Video Article

Measuring the Flight Ability of the Ambrosia Beetle, *Platypus Quercivorus* (Murayama), Using a Low-Cost, Small, and Easily Constructed Flight Mill

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Abstract

The ambrosia beetle, *Platypus quercivorus* (Murayama), is the vector of a fungal pathogen that causes mass mortality of *Fagaceae* trees (Japanese oak wilt). Therefore, knowing the dispersal capacity may help inform trapping/tree removal efforts to prevent this disease more effectively. In this study, we measured the flight velocity and duration and estimated the flight distance of the beetle using a newly developed flight mill. The flight mill is low cost, small, and constructed using commonly available items. Both the flight mill arm and its vertical axis comprise a thin needle. A beetle specimen is glued to one tip of the arm using instant glue. The other tip is thick due to being covered with plastic, thus it facilitates the detection of rotations of the arm. The revolution of the arm is detected by a photo sensor mounted on an infrared LED, and is indicated by a change in the output voltage when the arm passed above the LED. The photo sensor is connected to a personal computer and the output voltage data are stored at a sampling rate of 1 kHz. By conducting experiments using this flight mill, we found that *P. quercivorus* can fly at least 27 km. Because our flight mill comprises cheap and small ordinary items, many flight mills can be prepared and used simultaneously in a small laboratory space. This enables experimenters to obtain a sufficient amount of data within a short period.

Video Link

The video component of this article can be found at https://www.jove.com/video/57468/

Introduction

Animals migrate long distances in search of food and mates. Migrating animals might sometimes carry undesirable companions. The female ambrosia beetle, *Platypus quercivorus* (Murayama), is a known vector of the fungal pathogen, *Raffaelea quercivora* Kubono et Shin-Ito. This pathogen causes mass mortality of *Fagaceae* trees (Japanese oak wilt) and a high level of mortality[1]. Since 1980, this disease has been expanding throughout Japan, and has become a serious problem[2].

P. quercivorus is a small insect (4-5 mm in body length and 4-6 mg in body weight), and yearly expansion of the disease suggests that they are capable of flying up to several km[3,4]. The male *P. quercivorus* locates a host tree and releases an aggregation pheromone that attracts both males and females[5]. Consequently, the host tree is mass attacked by conspecifics, and eventually dies. The male bores a tunnel inside the tree after landing and a pheromone-attracted female enters the tunnel and lays eggs. The hatched *P. quercivours* grow in the tunnel until they become adults. Adults emerge and disperse to locate new hosts. Thus, expansion of the disease is possibly related to the migratory ability of this beetle. However, the extent to which the beetle can fly is still unclear. In addition, females are bigger than males[6] (female: 4.6 mm, and male: 4.5 mm) and male beetles search for a target tree, enter the tunnel inside the tree, and then attract the female. Considering these sexual differences in the body size and role of flight in their life, sexual differences may exist in flight ability, but the difference in ability remains unclear.

In general, it is extremely difficult to measure migratory ability in the field, especially flight ability, due to the wide range of the migratory area. Migratory ability has been measured in laboratories under tethered conditions, such as a flight mill system, for over 60 years[7,8,9,10,11,12,13]. Flight mill systems have shown that some insects have the ability for long distance flight. For example, the longest flight distance of the mountain pine beetle in a flight mill was over 24 km[14], and *Tetrastichus planipennisi* Yang flew maximally over 7 km[15]. Although the flight mill is a commonly available tool, biological assays with a living animal often result in considerably large individual differences. To overcome this, many measurements, repeated multiple times, are required to obtain reliable estimates of mean dispersal capacity. Therefore, multiple individuals should be used at the same time for the quick collection of a sufficient amount of data. However, simultaneous experiments require a larger space, multiple experimental setups, and are costlier when compared to a single measuring system. Hence, the flight mill must be low cost, should be easily built with commonly available items, and compact in size. Furthermore, the experimental procedure should not be complicated or need a skillful operator.

In this study, we assembled a small, low-cost flight mill (**Figure 1** and **Figure 2**) that could be easily used in experimentation, and measured the flight ability of the ambrosia beetle, *P. quercivorus.*

