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Fly Roller: Development of an Instrument to Exercise Fruit Flies

Reiner Dizon, Angel Solis, Ameera Essaqi, Michael Isaacs, Austin McKenna, Allen Gibbs, David Lee, and Sarah L. Harris

Abstract

Fruit flies (*Drosophila melanogaster*) are a widely studied model species for addressing basic and applied biological questions, including the obesity epidemic (Hardy, Physiology and genetics of starvation-selected ħ. Las Vegas: Digital Scholarship@UNLV, 2016). Biologists investigate the effects of obesity through studying the physiology and behavior of normal and obese flies in response to exercise, diet, and other experimental conditions. In this paper, we propose an instrument, called "Fly Roller", for exercising flies in biological experiments with or without measurement of metabolic rate. Fly Roller comprises two parts: a roller mechanism and a controller circuit. The roller mechanism supports and slowly rotates a plastic tube containing fruit flies, which reflexively walk along the inner wall of the tube. When metabolic measurements are desired, a gas analyzer can be coupled to a modified tube design with air valves at the ends of the tube, allowing the air lines to remain stationary in low-friction bearings while the tube containing the flies is rotated.

Keywords

Physiology · Exercise · Obesity · Metabolic · Experimental instrument · *Drosophila melanogaster* · Fruit fly

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59.1 Introduction

Some of the most widely used species for modeling certain human diseases are fruit flies (*Drosophila melanogaster*). This model has been used in studies of Alzheimer's, Parkinson's, cancer, and heart disease. In recent years, this particular species is becoming instrumental in the investigation of genetic and physiological aspects of obesity. For this reason, biologists can study these flies' responses to various conditions, such as exercise, dietary change, or stress. Through changes in genes, diet, or the environment, fruit flies can become obese. Such flies share similar phenotypes as obese humans, like cardiac dysfunction [1–3] and disrupted sleep behavior [4].

Some existing automated methods exist to induce exercise in *Drosophila* include taking advantage of the flies' negative geotactic response and taking advantage of flies' natural attraction to bright light. One such solution, the "Power Tower," repeatedly lifts a tray of vials containing flies slowly for 15 s and then drops into free-fall and causes the flies to be startled. Another solution is the modified TreadWheel, a device used to slowly mix the contents in a test tube. The modified TreadWheel slowly rotated vials full of *Drosophila* instead [5]. A further modified version of the device named the Rotating Exercise Quantification System (REQS) was later created which allowed the flies' movement to be detected when they crossed the center of the tube [6]. All the above solutions come with their own problems. The Power Tower would repeatedly cause damage to the flies during each drop when the flies hit the bottom of the housing. This small but consistent trauma would build up throughout the experiment. The REQS can only detect movement when the fly crosses the center of the tube. This mean that all activity that occurs on either edge of the test tube is not recorded by the system. Despite the creative designs to stress flies, none of

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the current methods enable researchers to measure metabolic activity during exercise.

In this paper, we propose the Fly Roller system as a solution to examine the differential effects of exercise on fruit flies. Our system enables researchers to exercise fruit flies within their storage vials at different durations and speeds (both fixed and varying). We constructed a roller mechanism, chassis, and controller which, together, act as a speed-controlled treadmill for fruit flies.

In the rest of this paper, we highlight relevant background information (Sect. 59.2), describe the primary methodologies and components used in our system (Sect. 59.3), and discuss the experimental setup and testing results as well future work (Sect. 59.4). We discuss our conclusions and some of the issues resolved throughout the design and implementation process in Sect. 59.5.

59.2 Background

Obesity has become the leading cause of type-II diabetes, certain types of cancer, and cardiovascular disease, and, as such, measuring how exercise can change the course of these diseases is paramount [7]. When simplified, obesity is caused by an excess of calories taken in versus the number of calories expended. The caloric excess is stored in the body as lipids in fat tissue. Thus, obesity can usually be counteracted by decreasing caloric intake and/or increasing caloric expenditure to promote a calorie deficit that consumes energy from excess fat stores. Fruit flies can be used to model the effects exercise has on organisms. Such effects include changes in biochemistry, psychology, and even genetic changes that regulate animal physiology.

