

Flight and Walking Performance of Dark Black Chafer Beetle *Holotrichia parallela* (Coleoptera: Scarabaeidae) in the Presence of Known Hosts and Attractive Nonhost Plants

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Abstract

Holotrichia parallela damages seriously on peanut (*Arachis hypogaea*) pods. Elucidation of its flight and walking performance in the presence of different plants may provide an insight in its host selection process and an explanation to its strong olfactory preference to an attractive nonhost, castor bean (*Ricinus communis*). We determined the relationships among flight performance, mate choice, and body weight of *H. parallela* beetles, and then investigated their flight and walking patterns in the presence of known hosts and attractive nonhost plants using a flight mill and a locomotion compensator, respectively. Body weights were not related to mating success, regardless of sex. The flight proportion of selected females drastically decreased compared with nonselected females, nonselected males, and selected males. Within mated males, heavier individuals exhibited poorer flight performance than lighter ones. In flight bioassay, peanut showed an arrestment effect on virgin females. For walking activity factors (distance, time, and speed), the host plants velvetleaf (*Abutilon theophrasti*) and Siberian elm (*Ulmus pumila*) elicited the strongest responses in females and males, respectively. Interestingly, the most preferred adult host, Siberian elm, and the nonhost, castor bean, elicited the highest values of two orientation factors (orientation and upwind length) in females. The chemical similarity hypothesis, which states that feeding or oviposition of insects mistakenly on nonhost can be traced to their chemical similarity to actual hosts, could explain the attraction of *H. parallela* to castor bean.

Key words: scarab, castor bean, flight mill, locomotion compensator

The dark black chafer beetle, *Holotrichia parallela* Motschulsky, is one of the most severe soil pests in peanut cultivation areas of North China (Zhang et al. 2018). The larvae (so called ‘grubs’) feed on the peanut pods and result in 10–20% yield loss in a normal year (Ju et al. 2017). Recently, the ban of straw combustion in conjunction with the application of mechanical harvest technology in China, abundant immature straw powder has been directly returned to the field, which creates an environment conducive to *H. parallela* population growth and persistence (Li et al. 2018) and aggravating its damage. Traditional soil chemical treatments such as seed-coating or soil drenching with chlorpyrifos pollute the soil and decrease the quality of peanut products (Li et al. 2013).

For *H. parallela*, plants can be grouped into three classes: 1) host plants for eggs or larvae of *H. parallela*. They are mainly damaged by the below-ground larvae (on roots), to a lesser extent, by the aboveground adults (on leaves), including many important crops

such as peanut (*Arachis hypogaea*), sweet potato (*Ipomoea batatas*), soybean (*Glycine max*), and so forth; 2) host plants for adult *H. parallela*. The leaves are served as supplemental food of the adult beetles but no larval damage has been reported on their below-ground roots, including many woody plants (e.g., glossy privet [*Ligustrum lucidum*] and Siberian elm [*Ulmus pumila*]) and a few grassy plants (e.g., velvetleaf [*Abutilon theophrasti*]); and 3) nonhost plants, including a vast number of naturally unacceptable plants, and very scarcely, acceptable but detrimental plants (e.g., the castor bean, *Ricinus communis*) (Zhang et al. 2018).

At sunset, the emerged adults fly to the adult host plants to mate (commonly lasts 7–11 min) and feed. At the end of the scotophase, mated females fly to fields to lay eggs in the soil (about 5–12 cm deep) close to the ovipositional host plants (Xu et al. 2002). Castor bean (*R. communis*) is a potential trap crop of *H. parallela*, as previous olfactometry studies showed that the adults prefer this nonhost plant

to other major host plant species (Zhang et al. 2018). Additionally, the beetles cannot use castor bean leaves as a supplemental food, as they may suffer temporary paralysis after some sampling bites (Li et al. 2010). This detrimental effect could be balanced by the enhancement of male mate-location and copulation rate in the presence of castor bean plants, suggesting that this nonhost plant may provide an attractive but risky mating site for these beetles at night (Zhang et al. 2018). However, detailed information on the behavioral process of *H. parallela* attraction to this nonhost plant remains largely unknown. Therefore, this study tries to understand its mating-related traits (e.g., body weight and flight capacity) and movement pattern in order to provide an insight into the strong preference of this herbivore to its nonhost plant species. Additionally, this study will promote the effective application of this potential trap crop.

Flight mills have been used to explore the effects of sex, age, mating status (Schumacher et al. 1997, Sarvary et al. 2008), temperature, or humidity (Zhang et al. 2008) on the flight capacity of various insects, including Diptera, Lepidoptera, Hemiptera, and Coleoptera (Hahn et al. 2017). The locomotion compensator is a powerful tool for investigating the orientation response of beetles to different odor sources because it allows them to walk freely without any barrier in an air stream (Heisswolf et al. 2007). According to our observation, *H. parallela* moves to a suitable habitat from a distance depending on its limited flight capacity. After reaching a suitable habitat, it locates biologically relevant resources mainly by walking (Zhang et al. unpublished data). Based on this movement pattern, we first determined the relationships among flight performance, mate choice, and body weight of *H. parallela* beetles, and second conducted two experiments on the flight and walking performances of virgin beetles in the presence of different plant species using a flight mill and a locomotion compensator, respectively. To our knowledge, this is the first study to test the flight and walking movement behaviors simultaneously in a scarab species.

