Applied Entomology and Zoology
https://doi.org/10.1007/s13355-025-00893-0

ORIGINAL RESEARCH PAPER

Effects of caffeine on the longevity and locomotion activity of the common green bottle fly, *Lucilia sericata* (Diptera: Calliphoridae)

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Received: 15 May 2024 / Accepted: 11 January 2025

Abstract
The common green bottle fly, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), is a promising and useful managed pollinator for greenhouse agricultural crops. The fly can pollinate at lower and higher temperatures than European honeybee. However, management of the longevity of pollinators is important for growers using greenhouses. Previous studies using other insects showed that caffeine affects insect longevity and behaviors. For instance, European honeybee live longer and have increased memory after caffeine consumption. How caffeine affects the longevity and behavior of pollinators is worth investigating because it can affect pollinator's behavior, extend longevity, or be an insecticide against pollinators. In the present study, therefore, the longevity and locomotion of *L. sericata* were investigated when they were given different caffeine concentrations. First, the longevity of *L. sericata* with five different caffeine concentrations was compared to the control. The results showed that higher concentrations of caffeine (2%, 1%, and 0.5%) significantly decreased the life span compared to lower concentrations (0.05% and 0.01%). Second, the locomotion activities of *L. sericata* were examined at those two caffeine concentrations with treated and control male and female flies utilizing a Drosophila Activity Monitor (DAM). Treatment with 0.05% caffeine dramatically reduced locomotion, but treatment of 0.01% caffeine did not. We also compared lipid concentrations of flies: flies treated with 0.05% caffeine had a lower lipid concentration compared to flies treated with 0% and 0.01% caffeine. These results indicate that caffeine had negative effects on the longevity and locomotion activities of the pollinator *L. sericata* in laboratory conditions.

Keywords Caffeine · Life span · Locomotor activity · Pollinator

Introduction

Insect pollination is particularly useful in agriculture to increase the productivity and quality of various crops (Bartomeus et al. 2014). Plant-produced alkaloids like caffeine can pharmacologically manipulate a pollinator's behavior (Strachecka et. al. 2014; Wright et al. 2013). Recently, the number of European honeybee, *Apis mellifera*, is significantly decreasing around the world due to intensive use of agricultural chemicals and global warming (Decourtye et al. 2019). In Japan, the common green bottle fly, *Lucilia sericata* (Meigen 1826) (Diptera: Calliphoridae), is attracting great attention as a new pollinator due to its effectiveness in the pollination of agricultural crops, such as strawberries (Hanada et al. 2016), mangoes (Mohsen 2019), and onions (Currah and Ockendon 1983, 1984). Advantages to using *L. sericata* rather than European honeybee in the greenhouse are that these flies can easily adapt under low or high temperatures, cannot sting humans, and are low cost because there is no need to prepare special facilities like hives for European honeybee (Nara Prefecture Agricultural Research and Development Center 2019, NPAR 2019). However, *L. sericata* has a shorter life span compared to European honeybee. Generally, the longevity of *L. sericata* used as managed pollinators is estimated to be about 2 weeks in greenhouses (NPAR 2019) and about 40 days in laboratories (Shimomae et al. 2022). Users needed to introduce newly emerged flies into greenhouses for strawberry cultivation every 7–10 days (NPAR 2019) according to the user's manual to ensure their effectiveness and performance. Management of the longevity of pollinators is important for growers.

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Published online: 09 February 2025

Caffeine (1, 3, 7-trimethylxanthine) is not only one of the bioactive stimulants from the family methylxanthines but also a psychoactive substance that is commonly used around the world (Fredholm et al. 1999). Caffeine can be found naturally in coffee, cocoa, plant leaves, fruits, citrus plants, and even floral nectar (Wright et al. 2013). Many studies indicated that caffeine not only extends the life span of invertebrates but also affects their behavior. For instance, caffeine extended the lifespan of adults for *Vespa orientalis* and *A. mellifera* (Ishay and Paniry 1979; Strachecka et al. 2014). Adnan et al. (2020) showed that feeding caffeine to Queensland fruit fly males accelerated sexual maturity. Caffeine also affects male courtship behavior of the red flour beetle *Tribolium castaneum* (Yuhao et al. 2020).

