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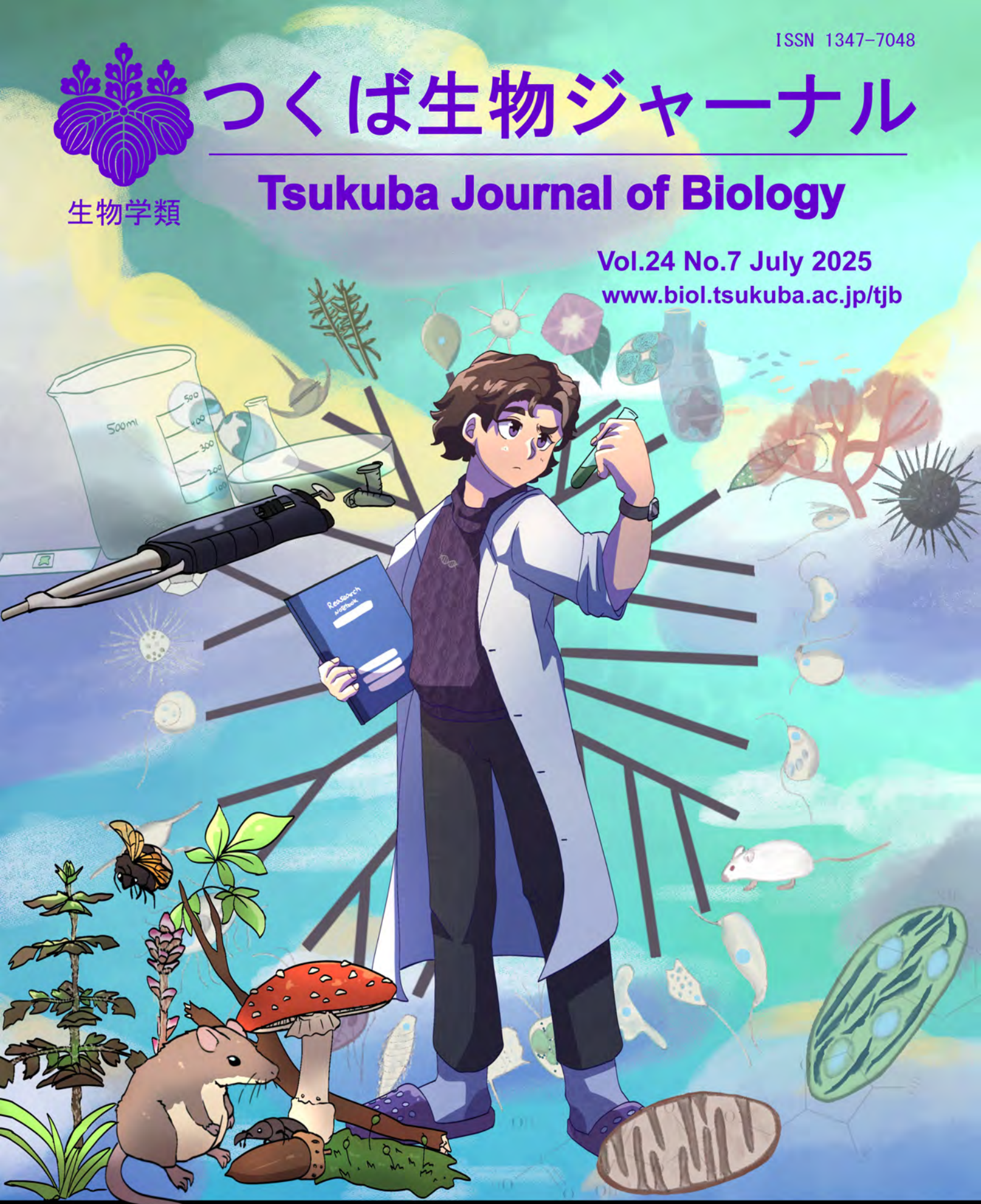

THE WORLD

つくば生物ジャーナル

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Abstracts of Graduation Research Presentations, July 2025

July 4th 2025

College of Biological Sciences
University of Tsukuba

表紙画　Cover art

上西　慧莉紗　Erisa Uenishi

古橋　夢摘　Mutsumi Furuhashi

表紙画の解説　Explanation of the cover art：

テーマは「世界」。生物は我々が住む世界を作り上げており、それの学問である生物学はその世界について追及する学問になります。我々が学んでいる生物学の重要さを表現するためにタロットカードの「世界 (The World)」をモチーフに表紙画をデザインしました。四隅には情熱・理性・陸・水を示した、オルガネラ・実験器具・陸域生態・水圏生態を置き、円環は真核生物の大系統で表現しました。