Materials and Methods

Insects

Holotrichia parallela is a univoltine species. In nature, the pupation of the overwintered larvae peaked at mid-May. The pupal stage lasts 20–25 d. *Holotrichia parallela* adults were originally collected by black light trap from Scientific & Education Yard, Henan Agricultural University, Zhengzhou, Henan Province, China, and reared on fresh Siberian elm leaves in the laboratory (24°C, 16 h light: 8 h dark). Deposited eggs were maintained in sterile soil (approximately 20% relative humidity). After 8–10 d, the hatchlings were maintained on moistened peanut pods. Under the above-mentioned laboratory conditions, the larvae pupated after 9–10 wk. Soil conditions for maintaining the pupae were the same to the larvae except that food supply was ceased. Emerged adults were grouped according to sex and the two sexes were reared in separate containers. After 20–25 d after emergence, they were used for subsequent bioassays. Each beetle was tested only once in a specific flight or walking bioassay. It was almost impossible to record how many mating families that test beetles were from. First, the mortality in the larval stages was high, and once the dead larvae were found, it was separated from laboratory cultures as soon as possible to prevent pathogen infection; and second, the fecundity of mated females were relatively low.

Plants

Seeds of castor bean (*R. communis*, var. ‘No. 6 Zibima’) and peanut (*Arachis hypogaea*, var. ‘No. 9326 Yuhua’) were purchased

from Zibo Academy of Agricultural Sciences and Henan Academy of Agricultural Sciences, respectively. Castor bean and peanut were planted at mid-March and mid-April, respectively, in an experimental field in the third community of Henan Agricultural University. These plants were suitable for bioassay at late-May. At this time, the peanut was in its blossom stage, and the castor bean plant was 40 cm in height. No pesticide or fertilizer was used, but care was always taken to select plants without visible pathogen-infection or herbivore-infestation for laboratory bioassays. Seeds of velvetleaf (*A. theophrasti*) were collected in the field and planted in pots. Shoots of the Siberian elm (*U. pumila*), together with the leaves, were directly cut from the trees and used in the experiments.

General Procedure of the Flight Mill Bioassay

Flight performance was measured in a computerized flight mill with a 10-cm radial horizontal arm (Jiaduo Scientific, Industry, and Trade Co., Ltd., Hebi, China). From 10: 00 h to 11: 00 h, beetles aging 20–25 d were individually attached to the radial arm by an iron wire (10 cm length, 1.5 mm diameter) glued to the prothorax using a nontoxic instant adhesive at one end. The other end was attached to a gimbal system. Each flight mill was performed in a static-air arena (200 × 200 × 80 cm) at the conditions of 25°C, 45–55% relative humidity, in total darkness. The test beetles were deprived of food and water during flight. The flight bioassay was terminated after 24 h or when the beetle had died (monitored by an infrared video system). Data were collected by Arduino Microcontroller unit which could monitor 30 flight mills, and seven parameters (flight proportion, flight distance, flight time, flight speed, maximal instantaneous flying speed [FSM], maximal flying duration in a single flight event [FTM], and maximal flying distance in a single flight event [FDM], the upper part in Table 1) were calculated using a hand-made program based on Matlab software (Cui et al. 2016).

Flight Performance, Mate Choice, and Body Weight of *H. parallela*

To analyze the relationships among mate choice, body weight and flight performance of *H. parallela* beetles, the beetles tested were sequentially subjected to the following three tests: mate choice, body weight measurement, and flight bioassay. In each test, 30 beetle pairs aging 20–25 d were released in a Perspex cylinder (50 cm diameter × 70 cm height) at 20: 00 h. Copulation behavior of the beetles was observed under dim diffused light. We immediately separated the couples which had completed copulation from the group, and measured their body weights using an electronic balance (nearest to 0.1 mg). After that, they were placed in separated Petri dishes. At the end of the scotophase (4: 00 h), selected females, nonselected females, selected males, and nonselected males were counted. From 10: 00 h to 11: 00 h in the same day, they were subjected to flight bioassay which lasting 24 h, using the methods outlined in flight mill bioassays above. Another batch of 30 beetle pairs was operated in the same way but on a different day. A total of 74 nonselected beetles (37 females and males each) and 23 pairs of selected beetles were obtained. Five individuals of nonselected females were found dead and were excluded from the analysis. Therefore, the sample sizes of the four groups with available flight parameters (see Table 1) were: nonselected females ($n = 32$), selected females ($n = 23$), nonselected males ($n = 37$), and selected males ($n = 23$). Flight proportion in each group was calculated, that is, the proportion of active fliers (lived after flight test and produced non-zero flight parameters) in the total available tested insects, including active fliers and nonactive beetles (failed to fly for unknown reasons but lived after the flight test).

Table 1. Flight and walking parameters of *H. parallela* beetles and corresponding definitions

Movement pattern	Parameters	Definition
Flight	Flight proportion (%)	Percentage of active fliers in a given group.
	Flight distance (m)	Total flight distance of active fliers during 24 h testing period.
	Flight time (s)	Sum of active flying time during 24 h testing period.
	Flight speed (m/s)	Flight distance divided by active flying time.
	FSM (m/s)	Maximal instantaneous flying speed.
	FTM (s)	Maximal flying duration in a single flight event.
Walking	FDM (m)	Maximal flying distance in a single flight event.
	Walking distance (mm)	Total distance that a beetle had traveled during 240 s testing period.
	Walking time (s)	Sum of active walking time of a beetle during 240 s testing period.
	Walking speed (mm/s)	Walking distance divided by the sum of active walking time.
	Straightness	Ratio of the length of the final vector to the total distance walked, ranging from 0 to 1.
	Orientation (%)	Percentage of track points of a beetle within the range of -30° and $+30^\circ$ (0.52 rad) relative to the odor source.
	Upwind length (mm)	Net distance from the origin toward the odor source along a straight line.

