

RESEARCH ARTICLE

# Adult Tea Green Leafhoppers, *Empoasca onukii* (Matsuda), Change Behaviors under Varying Light Conditions

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**Citation:** Shi L, Vasseur L, Huang H, Zeng Z, Hu G, Liu X, et al. (2017) Adult Tea Green Leafhoppers, *Empoasca onukii* (Matsuda), Change Behaviors under Varying Light Conditions. PLoS ONE 12(1): e0168439. doi:10.1371/journal.pone.0168439

**Editor:** Arash Rashed, University of Idaho, UNITED STATES

**Received:** June 28, 2016

**Accepted:** December 1, 2016

**Published:** January 19, 2017

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**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** Funded by the Ministry of Science & Technology in China with a program on International Cooperation between Fujian Agriculture and Forestry University and Tea Research Institute of Sri Lanka (No. 2010DFB33030), <http://www.most.gov.cn/> (MY), by the Ministry of Agriculture in China with a project on (No. 2011 - Z26), <http://www.moa.gov.cn/> (MY), and the Fujian Provincial Department of

## Abstract

Insect behaviors are often influenced by light conditions including photoperiod, light intensity, and wavelength. Understanding pest insect responses to changing light conditions may help with developing alternative strategies for pest control. Little is known about the behavioral responses of leafhoppers (Hemiptera: Cicadellidae) to light conditions. The behavior of the tea green leafhopper, *Empoasca onukii* Matsuda, was examined when exposed to different light photoperiods or wavelengths. Observations included the frequency of locomotion and cleaning activities, and the duration of time spent searching. The results suggested that under normal photoperiod both female and male adults were generally more active in darkness (i.e., at night) than in light. In continuous darkness (DD), the locomotion and cleaning events in Period 1 (7:00–19:00) were significantly increased, when compared to the leafhoppers under normal photoperiod (LD). Leafhoppers, especially females, changed their behavioral patterns to a two day cycle under DD. Under continuous illumination (continuous quartz lamp light, yellow light at night, and green light at night), the activities of locomotion, cleaning, and searching were significantly suppressed during the night (19:00–7:00) and locomotion activities of both females and males were significantly increased during the day (7:00–19:00), suggesting a shift in circadian rhythm. Our work suggests that changes in light conditions, including photoperiod and wavelength, can influence behavioral activities of leafhoppers, potentially affecting other life history traits such as reproduction and development, and may serve as a method for leafhopper behavioral control.

## Introduction

Insects can be highly influenced by light conditions including photoperiod, light intensity, and wavelength [1, 2, 3]. Apart from the widely known diapause phenomenon [4, 5], photoperiod

Science and Technology (No. 201610018), <http://www.fjkjt.gov.cn/> (HH). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

influences various behaviors such as egg hatching, flight, locomotion, feeding, courtship, and mating [6, 7]. For instance, walking, foraging, and oviposition of *Frankliniella occidentalis* Pergande are increased with increasing time of illumination but reduced in continuous darkness [8]. Many *Drosophila* species and the tephritid fly *Anastrepha ludens* have their mating success reduced when exposed to continuous darkness [9, 10]. The influence of wavelength varies among insect species. Insects tend to be most sensitive to blue or blue–green regions of the spectrum (400–550 nm) [3]. Short wavelengths stimulate the flight and inhibit landing of the whitefly *Trialeurodes vaporariorum*, while long wavelengths stimulate landing and inhibit flight [11]. Reproduction and development of ladybird beetles, ants, and honeybees are negatively affected by red light [12]. Meanwhile, green light has been found to reduce reproduction in *Propylea japonica* [13]. In Japan, as a pest management approach, yellow and green lights have been used at night to suppress behavioral activities of nocturnal moths [6].

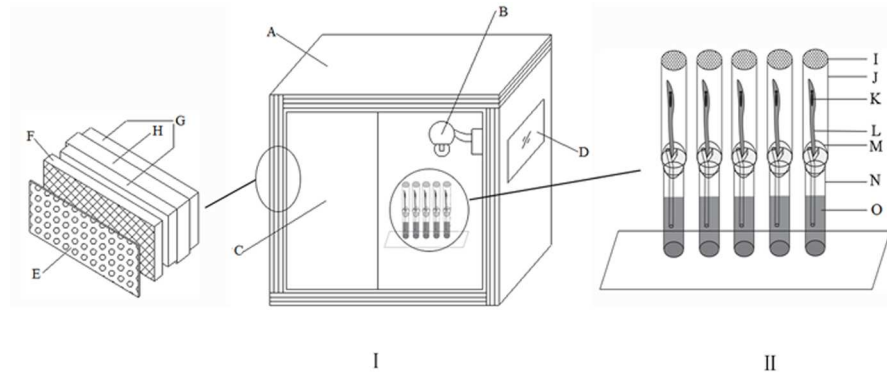
The tea green leafhopper, *Empoasca onukii* Matsuda (Hemiptera: Cicadellidae), is one of the main insect pests in tea plantations in Asia [14, 15]. There are five nymphal instars. Courtship and mating occur 4–5 days after emergence. Usually, the adult females are larger than the males and live longer [16]. All stages can cause serious damage to tea plants. Nymphs and adults suck sap from young tea shoots and leaves and the eggs are laid into the branches, causing damage called hopperburn [17]. In China, *E. onukii* has 9–17 generations per year depending on regional climatic conditions. The most serious outbreaks normally occur in June and September, resulting in loss of tea production [16, 18]. So far, little is known about the behavior of the tea green leafhopper, especially the influence of changing light conditions. Variation in photoperiod can lead to changes in circadian rhythm affecting behavior and potentially fitness [18]. In adult tea green leafhoppers, three major behavioral activities can be observed: cleaning, locomotion, and searching. Cleaning is an essential behavior that enhances hydrophobicity through the coating of their integuments with brochosomes, thus protecting the adults from being washed off from leaves by water [19, 20]. Locomotion activities, such as walking, jumping and flying, are considered important elements of survival as they are usually related to avoiding danger (for instance, enemies), foraging, finding places to stand and social interactions [21, 22]. Searching is crucial especially during courtship as the male leafhopper continually flies among plants until it finds a mate. When in proximity of a female, the male initiates its courtship behavior using vibration signals produced from the abdomen. A receptive female will then respond to the vibration signals and allows the male to move closer to her. In this period of time, females barely move [23, 24, 25]. Investigating the adaptive behavioral responses of leafhoppers to the influence of light can help better understand its adaptive capacity to respond to environmental changes and gradually develop alternative insect control methods.

