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RESEARCH ARTICLE

Differential Spectral Sensitivity Influences Mating, Development, and Reproduction in Group-Housed *Drosophila melanogaster*

Rui Han[1] | Jun Zhang[2] | Yi-Han Xu[2] | Zeng-Xingyue Xiao[2] | Miao-Ling Xie[2] | Hao-Ru Xin[2]

[1]Tan Kah Kee College, Xiamen University, Zhangzhou, China | [2]School of Life Sciences, Xiamen University, Xiamen, China

Correspondence: Rui Han (hanrui901120123456@gmail.com) | Jun Zhang (jzhang@xmu.edu.cn)

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ABSTRACT

Light is an important factor affecting the behavior and physiology of organisms. As a phototactic organism, studying the effects of different colors of light on the mating, development, and reproduction of *Drosophila melanogaster* helps elucidate the important influence of spectral sensitivity on organisms. This study explored the effects of white light (400 lux, control), green light (400 lux, wavelength to which fruit flies are most spectrally sensitive), red light (400 lux, wavelength to which fruit flies are least spectrally sensitive), and dim-green light (225 lux, green-band irradiance matched to the white control) on the sexual vitality, development, and reproductive capacity of fruit flies under group-housed conditions. The results revealed that 1 h of green light exposure per day significantly shortened mating latency and mating duration, accelerated pupation time, raised the egg-to-pupa conversion rate, and increased the number of offspring. In contrast, there was no significant difference in various indicators between the white, red, and dim-green light groups. This study explored the important role of light intensity and spectral sensitivity in regulating the mating and reproduction processes of fruit flies and provides more evidence for comparative research on the behavioral and physiological effects of light on different organisms.

1 | Introduction

Organisms in nature rely on their powerful visual systems to complete various key life activities such as foraging, avoiding enemies, and reproducing (Lima and Dill 1990; Stephens 2008; Théry and Gomez 2010; Fellowes et al. 2023). Vision not only guides a series of visual based behaviors in organisms (Gibson 1958; Hein 1981; Ryu et al. 2022; Han, Tan, et al. 2024) but is also directly related to multiple life processes such as growth, development, and aging (Riesen 1950; Fernald 1989; Hedden and Gabrieli 2004). Studying how visual information regulates the behavior of organisms is highly important for understanding their survival and adaptation mechanisms.

Wavelength, as an important key visual characteristic, has always been a major focus of vision research. The range of wavelengths that different organisms can perceive and process shows obvious differences (Yokoyama 2000; Kelber et al. 2003), and for relatively lower organisms such as insects (Briscoe and Chittka 2001; Cronin et al. 2014), different wavelengths of light often have more significant effects on their behavior and physiology. Exploring the effects of light with different wavelengths on organisms is important for understanding the ecological and physiological significance of light perception.

As a classic model organism, *Drosophila melanogaster* is widely used in visual, behavioral, and genetic research because its

Summary

- Green light shortens mating latency and accelerates pupation in *Drosophila melanogaster*.
- Spectral sensitivity modulates reproductive behavior across generations in group-housed flies.

behavior is readily observed and its nervous system is comparatively simple (St Johnston 2002). The fly exhibits robust phototaxis (Wehner 1972; Götz 1980; Hengstenberg 1993; Tammero and Dickinson 2002; Yen et al. 2019; Han, Huang, et al. 2021, Han, Wei, et al. 2021b), yet its sensitivity to light depends strongly on wavelength. Electrophysiological and molecular studies show that the fruit fly can perceive ~330–600 nm, covering ultraviolet, blue, green, and yellow-green light (Salcedo et al. 1999; Sharkey et al. 2020). In the compound eyes, six opsins (Rh1–Rh6) are distributed among outer and inner photoreceptors, providing color discrimination across this range (Stavenga and Arikawa 2008; Keene and Sprecher 2012; Garbers and Wachtler 2016; Schnaitmann et al. 2020; Sharkey et al. 2020). Additional light-sensing organs expand the fly's photic input: the three dorsal ocelli express UV-sensitive Rh2 (Pollock and Benzer 1988; Saint-Charles et al. 2016; Jean-Guillaume and Kumar 2022); the Hofbauer–Buchner eyelets project Rh6 signals directly to circadian clock neurons (Veleri et al. 2007); and deep-brain neurons detect blue/UV light via Cryptochrome and violet light via Rh7 (Ni et al. 2017). Functionally, this arrangement renders the fly highly sensitive to green light but markedly insensitive to red light (Humberg and Sprecher 2017; Little et al. 2019). However, it is worth noting that exposure to red light alone can still entrain circadian rhythms in fruit flies through Rh1- and Rh6-mediated input from the compound eyes, suggesting that flies retain some ability to detect red light despite their overall insensitivity to it (Hanai et al. 2008). Nevertheless, most previous work on light–behavior interactions in fruit flies has examined locomotion or cognition, manipulating light intensity or duration (Yamaguchi et al. 2008; Nash et al. 2019; Krittika and Yadav 2022). By contrast, how the flies' wavelength-dependent visual sensitivity influences their mating and reproductive processes remains largely unexplored.

To better understand how spectral sensitivity affects mating, development, and reproduction, it is essential to identify appropriate behavioral and physiological indicators that reflect mating success and developmental outcomes. Previous studies have often used a variety of behavioral indicators to evaluate mating and reproductive ability of fruit flies. Mating latency (the time from pairing to the start of mating) and mating duration are often used as indicators of sexual vitality in the fruit fly (Billeter and Levine 2013; Seong et al. 2023), while the process of egg laying, pupation time, and eclosion time mainly reflect the developmental ability of the fruit fly (Ramakrishnan et al. 2023), and the number of adult offspring reflects its reproductive capacity (Reis 2016). These indicators can comprehensively reflect the sexual behavior, development speed, and reproductive ability of flies under different visual conditions.

Therefore, this study aims to explore how spectral sensitivity affects the mating and reproductive capacity of fruit flies. We selected white light (400 lux, control), green light (400 lux, peak sensitivity for fruit flies), red light (400 lux, low sensitivity for fruit flies), and dim-green light (225 lux, green-band irradiance-matched control) to evaluate the sexual vitality, developmental speed, and reproductive capacity of flies under group-housed conditions. This study explores the different effects of light of different colors on fruit flies and provides comparative significance for studies on the effects of light on different organisms.

2 | Materials and Methods

2.1 | Fly Strains

The fruit flies used in this study were wild-type *Drosophila melanogaster* of the *Canton-S* strain purchased from the Bloomington Drosophila Stock Center (BDSC), with the resource number RRID BDSC_64349. During the experiment, the flies were maintained at a constant temperature of 25°C and a relative humidity ranging from 40% to 60%. A 12-h light/12-h dark cycle was used, with the light provided from 8:00 a.m. to 8:00 p.m. and darkness from 8:00 p.m. to 8:00 a.m. to simulate natural circadian rhythms. The nutritional requirements of the flies were met via a culture medium containing cornstarch, yeast, and sucrose.

2.2 | Light Illumination Setting

All illumination in this study comes from a Honeywell eye-care desk lamp (model HWX-02B Pro, diameter 450 mm, height 50 mm, rated power 45 W, nominal luminous flux 3200 lm). We measured the spectral power distribution (SPD) of this lamp in the 380–780 nm range with a spectrometer (Figure 1A). The results show that its spectrum is continuous and relatively flat, without the three-peak structure common in traditional RGB white LEDs. We adjusted the distance between the desk lamp and the rearing vials, keeping the actual illuminance received by flies in the white-light group at about 400 lux using a lux meter.

