To further survey the fidelity of regenerated appendages, in addition to the regenerative ratio measurements, the macroscopic morphology and microanatomical sensilla of regenerated appendages (RG) were compared with contralateral wild type (WT) ones. Each kind of appendages on one body side were removed at N3 instar, and observed at adult stage. There were no obvious differences for the general morphologies between RG and WT groups for l. papls (Fig. 7A1), m. palps (Fig. 7B1), female antennae (Fig. 7C1), male antennae (Fig. 7D1), legs (Fig. 7E1), and cerci (Fig. 7F1) when detected by light microscopy. All the podomeres of each kind of appendages restored to their original shapes. Besides, the sensory bristles and sensilla which form part of the epidermis provide exquisite markers for assessing the accuracy of regeneration. The diversity, morphology, and spatial distribution of these sensory organs on five kinds of exteroceptive appendages (l. papls, m. palps, antennae, legs, and cerci) were detected by SEM. Three main kinds of sensilla, including SC I (sensilla chaetica subtype I), SC II (sensilla chaetica subtype II) and SP (sensilla palmatum), on both l. palps and m. palps were restored (Fig. 7A and 7B). Three main kinds of sensilla, including SC I, SC II and SB (sensilla basiconica), on both female and antennae were restored (Fig. 7C and 7D). Three main kinds of sensilla, including Br (bristle), SB, and ST II (sensilla trichoid subtype II), on legs were restored (Fig. 7E). Four main kinds of sensilla, including Br, SC II, SB and AS (acoustic sensilla), on cerci were restored (Fig. 7F). These data mentioned above show that all these kinds of appendages of German cockroach can be restored with strong regenerative ability, including both macroscopic morphology and microanatomical sensilla on them.

In addition, even wing pads have relative weaker regenerative capacity than the other five appendages, amputation of wing pads yielded well-shaped yet smaller adult wings. However, it appears that their complex sensilla of multiple appendages is able to be largely regenerated. Detailed microscope observation is still in progress.