In this study, we investigated the behavioral responses of *E. onukii* under different indoor light environments. Adult females and males were exposed to different photoperiods and light wavelengths (normal photoperiod, continuous illumination, continuous darkness, and yellow and green light during the night). The objective of this study was to determine whether variation in light conditions could affect the behavior of tea green leafhopper adult females and males.

## Materials and Methods

### Insect rearing

The leafhoppers were collected from a tea plantation (Fuzhou, Fujian Province, China, 119.2°E, 26.1°N) belonging to the Fujian Agriculture and Forestry University (FAFU) (We received permission from FAFU to conduct this study). We cut several tea branches with leaves that contained eggs of *E. onukii*. The branches were brought back to the laboratory and



**Fig 1. System used for leafhopper behavior observations.** A: observation room; B: infrared camera (two in total, the figure shows one); C: door; D: observation window (hollow glass); E: deadening felt (inside the room); F: copper mesh; G: composite board; H: sound proof cotton; I: plastic wrap (with ventholes); J: glass tube B (10mm in diameter, 100mm in high); K,S: tested leafhopper; L: tea tip; M: cotton; N: glass tube B.

doi:10.1371/journal.pone.0168439.g001

the stems were immersed in water under conditions of 12:12 photoperiod (7:00–19:00 light by quartz lamp, 19:00–7:00 with darkness, China standard time),  $27 \pm 1^\circ\text{C}$ . The light intensity during the day was around 2000 lux (see [S1 Fig](#) for spectral characteristics of the quartz lamp). Once the eggs hatched, nymphs were monitored until they reached developmental stages (instars) 4–5. They were then carefully transferred into a tube containing fresh tea tips until their emergence as adults. The new adults were sexed and then used in the following experiments.

### Video recording system

The *E. onukii* adult has a mean body length (with wings) of approximately 2.5 mm, which makes behavioral activities difficult to be observed. A new system was designed to observe the behavior of the leafhoppers under the various light treatments, in which tubes containing newly emerged adults were placed in the center of an observation chamber (1.2 m × 1.2 m × 1.2 m, [Fig 1](#)). The observation chamber contained two high-speed infrared cameras (1/4 SONY CCD, 24 zoom, fast automatic focusing, with cradle control) were positioned on the two opposite sides of the chamber. To reduce outside noise and vibrations, the chamber walls were soundproofed with cotton pressed between two wooden planks (each 5cm thick). To further reduce noise and the interferences of radio waves, the interior of the room was covered with a black sound deadening felt maintained in place with copper wire mesh (100 meshes per inch) (see [Fig 1](#) for details). The temperature of the observation chamber was kept constant at  $27 \pm 1^\circ\text{C}$ .

### Behavioral observations

Five different light treatments were used: A) same photoperiod as the rearing conditions, i.e. 12 hours of normal illumination (called Period 1 (day) between 7:00–19:00) and 12 hours of darkness (Period 2 (night) between 19:00–7:00), representing the normal conditions in the field (LD); B) 24 h of continuous illumination (LL); C) 24 h of continuous darkness (DD); D) Period 1 with normal illumination (quartz lamp) and Period 2 illuminated with yellow LED light (dominant wavelength: 569.0 nm, see [S2 Fig](#) for details) (LY); and E) Period 1 with normal illumination (quartz lamp) and Period 2 illuminated with green LED light (dominant wavelength: 508.0 nm, see [S3 Fig](#) for details) (LG). Light intensity remained constant at 2000 lux.

Each treatment included 20 replicates, i.e. 10 virgin adult females and 10 virgin adult males, which had emerged within 12 hours. For each observation session, only one newly emerged

(within 12 h) adult was transferred into a tube (diameter 1cm, height 10cm, made of transparent borosilicate glass, which displays high transmission of light) containing a tea tip maintained alive with water at the bottom (see Fig 1). The tea tips were picked from the tea plants in the greenhouse, not previously exposed to tea green leafhopper. The tea tips were replaced every other day. Very few leafhoppers (2 females and 5 males) were found dead during the experiments; in those cases, data were discarded and new leafhoppers were used to obtain 10 replicates per treatment.

The cameras began continuous recording at 0:00 h of the second (one day acclimation) day for the next 10 consecutive days. To analyze the frequencies and lengths of the different types of behavior, the videos were repetitively played using various speeds (maximum eightfold). The three types of behavior were reported as follows: 1) the number of locomotion events per hour, including walking, jumping, and flying. A locomotion event was considered as a fast move with the distance being equal or longer to the body length of the tested leafhopper, 2) the number of cleaning events per hour, where leafhoppers used their legs to clean their body, mainly their head and wings, 3) the number of minutes of searching activities per hour, which was described as any time a leafhopper left the tea tip and stayed or moved along the tube, as if it was to searching something (such as mate, new food, etc.).

## Data analyses

For each treatment and sex, charts of activities per hour were produced, and then the total number of activities (locomotion and cleaning) or minutes of searching activities over each Period (1 (day) and 2(night)) and for 24 hours was calculated for each of the 10 days. Data were compared among different photoperiod treatments (Treatment A, B and C) and wavelength treatments (Treatment B, D and E). Repeated-measures analyses of variance were used to determine whether the variation over the 10 days was significant (within subject variation) and if these results significantly differed among treatments and sexes (between subject variation). To examine the potential effect of light on day versus night activities, a ratio for each type of activities was calculated (Period 1 activities / total activities for a period of 24 hours) followed by repeated-measures analysis of variance. Normality and homogeneity of variances were satisfied and since the chamber effect as experimental run was *a priori* tested and was not significant, it was not further carried as a variable in the analyses.

Finally, the total number of activities (locomotion and cleaning) or minutes of searching activities in Periods 1 and 2 over the ten days was calculated for each individual. Two-way analysis of variance was used to compare treatment (photoperiod and wavelength separately), sex and their interaction.