Protocol

1. Construction of a Flight Mill

1. **Construction of a flight mill apparatus**
    1. Cut off the plastic part from a needle (metal part: 40 mm in length and 0.25 mm in diameter; plastic part: 22 mm in length and 2 mm in diameter) with nippers (**Figure 3**).
    2. Fix this needle with an untreated needle in the shape of a cross with epoxy resin adhesive (**Figure 3**), referring them as a flight mill arm and an axial needle.
    NOTE: For an axial needle, the untreated side should be a bottom side. The uncovered tip of the flight mill arm is for gluing a beetle (**Figure 1B** and **Figure 3**).
2. **Construction of the base**
    1. Make a small dimple on the surface of a thin stainless metal plate (5 cm x 5 cm) by hammering a nail to prevent the axial needle from sliding horizontally (**Figure 4**).
    NOTE: The actual dimensions of the metal plate are not critical, and another material is possible, but avoid using any soft material; otherwise, the needle will be stuck, preventing the mill from revolving.
    2. Place and fix the metal plate on the wooden board (wooden base) with adhesive tape.
    3. Bend a steel plate to make it double L-shaped (**Figure 1C** and **Figure 2A**).
    NOTE: It was convenient to use an L-shaped metal plate for fixing furniture on the wall. Another convenient point in support of using this kind of plate was that the plate already had many holes. Holes were used for screwing, and also fixing a snap button (**Figure 1A** and **Figure 4**).
    4. Make a cylinder by cutting the tip of a disposable plastic pipette (height = 1 cm, outside diameter (o.d.) = 4 mm, interior diameter (i.d.) = 2 mm) for guiding an axial needle (**Figure 2A** and **Figure 4**).
    5. Put and fix the double L-shaped plate and the cylinder on the metal plate (**Figure 2A** and **Figure 4**).
3. **Construction of the sensing apparatus**
    1. Bend a metal plate to make it L-shaped to make a top plate.
    NOTE: It was convenient to use an L-shaped metal plate for fixing furniture on the wall (**Figure 5B-C**). If so, you can skip this step.
    2. Put a small metal cap (5 mm in length and 1 mm in diameter) on the top plate (**Figure 2D-E**, **Figure 4**, and **Figure 5A**).
    NOTE: As a cap, we used a snap button. It passed through a hole in the L-shaped plate (**Figure 4**).
    3. Fix a photo sensor on the L-shaped plate (**Figure 4** and **Figure 5A**). Screwed a circuit substrate for the sensor on the L-shaped plate to save space (**Figure 2D-E**, and **Figure 4**).
    4. Glue an infrared LED (150 mW) on a small magnet together with a circuit substrate for the LED (**Figure 1A** and **Figure 2A**).
    5. Place the LED (150 mW) on the base plate beneath the photo sensor (**Figure 1A** and **Figure 2A**).
4. **Construction of the holder**
    1. Bend a metal plate to make it L-shaped.
    NOTE: It was convenient to use an L-shape metal plate for fixing furniture on the wall (**Figure 5B-C**). If so, you can skip this step.
    2. Fix the plate on a wooden board (wooden wall) with screws (**Figure 1C**, **Figure 4**, and **Figure 5B**). The height of the wooden board is not critical, it was 7 cm in this study.
5. **Connecting cables**
    1. Connect the photo sensor to an analog input channel (AIN) of an A/D converter via normal electric cables.
    NOTE: It is helpful if all the cables are bundled and fixed on the L-shaped plate (**Figure 5B-D**) because a messy workspace often prevents fine manipulation throughout the experiment.
    2. Connect the A/D converter to a personal computer (PC) via a USB cable.

2. Experimental Procedure

1. Collect **all** freshly emerged *P. quercivorus* adults from a dead *Quercus crispula* Blume (*Fagales: Fagaceae*) tree in the morning (7-9 am) of the day on which the experiment is to be performed.
NOTE: Do not use beetles collected in the previous day. More than 100 beetles came out every day and newly emerged beetles were checked daily. See a reference[16] for detailed methods on collecting beetles.
2. Put a beetle on ice for anesthetization. Avoid getting the beetle wet; otherwise, it will be difficult to complete the following procedure. Perform all subsequent procedures on ice.
3. Place a small amount of one component of the instant glue (jellylike glue) on the beetle's pronotum with the mill arm, and keep the mill arm in contact with the pronotum.
NOTE: The jellylike glue will dry slowly if this glue is used alone. However, this glue functions quickly when two components are mixed (**Table of Materials**). The other component (liquid glue) will be used in the next step.
4. Add a small amount of the other component of the glue (liquid glue) using a fine needle or stick. Ensure that the wings are free from glue (**Figure 1B**). The liquid glue is used to facilitate the hardening of the jellylike glue.

5. Adjust the cross-shaped needle into the flight mill (**Figure 6**) by using a magnet to hold the L-shaped plate (top plate) on the other L-shaped plate. Simply slide the top plate when adjusting the height of the top plate for the needle. Insert the upper tip of the axial needle into the hole of the snap button on the top plate (**Figure 5A**), and place the other tip into the guide on the base plate (**Figure 6**).
6. Adjust the position of an IR LED beneath the sensor.

3. Obtain and Analyze Data

1. Record the amplified output signal from the photo sensor and store it in the PC through the A/D converter by using commercial software with a sampling rate of 1,000 points/s (**Figure 7A**) (for A/D converter and software, **Table of Materials**).
2. Start the software DAQFActoryExpress.
3. Click a cross (+) mark on the **LOGGING** icon in the **Workspace** window.
4. Right click the logging set name and select **Begin Logging Set**.
   NOTE: The software continues logging and saving data.
5. To stop recording, right click the logging set name and select **End Logging Set** to save a .csv file.
6. Extract the passing time of the flight mill arm above the IR LED using an appropriate software by detecting times only when the recorded voltage exceeded the threshold (0.5 V).
   NOTE: Because some software (*e.g.*, MS Excel) can read a created .csv file, use a familiar software depending on the study's purpose. If necessary, download the custom-made programs available via Github, https://github.com/HidetoshiIkeno/FlightMill. For further information about our programs as well as instructions to use the program, see the README file which is accompanied with the main program.

