



The Impact of Different Mating Systems on Locomotion in *Drosophila melanogaster*

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Abstract The impact of mating systems on individual behavior and physiology is a significant topic within evolutionary biology and behavioral ecology. This study aimed to explore the effects of different mating systems on locomotion in *Drosophila melanogaster*. By employing various mating contexts such as virginity, monogamy, polygamy with mated partners (PMP, where mated partners are rotated daily), and polygamy with virgin partners (PVP, where virgin partners are rotated daily), this study measured the locomotion levels, including the activity level and the average movement speed, of both male and female fruit flies. The results indicate that virgin males exhibit greater locomotion, PMP males show a significant decrease in locomotion, and the locomotion of PVP males decreases over time. In contrast, female locomotion does not significantly differ across mating systems. These findings highlight the influence of

mating behavior on male physiological states and suggest potential implications for lifespan related to mating systems. This research provides novel insights into the impact of mating systems on behavioral and physiological mechanisms in animals.

Keywords Virgin · Monogamy · Polygamy · Sex differences · Behavioral analysis · Fruit fly

Introduction

In evolutionary biology and behavioral ecology, examining the effects of different mating systems on physiological and individual behavior is crucial. The insect world exhibits an immense variety of mating phenomena, where diverse mating systems can coexist within a single species but demonstrate significant diversity across natural populations (Alexander et al. 1997; Gillott 2018). This diversity implies that various mating systems can have a profound effect on the physiological health and behavioral patterns of insects. For example, mating systems that vary from monogamy to polygamy can influence the reproductive strategies of individuals (Clutton-brock and Parker 1995; Shuker and Simmons 2014), lifespan (Partridge and Farquhar 1981; Iliadi and Boulianne 2010; Túler et al. 2018; Koppik et al. 2018), and social interactions (Shuker and Simmons 2014; Klibaite and Shaevitz 2020), thereby influencing population structure and the dynamics of evolution (Vartak

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et al. 2015). Understanding these impacts is critical for unraveling the mechanisms sustaining biodiversity and adaptive evolutionary processes.

The fruit fly *Drosophila melanogaster*, widely used as a model organism owing to its unique biological characteristics—including high genetic variability, short lifespan, and complex behavior patterns—serves as an ideal subject for these studies. In natural environments, fruit flies exhibit a wide range of mating systems, including monogamy and polygamy, which may be related to the mating success of both sexes (Baxter et al. 2018). Female fruit flies tend to achieve their maximum reproductive potential through selective mating, often requiring only a limited number of copulations; in contrast, male fruit flies increase their mating frequency to increase reproductive success (Vartak et al. 2015). Additionally, fruit flies display sex differences in wing expansion, first feeding, first excretion, and courtship during the period from eclosion to first mating (Seong et al. 2023). Moreover, previous research has revealed substantial sex differences in the propensity of fruit flies to remate following their initial copulation (Long et al. 2010). Females exhibit a markedly extended refractory period post mating (Manning 1967; Aigaki et al. 1991; Chapman et al. 1995), which could lead to a higher rate of sexual rejection for males in a cohabiting environment (Dukas and Dukas 2012). These differences in reproductive behavior not only highlight the distinct strategies employed by each sex but also potentially impact their locomotive abilities (Qiu et al. 2021).

In assessing the vitality of fruit flies, locomotion is considered a key indicator of the physiological state and ecological adaptability, impacting resource acquisition (Nakazato et al. 2001), predator avoidance (Hendrichs et al. 1991; Herberholz and Marquart 2012), mate seeking (Nilsen et al. 2004; Yamamoto and Koganezawa 2013), and territory defense (Kravitz and Fernandez 2015), thus directly influencing reproductive success (Clutton-brock and Parker 1995; Qiu et al. 2021) and survival competition (Hendrichs et al. 1991). However, despite studies exploring the impact of mating systems on fruit fly reproductive strategies and survival rates (Economos et al. 1979), there is still a lack of understanding regarding how these systems intricately affect the basic physical abilities of fruit flies, especially their locomotive capabilities. In particular, changes in locomotive

ability following sexual rejection have emerged as critical areas for further investigation and deserve comprehensive research.

This study is dedicated to systematically examining the specific impacts of different mating systems on the locomotive abilities of both male and female fruit flies, aiming to reveal the interactions between reproductive behaviors and individual physiological states and to explore how these mating systems are shaped and optimized through evolution. We hypothesize that mating behaviors and subsequent reproduction may significantly affect the locomotion of male fruit flies, especially under polygamous systems where males may expend more energy seeking and competing for mates, potentially reducing their overall locomotion. This impact might be less pronounced in females than in males because of biological differences in reproductive behaviors and strategies. By investigating these hypotheses, we selected four common mating systems of fruit flies in nature, modified from the experimental setups used in a previous study (Vartak et al. 2015): virgin (no mating experience), monogamy (only a single partner), polygamy with mated partners (PMP, with different mated partners rotated daily), and polygamy with virgin partners (PVP, with different virgin partners rotated daily). We analyzed the locomotion levels of both sexes across these four mating systems, aiming to offer fresh insights into the impact of mating systems on fruit flies. This research enriches our understanding of the interaction between behavior and physiology and provides a theoretical foundation for broader biological fields, particularly in understanding the interactions between mate choice, mating behavior, and the physiological states of individuals. Furthermore, the findings of this study may also provide theoretical support for similar phenomena observed in other species, thereby enriching our overall understanding of biodiversity and behavioral adaptability.

