

ORIGINAL ARTICLE

JumpDetector: An automated monitoring equipment for the locomotion of jumping insects

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Abstract Continuous jumping behavior, a kind of endurance locomotion, plays important roles in insect ecological adaption and survival. However, the methods used for the efficient evaluation of insect jumping behavior remain largely lacking. Here, we developed a locomotion detection system named JumpDetector with automatic trajectory tracking and data analysis to evaluate the jumping of insects. This automated system exhibits more accurate, efficient, and adjustable performance than manual methods. By using this automatic system, we characterized a gradually declining pattern of continuous jumping behavior in 4th-instar nymphs of the migratory locust. We found that locusts in their gregarious phase outperformed locusts in their solitary phase in the endurance jumping locomotion. Therefore, the JumpDetector could be widely used in jumping behavior and endurance locomotion measurement.

Key words bio-artificial interaction; endurance locomotion; EthoVision; jumping behavior; *Locusta migratoria*

Introduction

Endurance locomotion, which is characterized by the extreme and persistent state of movement (Joyner & Coyle, 2008; Kenney *et al.*, 2011), is essential for insects' environmental adaptation and survival, such as long-distance migration (Pedgley *et al.*, 1995), predator avoidance (Kirkton & Harrison, 2006), and foraging (Lighton *et al.*, 1987). Endurance locomotion generally occurs under forced conditions by means of continuous external stimuli for a long period of time (Lamou *et al.*, 2016). Thus, endurance locomotion can reflect body tolerance, exhaustion, or ultimate movement capacity (Jenni-Eiermann *et al.*, 2002; Joyner & Coyle, 2008; Mach & Fuster-Botella, 2017).

In animal behavioral studies (e.g., including researches that investigate animal endurance locomotion), the desired

display is usually triggered by external stimuli produced from an artificial system, in which specialized engines or devices were generally embedded to interact with animals (Webb, 2000; Krause *et al.*, 2011). With the help of these artificial interactors, locomotion has been extensively explored in many species (Romano *et al.*, 2018); examples include forced wheel running (Chen *et al.*, 2014) and treadmill running (Moraska *et al.*, 2000) for vertebrates' walking, wind tunnel (Nebel *et al.*, 2012) and swim tunnel devices (van den Thillart *et al.*, 2004) for birds flying and fish swimming. For insects with various movement strategies, such as crawling, jumping, or flying (Sane, 2003; Burrows & Sutton, 2012; Gunther *et al.*, 2016), the strategy for insect-artificial interaction is complicated, such as the arena for crawling (Roessingh *et al.*, 1993) and flight mill for flying (Attisano *et al.*, 2015). Recently, the predator-mimicking robot has also been applied for interacting with insects' jumping escape (Romano *et al.*, 2017). Although many systems have been developed to investigate successfully a number of behaviors in different animal species, a dedicated system to trigger jump behavior to test the endurance locomotion remains unavailable.

The migratory locust, *Locusta migratoria*, is a worldwide agricultural pest with large aggregation and

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long-distance migration (Wang *et al.*, 2014). In response to the changes in population density, locusts can develop distinct ecological phases, for example, gregarious and solitary phases, which exhibit remarkable locomotor plasticity (Pener & Simpson, 2009; Wang & Kang, 2014). Locusts have a typical jumping behavior and adopt a catapult mechanism, in which the extensor tibiae muscles of the hind legs contract to store energy in distortions of the exoskeleton, and then suddenly release these stores to power the hind legs' rapid movement (Sutton & Burrows, 2008; Burrows, 2016). The jumping behavior can help adult locusts to escape danger or initiate flight (Kutsch & Fuchs, 2008; Burrows, 2016). Jumping is the main form of locomotion for juvenile locusts with low power output and high endurance as a marathoner (Kirkton & Harrison, 2006). The difference of velocity performance in a single jump has been reported between solitary and gregarious locusts (Rogers *et al.*, 2016). The properties, kinematics, influencing factors, and related physiological mechanisms of the locusts' jumping behavior have been extensively investigated (Bennet-Clark, 1975; Snelling *et al.*, 2011; Beck *et al.*, 2017) by using various manual methods or traditional observations. For example, visual stimulation from moving objects (Romano *et al.*, 2019) and tactile stimulation from a paintbrush (Kirkton *et al.*, 2005) were used to elicit locust jump. High-speed cameras or audiotapes have been used to record the location of the locust jump (Sutton & Burrows, 2008; Kirkton *et al.*, 2012). However, an automatic method to elicit the jumping behavior for biomechanics investigation is still unavailable and this makes the investigation of jumping behavior difficult.

In this study, to develop a universal method for the detection of insect jumping behavior, we designed detection equipment with automatic video tracking of the trajectory and recording of data. We further evaluated the effectiveness of the system in detecting the jumping behavior of the migratory locust and efficiently characterized the traits of endurance jumping in locust nymphs. Our study provides an automatic method for monitoring the jumping behavior and endurance locomotion.

Materials and methods

Method overview

A detection equipment named JumpDetector was designed to investigate the jumping behavior of insects under forced conditions. Our equipment could trigger the continuous jumping behavior of insects in custom-designed devices, namely, forced device, through an

electric motor-driven plastic rotating brush. The locomotion processes of insects were recorded by using a camera with related softwares, and video data from the jumping insects were further analyzed for trajectory tracking by using EthoVision. The locomotor parameters, namely, total frequency and accumulated jumping distance within an observation period, were recorded by using personalized computerized script (J-Recorder, Supplementary file **Script 1**).