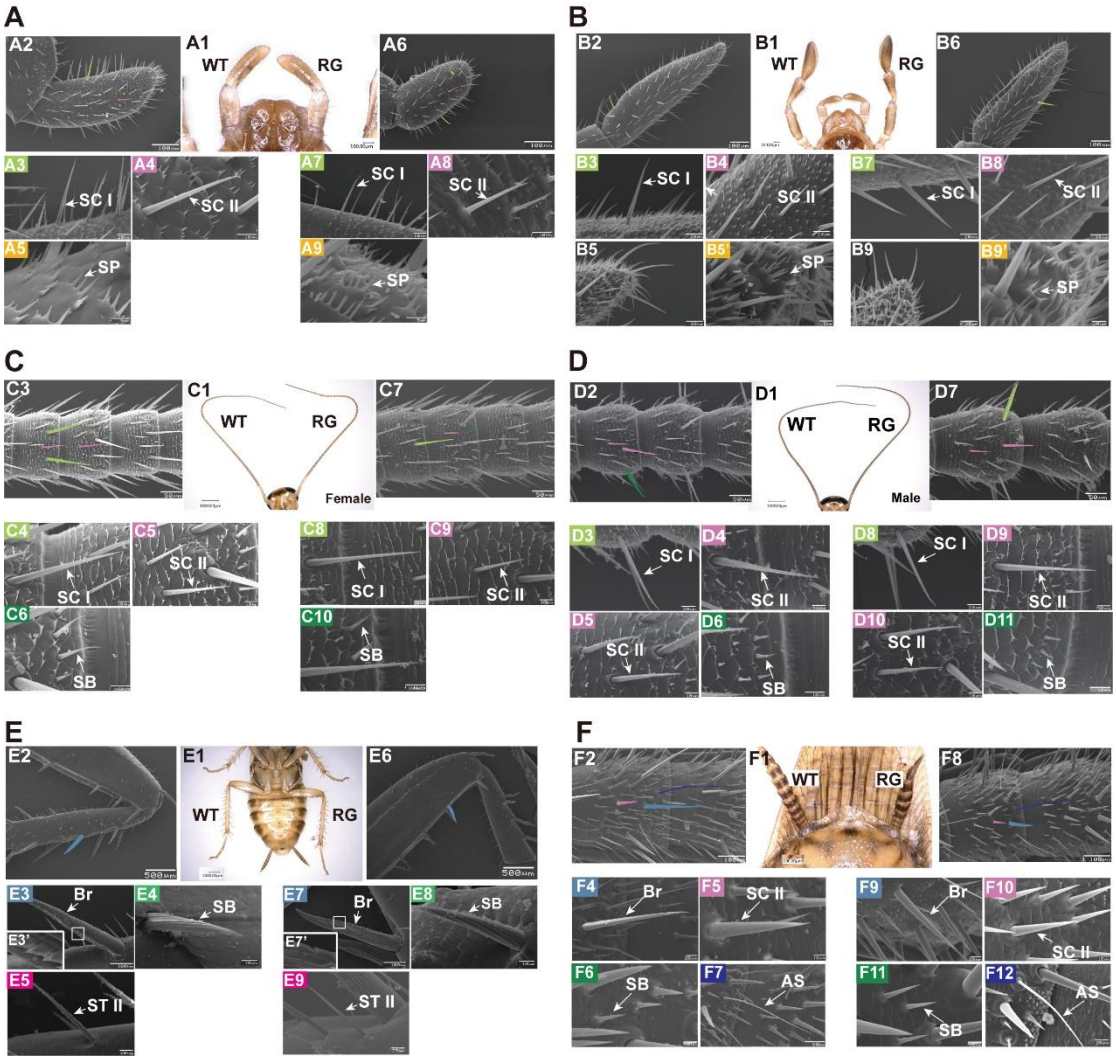

Figure 7: The integral shape and complex sensilla of multiple appendages can be regenerated. (A-F). The macroscopic morphology and microanatomical sensilla of regenerated (RG) and contralateral wild type (WT) appendages were observed by light microscopy and SEM. The l. palps (A), m. palps (B), female antennae (C), male antennae (D), legs (E), and cerci (F) were used, and different colors correspond to different sensilla.

Transcriptional profiles amongst appendages are different between homeostasis and regeneration

Some insect appendages are considered to be serially homologous structures exhibiting similar transcriptional profiles, they retain anatomical and developmental aspects of their common evolutionary origin [6-8]. The shape and size of nymphal appendages are determined during embryonic stage, while little is known about their hierarchical relationships of gene expression patterns among these appendages during homeostasis and regernation. To reveal the source of morphological and molecular diversity of six different kinds of appendages under homeostatic nymphal and regenerative conditions, tissues around the regenerating areas and uninjured contralateral tissues were harvested for RNA-Seq (Fig. 4). Transcriptional similarity between relevant appendages can be modelled as character trees depicting which appendages are mostly similar to each other. When placing emphasis on homeostasis, the two mouthparts, l. palp and m. palp, are clustered together as a main branch (95%), functioning in feeding. In another main branch, the antenna clusters most closely with the cercus (96%), functioning in sensing; while the leg clusters most closely with the wing pad (75%), functioning in mobility (Fig. 8A). Thus, the cluster pattern of homeostasis corresponds to the appendages' biological functions at a certain degree. Meanwhile, the regenerative character tree is made based on the gene expression of regenerating tissues, and a much different branch type turned out compared with homeostatic clusters. Three cephalic appendages antenna, l. palp, and m. palp are clustered together (98%), while the leg itself clustered as a subbranch (90%); the wing pad and cercus are clustered together as another main branch (71%) (Fig. 8B). Correspondingly, the cluster pattern of regeneration largely reflects the spatially appendage-bearing segments. Conclusively, there is a significant shift from a function-based homeostatic profile to a spatially segment-related regenerative profile, indicating that a program involved in embryonical development is reactivated during multiple appendages regeneration.