The theme is “The World”. Living organisms make up the world we live in, and the study of biology is the study of that world. To express the importance of the biology we are studying, we designed the cover art based on the motif of the tarot card “The World”. The four corners represent passion, reason, land, and water, with the organelle, laboratory equipment, terrestrial ecosystem, and aquatic ecosystem, and the circle is represented by major eukaryotic lineages.

July 4th 2025 @Sogo-Kenkyuto room A110

13:05–13:20 **小村 心人**

ソリトン様細胞集団運動を示す非走化性変異株における走化性物質合成様式の解析

1

13:20–13:35 **Suzuki Tatar Derin**

No food, no fight? The effect of host plant quality on male alternative reproductive tactics in the two-spotted spider mite

2

13:35–13:50 **Al-Janahi AlMaha Abdulaziz M A**

Identifying Genes That Regulate Gut-Derived Hormone Release in Adult Female Fruit Fly

3

13:50–14:05 **Gushiken Anna**

Binding protein-mediated neuronal transport of insulin to endocrine targets in the fruit fly

4

14:05-14:20 **Tastanbekova Aisana**

Cancer cachexia is mediated by dysbiosis and midgut pH dysregulation in *Drosophila*

5

ソリトン様細胞集団運動を示す非走化性変異株における走化性物質合成様式の解析

小村心人（筑波大学 生物学類）　　指導教員：桑山秀一（筑波大学 生命環境系）

Introduction

細胞性粘菌Dictyostelium discoideumはアメーバゾアに属する単細胞アメーバで土壌中においてバクテリア等を餌としながら単純2分裂で増殖する。しかしながら、飢餓状態に陥るとcAMPを細胞自ら分泌してすることにより cAMP に対する走化性能を有する周囲の細胞を誘引し、凝集して集合体を形成する。この集合体は後に、ナメクジ状の形態を経て頭頂部の胞子塊を柄細胞で支える子実体と呼ばれる多細胞体構造を形成する。

所属研究室では走化性能を欠損するため飢餓状態においても細胞凝集を行えない株を所有しており、それらのうち飢餓状態においてこれまで報告されたことのない細胞集団運動を行う株(以下KI-5)を発見している。その細胞集団運動は、野生株のように1点に集合することなく波状の構造物を形成し、構造物が集団として運動することが解析により判明した。この波状の形態が一様に移動する現象はソリトンと呼ばれる物理現象と酷似しており、一定の速度で移動し複数の波状構造が衝突してもすり抜ける特徴があることを報告した(Kuwayama and Ishida, Scientific Reports, 2013)。

ソリトンとは自然界において日常的に観察される波動に関する物理現象の一種であり、波の速度が一定であり衝突しても互いの波動運動に干渉作用をしない特殊な性質を有する孤立波である。

これまで所属研究室の研究により KI-5 が示す孤立波様の細胞集団運動の維持は、波状細胞集団運動が移動する際、進行方向前方にある細胞を取り込み、後方で取り残すことでその波に含まれる細胞集団の量を一定に保つことがわかっている。また、複数の細胞集団運動同士が衝突した際には細胞集団運動の形状、大きさ、運動方向は維持され一見互いにすり抜けるように観察されるが、衝突時において細胞の集団の混合が起こり分離時に混合されたまま波状細胞集団運動が分離することが示されている。

先行研究では KI-5 が示すソリトン様細胞集団運動は細胞自身が分泌する細胞外の cAMP に影響されないことがわかっているが、細胞内部で合成される cAMP とソリトン様集団運動との関連性については明らかになっていなかった。そこで、私は細胞内部で合成されるcAMPとKI-5細胞のソリトン様細胞集団運動の時間的空間的動態の関係を観察することを目的に、cAMPが結合するとGFP蛍光を発する遺伝子産物Flamind2をKI-5細胞に発現させ、蛍光顕微鏡により観察することを卒業研究のテーマとした。

