Light Intensity Affects the Reproductive Success of *Danaus chrysippus* (Lepidoptera: Danaidae) by Influencing Flight Behavior[1]

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Abstract Butterflies (Lepidoptera) can absorb heat from sunlight to increase their thoracic temperature above the threshold for flight. Autonomous flight is closely related to the reproductive success of butterflies; however, it is unclear whether light intensity controls the ability of butterflies to absorb heat and alter flight activity and, thus, affect reproduction. Such insight would provide an improved understanding of the utilization of solar heat by insects. In this study, we investigated the flight behavior of *Danaus chrysippus* (L.) (Lepidoptera: Danaidae) in the field and measured the thoracic temperature of butterflies when flying and perching. We determined the effects of thoracic temperature on flight frequency and reproductive success under a light intensity range of 2,000–45,000 luminous flux per unit area (lx). Within this range, strong sunlight significantly increased the flight activity of butterflies in the field. Adults absorbed heat from sunlight to maintain a thoracic temperature that was 4–4.5°C higher than the ambient temperature to allow autonomous flight. Light intensity between 15,000 and 45,000 lx enabled butterflies to absorb more heat to reach an equilibrium temperature, increasing the frequency of autonomous flight and mating. In summary, light intensity influences the autonomic flight activity of butterflies and then significantly affects mating frequency and reproduction. Thus, light intensity manipulation can be used to regulate butterfly reproduction for their conservation and utilization in laboratory breeding facilities.

Key Words *Danaus chrysippus*, light intensity, autonomous flight, heat absorption, reproductive success

Sunlight is the most important source of energy for most creatures to survive and reproduce. Ectotherms utilize heat from the sun directly, as exemplified by insects, particularly lepidopterans. Previous studies showed that butterflies must absorb heat from sunlight to increase their thoracic temperature above the threshold for flight (Barton et al. 2014; Bonebrake et al. 2014; Kemp and Krockenberger 2004; Kleckova et al. 2014; Liao et al. 2017; Mattila 2015). For example, in the genus *Erebia*, when the habitat temperature was 13.4–31.0°C the body temperature of the butterflies in flight was 20.9–38.3°C (Kleckova et al. 2014), which was $\geq$7°C higher than the ambient temperature.

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Butterflies absorb heat mainly via their wings (Han et al. 2016; Niu et al. 2016; Shanks et al. 2015). When adult butterflies require heat for autonomous flight, they adjust their body posture and either fully expand their wings or angle them for optimal absorption of sunlight; the heat absorbed is then transferred to the thoracic muscles to enable flight (Heinrich 1990; Huey et al. 2003). Light intensity was shown to significantly influence the ability of the butterfly *Tirumala limniace* (Cramer) to absorb heat and, thus, impact its resulting flight activity (Liao et al. 2017). According to the results of a study on a new mechanism of behavioral thermoregulation, Kingsolver (1985) suggested that *Pieris* butterflies use their wings as solar reflectors to reflect solar radiation onto the body to increase their body temperature.

Autonomous flight is directly involved in the mating and reproductive behavior of butterflies (Bennet et al. 2012; Chou 1999; Shreeve 1992). Therefore, autonomous flight is closely related to the reproductive success of butterflies (Gibbs et al. 2010, 2018; Westerman et al. 2014). However, the relationship between light intensity, heat absorption, flight activity, and reproductive success in butterflies has been unclear. Such an understanding would provide further insight into the use of solar heat by butterflies and other insects. As economically important insects, butterflies are valuable because of their colorful wings and graceful flight. In addition, they are nutritionally valuable because they are rich in protein. They can also be used as indicators of environmental quality (Chen et al. 2008; Chou 1999; Feng et al. 2016). The intensity of light sources in a culture room could be adjusted to levels that are suitable for butterfly reproduction. Shading nets and lighting equipment can be used to adjust the light intensity in greenhouses in the field on sunny days, and cloudy and rainy days, respectively. Such measures could be used to regulate increases in butterfly populations. Such measures would not only enable the production of a sufficient source of insects for the market but also increase the numbers of threatened butterflies, thus aiding their population growth. Therefore, a clear understanding of the relationship between light intensity, flight, and reproductive success could provide theoretical guidance for butterfly conservation and utilization as well as improving their value as an economic resource.

In China, *Danaus chrysippus* (L.) (Lepidoptera: Danaidae) is mainly distributed in the dry hot valleys of south Yunnan, Guangxi, and Guangdong provinces. It is an important species for ecological and environmental quality monitoring in southern China. Its optimum temperature range for survival and flight is 25–36°C. In general, male butterflies fly more actively than do females. Both female and male butterflies are polyandrous and can mate multiple times. Male butterflies also produce spermatophores that are rich in nutrients and sperm (Chen et al. 1999).

The present study, therefore, was conducted to (a) clarify the effects of sunlight intensity on the flight behavior of adult *D. chrysippus* under natural conditions, and (b) determine the relationship among light intensity, flight behavior, mating behavior, and fecundity of *D. chrysippus*.

Materials and Methods

Insects. Pupae of *D. chrysippus* were bought from the Ornamental Insect Technology Development of Kunming Zhonglin Co., Ltd, Kunming, China. The pupae were placed on a cylindrical net (height65 cm; diameter 50 cm) on top of a towel so that they could easily spread their wings after emergence. Subsequently, the pupae were transferred to a room at 25 ± 1°C with a relative humidity of 75 ± 5% under a 14:10 h light:dark photoperiod. Once the adults had emerged, the females and males were reared separately in two different cages to prevent mating. Three-day-old unmated adults were used in this study.

Study site. The field study was conducted at the Yuanjiang Experiment Station (N 23°36′ 11″, E 102°00′ 46″) of the Research Institute of Resource Insects, Chinese Academy of Forestry. Yuanjiang County is well suited as a butterfly-breeding location, given its annual temperature of 19–20°C and an average annual precipitation of 770–2,400 mm.