The effect of caffeine on activity has been observed in some invertebrates. In *Drosophila melanogaster*, caffeine-treated flies become more active than control flies depending on the caffeine dose (Shaw et al. 2000). If caffeine has the effect of extending the life span of *L. sericata* as it did in other insect taxonomic species described above, it would allow farmers to utilize the pollinator more efficiently. In considering the use of a pollinator, it is important to investigate the effects of caffeine on longevity and locomotion activities of flies. The survival time of caffeine injected fruit flies was not only related to its dosage but also to the sex (Carrillo and Gibson 2002). On the other hand, caffeine shortens the lifespan of invertebrates, including insects, suggesting that it may be an insecticide (e.g., Hollingsworth et al. 2002; Moon et al. 2006; Qush et al. 2023; Suh et al. 2017). We investigated the effects of caffeine on flies and especially the dosage. Moreover, caffeine can break down triglycerides and release free fatty acids into the bloodstream through its effects on adipose tissue metabolism (Barcelos et al. 2020) which may change lipid metabolism in other organisms, and we hypothesize similar metabolic impacts in *L. sericata* in this study. In insects, lipid levels are influenced by various factors, including developmental stage, nutritional status, sex, and environmental temperature (Ryan and van der Horst 2000). Therefore, this lipid measurement may reveal insights into how caffeine influences altering physiological functions, especially in locomotion activity and longevity of *L. sericata*. Further, caffeine treatment might affect the body fat of flies because previous studies using ants showed that the fat concentration is associated with reproductive activity in ant species (Kishino et al. 2024). Therefore, we examined whether caffeine affected the longevity, locomotion activity, and lipid concentration of *L. sericata* in laboratory conditions.

Materials and methods

Longevity

Pupae of *Lucilia sericata* were supplied by Japan Maggot Company. We placed the adult flies that emerged from pupae in adult-rearing cages (height 25 cm × width 25 cm × depth 30 cm), for experiments on longevity in the laboratory. All flies were provided sugar and were kept at 25 ± 2ºC and 16L:8D.

Before the longevity experiment, suitable sugar concentrations for the flies were explored to overcome the stickiness of sugar during the experiments. Six different sugar concentrations (2%, 3%, 4%, 5%, 6%, and 7%) were provided to flies, and their longevity was monitored. The longevity of six treatments were: average 19.24 ± standard deviation 6.86 d for 2% ($n = 25$), 22.29 ± 8.24 for 3% ($n = 24$), 24.57 ± 10.50 for 4% ($n = 22$), 14.63 ± 5.29 for 5% ($n = 23$), 18.34 ± 8.36 for 6% ($n = 25$), and 19.29 ± 7.70 for 7% ($n = 30$) (see Suppl. Figure). Because the flies fed 4% sugar water lived longer than flies exposed to other concentrations, 4% sugar was used for the following experiments.

Caffeine at 2%, 1%, 0.5%, 0.05%, and 0.01% concentrations including 4% sugar were prepared. Adult flies were provided one of the five different caffeine concentrations after they emerged from the pupae. For the control, we gave the flies only 4% sugar solution as food. Three replications were conducted for each caffeine concentration and control. For each replication, 50 pupae were placed into a small plastic container (diameter 8.6 cm × height 4.1 cm) in an insect rearing cage (height 25 cm × width 25 cm × depth 30 cm) in the chamber described above until the pupae became adults. The caffeinated solutions were provided with cotton in a plastic cup (diameter 8.6 cm × height 4.1 cm) inside the insect rearing cage. The solutions and cotton were changed every 5 days, and the number of dead flies was counted every day.

Locomotor activity

Caffeine (0.01% and 0.05%) and sugar-treated flies of *L. sericata* were used in this experiment to understand the effects of caffeine on their locomotion activities. Two different concentrations of caffeine were added to 4% sucrose water: 0.01% and 0.05%. Then young flies (1 to 4 days old) were treated with 0.01% or 0.05% caffeine after they hatched from the pupae. Treated flies were randomly selected from the insect rearing cages and anesthetized by CO_2 gas for 1 min on an Icenon cooling pad (Hakugen Earth, Tokyo). Then flies were transferred to glass tubes.

One end was closed with cotton. The other end was closed with a cap and cotton which was soaked with 0.01% or 0.05% caffeine solution or sugar solution (control) during the locomotion activities experiment. These glass tubes were placed in the locomotor activity monitoring system, model DAM, manufactured by TriKinetics Inc. USA. These monitors were set up in the incubator (25ºC, 16:8 h L:D, 65% RH). The number of times that a fly crossed over the infrared beam was recorded for every 10 s for 7 d. The first and last days of activity measurement were discarded before analyzing the data due to fly hyperactivity after handling.