Material & Methods

(1)プラスミドの取得と増幅

まず細胞性粘菌における Flamind2 の発現ベクターを細胞性粘菌ナショナルバイオリソースプロジェクト（NBRP nenkin）から取得した。大腸菌 DH5α コンピテントセルに導入しベクターを大腸菌内で増幅、その後プラスミドプレップを行い細胞性粘菌の形質転換に十分な量のプラスミドを取得した。

(2)形質転換

研究標準株(以下AX2)とKI-5をそれぞれHL5液体培地で培養し、形質転換効率が最も高い対数増殖期初期（1-2 × 106 cells/mL）の細胞をそれぞれ取得した。それぞれの細胞は、最終細胞密度が5 x 107 cells / mLになるようにリン酸緩衝液を主成分としたエレクトロポレーション緩衝液に懸濁した。この懸濁液800 μLにプラスミドを15 μL（約 1 μg プラスミドDNAに相当）加え、幅 4 mm のエレクトロポレーション用キュベットに封入し、BioRad社GenePulser Xcellを用いて、 500 V/ mm, 1 msec × 2で電気刺激を与えプラスミド導入を行った。

それぞれの細胞は HL5 培地を含む培養シャーレにおいて細胞性粘菌の標準培養温度である21℃で一晩静置した。その後、ネオマイシン耐性である本プラスミドが導入された細胞のみを生育されるよう選択薬剤G418 (最終濃度20 μg)を加えた。2週間程度後に形質転換されたと考えられる生育株を観察することができた。以下、これらの株をそれぞれAX2-Fla2及びKI-5-Fla2とする。

(3)表現型の観察

Flamind2 の発現により表現型が変化しないことを確認するため、一晩振盪培養した Ka (800 μL) と形質転換した細胞株 (100 μL)を 5LP 寒天培地上に撒き、数日 21℃で静置培養し、AX2-Fla2及びKI-5-Fla2の飢餓状態における細胞の振る舞いを観察した。

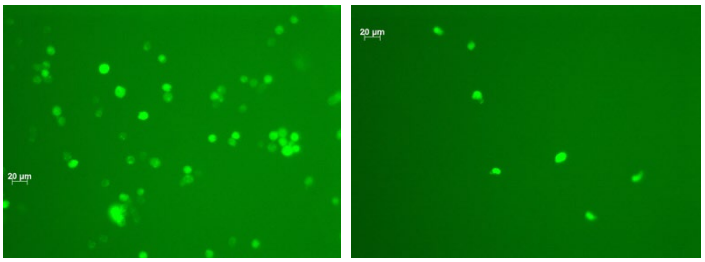
(4)蛍光観察

作製したAX2-Fla2及びKI-5-Fla2、コントロールとしてAX2とKI-5について蛍光顕微鏡でGFPの蛍光を確認した

Result &Discussion

生育株を獲得するまでに形質転換を7回失敗し、8回目で生育株の獲得に至った。8 回目の形質転換では、どちらの株も電気刺激から 5 日後を目安に薬剤耐性を獲得した細胞クラスターが発生した。AX2-Fla2 についてはクラスターから細胞が順調に増殖したが、KI-5-Fla2は細胞が十分に増殖するまで2週間を要した。

また、HL5液体培地上で培養したそれぞれの細胞株の平常時における GFP 蛍光が以下のように観察された。蛍光が観察されたことから目的の形質が細胞内で発現していると考えられる。現在は寒天培地上で培養を行い、飢餓状態での蛍光観察を試みている。それらの内容について発表会で報告したい。



AX2-Fla2(左図)、KI-5-Fla2(右図)のGFP蛍光 Scale bar=20μm

Reference

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No Food, No Fight? The Effect of Host Plant Quality on Male Alternative Reproductive Tactics in the Two-spotted Spider Mite

Derin Suzuki Tatar（筑波大学 生物学類）　　指導教員：Yukie Sato（筑波大学 生命環境系）

Introduction

Male-male competition is a fundamental driver of evolution in sexually reproducing organisms. They lead to diverse reproductive strategies, including Alternative Reproductive Tactics (ARTs). A model organism for studying ARTs is the two-spotted spider mite, *Tetranychus urticae* (hereby referred to as *T. urticae*). For *T. urticae* males, competition for virgin females is driven by sperm precedence, where the first male to mate fertilizes all her eggs. *T. urticae* males usually employ a 'fighter' tactic, guarding females during the teleiochrysalis stage (just before adulthood). By repelling other males, they ensure to mate with the virgins. However, the Houten-1 strain also exhibits a 'sneaker' tactic, where males mount females like the 'fighter' tactic, but instead avoid combat by not reacting to any male interactions. The occurrence of sneaking especially among younger males aligns with the life history strategy hypothesis, suggesting an age-dependent risk aversion. On the other hand, ARTs are often explained by the Resource Holding Potential (RHP) hypothesis, which suggests an individual's ability to win conflicts depending on the resources it possesses. Therefore, the RHP hypothesis supposes that individuals with low amounts or quality of resources would not choose the 'fighter' tactic. However *T. urticae* males have not shown any significant size differences between the tactics, leaving RHP unconfirmed by their morphology alone.