Effects of sunlight intensity on flight behavior in the field. In this experiment, we investigated the effects of sunlight intensity on the flight behavior of adult *D. chrysippus* in the field. The date of 12 September 2017 was chosen as the study day because the skies were clear and cloudless. Three-day-old female and male butterflies were placed separately in cages (200 × 200 × 200 cm) at 7:00 a.m. The cages were placed in a 25°C transparent glasshouse (300 × 300 × 300 cm) to prevent any influence of wind on the flight of the butterflies. Each of the pots (height 36 cm; diameter 32 cm), containing three, 2-year-old flowering milkweeds (*Asclepias curassavica* L.; height 50–60 cm), were placed in the middle of each cage in four rows of five pots to provide a source of nectar for the adult *D. chrysippus*. The flight behavior was recorded with cameras (CS-C5C-3B1WFR, Hangzhou Hikvision Digital Technology Co., Ltd., Hangzhou, China) from 7:00 a.m. to 8:00 p.m. The light intensity in the glasshouse during the same time period (7:00 a.m. to 8:00 p.m.) was measured every 10 min with an illuminometer (TES-1334A LIGHTMETER, Taiwan TES Co., Ltd., Taiwan). The flight frequencies were determined from analysis of the video recording. Flight was defined as a butterfly taking off from one point and landing at another, and the flight frequency was determined every 30 min. The light intensity per 30 min was the average of the three measurements made per 30-min interval. The observation of male and female flight behaviors was repeated five times, and each time included 13–20 new 3-day-old male or female butterflies; in total, 45 male butterflies and 78 female butterflies were investigated.

Thoracic temperatures of adults in the field when perching and during flight. To determine whether butterflies absorb heat from sunlight for autonomous flight, the thoracic temperature of butterflies when flying and perching was measured in the field on 13 September 2017. The new and unmated 3-day-old female and male butterflies were placed separately in the cages at 0800 h. Butterflies in flight, and those perching on the net with the wings closed and not flapping, were caught and the thoracic temperatures were measured immediately using a thermocouple thermometer (Thermometer GM1312, Shenzhen Jumaoyuan Science and Technology CO., Ltd., Shenzhen, China). Once the butterfly wings had opened, they began to absorb heat. When the body temperature had elevated enough to take off, the butterfly would flap its wings and take off immediately. Thus, the butterflies that perched on the net with closed wings were selected as 'perching' butterflies. To record the thoracic temperature, the thermistor was inserted through the mesoscutellum and placed in or near the dorsal longitudinal muscles. The difference in temperature between the thorax and the ambient temperature (ΔT)

was calculated. In total, temperatures were measured in 28 female and 24 male butterflies in flight and 30 female and 36 male perching butterflies.

Effects of light intensity on heat absorption. To ascertain how light intensity affected the ability of butterflies to absorb heat, we investigated whether the thoracic temperature increased under different light intensities. Based on the natural light-intensity levels from 7:00 a.m. to 8:00 p.m. on 12 September 2017, five light-intensity levels (2,000, 8,000, 15,000, 30,000, and 45,000 lx) were selected for this study. A 3-day-old adult butterfly was anesthetized using ethanol steam. The butterfly with wings fully opened was fixed to a square white plastic foam board (8 × 8 cm). In the wild, it has been observed that when butterflies need to absorb heat for autonomous flight, their wings are fully opened. Once a suitable body temperature has been achieved, they immediately take off.

An artificial light was suspended at 50 cm above the butterfly. Before the lamp was turned on, the butterfly was kept in dark environment until its body temperature stabilized. After the light was turned on, the thoracic temperature was recorded using an infrared imaging device (FILIR ET320, FLIR Systems, Inc., Wilson, OR, USA) until it remained at a set temperature for 120 s, indicating that the thoracic temperature had reached an equilibrium under the light intensity. The equilibrated thoracic temperature, rate of thoracic temperature increase, ΔT, and time to reach equilibrium were recorded during the experiment. For each light treatment (2,000, 8,000, 15,000, 30,000, and 45,000 lx), five female and five male butterflies were examined.

Effects of light intensity on reproductive success. In this experiment, the direct effect of light intensity on flight behavior and, thus, its indirect effects on mating behavior and fecundity were investigated. An artificial light was suspended 20 cm above the net cage (200 × 200 × 200 cm), allowing it to illuminate the entire cage. At the same time, the light intensity of the artificial light was adjusted by a potentiometer. A 50-cm–wide opening was cut on one side of the cage to place the butterflies inside the cage. Five levels of light intensity (2,000, 8,000, 15,000, 30,000, and 45,000 lx) were selected for this study. Each treatment (2,000, 8,000, 15,000, 30,000, and 45,000 lx) had five repetitions; each repetition had 16–18 pairs of 3-day-old unmated adult butterflies. Generally, within 1–2 d after adult emergence, the butterfly wings are still developing, and the flight ability of such butterflies was poor. On the third day after emergence, the wings have fully developed and the butterflies begin to fly, visit flowers, undertaking courtship, and other activities. The flight ability of the mated adult butterflies is weaker than that of unmated butterflies. Mating behavior is an energy-consuming process; therefore, butterflies need to consume a large amount of energy during the mating process. After mating is ended, the butterflies require some time to recover, especially male butterflies. Thus, 3-day-old unmated adult butterflies were the most appropriate to use for this experiment.

Three-day-old unmated adult butterflies were placed in the cages at 6:30 a.m. Ten pots, each containing three flowering milkweeds, were placed in each cage as sources of nectar for the butterflies. The artificial lights were turned on at 7:00 a.m. and the flight and mating behaviors were recorded by cameras between 7:00 a.m. and 8:00 p.m. over a period of 4 d. The temperature inside the cage was maintained at 26–28°C by an air conditioner. A female was recorded as having mated once if the male and female butterflies had copulated for more than 5 min. The number of times all female butterflies in a cage had mated within 4 d was counted, and the average number of times each female mated over 4 d was then used as the mating frequency.