Methods

Fly Strains

Fruit flies were cultured in incubators at a temperature of 25°C with a humidity ranging between 40 and 60% under a 12-h light–dark cycle. The lights were on from 8:00 am to 8:00 pm and off from 8:00 pm

to 8:00 am. The wild-type strain w^{1118} flies, obtained from the Vienna Drosophila Resource Center (VDRC), were used as a standard wild-type strain, with the FlyBase ID: FBgn0003996 CG2759.

The Tracking System

To track the locomotion levels of fruit flies under different test conditions, we captured their movement trajectories via our custom-developed system. We employed the Mrobo D3 night vision HD sports camera to record the movement of the fruit fly, with video output in AVI format, a resolution of 1920 * 1080P, and a frame rate of 24 fps. The video setup included eight evenly spaced test tubes, each containing a single fly, separated by dividers to prevent interference. For efficient tracking, videos were edited into eight separate clips, each featuring one test tube, converted to 1920i resolution in MP4 format, and muted. Movement tracking was subsequently conducted via a Python 3.5 script that uses optical flow techniques to track and record the trajectory of the fruit fly. This was achieved by analyzing the visual displacement of objects between two consecutive frames, capturing the position changes and accurately plotting their movement paths as x and y coordinates in pixels.

The Experimental Design

To explore the impact of various mating systems on fruit fly locomotion, we carefully selected healthy virgin male and female fruit flies from the w^{1118} white-eyed strain, which is known for its standard genetic background, and placed them in conical flasks filled with fresh culture media for breeding. After the initial batch of pupae emerged, the adult flies were removed, and the newly hatched virgin flies were sorted into fresh test tube cultures every six hours. Each fly was placed in its own tube and labeled. This process continued until there were no further observations of emerging virgin flies.

In the preliminary phase of our experiment, we selected 4 virgin male and 4 virgin female flies. The preexperiment phase started one day after the flies had emerged, with each group being carefully transferred to individual test tubes. The tubes used were round-bottom glass test tubes with a diameter of 15 mm and a length of 100 mm. These tubes were then placed in an incubator (see the Fly Strains section for

detailed incubator setups) designed to simulate natural environmental conditions, with a lighting schedule regulated by a timer to mirror the natural day cycle. We tracked the flight paths within these tubes over a 24-h period. We subsequently utilized a Python 3.5 script to quantify their activity levels and movement speeds, specifically identifying the 1-h window where fly activity was at its peak for closer observation in subsequent experiments.

For the main experimental phase, the flies were grouped according to their mating system into six conditions: male virgin (males housed in pairs without females), female virgin (females housed in pairs without males), monogamy (one male to one female), PMP (males and females rotated among partners), PVP male (one male with multiple virgin females), and PVP female (one female with multiple virgin males) (Fig. 1). Each group was placed under the same environmental conditions and cultured for seven days, with daily changes to the test tubes.

Specifically, the virgin male group included four virgin male flies paired and cultured in two test tubes, and the virgin female group comprised four virgin female flies paired and cultured in two test tubes. The monogamy group consisted of four pairs of virgin male and female flies cultured in four test tubes. The PMP group included eight pairs of virgin male and female flies cultured in eight test tubes. The PVP male group and the PVP female group each had six virgin male and female flies, with each group's six flies cultured individually in six test tubes. These are the sample sizes after the removal of outliers.

During the seven-day period, the virgin male group, the virgin female group, and the monogamy group received no additional treatments. For the PMP group, female flies were transferred daily to a new test tube, effectively changing their male partners; for example, the female in tube 2 was moved to tube 1, the female in tube 3 was moved to tube 2, and so on. In the PVP male group, a new virgin female was introduced to each of the six test tubes daily, and the previous day's female was removed. Similarly, in the PVP female group, a new virgin male was introduced to each of the six test tubes daily, and the previous day's male was removed.

Our study aimed to explore the effects of different mating systems on the locomotion of fruit flies. Therefore, on the basis of previous studies (Chi et al. 2020; Han et al. 2021a, b, 2024), we designed