To obtain narrow-band monochromatic light, we installed interference filters in front of the desk lamp. After filtering, the green-light condition obtained a 465–565 nm band-pass window with a full width at half maximum (FWHM) of 490–540 nm; after filtering, the red-light condition obtained a 600–700 nm band-pass window with an FWHM of 625–675 nm. By adjusting the distance between the flies in the green-light and red-light groups and the lamp, we kept the illuminance of green light and red light at 400 lux.

Under the 400-lux white-light condition, about 225 lux of green-light component is contained in the 465–565 nm band. To match this intensity, we set up a “dim-green group”; we kept the green-light filter combination unchanged and adjusted the distance between the flies and the lamp, reducing the illuminance of the green-light band to 225 lux.

For the green, red, and dim-green groups, illumination was applied every day from 12:00 to 13:00 (ZT4–ZT5). During this 1-h period, the corresponding filter was placed on the white lamp, and the distance between the flies and the lamp was

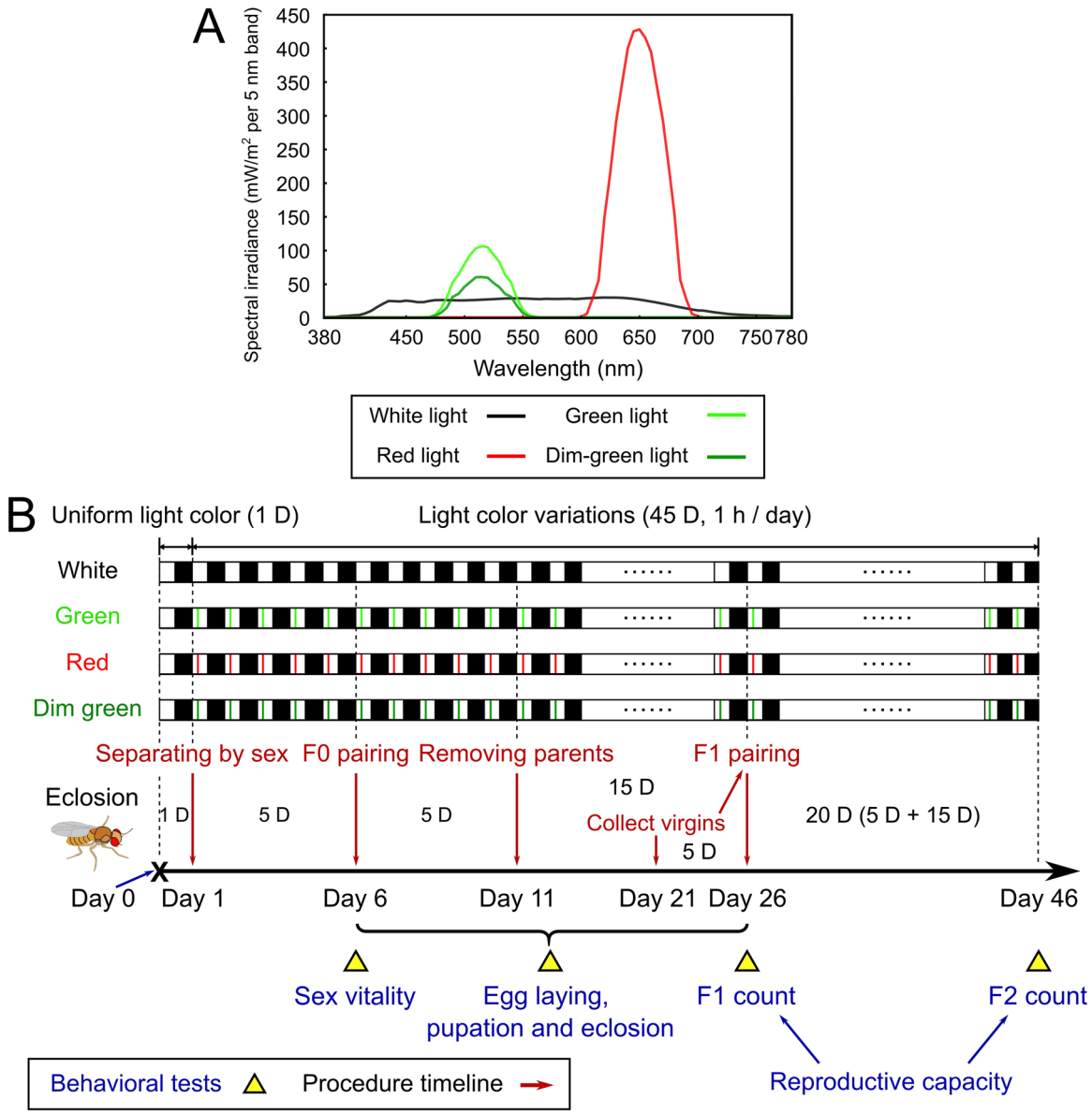

FIGURE 1 | Experimental light conditions and behavioral timeline. (A) Spectral power distributions (380–780 nm, 5 nm resolution) of the four illumination treatments: broad-spectrum white (400 lux), narrow-band green (465–565 nm, 400 lux), narrow-band red (600–700 nm, 400 lux), and dim green (465–565 nm, 225 lux). (B) Schematic of the protocol used to test sexual vitality and reproductive capacity. On the first day after eclosion (Day 0), all flies were kept under identical white light and virgin males and females were isolated. From Day 1 to Day 5, each group received its assigned light treatment once per day from 12:00 to 13:00 (ZT4–ZT5): the green, red, or dim-green groups were exposed for 1 h by placing the corresponding filter in front of the lamp, whereas the white-light group remained under continuous white light. Outside this 1-h window, all groups experienced the standard 12 h light/12 h dark cycle. On Day 6, males and females were paired and their courtship behavior was recorded. Beginning on Day 6, egg laying in each vial was recorded every 8 h through Day 10, whereas pupation and adult eclosion were recorded every 8 h up to Day 25. Parental flies were removed at the end of Day 10. On Day 21, 40 newly eclosed virgin males and 40 virgin females were collected from each group and kept separately to prepare for F1 pairing. The total number of first-generation (F1) adults was counted at the end of Day 25. The virgins separated on Day 21 were then paired, and pupae and adults of the second generation (F2) were counted during the subsequent 20 days.

adjusted so that the flies received the required illuminance for the designated color. When the exposure ended at ZT5, the flies were returned to the same position as the white-light group and continued to be reared under white light for the remainder of the day.

Spectral irradiance for each condition was also recorded with a spectrometer at 5 nm intervals and is shown in Figure 1. Each of the above illumination conditions was measured three times for the spectrum and averaged, ensuring that the target intensity was precise and consistent. Under the existing conditions, with a cold-light lamp as the light source and the room temperature set to 25°C by air conditioning, we measured the local temperature at the position of the flies. After a 1-h switch from white light (25.0°C) to green light (25.1°C), dim-green light (24.9°C), or red light (25.1°C) and then back to white light

(25.0°C), the maximum difference observed was 0.2°C. Therefore, we consider temperature variation across lighting conditions to be negligible, while humidity and photoperiod were also kept constant.