After the 4-day experimental period, female adults were moved to a cage in a culture room at a constant temperature of 27°C with the same light conditions as previously described. Each cage contained 10 pots of milkweed, without flowers, as food for any newly hatched larvae. All females in the same repetition were transferred into the same new cage. To prevent any nutritive supplementation inducing the secondary development of ovaries, and thereby affecting fecundity, the female butterflies were fed with distilled water. The females laid their eggs on the back of milkweed leaves.

At 6:00 p.m. daily, the old milkweeds were replaced with new specimens. The plants with eggs attached were transferred to a 27°C constant temperature culture room. The number of eggs was counted and the leaves with eggs attached were then covered by 200-mesh bags. Larval hatching requires low light conditions; therefore, the light source in the culture room comprised a fluorescent lamp with a light intensity less than 2,000 lx. The number of newly hatched larvae was recorded at 8:00 a.m. daily, and the percentage of eggs that hatched was used as the hatchability rate. The number of eggs laid by the females and their hatchability were examined every day until all females in the cages had died. The date on which females died was recorded to calculate their lifespan.

Statistical analysis. A mixed model for repeat measurement data was used to analyze the frequency differences in flight at different times on 12 September 2017. Differences in flight frequency between males and females were analyzed with the Student's *t*-test. Binominal regression analysis was used to assess the relationship between flight frequency and sunlight intensity on 12 September 2017. Differences in ΔT between perching and flying butterflies, and differences between air temperature and thoracic temperature of adults in flight and perching, were analyzed through the Student's *t*-test. The ΔT, thoracic temperature at equilibrium, rate of thoracic temperature increase, the time to reach equilibrium, flight frequency per adult per day, mating frequency per female, fecundity, egg hatchability, and female lifespan under different light intensities were analyzed using a one-way analysis of variance (ANOVA). Tukey's honestly significant difference was used to compare the multiple means when significant differences existed. Pearson correlation analysis was used to assess the linear relationships between mating frequency per female, fecundity, and egg hatchability with flight frequency per adult per day to examine how flight activity affected mating and fecundity. Pearson correlation analysis was used to assess the linear relationships between fecundity and egg hatchability with mating frequency to examine how mating frequency affected fecundity. All statistical analysis was performed using Statistical Analysis System, vers. 9 software (SAS Institute Inc. 2002).

Results

Effects of sunlight intensity on flight behavior in the field. The flying frequencies of adult *D. chrysippus* and daytime sunlight intensity on 12 September 2017 at the Yuanjiang Experiment Station are shown in Figure 1. The temporal flight

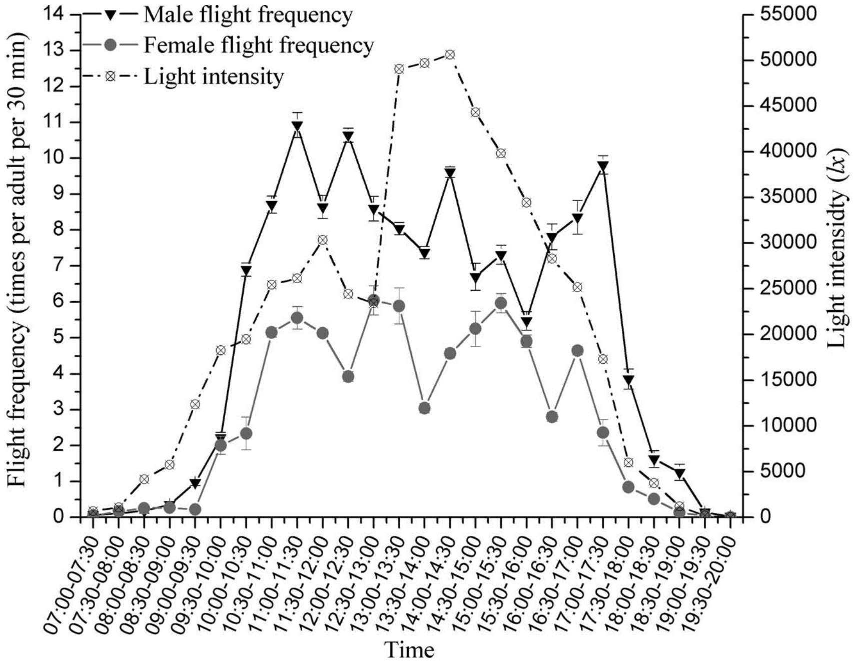

Fig. 1. Flying frequency of male and female adult *D. chrysippus* in relation to sunlight intensity on 12 September 2017 at the Yuanjiang Experiment Station.

pattern of the butterflies was similar to the temporal pattern in sunlight intensity (Fig. 1). In both sexes, the flight frequency significantly increased with increasing sunlight intensity (male: $F = 271.58$, df $= 25, 100$, $P < 0.0001$; female: $F = 108.07$, df $= 25, 100$, $P < 0.0001$). When the sunlight intensity was >15,000 lx (10:00 a.m. to 5:30 p.m.), the males took flight >4 times per 30 min whereas females took flight >3 times. When the sunlight intensity was <5,000 lx (7:00–9:00 a.m. and 6:00–8:00 p.m.), the butterflies took flight less than twice per 30 min period, if at all (Fig. 1). Thus, sunlight intensity significantly influenced butterfly flight activity.

Based on the above results, a binominal regression was used to analyze the relationship between light intensity and flight frequency (Fig. 2). The results showed that male and female flight frequencies were both significantly affected by light intensity. The flight frequency and the intensity of light showed a significant quadratic correlation (binomial regression model $R^2 > 0.5$, $P < 0.01$) (Fig. 2). The regression models of male and female butterflies were $y = -7\text{E-}09x^2 + 0.0005x - 0.3716$ ($R^2 = 0.7467$, $P < 0.0001$) and $y = -3\text{E-}09x^2 + 0.0003x - 0.5005$ ($R^2 = 0.7834$, $P < 0.0001$), respectively. The optimum light intensity for male butterfly flight was 35,714 lx and 50,000 lx for female butterflies.