Lipid measurement

Caffeine solutions (0.01% and 0.05% with water) and sugar-treated flies were subjected to fat content analysis based on the methanol–chloroform method after their death (Barnes and Blackstock 1973; Idogawa et al. 2017; Kishino et al. 2024). Whole bodies were individually put into glass vials, then dried at 80 °C for more than 24 h, and the dry mass was weighed with an ultramicrobalance. Subsequently, fat was extracted using a solvent (2:1 mixture of chloroform and methanol, Wako, Japan) by soaking the dried samples for more than 24 h in 2 ml of solvent at room temperature. After fat removal, the flies were dried at 80 °C more than 24 h and weighed again to measure their lean mass. The body fat concentration was calculated as the body fat percentage as follows: (dry mass − lean mass) × 100/dry mass. We only used 4-day-old *L. sericata* to measure lipid concentrations.

Statistics

To analyze the data of the longevity of *L. sericata*, first a one-way ANOVA *F* test was carried out to determine the difference among the groups. Then Tukey's honest significant difference (HSD) test at 95% level was used to compare the difference of caffeine effects on the longevity of the flies between groups.

For the locomotion activity data analysis, a linear model (LM) was performed to examine the effect of sex (male or female), caffeine (0%, 0.01%, or 0.05%), and age (1-day-old or 4-day old). For the lipid amount data analysis, a linear model (LM) was performed to examine the effect of sex (male or female), and caffeine (0%, 0.01%, or 0.05%). Analyses and plots were performed using R studio 2023.12.1 (package: ggplot2). RStudio software version 4.3.0 was used for statistical analyses.

Results

Longevity

The longevities of *Lucilia sericata* at five different caffeine concentrations (2%, 1%, 0.5%, 0.05%, and 0.01%) and control are shown in Fig. 1. The longest longevity of flies in the control group was 49 days after emergence, and the mean longevity was 24 days. Among flies in caffeine-treated groups, high caffeine concentrations, 2%, 1%, and 0.5%, affected the flies' longevity negatively and all flies died within 15 days. Flies treated with high concentrations

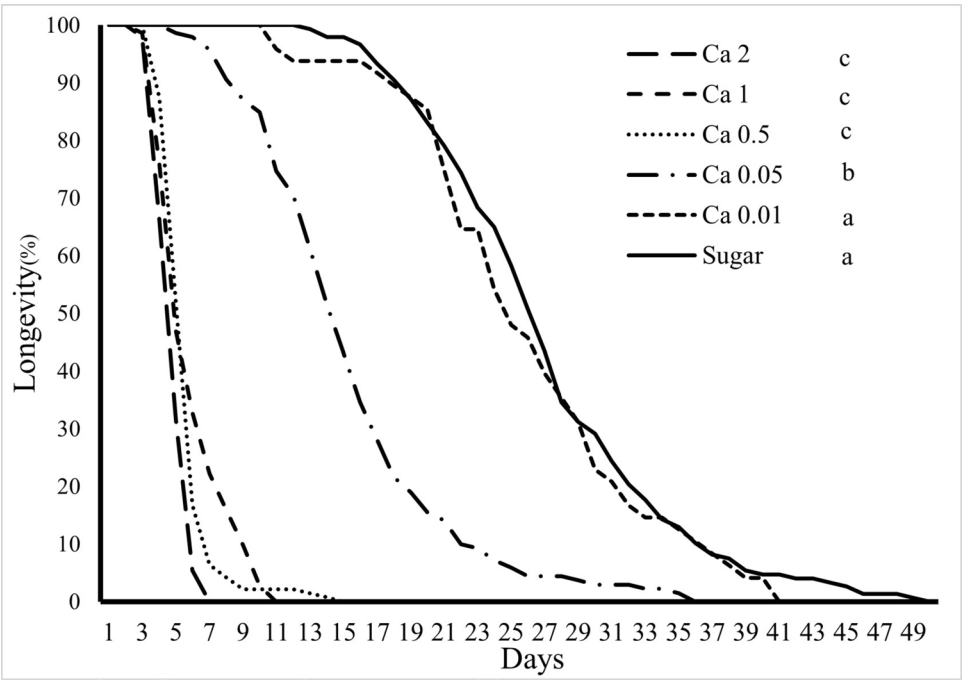

Fig. 1 Longevity of *L. sericata* treated with five different caffeine concentrations (2%, 1%, 0.5%, 0.1%, and 0.01%) and sugar (control). The same letters are not significantly different at 5% level by Tukey's honest significant difference (HSD) test

(2%, 1%, 0.5%) started to die quickly from 4 days after they emerged from pupae. After 9 days, 90% of the population had died and only 10% of its population lived the remaining 6 days. The longevity of flies treated with caffeine 2%, 1%, and 0.5% was significantly shorter than other that of files treated with caffeine 0.05%, 0.01%, and sugar (control) (Tukey's HSD test, $p = 0.05$). On the other hand, flies treated with low concentrations of caffeine (0.05% and 0.01%) lived up to 35 and 40 days, respectively. The longevities of flies treated with 0.01%, 0.05% caffeine and sugar (control) were significantly longer than the others among the group (Fig. 1, Tukey's HSD test, $p = 0.05$).