Representative Results

In these experiments, about 50% of the beetles applied to the flight mill showed one or more revolutions. When the plastic part passed a virtual line between the sensor and the LED, the recorded voltage changed from about 0 V to about 6.5 V, and the duration of a passing was within 10-20 ms, depending on the flight velocity. Therefore, a spike-like voltage change is observed as one revolution (**Figure 7B**). We defined flight as when the flight mill arm revolved, *i.e.*, voltage exceeded the threshold (0.5 V), irrespective of the number of revolutions in a bout, the revolution velocity, or the revolution duration. We also defined flight time as a time only when the recorded voltage exceeded the threshold. Thus, only one time point was extracted for each passing of the plastic part. Consequently, 50% of the beetles applied to the flight mill "flew". Some beetles tended to open and close their wings repeatedly before a flight, although in most cases beetles started flying without showing any signs beforehand. Typically, a beetle kept flying for a certain period (partial flight) and then flew again after an interval (**Figure 7C-D**). It was not possible to predict whether or not the beetle would resume flight. Thus, we designated a measurement as being complete when the interval was over 60 min.

In most cases, the beetles flew with a speed of 3-6 revolutions per second (0.75-1.50 m/s). We estimated the total flight distance by multiplying the distance of one revolution (*i.e.*, circumference of the flight path), which will be approximately 25.1 cm, and depend on the radius of the flight mill arm, with the total number of revolutions. To avoid underestimation of the beetles' flight capacity, we omitted beetles that showed a short flight (less than 1 km) from this analysis. Finally, we obtained 16 beetles (7 males and 9 females) from 35 beetles that performed at least one revolution.

We defined flight duration as the total time spent flying, and flight distance as the summed distance of partial flights. 16 beetles showed a 1.26 hour (3.24 km) or longer flight without energy intake. The maximum duration and distance were 7.5 h and 27.1 km, respectively. Because flight durations and distances were largely different among individuals in these experiments, the median values were more informative than the mean values.

To examine sexual differences in flight ability, we grouped data depending on sex and found that the flight distance was similar for males (median: 10.2 km, average: 13.4 ± 3.11 km, min: 3.3 km, max: 27.1 km) and females (median: 17.2 km, average: 17.2 ± 2.16 km, min: 8.7 km, max: 25.4 km). The Wilcoxon rank sum test showed no significant difference (p = 0.211) in flight distance between males and females (**Figure 8**) nor in flight durations between males and females (males: 3.8 h, females: 4.7 h, p = 0.142). Thus, we conclude that both sexes of beetles may have the same ability of flight in distance and duration.