## Results

Overall, the different light conditions resulted in significant changes in behavioral activities during the day and night as well as circadian patterns. Line charts of the mean values of locomotion, cleaning, and searching activities for each hour of the day for the 10 days of experimentation are shown in S4, S5 and S6 Figs. In the following sections, we described the general trends of behavioral patterns under the various photoperiods (LD, LL and DD) and wavelength treatments (LL, LY and LG).

### The effects of photoperiod

**Locomotion behavior.** Under LD, LL and DD, locomotion activities and the proportion of locomotion activities in Period 2 (Period 2 / (Period 1 + Period 2)) of both *E. onukii* males and females significantly varied over the 10 days and among sexes and treatments (Table 1).

**Table 1. Comparison of the behaviors of *E. onukii*, a) locomotion, b) cleaning, and c) searching, over time, sex and photoperiod treatments (LD, LL, DD) using a two factor repeated measures analysis of variance.**

Source of variation	Daily amount			Daily ratio of Period 2		
	df	F-value	P-value	df	F-value	P-value
<b>a) Locomotion events</b>						
<b>Within subject variation</b>						
Day	9, 46	15.59	<0.001	9, 46	2.36	0.013
Day*Treatment	18, 94	12.48	<0.001	18, 94	3.32	<0.001
Day*Sex	9, 46	5.16	<0.001	9, 46	2.02	0.037
Day*Treatment*Sex	18, 94	5.33	<0.001	18, 94	2.06	0.007
<b>Between subject variation</b>						
Treatment	2, 54	43.54	<0.001	2, 54	259.91	<0.001
Sex	1, 54	10.38	0.002	1, 54	2.08	0.155
Treatment*Sex	2, 54	2.18	0.123	2, 54	10.68	<0.001
<b>b) Cleaning events</b>						
<b>Within subject variation</b>						
Day	9, 46	4.99	0.001	9, 46	0.87	0.541
Day*Treatment	18, 94	7.26	<0.001	18, 94	0.77	0.721
Day*Sex	9, 46	2.55	0.039	9, 46	2.24	0.023
Day*Treatment*Sex	18, 94	4.75	<0.001	18, 94	1.25	0.225
<b>Between subject variation</b>						
Treatment	2, 54	66.63	<0.001	2, 54	77.59	<0.001
Sex	1, 54	0.41	0.527	1, 54	0.01	0.924
Treatment*Sex	2, 54	11.34	<0.001	2, 54	0.83	0.443
<b>c) Searching duration</b>						
<b>Within subject variation</b>						
Day	9, 46	18.53	<0.001	9, 46	3.74	<0.001
Day*Treatment	18, 94	6.13	<0.001	18, 94	3.31	0.003
Day*Sex	9, 46	2.52	0.015	9, 46	2.06	0.039
Day*Treatment*Sex	18, 94	5.89	<0.001	18, 94	3.12	<0.001
<b>Between subject variation</b>						
Treatment	2, 54	13.60	<0.001	2, 54	45.23	<0.001
Sex	1, 54	40.78	<0.001	1, 54	0.31	0.578
Treatment*Sex	2, 54	1.90	0.159	2, 54	2.57	0.086

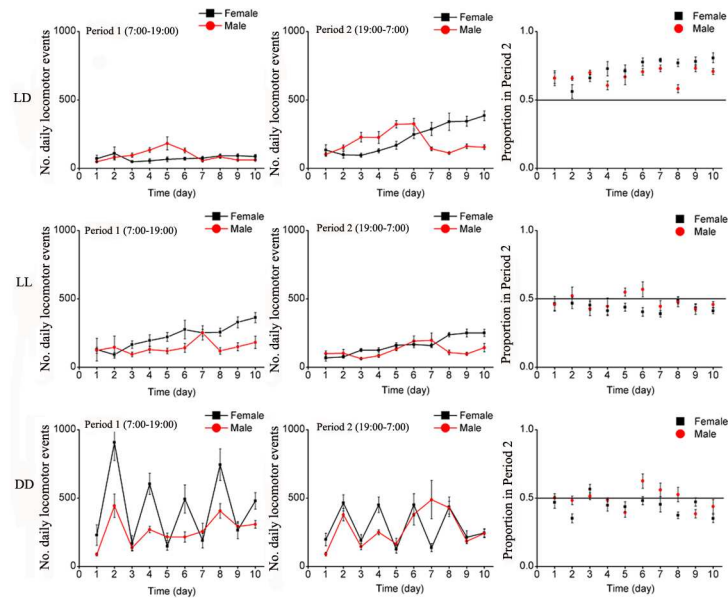
Treatments: A) Period 1 with light of quartz lamp, Period 2 with darkness; B) continuous illumination by quartz lamp; C) continuous darkness. Each day (24H) was divided as Period 1(7:00–19:00) and Period 2(19:00–7:00). Repeated-measures ANOVAs were used, since the sphericity assumption was not satisfied ( $P<0.05$ ), Huynh-Feldt was used for adjusting.

doi:10.1371/journal.pone.0168439.t001

Under normal light condition (LD), leafhoppers moved little during the day (Period 1) (Fig 2). At night, the insects were more active and values tended to increase over the 10 day-period, especially for males on Day 5.

The locomotion behavior of leafhopper was greatly affected by changes in light condition. Under continuous illumination (LL), both males and females were continuously active and activities slightly increased over time (Fig 2). However under complete darkness (DD), the rhythm of locomotion activities completely changed with a two-day cycle pattern and females showed a stronger signal than males (Fig 2).

The total numbers of locomotion events over 10 days were calculated for each sex and treatment. In Period 1, although there were significant differences between males and females, both



**Fig 2. Mean number of daily locomotion events of *E. onukii* adults under varying photoperiod treatments for the 10 days of observation, during Period 1 (left side) and Period 2 (center graphs), and the average proportion of locomotion events in Period 2 (Period 1 / (Period 1 + Period 2), right side). Each day (24h) was divided as Period 1 (7:00–19:00) and Period 2 (19:00–7:00). LD: Period 1 with light of quartz lamp, Period 2 with darkness; LL: continuous illumination by quartz lamp; DD: continuous darkness.**

doi:10.1371/journal.pone.0168439.g002

were the most active in complete darkness (DD), followed by continuous illumination and finally normal photoperiod (Table 2, Fig 3). In Period 2, only treatment effect was significant (Table 2) with more locomotion events in DD than LD and LL. Overall, both males and females were more active during the night than during the day under normal photoperiod compared to LL and DD (Fig 3).