While individual differences and evolved strategies influence reproductive tactics, an organism's environmental context and physiological condition also play a critical role in its expression. However, the specific influence of male nutritional state, mediated by host plant quality on *T. urticae* ARTs remains largely understudied. This study investigates how the manipulation of male nutritional status influences these tactics, thereby enhancing our understanding of factors shaping male life history strategies.

Materials and Methods

Tetranychus urticae (Houten-1 strain, University of Amsterdam) were reared on common bean (Phaseolus *vulgaris)* leaves using the leaf disk method at 25°C under a 15L9D photoperiod. To influence male nutritional status, experimental leaves were prepared as "Good" (high chlorophyll, grown in UV light) or "Bad" (low chlorophyll, grown in shade). Five teleochrysalis females were placed on "Good" and "Bad" leaf disks (φ25mm) as oviposition arenas. After 4-5 days, the adult females were removed, and eggs were standardized to 30 eggs per arena. The resulting males (produced via arrhenotokous parthenogenesis) were transferred to mating leaf arenas (φ15mm), with five males per arena. A single teleiochrysalis female was then introduced, and tactic choice was observed hourly for 4 hours.

Results and Discussion

Our experimental manipulation successfully created host plant conditions that were distinct from each other, thereby impacting male *T. urticae's* nutritional status. Male reproductive tactics were significantly affected by host leaf quality (Figure 1). Males on "Good" leaves were significantly less likely to exhibit the 'sneaker' tactic compared to those on "Bad" leaves ($P < 0.05$). Specifically, in the "Bad" leaf group, approximately 23.4% of observed males employed the 'sneaker' tactic, while only 14.7% did so in the "Good" leaf group. This demonstrates that improved nutritional conditions lead to a reduced choice of the 'sneaker' tactic in males.

Increased 'sneaking' in males from nutritionally "Bad" quality leaves agrees with the RHP hypothesis. This study supports the RHP hypothesis as a mechanism underlying ARTs in *T. urticae.* These findings do not contradict life history theory; while most sneakers are young males, not all adopt the 'sneaker' tactic. RHP may serve to help determine which individuals among the young males show the 'sneaker' tactic.

These findings on *T. urticae* shows us that the evolution of ARTs is likely shaped by a web of various mechanisms. This complexity is important for understanding the diverse reproductive strategies observed not only in *T. urticae,* but also across a wide range of taxa.

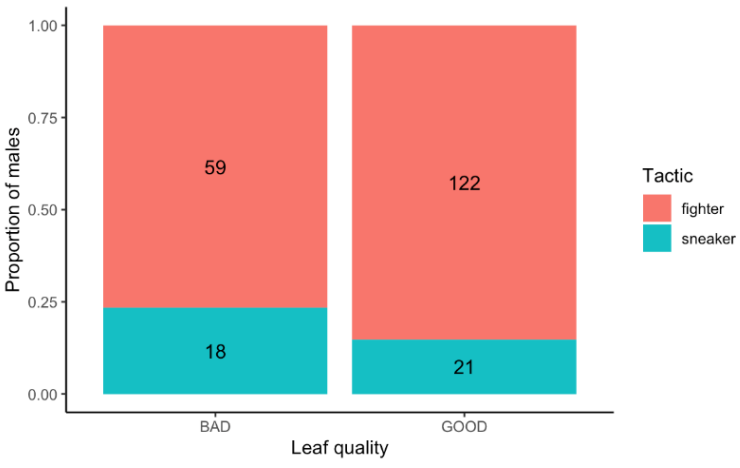

Figure 1: Male reproductive tactics ('fighter' and 'sneaker') as influenced by host plant quality in *Tetranychus urticae.* Numbers in bars indicate the number of males.