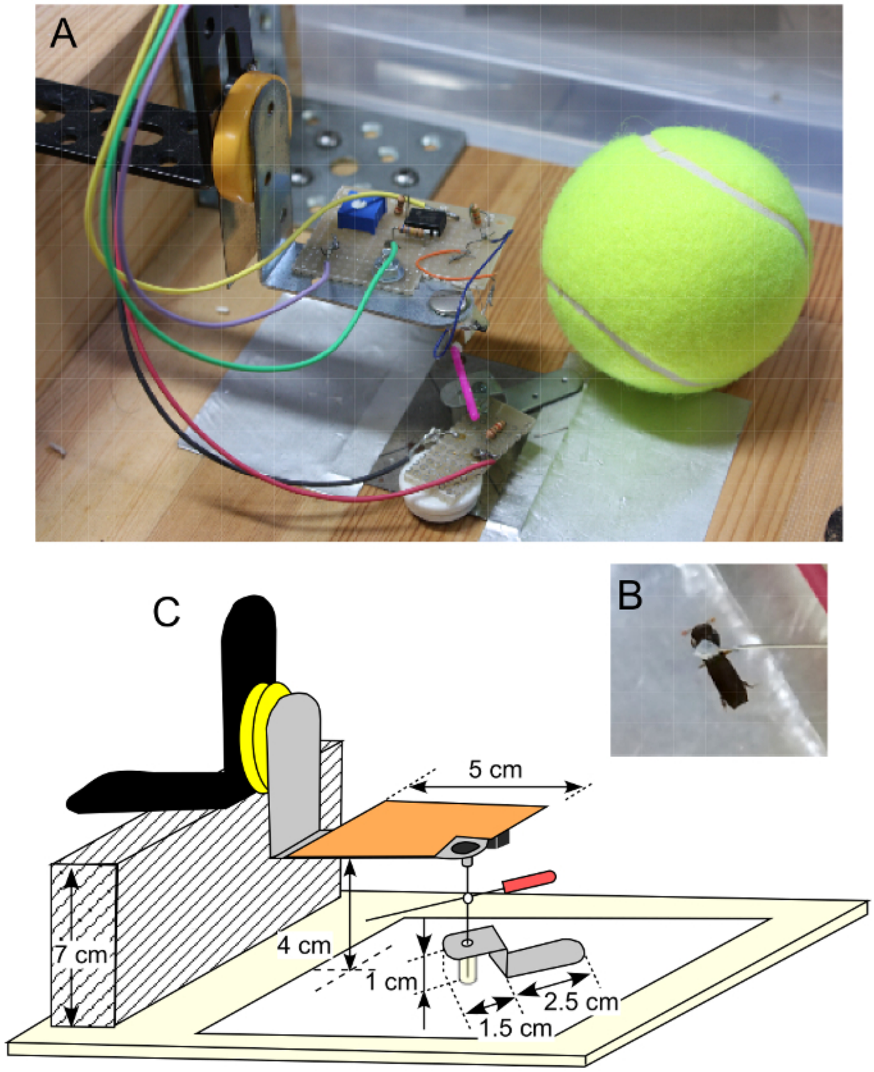

Figure 1: Overviews of the flight mill. (A) The oblique view of the flight mill. Compare to a tennis ball for scaling. **(B)** A beetle attached to the flight mill. **(C)** Scales of a flight mill. See **Figure 2** for more detail of each parts. Please click here to view a larger version of this figure.

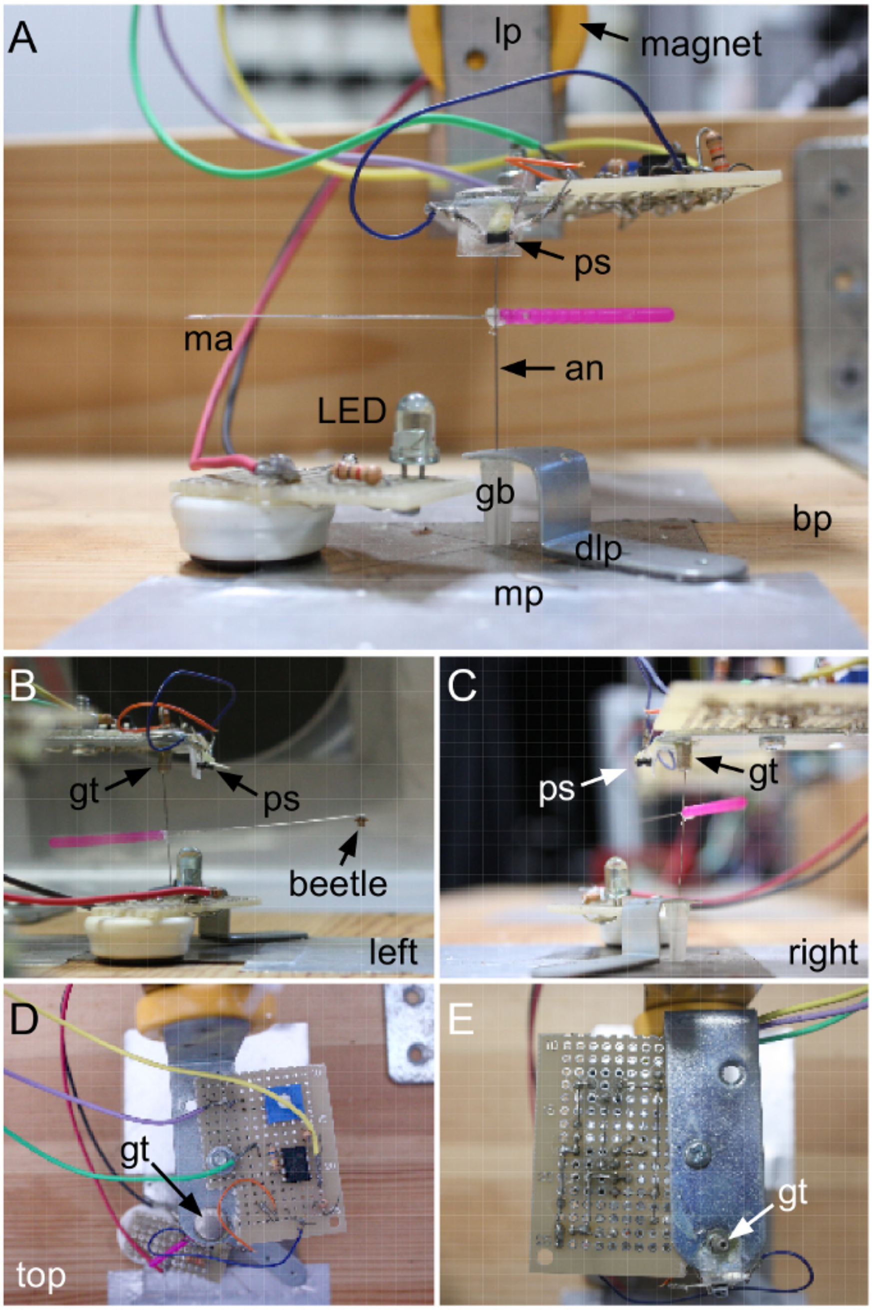

Figure 2: Flight mill from different view angles. A frontal **(A)**, left-side **(B)**, right-side **(C)**, and top **(D)** view of the flight mill. **(E)** The bottom view of the top plate. For photographing, the LED was moved from beneath the photo sensor. an: axial needle, bp: base plate, dlp: double L-shaped plate, gb: guide on the base plate, gt: guide on the top plate, LED: IR LED, lp: L-shaped plate, ma: flight mill arm, mp, metal plate on the base plate, ps: photo sensor. Please click here to view a larger version of this figure.