Main components and configurations of the detection equipment

An image of the JumpDetector is shown in Figure 1A. This equipment was composed of four parts: a forced system, a video recording system, a behavior tracking system, and a data recording system (Fig. S1). The forced system included a group of forced devices, a variable voltage power supply (Shenzhen Pin-Cheng Motor Co., Ltd., Shenzhen, China), and a central controller (Shenzhen Pin-Cheng Motor Co., Ltd., Shenzhen, China). The video recording system comprised a digital video recorder (HDR-CX760E, Sony, Tokyo, Japan) and video capture software (Corel Video Studio) installed in a computer. The digital video recorder configuration should exceed 1080p and 50 fps to ensure high video resolution, and the distance between the digital video recorder and the forced devices was kept in the range of 600–800 mm to achieve a high video quality. An EthoVision XT behavior tracking system (Noldus Information Technology, Wageningen, Netherlands) was used to track the trajectory of the jumping insects. Data were preliminarily recorded by using EthoVision XT. Then the jumping events were automatically recognized, and the parameters were computed in J-Recorder. For a successful data collection and analysis, the computer configuration was as follows: Intel (R) Core (TM) i5-7200U @2.50 GHz CPU, 8.00 GB memory, and 1 T hard disk. A filament lamp (22 W, 4100 K, white reflector) was used as an accessory component of the forced system or the video recording system to fabricate homogeneous and bright illumination conditions and thus to obtain clear video data.

Forced device structure

The forced device was the core part of the JumpDetector. Each forced device comprised a cylindrical chamber called the forced chamber, a base, an electric motor, and a rotating brush (Fig. 1B). The forced chamber was a transparent glass material with a diameter of 50 mm and a height of 150 mm to allow the insects to jump. It also