Identifying Genes That Regulate Gut-Derived Hormone Release in the Adult Female Fruit Fly
(成虫雌ショウジョウバエにおける腸由来ホルモン分泌の制御遺伝子の特定)

AlMaha Al-Janahi（筑波大学 生物学類）　指導教員：Ryusuke Niwa（筑波大学 生存ダイナミクス研究センター）

Introduction

Neuropeptide F (NPF), the homolog of Neuropeptide Y in vertebrates, is an essential gut hormone, secreted from enteroendocrine cells (EEC) that regulates key physiological and behavioral processes in insects, including the fruit fly *Drosophila melanogaster*. The secretion of NPF is vital for various physiological processes, such as the regulation of adult feeding behaviours[1], germline stem cell proliferation[2], and lipid metabolism[3]. It has further been confirmed that gut-derived NPF secreted by EECs is released into the adult circulation, responding to dietary cues[4].

As a vital signaling molecule in interorgan communication, gut-derived NPF was selected to better understand the mechanisms behind its release by EECs. However, despite its importance, the genetic mechanisms underlying the regulation of NPF release from EECs in adult *Drosophila* remain largely unknown. In this study, we aimed to identify the genes responsible for the release of NPF from the gut of adult female *Drosophila* into the hemolymph, in an attempt to uncover how this process is tightly controlled. To investigate this, we conducted a targeted genetic screen using the GAL4/UAS system to achieve transgenic RNA interference (RNAi) selectively in NPF^+ EECs, allowing us to knock down candidate genes and observe their roles in NPF release. This approach enabled us to gain a deeper understanding of the functional significance of EECs in regulating endocrine signaling, providing insight into how gut hormones influence systemic physiology.

Material & Methods

GAL4/UAS system

To identify the genes involved in regulating NPF release from the midgut, we performed a transgenic RNAi screen of candidate genes in EECs of adult female *Drosophila* by utilizing the GAL4/UAS system. The GAL4/UAS system achieves tissue-specific knockdown through the use of a GAL4 driver line and a UAS-linked responder line. In this study, the Tkg-GAL4 driver line, which expresses GAL4 in EECs of the adult midgut, was crossed to UAS-RNAi lines to silence targeted genes, allowing us to assess the role of each gene in regulating NPF release. Virgin female offspring carrying the Tkg-GAL4 driver and UAS-RNAi transgene were collected and dissected to isolate the gut.

We selected candidate genes from a public single-cell RNA sequencing (scRNA-seq) dataset of EECs, focusing on those enriched in the NPF^+ cell cluster[5]. Amongst the list of genes, those with human homologs were prioritized for initial testing.

Rearing and Gut Dissection Protocol

Virgin female flies from Tkg-GAL4 crossed either with UAS-RNAi lines (Tkg-GAL4 > UAS-RNAi) or with w^{1118} males for the positive control (Tkg-GAL4 > +) were collected on Day 1 and maintained for 5 days to allow full gut maturation. On Day 5, the virgin females from the two genotypes were divided into two groups: one group remained unmated (virgin group), and the other was mated with w^{1118} (mated group), resulting in four experimental groups: (1) control virgins (**Tkg-GAL4 > +**, V), (2) control mated (**Tkg-GAL4 > +**, M), (3) RNAi virgins (**Tkg-GAL4 > UAS-RNAi**, V), and (4) RNAi mated (**Tkg-GAL4 > UAS-RNAi**, M). On Day 6, all four groups were dissected after allowing the mated groups to mate for 16-18 hours. The guts of 10-15 flies per group were dissected and stained.

Immunohistochemistry

The guts were dissected in 1×phosphate-buffered saline (PBS) and fixed in 4% formaldehyde in PBS. The gut tissue was then dehydrated in ethanol and then blocked with 2% bovine serum albumin in PBS for one hour. To visualize the gut tissue, primary antibodies – mouse anti-Pros and guinea pig anti-NPF – were used for staining, followed by Alexa Fluor-conjugated secondary antibodies (488 and 555). The Zeiss 700 confocal microscope (Carl Zeiss) was used for gut imaging.

Quantification of NPF intensity in EECs

The fluorescence intensity of gut-derived NPF in EECs was quantified using ImageJ. Statistical analysis was used to test for any significant differences in the NPF intensity between virgins and mated flies within the two groups (Tkg-GAL4 > UAS-RNAi and Tkg-GAL4> +).