The Student's *t*-test we used to analyze the differences in flight frequency between male and female butterflies showed that males were more active than females under similar sunlight conditions (i.e., similar light intensities; Table 1). The

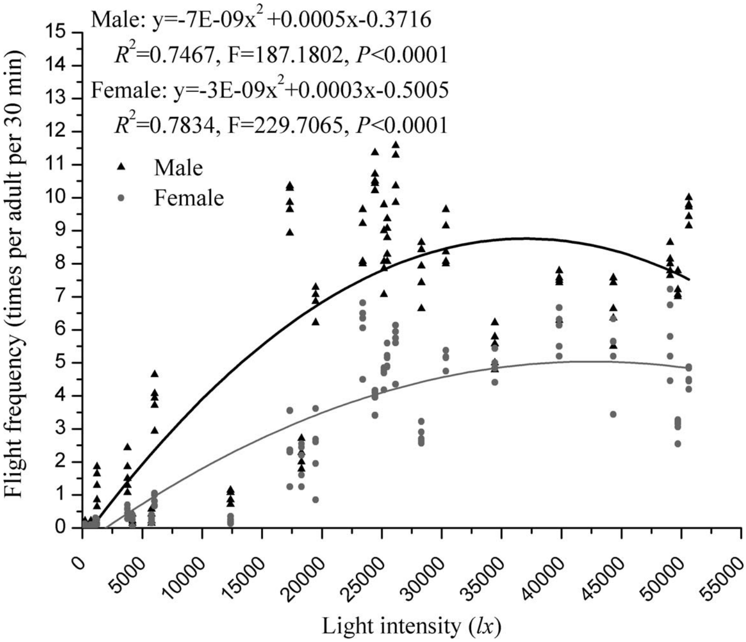

Fig. 2. Effects of light intensity on the flight frequency of adult butterfly *D. chrysippus*, as analyzed by binominal regression analysis. The correlation between flight frequency and light intensity is indicated by the R^2 values.

flight frequency of male butterflies was significantly higher than for female butterflies at 9:00–9:30 a.m., 10:00 a.m.–3:30 p.m., and 4:00–7:00 p.m. (Table 1). Thus, females generally made fewer flights than did males at most time points during the study (Fig. 1). When the sunlight intensity was ≥50,000 lx (1:00–2:30 p.m.), the flight frequency of males decreased until the light intensity was lower than 50,000 lx (Fig. 1). The curve of the binomial regression model between female flight frequency and light intensity was also lower than that of the males (Fig. 2). The flight frequency of females increased slowly with increasing light intensity compared with males. Thus, male butterflies were more active than were female butterflies (Table 1; Figs. 1, 2).

Thoracic temperatures of adult butterflies during flight and perching. The ΔT of adult butterflies in flight was significantly higher than in perching adult butterflies (Fig. 3). The ΔTs of male and female adults in flight were significantly higher than perching adults (male: $t5_8 = 15.18$, $P < 0.0001$; female: $t_{56} = 11.69$, $P < 0.0001$). However, there was no significant difference in the ΔTs of male and female adults, regardless of flight or perching (in flight: $t_{50} = 0.53$, $P = 0.5967$; in perching: $t_{64} = 0.81$, $P = 0.4193$). The thoracic temperatures of male and female adults in flight were 4–4.5°C higher than the ambient temperature (male: $t_{46} = 8.53$, $P < 0.0001$; female: $t_{54} = 10.03$, $P < 0.0001$). By contrast, the thoracic temperatures of male and female adults when perching were similar to the ambient temperature (male: $t_{70} =$

Table 1. Differences in flight frequency between female and male adult *D. chrysippus.*

Time	t	P	Time	t	P
07:00–07:30 a.m.	0.451	0.664	13:30–14:00	20	<0.0001**
07:30–08:00 a.m.	1.045	0.327	14:00–14:30	25.81	<0.0001**
08:00–08:30 a.m.	0.786	0.455	14:30–15:00	2.347	0.047*
08:30–09:00 a.m.	0.943	0.373	15:00–15:30	3.587	0.007**
09:00–09:30 a.m.	8.485	<0.0001**	15:30–16:00	1.835	0.104
9:30–10:00 a.m.	0.694	0.507	16:00–16:30	13.204	<0.0001**
10:00–10:30 a.m.	9.256	<0.0001**	16:30–17:00	7.628	<0.0001**
10:30–11:00 a.m.	13.109	<0.0001**	17:00–17:30	16.664	<0.0001**
11:00–11:30 a.m.	11.441	<0.0001**	17:30–18:00	10.413	<0.0001**
11:30 a.m.–12:00 p.m.	10.42	<0.0001**	18:00–18:30	4.468	0.002**
12:00–12:30 p.m.	28.302	<0.0001**	18:30–19:00	4.848	0.001**
12:30–1:00 p.m.	4.805	0.001**	19:00–19:30	2.158	0.063
1:00–1:30 p.m.	4.057	0.004**	19:30–20:00	1.633	0.141

*, ** Indicate levels of significance of $P < 0.05$ and $P < 0.01$, respectively, according to the Student's *t*-test.

0.32, $P = 0.7484$; female: $t_{58} = 0.52$, $P = 0.6020$). Thus, for autonomous flight, adult butterflies need to absorb heat from the sun to obtain a high thoracic temperature.

Effects of light intensity on ability of butterflies to absorb heat. Light intensity significantly affected the ability of adult *D. chrysippus* to absorb heat (Figs. 4, 5). As the light intensity increased, the thoracic temperature significantly increased (male: $F = 127.97$, df = 4, 25, $P < 0.0001$; female: $F = 259.31$, df = 4, 25, $P < 0.0001$; Fig. 4A). The ΔT also increased significantly (male: $F = 121.41$, df = 4, 25, $P < 0.0001$; female: $F = 225.65$, df = 4, 25, $P < 0.0001$; Fig. 4B). The time to reach equilibrium was shorter at higher light intensities (male: $F = 200.78$, df = 4, 25, $P < 0.0001$; female: $F = 231.30$, df = 4, 25, $P < 0.0001$; Fig. 5A), and the rate of thoracic temperature increase of both males and females also significantly increased (male: $F = 36.22$, df = 4, 25, $P < 0.0001$; female: $F = 58.52$, df = 4, 25, $P < 0.0001$; Fig. 5B). Thus, *D. chrysippus* exposed to stronger light intensities were able to absorb more heat for autonomous flight with a faster rate of heat absorption (Figs. 4, 5).