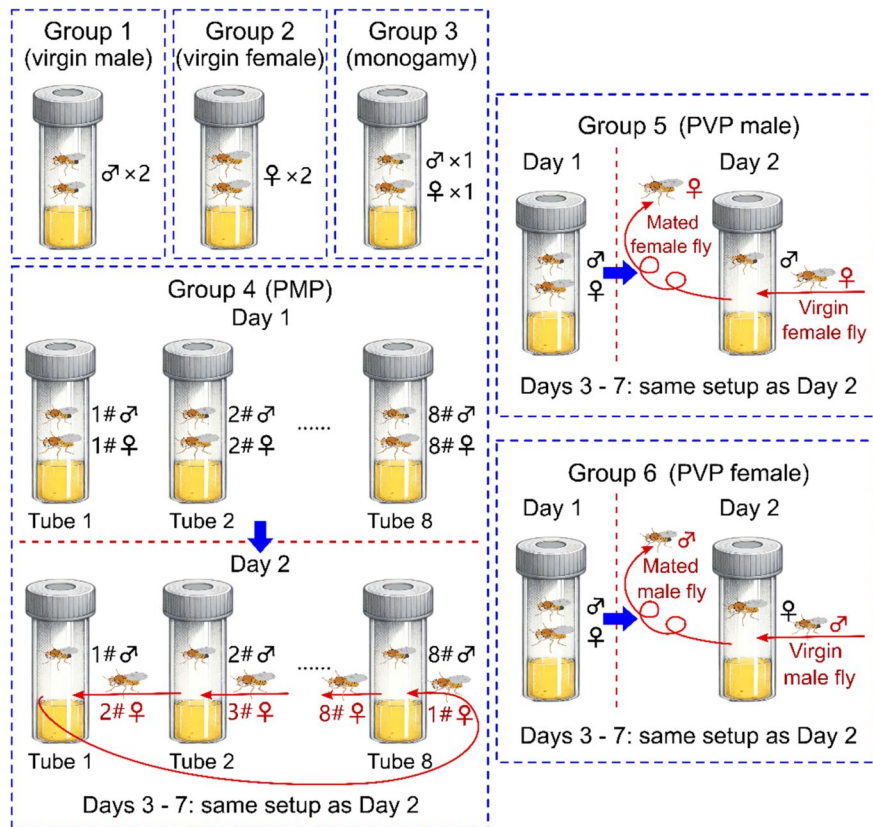


Fig. 1 Schematic representation of the experimental protocol for different mating systems in *Drosophila melanogaster*. Group 1, the male virgin group, had two male virgin flies per tube. Group 2, the female virgin group, contained two female virgin flies per tube. Group 3, the monogamy group, contained one male fly and one female fly per tube. Group 4, the polygamy with mated partners (PMP) group, started with one unmated male and one unmated female per tube, with the female flies rotating to the next tube daily. Group 5, polygamy

with virgin partners for males (PVP males), started with one unmated male and one unmated female per tube. Each day, a new unmated female was introduced, while the female from the previous day was removed. Group 6, polygamy with virgin partners for females (PVP females), followed a similar protocol with unmated males introduced daily. All the groups were cultured under identical conditions for 7 days with daily tube changes.

behavioral measurement and analysis methods to quantify locomotor indices. We first recorded the activity of virgin male and female flies in test tubes over a day via our custom-developed system and employed a Python 3.5 script to capture the position of fruit flies in every frame of the video. The positions were recorded as x and y coordinates in pixels to track their movement trajectories, with periods of inactivity lasting over five minutes being noted as sleep duration. Furthermore, we documented the total sleep duration of each virgin fly every 30 min for statistical analysis.

After the seven-day culture period, each fruit fly was isolated in individual test tubes and cultured for

an additional six days, after which the culture medium was changed every 48 h. All the treatments and sexes were measured simultaneously to ensure consistency in the environmental conditions and minimize variability due to time differences. During this period, we recorded the daily activity levels and average movement speeds of each fly.

Data Analysis

To analyze the locomotion level of fruit flies, we defined the activity level and average movement speed, which quantify the ability of fruit flies to move over time. The activity level is defined as the

percentage of points at which the fruit fly moves within a unit of time relative to all captured points of the fruit fly, whereas the average movement speed is calculated as the total distance moved divided by the unit of time.

Statistical Analysis

During the data analysis, we identified outliers based on each fruit fly's activity levels and average movement speeds. In each group, if a fruit fly exhibited activity levels or movement speeds that were more than two standard deviations below the group mean on any given day, all six days of data for that fly were considered outliers and excluded from the analysis. For male fruit flies, the number of excluded flies in each group was: 2 in the virgin group, 2 in the monogamy group, 1 in the PMP group, and 1 in the PVP group. For female fruit flies, the number of excluded flies was: 1 in the virgin group, 3 in the monogamy group, 2 in the PMP group, and 1 in the PVP group. This strategy was used to ensure that the exclusion of individuals would not interfere with the overall interpretation of the data.

All the statistical analyses were performed via SPSS 22.0 (Statistical Product and Service Solutions version 22.0) software. Descriptive statistics were used to examine the sleep duration of fruit flies at various times throughout the day.

Since the activity level data were expressed as percentages, a logit transformation was applied to ensure normality, which is required for parametric tests. Additionally, a generalized linear mixed model (GLMM) was employed to analyze the activity level data using a binomial distribution. GLMM was chosen instead of a generalized linear model (GLM) because the data involved repeated measurements for each individual fruit fly, leading to correlations within subjects. By incorporating individual ID as a random effect, GLMM accounts for within-subject correlations and between-individual variability, providing a more accurate representation of the data structure compared to GLM, which assumes independence between observations. To model the activity level data, the number of active events (the number of movement points recorded within a unit of time) was used as the outcome variable, and the total trial counts (the total number of

points recorded within a unit of time) were used as the denominator in the binomial distribution, ensuring the correct handling of the proportion data. We used GLMM to analyze the main effects of mating system and sex on activity levels, as well as the interaction effects of mating system, sex, and time. Post-hoc tests were conducted to compare specific group differences.