Locomotor activity and lipid concentration

We measured locomotor activity of *L. sericata* with long-term exposure to low concentrations of caffeine 0.01% and 0.05%, to determine whether their locomotor activities were changed or not. Figure 2 shows that total locomotor activity for 5 days treated with caffeine 0%, 0.01%, and 0.05% caffeine. Figures 3 and 4 present actograms illustrating the locomotion activities of 1-day-old and 4-day-old male and female *L. sericata*, respectively, treated with varying concentrations of caffeine 0% (control), 0.01%, and 0.05% over 6 days. For 1-day-old males and females, locomotion activity significantly decreased under the 0.05% caffeine treatment compared to the 0.01% and control groups (male and female, $p < 0.001$; Figs. 2 and 3; Table 1). However, for 4-day-old males and females, no statistically significant differences in locomotion activity were observed across the caffeine treatments (male $p = 0.33$ and female $p = 0.67$, Figs. 2 and 4; Table 1). These results indicate that the effects of caffeine on locomotion activity are age-dependent, with younger individuals showing greater sensitivity to higher caffeine concentrations.

Moreover, a linear model (LM) showed that the locomotor activities were significantly affected by caffeine 0.05% (estimate = − 2361.7 ± 692.5, $p < 0.001$; Table 1). With increasing caffeine intake, activity level decreased on day 1 after caffeine exposure but did not change on day 4 (Fig. 2). Age also affected locomotor activity (estimate = − 963.1 ± 191.5, $p < 0.001$; Table 1), but sex did not affect locomotor activity (estimate = 407.1 ± 560.1, $p = 0.468$; Table 1).

The lipid concentrations of flies treated with 0.01% and 0.05% caffeine at 1 day after exposure to caffeine were significantly decreased compared to flies treated with 0% caffeine as control (Fig. 5, Table 2, LM: $p < 0.01$). There were no differences on lipid amount between male and female (Table 2, LM: $p > 0.05$).

Discussion

NPAR (2019) showed that *L. sericata* survives only 1 to 2 weeks in greenhouses, but Shimomae et al. (2022) showed that the longevity of *L. sericata* was significantly extended (ca. 40 days) in the laboratory when sugar in water was supplied compared to the cases without sugar and/or water. In the present study, caffeine did not prolong the lifespan of *L. sericata* as it did in previous studies of other species; rather, high concentrations of caffeine significantly shortened the lifespan. According to Abdelkader et al. (2013) and Wang et al. (1998) findings, there is toxic effects of caffeine on insects. Caffeine may inhibit phosphodiesterase (PDE) activities and prevent the breakdown of cyclic adenosine monophosphate (cAMP), leading to increased intracellular