Effects of light intensity on the relationship between flight frequency and reproductive success. Light intensity significantly affected flight frequency which, in turn, affected mating frequency, fecundity, and egg hatchability (flight frequency: $F = 136.81$, df = 4, 20, $P < 0.0001$; mating frequency: $F = 146.46$, df = 4, 20, $P < 0.0001$; and fecundity: $F = 1389.89$, df = 4, 20, $P < 0.0001$; Figs. 6, 7). Mating frequency, fecundity, and hatchability increased with flight frequency in adult *D. chrysippus* (Fig. 7). At light intensities below 15,000 lx, most females

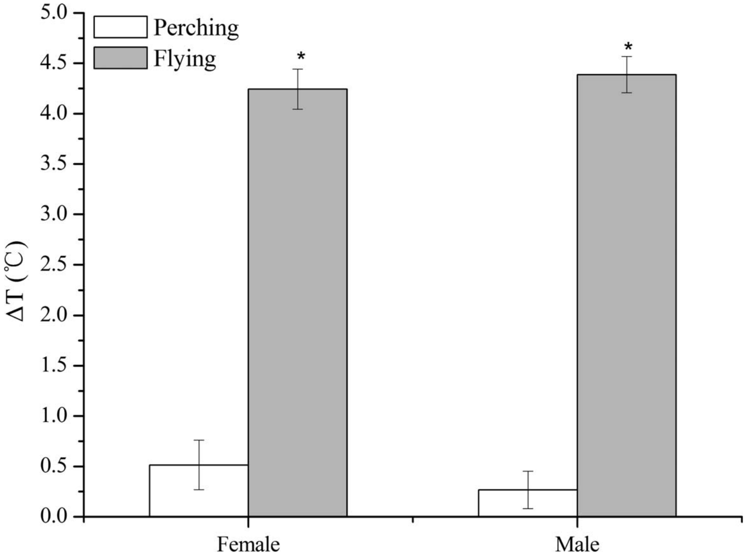

Fig. 3. Thoracic temperature excess (ΔT) of male and female adult *D. chrysippus* when perching and flying under natural sunlight conditions on 13 September 2017. *Indicates a significant difference (at the $P < 0.05$ level) in increased temperature between adults when perching versus flying.

failed to mate. At 15,000 lx, on average females mated once and laid 41 eggs with 45.35% hatchability. At 30,000 lx, on average females mated twice and laid 91 eggs per female with 79.82% hatchability. At 45,000 lx, on average females mated 2.5 times and laid 99.29 eggs with 80.18% hatchability (Fig. 8). There was no significant difference in female lifespan at 15,000, 30,000, or 45,000 lx, but it was much longer at these intensities than at 2,000 and 8,000 lx ($F = 3.059$, df $= 4, 215$, $P = 0.018$; Fig. 9). Stronger light intensities resulted in higher reproductive success (Figs. 6, 7, 8, 9).

Discussion

Effects of light intensity on flight activity. The current findings suggest that light intensity regulates the flight activity of adult *D. chrysippus*, both males and females, given the positive quadratic correlation recorded between flight frequency and sunlight intensity. Similar results were recorded in the field when the butterflies were exposed to a range of light intensities 2,000–45,000 lx. These results are in agreement with a previous study on the effect of light intensity on the flight activity of adult *T. limniace*, which showed that the flight frequency and flight activity of male and female butterflies significantly increased with increasing light intensity 243–2,240 lx (Liao et al. 2017). Thus, high light intensity can significantly increase butterfly flight activity. However, this study also showed that when sunlight intensity was >45,000 lx, butterflies made fewer flights than at 45,000 lx. Thus, butterfly flight

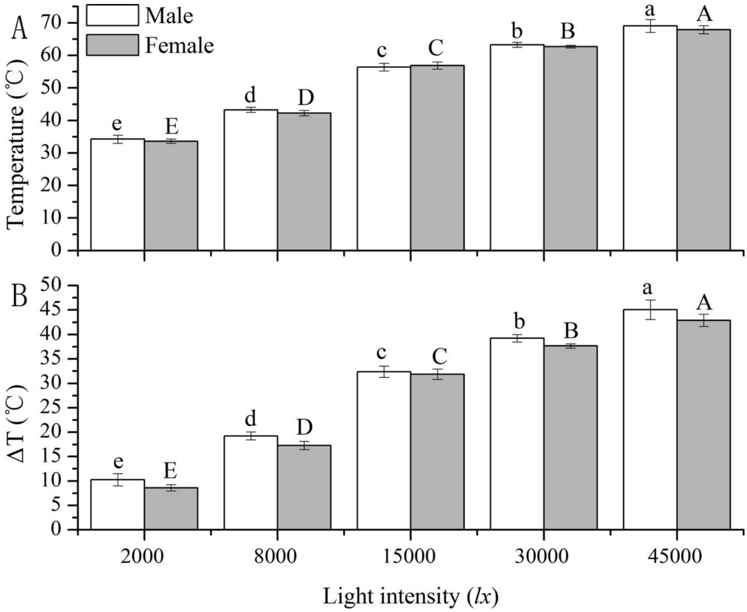

Fig. 4. (A) Equilibrium thoracic temperature and (B) thoracic temperature excess (ΔT) of adult *D. chrysippus* exposed to five light-intensity levels. Different lowercase and capital letters indicate significant differences (at the $P < 0.05$ level) in the data from males and females among the five light intensities.

usually occurs within a range of light intensity. A light intensity that is too high or too low will not be conducive to autonomous flight. Therefore, within the appropriate range of light intensity, high light intensity can significantly increase butterfly flight activity.