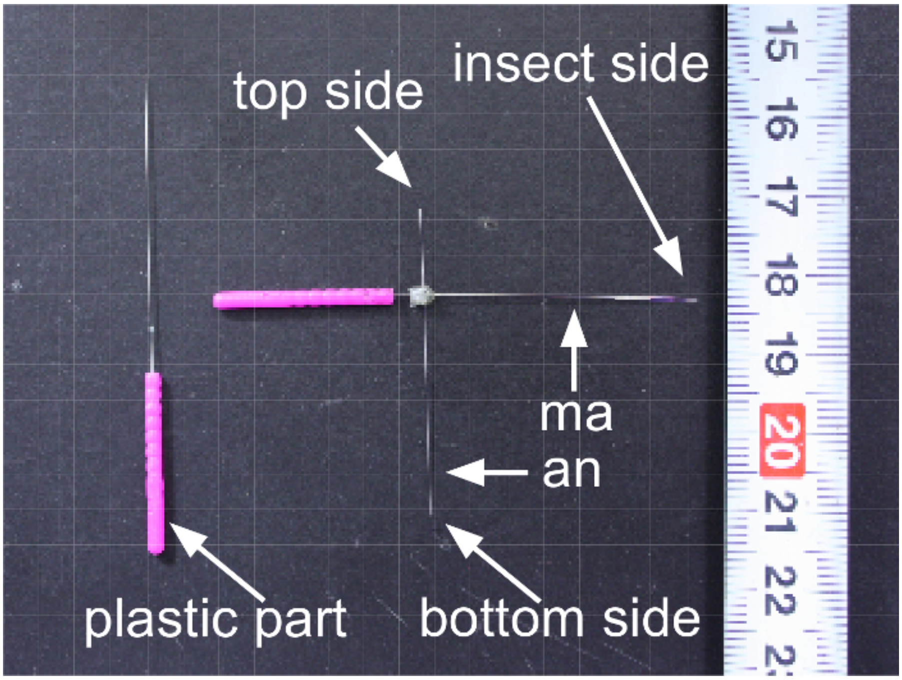

Figure 3: Needle used for the flight mill. An original needle (left) and a cross-shaped needle (right). A needle size is: metal part: 40 mm in length and 0.25 mm in diameter, plastic part: 22 mm in length and 2 mm in diameter. an: axial needle, ma: flight mill arm. Please click here to view a larger version of this figure.

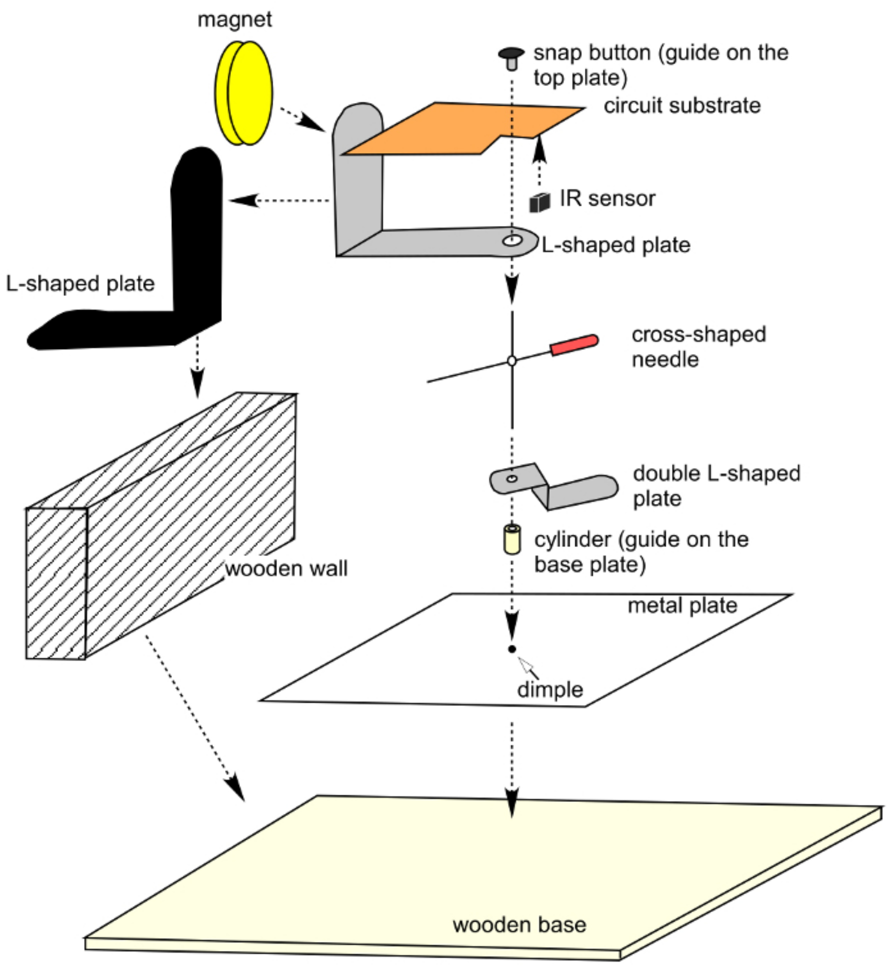

Figure 4: How to construct the flight mill. Please see text for details. Please click here to view a larger version of this figure.