2.3 | Tracking Apparatus

We utilized multiple smartphones to record mating and reproduction behavior simultaneously, with a video resolution of 1920 × 1080 P. Through video playback, we can clearly observe mating behavior: during mating, the male fruit fly mounts the female's back, and their bodies remain relatively still; after mating, the male quickly dismounts. These actions are distinct and easy to identify. We counted the number of mated pairs per tube, recorded the start time of each mating pair, and measured the duration of each mating event from start to finish by reviewing the videos.

2.4 | Fly Collection and Crossing

F0 collection (Day 0): Within 8 h after eclosion, adults were briefly anesthetized with ether, and virgin males and females were separated under a stereomicroscope. Eight males or eight females were placed in each transparent vial (24 × 95 mm). From Day 1 onward, flies were assigned to four light-treatment groups—white, green, red, and dim green—each consisting of five male vials and five female vials (40 virgin males and 40 virgin females per group; Figure 1B).

Light pre-exposure (Days 1–5): From Day 1 to Day 5, every group received its designated light for 1 h per day, 12:00–13:00 (ZT4–ZT5). For the remaining time, the regimen was 11 h white light and 12 h darkness (see Section 2.2).

Mating assay (Day 6–10): On Day 6, one male vial and one female vial from the same treatment were combined (eight males + eight females). Video recording with multiple smartphones began to score mating latency and mating duration. From this point onward, egg laying in each vial was recorded every 8 h through Day 10, while pupae and adults were counted every 8 h through Day 25. All groups received their 1 h color-specific light exposure from 12:00 to 13:00. Parental flies were removed at the end of Day 10.

F1 and F2 procedures (Days 11–45): Newly eclosed F1 adults were collected every 8 h and kept under the same light regimen as their parents (e.g., F1-Green flies were exposed only to green light); light-group populations were never mixed. Pupae and adults in each vial continued to be counted every 8 h, while each group received its daily 1 h color-specific exposure at 12:00–13:00. The same schedule was applied to the F2 generation after F1 pairing.

Ten days after the removal of the parental flies (Day 21), 40 newly emerged virgin males and 40 newly emerged virgin females were selected from each group and subjected to daily 1 h color-specific pre-exposure. At the end of Day 25 of the experiment, the total number of F1 adults in each group was recorded, and these pre-exposed virgin flies were paired under the same light regimen as their parents. The total number of F2 adults was counted 20 days after F1 pairing. Throughout the entire experiment, every stage followed a uniform schedule of a daily 1 h color-specific exposure plus a 12 h light/12 h dark cycle.

2.5 | Data Analysis

We quantified the sexual vitality, development, and reproductive capacity of fruit flies through a series of defined indicators. For the sexual-vitality assay, mating latency (the interval from pairing to the onset of copulation) and mating duration were measured for every one of the eight pairs in each vial. For development, each vial was inspected every 8 h. Egg production—visually counted on the food surface—was recorded from Day 6 to Day 10. Pupae and subsequently eclosed adults were enumerated at the same 8-h resolution from Day 6 to Day 25.

For conversion rate, the pupation rate for each vial was obtained by dividing the total number of pupae counted by the total number of eggs laid in that vial, expressed as a percentage. The eclosion rate was calculated by dividing the total number of adults eclosed by the total number of pupae in the same vial. The metrics were computed with the following formulas:

$$\text{Pupation rate} = \frac{\text{Number of pupae}}{\text{Number of eggs}} \times 100\% \quad (1)$$

$$\text{Eclosion rate} = \frac{\text{Number of adults}}{\text{Number of pupae}} \times 100\% \quad (2)$$

For reproductive capacity, the total number of eclosed adults in the F1 and F2 generations were counted to directly reflect the reproductive success of the parental flies. In addition, the reproductive capacity of the F1 and F2 generations was expressed as a percentage change relative to the control group, calculated with the following formula:

$$\text{Reproductive capacity} = \frac{\text{Number of eclosed flies in experimental group} - \text{Number of eclosed flies in control group}}{\text{Number of eclosed flies in control group}} \times 100\% \quad (3)$$

2.6 | Statistical Analysis

All data analyses and graphing were performed via Python 3.5 and Statistical Product and Service Solutions version 22.0 (SPSS 22.0). The experimental data are presented as the mean ± standard error of the mean ($\overline{X} \pm \text{SEM}$), and individual data points are displayed as scatter plots on the graphs to visualize the data distribution.

To examine the differences among fruit flies under four light conditions (white light, green light, red light, and dim-green light), one-way analysis of variance (ANOVA) was performed. This method was used to assess whether there were statistically significant differences in the means

of the measured variables among the four light treatment groups.

In addition, post hoc tests were conducted to compare differences between each pair of groups. The Least Significant Difference (LSD) method was used for pairwise comparisons under the condition that the overall ANOVA showed significant results. This allowed us to further analyze specific differences between groups (white vs. green, white vs. red, white vs. dim green, green vs. red, green vs. dim green, and red vs. dim green). Before ANOVA, all the data were tested for normality and homogeneity of variance. A p value less than 0.05 was considered statistically significant.

Finally, to determine whether adult yield differed between generations within the same light treatment, a two-way ANOVA was conducted with "generation" (F1, F2) and "light condition" as between-subject factors.

2.7 | No AI Use Declaration

The authors confirm that no Artificial Intelligence Generated Content (AIGC) tools were used in the preparation of this manuscript.

3 | Results

3.1 | Green Light Exposure Enhances Sexual Vitality

To understand the effects of differential light sensitivity on the mating behavior of flies, we first analyzed their sexual vitality (Figure 1). Previous studies have indicated that, in group-housed fruit flies, females may delay mating while waiting for the strongest male (Markow 1987; Billeter and Levine 2013), which could increase the mating latency of certain individuals. Moreover, repeated mating by males may affect mating duration during subsequent copulations. These findings suggest that the group setting may alter both mating latency and mating duration, but the magnitude of this effect within our experimental model remains unclear. To provide a comprehensive assessment of how the different light conditions influence mating, we therefore report and analyze the latency and duration of the first mating pair in each vial, and the latency-and-duration distributions for all 40 females subjected to that light treatment (Figure 2). One-way ANOVA revealed a significant main effect of light treatment on the mating latency of the first female in each vial ($F(3, 16) = 4.712$, $p < 0.05$, partial $\eta^2 = 0.469$) (Figure 2A). Post-hoc comparisons showed that the first-pair latency in the green-light group was significantly shorter than that in the white-light group (mean difference = −7.720, SEM = 2.807, $p < 0.05$), the red-light group (mean difference = −9.740, SEM = 2.807, $p < 0.01$), and the dim-green group (mean difference = −7.760, SEM = 2.807, $p < 0.05$). Although the latency in the red-light group was slightly longer than in the white-light group, the difference was not statistically significant (mean difference = 2.020, SEM = 2.807, $p = 0.482$).