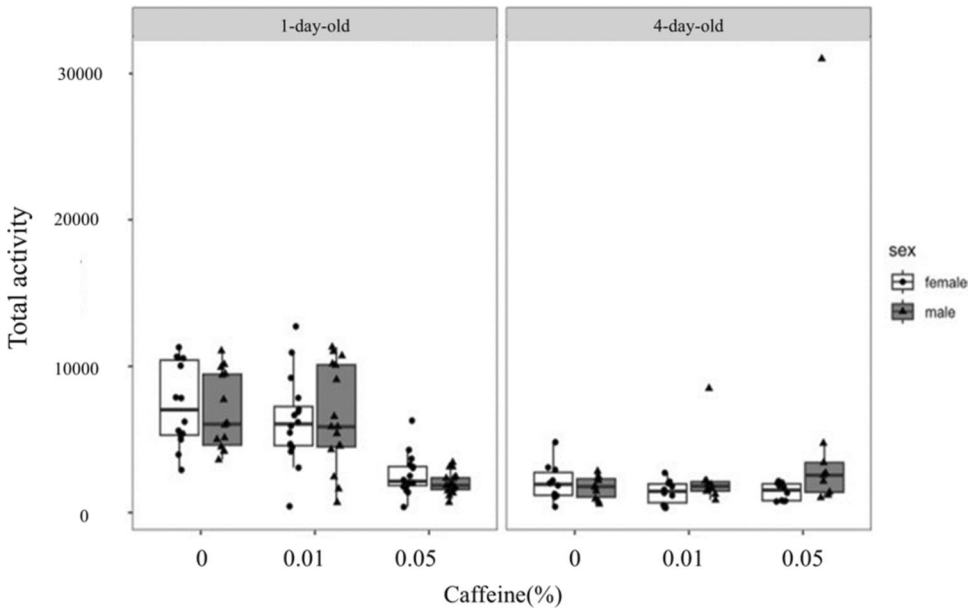

Fig. 2 Locomotion activity of 1-day old (left graph) and 4-day old (right graph) for both sexes of *L. sericata*. *L. sericata* was treated with caffeine 0%, 0.01%, and 0.05% caffeine in the DAM system for 5 days. Circle and triangle indicate male and female, respectively

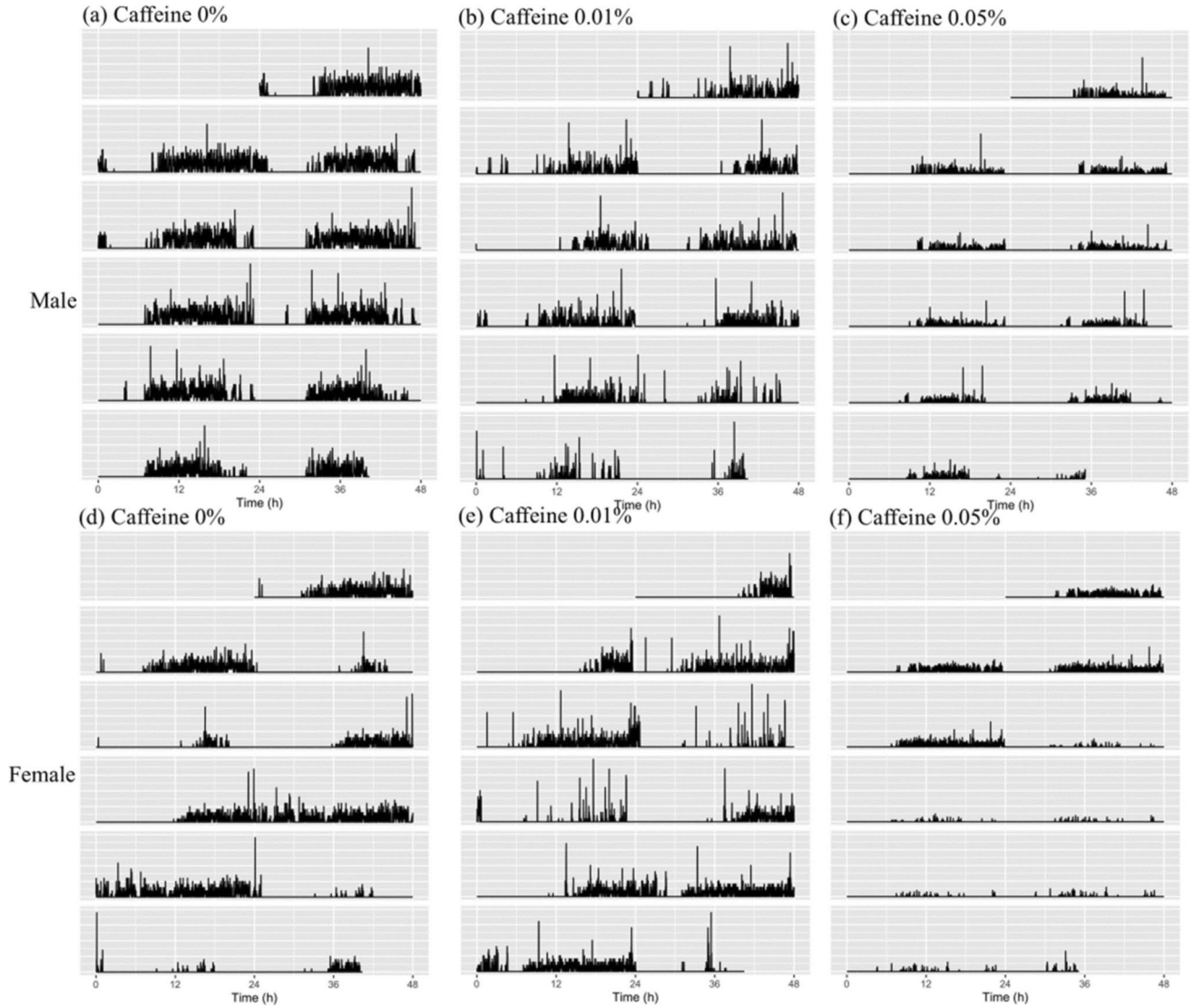

Fig. 3 Representative actograms of locomotion activities of 1-day-old male (above) and female (bottom) *L. sericata* over 6 days under three caffeine treatments: 0% (**a**, **d**), 0.01% (**b**, **e**), and 0.05% (**c**, **f**). The experiments were conducted under a light–dark (LD) cycle of 16:8 at constant 25℃

levels of cAMP. Those might disrupt normal physiological processes, including metabolic functions, potentially causing toxicity and leading to decrease the longevity in *L. sericata*.