Differences in flight activity between male and female butterflies. The present results showed that male *D. chrysippus* were more active than were females under similar sunlight conditions. A similar result was reported for *T. limniace* whereby the male butterflies exhibited higher flight activity than the females as the light intensity increased (Liao et al. 2017). The mating strategy of most butterfly species is to 'wait and seek' (Bennett et al. 2012; Tiple et al. 2010). Dominant males adopt a wait strategy by monopolizing large sunspots on the forest floor and then quickly rise to intercept and mate with passing females. By contrast, subdominant males adopt a seek strategy and must continuously search for females to mate with (Bergman and Wiklund 2009a,b). *Bicyclus anynana* (Butler) males had higher courting rates than did females and flew more often than did females (Westerman et al. 2014). In the current study, a high light intensity significantly increased the mating frequency of adult *D. chrysippus* by elevating the flight activity, with males being more active than the females. Thus, the mating strategy described above determines that male butterflies are more active than females.

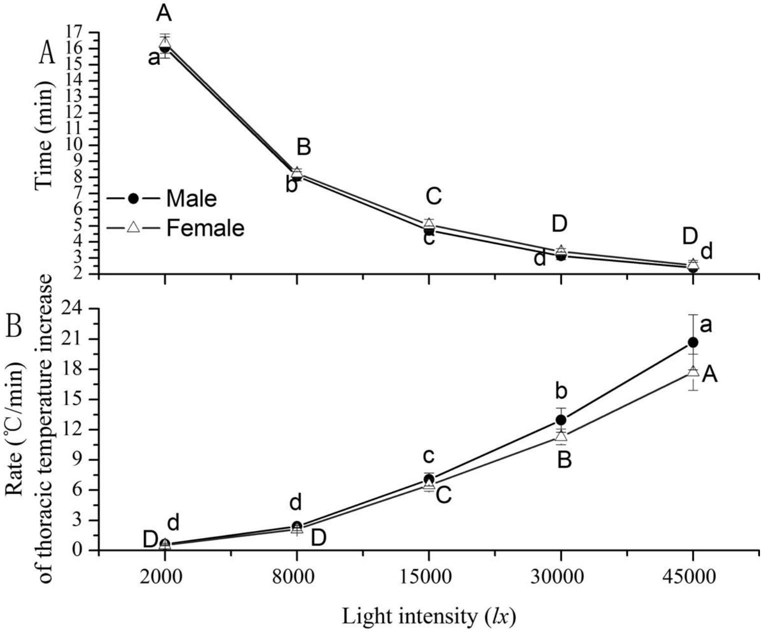

Fig. 5. (A) Time taken to reach equilibrium temperature and (B) rate of thoracic temperature increase of adult *D. chrysippus* exposed to five light-intensity levels. Different lowercase and capital letters indicate significant differences (at the $P < 0.05$ level) in the data from males and females among the five light intensities.

Some studies considered that the difference in flight activity between sexes results from the differences in body mass allocation between females and males (Berwaerts et al. 2002, 2008; Berwaerts and Van Dyck 2004). Given the position of the male in the mating strategy, it is required to have a greater flight ability compared with the female. Thus, males allocate more body mass to the thorax whereas females allocate more to the abdomen. Males with a heavier relative thorax mass have a stronger ability to fly because the relative thorax mass is closely related to flight ability, and flight muscle investment is associated with relative thorax mass. In the current study, females showed a slower rate of flight frequency increase with increasing light intensity compared with males. Therefore, it is reasonable that male *D. chrysippus* flew more actively compared with females. Further research is required to determine differences in flight activity between females and males of this species.

Effects of light intensity on the ability of butterflies to absorb heat. The current results showed that, for autonomous flight to occur, the butterflies increased their thoracic temperature to 4–4.5°C above the ambient temperature, and they did so by absorbing heat from the sun, which is in agreement with previously published results (Kleckova et al. 2014; Liao et al. 2017; Mattila 2015). When adults were in flight, the ΔTs of male and female adults were significantly

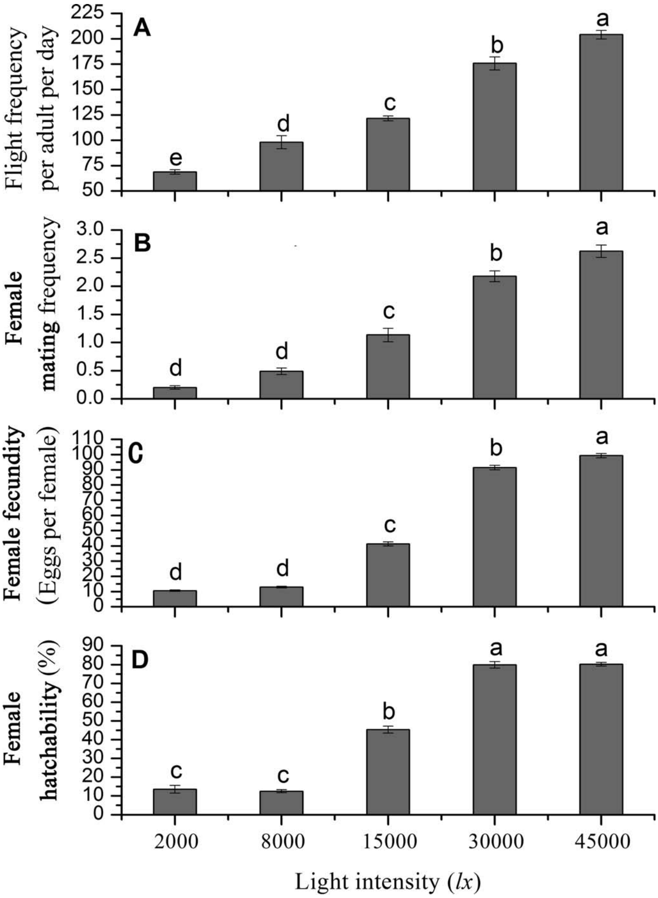

Fig. 6. (A) Flight frequency, (B) mating frequency, (C) fecundity, and (D) egg hatchability of adult *D. chrysippus* exposed to five light-intensity levels. Different lowercase letters on the bars in the same subfigure indicate significant differences (at the $P < 0.05$ level) in the data for flight frequency, mating frequency, fecundity, and egg hatchability among the five light intensities.