Result and Discussion

Amongst the list of genotypes that we assessed, our quantitative analysis of NPF intensity in EECs between virgin and mated female *Drosophila* revealed RNAi hits of interest. These hits were characterized by an absence of the difference in NPF intensity between virgin and mated flies, in contrast to the positive control, in which mated females consistently exhibit reduced NPF intensity, suggesting that these hit genes are involved in midgut NPF release. However, further investigations need to be carried out to further elucidate their molecular mechanisms.

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Binding protein-mediated neuronal transport of insulin to endocrine targets in the fruit fly

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Introduction

Insulin-like peptides (ILPs) are crucial regulators of diverse physiological processes, including growth, metabolism, nutrient sensing, stress responses, and reproduction (Nakae et al., 2001; Antonova et al, 2012). In insects, ILPs are primarily produced by neurosecretory cells known as insulin-producing cells (IPCs) in the brain and share functional similarities with insulin and insulin-like growth factors (IGFs) in mammals (Okamoto & Yamanaka, 2015; Nässel & Broeck, 2016). In the fruit fly *Drosophila melanogaster*, genetic ablation of IPCs leads to reduced body size and elevated hemolymph sugar levels (Rulifson et al., 2002), highlighting the essential role of ILPs in growth and metabolic regulation. One key target tissue of IPC-derived ILPs is the A-organ (tentative name). In adult insects, ILP signaling in the A-organ is known to promote ovary maturation. However, the mechanism by which brain-derived ILPs are transported to the A-organ remains unclear. In this study, we utilized the powerful genetic tools available in *D. melanogaster* to investigate the transport mechanism of *Drosophila* ILPs (DILPs) from the IPCs to the A-organ.

Material & Methods

Gal4/UAS system

The Gal4/UAS system was used to spatially control gene expression in specific cell populations (Brand & Perrimon, 1993). Various Gal4 driver lines and UAS-RNAi strains were obtained from Bloomington Drosophila Stock Center (USA), Vienna Drosophila Research Center (Austria) and National Institute of Genetics (Japan). The *DILP-binding protein* (tentatively named *DBP*) mutant line was kindly provided by Takashi Nishimura (Gunma University).

Immunostaining

Wandering third instar larvae were dissected in 1x phosphate-buffered saline (PBS). Tissues were fixed with 4% paraformaldehyde in PBS containing 0.1% Triton-X100 (PBST) for 20 minutes at room temperature (RT) and washed three times with PBST. Tissues were then blocked in PBST containing 5% normal goat serum for at least one hour at RT. Primary antibody incubation was carried out overnight at 4°C. Followed by three PBST washes, the tissues were then incubated with the secondary antibody mix for 2 hours at RT and again washed thoroughly before mounting in a drop of FluorSave (Merck Millipore) mounting medium. Samples were imaged on Zeiss 700 or Zeiss 900 confocal laser scanning microscope (LSM) and analyzed using ImageJ. The primary antibodies used were rabbit anti-DILP2 (1:2000) and chicken anti-GFP (1:2000) antibodies. Alexa fluorescent-labelled antibodies were used as secondary antibodies.

Ovarian development analysis

Newly eclosed virgin female flies were collected and kept in 25° C incubation for 4 days before dissection. Ovaries were dissected in 1x PBS, and the number of mature eggs (stage-14 oocytes) were counted under a stereomicroscope.

Results & Discussion

To investigate the transport mechanism of DILPs from IPCs to the A-organ, I performed immunostaining using anti-DILP2 antibodies. DILP2 produced and secreted from IPCs was found to be taken up by a distinct neuronal population (tentatively named B-neurons). Furthermore, these B-neurons were shown to project to the A-organ. In *DBP* mutant flies, DILP2 immunoreactivity in B-neurons and their axons was abolished, suggesting that IPC-derived DILP2 is taken up by B-neurons in a *DBP*-dependent manner and subsequently transported to the A-organ. This offers insights into how systemic peptide signals reach distant endocrine targets without direct neuronal connections, a previously unrecognized relay mechanism for hormone signalling in *D. melanogaster*.

Despite consistent environmental conditions, the number of mature eggs fluctuated considerably across samples. Notably, control flies exhibited an unexpectedly low mature egg number (20-25), well below the typical range of 35-40. Given this variability and lack of statistical significance, alternative phenotypic marker beyond egg maturation may be needed for a more robust analysis.