Flight Assays in the Presence of Host and Nonhost Plant Species

We tested the flight performance of *H. parallela* virgin beetles aged 20–25 d postemergence in the presence of different treatment materials. General test procedure was mentioned above, except that in each test, a plastic pot containing a specific treatment material was placed in one of the corners of the arena, at about 1-m distance from the flight mill. Treatment plant materials included a flowering peanut plant, a castor bean plant (40 cm in height), a velvetleaf plant (40 cm in height), and a bouquet of cut Siberian elm shoots (40 cm in length). A pot filled with soil alone was used as a control. Flowering peanut was used because this stage is in accordance with the emergent season of *H. parallela* adults in the nature. The Siberian elm is a woody plant whose leaves are mainly used by adults as supplemental food, so the shoots were used. Test stages of castor bean and velvetleaf were determined mainly by their approximately equal biomass to the Siberian elm shoots. Thus, this bioassay includes four plant treatments and a control treatment. Within each sex, each treatment was replicated with 10–17 individuals (Dataset 2, [Supplementary Information](#)). At the end of each run, both the tested beetle and the pot with a tested material were removed from the arena, and the odor residue was thoroughly drawn out from the arena using a 30-cm-diameter fan.

Walking Bioassays in the Presence of Host and Nonhost Plant Species

The walking behavior response of *H. parallela* virgin beetles 20–25 d postemergence to volatiles from different sources borne in an airstream was tested on a locomotion compensator with a 300-mm diameter servosphere (Tracksphere LC 300; Syntech, Germany). The bioassay was conducted from 20:00 h to 22:00 h (in accordance with natural activity rhythm of *H. parallela* beetles) at a condition of 25°C and in total darkness. The time in which the walking bioassay being conducted was different to that of the flight bioassay, since that a flight test lasted 24 h, while a walking test lasted only 5 min (see below). Airstream was generated by an air sampler (QCD-1500, Yancheng Galaxy Science & Technology Co., Ltd., China) at rate of 0.5 liter/min and was charcoal-filtered and distilled water-moistened before blowing on to a test beetle that was released at the top of the servosphere. Test odor(s) was/were mixed into the airstream through a Pasteur pipette connected to a glass jar (25 cm ID and 40 cm depth) with Teflon tubing (1 cm ID). The outlet of the mixed airstream was 40 mm away from the top of the sphere.

Tested odor sources were as follows: 1) a potted castor bean plant (40 cm in height), 2) a flowering peanut plant, 3) a velvetleaf plant (40 cm in height), 4) a bouquet of cut Siberian elm shoots (40 cm in length), and 5) a pot containing only soil. The last treatment was served as control. Within each sex, each treatment was replicated with 10 individuals. The beetles were starved 24 h prior to testing. In each trial, a beetle was allowed to acclimatize on the sphere for 60 s, and then one of the five odor sources was applied for 240 s. According to our observation, this acclimation time (60 s) was long enough for the beetles to stop spontaneous movement due to the novel conditions (e.g., servosphere surfaces and clean air stream), but short enough to avoid random movement due to hunger. The ‘TrackSphere’ program (Version 2.2) was used to collect and store the displacement data, and to reconstruct walking paths. Each walking path was sampled at 10 data points and saved as *csv* files. According to the walking characteristics of *H. parallela* beetles, we resampled the data (1 data point/10 s) from the *csv* files and calculated six parameters as walking distance, walking time, walking speed, straightness, orientation, and upwind length (Table 1, lower).

Statistics

Within each sex, to examine the relationship between mating choice and body weight, we used Two-samples *t* tests to analyze the differences in body weights between: 1) selected ($n = 23$) and nonselected ($n = 32$) females; 2) selected ($n = 23$) and nonselected ($n = 37$) males; 3) active fliers ($n = 14$) and nonactive beetles ($n = 18$) within nonselected females; 4) active fliers ($n = 17$) and nonactive ($n = 20$) beetles within nonselected males; 5) active fliers ($n = 3$) and nonactive beetles ($n = 20$) within selected females; and 6) active fliers ($n = 12$) and nonactive beetles ($n = 11$) within selected males. In each comparison, the body weight data were checked for normality and homogeneity of variances by a Shapiro–Wilk test and a Levene’s test (Dataset 4, [Supplementary Information](#)). Data were transformed, where necessary, or tested by using Satterthwaite’s corrected Two-samples *t* tests. To examine the factors (sex, mating status, or their combination) influencing flight proportions (binomial-typed variable), we used Pearson’s χ^2 tests to compare the flight proportions between: 1) males versus females; 2) selected beetles versus nonselected beetles; 3) selected males versus nonselected males; 4) selected females versus nonselected females; 5) selected males versus selected females; and 6) nonselected males versus nonselected females. Yate’s correction for continuity was used, since that each comparison had 1 degree of freedom.

Since that normality and homogeneity of variance could not be reached simultaneously (as tested by Shapiro–Wilk test and Levene’s test, respectively) by any data transformations, nonparametric Mann–Whitney U tests were used to statistically analyze sexual difference in the presence of the same treatment material (flight bioassay) or toward the same odor source (walking bioassay). Within each sex, Mann–Whitney U tests were also used to compare the difference of beetle responses between each plant and the blank. During these analyses, dead beetles and nonactive beetles were excluded from the statistics of flight parameters (<6%). Beetles dropped from the servosphere (<9%) and no beetles had to be excluded due to too short walking distance (<60 mm) during the 240-s bioassay period. In the walking bioassay, all statistical analyses were performed using SPSS 19.0 for Windows. Significance was set at $P \leq 0.05$. Boxplot graphs were prepared in an Origin 2018 software.

Results

Relationships Among Flight Performance, Body Weight, and Mate Choice

No significant differences were found in the body weights between selected and nonselected beetles, regardless of sex (Females: two-samples t test with Satterthwaite’s correction: $t = 2.021$, $df = 31.081$, $P = 0.052$; Males: two-samples t test: $t = 0.615$, $df = 58$, $P = 0.541$, Fig. 1). Nonactive flying selected males were significantly heavier than active flying selected males (566.87 ± 21.01 vs 485.60 ± 26.18 mg, two-samples t test: $t = 2.393$, $df = 21$, $P = 0.026$; Fig. 2). Flight proportion of selected females was significantly lower than the other three groups (selected females vs selected males: $\chi^2 = 6.331$, $df = 1$, $P = 0.012$; selected females vs nonselected females: $\chi^2 = 4.56$, $df = 1$, $P = 0.033$; selected females vs nonselected males: $\chi^2 = 5.508$, $df = 1$, $P = 0.019$). In fact, of 23 selected females, only 3 individuals had flight capacity after mating (Fig. 3).