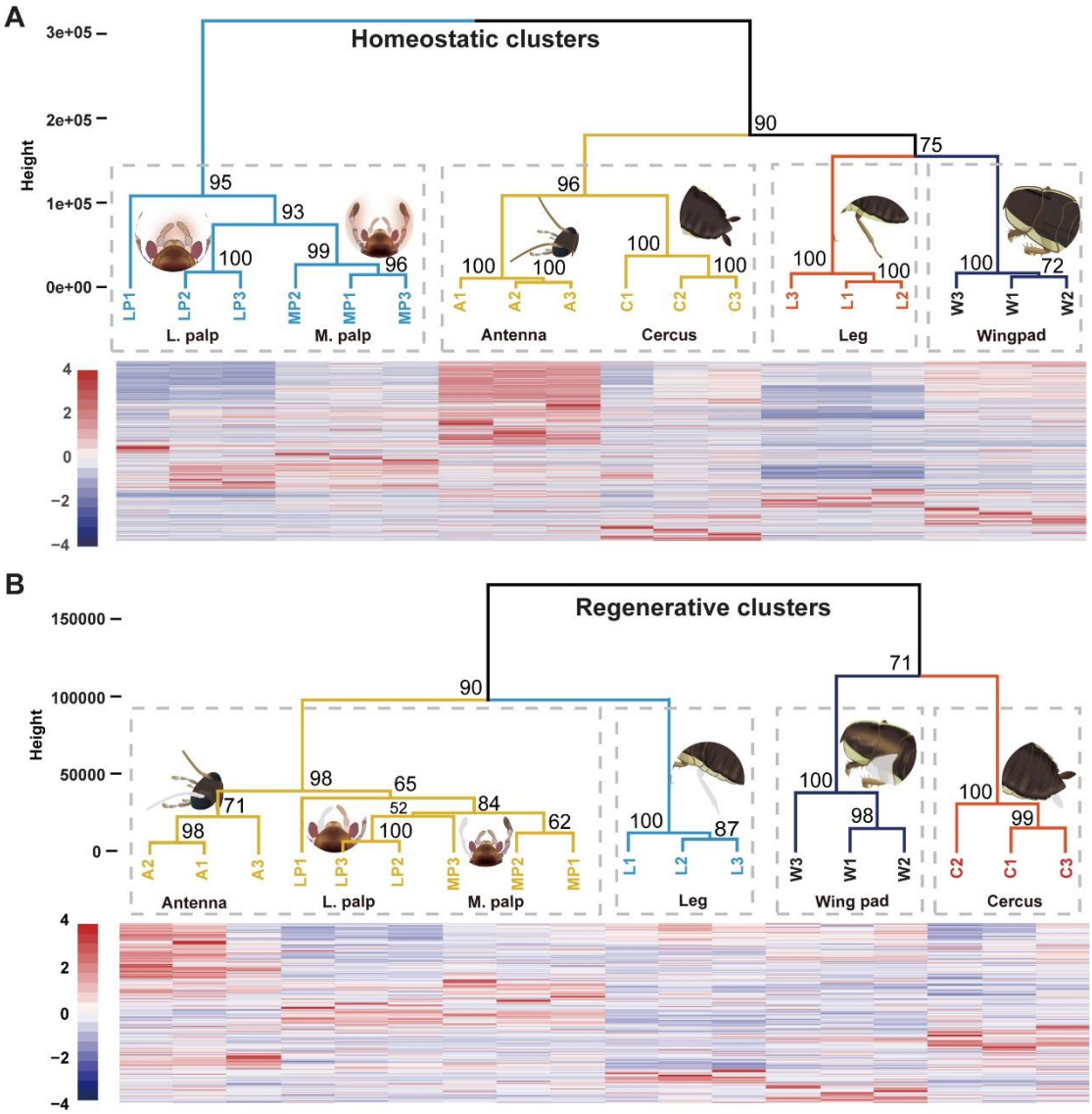

Figure 8: Character trees of gene transcripts across different appendages in both homeostatic and regenerative stages. (A). Hierarchical clustering of samples and expression heatmap of genes in the homeostatic stage. (B). Hierarchical clustering of samples and expression heatmap of genes in the regenerative stage. Numbers at nodes are approximately unbiased multi-scale bootstrap support values. The samples in same dashed boxes are much closer with each other.