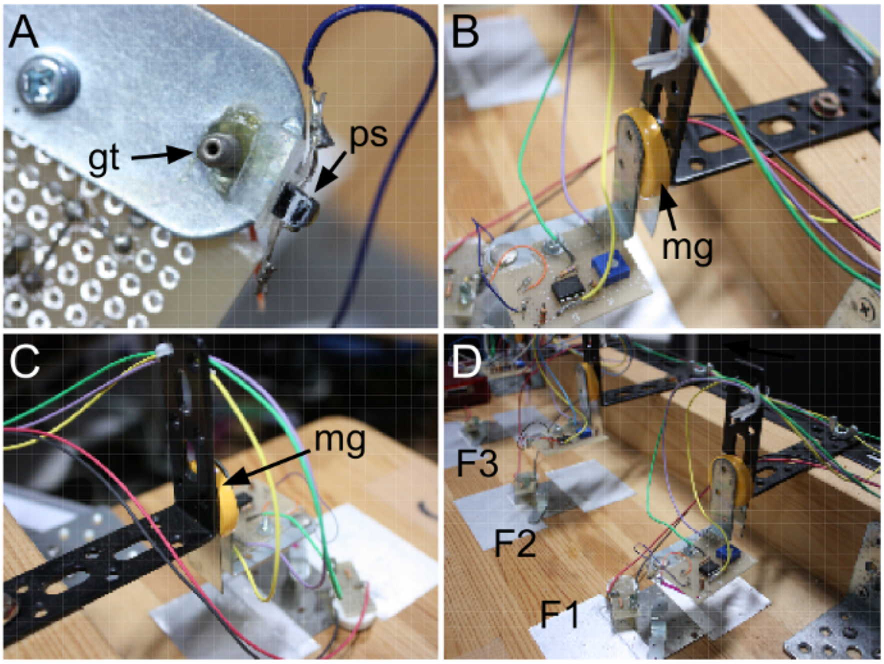

Figure 5: Flight mill parts. (A) A guide on the top plate and a photo sensor were fixed on the L-shaped plate with epoxy resin adhesive. The diameter of the hole of the snap button was 1 mm and the length was 5 mm. **(B)** An oblique view of the flight mill from an upper right angle. A magnet connects two L-shaped plates. A black L-shaped plate screwed on the wooden plate. **(C)** A back view of the flight mill. Electric cables were bundled and fixed on the L-shaped plate that was screwed on the wooden board. **(D)** Three flight mills (F1-F3) are arrayed in a small space (45 cm x 20 cm). gt: guide on the top plate, mg, magnet, ps: photo sensor. Please click here to view a larger version of this figure.

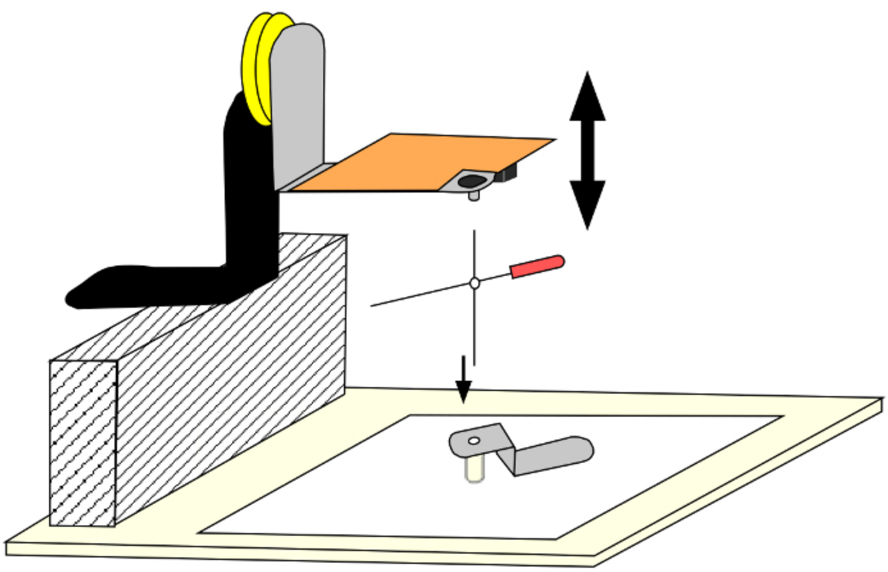

Figure 6: How to adjust the flight mill. Slide the top plate vertically and insert the top tip of the axial needle into the guide on the top plate, *i.e.*, the hole of the snap button. Insert the cross-shaped needle into the hole of the double L-shaped plate. Please click here to view a larger version of this figure.