Obese fruit flies have become a useful model for human exercise because, like humans, through dietary changes and exercise their fat levels can be altered [6, 8–11]. The flies used are a specific population that has been selectively bred to be starvation resistant. The evolution experiment resulted in populations of flies that have up to three times more lipid content than their control cohort population. In addition to their obese phenotype, the starved flies exhibit cardiac dysfunction, lower metabolic rates, and disrupted sleep behavior [3, 4, 12].

In the Gibbs lab, fruit flies' metabolic information is gathered using flow-through respirometry, which passes carbon dioxide (CO_2)-free oxygen into an airtight container that holds flies and then collects the gasses on the other side. This allows for the detection of the resulting Oxygen (O_2) or CO_2 levels. CO_2 measurements are used for a single fly and O_2 measurements for a group of flies. Both gas measurements are linked to metabolic rate and can thus be used to find caloric expenditure.

59.3 Methods

This section describes our design methodology for building the Fly Roller system (including the first iterations). The Fly Roller consists of two main components: (1) the roller mechanism and (2) the controller unit.

59.3.1 Preliminary Versions

Figure 59.1 shows the original Fly Roller design, where a single test tube is rotated by a large rubber wheel. To decrease the system cost, the second version rotated multiple test tubes on each wheel (see Fig. 59.2).

The acrylic chassis was designed in SolidWorks and provided cutouts to hold the wheel axles and test tubes. Each wheel had one direct drive motor and all motors were controlled by the same printed circuit board (PCB). This design proved to have a few shortcomings. First, the rubber on plastic interface caused static electricity to be generated and build up around the tube. The tube then had enough charge to

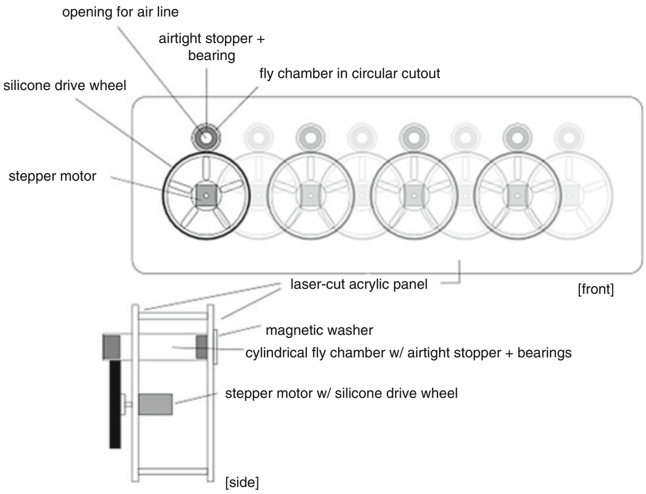


Fig. 59.1 Rendering of roller mechanism version 1

Fig. 59.2 Roller mechanism version 2

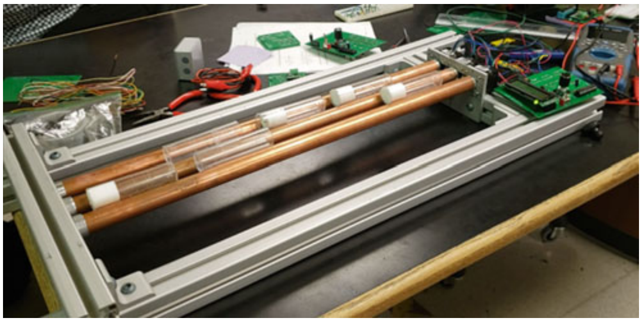


Fig. 59.3 Roller mechanism version 3 (with controller circuit)

hold some of the test flies that were used in the roller against the wall of the test tube. Secondly, the tubes would rub against their acrylic slots, causing the tubes to screw either inwards or outwards and bind up or fall out. Lastly, the acrylic would begin to cut into the plastic test tubes with use, which caused damage and even holes in the tubes.