Between-Sex Comparison of Flight Performance in Each Treatment

In the presence of castor bean, the parameters of flight distance, flight time, and FTM of males were significantly larger than those of females ($U_{\text{Flight distance}} = 2.109$, $P = 0.035$; $U_{\text{Flight time}} = 2.791$, $P = 0.005$;

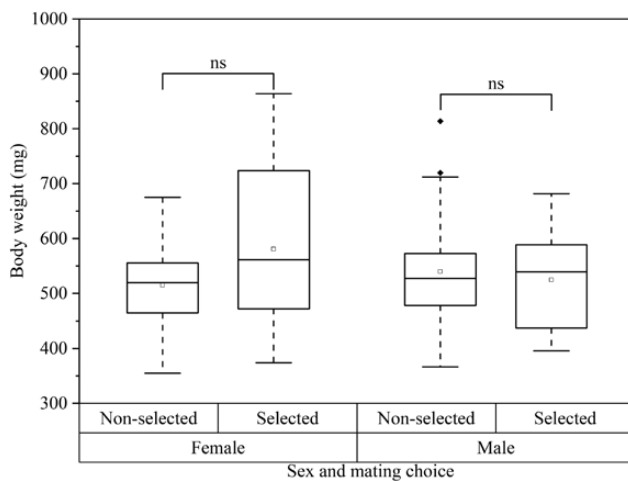


Fig. 1. Body weights of nonselected females ($n = 32$), selected females ($n = 23$), nonselected males ($n = 37$), and selected males ($n = 23$) of *Holotrichia parallela* adults. ‘***’ indicates significant difference at $P = 0.01$ level, ‘ns’ means no significant difference. Female body weight was compared by Two-samples t test with Satterthwaite’s correction, while male body weight was compared by Two-samples t test directly.

$U_{\text{FTM}} = 2.543$, $P = 0.011$; Fig. 4A,C,D). Males flew significantly a longer time than females in the presence of peanut ($U = 2.013$, $P = 0.044$; Fig. 4C). In the absence of plant materials (blank), FSM value of the females was larger than that of the males ($U = 1.985$, $P = 0.047$; Fig. 4F). All flight parameters did not differ between sexes in the presence of Siberian elm or velvetleaf (Fig. 4).

Within-Sex Comparison of Flight Performance Between Each Plant Treatment and Blank

In the presence of peanut, the parameters of flight distance, flight time, FDM , and FTM of females were significantly lower than those of blank ($U_{\text{Flight distance}} = 2.547$, $P = 0.011$; $U_{\text{Flight time}} = 2.628$, $P = 0.009$; $U_{\text{FDM}} = 2.464$, $P = 0.014$; $U_{\text{FTM}} = 2.354$, $P = 0.019$; Fig. 4A–D). Both flight time and FTM of males were significantly lower in the presence of velvetleaf than in the absence of plants ($U_{\text{Flight time}} = 2.258$, $P = 0.024$; $U_{\text{FTM}} = 2.170$, $P = 0.030$; Fig. 4C,D). FSM of males in the presence of castor bean was significantly higher than blank ($U = 2.035$, $P = 0.033$; Fig. 4F).

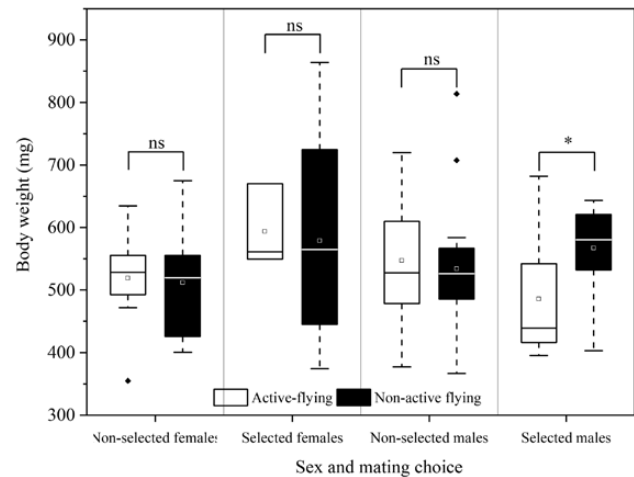


Fig. 2. Body weight difference between active flying and nonactive flying individuals of *Holotrichia parallela* within each of the following groups: nonselected females, selected females, nonselected males, and selected males. ‘*’ indicates significant difference at $P = 0.05$ level, and ‘ns’ means no significant difference. Data from nonselected males were log-transformed and before two-samples test (presented in original data in this figure), while data from the other three groups were tested by two-samples t tests directly.

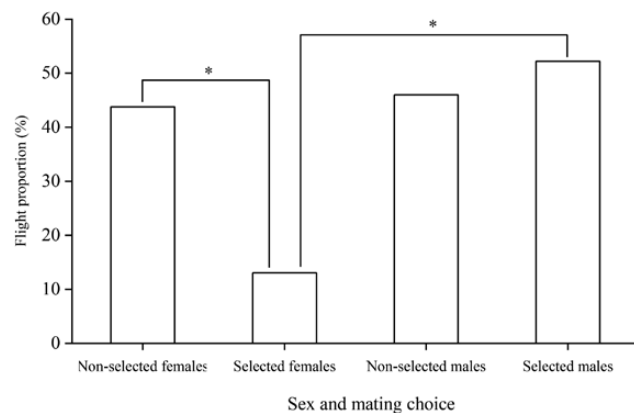


Fig. 3. Flight proportion of both sexes of nonselected and selected *Holotrichia parallela* adults on the flight mill. ‘*’ indicates significant difference at $P = 0.05$ level, as tested by a χ^2 test.