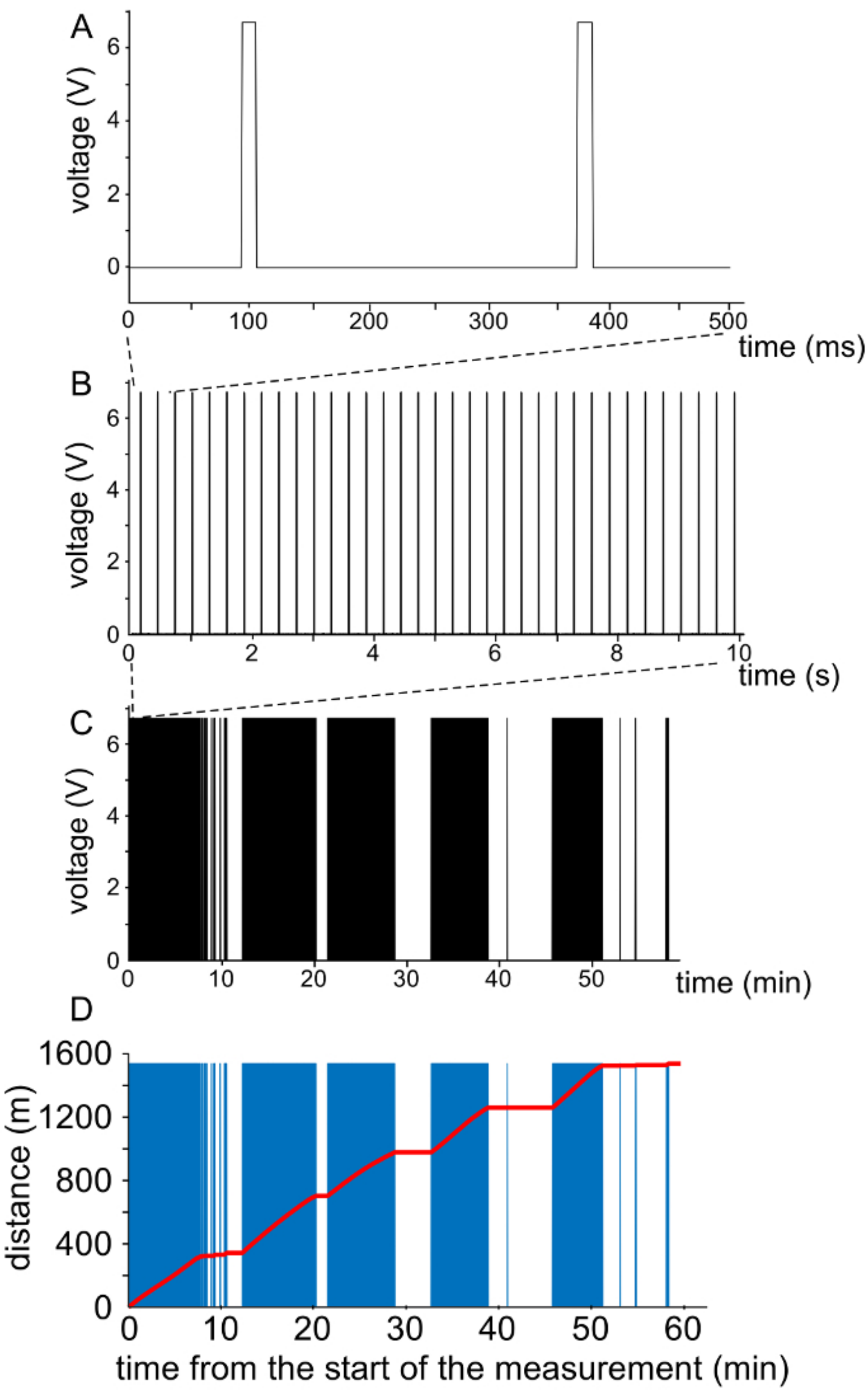

Figure 7: Representative time trace of revolutions. (A) An example of a voltage output during a 500 ms (**A**), a 10 s (**B**), and a 1-hour (**C**) flight activity. Trace is temporally expanded from (**C**) to (**A**). The dotted lines indicate the periods which are expanded. Voltage output was sampled every 1 ms (1,000 points per second). When the arm passed above the IR LED, the output voltage of the sensor increased from 0.01 V to about 6.7 V. A spike-like voltage change was observed as one revolution (**B**). When the time scale is minute like in panel C, a long-lasting flight is observed as like a black rectangle (**C**). Typically, the flight activity has two phases: one is an intensive-flight phase, the other one is the pause phase. The length of the interval between intensive-flight phases is not predictable. During the intensive-flight phase, the beetle flew with constant speed. **(D)** Superimposition of the voltage output and corresponding accumulated flight distance. The voltage output is the same as with panel (**C**). Blue: voltage output of a 1-h flight, red: accumulated flight distance. Please click here to view a larger version of this figure.