For the activity levels and average movement speeds of virgin male and virgin female fruit flies, independent sample T-tests were used for comparison. The initial measurements of activity levels were logit-transformed to ensure normality, while the initial measurements of average movement speeds were not transformed. In addition, for changes in the average movement speeds of fruit flies under different mating systems, repeated measures ANOVA was used to analyze the main effects and interaction effects of mating system, sex, and time. Similarly, the average movement speed data were not transformed. The specific analysis steps are as follows:

Main effects analysis: Main effects analysis was used to examine the effects of different mating systems (virgin, monogamy, PMP, and PVP) on the activity levels (analyzed using GLMM) and average movement speeds (analyzed using ANOVA) of male and female fruit flies. The main effect of sex was also analyzed to determine overall differences between male and female fruit flies.

Interaction effects analysis: GLMM was used to analyze the interaction effects of mating system, sex (male or female), and time (six days) on activity levels, while repeated measures ANOVA was used to analyze the interaction effects on average movement speeds.

Model simplification: If the interaction effects are not significant, the model will be simplified by removing nonsignificant interaction terms.

Post-hoc test: Since multiple post-hoc tests were conducted in this study, we applied the Bonferroni correction method to adjust for the bias in significance levels caused by multiple testing and to reduce the risk of false positives. This method was used to analyze the differences between different time points within each mating system, as well as the specific differences between the sexes within each mating system.

Results

The Sleep/Locomotor Rhythm of Virgin Fruit Flies

To understand the time with the most significant changes in locomotion across different mating systems in fruit flies (Fig. 1, see Methods for details), we initially documented the daily sleep duration of both virgin male and virgin female flies, following previously described methodology (Ho and Sehgal 2005; Harbison and Sehgal 2009; Negel-spach et al. 2022). For the w^{1118} strain, we observed

longer sleep durations in virgin female flies than in virgin male flies, indicating greater locomotion levels in males (Fig. 2a). Both sexes presented shorter total sleep durations per half-hour at the light–dark transition, indicating "morning peaks" and "evening peaks" in locomotion. This pattern aligns with the literature, suggesting that w^{1118} strain flies prefer mating during the "evening peak." We chose the hour before lights were turned off for daily tracking of fly movement across groups. To quantify locomotion, we defined "activity level" and "average movement speed" (Fig. 2b–c; see Methods for details). In

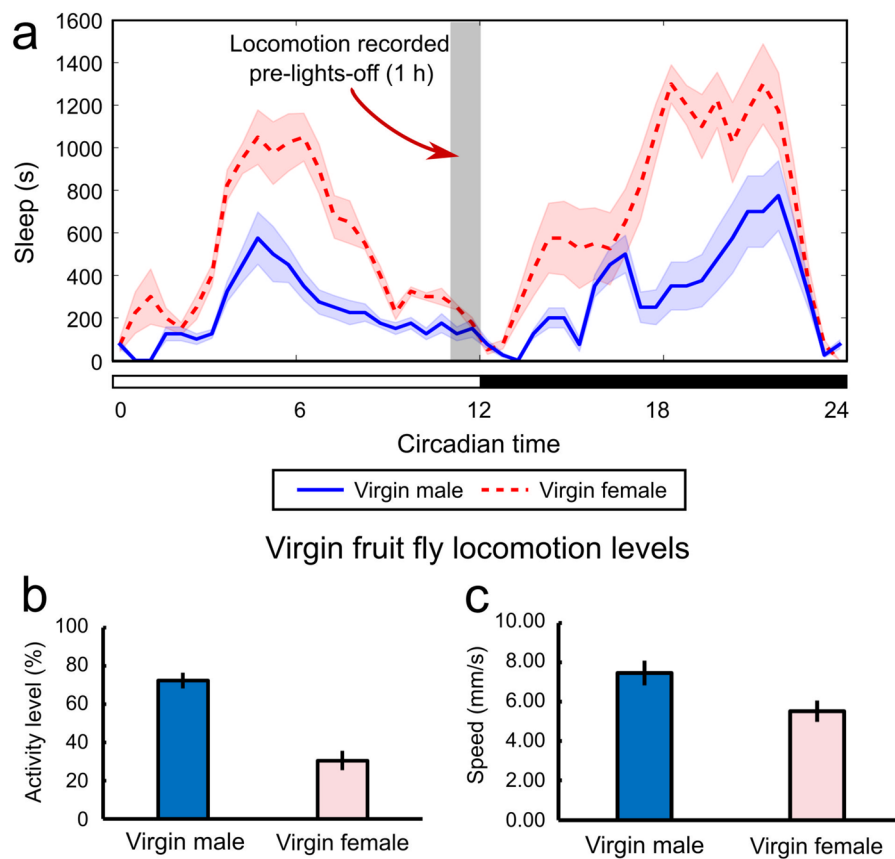


Fig. 2 Sleep duration and locomotion level of virgin male and virgin female *Drosophila melanogaster* over a 24-h period. **a** The sleep duration (seconds) of virgin males (blue solid line) and virgin females (red dashed line) was recorded throughout a 24-h period via a custom-developed tracking system. Sleep duration was captured at 30-min intervals. The black and white alternating bars below the graph indicate the circadian time of light (12 h) and dark (12 h), respectively. The blue and red shaded areas indicate the standard errors of the means for virgin males and virgin females, respectively. The gray shaded