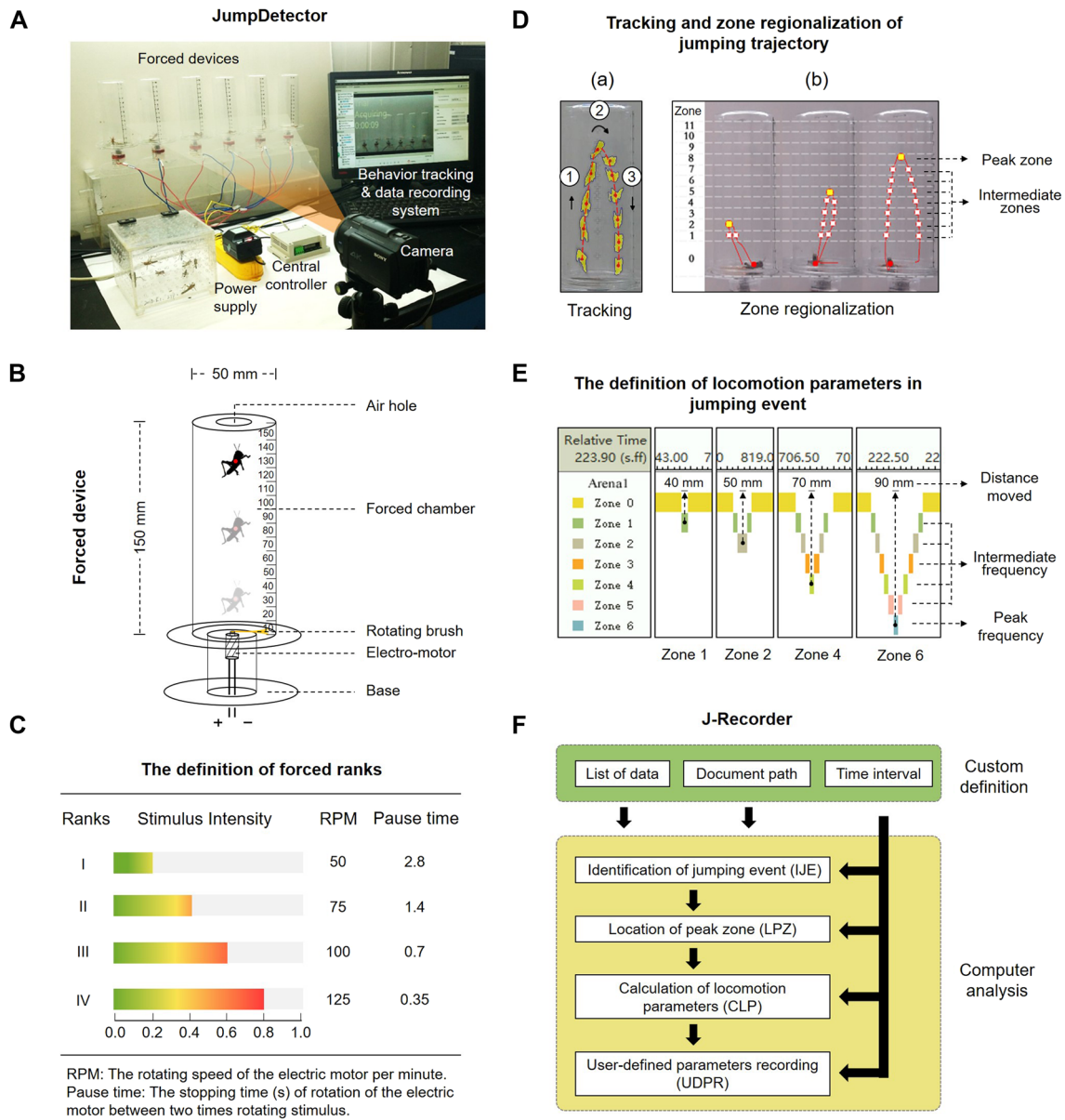


Fig. 1 Schematic and detection method of JumpDetector. (A) Image of JumpDetector. (B) Structural composition of a forced device. (C) Forced stimulus intensities provided by our detection platform and defined as forced ranks from I to IV. (D) Tracking and zone regionalization of jumping trajectory, (a): trajectory tracking (1: upward, 2: peak, 3: downward), (b): zone regionalization of jumping trajectory (yellow circle represents the peak zone; white circle displays the intermediate zones). The red line indicates the jumping trajectory, and the red spot represents the center of the recognized target). (E) Definition of locomotion parameters in a jumping event (the frequency in the peak zone (peak frequency) indicates the frequency of movement, and the height of the peak zone shows the distance moved). (F) Flowchart demonstrating the data recording process by using an automatic script J-Recorder.

had a hole with a diameter of 15 mm at the top to ensure air circulation. The base of the forced device was made of plastic and had an electric motor fixed at the center. The plastic rotating brush was fixed to the motor shaft to produce stimulation when the motor was rotating. The

power lines of the electric motor in each forced device were connected to the central controller for unified control. The forced chamber and the base could be separated freely from each other to ensure the input or removal of insects.

Methods of controlling the forced devices

The electric motor in the forced devices was controlled by a variable voltage power supply and a central controller. The variable voltage of the power supply could be adjusted manually, and the electric motor could be controlled at different rotating speeds, such as 50, 75, 100, 125 r/min. The central controller could regulate the rotation mode of the electric motor by producing different signals of rotation direction, rotation time, and pause time. The frequently used mode was set on the LED screen of the central controller. The variable voltage power supply and the central controller were set to cooperate with each other, producing different stimulus intensities, which were defined as different forced ranks (Fig. 1C).

Tracking and zone regionalization of jumping trajectory in EthoVision

For the successful trajectory tracking of jumping behavior, the EthoVision system was equipped with accurate identification and optimized tracking methods. Target discrimination and tracking methods were chosen on the basis of background noise and signal loss (Fig. S2, Movie S1). Color mode was recommended to display different signals for target recognition. For example, the yellow area was defined as the recognition region of the target object, the blue line indicated the silhouette of the target object, and the orange regions corresponded to the noise signals (Fig. S2, Movie S1).

Individualized arena and active zones were defined to quantify trajectory tracking. An arena was created in the jumping region of each forced chamber, and each arena was divided into multi-zones (i.e., one zone per centimeter of height) in the vertical direction. Each zone represented the insect location as it was jumping, and the zones were defined in terms of the height reached by the insects jumping in the forced chamber.

The tracked location of the insects sequentially appeared in three stages, namely, upward, peak, and downward, during a typical jumping behavior (Fig. 1D). Zone regionalization was conducted for the tracked trajectory at the three stages to characterize the jumping events. The zone located at the upward or downward stage was defined as the intermediate zone of the jumping events, and the zone located at the peak stage was defined as the peak zone (Fig. 1D). EthoVision XT could track the trajectories of a jumping insect and record the zones of the trajectory passed in every jumping event. The zone regionalization of the trajectories substantially facilitated the definition of locomotion parameters and the recording of data.

Definition and automatic data recording of the locomotion parameters

Each passed zone of the jumping trajectory could be recorded as the frequency moved in a typical jumping event. The frequency of the peak zone was defined as the peak frequency, while the frequency of the intermediate zone was denoted as the intermediate frequency. Thus, only one peak frequency and more than one pair of intermediate frequencies were generally produced in every jumping event. The peak frequency was used to represent the frequency of movement in every jumping event, and the height of the related peak zone was used as the distance moved (Fig. 1E). Multiple jumping events produced numerous movement frequencies during an observation period, and the total number of frequencies was defined as the total frequency of movement (TFM). Similarly, the sum of the distances moved was defined as the total distance moved (TDM). TFM and TDM were used as primary parameters to illustrate the locomotor ability of the tested insect in a forced environment.

To evaluate and calculate TFM and TDM more efficiently, we developed a J-Recorder to distinguish jumping events and calculate locomotor parameters. J-Recorder was a script in R language and composed of four steps: identification of the jumping event (IJE), location of the peak zone (LPZ), calculation of the locomotion parameters (CLP), and user-defined parameters recording (UDPR) (Fig. 1F). In IJE and LPZ, all positive jumping events and the related peak zones were determined. In CLP, TFM and TDM were calculated for each jumping event. In UDPR, the locomotion parameters were identified and exported in a custom-defined manner (e.g., individualized time interval). J-Recorder was fully automated and used for batch data processing. Hence, this equipment was convenient and efficient for high-throughput data analysis.

Experimental procedures of locomotion detection

The detection method consisted of three major steps, namely, preparation, testing, and post-processing. In the preparation step, the candidate insects were prepared and reared at 22 W light and 30 ± 2 °C. The software related to the equipment was properly installed and subjected to preliminary tests. In accordance with the requirement, the working mode of the motor was set to generate a proper forced stimulus. And then the time of video recording, data format, and other parameters of the video files were also set. In the testing step, the candidate individuals were placed into one of the six forced chambers, and the motor was switched on to force the insects to continuously jump.