We also analyzed the effect of light treatment on the mating latency of every female within each group (Figure 2B). The main effect of light treatment was significant ($F(3, 156) = 8.346$, $p < 0.001$, partial $\eta^2 = 0.138$). Post-hoc tests revealed the same pattern as for the first pair: the green-light group showed markedly shorter latencies than the white-light group (mean difference = −13.855, SEM = 4.321, $p < 0.01$), the red-light group (mean difference = −21.015, SEM = 4.321, $p < 0.001$), and the dim-green group (mean difference = −14.330, SEM = 4.321, $p < 0.01$). Again, the latency in the red-light group exceeded that of the white-light group, but the difference did not reach statistical significance (mean difference = 7.160, SEM = 4.321, $p = 0.100$).

By contrast, although the mean differences in mating duration for the first female in each vial followed a trend similar to that observed for mating latency, the main effect of light treatment was not significant ($F(3, 16) = 0.994$, $p = 0.421$, partial $\eta^2 = 0.157$) (Figure 2C). However, when the mating duration of every female was analyzed, the main effect became significant ($F(3, 156) = 4.702$, $p < 0.01$, partial $\eta^2 = 0.083$) (Figure 2D). Post-hoc tests showed that the green-light group exhibited significantly shorter durations than the red-light and dim-green groups, while the difference between the green- and white-light groups did not reach statistical significance (green vs. white: mean difference = −1.585, SEM = 1.060, $p = 0.137$; green vs. red: mean difference = −2.488, SEM = 1.060, $p < 0.05$; green vs. dim green: mean difference = −3.875, SEM = 1.060, $p < 0.001$). In addition, mating duration in the dim-green group was slightly longer than in the white-light group (mean difference = 2.290, SEM = 1.060, $p < 0.05$). Taken together, these results indicate that exposing flies to 1 h of green light per day for 5 consecutive days enhances sexual vitality, principally by reducing mating latency.

3.2 | Green Light Exposure Accelerates Developmental Progression

To further investigate how different light conditions affect development, we tracked oviposition, pupation, and eclosion in each group (Figures 3 and 4, Figures S1 and S2). One-way ANOVA showed that light treatment had no significant effect on the total number of eggs laid per vial ($F(3, 16) = 1.351$, $p = 0.293$, partial $\eta^2 = 0.202$) (Figure 3A) and likewise no effect on the latency to the first egg in each vial (F $(3, 16) = 0.167$, $p = 0.917$, partial $\eta^2 = 0.030$) (Figure 3B). The temporal distribution of egg-laying was virtually identical across treatments (Figure 4A; Figures S1A and S2A).

In contrast, analysis of pupation revealed a significant main effect of light on the total number of pupae per vial ($F(3, 16) = 7.338$, $p < 0.01$, partial $\eta^2 = 0.579$). Post-hoc tests showed that the green-light group produced markedly more pupae than every other group (green vs. white: mean difference = 18.200, SEM = 4.521, $p < 0.01$; green vs. red: mean difference = 17.000, SEM = 4.521, $p < 0.01$; green vs. dim green: mean difference = 16.600, SEM = 4.521, $p < 0.01$) (Figure 3C). Latency to the first pupa also displayed a significant main effect ($F(3, 16) = 4.756$, $p < 0.05$, partial $\eta^2 = 0.471$), with the first pupa appearing sooner in the green-light group than in the other treatments (green vs. white: mean difference = −1.266,

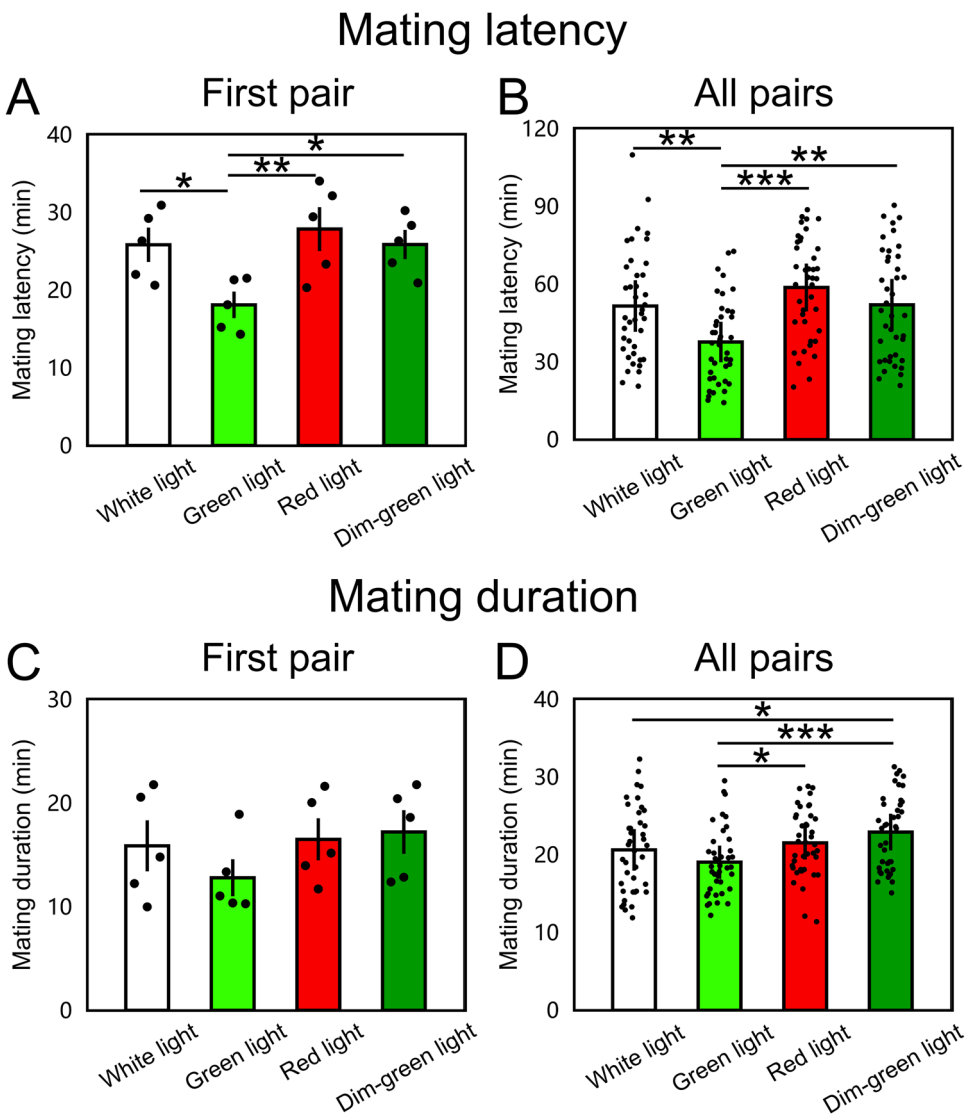

FIGURE 2 | Effects of different light conditions on sexual vitality. (A, B) Mating latency of the first pair in each vial (A) and mating latency calculated from all females in each vial (B) under white, green, red, and dim-green light conditions. (C, D) Mating duration of the first pair (C) and mating duration calculated from all females (D) under the same four light conditions. *Canton-S Drosophila melanogaster* was used in all experiments. The white, green, red, and dim-green bars represent the corresponding light conditions. Error bars indicate the standard error of the mean (SEM), and each black dot represents an individual data point. One-way ANOVA was used for statistical analysis (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

SEM = 0.369, $p < 0.01$; green vs. red: −0.866, SEM = 0.369, $p < 0.05$; green vs. dim green: −1.132, SEM = 0.369, $p < 0.01$) (Figure 3D). Histogram plots showed that, between Days 4 and 7 after mating, the number of pupae was higher in the green-light group than in all other groups (Figure 4B; Figures S1B and S2B), indicating that green light accelerates the time to reach the pupal stage.