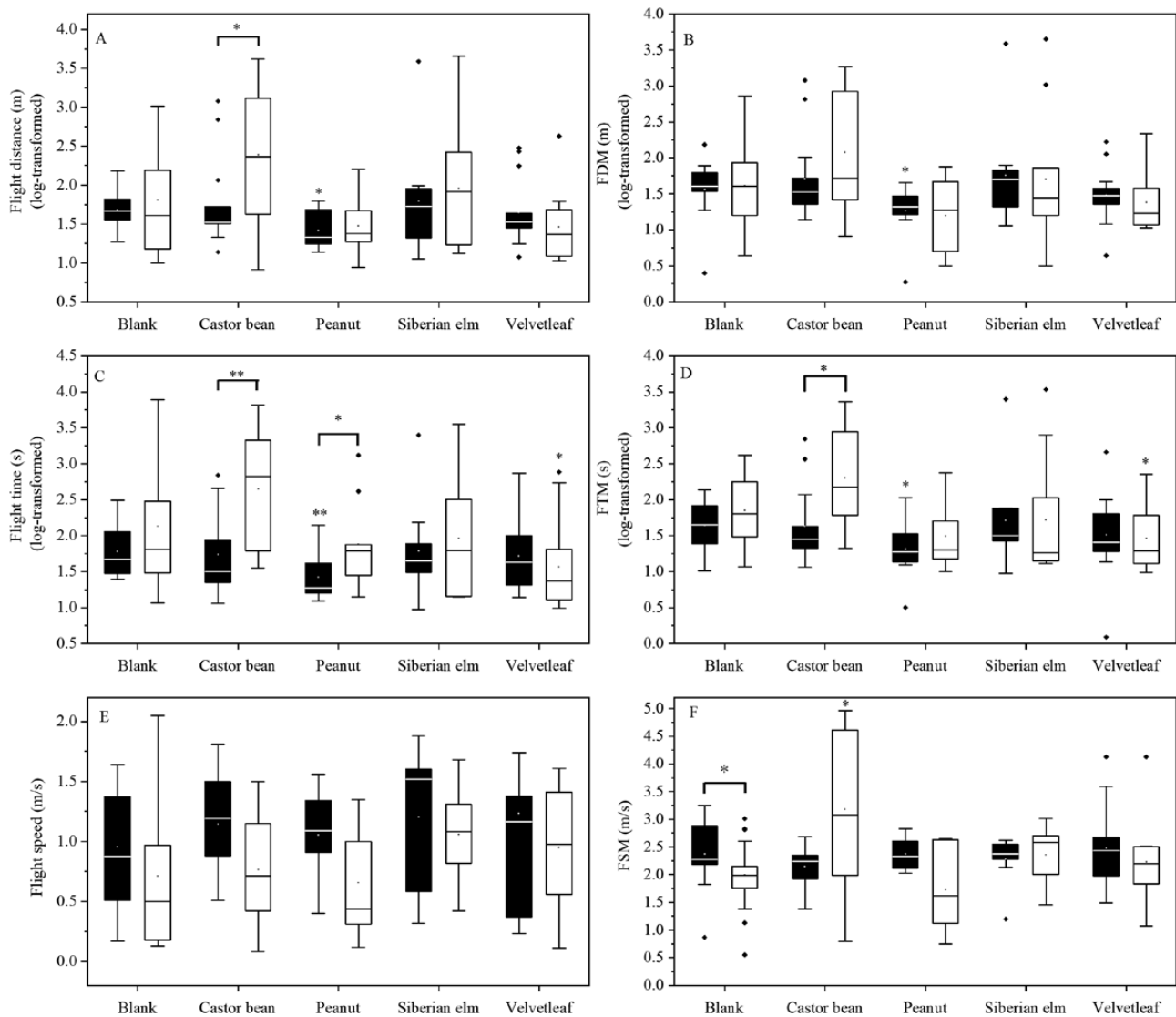


Fig. 4. Flight performance of male and female *Holotrichia parallela virgin* beetles in the presence of known hosts and attractive nonhost plants on the flight mill. (A) flight distance; (B) flight time; (C) flight speed; (D) maximal flight distance in a single flight event (FDM); (E) maximal flight time in a single flight event (FTM); (F) maximal flight speed in a single flight event (FSM). Solid boxes: females; Open boxes: males. '*' and '**' attached above a box indicate significant difference between the responses of corresponding plant material and control within a given sex, while '*' across a pair of boxes within a treatment indicates significant sexual difference at $P=0.05$ level, '**' across a pair of boxes within a treatment indicates significant sexual difference at $P=0.01$ level Mann-Whitney U test.

Between-Sex Comparison of Walking Performance Toward Each Odor Source

In the absence of plant odors, males walked faster than females ($U = 2.117, P = 0.034$), while females walked straighter than males ($U = 2.117, P = 0.034$; Fig. 5A,B). Males walked a longer upwind length than females toward velvetleaf ($U = 2.343, P = 0.019$; Fig. 5C). In the presence of castor bean, both upwind length and orientation of females were significantly higher than those of males ($U_{\text{Upwind length}} = 2.648, P = 0.008$; $U_{\text{Orientation}} = 3.288, P = 0.001$; Fig. 5C,D). In the presence of Siberian elm odor, females walked straighter than males ($U = 2.117, P = 0.034$; Fig. 5B). All walking parameters did not differ between sexes in the presence of peanut (Fig. 5).

Within-Sex Comparison of Walking Performance Between Each Plant Odor Source and Blank

Within females, castor bean and velvetleaf elicited faster walking speed than blank ($U_{\text{castor bean vs blank}} = 2.268, P = 0.023$;

$U_{\text{velvetleaf vs blank}} = 2.873, P = 0.004$; Fig. 5A). Both walking times and path straightness were not affected by any plant odor sources (Fig. 5B,E). Females walked a longer upwind length in the presence of castor bean than blank ($U = 2.343, P = 0.019$; Fig. 5C). Castor bean and Siberian elm elicited the highest and equal orientation rates, which were significantly higher than blank ($U_{\text{Siberian elm vs blank}} = 3.573, P < 0.001$; $U_{\text{castor bean vs blank}} = 3.831, P < 0.001$; Fig. 5D). Females walked a longer distance in the presence of velvetleaf than blank ($U_{\text{velvetleaf vs blank}} = 2.192, P = 0.028$; Fig. 5F).