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Cancer cachexia is mediated by dysbiosis and midgut pH dysregulation in *Drosophila*

Aisana Tastanbekova（筑波大学 生物学類）　指導教員：Yuya Sanaki（筑波大学 生存ダイナミクス研究センター）

Introduction

Cachexia is an advanced stage of cancer leading to strong muscle and/or fat mass loss due to a dysregulation of metabolism. It is known that metabolism is largely controlled by microbiota, which consists of bacteria colonized in the gut[1].

Our group previously found that a dysbiosis characterized by increased *Escherichia coli*, a non-pathogenic Gram-negative bacterium, caused cancer cachexia in *Drosophila melanogaster*[2]. In healthy flies, *E. coli* distribution is strongly limited in the anterior midgut by the acidic region located in the middle midgut, thus constraining its abundance[3]. To understand how *E. coli* expands in the flies with cancer, I asked if there are any compromises in the low pH boundary in the tumor-bearing flies.

Material & Methods

1. *in vivo* Cancer Model

Wild-type *Drosophila melanogaster* Oregon-R were reared on a normal food (wheat flour, dried yeast, sugar, agar, propionic acid, methylparaben) at 25°C. Virgin female flies were treated with antibiotic-containing food on the day and after 10-15 days of hatching (Ampicillin-Gentamicin-Levofloxacin) for 3 to 7 days to eliminate the gut microbiota. The flies were injected with Ras^{V12} tumor cells (500 cells/fly) and then recovered in antibiotics or conventional food for one day.

2. pH Monitor

7 and 10 days after injection, gut dissection was performed in PBS, followed by fixation, washing, and staining experiments. The pH acidity was monitored by feeding fluorescein isothiocyanate-dextran (FITC-dextran) containing food. Gut samples were analyzed using the Axio Imager Z2 microscope (ZEISS). All image analyses were performed on FIJI.

3. mCherry-Expressing *E. coli*

To observe the distribution of *E. coli* in the gut, tumor-injected flies were fed a non-pathogenic mCherry-expressing *E. coli* strain. For the control experiment, flies without tumor injections were first reared in the antibiotic-containing food for at least 3 days. The next day, flies were transferred into the mCherry-expressing *E. coli* inoculated food and reared for at least 7 days. Gut tissues from both samples were dissected and examined using the Axio Imager Z2 microscope (ZEISS).

Results & Discussion

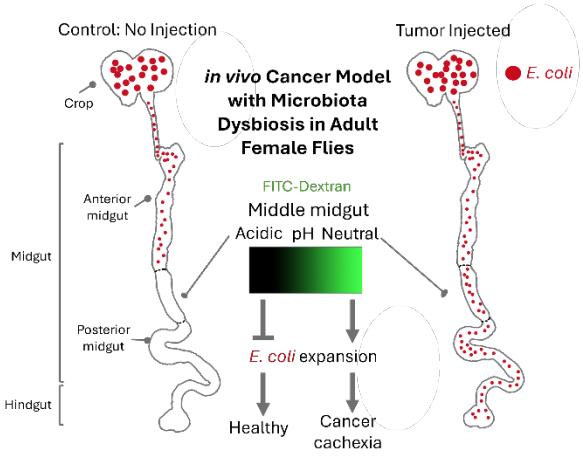

Figure 1. Diagram of a cancer cachexia model with microbiota dysbiosis in the adult female fly

Our results show that in control flies without tumour injection, mCherry-expressing *E. coli* was highly enriched in only the anterior midgut. On the other hand, after 7 and 10 days of tumor cell injection, tumor-bearing female flies had *E. coli* expanded to the entire midgut. These results show that the colonization area of *E. coli* expanded in flies with advanced stages of cancer (**Figure 1**).

Next, we examined the pH changes in the middle midgut between control and tumor-bearing flies to check if the low pH region was compromised in advanced stages of cancer. We found that control flies retained the acidic region of the middle midgut. In contrast, the middle midgut lost its acidity in the cancerous state, both in axenic and microbiota-associated conditions.

Our findings may suggest that the presence of tumors disrupts the acidic region in the middle midgut, indicating a potential link between cancer progression and compromised microbiota control in the gut. This disruption facilitates microbiota overgrowth, contributing to the development of cachectic phenotypes in tumor-bearing flies. These results provide a potential mechanistic background in the relationship between microbiota dysbiosis and cancer cachexia.

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