A similar pattern emerged for adult eclosion. Light treatment exerted a significant main effect on the total number of adults per vial ($F(3, 16) = 6.968$, $p < 0.01$, partial $\eta^2 = 0.566$); post-hoc comparisons showed that the green-light group produced significantly more adults than all other groups (green vs. white: mean difference = 16.400, SEM = 4.084, $p < 0.01$; green vs. red: 14.800, SEM = 4.084, $p < 0.01$; green vs. dim green: 14.200, SEM = 4.084, $p < 0.01$) (Figure 3E). Latency to the first adult also differed significantly among treatments ($F(3, 16) = 3.913$, $p < 0.05$, partial $\eta^2 = 0.423$); the first adult emerged earlier under green light (green vs. white: mean difference = −1.602, SEM = 0.568, $p < 0.05$; green vs. red: −1.468, SEM = 0.568, $p < 0.05$; green vs. dim green: −1.668, SEM = 0.568, $p < 0.05$) (Figure 3F). Time-series data revealed that the number of eclosed adults was consistently higher in the green-light group than in other groups on Days 13–16 after mating (Figure 4C; Figures S1C and S2C).

Next, we calculated the pupation rate (eggs to pupae) and eclosion rate (pupae to adults) for each vial (Figure 5). Light treatment had a significant main effect on pupation rate ($F(3, 16) = 3.729$, $p < 0.05$, partial $\eta^2 = 0.411$); post-hoc tests showed that green light yielded a higher conversion than both white and red light and also exceeded dim-green, although the difference did not reach statistical significance (green vs. white: mean difference = 4.571, SEM = 2.073, $p < 0.05$; green vs. red:

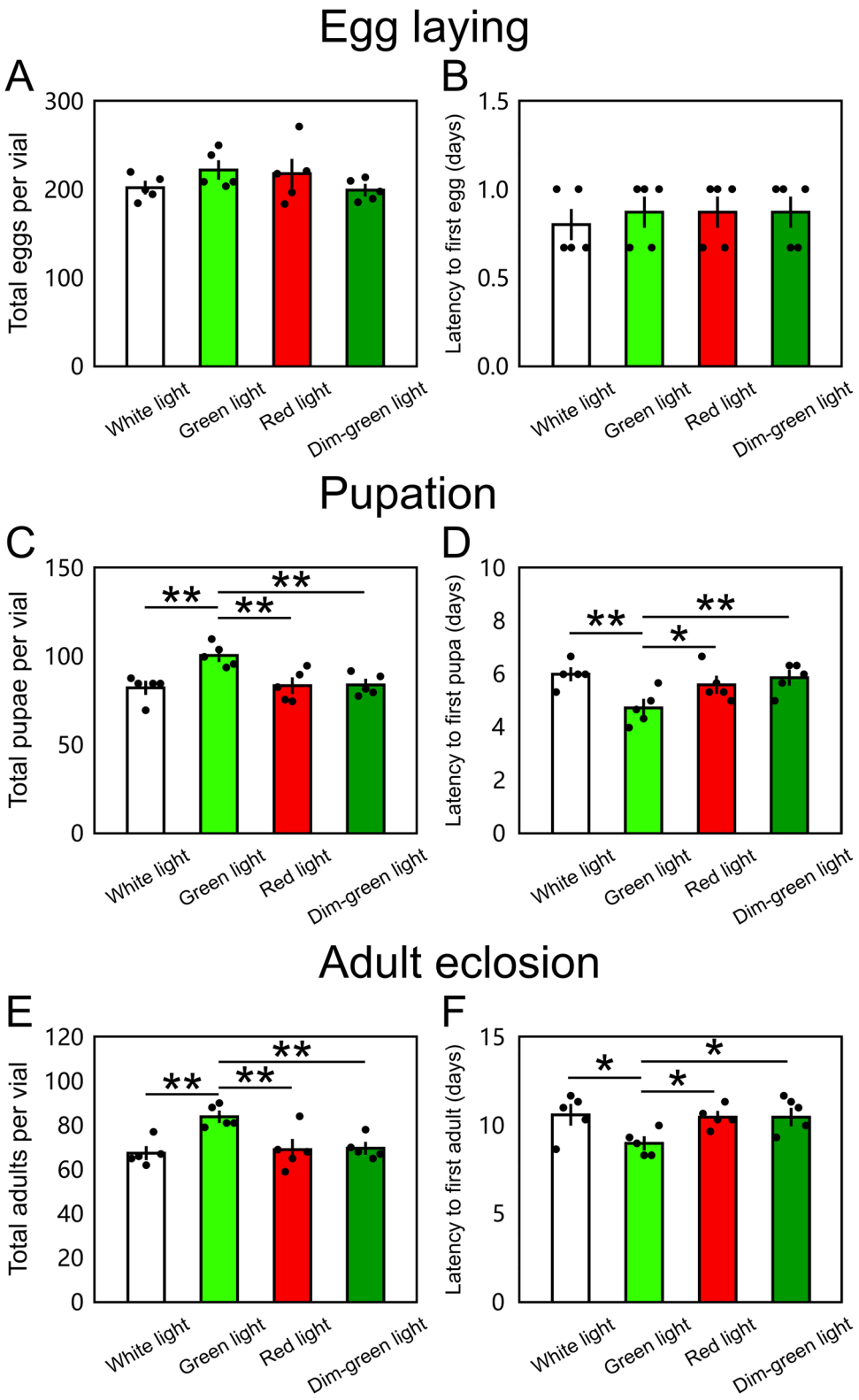

FIGURE 3 | Effects of different light conditions on development. (A, B) Total egg number per vial accumulated from Day 6 to Day 10 (A) and latency to the first egg in each vial (B). (C, D) Total pupae per vial accumulated from Day 6 to Day 25 (C) and latency to the first pupa (D). (E, F) Total adults eclosed per vial accumulated from Day 6 to Day 25 (E) and latency to the first adult (F). The graphical representations, data markers, statistical methods, and significance notations follow the same conventions as those in Figure 2.

6.768, SEM = 2.073, $p < 0.01$; green vs. dim green: 3.273, SEM = 2.073, $p = 0.134$) (Figure 5A). By contrast, the eclosion rate showed no main effect ($F(3, 16) = 0.034$, $p = 0.991$, partial $\eta^2 = 0.006$) (Figure 5B).

Collectively, these findings indicate that green light accelerates development primarily by shortening the time to reach the pupal stage, while the increased egg-to-pupa conversion rate contributes to enhanced reproductive capacity.