Within males, Siberian elm and velvetleaf elicited significantly faster walking speed ($U_{\text{Siberian elm vs blank}} = 2.420, P = 0.016$; $U_{\text{velvetleaf vs blank}} = 2.041, P = 0.041$; Fig. 5A). Siberian elm elicited significantly less straight walking path ($U = 2.797, P = 0.005$; Fig. 5B). Velvetleaf elicited significantly a longer upwind length ($U = 2.495, P = 0.013$; Fig. 5C). Castor bean and Siberian elm elicited the highest and equal orientation rates ($U_{\text{Siberian elm vs blank}} = 2.184, P = 0.029$; $U_{\text{castor bean vs blank}} = 2.614, P = 0.009$; Fig. 5D), in consistent with the results obtained from females. Walking times of males were unaffected by

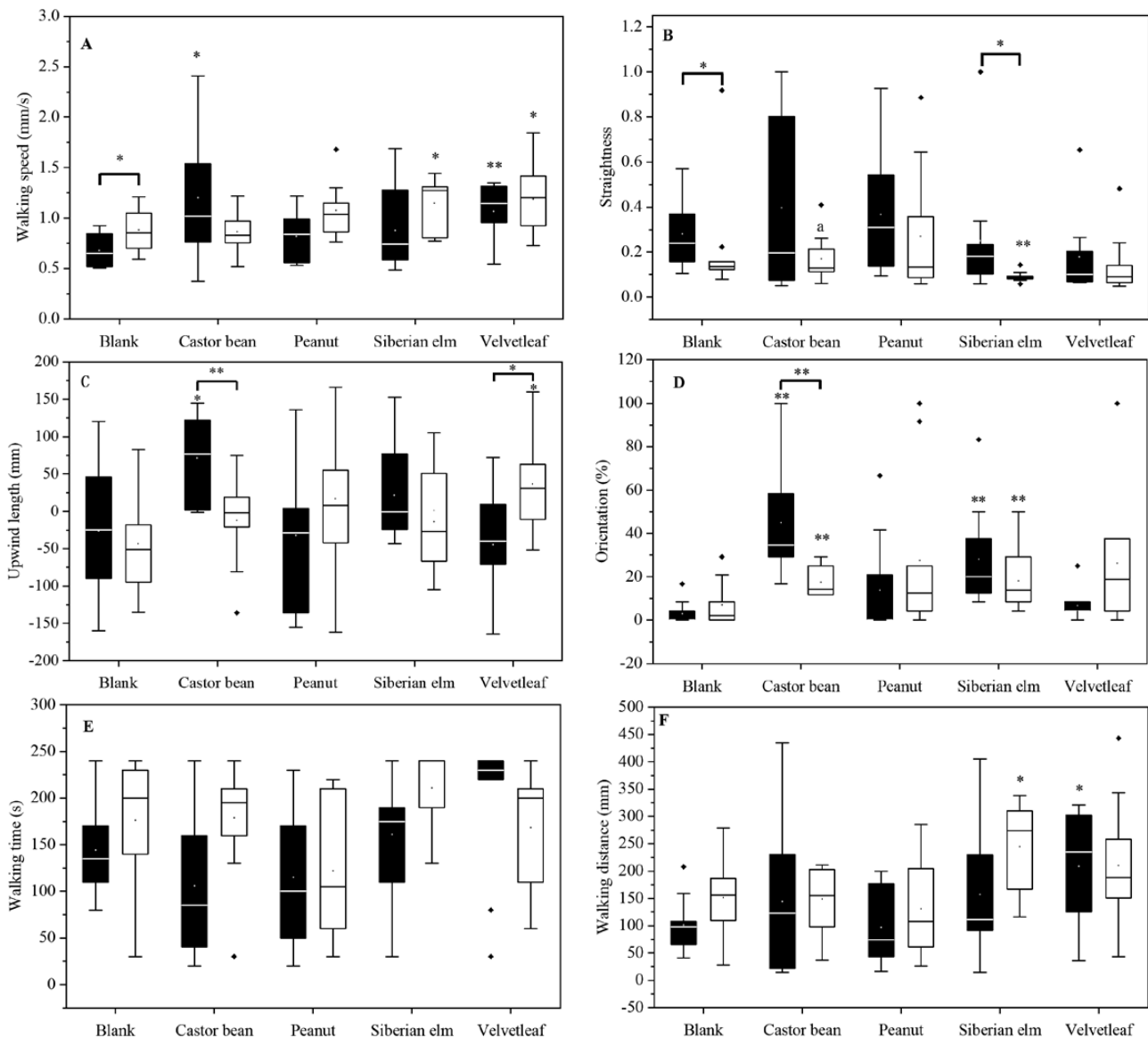


Fig. 5. Walking performance of male and female *Holotrichia parallela* virgin beetles in the presence of known hosts and attractive nonhost plants on a locomotion compensator. (A) walking speed; (B) straightness of the walking path; (C) upwind length; (D) orientation rate; (E) walking time; (F) walking distance. Solid boxes: females; Open boxes: males. '*' and '**' attached above a box indicate significant difference between the responses of corresponding plant material and control within a given sex, while 'a' and 'b' across a pair of boxes within a treatment indicates significant sexual difference at $P = 0.05$ level, '**' across a pair of boxes within a treatment indicates significant sexual difference at $P = 0.01$ level Mann-Whitney U test.

any plant odor sources (Fig. 5E). Males walked a longer distance in the presence of castor bean ($U = 2.343$, $P = 0.019$; Fig. 5F).