DEGs contribute to the shift of transcriptional patterns from homeostasis to regeneration

To elucidate the mechanisms underlying the regenerative capacity of multiple appendages and the reasons for profile shift from homeostasis to regeneration, the DEGs in each kind of appendages and the intersections among these appendage types during regeneration are analyzed. For each kind of appendage types, there are 315, 335, 484, 2097, 2308, and 2334 DEGs detected in cercus, antenna, wing pad, leg, m. palp, and l. palp groups, respectively. Focusing on the tissue specific genes, there are 659, 644, 395, 69, 57, and 23 tissue specific DEGs are found in leg, l. palp, m. palp, wing pad, cercus, and antenna groups, respectively (Fig. 9A). As shown with Venn diagram, the antenna, m. palp, and l. palp with strong intersection relationship compared with others. To clarify the drives for the shift of gene transcription patterns between homeostatic and regenerative conditions (Fig. 8), another hierarchical clustering was made using DEGs from each kind of appendages. Intriguingly, this pattern is much similar to that of regenerative clusters, and a spatially segment-related regenerative profile turned out again for the DEGs (Fig. 8B and 9B): the antenna, m. palp, and l. palp were tightly clustered together (97%), leg itself clustered as a subbranch (93%), and wing pad and cercus are clustered together as another main branch (82%) (Fig. 9B). Altogether, DEGs contribute to the shift of transcriptional shift from homeostasis to regeneration, shedding light to gene expression changes results in a reactivated program of embryonical development during multiple appendages regeneration.