higher than those of perching adults. By contrast, the thoracic temperatures of male and female adults when perching were almost the same as ambient temperatures. Unlike other heat-absorbing insects, the increase in the body temperature of butterflies results mainly from the absorption of solar radiation (Barton et al. 2014; Bonebrake et al. 2014). As opposed to butterflies, the thoracic temperatures of moths are always similar to those of the surrounding environment. Moths increase their thoracic temperature to above that of the environment by wing movement. They also increase their oxygen consumption to enable flight to occur (Hanegan and Heath 1970). Thus, *D. chrysippus* adults must absorb heat from sunlight to enable autonomous flight.

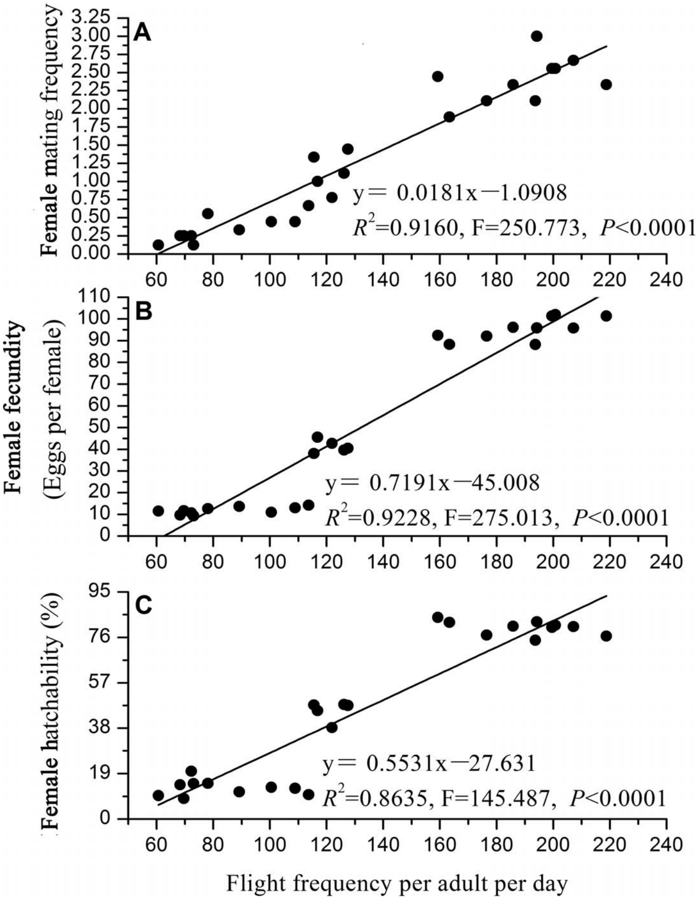

Fig. 7. Relationship between adult *D. chrysippus* flight frequency and (A) mating frequency (B) fecundity, and (C) egg hatchability as analyzed by linear regression. The correlation between flight frequency and mating frequency, fecundity, and egg hatchability is indicated by the R^2 values.

Butterflies exposed to higher light intensities (up to 45,000 lx) were able to absorb more heat for autonomous flight, the time for heat absorption was shorter, and the absorption rate was also faster. Butterfly wings absorb heat directly from sunlight and transmit it to the thorax for autonomous flight. For example, Kingsolver (1985) suggested that, in *Pieris* butterflies, their wings act as solar reflectors to reflect solar radiation onto the body to increase its temperature. The wings might reflect more heat onto thorax when the butterflies are exposed to higher light intensities. However, this method of heat transfer has not been effectively demonstrated by other studies.

An optimal light intensity can improve the ability of a butterfly to gain heat from a light source as well as to gain more heat in less time, and at a faster rate, thus

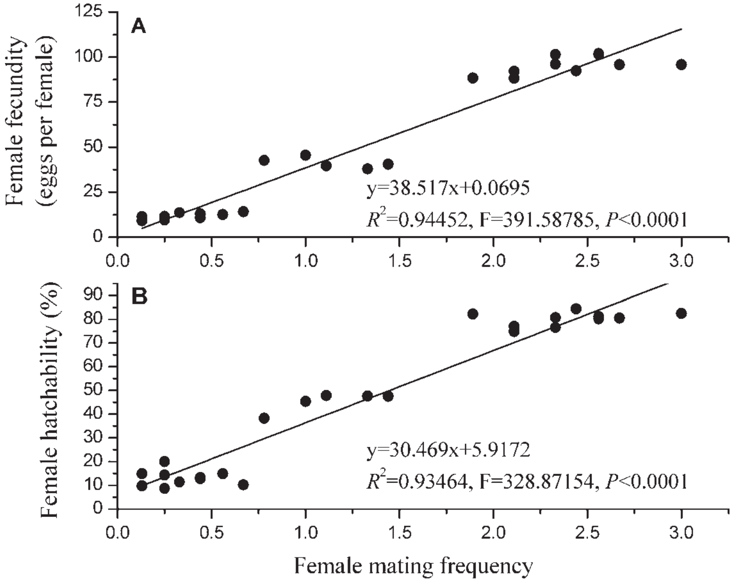

Fig. 8. Relationship between adult *D. chrysippus* (A) mating frequency and fecundity, and (B) egg hatchability as analyzed by linear regression. The correlation between mating frequency and fecundity, and egg hatchability, is indicated by the R^2 values.

enabling them to initiate flight more quickly. For example, in *T. limniace*, at 243–2,240 lx, adult butterflies were able to absorb more heat quicker than at low light intensities to increase thoracic temperature to above the environmental temperature and to take off earlier (Liao et al. 2017). Similarly, the body temperature excess of adult *Heteronympha merope* (F.) increased with increasing solar radiation (Barton et al. 2014).

