BMC Biology

RESEARCH ARTICLE

Open Access

CRY1 is involved in the take-off behaviour of migratory *Cnaphalocrocis medinalis* individuals

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Abstract

Background Numerous insect species undertake long-distance migrations on an enormous scale, with great implications for ecosystems. Given that take-off is the point where it all starts, whether and how the external light and internal circadian rhythm are involved in regulating the take-off behaviour remains largely unknown. Herein, we explore this issue in a migratory pest, *Cnaphalocrocis medinalis*, via behavioural observations and RNAi experiments.

Results The results showed that *C. medinalis* moths took off under conditions where the light intensity gradually weakened to 0.1 lx during the afternoon or evening, and the take-off proportions under full spectrum or blue light were significantly higher than that under red and green light. The ultraviolet-A/blue light–sensitive type 1 cryptochrome gene (*Cmedcry1*) was significantly higher in take-off moths than that of non-take-off moths. In contrast, the expression of the light-insensitive *CRY2* (*Cmedcry2*) and circadian genes (*Cmedtim* and *Cmedper*) showed no significant differences. After silencing *Cmedcry1*, the take-off proportion significantly decreased. Thus, *Cmedcry1* is involved in the decrease in light intensity induced take-off behaviour in *C. medinalis*.

Conclusions This study can help further explain the molecular mechanisms behind insect migration, especially light perception and signal transmission during take-off phases.

Keywords *Cnaphalocrocis medinalis*, Migratory insect, Light intensity, Take-off behaviour, Cryptochrome

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Background

Migration is a behavioural strategy evolved by insects during long-term adaptation to unstable environments, allowing them to avoid unfavourable environments and expand their distribution areas in space as well as to prolong their occurrence periods and maintain population growth in time [1–4]. The substantial movement of migratory insects vastly affects communities through predation and competition [5]. Moreover, the energy, propagules, pathogens and parasites accompanying insect migration can exert substantial and intricate effects on ecosystems and agricultural productivity [5, 6]. Take-off is the initial stage of migration and serves as the beginning of all subsequent behaviours [7]. However, numerous environmental factors trigger insect take-off behaviour [8–11]. When and how they decide to take off and start their journey remains unknown. Many studies have found that most nocturnal migratory insects take off at dusk (when the Sun is 6° below the horizon) [5, 12–14]. Nevertheless, it is not clear whether take-off behaviour of nocturnal insects is caused by light intensity changes at sunset or is regulated by endogenous circadian rhythms; it is also possible that both are involved in this regulation process.

Cryptochrome (CRY) is an important non-imaging visual sensor involved in mediating insect light attraction/avoidance, light awakening and other behaviours [15–19]. As a light-sensing flavin-based photopigment, it can detect blue light in its flavin adenine dinucleotide (FAD)-oxidised and FAD-anionic semiquinone semi-reduced states [20–22]. CRY transduces light information to this system by providing a light-dependent attenuation of period (PER) and timeless (TIM) activity [23–25]. As a typical seasonal response, migration flight in insects is widely considered to be regulated by the circadian clock [26]. The circadian clock is a group of 24-h transcription–translation feedback loops (TTFLs), which operate using self-inhibition via transcriptional repressors TIM and PER in one of the loops [27, 28]. When PER and TIM are degraded, transcriptional activators clock (CLK) and cycle (CYC) can activate the transcription of PER and TIM mRNAs, thus restarting the circadian rhythm [28, 29]. The rhythmic behaviour of insects is regulated by the biological clock and *crys* [24, 26, 30]. *Drosophila* shows rhythmic and active behaviour at the turn of day and night, while it shows behavioural arrhythmicity rapidly in continuous light environments [31]. In strains silencing *cry*, *Drosophila melanogaster* showed stable rhythmic and active behaviour under continuous light [32]. Therefore, if the take-off behaviour of insects is triggered by changes in light intensity, CRY is likely to be involved in sensing the light signals. Conversely, if take-off is a rhythmic behaviour, the normal functioning of the circadian clock may be essential for the take-off to occur.

The rice leaf folder, *Cnaphalocrocis medinalis* (Guenée), is a major migratory insect pest of rice with strong migration ability; there have been serious outbreaks in many Asian countries in recent decades, especially in China [33, 34]. *C. medinalis* can migrate over long distances over many nights; in China, it migrates from the south to the north every spring and summer and back to the south in autumn and winter [35, 36]. As a nocturnal migratory insect, *C. medinalis* moths take off only at dusk, then continues its migration until the dawn of the following day, taking off again at the next dusk [37]. Previous studies on the take-off behaviour of *C. medinalis* found significant light dependence [38]. Considering that light intensity, spectral composition, circadian timing and different light input channels may contribute to light-modulated behaviours, we put forward the following questions about the take-off behaviour mechanism of *C. medinalis*: (i) What are the necessary lighting conditions to induce take-off behaviour in *C. medinalis*? (ii) Is the take-off behaviour of *C. medinalis* rhythmic? Is the take-off behaviour regulated by the circadian rhythm? (iii) What is the photoreceptor responsible *C. medinalis* for receiving the light signal and inducing take-off?

To address these issues, we conducted a series of experiments from multiple perspectives, including insect ecology, behavioural and molecular biology. We observed the take-off behaviour of field populations and investigated the optical signal parameters that trigger take-off behaviour in *C. medinalis* by conducting monochromatic light induction experiments to identify its sensitive wavelength. Additionally, we determined differences in the relative expression of *Cmedcrys* and circadian rhythm genes (*Cmedper* and *Cmedtim*) in different light wavelengths in take-off and non-take-off individuals and observed changes in the take-off behaviour and relative expression of other genes in full-spectrum and blue light after interfering with the expression of *Cmedcry1* via RNA interference (RNAi). The results of this study enhance our understanding of the regulatory mechanisms of photoinduced migration in *C. medinalis* and shed light on the development of novel control measures for outbreaks of migratory insect pests.

Results

Field population took off under gradual darkening conditions

Take-off behaviour of 360 *C. medinalis* moths were observed in a cage located in rice fields from 19:00 to 21:00 h Beijing time (same thereafter) from 14 July to 6 August 2019 in Yongfu County, while sunset occurred around 19:30 h (Additional file 1: Table S1). When a

moth took off and spiralled vertically at a height greater than 100 cm, this was considered as a migratory take-off event in this study [39]. Two hundred forty two of these moths (67.2%) were observed to take off under evening twilight conditions (light intensity below 10 lx) after sunset, with 96.6% (234/242) taking off during the period of 19:45–20:15 h, which was significantly higher than during other periods ($\chi^2 = 416.0$, df = 6, $P < 0.001$) (Fig. 1A). Very few moths (3.4%, 8/242) took off after 20:15 h when the light was very weak (less than 0.1 lx) (Fig. 1A). These results indicated that take-off behaviour in *C. medinalis* usually occurs within 30 min after sunset and requires twilight as a trigger.