The locomotor activities of 1-day-old *L. sericata* were significantly decreased by 0.05% caffeine but not by 0.01% caffeine. Adult locomotor activity is crucial for effective insect pollination in greenhouses. Our findings reveal that 1-day-old *L. sericata* exhibited significantly higher locomotion activity compared to 4-day-old flies. This suggests that 4-day-old flies might have developed mechanisms to metabolize or adapt to caffeine exposure. In 1-day-old flies, locomotion activities slightly increased with prolonged exposure to low caffeine concentrations (0.01%), supporting previous studies showing hyperactivity induced by caffeine in both mammals and *Drosophila* (Matsuoka et al. 1987; Shaw et al. 2000). However, 1-day-old flies treated with 0.05% caffeine showed a significant reduction in locomotion activity, aligning with biphasic caffeine effects reported in honeybee and mice. For instance, Malechuk (2009) observed enhanced locomotion at low caffeine doses but reduced activity at high doses in honeybees. Similarly**,** Yacoubi et al. (2000) demonstrated decreased locomotion in mice at high caffeine doses. Unexpectedly, locomotion activities in 4-day-old flies treated with either 0.01% or 0.05% caffeine were not significantly different from the control (sugar-fed) group, suggesting age-dependent metabolic or behavioral responses to caffeine.

We investigated how caffeine affects lipid metabolism in *L. sericata*. The lipid concentrations of flies treated with