The ability of butterflies to absorb heat might be affected by factors such as wing morphology, basking posture, and thorax size (Berwaerts et al. 2001, 2002; Kingsolver 1987, 1988; Schmitz 1994). In the butterfly *Pararge aegeria* (L.), those with large-sized wings heated up more slowly compared with those with small-sized wings, but the differences decreased as the temperature increased, and butterflies with fully-opened wings were able to absorb more heat and cool faster compared to those with half-opened wings (Berwaerts et al. 2001). The butterfly *Parnassius phoebus* (F.), which lives at high altitudes and latitudes, uses the finned black strategy to obtain heat from solar radiation. With increases in altitude and latitude, the color of its wings becomes darker (Guppy 1986). Air or ambient temperature could also affect heat absorption by butterflies. For example, within the 22–48°C temperature range, *Hipparchia semele* (L.) males maximize their heat load by exposing the maximum body area possible for sun-basking at low temperatures and fold their wings to minimize heat absorption at higher temperatures (Dreisig 1995). The present results showed that light intensity can significantly influence the ability of adult *D. chrysippus* to absorb heat. At the range of light intensity 2,000–45,000 lx,

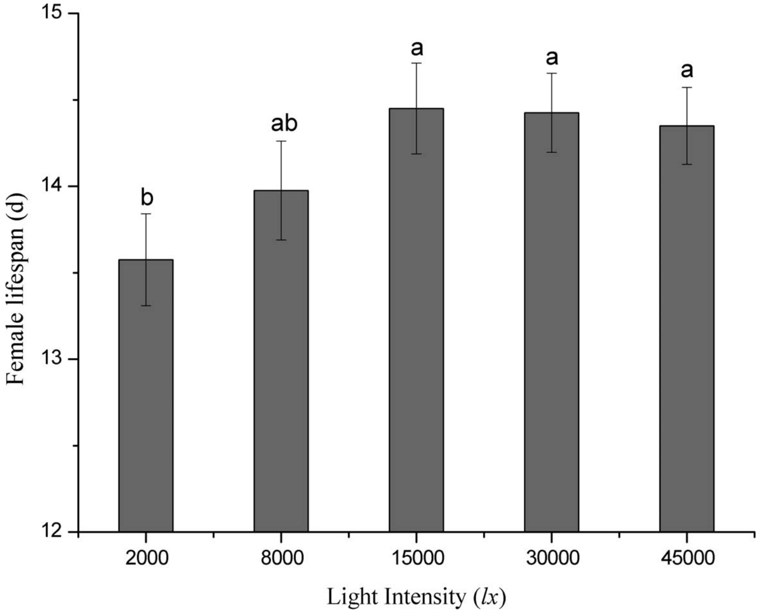

Fig. 9. Female lifespan of adult *D. chrysippus* exposed to five light-intensity levels. Different lowercase letters on the bars indicate significant differences (at the $P < 0.05$ level) in female lifespan among the five light intensities.

a higher light intensity could result in a higher thoracic equilibrium temperature, a higher ΔT, and a shorter time and a quicker rate of thoracic temperature increase to reach the equilibrium temperature.

The colors of a butterfly wing mainly comprise iridescence and structural colors (Han et al. 2016; Michielsen et al. 2010; Nijhout 1991). The part of the wing that has a structural color is used to absorb heat from the sun (Bosi et al. 2008), which is made possible by photonic crystal structures contained within the scales rather than by the wings reflecting the sunlight (Han et al. 2013, 2015; Li et al. 2004; Luohong 2014). In the current study, a higher light intensity would have enabled the photonic crystals to absorb more heat from the light sources compared with lower light intensities. Thus, heat transfer does not depend on reflectance by the wings but on their internal tissues. However, further studies are required to determine how heat is transferred from the wings to the thoracic muscles in butterflies.

Effects of light intensity on reproductive success in butterflies. The current findings showed that at light intensities between 15,000 and 45,000 lx, higher light levels increase flight activity, which in turn influences female mating frequency. Females with a higher mating frequency lay more eggs per day, and their eggs have a higher egg hatching success. Previous studies reported that flight behavior directly participates in mating and reproductive behavior (Almbro and Kullberg 2009; Chou 1999; Shreeve 1992,). Thus, within an appropriate range of light intensity, the higher the flight activity the higher the reproductive success. In *B.*

anynana, higher flight activity increased the chances of males successfully mating with females (Westerman et al. 2014). In *Pararge aegeria* (L.), males that were forced to fly in the laboratory produced more eupyrene sperm bundles than did resting males (Vande Velde et al. 2012). In the present study, with an increase in mating frequency the fecundity and egg hatchability also increased. Higher light intensity (up to 45,000 lx) enhanced the flight frequency to increase mating frequency. Males are able to transfer spermatophores that are rich in nutrients and sperms to the females, which could result in the increased fertility of females recorded in this study. Further research is required to determine the mechanism of flight affecting reproduction in this species.

In summary, strong light enables butterflies to quickly absorb more heat from a light source to reach an equilibrium temperature, thereby increasing the frequencies of autonomous flight and mating and improving their reproductive success. Butterflies have high economic and ecological value and can also be used to evaluate the ecological quality of environments (Chen et al. 2008; Chou 1999; Feng et al. 2016). However, reproductive success is a key factor in butterfly conservation and utilization. Given that we have shown here that light intensity could regulate autonomous flight activity to control mating frequency, thus affecting reproductive success, it might be possible to regulate butterfly reproduction by adjusting the light intensity. The present results indicate that the light intensity in culture rooms or greenhouses in the field could be adjusted to levels that are suitable for butterfly reproduction to improve the population growth of butterflies, not only resulting in a sufficient source of butterflies with high economic value but also expanding the population size of butterflies threatened with extinction, which could then be released in the field. Thus, our results provide theoretical guidance for the protection and utilization of butterflies in both the field and breeding facilities.

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