Simultaneously, the video recording system was used to record the video data of the insects, and the video files were stored in a computer. In the post-processing step, the video files were input into EthoVision XT and the trajectory of the jumping insects was tracked. The locomotion parameters of the jumping behavior were analyzed with J-Recorder on the basis of the trajectory data.

Throughput and extensibility of the detection platform

The tested throughput could be changed in the number of the forced devices and the distance between the digital video recorder and the forced devices. An increase in throughput was required to combine the video quality and workload capacity of the central controller because a high throughput would lead to a low resolution of individual objects in the video data and a high load for the central controller. The equipment was operated in a medium-throughput mode consisting of six forced devices. The height and diameter of the forced chambers or the type of rotating brushes could be changed for large individuals or for other insect species.

Insect husbandry

The migratory locusts used in the experiments were obtained from laboratory colonies as previously reported (Kang *et al.*, 2004), and were reared in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China. Gregarious locusts were cultured in large, well-ventilated, cages (400 × 400 × 400 mm) with a density of 500–1000 individuals per cage. Solitary locusts were cultured by rearing gregarious locusts under physical, visual, and olfactory isolation. These conditions were achieved by rearing locusts alone in a ventilated cage (100 × 100 × 250 mm) supplied with charcoal-filtered compressed air. Both colonies were fed with a diet consisting of fresh wheat seedlings and bran in a 14 h light:10 h dark cycle at 30 ± 2 °C. Two phase locusts were used for the behavioral experiment at day 3 of the 4th-instar nymphs which were characterized by the wing buds flip and the inner surface becoming the outer surface (Baker, 1984). This remarkable feature of wing buds was different from the spade shape in the 3rd-instar nymphs and the sufficient length in 5th-instar nymphs in which the wing buds were about 1/2 the length of the abdomen and obscure tympanic organ. When 4th-instar nymphs were newly molted, their thoraxes were marked with a colored pen.

Statistical analysis

Student's *t*-test was used to compare the locomotor abilities of two locust groups, namely, forced (with

forced conditions) and control (without forced conditions) groups. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was adopted for the multigroup comparisons of the locomotor ability data detected at different time intervals or under different forced conditions. Repeated measures ANOVA was used to compare the locomotor abilities between gregarious and solitary locusts with different time intervals. The values from each independent experiment were presented as mean ± standard error (SEM). Differences were considered significant at $P < 0.05$. Data were analyzed by using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Evaluation of the forced device effectiveness and safety

A device was designed to trigger the continuous jumping behavior of the insects in a cylindrical chamber via an external stimulus, which was produced by a stepper motor. Juvenile locusts were used as the sample in the test. When the motor started to rotate at a definite rotation rate (100 r/min, pause time 0.7 s), the locusts as the study subjects started to continuously jump (Movie S2), and the average jumping frequency was 19.5 times ($n = 30$) within the forced time of 60 s. By contrast, the locusts jumped 0.5 times ($n = 25$) in the control group without motor rotation (Student's *t*-test, $P < 0.001$). Therefore, the forced device was effective in inducing the continuous jumping of the locusts. These insects also had unharmed limbs or bodies after they underwent a high-intensity exercise under the forced conditions, displayed normal behavior, and rapidly escaped from the stick stimulus (Movie S3). These results indicated that the device was efficient and noninvasive for detecting the jumping behavior of insects.

Accuracy and efficiency of automatic video tracking and data recording of JumpDetector

The jumping behavior of many insects occurred rapidly, continuously, and repetitively under the forced conditions. Consequently, the manual scoring of the locomotor parameters was difficult and time consuming. As such, automatic video tracking was adopted by using EthoVision, and data were recorded with J-Recorder. When the candidate locusts as an example were tested, our results revealed that the automatic method excellently agreed with the manual operation. TFM exhibited a correlation coefficient of 0.99, a bias of 0.5, and a SEM of 0.82, and TDM showed a correlation coefficient of 0.98, a bias of 9.8, and a SEM of 0.58 (Fig. 2A). This automatic counting was

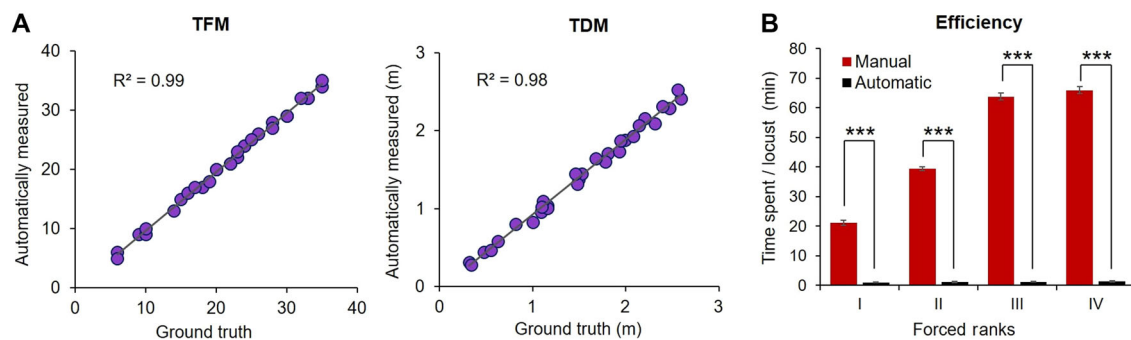


Fig. 2 Accuracy and efficiency of the automatic video tracking and data recording methods. (A) Comparison of TFM and TDM between the automatic method and the ground truth for each locust in 1 min movies ($n = 30$). (B) Comparison of the efficiency between the automatic and manual operations. TFM, total frequency of movement; TDM, total distance moved. *indicates a significant difference between automatic method and manual operation (Student's t -test, *** $P < 0.001$).