Between-Plant Comparison of Flight and Walking Performance

Statistical results of between-plant comparison of flight and walking parameters are summarized in Table 2. All the six flight parameters of virgin female beetles in the presence of the nonhost plant, castor bean, were not lower than any parameters produced by any host plant species. Two parameters (flight time and FDM) produced by castor bean were even higher than those produced by peanut ($U_{\text{flight time}} = 2.057$, $P = 0.040$; $U_{\text{FDM}} = 2.117$, $P = 0.034$). This trend was more pronounced in the flight parameters of males, as there are four, two, and one parameters showed significant difference when castor bean treatment was compared with velvetleaf, peanut, and Siberian elm, respectively (Castor bean vs velvetleaf: $U_{\text{flight distance}} = 2.374$,

$P = 0.018$; $U_{\text{flight time}} = 3.033$, $P = 0.002$; $U_{\text{FTM}} = 2.769$, $P = 0.006$; $U_{\text{FDM}} = 2.045$, $P = 0.041$; Castor bean vs peanut: $U_{\text{flight distance}} = 2.369$, $P = 0.018$; $U_{\text{FTM}} = 2.613$, $P = 0.009$; Castor bean vs Siberian elm: $U_{\text{FTM}} = 2.042$, $P = 0.041$).

Generally, in the presence of velvetleaf, walking distance, walking time, and walking speed of females were not lower than any other plant species. Females walked significantly remoter, longer, and faster in the presence of velvetleaf than in the presence of the main ovipositional host plant ($U_{\text{walking distance}} = 2.268$, $P = 0.023$; $U_{\text{walking time}} = 2.405$, $P = 0.016$; $U_{\text{walking speed}} = 2.117$, $P = 0.034$), and walked significantly longer than in the presence of castor bean ($U = 2.287$, $P = 0.022$). In contrast, two walking parameters reflecting orientation level of females elicited by the nonhost castor bean were significantly higher than those elicited by velvetleaf ($U_{\text{orientation}} = 3.694$, $P < 0.001$; $U_{\text{upwind length}} = 2.943$, $P = 0.003$) and peanut ($U_{\text{orientation}} = 2.609$,

Table 2. Between-plant comparison of flight and walking parameters of both sexes of *H. parallela* beetles

Sex	Parameter	Probability values of difference between-plant treatments					
		Castor bean vs Velvetleaf	Castor bean vs Peanut	Castor bean vs Siberian elm	Siberian elm vs Velvetleaf	Siberian elm vs Peanut	Peanut vs Velvetleaf
Female	Flight distance	0.716	0.068	0.901	0.682	0.130	0.147
	Flight time	0.923	0.04 (>)	0.710	0.725	0.067	0.049
	Flight speed	0.627	0.582	0.642	0.349	0.260	0.935
	FSM	0.190	0.213	0.238	0.770	0.778	0.827
	FTM	0.846	0.087	0.535	0.349	0.067	0.139
	FDM	0.610	0.034 (>)	0.756	0.320	0.045 (>)	0.125
Male	Flight distance	0.018 (>)	0.018 (>)	0.260	0.042 (>)	0.239	0.670
	Flight time	0.002 (>)	0.050	0.078	0.124	0.970	0.118
	Flight speed	0.391	0.624	0.193	0.601	0.057	0.135
	FSM	0.156	0.060	0.342	0.295	0.063	0.355
	FTM	0.006 (>)	0.009 (>)	0.041 (>)	0.389	0.790	0.570
	FDM	0.041 (>)	0.050	0.398	0.230	0.254	0.618
Female	Walking distance	0.151	0.821	0.734	0.290	0.199	0.023 (<)
	Walking time	0.022 (<)	0.596	0.095	0.157	0.111	0.016 (<)
	Walking speed	0.821	0.131	0.174	0.226	0.821	0.034 (<)
	Straightness	0.290	0.705	0.791	0.326	0.257	0.049
	Orientation	0.000 (>)	0.009 (>)	0.074	0.002 (>)	0.054	0.555
	Upwind length	0.003 (>)	0.021 (>)	0.059	0.041 (>)	0.198	0.940
Male	Walking distance	0.290	0.545	0.019 (<)	0.364	0.010 (>)	0.174
	Walking time	0.732	0.139	0.171	0.074	0.006 (>)	0.239
	Walking speed	0.028 (<)	0.082	0.016 (<)	0.821	0.496	0.450
	Straightness	0.131	1.000	0.007 (>)	0.650	0.082	0.174
	Orientation	0.593	0.620	0.675	0.649	0.879	0.704
	Upwind length	0.121	0.496	0.734	0.140	0.406	0.597

'>' or '<' in the parentheses followed by the probability values in the table indicate significantly higher or lower order corresponding to the comparison options in the header, and probability values without symbols in parentheses indicate no significant difference between corresponding plant treatments. Tested by Mann–Whitney *U* tests.

$P = 0.009$; $U_{\text{upwind length}} = 2.306$, $P = 0.021$). Orientation and upwind length elicited by Siberian elm were also significantly stronger than those elicited by velvetleaf ($U_{\text{orientation}} = 3.072$, $P = 0.002$; $U_{\text{upwind length}} = 2.041$, $P = 0.041$). These two parameters between the nonhost plant (castor bean) and the most preferred adult host plant (Siberian elm) did not differ significantly ($U_{\text{orientation}} = 1.787$, $P = 0.074$; $U_{\text{upwind length}} = 1.891$, $P = 0.059$). Males walked significantly remoter and faster in the presence of Siberian elm than in the presence of castor bean ($U_{\text{walking distance}} = 2.343$, $P = 0.019$; $U_{\text{walking speed}} = 2.42$, $P = 0.016$), and significantly remoter and longer than in the presence of peanut ($U_{\text{walking distance}} = 2.57$, $P = 0.010$; $U_{\text{walking time}} = 2.76$, $P = 0.006$). Walking speed of males in the presence of velvetleaf was significantly higher than that in the presence of castor bean ($U = 2.192$, $P = 0.028$). Orientation and upwind length of males did not differ between any compared plant treatments.