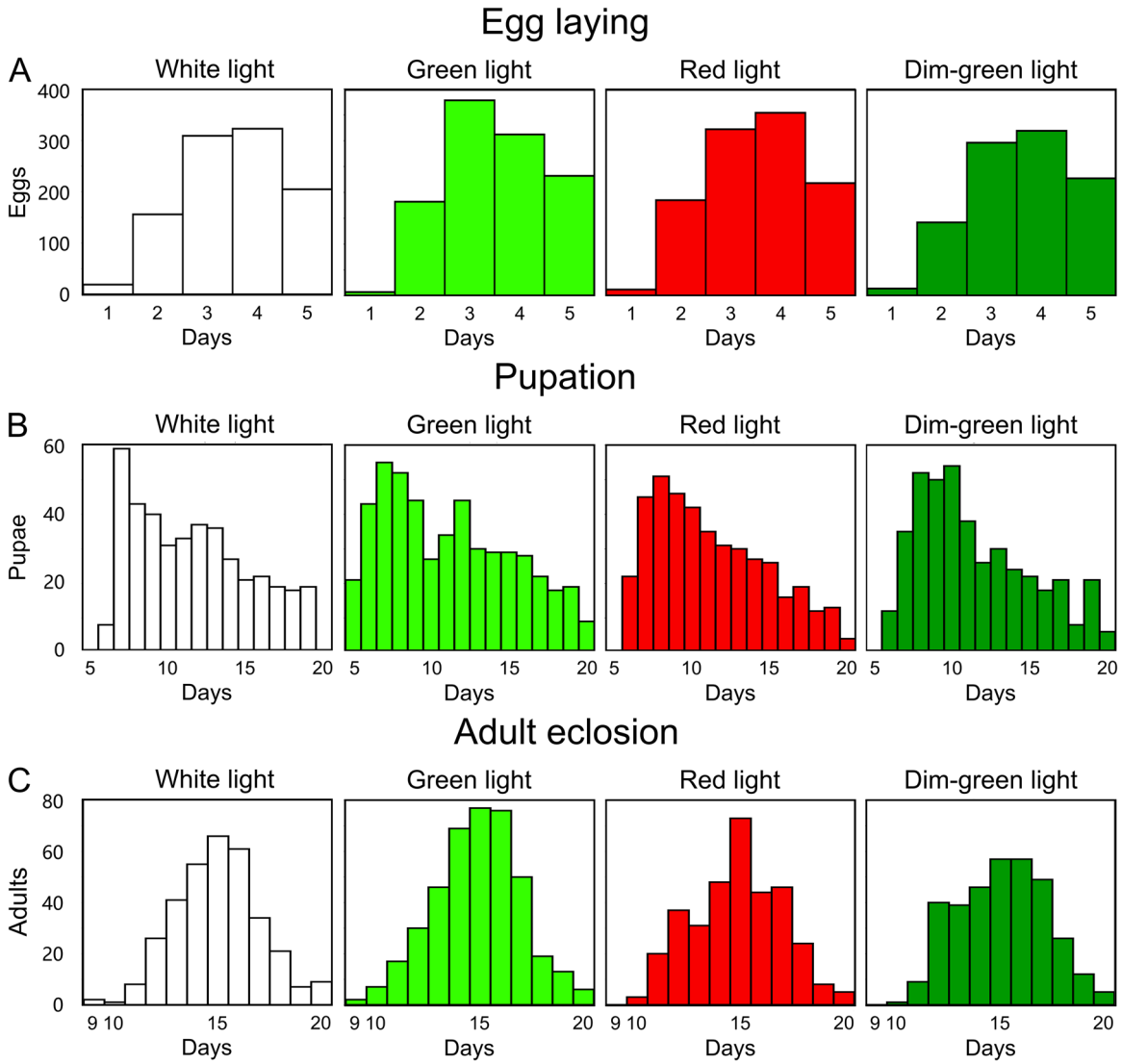

FIGURE 4 | Daily distributions of egg laying, pupation, and eclosion under different light conditions. (A) Histogram (1-day bins) of egg-laying events in the white-, green-, red-, and dim-green-light groups. (B) Histogram (1-day bins) of pupation events for the same four light treatments. (C) Histogram (1-day bins) of adult eclosion events for the same four light treatments. Counts were collected every 8 h and pooled into 1-day bins. The *x*-axis values represent days postcopulation, with Day 1 corresponding to the day of mating (Day 6).

3.3 | Green Light Exposure Enhances the Reproductive Capacity of Female Fruit Flies

We took the total number of adults that emerged within 20 days after mating—that is, the values plotted in Figure 3E—as the first-generation (F1) yield (Figure 6A). Virgin flies were reared under the same light treatment conditions for 5 days after pairing, then allowed to mate, and the number of second-generation (F2) adults was counted 20 days later (Figure 6B). Because the F1 results have already been described above, only the F2 data are interpreted here. One-way ANOVA revealed a significant main effect of light treatment on F2 counts ($F(3, 16) = 3.728$, $p < 0.05$, partial $\eta^2 = 0.411$). Post-hoc comparisons showed that the green-light group produced significantly more F2 adults than every other group (green vs. white: mean difference = 14.200, SEM = 5.942, $p < 0.05$; green vs. red: 15.200, SEM = 5.942, $p < 0.05$; green vs. dim green: 18.200, SEM = 5.942, $p < 0.01$).

We also analyzed whether the total adult yield differed between F1 and F2 under each illumination regime (Figure 6C). A two-way ANOVA revealed no significant simple effects of generation within any group (white: $F(1, 32) = 0.679$, $p = 0.416$, partial $\eta^2 = 0.021$; green: $F(1, 32) = 0.154$, $p = 0.697$, partial $\eta^2 = 0.005$; red: $F(1, 32) = 0.098$, $p = 0.756$, partial $\eta^2 = 0.003$; dim green: $F(1, 32) = 0.154$, $p = 0.697$, partial $\eta^2 = 0.005$), indicating that each light treatment exerted a stable effect across generations. In terms of reproductive capacity, the green-light group consistently exhibited an upward shift in both generations, yielding the highest relative reproductive capacity (Table 1), whereas the other treatments differed little from white light. These findings demonstrate that green light enhances the reproductive output of fruit flies and does so consistently across two generations.

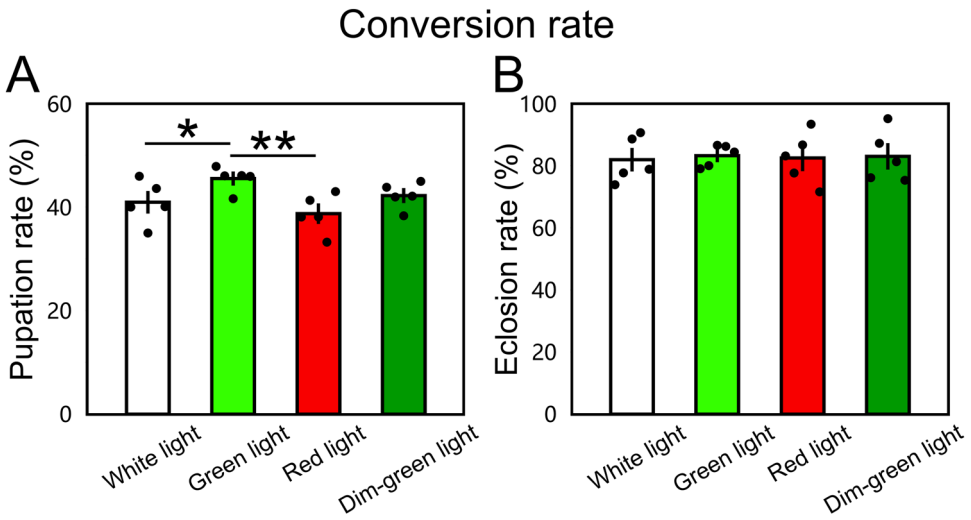

FIGURE 5 | Conversion rates under different light conditions. (A) Egg-to-pupa conversion rate (pupation rate) and (B) pupa-to-adult conversion rate (eclosion rate) for the white-, green-, red-, and dim-green-light groups. The graphical representations, data markers, statistical methods, and significance notations follow the same conventions as those in Figures 2 and 3.