very close to the base truth both in large and small values. In terms of efficiency, the automated video tracking method saved a considerable amount of time. At least 20–60 min was necessary to score the locomotion parameters manually for each locust movement in a 10 min movie. Even the time spent was extended for scoring under the enhanced intensity of the forced stimulus. By contrast, only 1–2 min was required to complete the detection through the automatic analysis system, thereby increasing the efficiency by 20- to 50-fold (Student's t -test, $P < 0.001$, Fig. 2B). Therefore, the automatic video tracking and data recording method of JumpDetector performed excellently, eliminating the need for difficult manual labor.

Adjustable performance of JumpDetector

The performance of this equipment was further evaluated under the adjustable intensity of the forced stimulus. The locusts continuously jumped under different forced stimuli compared with those in the control group. TFM and TDM significantly increased as the forced rank increased from I to III (one-way ANOVA, $P < 0.05$) but stopped increasing at forced rank IV (Fig. 3A). Activity thermography analysis revealed that the covered area of the movement widened, when the forced ranks increased (Fig. 3B). Therefore, the proposed equipment was competent under the adjustable intensity of the forced stimulus. By using this adjustable stimulus, we found that there was the same optimal forced condition with 100 r/min of rotating speed and 0.7 s pause time of rotating motor for continuous jumping locomotion in 3rd, 4th and 5th-instar nymphs of migratory locusts (Fig. S3-S4).

The performance of JumpDetector was evaluated with different forced times under the unified forced stimulus of rank III. The TFM and TDM of the locusts significantly

increased with prolonged time (one-way ANOVA, $P < 0.05$). By contrast, the locusts revealed an unusual jumping behavior in the absence of the forced stimuli (one-way ANOVA, $P = 0.87$, Fig. 3C). Thus, the detection equipment was suitable for monitoring the jumping behavior in different observation durations.

Characterization of the endurance locomotion of locust nymphs

The locomotion ability of the 4th-instar nymphs of the migratory locusts was observed under forced rank III for 1 h, and the dynamic pattern of the locomotion parameters was characterized via sliding window analysis with 15, 10, 5, and 1 min time intervals. At the window size ranging from 15 min to 5 min, TFM and TDM gradually decreased after the first time interval (Fig. 4A). When the window size of the time interval decreased to 1 min, the locomotion parameters per time unit revealed that the average TFM and TDM could reach 20 times and 1.3 m at the start of 1 min, respectively. Both values gradually decreased over time, and the downward trend indicated a remarkable periodicity from fast to slow (Fig. 4B). This tendency appeared sequentially at three stages, that is, drastic (stage I: 0–10 min), continued (stage II: 10–30 min), and slow (stage III: 30–60 min) decline. Therefore, the endurance jumping locomotion of the locust nymphs was characterized by the gradual decline with fast-to-slow in TFM and TDM (Fig. 4B).

Different performances of endurance locomotion between gregarious and solitary locusts

We measured the endurance locomotion of gregarious and solitary locusts by using the JumpDetector system.