**Cleaning Behavior.** Both males and females exhibited similar cleaning patterns over the period of 10 days and among treatments, (Table 1). In normal light condition (LD), the number of cleaning events was greater during Period 2 than Period 1 (Fig 4).

Under continuous illumination (LL), both males and females exhibited constant cleaning behavior for both Periods 1 and 2 (Fig 4). In continuous darkness (DD), females and males (however to a lesser extent) exhibited a pattern of activities with peaks occurring every 48

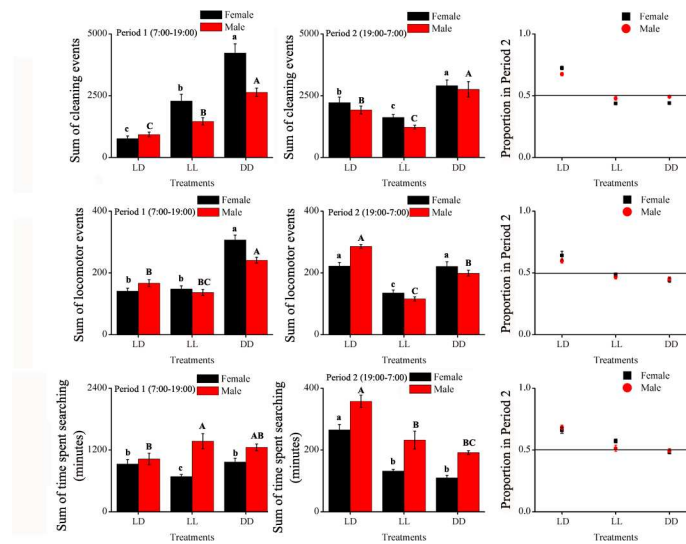
**Table 2. Results of a two-way ANOVA comparing the total number of locomotion and cleaning events and total amount of searching activities over the 10 days between sexes and treatments for a) Period 1 and b) Period 2.**

Period	Source of variation	Locomotion events			Cleaning events			Searching duration		
		df	F-value	P-value	df	F-value	P-value	df	F-value	P-value
Period 1	Treatment	2, 54	70.60	<0.001	2, 54	86.76	<0.001	2, 54	1.03	0.363
	Sex	1, 54	17.51	<0.001	1, 54	3.58	0.064	1, 54	21.598	<0.001
	Treatment*Sex	2, 54	8.11	<0.001	2, 54	8.67	0.001	2, 54	5.19	0.009
Period 2	Treatment	2, 54	24.50	<0.001	2, 54	81.62	<0.001	2, 54	52.29	<0.001
	Sex	1, 54	2.99	0.089	1, 54	0.74	0.393	1, 54	45.15	<0.001
	Treatment*Sex	2, 54	0.18	0.831	2, 54	11.31	<0.001	2, 54	0.16	0.853

Treatments: A) Period 1 with light of quartz lamp, Period 2 with darkness; B) continuous illumination by quartz lamp; C) continuous darkness. Each day (24h) was divided as Period 1 (7:00–19:00) and Period 2 (19:00–7:00). Two-way ANOVAs were used.

doi:10.1371/journal.pone.0168439.t002

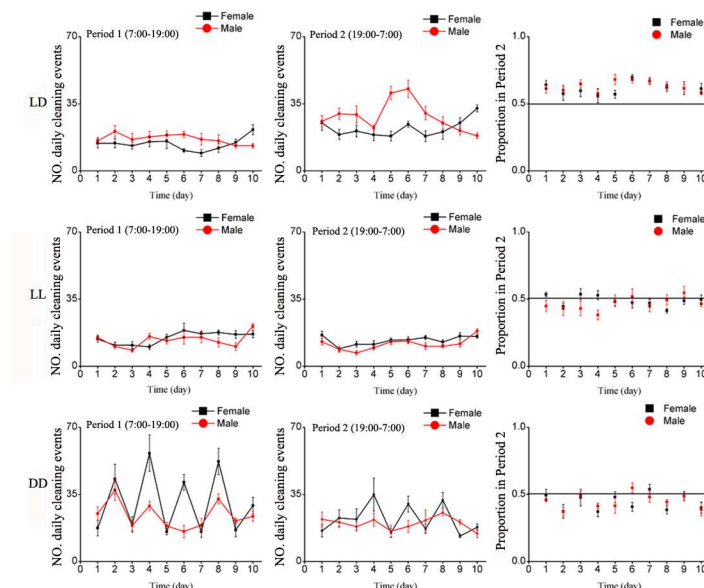




**Fig 3. Total amount of the three behaviors, cleaning, locomotion and searching activities, for the 10 days during Period 1 (left side) or Period 2 (center) of *E. onukii* males and females total ratio in Period 2 (Period 1 / (Period 1 + Period 2), right side), under varying photoperiod treatments.** Significant differences among the three treatments are marked with different letters (LSD,  $P < 0.05$ , females marked with lowercase letters, males marked with capital letters).