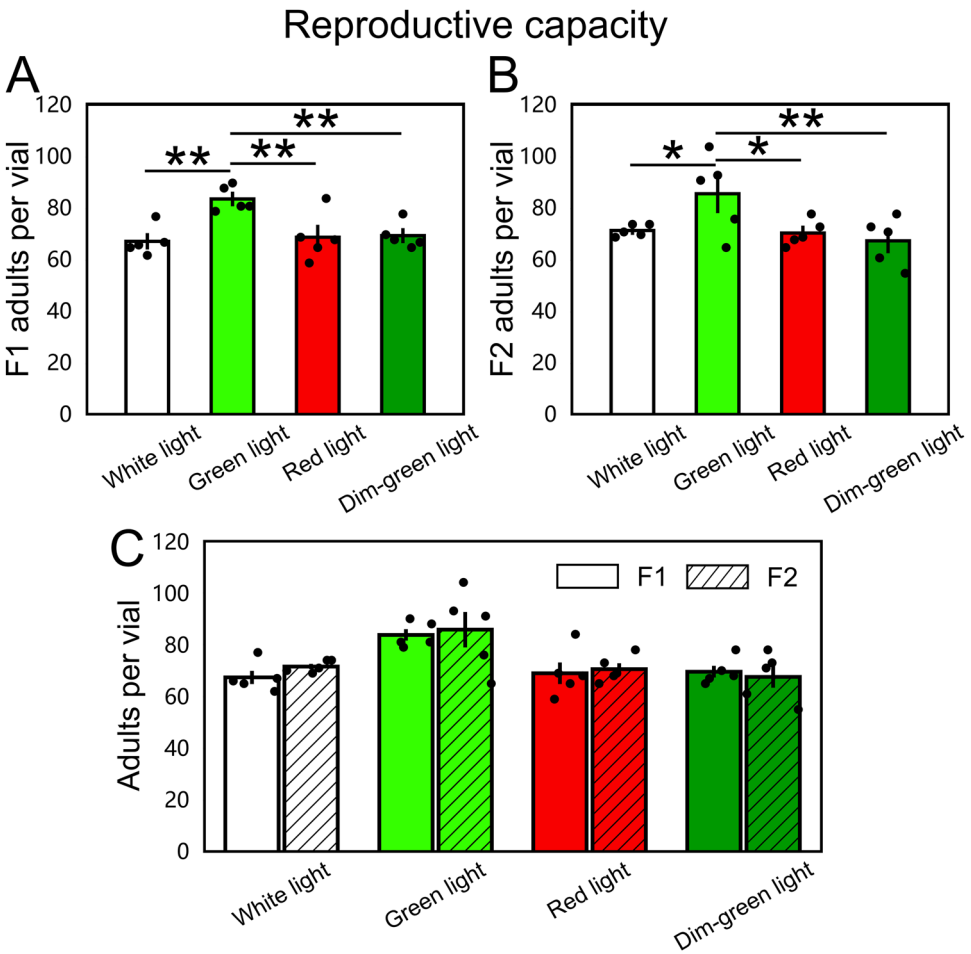

FIGURE 6 | Effects of different light conditions on reproductive capacity. (A, B) Total number of F1 adults (A) and F2 adults (B) produced per vial under white, green, red, and dim-green light. (C) Comparison of F1 and F2 adult counts within each light treatment (paired bars: solid fill for F1, hatched fill for F2). The graphical representations follow the same conventions as those in Figures 2–4.

TABLE 1 | Reproductive capacity of fruit flies under different light conditions.

Light conditions	F1 count	F1 reproductive capacity (%)	F2 count	F2 reproductive capacity (%)
White light	337	–	358	–
Green light	419	24.33	429	19.83
Red light	345	2.37	353	−1.40
Dim-green light	348	3.26	338	−5.59

4 | Discussion

In the present study, we tested the effects of different light conditions on the sexual vitality, development, and fertility of *Drosophila melanogaster*. The results showed that 1 h of green light exposure per day promoted the sexual vitality of the fruit fly, which manifested mainly as a reduction in mating latency and a shortening of mating duration. At the same time, compared with those in the white light group, the pupation time and eclosion time of the flies in the green light group were significantly shorter. Finally, green light exposure significantly increased the number of pupae, raised the egg-to-pupa conversion rate and the yield of the fruit fly, and this effect remained stable across two consecutive generations. In contrast, red light exposure did not significantly differ from white light exposure. Furthermore, the little to no difference between the dim-green and white light groups across all measures suggests that the promotive effects of green light on mating, development, and productivity become evident only when green-light intensity rises above a certain threshold. This finding also helps rule out the possibility that the effects of green light were simply due to the absence of red and blue components in full-spectrum white light. Overall, these findings suggest that wavelength-specific spectral sensitivity plays a key role in regulating developmental timing and reproductive behavior in *Drosophila melanogaster*.

Taken together with these observations, we found that green light has a positive effect on the mating, development, and yield of fruit flies. This wavelength-dependent effect may first be understood from a hormonal perspective. Activation of the green-sensitive photopigment Rh6 triggers release of prothoracicotropic hormone (PTTH) via ventral lateral neurons (LnVs) (Ramakrishnan et al. 2023). PTTH in turn promotes ecdysone synthesis; elevated ecdysone accelerates oocyte maturation and enhances sexual receptivity in virgin females (Sieber and Spradling 2015; Li et al. 2023), which could help explain reducing mating latency and shortening larval development. Consistent with this pathway, daily 12-h green-light exposure from the fertilized-egg stage has been shown to boost ecdysone secretion and advance pupation (Ramakrishnan et al. 2023).

From a neural viewpoint, Rh6 photoreceptors in the compound eyes initiate the hormonal cascade described above and are excited far more strongly by green than by red light, mirroring the behavioral differences we observed. Although wavelength-specific light may also influence general health and arousal state, an even clearer asymmetry emerges when the fly's extra-retinal photoreceptors are taken into account. *Drosophila melanogaster* possesses several such pathways: the Hofbauer–Buchner eyelets, which express the same green-sensitive Rh6 opsin and project directly to circadian clock neurons in the accessory medulla, while red light, lying outside the Rh6 action spectrum, likely induces minimal activation (Veleri et al. 2007). By contrast, the three dorsal ocelli, whose receptors express the UV-sensitive Rh2, remain essentially silent at the wavelengths used here (Pollock and Benzer 1988; Saint-Charles et al. 2016). Deep-brain neurons co-expressing Cryptochrome and the violet-sensitive opsin Rh7 extend photosensitivity into the blue–green range and likewise respond only weakly to red light (Ni et al. 2017). Together, these compound-eye, eyelet, ocellary, and deep-brain circuits create an intrinsic spectral bias that amplifies green-light input while transmitting little information in the far-red region, providing a mechanistic basis for the enhanced sexual vitality, accelerated development, and increased fecundity elicited by green illumination in the present study.