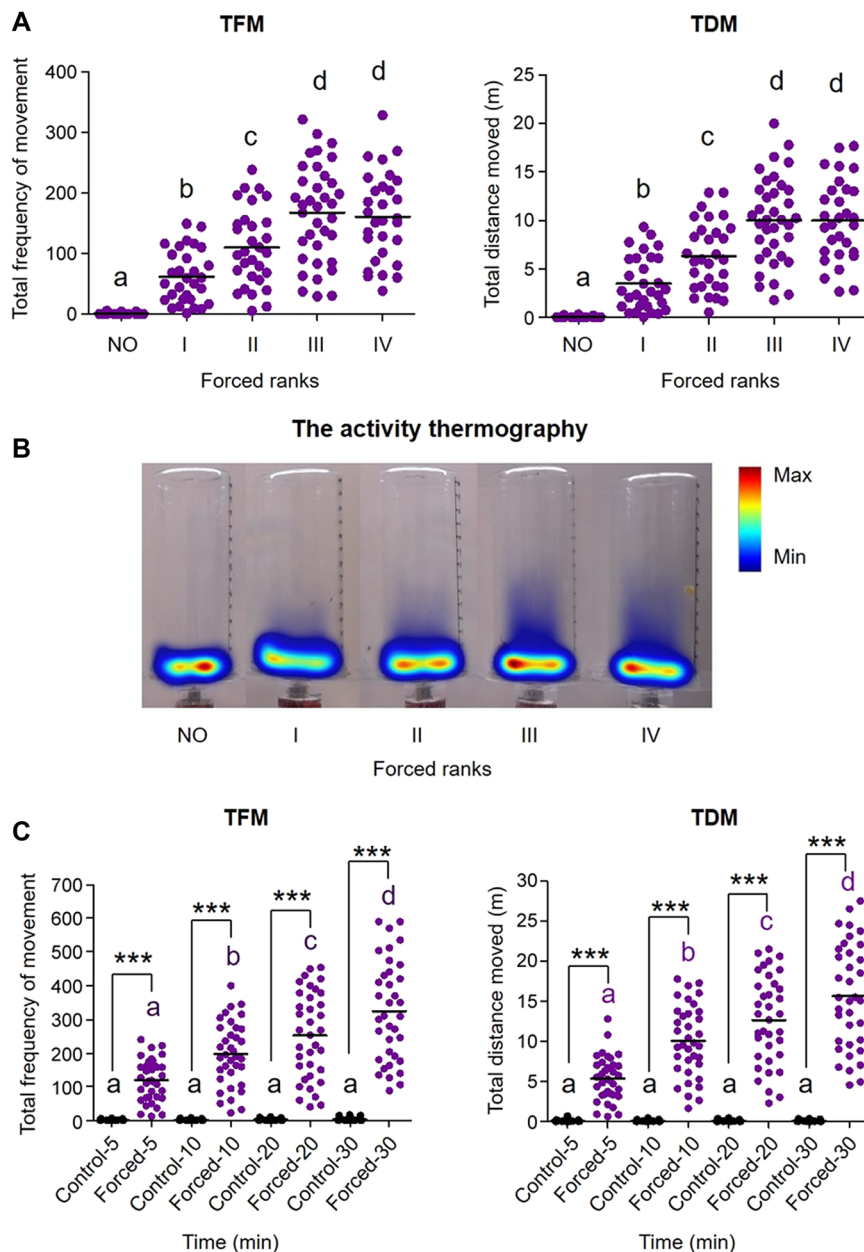


Fig. 3 Performance evaluation of JumpDetector. (A) TFM and TDM significantly increased as the forced rank increased from I to III and stopped increasing at forced rank IV (within 10 min forced movement). NO indicates group without forced conditions. (B) Activity thermography analysis showed the increasing tendency of the moving area and the increasing forced rank from I to III. (C) TFM and TDM in the forced group significantly increased over time (30 min) compared with those of the control group. Significant differences at different points are denoted by letters with different colors ($n = 31$ to 36 animals per point, one-way analysis of variance (ANOVA), $P < 0.05$). *indicates significant difference between “Control” (without forced conditions) and “Forced” (with forced conditions) group locusts (Student’s t -test, $***P < 0.001$). TFM, total frequency of movement; TDM, total distance moved.

The results showed that both TFM and TDM values were significantly higher in gregarious locusts than those in solitary locusts within 1 h of forced movement (Student’s t -test, $P < 0.01$, Fig. 5A–B). We further analyzed TFM

and TDM values at 1 min time intervals to explore dynamic patterns of locomotion parameters between the two locust phases. We found that both values displayed a gradually decreasing pattern in both locust phases and were

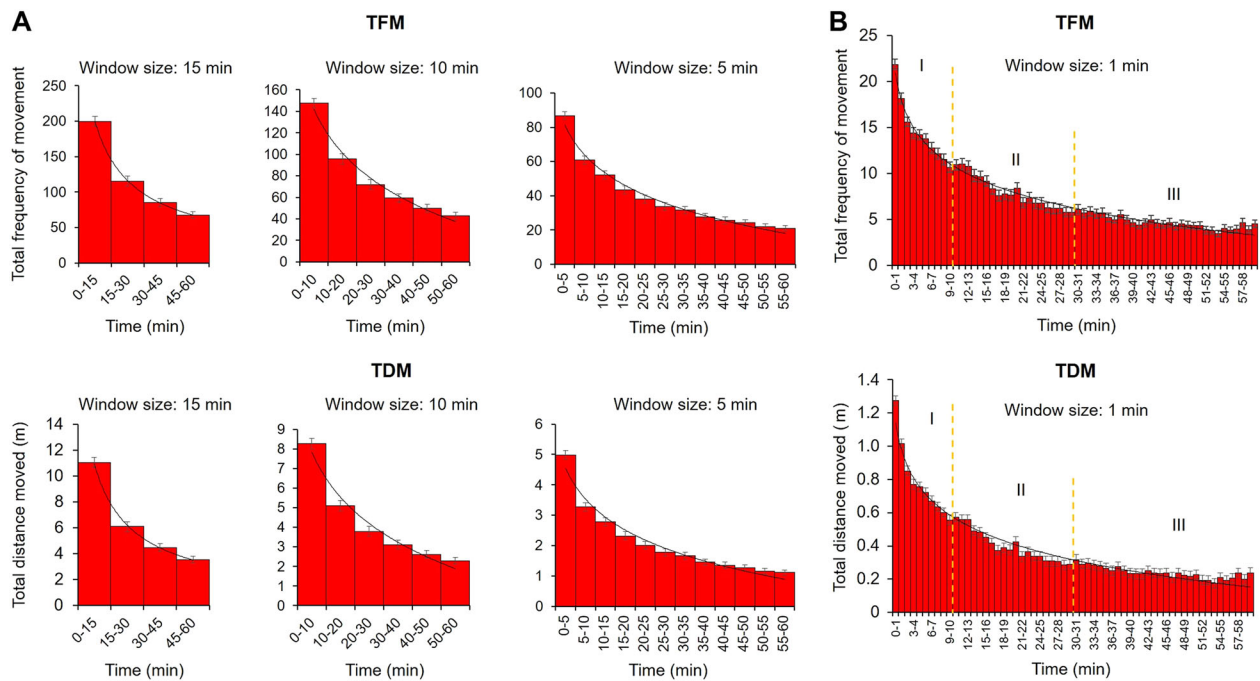


Fig. 4 Characterization of the endurance locomotion of 4th-instar locust nymphs. (A) Dynamic patterns of TFM and TDM gradually declined after the first time interval under different window sizes at the 15, 10, and 5 min time intervals. (B) Dynamic patterns of TFM and TDM gradually decreased with drastic (stage I: 0–10 min), continued (stage II: 10–30 min), and slow (stage III: 30–60 min) decline under the window size at 1 min. TFM, total frequency of movement; TDM, total distance moved.