59.3.2 Fly Roller

The final version of the Fly Roller system used copper tubes, also referred to a rollers, and a single motor to rotate a set of test tubes at a uniform, possibly varying, rate (see Fig. 59.3). Each copper tube is *20 in. long* with a *diameter of 0.25 in.*, which is smaller than the radius of the test tubes it rotates. Each roller (copper tube) attaches to its own ball bearing on each end. This provides the connection between the rollers and the stepper motors. For the rollers to rotate in place, the tube-ball bearing combination sits on an aluminum housing made of 80/20. We also designed an aluminum housing to mount the in-line stepper motors that attaches to the 80/20 housing of the rollers.

The system also shines an ultraviolet (UV) light down onto the top of the system to encourage flies to move. Because fruit flies exhibit phototaxis, an attraction to light, they begin to walk towards the top of the tube, where position constantly changes as it rotates. This constant rotation causes the flies to exert themselves and exercise.

The Fly Roller accommodates four test vials between each pair of copper rollers. In total, the Fly Roller holds up to eight test vials of flies. Of course, this system can be easily expanded to include more copper rollers to accommodate more test vials, as needed.

59.3.3 Control Unit

The Fly Roller control unit is a custom designed printed circuit board (PCB) that includes three main modules: the microcontroller, user interface (UI), and the motor controller.

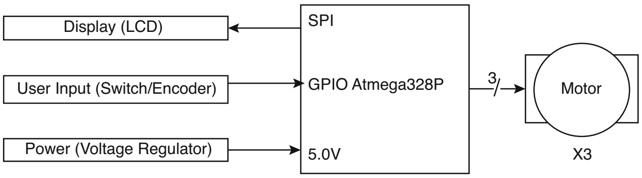
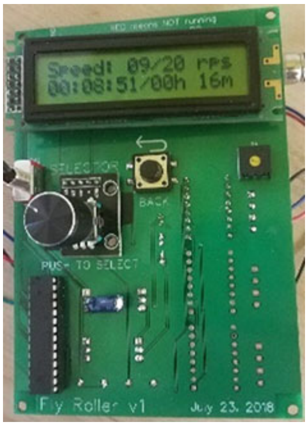


Fig. 59.4 High-level schematic of the controller

Fig. 59.5 Controller circuit PCB (front view)



The ATmega328p microcontroller, depicted in Fig. 59.4, acts as the brains of the system and controls the other two sub-modules, the motor controllers and the UI. The UI consists of a liquid crystal display (LCD) to prompt user input via menus and to display current settings, a knob-based encoder for option selection, a push button switch to turn the Fly Roller on and off, and a set of LEDs (light-emitting diodes) to indicate the current mode. The motor controller sub-unit consists of motor drivers and the motors. The control unit is powered by up to 20 volts from a wall transformer via a standard barrel jack connector and a power supply. The control system itself uses 5 V, which is produced by an LM7805 voltage regulator. Also, a further set of 8 V voltage regulators, *i.e.*, LM7808, power the stepper motors via their corresponding stepper driver chips. The motor encoder gives feedback to system to control how fast the motor, and thus rollers, rotate.

Figure 59.5 shows the control unit PCB. The top of the PCB shows all UI components, most prominently the liquid crystal display (LCD). All motor controller parts are located on the backside of the PCB and below the UI parts so that the user sees only the UI parts, which simplifies the interface seen by the end user. The PCB is 3 × 2.8 in., which size is based on the size of the roller mechanism.

The LCD connects to the board using header pins positioned near the top of the PCB. This display prints messages to the end user about various configuration options for the Fly Roller motors. The user responds to these messages using the encoder and the push button. During the Fly Roller configuration process, the user turns the encoder to set the rotation speed, rotation profile, and duration in response to messages displayed on the LCD. Users confirm their selection by pushing down on the encoder. To return to a previous screen, the user can press the push button, which is clearly labeled as the "BACK" button. Additionally, a red-green LED indicator above the LCD screen indicates if the motors are operating or not. A red LED indicates the system is stopped or paused. A green LED means the motors are currently running. The FlyRoller UI enables the end user, *i.e.*, a researcher, to change the configuration of the Fly Roller and to notify them of the motors' state, without the need for an attached computer.