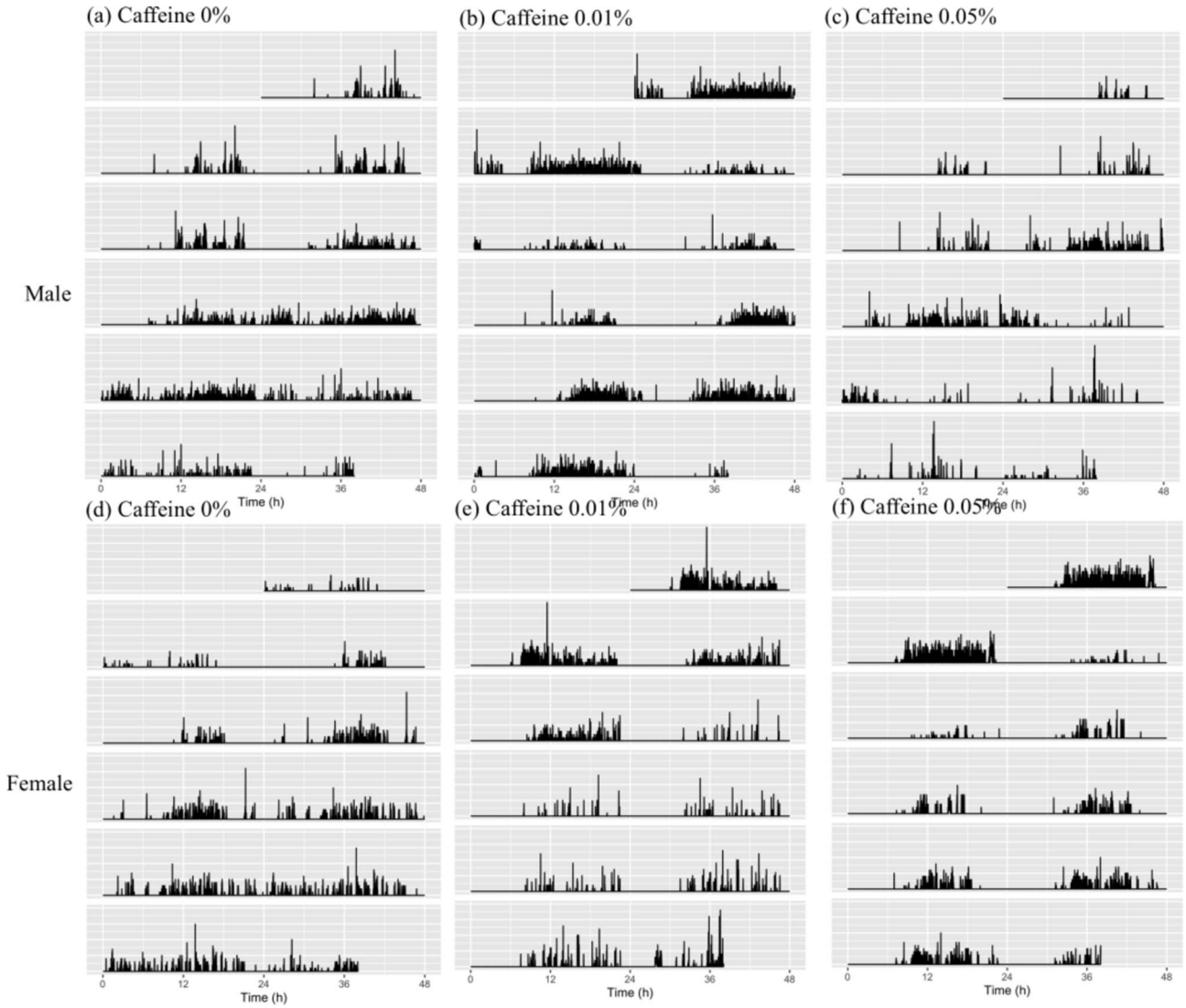

Fig. 4 Representative actograms of locomotion activities of 4-day-old male (above) and female (bottom) *L. sericata* over 6 days under three caffeine treatments: 0% (**a**, **d**), 0.01% (**b**, **e**), and 0.05% (**c**, **f**). The experiments were conducted under a light–dark (LD) cycle of 16:8 at constant 25 ℃

Table 1 Results of a linear model for the effects of sex, age, and caffeine concentrations on locomotor activity

Variables	Estimate	Standard error	*t* value	*p* value
(Intercept)	6955.7	637.1	10.918	< 0.001
Sex	407.2	558.3	0.729	0.466
Caffeine	− 47,882.4	12,943.4	− 3.699	< 0.001
Age	− 963.6	190.8	− 5.051	< 0.001

0.01% and 0.05% caffeine were significantly decreased compared to flies with 0% caffeine as control (Fig. 5). These data indicate that *L. sericata* flies suffered from a high dose of caffeine by shortened longevity, lower activity, and decreased lipid concentrations. Moreover, we only used 4-day-old *L. sericata* to measure lipid concentrations. No significant changes in locomotion activity were observed at caffeine concentrations of 0.01% and 0.05%, while lipid concentrations significantly decreased at these doses. This suggests that, although the flies' locomotion activity did not change significantly, caffeine still influences their metabolic processes, potentially altering energy stores without affecting locomotion activity. Since lipids serve as the primary energy reserves in insects, supporting critical processes such as embryogenesis, growth, development, metamorphosis, diapause, reproduction, and flight (Ryan and van der Horst 2000), the observed significant decrease in lipid content at 0.01% and 0.05% caffeine concentrations in our study may suggest a potential metabolic impact in *L. sericata*. This decrease might help explain caffeine's