significantly higher in gregarious locusts than in solitary locusts at each time interval (repeated measures ANOVA, $P < 0.01$). These results indicated that gregarious locusts have a high endurance locomotion ability.

Discussion

The novel equipment called JumpDetector was developed with automatic monitoring and data recording functions to measure the jumping behavior of insects. This device exhibited a high-throughput ability to detect continuous jumping behavior because it was designed to simultaneously monitor multiple individuals with one camera. In previous studies, several traditional methods, including the numbered grid system (Kirkton *et al.*, 2005), high-speed camera (Burrows & Morris, 2003), and cylindrical device (Kral, 2008), are used to investigate the properties and mechanisms underlying the jumping behavior of insects. However, these operations and interactive methods are time consuming or lacking in precision for behavioral detection. Recently, some scientists adopted a video to record jumping behavior, but the stimulation triggering jumping was still manually operated (Gvirsman *et al.*, 2016). Our equipment addressed the shortcomings of

these methods through automatic monitoring and precise stimulus control. Our device could also support parameter adjustment and dynamic analysis with automatic video tracking and was also cost-effective because it is easy to reproduce and manufacture by using spectral materials.

Our device could provide an adjustable stimulus in intensity and time by changing the speed and rotation mode of the electric motor. This adjustability could also induce the corresponding changes in the frequency and distance of the jumping behavior. Two parameters describing locomotor characteristics, namely, TFM and TDM, could be obtained through adjustable stimulation. The rotating motor used in our study was convenient for optimizing stimulus conditions by adjusting the speed and rotation mode, which are similar to the belt speed and incline of the forced treadmill (Lerman *et al.*, 2002; Cook *et al.*, 2013). Our equipment could accurately describe the locomotor characteristics through adjustable stimulation to determine the jumping behavior by optimizing the forced conditions (Fig. S3–S4).

The precision and efficiency in detecting jumping behavior were improved by developing an automated video tracking method. To the best of our knowledge, this study was the first to automatically detect the continuous jumping behavior of insects. Here, we used EthoVision

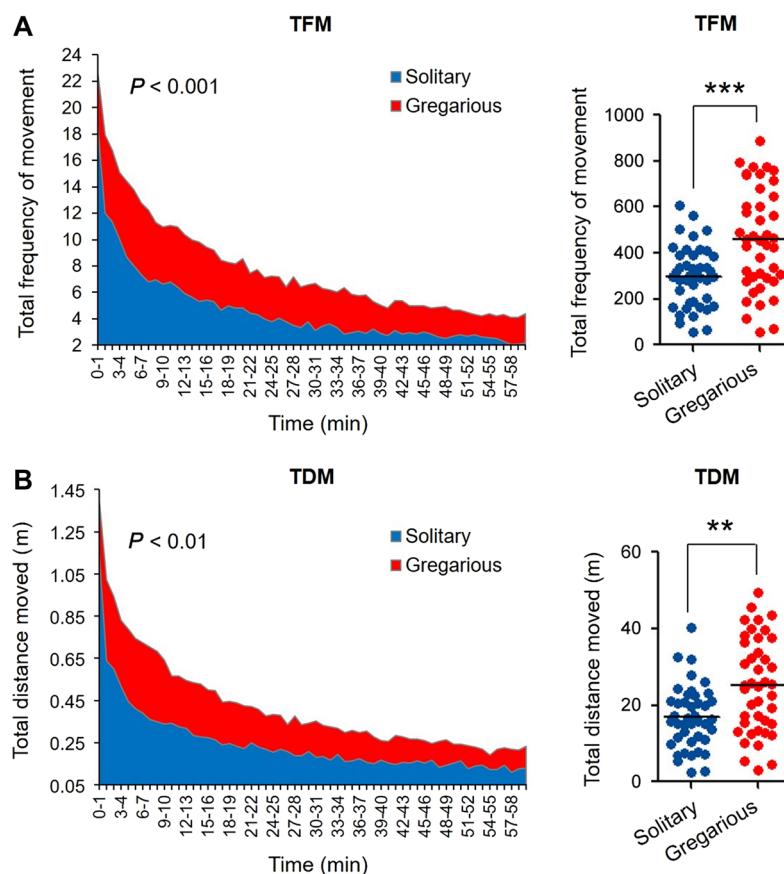


Fig. 5 Different performances of endurance locomotion between gregarious and solitary locusts. (A–B) Both TFM and TDM values displayed a gradually decreasing pattern in the two locust phases within 1 h forced movement. Both values were significantly higher in gregarious locusts than in solitary locusts at each time interval or total time. Repeated measures analysis of variance (ANOVA) is used to compare the locomotor abilities between the gregarious locusts and the solitary locusts at different time intervals ($n = 42$ animals per point, difference is considered significant at $P < 0.05$). *indicates a significant difference between solitary and gregarious locusts ($n = 42$ animals per group, Student's t -test, ** $P < 0.01$, *** $P < 0.001$). TFM, total frequency of movement; TDM, total distance moved.