From September 1–30, 2019, 427 moths were collected from rice fields and observed indoors in simulated sunset conditions. The take-off proportion within the 30 min before sunset was only 9.1% (39/427), but that within the 15 min after sunset was as high as 43.6% (186/427), after which the take-off proportion of *C. medinalis* gradually decreased (Fig. 1B). In total, 70.7% (302/427) of moths took off within 30 min after sunset, which was significantly higher than in the other time periods ($\chi^2 = 254.0$, df = 1, $P < 0.001$) (Fig. 1B). Therefore, observations in both the field and laboratory showed that the peak in *C. medinalis* take-off activity appeared within the 30 min after sunset. Since the laboratory observations excluded other environmental factors and only simulated the changes in light intensity at sunset, we thus speculated that the decrease in light intensity was the major factor that induced take-off behaviour in *C. medinalis*.

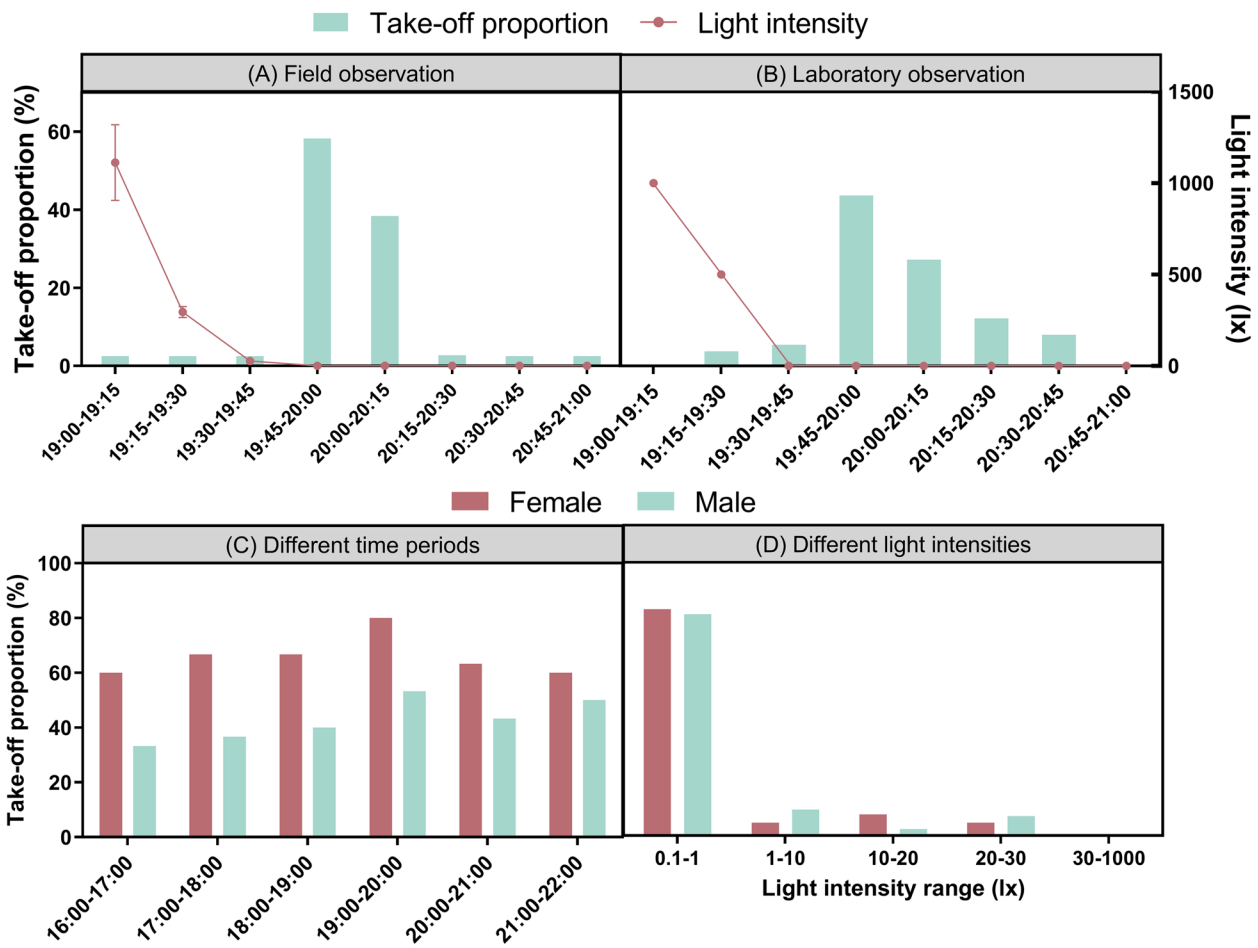

Fig. 1 Take-off behaviour of field populations at different time periods under field conditions (**A**) and laboratory simulated environments (**B**) and laboratory populations at different time periods (**C**) and light intensities (**D**). Red lines with circles mean light intensity in different time periods, and the data are presented as average SEM. During the non-treatment time period, the photoperiod was set according to the conditions when *C. medinalis* was reared, that is, 5:30–19:30 was the light period

Take-off dependent on low light intensity but not time period

To determine whether the take-off behaviour in *C. medinalis* moth is governed by the circadian rhythm, 30 lab-reared moths (2 d old) were observed in cages for 1-h periods from 16:00–22:00 with simulated sunset light changes (1000 to 0.1 lx). The take-off proportions of females and males ranged from 60.0% (18/30) to 80.0% (24/30) and from 40.0% (12/30) to 53.3% (16/30), respectively. There were no differences in take-off proportions among all the time periods in both males and females (female: $\chi^2 = 3.7$, df = 5, $P = 0.594$; male: $\chi^2 = 3.7$, df = 5, $P = 0.600$) (Fig. 1C). This result indicated that moths took off by perceiving the decreasing light intensity rather than this being controlled by circadian rhythms.

Further, the take-off proportion of moths under different light intensities ranging between 1000 and 0.1 lx were analysed. The results showed that *C. medinalis* took off at specific light intensities (females: $\chi^2 = 16.8$, df = 3, $P < 0.001$; males: $\chi^2 = 9.4$, df = 3, $P = 0.024$). When the light intensity was greater than 30 lx, female and male moths did not take off (Fig. 1D). When the light intensity decreased to 20–30 lx, a few moths (females: 4.68%, 3/64; males: 7.14%, 3/42) began to show take-off propensity (Fig. 1D). However, when the light intensity was reduced to below 1 lx, the take-off proportion of moths reached its highest, with 82.81% (53/64) and 80.95% (34/42) of female and male moths, respectively, taking off (Fig. 1D). Thus, *C. medinalis* took off under low light intensities ranging from 0.1 to 1.0 lx.

Upregulation of *Cmedcry1* in migratory moths

Compared to non-take-off moths (resident moths), the relative expression of ultraviolet-A (UA)/blue light–sensitive *Cmedcry1* was significantly higher in take-off moths ($t = 7.59$, df = 4, $P = 0.016$) (Fig. 2). However, there was no difference in the expression of *Cmedcry2* ($t = 0.98$, df = 4, $P = 0.381$) and *Cmedtim* ($t = 1.13$, df = 4, $P = 0.323$) and *Cmedper* ($t = 0.04$, df = 4, $P = 0.972$) among take-off and non-take-off moths (Fig. 2).