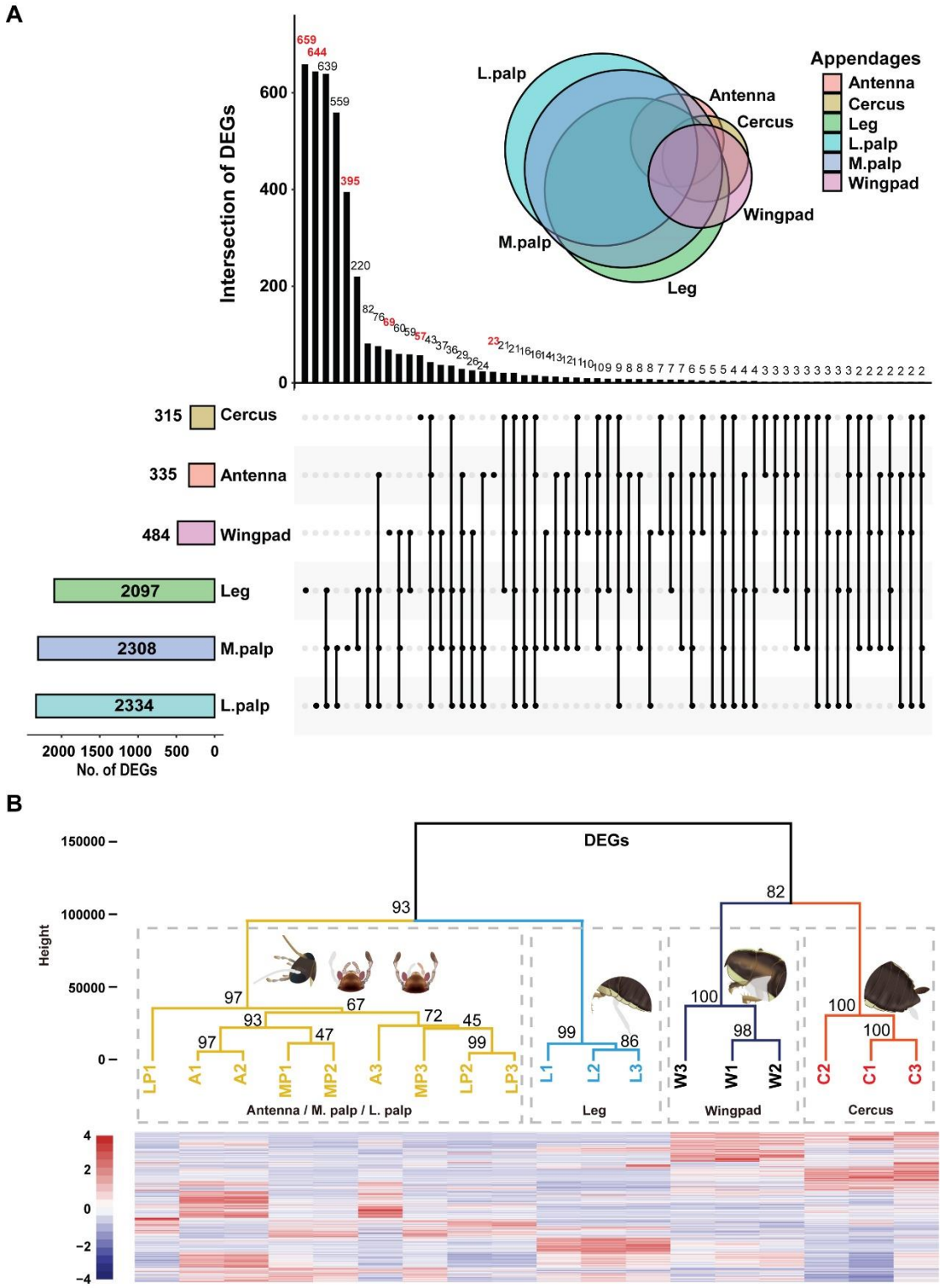

Figure 9: Multiple appendage specific genes involved in regenerations of different appendages. (A). Tissue specific DEGs in each kind of appendages and the intersections among these appendage types during regeneration are analyzed using upset. (B). Hierarchical clustering of samples and expression heatmap of different expressed genes between homeostatic and regenerative stages.

Summary and perspectives

Here, choosing the German cockroach as our research object, we discover that five kinds of appendages, including antenna, l. palp, m. palp, leg, and cercus, with strong regeneration capacity, while wing pad with relative weaker regeneration capacity. In each appendage, the regeneration capacity tightly depends on both developmental ages and damage degrees. The appendages can precisely restore the complex microanatomy and spatial distribution of sensilla if incomplete amputation was performed at early developmental age. Using RNA-Seq strategy, we found the hierarchical relationships of gene expression pattern in multiple appendages are different between homeostasis maintenance and regenerative conditions. More importantly, there is a significant shift from a function-based homeostatic profile to a spatially segment-related regenerative profile. The DEGs in each kind of appendages contribute to the shift of transcription profiles. This study gives a comprehensive and comparable understanding on multiple appendages regeneration of German cockroach, indicating that multiple appendages should be coordinately regenerated to enable the cockroach survival as a complex organism.

References:

1. Bely, A.E., and Nyberg, K.G. (2010). Evolution of animal regeneration: re-emergence of a field. Trends Ecol Evol *25*, 161-170.
2. Panganiban, G., Irvine, S.M., Lowe, C., Roehl, H., Corley, L.S., Sherbon, B., Grenier, J.K., Fallon, J.F., Kimble, J., Walker, M., et al. (1997). The origin and evolution of animal appendages. P Natl Acad Sci USA *94*, 5162-5166.
3. Tanaka, E.M. (2016). The Molecular and Cellular Choreography of Appendage Regeneration. Cell *165*, 1598-1608.
4. Slack, J.M.W. (2017). Animal regeneration: ancestral character or evolutionary novelty? Embo Rep *18*, 1497-1508.
5. Konstantinides, N., and Averof, M. (2014). A Common Cellular Basis for Muscle Regeneration in Arthropods and Vertebrates. Science *343*, 788-791.
6. Budd, G.E., and Telford, M.J. (2009). The origin and evolution of arthropods. Nature *457*, 812-817.
7. Angelini, D.R., and Kaufman, T.C. (2006). Insect appendages and comparative ontogenetics (vol 286, pg 57, 2005). Dev Biol *291*, 207-207.
8. Morata, G. (2001). How Drosophila appendages develop. Nat Rev Mol Cell Bio *2*, 89-97.
9. Averof, M., and Akam, M. (1995). Hox Genes and the Diversification of Insect and Crustacean Body Plans. Nature *376*, 420-423.
10. Hughes, C.L., and Kaufman, T.C. (2002). Hox genes and the evolution of the arthropod body plan. Evol Dev *4*, 459-499.
11. Zhong, J., Jing, A., Zheng, S., Li, S., Zhang, X., and Ren, C. (2023). Physiological and molecular mechanisms of insect appendage regeneration. Cell Regen *12*, 9.
12. Li, S., Zhu, S.M., Jia, Q.Q., Yuan, D.W., Ren, C.H., Li, K., Liu, S.N., Cui, Y.Y., Zhao, H.G., Cao, Y.H., et al. (2018). The genomic and functional landscapes of developmental plasticity in the American cockroach. Nat Commun *9*.
13. Gerber, T., Murawala, P., Knapp, D., Masselink, W., Schuez, M., Hermann, S., Gac-Santel, M., Nowoshilow, S., Kageyama, J., Khattak, S., et al. (2018). Single-cell analysis uncovers convergence of cell identities during axolotl limb regeneration. Science *362*, 421-+.
14. Roensch, K., Tazaki, A., Chara, O., and Tanaka, E.M. (2013). Progressive Specification Rather than Intercalation of Segments During Limb Regeneration. Science *342*, 1375-1379.
15. Marsh, J.L., and Theisen, H. (1999). Regeneration in insects. Semin Cell Dev Biol *10*, 365-375.

