A High-Resolution System for Recording the Daily and Lifetime Behavioral and Movement Patterns of Individual Tephritid Fruit Flies

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ABSTRACT

We developed a system in which the lifetime behavior and movement patterns of *Anastrepha ludens*—a Tephritid fruit fly slightly larger than a housefly and commonly known as the Mexican fruit fly—maintained in 27 cubic cm cages is recorded every fifth of a second for one minute three times per hour for 24 hours each day throughout their lives. The data generated from the behavioral monitoring system (BMS) include date, time, 1-of-6 behaviors, and locations in 3-dimensional space (XYZ location). This yields over 12 million data-points for a single fly that lives 100 days. The raw data enable researchers to analyze: (1) behavior including frequency, sequence, age and diel patterns, and circadian rhythm; (2) movement including age and diel patterns, rates, and pathways; and (3) location within the cage by age and time-of-day.

BEHAVIORAL MONITORING SYSTEM

The hardware of the BMS for monitoring behaviors of mexfly consists of a pair of cameras to acquire left and right video images in real time, a nine-cage tray to house individual flies in each cage, visible and infrared lights. The cameras are connected to an image processor for short term storage of video images, which are fed to a computer for behavior detection and long-term data storage. The system is configured as follows at the beginning of the experiments and then each time after fresh water and food are provided to flies. The cameras and lighting are adjusted to capture clear left and right images of each cage in the center of video. The fixed positions of water and food in the images are captured respectively and recorded into the system. The fly is recognized as a white image in the dark background of the cage. The rate of sampling by cameras is 5 frames or images per second. The sampling time per recording can be adjusted between the ranges of 10 to 60 seconds. The quantity of images or frames in the video will be a function of the sampling time per recording. Once the positions of water and food, sex of the flies, date of birth, comments, and sampling time are loaded, the recording can be initiated. The BMS records 3-D coordinates of individual flies from each video frame and automates classification of six behaviors, termed as resting, moving, walking, flying, feeding and drinking, for each bout or image frame, based on changes of 3-D coordinates between two constitutive frames and relative positions from the food and water sources. The BMS generates two ethograms in the text format every day, a behavior file containing date, time, XYZ coordinates, speed and behavior classification at each bout of imaging, and a statistics file containing date, time, total activity level, frequency and activity level of each behavior per sampling period.

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PROOF OF CONCEPT

Behavioral Experiments

Two BMS were used to monitor lifetime behaviors of 16 mexfly females with each BMS holding four females on the full diet and four females on sugar only diet. The flies were randomly assigned to and individually housed in a cage of the 9-cage tray in each BMS. Fresh food and water were provided to flies from the bottom of each cage once a week. The recording was initiated at the eclosion of the flies and stopped at day 134 when approximately half of the flies were dead. The video sampling time for each cage was 60-second long.

Lifetime Behavior Patterns and Dietary Effects

We used two BMS systems to continuously track eight mexfly females on the full diet and eight females on sugar only diet for 134 days when more than half of the mexflies were dead. The lifetime recording was disrupted for short periods of time when the system needed re-adjustment due to introduction of fresh food and water once a week or occasionally power outage. For each fly, we used the behavior ethograms from the BMS to compute the frequency and activity level of each of the six behaviors in every hour of the day in function of the age in days.

Using these hourly behavior patterns, we first computed frequency of each of the six behaviors over the age or over the time of the day to assess age-related or circadian rhythm patterns respectively. Several age-related patterns could be identified from the data depicted in the figures based on the outcome of recordings for 16 females on two different diets. Flies on both diets spend more than 60% of their time on resting every day over their lifetime. The next most frequent daily behaviors are walking and moving, which, combined, count approximately 25% of a fly's daily time. Flies on sugar diet have significantly higher walking frequency than those on the full diet. Flying, feeding and drinking occur at very low frequencies, which counts less than 5% of all the behaviors in most of the days. The frequency of daily resting and moving remain relatively constant with little fluctuation over the lifetime of flies, suggesting that there are no age-associated changes for these two behaviors. The frequency of daily walking reaches the peak around the age of 20 days and then gradually decreases with age for flies on both diets, suggesting that walking frequency is an age-related parameter. Overall, the frequency of daily flying declines with age after reaching the peak around the age of 20 days, while the frequency of daily feeding and drinking appears to increase with age.

We also analyzed the circadian rhythm patterns for the hourly frequency of each behavior. During night time between 7 pm when the light is off and 7 am when the light is on, flies spend almost all of their time (>95%) on resting, presumably in sleep, while other behaviors are rare. During daytime, resting is still the most frequent behavior, which occupies more than 55% of the flies' time; walking is the next most frequent behavior (up to 40%), followed by moving (up to 20%); feeding, Drinking and flying are rare (less than 5% combined). Walking and flying show hour-dependent changes and reach the peak period in late afternoon from 3-7 pm, while the other behaviors display relatively constant rates during daytime. During daytime, flies on sugar diet walk more frequent that those on the full diet with the peak level differing by approximately 50%. However, the highest walking frequency occurs at 6 pm for both mexflies on sugar and the full diet.

Lifetime Activity Patterns

It is reasonable to assume that the daily total distance a fly travels reflects how active this fly is every day. To further assess the lifetime activity level of flies, we calculated the distance a fly travels in each hour every day. By averaging the hourly total distance values from individual flies, we generated age-related distance patterns and circadian distance patterns for flies on the full or sugar only diet. The daily total distance gradually increases in the first 15 days, reaches the peak at the age of approximately 20 days and then gradually declines with age for flies on both diets. This suggests that daily total distance is a good aging marker. The daily distance is generally higher for flies on sugar diet than those on the full diet with the peak distance levels differing by ~60%. The circadian distance patterns reveal that overall patterns are similar between flies on both diets with ~90% of the daily distance traveled is during daytime. Similar to the circadian walking frequency patterns, ~50% of the distance traveled is in late afternoon from 3-7 pm and the distance peaks at 6 pm for flies on both diets. In addition, the peak distance level for flies on sugar is ~40% higher than those on the full diet.

DISCUSSION

We described a behavior monitor system capable of automating lifetime behaviors. Each BMS tracks the 3-D positions of nine individually housed mexflies once every few minutes over their lifetime and generates ethograms with automated classification of six behaviors. This BMS can be easily adopted for a regular research lab as it is made with video cameras and a computer commonly found in a biology lab. The software converts video images of large file size into text files, which allows long-term continuous recording and storage by a standard lab computer. In addition, the software automates classification of six basic behaviors of mexflies, which makes it user-friendly for biologists to analyze data without much additional programming. Although many of the existing video systems have potentials to conduct lifetime long experiments, our BMS is the first high resolution machine vision system demonstrated to have the capacity to record lifetime 3-D positions of an animal and automatically classify lifelong behaviors for months.