Since migratory and non-migratory *C. medinalis* individuals cannot be distinguished by phenotypic differentiation before take-off, we used starvation treatment as a proxy, because starvation causes *C. medinalis* to exhibit a stronger propensity for migratory take-off behaviour [39]. To rule out the possibility that the change in *Cmedcry1* expression was caused by take-off behaviour, we used moths under starvation (only fed with water) and feeding (fed with 5% honey solution) treatments as migratory and non-migratory populations to analyse the difference in *Cmedcry1* expression levels during the 6 h before and after the transition between light and dark periods. Significant differences were found in the expression of *Cmedcry1* between them before the light and dark transition ($F_{1, 16} = 38.62$, $P < 0.001$) (Additional file 1: Fig. S1). The relative expression of *Cmedcry1* was higher in the starvation treatment group, showing a significant upregulation trend after sunset (Additional file 1: Fig. S1). In contrast, *C. medinalis* in the feeding treatment group maintained low expression levels and showed no significant differences in response to changes in light cues (Fig. S1). Particularly around sunset (17:00 and 21:00), the expression levels of *Cmedcry1* differed significantly

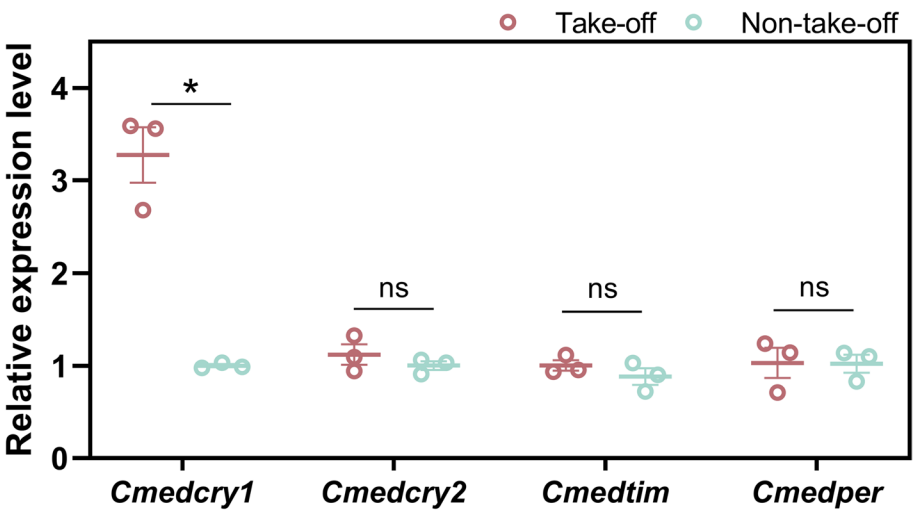

Fig. 2 The relative expression levels of *Cmedcry1*, *Cmedcry2*, *Cmedtim* and *Cmedper* in take-off and non-take-off individuals. The long horizontal lines represent the means, the upper and lower lines represent the average SEM, and * indicating significant differences (*t*-test—$P < 0.05$), while 'ns' indicates non-significant differences

between starved and fed moths (Additional file 1: Fig. S1), indicating that individuals with a higher propensity for migration exhibit higher levels of *Cmedcry1* expression.

More moths took off under full-spectrum or blue light than under green and red light

The take-off proportion of *C. medinalis* moths was expected to be higher under UA/blue light conditions if their take-off behaviour is induced by the UA/blue light–sensitive CRY1. Therefore, 2-day-old female moths were observed taking off under four different light wavelengths (full spectrum, blue light, green light, red light). There was a significant difference in the take-off proportion under different wavelengths ($\chi^2 = 55.11$, df = 3, $P < 0.001$) (Fig. 3A). The take-off proportion of female moths under the full-spectrum and blue light treatments were 76.3% (58/76) and 78.0% (103/123), respectively, and were not significantly different (Fig. 3A). However,

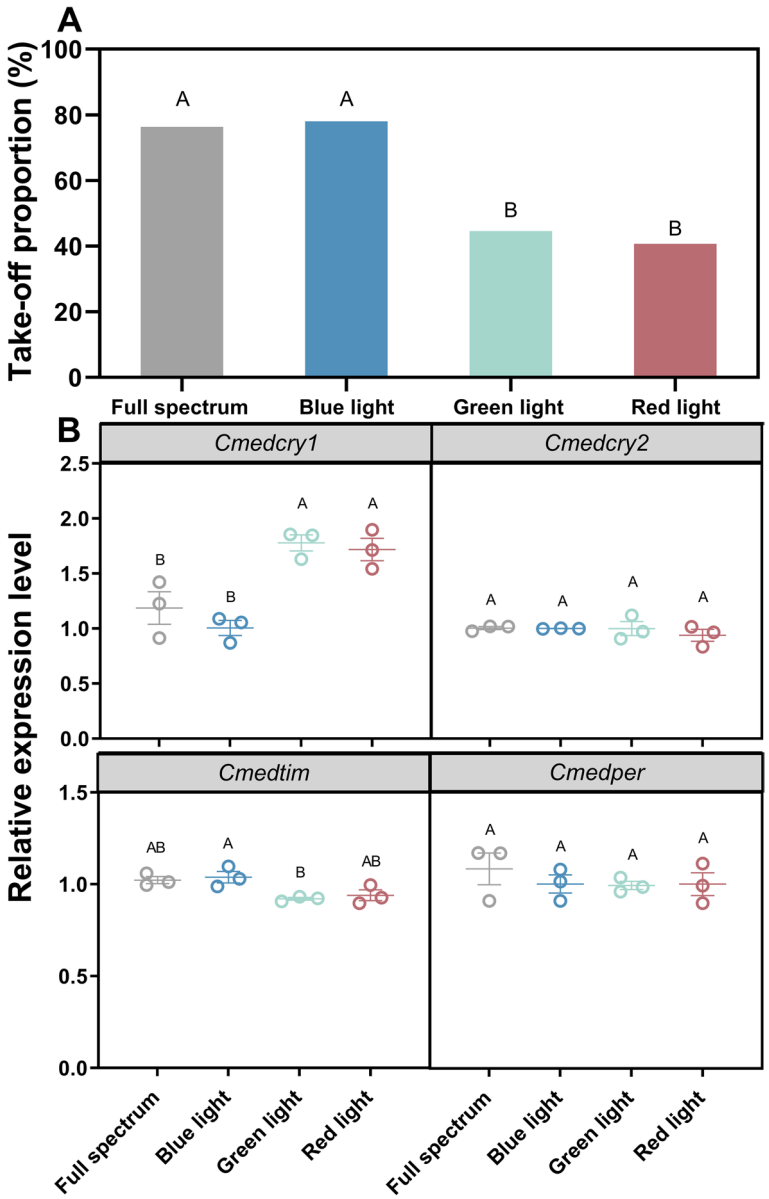

Fig. 3 Take-off proportion (**A**) and the relative expression of *Cmedcry1*, *Cmedcry2*, *Cmedtim* and *Cmedper* under full-spectrum, blue, green and red light (**B**). The circles represent the individual measured values of the samples, the long horizontal lines represent the means, the upper and lower lines represent the average standard error of the mean and significant differences in take-off proportion (chi-squared test—$P < 0.05$) and relative expression levels (Tukey's HSD—$P < 0.05$) were observed among different letters

the take-off proportion of female moths under green (44.6%, 45/101) or red light (40.6%, 50/123) was significantly lower than those under full-spectrum and blue light (Fig. 3A).