area indicates the hour preceding lights-off, which was selected for detailed locomotion analysis. **b–c** Activity level (%) and average movement speed (mm/s) of virgin males (blue bar) and virgin females (pink bar) during the hour preceding lights-off. The activity level is defined as the percentage of time points at which the fly moves within a unit of time relative to all captured points (independent sample T-test). The average movement speed is calculated as the total distance moved divided by the unit of time. Both the activity level and average movement speed are presented as raw data without any transformation

the statistical analysis, the activity levels were logit-transformed due to being percentage data, to ensure normality (see Methods for details). Observations revealed that virgin male flies exhibited greater activity levels than virgin female flies, although the difference approached but did not reach statistical significance ($t(6)=2.383$, Cohen's $d=1.685$, $p=0.055$). In terms of average movement speed, virgin males also showed higher values, but the difference was not statistically significant ($t(6)=1.023$, Cohen's $d=0.835$, $p=0.346$).

Mating System and Male Locomotion Level

Before analyzing the locomotion levels of male and female fruit flies separately, we first examined the overall sex differences. The analysis of the main effects showed that the effect of sex was not significant for either activity level (analyzed using the generalized linear mixed model (GLMM)) or average movement speed (analyzed using analysis of variance (ANOVA)) ($F(1, 256)=0.974$, $p=0.325$, activity level) ($F(1, 36)=0.418$, $p=0.523$, $\eta^2=0.015$, average movement speed) (see Methods for details). However, the interaction effect between sex and mating system was significant ($F(3, 256)=5.047$, $p=0.002$, activity level) ($F(3, 36)=8.225$, $p<0.001$, $\eta^2=0.468$, average movement speed).

Furthermore, the post hoc test results revealed that virgin male flies exhibited significantly greater locomotion levels than virgin female flies did (coefficient=1.903, $SEM=0.777$, $t=2.448$, $p=0.015$, 95% confidence interval (CI) [0.370, 3.435], activity level) (mean difference=3.351, $SEM=0.935$, $p=0.001$, average movement speed) (Table 1). However, after mating, the reduction in locomotion levels was greater in males than in females. Our results indicate that there was no significant sex difference

in activity levels under the PMP system (coefficient=-1.032, $SEM=0.808$, $t=1.278$, $p=0.203$, 95% CI [-2.624, 0.560]), and no significant sex differences in the average movement speed under the monogamy and PVP systems (monogamy: mean difference=1.490, $SEM=0.935$, $p=0.122$, PVP: mean difference=-1.444, $SEM=0.763$, $p=0.069$) (Table 1). These results indicate clear sex-specific differences. Based on this, we proceeded to analyze the performance of males and females separately under the different mating systems.

Next, we analyzed the activity level and average movement speed of male fruit flies under different mating systems. The main effects analysis revealed that different mating systems had a significant effect on the locomotion levels of male fruit flies ($F(3, 108)=45.942$, $p<0.001$, activity level) ($F(3, 18)=54.451$, $p<0.001$, $\eta^2=0.921$, average movement speed). Additionally, the interaction effect between different mating systems and time was significant ($F(15, 108)=2.041$, $p=0.021$, activity level) ($F(15, 90)=2.433$, $p=0.007$, $\eta^2=0.343$, average movement speed).

Further post hoc test results (where SEM denotes the standard error of the mean) revealed that, overall, the locomotion levels of virgin male flies was significantly greater than that of other mating systems (virgin vs. monogamy: coefficient=0.470, $SEM=0.562$, $t=0.846$, $p=0.405$, 95% CI [-0.648, 1.588], activity level; mean difference=3.426, $SEM=0.659$, $p=0.001$, average movement speed) (virgin vs. PMP: coefficient=3.733, $SEM=0.919$, $t=4.064$, $p<0.001$, 95% CI [1.907, 5.560], activity level; mean difference=7.879, $SEM=0.659$, $p<0.001$, average movement speed) (virgin vs. PVP: coefficient=2.504, $SEM=0.585$, $t=4.285$, $p<0.001$, 95% CI [1.342, 3.667], activity level; mean difference=5.826, $SEM=0.601$, $p<0.001$, average movement speed). The overall locomotion levels of the flies in the PMP group was significantly lower than that in the other groups (PMP vs. monogamy: coefficient=-3.263, $SEM=0.913$, $t=-3.574$, $p=0.001$, 95% CI [-5.079, -1.447], activity level; mean difference=-4.453, $SEM=0.659$, $p<0.001$, average movement speed) (PMP vs. PVP: coefficient=-1.229, $SEM=0.927$, $t=-1.326$, $p=0.188$, 95% CI [-3.072, 0.614], activity level; mean difference=-2.052, $SEM=0.601$, $p=0.025$, average movement speed). Additionally, the locomotion levels in the monogamous group was

Table 1 Post-hoc analysis results of the effect of sex (male vs. female) on locomotion levels of *Drosophila melanogaster* under different mating systems