Division of research work

In the process of the research, the supervisors helped us clarify the research ideas and provide the guidance on the methods of data analysis. All the help and guidance are free of charge. Ethan Li initiates this project and acts as a group leader. Ethan Li and Bernice Wang performed most of the experimental procedures, while Sichen Wu is in charge of bioinformatic analysis. Led by Ethan Li and supervised by Yuli Luo and Chonghua Ren, the three sophomores established an excellent team in bench work, data analysis, and paper writing.

Acknowledgements

We would like to extend sincere thanks to the two supervisors for their on-going encouragement and support, inspiring guidance and insightful comments on the research, without which the thesis would not have been in its present form.

Brief introduction to the contestants

Ethan Yihao Li, male, born in Maryland, America on March 12, 2007, is now a sophomore at the Affiliated High School of South China Normal University. Ethan has been interested in biology since childhood, especially the mysteries of insect regeneration. He won the First Prize (3 out of 300 projects) in the 38th Guangdong Provincial Youth Science and Technology Innovation Competition this April, and promoted to the 37th China Adolescents Science & Technology Innovation Contest which is the largest and highest youth technology competition in China. At the big event in August, he won the National Bronze Medal (15 out of 442 projects) and the Special Award of Youth Technology Innovation issued by Huazhong Agricultural University with bonus of 3000 yuan. Meanwhile, Ethan had been selected for the program of International Youth Cultural Exchange Activities and gave presentation as a group leader. Moreover, Ethan is a co-author in a scientific paper which is currently under revision in *Cell Reports* in terms of scientific research of cockroach leg regeneration. In addition, Ethan won the Gold Medal (top 10%) in 2023 USABO (USA Biology Olympiad) at China Division because of his solid biological knowledge.

Sichen Wu, male, born in Guangzhou, China on February 16, 2007. He is currently a sophomore at the Affiliated High School of South China Normal University. Sichen has achieved notable success in mathematics, winning the first prize (top 5%) and the special prize (0.3%) in the AMC Math Contest, which is recognized as the world's largest and oldest math competition. This competition attracted 1,450 students from 32 countries, and only five students from China have achieved this level of recognition. Furthermore, Sichen excels in both literature and science, earning recognition as an outstanding scholar. He has received numerous awards for his poetry and writing, and his works have been published. Additionally, Sichen has been honored with the title of Virtuous Teenager on multiple occasions.

Bernice YX Wang, female, born in Vancouver, Canada on Jun 13, 2007, is now a Grade Eleven student at the Affiliated High School of South China Normal University. She won the Distinction Award (Top 1% of the contestants) in 2023 American Mathematics Contest 10. In 2023, she took part in 2023 Pioneer Global-Problem Solving Institute-Disabling Disease and became one of the fellowship recipients of 2024 Pioneer Research Program. She also won the Individual Gold Award in 2023 Speech and Debate Pentation (ESDP) Guangdong. Besides, she took part in Biological Health and Medical Workshop held by King Lead Academician Forum.

Brief introduction to the Supervisors

Yuli Luo, a first-class teacher and Olympiad coach in Biology in the Affiliated High School of South China Normal University, has many years of experience in teaching and tutoring students to participate in competitions. He has guided students to win international and domestic awards in biology for many times.

Chonghua Ren, investigator, has been engaged in Animal science for more than ten years. His main research direction is to study the development, reproduction, and regeneration of animals, especially insects. He is currently working in the Key Laboratory of Insect Developmental Biology and Applied Technology in Guangdong Province, the College of Life Sciences, South China Normal University.