Consistent with the take-off proportion results, only the *Cmedcry1* receptor sensing UA/blue light showed significant differences in expression under different light conditions ($F_{3,\ 8}=13.92$; $P=0.002$), while there were no significant differences in expression of *Cmedcry2* ($F_{3,\ 8}=0.06$; $P=0.335$) and the circadian gene *Cmedper* ($F_{3,\ 8}=0.50$; $P=0.844$). The relative expression of *Cmedtim* under green light was significantly lower than that under blue light ($F_{3,\ 8}=6.04$; $P=0.019$) (Fig. 3B). Here, moths were placed in the specific spectral conditions for 4 h before experiments. As the relative expression level of *Cmedcry1* was significantly higher after being placed in darkness (Additional file 1: Fig. S2 & S3), the expression of *Cmedcry1* under green/red light conditions (possibly similar to dark conditions for CRY1) was significantly higher than that under full-spectrum or blue light conditions (Fig. 3B).

Silencing *Cmedcry1* reduced the take-off proportion

After injecting *dsCRY1* into newly emerged female *C. medinalis* adults, the expression of *Cmedcry1* was significantly reduced by over 50 and 60% under full-spectrum ($t=3.57$, df$=4$, $P=0.023$) and blue light ($t=2.892$, df$=4$, $P=0.0445$), respectively (Fig. 4A). After interfering with the expression of *Cmedcry1*, take-off proportions significantly decreased under both full-spectrum (reduced by 28.40%) ($\chi^2=3.9$, df$=1$, $P=0.048$) and blue light (reduced by 46.84%) ($\chi^2=6.9$, df$=1$, $P=0.008$) (Fig. 4A, B). However, compared to the group injected with *dsGFP*, there was no difference in the relative expression of *Cmedcry2* (full spectrum: $t=0.86$, df$=4$, $P=0.437$, blue light: $t=0.51$, df$=4$, $P=0.636$), *Cmedtim* (full spectrum: $t=0.72$, df$=4$, $P=0.511$, blue light: $t=1.96$, df$=4$, $P=0.121$) and *Cmedper* (full spectrum: $t=0.11$, df$=4$, $P=0.343$, blue light: $t=0.91$, df$=4$, $P=0.414$) after silencing *Cmedcry1*, whether in full spectrum or blue light (Fig. 4B). These results indicated that *Cmedcry1* was involved in the regulation of take-off behaviour in *C. medinalis*.

Discussion

Night-active insects exhibit a high sensitivity to light [32, 40]. Most nocturnal insects take off during the crepuscular period (when the Sun is 6° below the horizon after sunset) [41]. *C. medinalis* is a nocturnal pest, and its perception of changes in light is crucial for its migratory take-off behaviour [8, 33]. Herein, specific light signal parameters triggering take-off behaviour in *C. medinalis* were determined using field observations and laboratory experiments manipulating light intensity. Meanwhile, we investigated the role of *Cmedcry1* as a light receptor in migratory flight take-off behaviour induced by changes in light intensity, and we conducted preliminary validation of its possible involvement as an input element in the biological clock and seasonal response.

Field and laboratory observations suggested that *C. medinalis* took off after sunset when the light intensity decreased to nearly 0.1 lx, which was consistent with observations by Gao et al. [35] and Zhang et al. [42]. However, since the laboratory induction experiments were conducted at the same time as the field observation experiments, it is impossible to rule out that the take-off behaviour of *C. medinalis* is a rhythmic behaviour mediated by a circadian clock. The conducted take-off experiments at different time periods refuted this possibility; there was no significant difference in the take-off proportion of *C. medinalis* from 16:00 PM to 21:00 PM. This further reinforced the role of light signals in regulating take-off behaviour in *C. medinalis* while weakening the role of an internal clock. Even if the internal clock still plays a role, light-sensitive components are required to receive light signals to make the clock run in line with light cues [23, 43]. However, most evidence has suggested that the process of sensing light signals through the biological clock is slow, and the hypothesised responses to light signals within 10–30 min are not reasonable [26, 44].

The take-off behaviour of most insects is related to light intensity [41]; for example, *Laodelphax striatellus* [45], *Agrotis ypsilon* [46] and *Lymantria dispar* [47] all perform take-off behaviour under low light intensities. Our observational experiments showed similar results. Experiments with different light intensities revealed a minimum light intensity required to induce take-off behaviour of 10 lx. Only when the light intensity was reduced to 0.1 lx, did the take-off proportion match those observed in the field. This indicates that insect activity is only activated at light intensities below a threshold, which in the case of *C. medinalis*, may be as low as 0.1 lx. Compared to other nocturnal insects, the phototactic take-off behaviour of *C. medinalis* exhibit similarities, albeit with differences in the lowest threshold of light sensitivity. This variation could be attributed to differences in the sensitivity to light cues among different species. Nocturnal insects exhibit different light sensitivities compared to diurnal insects, with nocturnal insects having slower responses in their photoreceptor cells [48, 49]. This may be one of the reasons why nocturnal insects cluster for take-off after sunset. The clustering of take-off in insects after sunset may be due to slow responses of photoreceptor cells in nocturnal insects compared with diurnal insects [40]. This behaviour may enhance visual reliability