Mating systems Type	Virgin	Monogamy	PMP	PVP
Activity level	0.015*	0.048*	0.203	0.047*
Average movement speed	0.001**	0.122	0.023*	0.069

* $p<0.05$, ** $p<0.01$, post-hoc analysis

significantly greater than that in the PVP group (coefficient=2.034, $SEM=0.576$, $t=3.532$, $p=0.001$, 95% CI [0.889, 3.180], activity level; mean difference=2.401, $SEM=0.601$, $p=0.008$, average movement speed). Surprisingly, in the PVP group, the activity level gradually decreased over time after the end of the mating process, especially from day 1 to day 3 (coefficient=1.136, $SEM=0.432$, $t=2.631$, $p=0.010$, 95% CI [0.227, 1.995], activity level; mean difference=1.293, $SEM=0.503$, $p=0.022$, average movement speed) (Fig. 3).

Mating System and Female Locomotion Level

To better understand the locomotion patterns of male and female fruit flies under different mating systems, we further analyzed the activity level and average movement speed of females across different mating systems (Fig. 4). The main effects analysis revealed that different mating systems had no significant effect on the locomotion levels of female fruit flies ($F(3, 108)=0.595$, $p=0.620$, activity level) ($F(3, 18)=1.421$, $p=0.278$, $\eta^2=0.233$, average movement speed). Additionally, the interaction effect between

different mating systems and time was not significant ($F(15, 108)=0.494$, $p=0.937$, activity level) ($F(15, 90)=0.126$, $p=1.000$, $\eta^2=1.890$, average movement speed). The results indicate that different mating systems had no significant effect on the locomotion levels of female fruit flies.

Discussion

In the present study, we explored the impact of different mating systems on the locomotion of *D. melanogaster*. We discovered that virgin males display significantly greater activity levels and average movement speeds than males within other mating systems. Interestingly, the activity of males in PMP was notably lower than that in other mating systems, whereas the activity of monogamous males was greater than that of PVP males (Fig. 5). Conversely, female locomotion levels remained unaffected across various mating systems, highlighting the distinct differences in mating systems between male and female fruit flies.

Male fruit fly locomotion levels

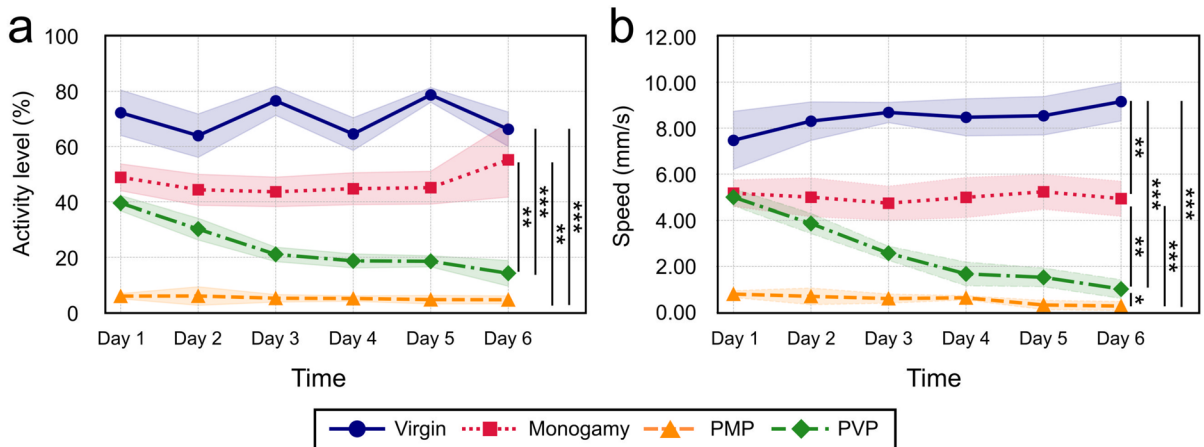


Fig. 3 Changes in locomotion levels of male *Drosophila melanogaster* over six days following different mating systems. **a-b** The activity level and average movement speed of male fruit flies under different mating systems over a six-day period. The y-axis represents the activity level (%) or the average movement speed (mm/s), and the x-axis represents the days. We used w^{1118} *D. melanogaster* as the study subject. The blue, red, orange, and green lines represent virgin males,

monogamous males, PMP males, and PVP males, respectively. The shaded area indicates the standard error for each mating system. During this period, we recorded the daily activity levels and average movement speeds of each fly. P values are indicated on the right side of each panel (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, generalized linear mixed model for activity level; repeated-measures ANOVA for average movement speed)