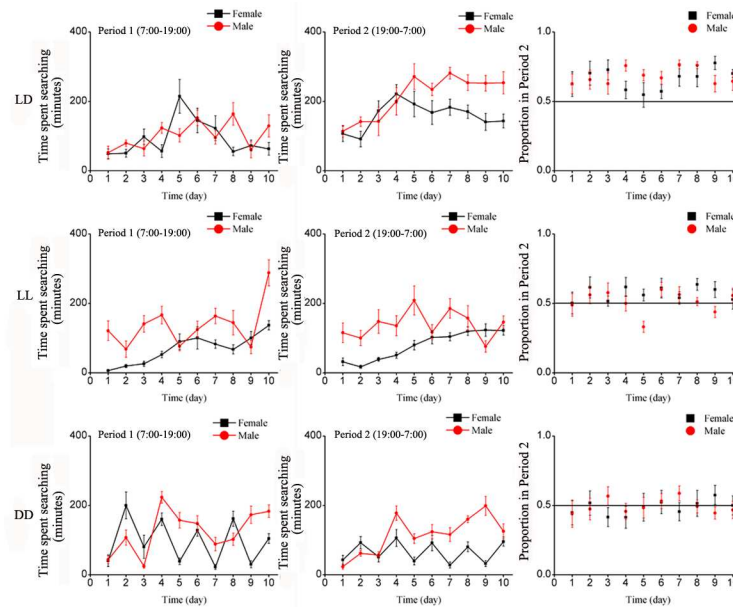
doi:10.1371/journal.pone.0168439.g003

hours. Females, however, tended to clean themselves more in Period 1 than in Period 2 (Fig 4). Overall, the total number of cleaning events over the 10-day period was significantly affected by treatment. The effects of various treatments however, were inconsistent between sexes as reflected in a significant treatment-by-sex interaction, while sex was not significant (Table 2).



**Fig 4. Mean number of daily cleaning events of *E. onukii* adults under varying photoperiod treatments for the 10 days of observation, during Period 1 (left side) and Period 2 (center graphs), and the average proportion of locomotion events in Period 2 (Period 1 / (Period 1 + Period 2), right side).** Each day (24H) was divided as Period 1(7:00–19:00) and Period 2(19:00–7:00). LD: Period 1 with light of quartz lamp, Period 2 with darkness; LL: continuous illumination by quartz lamp; DD: continuous darkness.

doi:10.1371/journal.pone.0168439.g004



**Fig 5. Mean number of daily searching events of *E. onukii* adults under varying photoperiod treatments for the 10 days of observation, during Period 1 (left side) and Period 2 (center graphs), and the average proportion of locomotion events in Period 2 (Period 1 / (Period 1 + Period 2), right side).** Each day (24H) was divided as Period 1 (7:00–19:00) and Period 2 (19:00–7:00). LD: Period 1 with light of quartz lamp, Period 2 with darkness; LL: continuous illumination by quartz lamp; DD: continuous darkness.

doi:10.1371/journal.pone.0168439.g005

In Period 1, leafhoppers were more active cleaning in DD than LD and LL. Like locomotion, under normal photoperiod (LD), both males and females were cleaning more in Period 2 than when exposed to LL or DD (Fig 3).

**Searching behavior.** Searching behavior of both males and females was significantly influenced by the variation in the type of wavelength illumination during Period 2 i.e. at night (Table 1). Under LD, the searching behavior of both males and females greatly varied over time. The time spent searching at night (Period 2) tended to be greater than in Period 1, especially for males (Fig 5). Under LL, females were less active than males although the searching activities tended to progressively increased over time. Females exposed to DD exhibited a two-day pattern of activities with higher peaks in Period 1 than Period 2. Overall, like for locomotion and cleaning, under normal photoperiod, both males and females tended to be more active searching during Period 2 than Period 1 (Fig 3). Overall, the total number of searching behavior over the 10-day period was not significantly affected by treatment, however, there was a significant treatment-by-sex interaction (Table 2).

### The effects of light wavelength

**Locomotion behavior.** Changes in wavelength at night (Period 2) significantly affected the locomotion behavior of *E. onukii* (Table 3). Compared to LL, locomotion of leafhoppers exposed to LY and LG was suppressed and this was especially true in LG (Fig 6). Under the three treatments, the number of locomotion events during Period 1 was relatively similar to those in Period 2 (Fig 6).

Locomotion behavior of both males and females was statistically non-significant when summed over the 10 days showing similar suppression of activities in Period 2 (Table 4). The



**Table 3. Comparison of the behaviors of *E. onukii*, a) locomotion, b) cleaning, and c) searching, over time, sex and wavelength treatments (LL, LY, LG) using a two way repeated measures analysis of variance.**

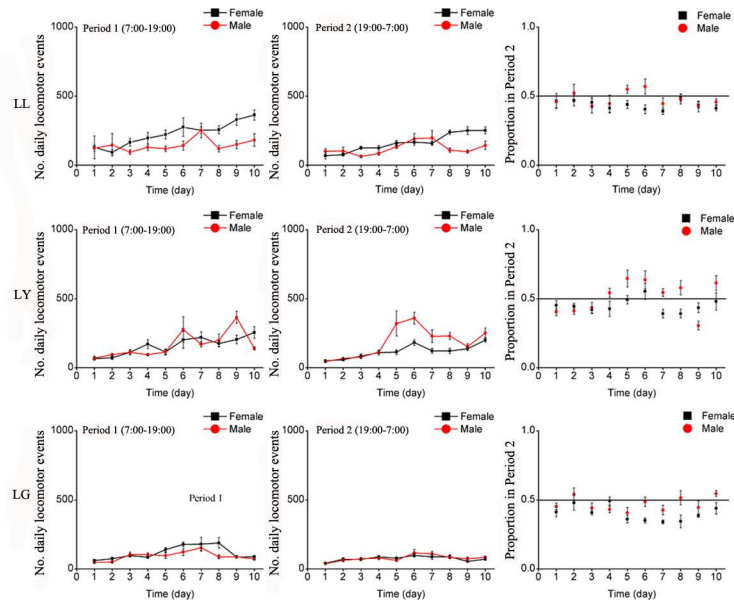
Source of variation	Daily amount			Daily ratio of Period 2		
	df	F-value	P-value	df	F-value	P-value
<b>a) Locomotion events</b>						
<b>Within subject variation</b>						
Day	9, 46	26.97	<0.001	9, 46	4.03	<0.001
Day*Treatment	18, 94	4.11	<0.001	18, 94	3.20	<0.001
Day*Sex	9, 46	2.39	0.033	9, 46	3.19	0.001
Day*Treatment*Sex	18, 94	3.91	<0.001	18, 94	1.57	0.067
<b>Between subject variation</b>						
Treatment	2, 54	17.66	<0.001	2, 54	5.14	0.009
Sex	1, 54	1.50	0.288	1, 54	24.28	<0.001
Treatment*Sex	2, 54	6.15	0.004	2, 54	0.51	0.603
<b>b) Cleaning events</b>						
<b>Within subject variation</b>						
Day	9, 46	14.28	<0.001	9, 46	1.68	0.105
Day*Treatment	18, 94	3.42	<0.001	18, 94	1.35	0.168
Day*Sex	9, 46	2.45	0.028	9, 46	1.70	0.102
Day*Treatment*Sex	18, 94	3.36	<0.001	18, 94	0.99	0.470
<b>Between subject variation</b>						
Treatment	2, 54	8.78	<0.001	2, 54	12.61	<0.001
Sex	1, 54	0.57	0.453	1, 54	0.25	0.622
Treatment*Sex	2, 54	4.14	0.021	2, 54	0.64	0.533
<b>c) Searching duration</b>						
<b>Within subject variation</b>						
Day	9, 46	37.00	<0.001	9, 46	1.47	0.177
Day*Treatment	18, 94	3.64	<0.001	18, 94	3.66	<0.001
Day*Sex	9, 46	2.47	0.026	9, 46	1.36	0.219
Day*Treatment*Sex	18, 94	2.76	0.002	18, 94	3.04	<0.001
<b>Between subject variation</b>						
Treatment	2, 54	4.42	0.017	2, 54	2.57	0.086
Sex	1, 54	8.29	0.006	1, 54	1.21	0.276
Treatment*Sex	2, 54	12.02	<0.001	2, 54	2.00	0.146