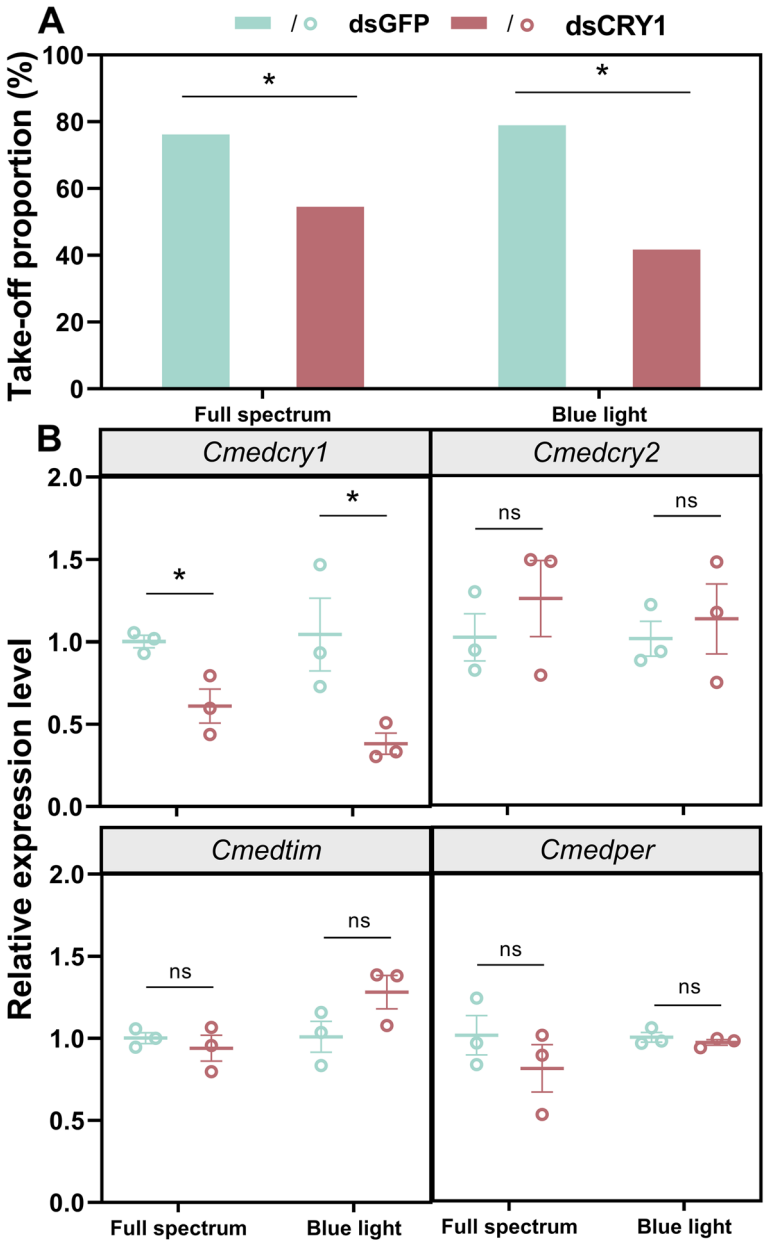

Fig. 4 Take-off proportion (**A**) and RNAi efficiency (**B**) of *C. medinalis* after interference with *Cmedcry1* expression and relative expression of *Cmedcry2, Cmedtim* and *Cmedper* under full-spectrum and blue light (**B**). The circles represent the individual measured values of the samples, the long horizontal lines represent the means, the upper and lower lines represent the average standard error of the mean and * indicates significant differences in the take-off proportion (chi-squared test—$P < 0.05$) and relative gene expression (*t*-test—$P < 0.05$) among the dsGFP and dsCRY treatment groups, while 'ns' indicates non-significant differences

and enable them to have visual activity under low light conditions [50]. The take-off of nocturnal insects under visually active light intensities at night may be associated with avoiding predators, navigating obstacles and orientation [40, 51].

The cryptochromes, as key non-visual photoreceptors in insects, detect ultraviolet and blue light in the oxidised and semi-reduced state of FAD and FAD-semiquinone [20, 21, 52]. *Cry1* has been found to mediate blue light sensitivity behaviours, such as the sensitivity of the monarch butterfly to changes in magnetic declination under blue light [53] and the special attraction of *Aedes aegypti* to blue–purple light [31, 32, 54]. CRY also participates in regulating *Drosophila*'s avoidance/attraction behaviour

under blue/ultraviolet light [31, 54, 55]. Consistent with reports on CRY1 in other Lepidoptera insects [43, 56], the present results showed significant differences in the take-off proportion of *C. medinalis* under different monochromatic light wavelengths, suggesting that *C. medinalis* is more sensitive to blue light. We speculate that this special sensitivity to blue light is mediated by the special structure of its photoreceptor, and we believe that it may be mediated by *Cmedcry1*. As expected, we found that the expression of *Cmedcry1* significantly increased in take-off individuals compared to the non-take-off individuals. This may be attributed to the upregulation of UV-A/blue light-sensitive CRY1, which triggers the take-off behaviour in migratory *C. medinalis.* However, due to the lack of phenotypic differentiation between migratory and non-migratory individuals, we were unable to differentiate between them before take-off observation to evaluate the expression of *Cmedcry1* to determine this causal relationship. Previous studies have found that starvation induced the migration of *C. medinalis*. Most starved *C. medinalis* were more inclined to take off, while those moths receive energy supplementation tend to remain stationary [39]. We found that starved moths exhibited higher expression levels of *Cmedcry1* before take-off compared to nutrient supplemented moths, and this difference became more pronounced after entering darkness. Meanwhile, the relative expression level of *Cmedcry1* in *C. medinalis* showed no significant difference between blue and full-spectrum light, but it was significantly upregulated under red and green light. Notably, the diurnal relative expression of *Cmedcry1* was upregulated after entering darkness. Moreover, the expression of *Cmedcrys* and circadian genes (*Cmedtim* and *Cmedper*) of moths under constant light at 1000 lx and gradually reduced light intensity to 0.1 lx also showed that *Cmedcry1* was upregulated after entering darkness, but these levels remained unchanged under constant light. In these experiments, moths were placed under different light wavelengths for 4 h to adapt. Due to the insensitivity of *Cmedcry1* to red and green light, the adaptation time under these two wavelength ranges was equivalent to causing the moths to enter darkness earlier, which led to a significant upregulation of *Cmedcry1* compared with exposure to full-spectrum light. However, the relative expression *Cmedcry2* did not differ under any treatments. More importantly, after interfering with *Cmedcry1*, the take-off proportion significantly decreased. The above results all indicate a close relationship between the expression of *Cmedcry1* and the take-off behaviour of *C. medinalis*. These results suggested that *Cmedcry1* has photosensitivity to specific wavelengths and is associated with take-off behaviour while *Cmedcry2* does not. However, the specific mechanism by which *Cmedcry1* influences the take-off behaviour of *C. medinalis* in this process remains uncertain.