Female fruit fly locomotion levels

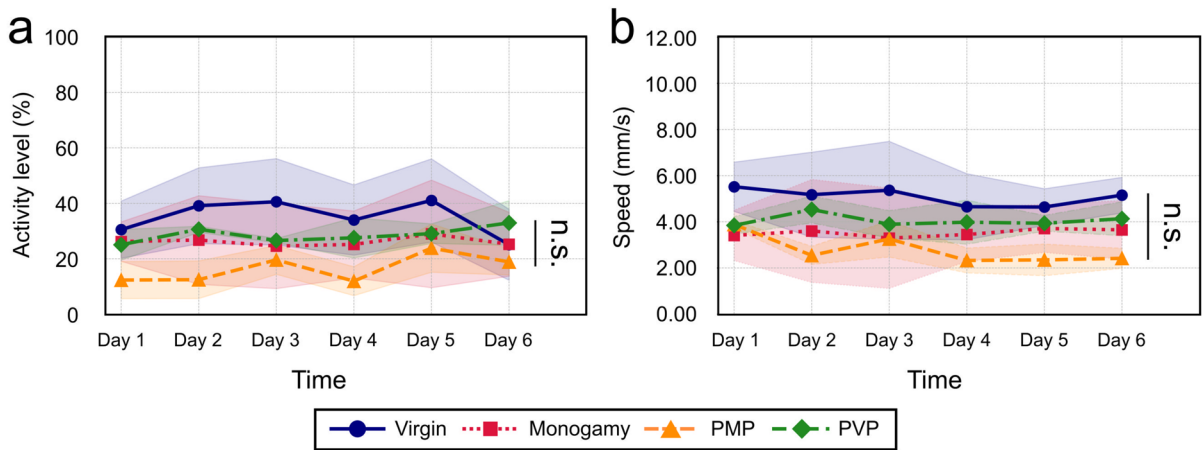
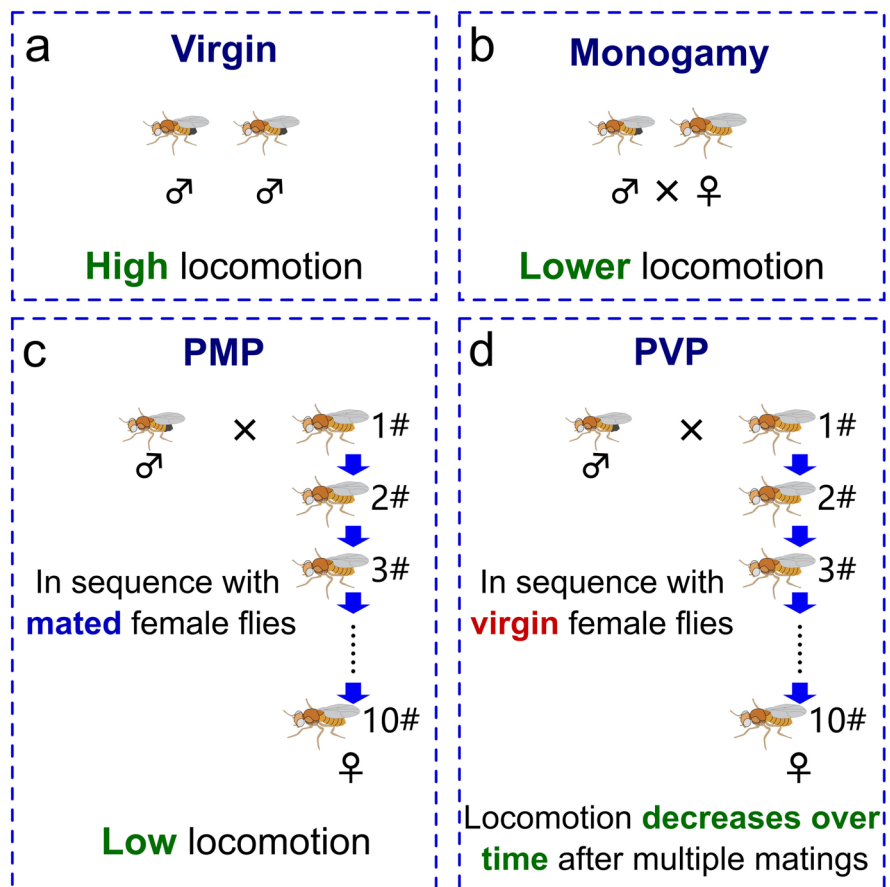


Fig. 4 Changes in locomotion levels of female *Drosophila melanogaster* over six days following different mating systems. **a-b** The activity level and average movement speed of female fruit flies under different mating systems over a six-day period.

These panels are generated following the same measurement as in Fig. 3 (n.s., no significance, generalized linear mixed model for activity level; repeated-measures ANOVA for average movement speed)

Fig. 5 Schematic diagram illustrating how different mating systems affect locomotion of male *Drosophila melanogaster*. **a** Virgin males display strong locomotion, **b** whereas monogamy reduces locomotion following mating. **c** PMP males, following successive interactions with previously mated females, exhibit significantly reduced locomotion. **d** In contrast, PVP males experienced a gradual decrease in locomotion after several days of encountering unmated females



We found that, overall, virgin males presented significantly greater locomotion levels than monogamous and PMP males did. We believe that this is related not only to the mating costs experienced by males (Fricke et al. 2008) during the mating process but also to courtship suppression (Goncharova et al. 2023). Research has demonstrated that following a single mating event, female fruit flies significantly decrease their likelihood of remating (Vartak et al. 2015). When female fruit flies do not accept mating, they exhibit behaviors such as fleeing and vibrating their wings to avoid copulation (Dukas and Scott 2015; Philipsborn 2020; Goncharova et al. 2023). In other words, male fruit flies are more likely to be rejected by mated female flies. Previous studies have also reported that male fruit flies exhibit courtship suppression when rejected by females, which can lead to a series of behavioral changes in males, such as reduced courtship behavior and decreased locomotion (Goncharova et al. 2023), which is consistent with our results.