On the other hand, with respect to physiological health status, studies have shown that low- to medium-intensity green light (100 lux and 600 lux) significantly extends the lifespan of fruit flies. This finding may be related to the gut microbiota or to the presence of photosensitive micronutrients (Shen et al. 2021). Therefore, we speculate that green light may have a positive effect on the overall health status of the fruit fly, thereby promoting earlier mating behavior and significantly enhancing reproductive capacity.

Notably, in our experiments, the egg-to-pupa conversion rates were approximately 40%–50%, which is lower than the > 80% survival rates typically reported for *Drosophila melanogaster* under optimal laboratory conditions (Olcott et al. 2010; Ormerod et al. 2017). Several factors may explain this discrepancy. Differences in larval density, food composition, or environmental variables such as light treatments could have reduced developmental success in our assays. In addition, methodological limitations likely contributed: pupae could be readily counted on the vial walls, whereas most eggs were deposited within the food medium and had to be counted by eye, which may have introduced errors. It is also possible that some larvae had not yet pupated at the time pupal counts were terminated, which would further lower the apparent conversion rate. We acknowledge these limitations. Future studies should employ more precise techniques for egg quantification (such as image-based counting) and extend the monitoring period to improve the accuracy of conversion-rate estimates.

Furthermore, the way light intensity was calibrated in this study represents another methodological constraint. We used a lux meter to adjust illumination to 400 lux for the white, green, and red treatments and to 225 lux for the dim-green treatment.

Because lux values are weighted according to the human photopic sensitivity curve, equal lux readings across colors do not correspond to equal physical irradiance. Consequently, the red-light condition required higher irradiance to reach 400 lux, which explains why its spectral peak in Figure 1 appears much higher than the green peak. Whether lux is an appropriate measure for insect photoreception remains uncertain, and this question cannot be resolved with the present experimental design. Future investigations should therefore consider controlling irradiance (mW/m^2) or other metrics that better capture the spectral sensitivity of the fruit fly.

Moreover, in our original plan we intended to expose the flies to 12 h of monochromatic light each day, mirroring the duration of the white-light phase. Previous studies have shown that a 12 h green-light cycle shortens lifespan, suppresses daytime activity, and shifts sleep toward the daytime while lightening nocturnal sleep compared with broad-spectrum white light (Krittika and Yadav 2022). A brief red-light pulse at dusk reduces night-time sleep, yet prolonged red illumination leaves activity peaks and survival virtually unchanged (Krittika and Yadav 2022; Bond et al. 2024). A small-scale pretest with 15 males and 15 females in our laboratory did show an abrupt drop in survival during the first 3 days under continuous 400 lux of green light, although this effect was not reproduced in a later repeat. (30-day survival curves for all four colors are provided in Figure S3.) Regrettably, we did not further dissect whether the lethality seen under continuous green illumination was driven by circadian and sleep disruption, nor can we exclude the possibility that the initial mortality spike was a chance event. Therefore, because the present study focuses on the effects of green and red light on courtship and reproduction rather than mortality, we adopted a more conservative regime: a single 1-h monochromatic pulse at 12:00 h (ZT4–ZT5) each day, following the reported protocol (Sakai et al. 2002). This approach minimizes potential confounding from light-induced lethality while still allowing us to test how color-specific sensitivity influences mating and reproductive output. Future research should examine the effects of prolonged green-light exposure and clarify the underlying mechanisms.

While our light exposure duration was considerably shorter than in some earlier studies, it still allowed for meaningful behavioral and developmental responses. It has been previously reported that daily 12-h green-light exposure from the fertilized-egg stage significantly accelerates pupariation relative to white light, as well as to blue, yellow, orange, red, and dark conditions (Ramakrishnan et al. 2023), and our results further indicate that 1 h of green light exposure per day also promotes development in the fruit fly. Moreover, during a preliminary pilot experiment, we tested the green-light group and the white-light control: virgin males and females were exposed to the assigned light for 1 h on the day after eclosion and then paired for mating. Under this regime no significant differences were detected between the two groups in sexual vitality, egg laying, pupation, and eclosion (see Figure S4), indicating that a single 1-h pulse was insufficient. Consequently, we revised the protocol to deliver a 1-h exposure each day for 5 consecutive days before mating, and this longer regimen produced statistically significant differences. These findings indicate that the effect of green light on sexual vitality is not immediate in the fruit fly.

In addition, previous studies have shown that mating behavior in the fruit fly is a typical social behavior (Dahanukar and Ray 2011; Corthals et al. 2017) and that social isolation can lead to a series of impacts, such as reduced locomotor ability and willingness to mate (Yost 2023; Han, Zhang, et al. 2024). Therefore, our study tested the effects of different light conditions on the mating and reproduction of group-housed flies. However, under group-housed conditions, female flies often simultaneously select the strongest male for mating, leading to a waiting behavior phenomenon (Markow 1987; Billeter and Levine 2013), which makes it difficult to measure the mating latency and mating rate of each individual, thereby reducing the precision of the data. Despite this limitation, our results still showed a consistent reduction in mating latency under green light, raising the question of which component of the mating process was accelerated. The concurrent reduction in both mating latency and mating duration indicates that this effect is unlikely to be explained solely by faster initiation of male courtship. Instead, it is more likely attributable to enhanced male courtship efficiency and/or increased female receptivity; in other words, males may have performed more effective courtship displays that were accepted more quickly, and females themselves may have exhibited a stronger willingness to mate. Future studies should disentangle these possibilities by separately quantifying male initiation time, courtship intensity, and female acceptance. In addition, experimental designs will be needed to assess individual-level responses of females under group-housing conditions and to examine the effects of light in monogamous mating contexts. Finally, previous studies have shown that fruit flies exhibit same-sex sexual behavior (Villella et al. 1997; Kimura et al. 2005; Bailey and Zuk 2009). Owing to the limitations of group rearing conditions, it is difficult to completely rule out interference from same-sex behavior, and future research is needed to clarify the effects of same-sex behavior under different light conditions.

5 | Conclusion

In our study, we explored the effects of lights with different spectral sensitivities on the mating and reproduction of *Drosophila melanogaster*. We found that green light significantly shortened mating latency, mating duration, and pupation time increased the pupation rate, and had long-term effects on reproductive capacity. Future research should further explore the long-term effects of different wavelengths of light on both group-housed and individually housed fruit flies. Such studies will not only deepen our understanding of phototactic responses in the fruit fly but also offer comparative insights into the behavioral and physiological impacts of light across different organisms.

Author Contributions

Rui Han: conceptualization, formal analysis, investigation, writing – original draft preparation, writing – review and editing, funding acquisition, resources, supervision. **Jun Zhang:** conceptualization, methodology, resources. **Yi-Han Xu:** methodology, formal analysis, investigation, supervision. **Zeng-Xingyue Xiao:** methodology, formal analysis, investigation, supervision. **Miao-Ling Xie:** methodology,

formal analysis, investigation, supervision. **Hao-Ru Xin:** methodology, formal analysis, investigation, supervision.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Figure S1: Daily and cumulative counts of egg laying, pupation, and eclosion under different light conditions (1-day bins). **Figure S2:** High-resolution (8 h-bin) temporal distributions of egg laying, pupation and eclosion under different light conditions. **Figure S3:** 30-day survival curves of adult flies under four light conditions. **Figure S4:** Single-day light-exposure experiment.