The UI module allows researchers to configure the Fly Roller via a program running on the microcontroller, and this same program drives the motor controllers to execute the user's desired settings for a given experiment. The motors used to operate the Fly Roller's roller mechanism are bipolar stepper motor with 200 steps per revolution. This small number of steps is not enough to observe a smooth roller rotation and, thus, could cause trauma or injury for the flies, thus disrupting the experiment. To resolve this issue and better control the stepper motors, we used DRV8825 stepper motor controllers. These controllers feature microstepping, which allows for up to $^{1}/_{32}$ of the original motor precision, thus enabling finer granularity of rotation. The system includes one motor controller chip per stepper motor, but all motor controllers are driven by the same control signals from the microcontroller.

Figure 59.6 shows the back side of the PCB, which mounts the non-UI parts—motor drivers and voltage regulators. Hence, the motor controller chips drive the Fly Roller motors and, in turn, the rollers, according to the user input processed by the microcontroller.

59.3.4 User Interface Program and Control Algorithm

At the core of the Fly Roller control unit is the ATmega328p microcontroller, which sends and receives information to/from the user via the UI and controls the motor control unit according to that user input. This section describes the microcontroller program, also referred to as firmware, that interfaces with the UI and controls the motors.

Figure 59.7 displays all the firmware's submodules as well as the interfaces between these submodules. Rather than having a single module for the UI, that module is further subdivided into two submodules, one for the LCD library we created (*i.e.*, LCD.h) and the other to interface with all other UI components (*i.e.*, user_int.h). The stepper motor header file contains all the necessary data and functions to drive the motors. The firmware's highest level of abstraction is in main.h and main.c.

Fig. 59.6 Controller circuit PCB (back view)

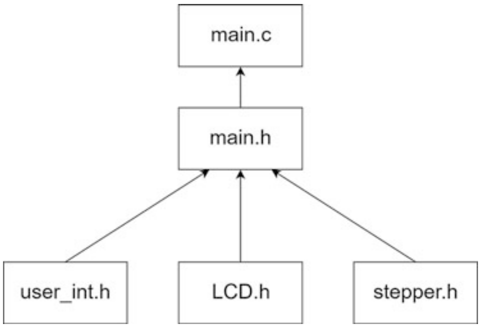


Fig. 59.7 File structure of firmware

The UI program enables user interactivity. The LCD submodule communicates with the on-board LCD screen using the 8080 interface. Even though the Fly Roller PCB contains many components that could use up most of the pins on the microcontroller, we made choices to eliminate redundant use of pins on the chip which allowed us to operate the 8080 interface in 8-bit mode. This pin configuration takes up 11 of the available 20 (total number) input/output (I/O) pins on the microcontroller. These 11 pins are defined in the LCD library as constants which allows us to abstract the pin names in the LCD library functions. The LCD library we wrote includes functions for initializing the LCD, printing to the LCD, and writing to a specific LCD location. These functions make up the overall LCD library used to display messages to the user for either prompting configuration input or display the current Fly Roller configuration and status.

The remainder of the UI firmware reads the pushbutton and encoder and drives the LEDs to indicate the current state of the Fly Roller. These remaining interfaces consume a total of six pins on the microcontroller. The pushbutton interface, which includes debouncing, allows the user to go back a screen on the display. Similarly, the built-in encoder pushbutton serves as the "NEXT/PROCEED" button. The encoder itself is a quadrature encoder, which means that each encoder rotation produces two square wave signals of

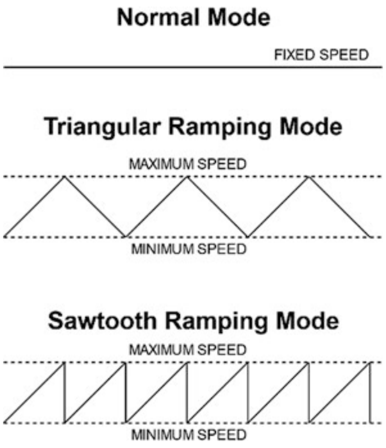


Fig. 59.8 Fly Roller's different speed modes

different phases. We check these signals for a clockwise rotation to indicate selection on the configuration selection screens for the Fly Roller. The red-green LED mentioned in Sect. 59.3.3, that informs the user whether the motors are running or not, requires two pins that are each controlled via pulse width modulation (PWM).