Due to the fact that CRY1 has been found to act as a photoreceptor in many insects, we propose the most straightforward assumption is that, *Cmedcry1* may perceive the attenuation of light intensity signals. The clock proteins TIM and PER are known downstream binding proteins of CRY1 and play an important role in regulating the circadian rhythm of insects [24, 28]. In some insects, such as *Plutella xylostella* and *Spodoptera litura*, silencing or knocking out *cry1* affects their rhythmic behaviour [57, 58]. Therefore, it is speculated that *tim* and *per* may be involved in the transduction of light intensity signals downstream of *Cmedcry1*. However, in *C. medinalis*, we did not find any significant changes in the relative expression level of *Cmedper* under different light wavelengths, whereas *Cmedtim* showed significantly differences in expression only under green and blue light. Moreover, no significant difference in the expression of *Cmedtim* and *Cmedper* was found among take-off and non-take-off individuals in *C. medinalis*, indicating that *Cmedtim* and *Cmedper* did not play a role during the take-off stage. The interaction between CRY1 and TIM was more evident in the perception of changes in circadian rhythms rather than the perception of light signals [25, 29, 59]. Interference with *cry1* changed the expression peaks and the time when the expression peaks appeared of *tim* and *per* in *Gryllus bimaculatus* [60]. However, the present results showed that silencing *Cmedcry1* did not significantly change the expression of *Cmedtim* and *Cmedper*, at least during the take-off phase. We believe that migration is a long-term regulatory effect that combines multiple factors, including changes in the circadian rhythm and starvation [39, 61]. Circadian rhythms may play a role in the formation of insect migration behaviour [26] whereas short photoperiod and starvation can promote the migration tendency of *C. medinalis* [60, 61]. Conversely, take-off is a short-term behaviour, which may be determined by environmental conditions (such as light, temperature and humidity) at the time of take-off. Due to the short reaction time, the correlation of circadian rhythm may be not significant. Importantly, considering that the peak response of *C. medinalis* to changes in light signals occurs approximately 10 min after the end of light exposure and the transmission of the signal is rapid [62], we speculate that if *C. medinalis* does rely on CRY1 to sense light signals, it may rely on neural signalling rather than the biological clock [54]. Many studies have confirmed that CRY photoactivation can cause rapid and sustained depolarisation and increased firing in large ventral–lateral clock neurons [31, 55, 62].

In summary, the present results indicated that take-off in *C. medinalis* was induced by the specific light

conditions under which the light intensity decreased to below 1 lx and was not completely regulated by circadian rhythms. *Cmedcry1* was involved in the process of sensing changes in light intensity and inducing take-off. The present study deepened our understanding of the effects of light cues on insect migratory behaviour and explored the molecular mechanisms and photoreceptor proteins involved in responding to light cues. This will contribute to further unravelling the regulatory mechanisms of take-off phases in insect migration. However, we have only explored the phenomenon at the mRNA level and have not elucidated the mechanism by which *Cmedcry1* senses light and its downstream pathways. It is necessary to conduct further experiments at the protein level and electrophysiology to clarify the complete pathway through which *Cmedcry1* transduces light signals.

Conclusion

Through field and laboratory observations of the take-off behaviour of *C. medinalis*, along with molecular biology experiments, it has been demonstrated that the take-off behaviour of *C. medinalis* is strongly induced by the reduction in light intensity. This process is closely related to the function of the photoreceptor protein CRY1. Our research enhances the understanding of the regulatory mechanisms of nocturnal migratory insect group take-off behaviour and is significant for a deeper comprehension of the patterns of long-distance insect migration.

Methods

Take-off behavioural observations in the field

Field observations were carried out in the early rice field of the Agricultural Science and Technology Institute of Yongfu County, Guangxi Zhuang Autonomous Region (24° 57′ N, 110° 01′ E). Before the start of the experiment, a fixed position of about 9 m^2 was selected, and a field cage (3 m length, 3 m width, 2 m height) was set up. In the field cage, about 30 rice plants (planting within a range of 1 m length and 1 m width) were reserved in the middle, and the rest were removed. According to the monitoring results of field population dynamics and ovarian development degree [63], from May to July 2011, July 11–22, 2011, was determined as the emigration period (Additional file 1: Table S2). During this period, 30 adult moths (the ratio of males to females was 1:1) caught in the field with insect nets were placed in the field cage every morning (about 06:00–07:00). Since *C. medinalis* usually takes off at sunset [42] and the sunset in Yongfu County is generally at 19:30 Beijing time during the observation period (Additional file 1: Table S1). Therefore, the field observation of *C. medinalis* take-off started at 19:00 every evening. Individuals that took off one time to a height of > 100 cm were identified as 'take-off individuals'. These take-off individuals were caught with a tube and placed into 500-mL transparent plastic cups to remove them from the field cage, and their number was counted every 15 min. At the same time, the light intensity was measured and recorded in the cage every 5 min using an illuminometer (TES-1330A; TES Electrical Electronic Corp, Taiwan, China) to calculate the average light intensity of each time period. The observations were ended at 21:00, and the remaining moths were caught and removed from the field cage. In this experiment, a total of 360 moths were observed in the field.

Take-off behaviour observations of field populations under laboratory simulation of sunset light

In order to exclude other environmental factors and further clarify the effects of light intensity changes on *C. medinalis* take-off, we simulated the light intensity changes at sunset in the field to observe the take-off behaviour of a field population. From July to October 2019, systematic field observations were conducted in the rice fields in Jiangyan District, Jiangsu Province, China (32° 31′ N, 120° 09′ E). According to the changes in the number of moths in the field and the degree of ovarian development [63], the migration period was determined to be from September 1 to September 30 (Additional file 1: Table S3). Therefore, we selected September 1–15 for take-off behavioural observation experiments. During the experiments, 20 moths were collected from the field every morning (about 06:00–07:00) and put into 500-mL transparent plastic cups (10 moths per cup). All females were fed distilled water to achieve near-field nutritional status, as starvation promotes take-off behaviour in *C. medinalis* [60]. According to the sunset time in Jiangyan District (Additional file 1: Table S4), the field-collected moths were transferred into a climate chamber (temperature 26 ± 1 °C, humidity 75–85%) 1 h before the observation of take-off behaviour at dusk [60]. Unless otherwise specified, the temperature and humidity during behavioural observation in the following text are the same. The moths were placed into the cylindrical take-off cage (50 cm in diameter, 120 cm in height) to observe the take-off behaviour. The take-off cage was made of highly transparent PVC films as described by Guo et al. [64], with a white plastic foam board at the bottom and a 500-mL transparent plastic cup as a take-off platform. A light source composed of 20 rows of fluorescent lamps (36 V/40 W) and two incandescent lamps (12 V/40 W) was placed 200 cm above the take-off platform to simulate the light condition of the sunset, with minimal effects on the temperature inside the PVC cage. The light intensity was changed by gradually extinguishing 20 parallel fluorescent lamps (two every 3 min) and connecting the incandescent lamp with a potentiometer

to create artificially simulated evening light. The indoor light intensity was gradually decreased from 1000 lx to 0.1 lx over a period of 45 min. The changes in light intensity during the observation period were simultaneously monitored with an TES-1330A illuminometer. The migratory take-off behaviour differs in take-off posture from take-off activities related to foraging, courtship and oviposition in previous studies on the field and laboratory observations [38, 39]. Moths were identified as 'migratory take-off' if the moth took off and spiralled vertically with a vertical distance greater than 100 cm or as 'non-migratory take-off' if a moth stayed still or hovered at a height less than 100 cm [60]. A total of 300 moths were observed, and their take-off times were recorded. After observation (20:00), migratory individuals and resident individuals (non-migratory take-off individuals) were collected immediately, frozen in liquid nitrogen and stored at − 80 °C for follow-up experiments.