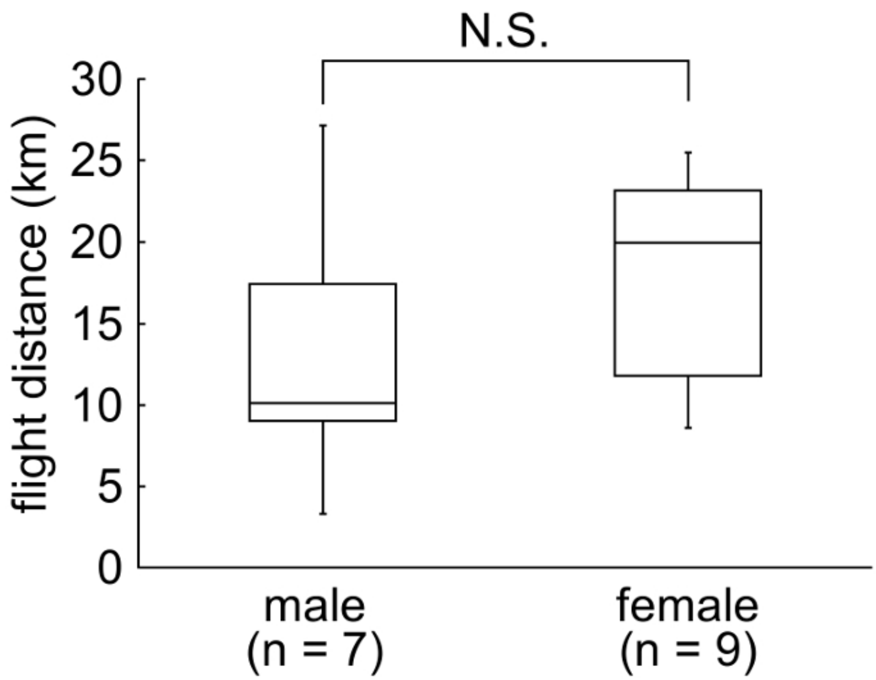

Figure 8: Comparison of the flight distance between males and females. A box plot of the flight distance. No significant difference in the flight distance is observed between males and females. The line in the box indicates the median, and the edges of the box indicate the higher and lower quartile, respectively. Maximum and minimum values are indicated by whiskers. Please click here to view a larger version of this figure.

Discussion

We developed a low-cost, easy-to-build, and compact flight mill for small insects such as *P. quercivorus* (4-5 mm in body length and 4-6 mg in body weight). Our flight mill comprised only ordinary items such as a needle, an IR LED, a photo sensor, instantaneous glue, *etc.*, and did not require any sophisticated, expensive, or rare items such as computer-controlled electric devices. This enabled the easy and quick collection of necessary items and reduced experimental costs. Indeed, it cost only 1,000 JPY (approximately 10 USD, 8 EUR, or 7 GBP) per flight mill (excluding non-flight mill specific items such as a PC, A/D converter, software, software license *etc.*) Furthermore, the presented flight mill was compact. Hence, it was possible to prepare and use multiple flight mills[16] without needing a large experimental space (**Figure 5D**). Those are the strongest points of this method with respect to other flight mills methodologies.

The propulsion power was very small in the case of this small beetle. Therefore, the friction resistance must be as small as possible, as mentioned in previous studies[15,17]. This is very critical for measurement. From this point of view, the use of a thin needle was very convenient for reducing the contact area of the flight mill base plate. For the same reason, the upper part of the flight mill must be also smooth. All potential contact points or places must be smooth.

When a target insect is small, the flight mill must be kept flat, although previous papers have not mentioned this clearly. Otherwise, the measuring results could be unexpectedly influenced by gravity-related effects. Gravity-related influences and large friction resistance could produce misleading results. Another critical point was the accuracy of the length of the flight mill arm. Because the number of revolutions exceeded over ten thousand when beetles flew long distances, an inaccurate measurement of the needle length provides misleading data. Measuring the radius of the mill arm revolution after construction is more practical than making the flight mill arm the precisely desired length.

For measuring the flight activity of *P. quercivorus*, this experiment suggests some practically important points. First, measurements must be performed by freshly emerged beetles. So far, we have noticed that starting an experiment in the morning provided a higher number of beetles that fly over 1 km, relative to starting in the afternoon. Hence, ideally as many beetles as possible must be measured in the morning. Second, the guides on the top and base plate may be critical for collecting substantial amounts of data. The experiment performed using the described procedure was quite easy and quick. From anesthetizing to completion of gluing, it took less than 1 min. Often, adjusting the cross-shaped needle to the flight mill is the rate-limiting step. If it takes too much time, only a few beetles can be measured. Those guides help to adjust the needle to the mill quickly. Third, the best conditions for measurement must be found, as well as the best procedure for handling insects. Ideally, all measured data must be used for analysis, although an exclusion was often used in the field of animal behavior[9,10,18]. We omitted insects that flew less than 1 km because we did not know whether short distance flyers have a poor capability to fly or some experimental faults caused the short distance flights. The best performance of the experiment would provide a more precise estimation of the beetles' flight capacity.

Another limitation might be simultaneously sampling of data from a lot of beetles. A high-powered PC is helpful in processing multiple flight mills' data simultaneously. Especially, while saving and writing data, the PC specifications are critical. Because the flight is high-speed and long-lasting, some data could go missing if an appropriate PC is not used. We found the 1 kHz sampling rate to be best for our set-up. However, the sampling rate must be adjusted to each specific flight mill apparatus.

Because the measurement of the flight of a beetle was terminated when a flight interval exceeded 60 min, we kept observing each beetle after 60 min had elapsed. In addition, our analysis was off-lined. Thus, it would be helpful if some behavioral states such as a flight interval, a flight duration, a flight distance, *etc*. are informed/displayed in real-time. To achieve real-time analysis, a new program must be developed in the future, and a high-powered PC should be used.

Disclosures

The authors have nothing to disclose.

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