The third module that main.h calls upon is "stepper.h" which handles the control for the stepper motors via the motor controller chip. As all the chips use the same three signals, their corresponding pins are defined in this header file to abstract away the actual pin name for the library functions. This header file also defines the physical characteristics of the motors, the roller mechanism, and the vial size for the flies' container. These constants and definitions enable the programmer to customize each firmware based on the dimensions and characteristics of the motors, rollers, and vials. Using these definitions, we have also created macros to define the appropriate speed and time constants for the experimental setup. These macros ensure that the input configuration matches what is observed during run time.

The system features two speed modes: (1) *Normal Mode* and (2) *Ramp Mode* as illustrated in Fig. 59.8. In normal mode, the user sets a fixed speed and duration. On the other hand, Ramp mode fixes the duration but has two sub-modes for speed: (1) *Triangular* and (2) *Sawtooth*. In triangular ramping mode, the motors oscillate between minimum (min) and maximum (max) speed linearly and without a sudden transition from extremes. Unlike the triangular mode, the sawtooth ramping mode drops immediately to the selected min speed just after reaching the max speed.

The main program calls the functions from the underlying submodules to implement the overall control. The main module also defines the state machine (states and state transitions) of the user interface program as shown in Fig. 59.9. Arrows indicate a state transition when the user presses enter during that state. The main program also includes initialization functions as well as reset functions that are called after each completed experimental run. When the system powers on, it executes an initialization sequence. Then, the system runs the finite state machine in an infinite loop. The user runs through various menu selections using the display, encoder, and push buttons. At any point, the user can return to a previous menu screen by pressing the pushbutton indicated by the label "BACK" and the loop back arrow that is printed on the PCB.

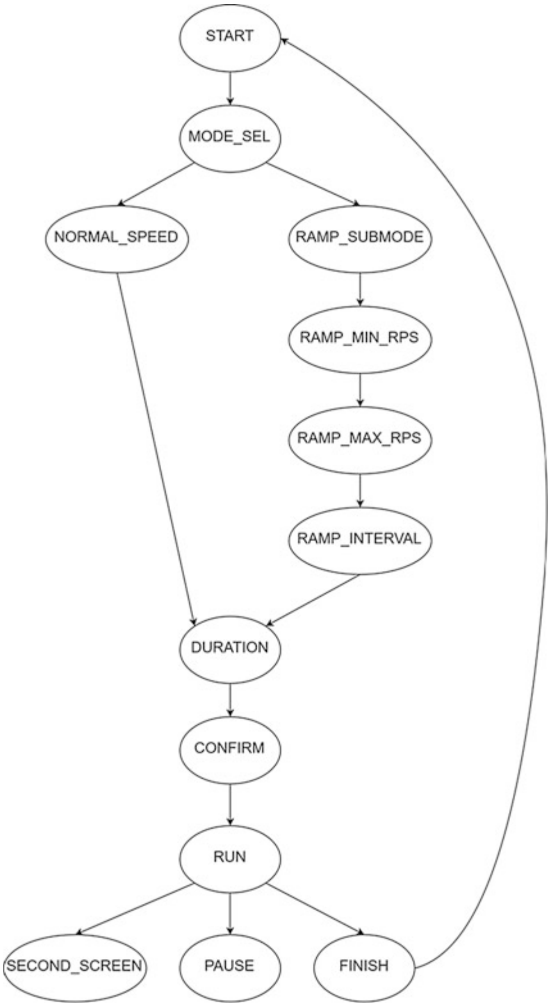


Fig. 59.9 Simplified state machine view for the menu screen

When the system starts after the initialization sequence or finishes an experimental run, the START state greets the user with a welcome screen that tells the user to press any button to get started. Once that occurs, the program moves to the MODE_SEL state where the user selects between the two main speed modes, Normal or Ramp. After mode selection—including submode selection when in Ramp mode, the system prompts the user to select a speed and duration for the experiment. The system's minimum revolutions per second (RPS) is $^{1}\!/_{20}$ RPS with a maximum of 1 RPS, which is displayed as $^{20}\!/_{20}$ RPS on the display (LCD). The precision for the speed is also $^{20}\!/_{20}$ RPS.