XT for automatic detection. EthoVision is generally applied to track and analyze the locomotion behavior of animals (Noldus *et al.*, 2001). However, the tracking signals of jumping are easily lost because of the fast action of jumping. In our study, we accurately tracked continuous jumping locomotion by optimizing the frame rate of the video recording and tracking parameters. Although some difficulties in detecting small insects were met in using our tracking method because of resolution limitation, infrared technology will be tested in the future because of its sensitivity in probing small insects (Miller *et al.*, 2002).

We could provide two parameters, namely, TFM and TDM, to describe the jumping events accurately by developing an automatic script J-Recorder. Two parameters, the frequency and the distance moved, are widely adopted by many studies to describe locomotion ability (Roessingh

et al., 1993), including jumping behavior (Kirkton *et al.*, 2005; Zaitsev *et al.*, 2015). However, EthoVision cannot directly define these two parameters because of the continuity and repetition of jumping. Therefore, we developed the J-Recorder to recognize the jumping events and record TFM and TDM via time window analysis. Additional secondary parameters are still under development.

TFM and TDM of locust nymphs gradually decreased with a fast-to-slow trait by using JumpDetector, thereby indicating the uniqueness of their endurance jumping locomotion. The gradual decline in jumping frequency but not in the distance jumped has been reported in other studies (Kirkton & Harrison, 2006). However, the patterns of the mean distance jumped in Kirkton and Harrison's work are inconsistent with our findings (Fig. S5), possibly because of the difference in the applied intensities and frequencies of the forced stimuli. Kirkton and Harrison

(2006) adopted manual physical stimulation to induce locust jumping and defined a 30 s pause between subsequent jumps, which were much longer than the 0.7 s pause in our automatic stimulus method. The long stimulus pause time may have caused the easy recovery of locusts from fatigue. As such, the decline pattern of the mean distance jumped was undetected in previous studies. In the present study, we optimized the pause time of 0.7 s between two jumps to achieve continuous jumping. Thus, the observed decreasing tendency of distance jumped was reasonable.

Our results showed that, compared with the solitary locust nymphs, the gregarious individuals displayed higher frequency and total distance moved in continuous jumping behavior but decreased distance moved in the first jump (Fig. S6), indicating an improved performance of endurance locomotion in gregarious locusts. Obviously, two locust phases adopted alternative strategies of jumping behavior, representing a trade-off relationship. Solitary locusts have been reported to improve their performance in a single jump, but more time would be spent in preparation of energy storage for the next jump (Rogers *et al.*, 2016). The improved performance of jumping velocity is better for solitary locusts in escaping from predators. Meanwhile, gregarious locusts are skilled in continuous jumping with the cost of decreased performance in a single jump. The improved endurance in continuous jumping is indispensable for gregarious locusts in foraging, dispersing, and long-distance movement (Ellis, 1951; Kuitert & Connin, 1952). The strategic divergence of jumping behavior between the two locust phases suggested that the locusts' jumping locomotion is an ideal model system for exercise studies related to strength and endurance training.

In this study, we developed an artificial interaction system in which the designed device directly triggered insects' endurance jumping behavior. The visual stimulation from robotic predators had been employed to produce a non-direct contact interaction with locusts and elicit their jumping escape behavior (Romano *et al.*, 2017, 2019). However, in our system, the mechanical stimuli from a rotating motor directly interacted with tactile system of insects with most stability and persistence. By means of this interaction system, we found that gregarious locusts displayed a higher performance of endurance jumping locomotion than that of solitary ones. Recently, a robotic cockroach (Halloy *et al.*, 2007) or chicken (Gribovskiy *et al.*, 2018) had been employed to explore the population behavior of shelter selection or imprinting, respectively. Because gregarious locusts have a typical collective behavior (Yates *et al.*, 2009; Ariel & Ayali, 2015), we supposed that a group of endurance jumping robots would be

very useful for understanding the interaction with locust individuals and swarm formation.

Our device could considerably support the extensibility of the jumping behavior for diverse purposes and in various insect species. JumpDetector will be useful for the molecular screening of locomotor disorders (Beall *et al.*, 2013), anxiety-related behavior (Walf & Frye, 2007), and circadian rhythm (Pastore & Hood, 2013) in insects as model systems.

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Disclosure

The authors declare no competing or financial interests.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Main components of JumpDetector.

Figure S2. Target discrimination on the insect jumping behavior in EthoVision XT.

Figure S3. The optimization of stimulus conditions for continuous jumping locomotion in 3rd, 4th, and 5th-instar nymphs of migratory locusts.

Figure S4. Comparison of continuous jumping locomotion among 3rd, 4th, and 5th-instar nymphs of migratory locusts.

Figure S5. Mean distance moved of the endurance jumping locomotion for the locust 4th-instar nymphs.

Figure S6. Performances of distance moved in the first jump in gregarious and solitary locusts.

Movie S1. Trajectory tracking of insect jumping by using EthoVision XT.

Movie S2. Forced device of JumpDetector was effective in inducing the continuous jumping of insects.

Movie S3. JumpDetector showed noninvasive detecting for insect jumping behavior.

Script 1. J-Recorder and the data analysis method.