Treatments: B) continuous illumination by quartz lamp; D) Period 1 with light of quartz lamp, Period 2 with yellow light (LED); E) Period 1 with light of quartz lamp, Period 2 with green light (LED). Each day (24H) was divided as Period 1(7:00–19:00) and Period 2(19:00–7:00). Repeated-measures ANOVAs were used, since the sphericity assumption was not satisfied ( $P<0.05$ ), Huynh-Feldt was used for adjusting.

doi:10.1371/journal.pone.0168439.t003

overall number of locomotion events under LG was less lower than those under LL and LY (Fig 7).

**Cleaning behavior.** The number of cleaning events significantly varied over the period of 10 days and among treatments, especially for Period 2 (LY) (Table 3, Fig 8). Cleaning activities under LG were suppressed in both Periods compared to LL and LY (Fig 7). Under LG leafhoppers exhibited less cleaning activities during Period 2 than under LL and LY (Fig 8). The total number of activities calculated over the ten days showed significant treatment and sexes by treatment interaction effects in Period 2 (Table 4), which was reflected being less active during Period 2 than Period 1, especially for the males.



**Fig 6. Mean number of daily locomotion events of *E. onukii* adults under varying wavelength treatments for the 10 days of observation, during Period 1 (left side) and Period 2 (center graphs), and the average proportion of locomotion events in Period 2 (Period 1 / (Period 1 + Period 2), right side).** Each day (24H) was divided as Period 1(7:00–19:00) and Period 2(19:00–7:00). LL: continuous illumination by quartz lamp; C) continuous darkness; LY: Period 1 with light of quartz lamp, Period 2 with yellow light (LED); LG: Period 1 with light of quartz lamp, Period 2 with green light (LED).

doi:10.1371/journal.pone.0168439.g006

**Searching behavior.** Unlike in LL, under LY and LG, females and males took a few days to become more active in searching but then the behavior pattern fluctuated in a similar way than under LL (Table 3). Searching behavior greatly varied during the course of the 10 days but with no or little significant differences among treatments and sexes (Fig 9 and Table 4).

## Discussion

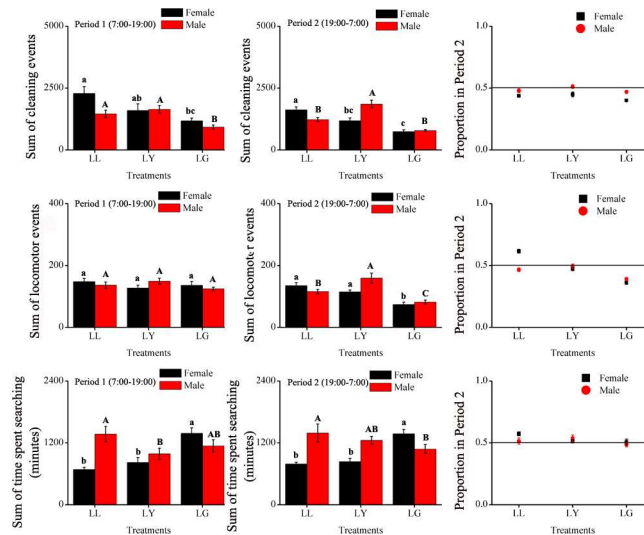
Our study showed that both photoperiod and light wavelength had significant effects on locomotion, cleaning and searching behaviors of *E. onukii* adults. A few studies on other leafhopper species have reported changes in behavior under changing light conditions. In *Graminella nigrifrons*, the males oriented toward light when searching for mates [26]. *Dalbulus maidis* displays more phototaxis to 560 nm than any other wavelength [27].

**Table 4. Comparison of the three behavioral values of *E. onukii* in total ten days’ observation in different light wavelength treatments, during Period 1 and Period 2 using a two way ANOVA.**

Period	Source of variation	Locomotion events			Cleaning events			Searching duration		
		df	F-value	P-value	df	F-value	P-value	df	F-value	P-value
Period 1	Treatment	2, 54	10.29	<0.001	2, 54	0.83	0.443	2, 54	1.03	0.363
	Sex	1, 54	5.16	0.027	1, 54	0.001	0.977	1, 54	21.60	<0.001
	Treatment*Sex	2, 54	2.80	0.070	2, 54	2.01	0.144	2, 54	5.19	0.009
Period 2	Treatment	2, 54	32.52	<0.001	2, 54	21.98	<0.001	2, 54	5.87	0.005
	Sex	1, 54	1.56	0.217	1, 54	2.07	0.156	1, 54	5.41	0.024
	Treatment*Sex	2, 54	13.70	<0.001	2, 54	5.84	0.005	2, 54	9.51	<0.001

Treatments: B) continuous illumination by quartz lamp; D) Period 1 with light of quartz lamp, Period 2 with yellow light (LED); E) Period 1 with light of quartz lamp, Period 2 with green light (LED). Each day (24H) was divided as Period 1 (7:00–19:00) and Period 2 (19:00–7:00). Two-way ANOVAs were used.