Furthermore, when we compared the differences between monogamy and PMP, we found that male fruit flies under PMP conditions presented extremely low levels of locomotion. In our experimental setup, monogamous males were only in contact with a single partner, meaning that each male only interacted with a familiar mated female fly. In contrast, PMP males frequently interacted with different unfamiliar mated female flies. The difference in locomotion levels between males in the monogamy and PMP systems suggests that the low activity levels in PMP males cannot be explained only by the fact that mated females are unwilling to remate and thus do not require much activity from the males. The difference might be related to whether the mated females are familiar or unfamiliar. We propose that being rejected by unfamiliar females has a greater effect on the locomotion of male fruit flies than being rejected by familiar females. This impact may influence their short-term behavior and potentially have long-term effects.

Additionally, studies indicate that courtship in fruit flies is a social behavior (Dahanukar and Ray 2011; Fernández and Kravitz 2013; Corthals et al. 2017; Klibaite and Shaevitz 2020). However, social isolation (Yost 2023) can lead to reduced locomotion in fruit flies, similar to what we observed in males under monogamy and PMP conditions. Due to the study's

design, locomotion was measured in isolation, raising the question: How much of the observed decrease in locomotion is due to courtship inhibition versus social isolation? Does isolation affect different mating systems differently? Is the impact of courtship inhibition exacerbated or mitigated by isolation? We believe that different mating systems play a crucial role for male fruit flies; otherwise, there would be no significant differences in locomotion between mating systems under isolation. Future research is needed to clarify how isolation affects locomotion in fruit flies under different mating systems.

Interestingly, we observed a decrease in the activity levels and average movement speeds of males in the PVP system over time after being kept individually, a phenomenon not observed in other mating systems. Research has indicated that male fruit flies engage in complex courtship behaviors before mating, including chasing, wing vibrating, and mating attempts (Goncharova et al. 2023; Seong et al. 2023), which consume a significant amount of energy (Fricke et al. 2008). Additionally, during the reproductive process, males face significant physiological costs, which in turn affect their lifespan (Partridge and Farquhar 1981), and there is a direct relationship between the number of mating events and aging in fruit flies (Iliadi and Boulianne 2010; Koppik et al. 2018). In our study, we believe that different mating systems may result in different mating frequencies for male fruit flies. However, why do different mating frequencies lead to a gradual decline in locomotion levels over time in male fruit flies in the PVP system after isolation, rather than causing lower locomotion levels from the beginning? We propose several possible explanations for this. First, although male fruit flies expend significant energy during mating, it is worth discussing whether there might be a delay in the effects of this energy depletion. While the activity levels of fruit flies in the PVP system gradually declined in the short term, it is also worth considering whether this decline might be compensated over a longer time span as the flies gradually store energy. Unfortunately, due to the limitations of our experimental design, we were unable to observe changes in the activity levels of fruit flies over a longer time span, which is one of the limitations of this study. Future research may need to explore the effects of energy depletion on fruit fly locomotion over a longer period. Second, during the mating period, male fruit

flies in the PVP system may have experienced higher mating frequencies, and after isolation, without further mating activities, it is worth considering whether their behavioral priorities may have shifted, gradually focusing on conserving energy. Additionally, the effect of social isolation should not be overlooked. Future studies should further quantify the long-term impact of mating frequency on male activity levels after isolation and explore the role of isolation in this process.

Moreover, we observed differences in the impact of various mating systems on male and female fruit flies; in particular, female fruit flies presented almost no significant variation in behavior under different mating systems. This phenomenon may be related to the sex-specific mating behaviors of fruit flies. Previous studies have indicated that mating has a significant effect on the physiology and behavior of female fruit flies. For example, after mating, female fruit flies increase their food intake and start laying eggs. These changes are caused mainly by seminal fluid proteins transferred from males to females during mating (Chapman and Davies 2004). Additionally, after mating, females exhibit behaviors that reject further mating attempts, both of which increase the mating costs for female fruit flies (Manning 1967; Aigaki et al. 1991; Chapman et al. 1995). High mating frequencies can significantly reduce the overall lifespan and the period during which female fruit flies can successfully reproduce (Edward et al. 2011). Thus, they need to reduce mating frequency and increase intervals between matings to protect their health and enhance egg laying success (Chapman et al. 1995; Mack et al. 2003; Wigby and Chapman 2005). As a result, in addition to virgin female fruit flies, the mating frequency of female fruit flies across different mating systems might not differ significantly. The above results indicate that the impact of mating on the locomotion levels of females may not be as significant as it is for males.

In this study, we explored the effects of different mating systems on fruit flies of different sexes. Notably, courtship suppression may significantly impact the locomotion levels of male fruit flies, especially when they interact with unfamiliar mated females. On the other hand, owing differences in mating costs and courtship behaviors, the effects of different mating systems on the locomotion levels of fruit flies also significantly differ by sex.

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Data Availability The data are available upon request from Rui Han.

Declarations

Competing Interests The authors declare no competing interests.

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