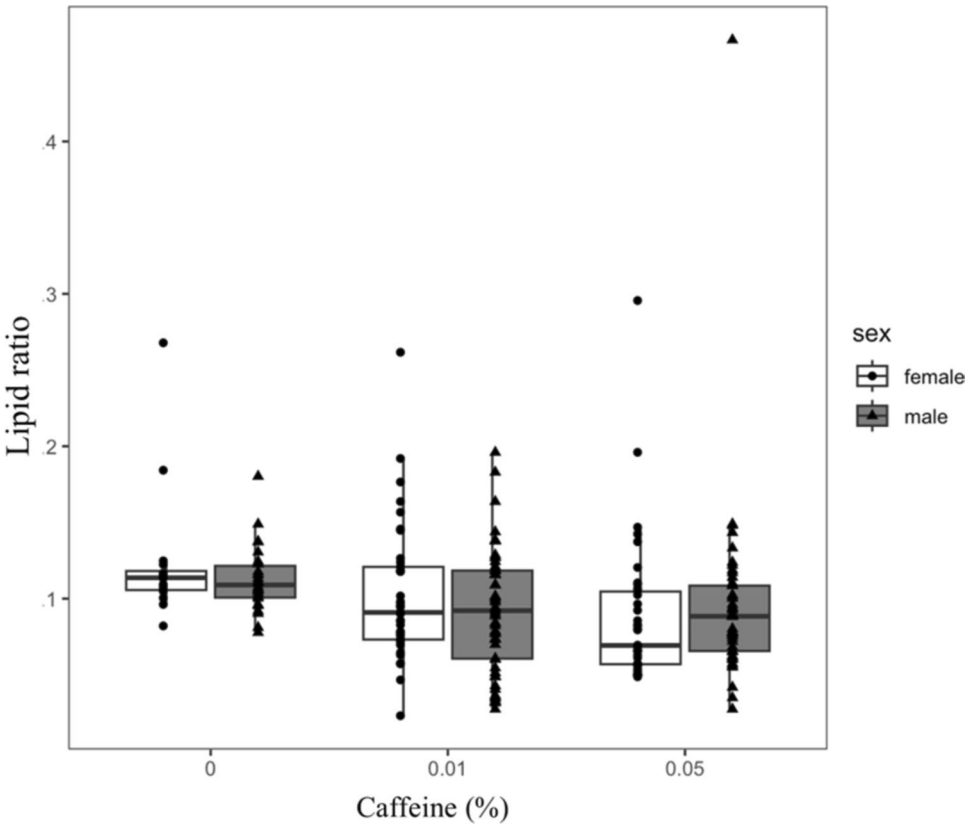

Fig. 5 Lipid amount per dry weight for both sexes of flies (*L. sericata*) treated with 0%, 0.01%, and 0.05% caffeine. Circle and triangle indicate male and female, respectively

Table 2 Results of a linear model for the effects of sex, and caffeine concentrations on lipid amount

Variables	Estimate	Standard error	*t* value	*p* value
(Intercept)	0.12553	0.01073	11.691	< 0.001
Sex	− 0.00399	0.00636	− 0.627	0.531
Caffeine	− 0.01132	0.00426	− 2.654	< 0.01

physiological influences, including alterations in longevity, locomotion activity, and lipid concentration, even at low concentrations like 0.01%. Furthermore, there is a correlation between changes in locomotor activity and a decrease in lipid concentration. Caffeine's effects on the neurological system temporarily increase activity, but supported depletion of insect's energy reserves eventually affects longevity and locomotion activity. In a cricket *Gryllus bimaculatus* and the tsetse fly *Glossina palpalis*, lipid concentrations are influenced on age (Adlington et al. 1996; Anand and Lorenz 2008). Also age and fat mass are related to activity level in the migratory locust *Locusta migratoria migratoriodes* (Strong 1968) and an ant *Diacamma cf. indicum* (Kishino et al. 2024). However, the effects of caffeine on lipid concentration have, to our knowledge, been little studied. In the present study, it was shown that lipid concentration was reduced with increasing doses of caffeine. It would be worthwhile in the future to investigate in detail the effects of caffeine on insect age, activity level, and lipid concentration.

It is interesting that caffeine at 0.05% significantly lowered activity and clearly shortened the lifespan of adults on day 5 post-hatching in the present study. The present results showed that caffeine had a negative effect on activity and longevity, suggesting treatment with caffeine may cause toxicity. Therefore, it may be assumed that a high concentration of caffeine could be used as an insecticide for pest control against *L. sericata*. Caffeine has a potential insecticide effect because it repels insects (Moon et al. 2006). In *Aedes aegypti*, caffeine toxicity has been shown to inhibit larval growth and serve as an adult control of field population (Laranja et al. 2006). Hollingsworth et al. (2002) reported that caffeine is effective in killing or repelling slugs and snails when applied to foliage. The present results were nearly identical to the results of *D. melanogaster* (Qush et al. 2023; Suh et al. 2017): higher concentrations of caffeine had a negative effect on the survival of fruit flies. The fact that caffeine had a negative effect on the longevity of *L. sericata*, as has been suggested for other organisms including insect (Hollingsworth et al. 2002; Moon et al. 2006), is a novel result of the present study. Therefore, the present results may contribute to future studies for insecticides and control because caffeine has a negative effect on lifespan, activity, and lipid concentration in Dipteran insects.

Contrary to the present results, caffeine has been found to increase the lifespan and memory of bees and wasps (Ishay and Paniry 1979; Strachecka et al. 2014). Future research into how chemicals, including caffeine, may prolong the lifespan and modify the behavior of insects.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13355-025-00893-0.

Acknowledgements We thank Takuya Sato (Japan Maggot Company, Co. Ltd) for providing us *Lucilia sericata*. This work was supported by a grant from the Japan Society for the Promotion of Science KAKENHI 21H02568, 21K19116, and 23K21343, (to TM). We are grateful to the reviewers for many useful suggestions.

Funding Open Access funding provided by Okayama University. Japan Society for the Promotion of Science, 23K21343, Takahisa Miyatake, 21K19116, Takahisa Miyatake, 21H02568, Takahisa Miyatake.

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