Discussion

In this study, we found that the body weights of *H. parallela* beetles were not related to mating success, regardless of sex. Generally, female fecundity in insects shows a strong positive relationship to adult body mass, and large body size of males can also be an advantageous attribute in mating (for a review, see Nylín and Gotthard 1998). Mate choice of *H. parallela* beetles was independent of body weight, suggesting that this nocturnal species may use other cues such as sex pheromone production as the indicator of a favorable mate.

Flight proportion of selected females was dramatically decreased when compared with selected males, nonselected females, and nonselected males. The decrease in the flight performance of *H. parallela* females after mating is similar to that of *Agrotis segetum*

(Guo et al. 2016) and *Phyllophaga cuyabana* (Oliveira and Garcia 2003), but contrary to a Coleoptera species, *Agrilus planipennis* (Wang et al. 2015) and a noctuid moth, *Autographa nigrisigna* (Hashiyama et al. 2013). This may be explained by two hypotheses. First, inactive females may be more easily selected as mates; second, mated females physiologically did not fly immediately after mating, as they may rest a short time for ovipositional preparedness and then fly into host plant fields in a different habitat. These two hypotheses are both somewhat resistant to testing. Here, we separated the mated beetles from virgin beetles in a free-mating situation and subsequently tested their flight performance, rather than comparing the flight capacity of the same female batches before and after mating. Alternatively, if we tested the flight capacity prior to testing mate choice, then the higher mating rate of nonactive fliers might be explained by two confounding effects: 1) motionless females may indicate a readiness to obtain a mate; and 2) they are more likely to mate just because they saved energy in previous flight bioassay.

Although body weight did not influence mate choice chance and flight performance of virgin males, it significantly influenced its flight performance after mating with a female, suggesting that heavier males invested more energy in copulation. Brown et al. (2017) reported that smaller beetles of *Batocera rufomaculata* have proportionally larger flight muscles and thus are better equipped to fly longer distances. However, this does not rationally explain our results, because the body weights of nonactive individuals and active fliers did not differ significantly in virgin males. We used body weight rather than elytra length as a potential trait affecting the mating choice of *H. parallela*, as it is a nocturnal species. However, elytra length maybe an important trait affecting flight performance. The

effects of elytra length on flight performance and mating success in *H. parallela* should be elucidated in the near future.

In the presence of difference plant treatments, all the flight parameters of females showing significant difference were detected in the comparisons between peanut and the other treatments (Siberian elm, castor bean, and velvetleaf). Moreover, flight response elicited by peanut treatment was always weaker than the other treatments. This may be explained by arrestment effect, that is, peanut may elicit landing of flying females after perceiving the volatiles emitted from this suitable ovipositional host plant. Flying females spinning around the flight mill may stop more frequently in an effort to recapture the cues from peanut chemostimuli. Similar result was obtained from the larvae of *Laspeyresia pomonella*, who showed faster walking at elevated levels of alpha-farnesene, but arrestment in the presence of apple extract (Hughes et al. 2003). In addition, all the flight parameters of both sexes produced by the nonhost plant, castor bean, were not lower than any other treatments, and this response pattern was more pronounced in males than in females. Flight parameters elicited by different plants may have no relation to their attractiveness, since that plant cues were nondirectional to a test beetle on the flight mill.

Bradley and Suckling (1995) classified the walking parameters of codling moth larvae into activity factors (such as walking distance, walking speed, and walking time) and orientation factors (such as orientation, upwind length, and path straightness). For the three walking activity factors (distance, time, and speed), velvetleaf elicited the strongest response in females, and Siberian elm elicited the strongest response in males, followed by velvetleaf. We believe that the most relevant parameters reflecting the motivation of scarabs to locate an odor source may be upwind length and orientation. The former is the net vector length along the line between the original releasing position of a beetle and an odor source, and the latter reflects the locating accuracy of a beetle in an odor plume. Interestingly, the most preferred adult host, Siberian elm, and the nonhost plant, castor bean, produced the highest and equal values of these two orientation factors in females. This result agrees with the approaching preference rank tested using a Y-typed olfactometer previously reported by our group (Zhang et al. 2018), suggesting that the motivation of *H. parallela* beetles walking toward a nonhost plant (i.e., castor bean) was at least equal to the most preferable food plant, the Siberian elm. Interestingly, the leaves of castor bean and Siberian elm share a major component (*cis*-3-hexenyl acetate) in their head-space (Sun and Lu 2006, Sun et al. 2008), suggesting that the chemical similarity hypothesis (Thompson 1988, Janz et al. 2009) could explain this phenomenon. In a peanut field in Qingdao, China, traps baited with synthetic sex pheromone of *H. parallela* beetles in combination with *cis*-3-hexenyl acetate caught significantly more beetles than the traps baited with sex pheromone alone (Ju et al. 2017). Recently, we also reported that castor bean plants may be an attractive yet risky mating site for *H. parallela* (Zhang et al. 2018). Now it seems that we can also not exclude feeding attraction of *H. parallela* adults to castor bean, although the toxins contained in its leaves impeded subsequent feeding, since that *cis*-3-hexenyl acetate is a ubiquitous green leaf volatile present in a number of food plants of various leaf-feeding beetles, including scarabs (e.g., Leal et al. 1994, Peacock et al. 2001, Cossé et al. 2006, Szendrei et al. 2011, Zhang et al. 2011, Sun et al. 2012). The parsimonious versatility of semi-chemicals may thus be broader in nature than previously thought, strongly suggesting that the biological significance of a potential semi-chemical obtained from separate investigations of feeding, oviposition, or mating should, at best, be applied with caution.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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Disclosure Statement

The authors have no competing financial interests to declare.

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