Laboratory insect rearing

Larvae of *C. medinalis* were originally collected from rice fields in Nanning, Guangxi Zhuang Autonomous Region (24° 57′ N, 110° 01′ E), and reared using wheat seedlings [64, 65]. Pupae were removed from the seedlings and transferred into a transparent plastic box (16 cm length, 24 cm width and 22 cm height) with wet cotton wool to maintain high relative humidity. After adult emergence, the male and female moths were separated and transferred into 500-mL clear plastic cups (5 moths per cup), fed with 5% honey solution [66], and the honey solution was changed daily during feeding. All test insects were placed in an incubator at a constant temperature of 26 ± 1 °C, a relative humidity of 80–90% and a photoperiod of 14 h:10 h light: dark (light period 05:30–19:30). Unless otherwise specified, *C. medinalis* were raised in these environmental conditions.

Effects of light intensity changes in different time periods on migratory take-off behaviour

Sunset light intensity changes (from 1000 lx to 0.1 lx) were simulated in different time periods to observe the migratory take-off behaviour of *C. medinalis*. Take-off observation experiments were conducted in the following six time periods: 16:00–17:00, 17:00–18:00, 18:00–19:00, 19:00–20:00, 20:00–21:00 and 21:00–22:00 h. The take-off device, light source, temperature, humidity and methods used in these experiments were the same as those of the laboratory observation equipment detailed above. The number of migratory take-off individuals was recorded, and the migratory take-off proportion of different treatment groups were calculated separately with no fewer than 30 moths per treatment. During the non-treatment time period, the photoperiod was set according to the conditions when *C. medinalis* was reared (04:30–19:30 was the light period).

Effects of different light intensities on migratory take-off behaviour

Take-off behaviour of 2-day-old *C. medinalis* (age of take-off peak [38]) was observed under different light intensity changes using the abovementioned take-off cage and light source. Six treatment groups with different light intensity changes were created. The light intensity was gradually reduced from 1000 lx to 1000, 500, 100, 50, 10 or 0.1 lx over 45 min and remained unchanged thereafter. The number of migratory take-off individuals under each treatment and the light intensity at the onset of taking flight were recorded separately, and the migratory take-off proportion of the different treatment groups was calculated. The observations began at 18:00 and ended at 20:00. The take-off device, light source, temperature, humidity and methods used in these experiments are the same as those used in the experiments described above. Each treatment was repeated at least 5 times with 5 moths per replicate (separate males and females, placed separately), and all test moths were not reused. A total of 385 moths were observed.

Effects of different light wavelengths on migratory take-off behaviour

Control (full spectrum), blue light (380–430 nm), green light (480–580 nm) and red light (635–680 nm) groups were created to observe whether 2-day-old female *C. medinalis* moths were sensitive to specific wavelengths of light. The light source consisted of 14 rows of monochromatic non-strobe LCD lamps (35 V/W; Ningbo Jiangnan instruments, Ningbo, China) with a total brightness of 1000 lx. In these experiments, the controller adjusted the light intensity to decrease from 1000 lx to 0.1 lx (100 lx/3 min) within 30 min to simulate the changes in night-time intensity. The starting time for experimental processing was 19:00 every day. Four hours before experiments, all moths were transferred to the climate room with a light intensity of 1000 lx to adapt to the lighting environment. According to the above method, the take-off behaviour of female *C. medinalis* moths was observed under full-spectrum, blue, green and red light. The number of take-off individuals was recorded and the take-off proportion under different light wavelengths was calculated. The sample sizes of female moths observed under full spectrum, blue light, green light and red light were 76, 123, 101 and 123, respectively. Migratory individuals under different light wavelengths were collected immediately after the conclusion of the take-off experiment (20:00), frozen in liquid nitrogen and stored at − 80 °C for subsequent experiments.

Expression profile of *Cmedcry1* and effects of light intensity reduction on expression of *Cmedcrys* and circadian genes

To determine the diurnal relative expression of *Cmedcry1*, female moths were collected in 4-h intervals starting from the day and night alternation point (05:00 h) on the second day after emergence until 05:00 h the next day. These moths were used to analyse the expression of *Cmedcry1* within 24 h. Additionally, starvation can induce *C. medinalis* to migrate, the take-off proportion of starved moths was significantly higher than that of nutrient supplemented moths [39]. To compare whether there are differences during the transition between light and dark periods of migratory and non-migratory *Cmedcry1*, we simulated migratory and non-migratory populations using feeding and starvation treatments. Starved and fed moths provided distilled water and 5% honey solution as food, respectively. Sampling was carried out at the 13:00, 17:00, 21:00 and 01:00.

In addition, in order to further determine the relationship between the expression of *Cmedcry1*, *Cmedcry2*, *Cmedtim* and *Cmedper* in light and dark during the take-off phase, 2-day-old female adults were collected before beginning the simulated sunset experiments (19:00) and were collected again during the peak take-off period (20:00) after experiencing a gradual decrease in light intensity to 0.1 lx, which was recorded as the light–dark (LD) treatment group. At the same time, female moths of the same adult age were collected at the same time as the LD treatment group after being treated with constant light intensity (1000 lx) for the same time, which were set as the light–light control group (LL). There were 3 female adults per sample and 3 replicates per sample. All samples were quickly frozen in liquid nitrogen and stored at − 80 °C for subsequent experiments.

Real-time quantitative PCR

The relative expression levels of *Cmedcry1*, *Cmedcry2*, *Cmedtim* and *Cmedper* in the migratory and resident individuals, moths collected under different light wavelengths, moths collected in the LD and LL treatment groups at two time points, as well as the expression profile of *Cmedcry1*, were all measured using real-time quantitative PCR. The wings and legs of adult *C. medinalis* were removed using anatomical scissors, and the scales on the body surface were removed using a small brush. Total RNA was extracted with 1000 mL RNA extraction buffer (100 mM Tris pH 7.5, 100 mM LiCl, 20 mM DTT and 10% sodium dodecyl sulphate; Tiosbio, Beijing, China). After chloroform purification, RNA was precipitated with isopropanol. Referring to the methods of Zhao et al. [67], all RNA samples were reverse transcribed using the Tiosbio Polestar 1st cDNA Synthesis kit (gDNA removed) (Beijing Baoying Tonghui Biotechnology Co., Ltd., Beijing, China) to obtain cDNA. Quantification of gene expression was performed on a QuantStudio™ 6 Flex Real-Time PCR System (Bio-Rad, Hercules, CA, USA) using 1 μL of cDNA template, iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) and primers. The primer of *Cmedcry1* (Gene ID: PP941966.1), *Cmedcry2* (Gene ID: Cmed043760.1), *Cmedtim* (Gene ID: Cmed094160.1), *Cmedper* (Gene ID: Cmed076210.1) and reference gene *actin* (Gene ID: JN029806.1) and *RPS3* (Gene ID: Cmed05991) [67] were designed based on the genome data of *C. medinalis* (http://v2.insect-genome.com/Organism/192, accessed on 6 June 2022) (Table 1). Individual responses were used to quantify each RNA level in a given cDNA sample, and the average Ct of 3-time repeated reactions in the same operation was used to quantify. Utilising the $2^{-\Delta\Delta CT}$ method for analysing relative gene expression levels [68].