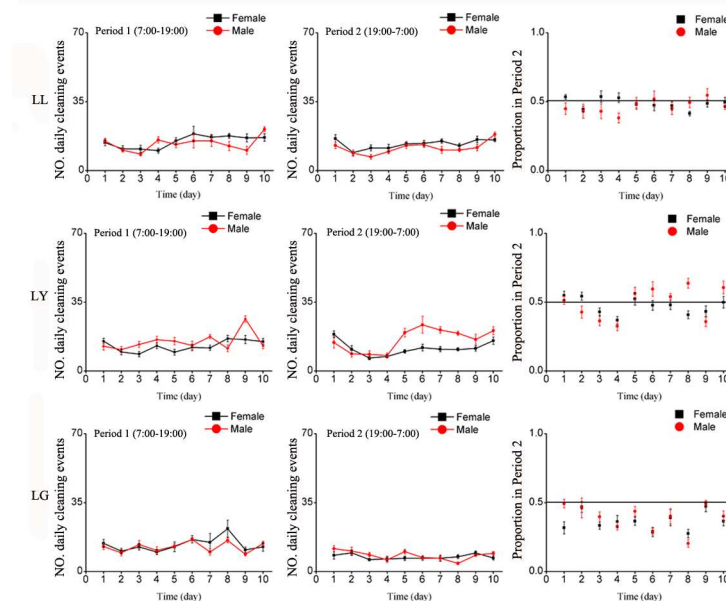
doi:10.1371/journal.pone.0168439.t004



**Fig 7. Total amount of the three behaviors, locomotion, cleaning and searching activities, for the 10 days during Period 1 (left side) or Period 2 (center) of *E. onukii* males and females total ratio in Period 2 (Period 1 / (Period 1 + Period 2), right side), under varying wavelength treatments.** Significant differences among the three treatments are marked with different letters (LSD,  $P < 0.05$ , females marked with lowercase letters, males marked with capital letters).

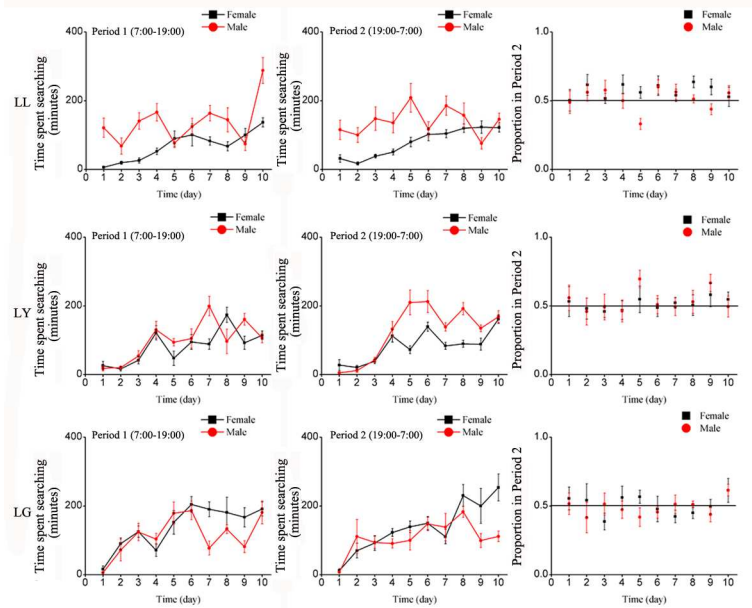
doi:10.1371/journal.pone.0168439.g007

The first important observation was that *E. onukii* displayed more active at night than during the day and could be considered either day neutral or slightly nocturnal. *E. onukii* reproduction is a long process that mainly occurs at night, possibly to reduce predation (Shi et al. in



**Fig 8. Mean number of daily cleaning events of *E. onukii* adults under varying wavelength treatments for the 10 days of observation, during Period 1 (left side) and Period 2 (center graphs), and the average proportion of locomotion events in Period 2 (Period 1 / (Period 1 + Period 2), right side).** Each day (24H) was divided as Period 1 (7:00–19:00) and Period 2 (19:00–7:00). LL: continuous illumination by quartz lamp; C) continuous darkness; LY: Period 1 with light of quartz lamp, Period 2 with yellow light (LED); LG: Period 1 with light of quartz lamp, Period 2 with green light (LED).

doi:10.1371/journal.pone.0168439.g008



**Fig 9. Mean number of daily searching events of *E. onukii* adults under varying wavelength treatments for the 10 days of observation, during Period 1 (left side) and Period 2 (center graphs), and the average proportion of locomotion events in Period 2 (Period 1 / (Period 1 + Period 2), right side).** Each day (24H) was divided as Period 1(7:00–19:00) and Period 2(19:00–7:00). LL: continuous illumination by quartz lamp; LY: Period 1 with light of quartz lamp, Period 2 with yellow light (LED); LG: Period 1 with light of quartz lamp, Period 2 with green light (LED).

doi:10.1371/journal.pone.0168439.g009

preparation). Unlike some other leafhoppers such as *Macrosteles fascifrons* and *G. nigrifrons* [28, 29], *E. onukii* does not migrate in daytime and can feed on the evergreen tea plants during the whole year (although populations and activities may be reduced during cooler winter conditions, suggesting possible diapause), it is possible that nocturnal activities represent an adaptive response to avoid predators, especially during reproductive periods.

Circadian rhythms have been detected in most organisms including insects [30, 31]. They can influence physiology and behavior such as locomotion (walking, flying, swimming, etc.), feeding, cleaning, courtship, and mating [18, 32, 33], and are regulated by clock genes that maintain cycles of around 24 hours. They can be influenced by external stimuli that reset the rhythm to a new period [32, 34]. Among these stimuli, light is considered one of the most important factors for insects [3, 35]. Continuous illumination can lead to behavioral arrhythmicity in insect such as *D. melanogaster* and *Calliphora vicina* [36, 37]. In our case, under complete darkness, the species modified its rhythm to a two-day cycle of activities.