Synthesis of dsRNAs and RNAi of *Cmedcry1*

DsRNA was synthesised based on each sequence of *Cmedcry1* and GFP using T7 RNAi Transcription kit (Vazyme, Nanjing, China) directly using PCR products as templates. Using the genes of female adult *C. medinalis* as the template, the *Cmedcry1* exon sequence of about 500 bp was obtained by linking the T7 promoter sequence TAATACGACTCACTATAGGG (Table 1) to the end of 5′ end of the primer and 2 × Taq Master Mix (TaKaRa, Beijing, China). The amplified products were analysed by 1% agarose gel electrophoresis and purified by E.Z.N.A. Gel Extraction kit (Omega, Norcross, Georgia, USA). The concentration of dsRNA was determined

Table 1 Primers for PCR amplification and dsRNA synthesis

Primer name	Sequence of primers (5′–3′)
Cmedcry1	F: TGCCACAGGACTATATACACGG
	R: ACAGGAAGGTTGGATACATCAC
Cmedcry2	F: AATTCTCGCCACAATATCTG
	R: ATGACACCGCAATCTCTT
Cmedtim	F: CGCCTTCACCAAAACAGTCG
	R: CCTGCGTCTCACCAGCATTA
Cmedper	F: CTCGACGATGGTATGTCGCA
	R: GCAAGAACGGCATGAAGGTG
actin	F: CACACAGTGCCCATCTACGA
	R: GCGGTGGTGGTGAATGAGTA
RPS3	F: AGGTTCAACATCCCCGAGCA
	R: CGGACACAACAACCTCGCAAC
dsCry1	F: taatacgactcactatagggTAGGGGAAAACAGGTTGCGATT
	R: taatacgactcactatagggAATTTGCACACCCACTGCTTC
dsGFP	F: taatacgactcactatagggATGGTGAGCAAGGGCGAGGAG
	R: taatacgactcactatagggCGGATCTTGAAGTTCACCTTG

using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Ds RNA was stored at − 20 °C.

Effects of silent *Cmedcry1* on the take-off behaviour of female moths

We injected 1000 ng/μL of dsRNA (1 μL) into female *C. medinalis* moths 2 h after emergence, and the mortality owing to injection damage was < 10%. After injection, the female moths were raised until 2 days after emergence (38 h after injection) for take-off behavioural observation in full-spectrum and blue light, and the take-off proportions were recorded. Take-off individuals were collected (3 female moths per group, 3 replicates) and further analysed for remaining transcript levels using real-time quantitative PCR. Using the dsGFP [69] (Table 1) treatment group as the control, the preparation method was the same as described above, and the same dose of dsRNA was injected. The observed number of female moths in each treatment group under full-spectrum and blue light exceeded 30 and 20, respectively.

Statistical analysis

Before analysis, data were tested for normal distribution and variance homogeneity using the Shapiro–Wilk and Levene tests, respectively. All data conform to normal distribution ($P > 0.05$) and homogeneity of variance ($P > 0.05$). Means of light intensities in different time periods, the relative expression of *Cmedcry1*, *Cmedcry2*, *Cmedtim* and *Cmedper* at different light wavelengths and 24-h expression profile of *Cmedcry1* were segregated using the Tukey's honestly significant difference test (Tukey's HSD test). The relative expression differences of *Cmedcry1*, *Cmedcry2*, *Cmedtim* and *Cmedper* among take-off and non-take-off female moths, female moths collected at two time points in the LD and LL treatment groups, and female moths treated with dsCRY1 and dsGFP at different light wavelengths were compared using *t*-tests. Differences in the take-off proportion among different treatments were tested using the chi-squared test. All statistical analyses were performed using IBM SPSS Statistics (V21; IBM Corp., Armonk, NY, USA) software.

Abbreviations

CRY1	Type 1 Cryptochrome
CRY2	Type 2 Cryptochrome
PER	Period
TIM	Timeless
Cmedcry1	*Cnaphalocrocis medinalis* type 1 Cryptochrome gene
Cmedcry2	*Cnaphalocrocis medinalis* type 2 Cryptochrome gene
Cmedper	*Cnaphalocrocis medinalis period* Gene
Cmedtim	*Cnaphalocrocis medinalis timeless* Gene
LD	Light-dark treatment
LL	Light-light treatment
RNAi	RNA interference
RPS3	Ribosomal protein S 3
actin	*β-actin*

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12915-024-01964-4.

Additional file 1. Table S1. Field sunset time in Yongfu County, Guangxi Zhuang Autonomous Region and initial time of *Cnaphalocrocis medinalis* take-off from July 11 to July 22, 2011; Table S2. Abundance of *Cnaphalocrocis medinalis* moths, female ovarian development and population characteristics in 2011 in Yongfu, China; Table S3. Abundance of *Cnaphalocrocis medinalis* moths, female ovarian development and population characteristics in 2019 in Jiangyan, China; Table S4. Field sunset time in Jiangyan County, Jiangsu province, China from September 1 to September 15, 2019; Figure S1. Differences in *Cmedcry1* expression levels between starvation and feeding treatments within 6 h before and after the light and dark transition; Figure S2. Change of relative expression of *Cmedcry1*, *Cmedcry2*, *Cmedtim* and *Cmedper* under LD and LL treatments; Figure S3. 24-hour expression profile of *Cmedcry1*. Gray background indicates dark phase.

Acknowledgements

We express our gratitude to the interns Yilin Liu and Wenqiang Li from Zhejiang Academy of Agricultural Sciences for their contributions to insect rearing, and we would also like to extend our thanks to the doctoral students Yuehui Diao and Chaomin Xu from Nanjing Agricultural University for their valuable recommendations on our work.

Declaration of AI use

AI has not been employed in any crucial research tasks. It might participate in information gathering and translation work in the form of search engines.

Authors' contributions

T. S.: formal analysis, investigation, methodology, writing—original draft, writing—review and editing; F. Y.: investigation, methodology; H. Z.: investigation, supervision; Y. Y.: investigation, supervision; Z. L.: funding acquisition, methodology, project administration; B. Z.: methodology, resources, supervision; H. X.: conceptualization, supervision, project administration, resources; J. L.: investigation, supervision; Y. L.: resources, supervision; Y. W.: methodology, writing—review and editing; J. G.: funding acquisition, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; G. H.: funding acquisition, conceptualization, project administration, supervision and writing—review and editing. All authors gave final approval for publication and agreed to be held accountable for the work performed therein. All authors read and approved the final manuscript.

Funding

This work was supported by Zhejiang Provincial Natural Science Foundation of China [LQ22C140006], the Fundamental Research Funds for the Central Universities, the National Natural Science Foundation of China [32202280], the earmarked fund for the China Agriculture Research System [CARS-01] and the State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products [2021DG700024-KF202308].

Availability of data and materials

All data needed to evaluate the conclusions in the paper are included in this published article and its supplementary information flies. Additional data related to this paper are available from the corresponding authors upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 9 January 2024 Accepted: 25 July 2024
Published online: 13 August 2024

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