Insects have different types of photoreceptors that are distributed in various tissues, leading to different levels of sensitivity to wavelengths [3, 38, 39]. Changes in wavelength trigger responses of the photoreceptors, which may affect physiology and behaviors. In our case, different wavelength exposure in Period 2 led to significant behavioral changes. For instance, under LG, locomotion and cleaning activities were seriously suppressed when compared to LL and LY.

Our study also showed that changes in behavior in function of photoperiod and wavelength could be sex-specific. Between-sex difference has already been observed in *E. onukii* where male adults are more attracted by yellow sticky cards than females [16]. We also observed that males were more active than females only under the yellow light treatment suggesting phototaxis response to this wavelength. Sexual dimorphism in phototaxis has been shown in other

leafhoppers, such as *E. vitis* and *Scaphoideus titanus* [40, 41]. Differences found between sexes may be attributed to sexual dimorphism of their photoreceptors leading to variation in response to different wavelengths [42]. Insects such as butterflies (e.g. species of *Lycaena* and *Colias erate*) and *D. melanogaster* display such sexual dimorphism in response to wavelength variation [43, 44, 45]. Further research is needed to confirm the differences in photoreceptors between male and female leafhoppers.

We have to acknowledge that this experiment was conducted under controlled laboratory conditions with rectangular light cycles and under constant temperature. This does not necessarily represent what leafhoppers experience in their natural environment where photoperiod and temperature can vary over the day and year. Experiments conducted with *D. melanogaster* under natural or semi-natural conditions show that additional daytime peaks can be found and be of importance [46, 47]. Moreover, some strains of *D. melanogaster* have been reported not to be day-neutral as first thought but exhibit an ovarian diapause under long nights and low temperatures [18]. *E. onukii* populations in China may also be influenced by these conditions. Previous observations show that populations tend to peak in July, when temperatures are 26°C or higher, and decline in the fall when temperatures drop to 15°C [16, 48]. Future studies should examine how natural photoperiod and temperature affect *E. onukii* behavioral patterns. Since photoperiodic responses can be complex as they involve several factors such as photoreception mechanisms, night length influence, and endocrine effectors that determine developmental steps, further genetic and molecular analyses should be included to better understand the roles of clock genes in the photoperiodic responses of the species [18].

Several of the 22,000 species of leafhoppers in the world are considered serious pests in agriculture and forestry [49]. Besides insecticides (including microbial insecticides), yellow sticky cards, natural enemies, and trap plants have been used as alternative management approaches [50, 51]. A previous study suggests that *E. onukii* male adults tend to be more attracted to yellow sticky cards than females [16]. Böll et al. (2004) also suggest that the difference in activity patterns between the two sexes of *E. vitis* may influence the level of attractiveness to the color of sticky card traps [40]. Considering that the present study showed that most behavioral activities such as locomotion and searching occur at night, to enhance pest control efficiency, it may be useful to supplement the control of sticky cards that are used during the day with a light control system at night. Although, light traps are now widely used for pest control [6, 52, 53], negative impacts on natural pest enemies remain a concern [54, 55]. Further research will be required to test such control systems to determine whether natural pest enemies, such as spiders, parasitoids, predatory mites, are also trapped leading to a decline in their populations as well as the pest. Further experiments of these lights (with or without trap) on leafhopper populations and pest enemies in the field will help determine their effectiveness as alternative control.

## Supporting Information

### S1 Fig. Spectral distribution of quartz lamp.

(TIF)

### S2 Fig. Spectral distribution of yellow LED light.

(TIF)

### S3 Fig. Spectral distribution of green LED light.

(TIF)

**S4 Fig. Line charts of the mean values of locomotion events (Mean  $\pm$  SEM).** Locomotion events (with standard errors) at each hour of the day for the 10 days of experimentation. For

each hour, the mean values of locomotion behaviour of 10 tested leafhoppers in each treatment are shown by line charts with standard errors. Every small chart got the same axis values. Treatments: A) Period 1 with light of quartz lamp, Period 2 with darkness; B) continuous illumination by quartz lamp; C) continuous darkness; D) Period 1 with light of quartz lamp, Period 2 with yellow light (LED); E) Period 1 with light of quartz lamp, Period 2 with green light (LED). Each day (24H) was divided as Period 1(7:00–19:00) and Period 2(19:00–7:00). (TIF)

**S5 Fig. Line charts of the mean values of cleaning events (Mean  $\pm$  SEM).** Cleaning events (with standard errors) at each hour of the day for the 10 days of experimentation. For each hour, the mean values of cleaning behaviour of 10 tested leafhoppers in each treatment are shown by line charts with standard errors. Every small chart got the same axis values. Treatments: A) Period 1 with light of quartz lamp, Period 2 with darkness; B) continuous illumination by quartz lamp; C) continuous darkness; D) Period 1 with light of quartz lamp, Period 2 with yellow light (LED); E) Period 1 with light of quartz lamp, Period 2 with green light (LED). Each day (24H) was divided as Period 1(7:00–19:00) and Period 2(19:00–7:00). (TIF)

**S6 Fig. Line charts of the mean values of searching duration (Mean  $\pm$  SEM).** Searching duration (with standard errors) at each hour of the day for the 10 days of experimentation. For each hour, the mean values of searching behaviour of 10 tested leafhoppers in each treatment are shown by line charts with standard errors. Every small chart got the same axis values. Treatments: A) Period 1 with light of quartz lamp, Period 2 with darkness; B) continuous illumination by quartz lamp; C) continuous darkness; D) Period 1 with light of quartz lamp, Period 2 with yellow light (LED); E) Period 1 with light of quartz lamp, Period 2 with green light (LED). Each day (24H) was divided as Period 1(7:00–19:00) and Period 2(19:00–7:00). (TIF)

## Acknowledgments

The authors are grateful to Mr Fasheng Huang for laboratory assistance.

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**Methodology:** LS LV MY.

**Project administration:** MY LV.

**Resources:** HH.

**Software:** LS LV.

**Supervision:** MY LV.

**Validation:** LS LV HH ZZ XL MY.



**Visualization:** LS.

**Writing – original draft:** LS.

**Writing – review & editing:** LS MY LV HH ZZ GH.

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