When Ramp Mode is selected, the user also chooses the rotation speed minimum and maximum and the ramp interval, that is, the duration from a minimum speed to the next instance of the minimum speed based on the ramping sub-mode. The minimum interval duration is 1 min with a maximum of 10 min, which can be set with 1-min precision. The total experiment duration is set in either speed mode, with the range of possible durations being 15 min to 2 days (i.e., 2880 min). Each turn of the encoder knob adds (clockwise) or subtracts (counterclockwise) time to the duration. Once completed, the user will then have the opportunity confirm all the settings, return to the previous menu, or return to the START screen if the entire experiment needs to change. Once the user confirms the settings, the motors are driven according to the user's specified settings during the RUN state. A countdown timer keeps track of timing information and the LED indicator switches from red to green to indicate the Fly Roller is running.

While the motor runs, the system can proceed to two possible states: the PAUSE state or the FINISH state. If the user presses back button, the system moves to the PAUSE state and prompts the user if the motors should be temporarily stopped. If the user presses on the encoder button, the LCD recalls the configuration of the Fly Roller during the current experiment, such as the mode, speed, and duration. Otherwise, when the experiment finishes, it will go to the FINISH state and lets the user know by printing "Exercise Over" as well as switching the LED indicator to green. From here, the system will return to the START state once any button is pressed.

59.4 Results, Discussion, and Future Work

Both versions of the Fly Roller—the initial designs and the final design—were tested in the Gibbs lab. Each version was tested using actual flies and for at least 10 h of total testing duration. Some of the issues uncovered in the initial designs were discovered during this process and led to the improvements resulting in the final Fly Roller design. This final design produced a repeatable, measurable, and consistent environment for motivating flies to exercise and measure metabolic rates.

The Fly Roller system provides a low-cost (Table 59.1), reliable experimental platform for testing metabolic rates of flies. The estimated cost for the Fly Roller can be seen in Table I, but this price would go down if multiple units were made. It includes an intuitive and user-friendly UI and precise speed/duration control. In future iterations, the Fly Roller will need to switch from regular test vials to test vials with a bearing end. These test vials would have a bearing on each end of the tube that would allow for the hose from the CO_2 measuring device to connect. This modified vial would allow for the CO_2 produced by the flies to be measured while disallowing the cables from binding and tangling due to the rotation of the test vials. Thus, the vials will enable researchers to monitor real-time metabolic changes.

Table 59.1 Fly roller parts cost

Modules descriptions	Unit cost	Qty	Total cost
Main microcontroller	4.30	1	4.30
LCD screen	7.95	1	7.95
Rotary encoder	2.14	1	2.14
Stepper motor	12.95	4	51.80
Stepper driver controller	16.08	4	64.32
Toggle switch	1.22	1	1.22
PCB	1.73	1	1.73
8 V/5 V regulators	0.75	4	3.00
Miscellaneous parts	X	X	13.5
Total			149.96

In addition to modifying the vials and their interfaces, we plan to further expand the controller unit. We will designate the existing control unit as the "leader" and then create a series of "follower" circuits that are smaller in dimension and only contain the motor controller module as well as power inputs. With these "followers," one can run more experiments in parallel using the same speed, mode, and duration settings. This expansion of the current controller unit will allow more exercise units to be run at one time per experiment.

59.5 Conclusion

In this paper, we presented the Fly Roller, a test system for exercising fruit flies and eventually measuring their metabolic rates. This test platform contains a set of mechanical rollers and a control unit - which consists of a microcontroller, motors, and UI. During the design and implementation phase, we faced several challenges in both aspects of the Fly Roller. The mechanical setup of the rollers faced challenges of non-uniform speed, static charge build-up, and binding, but the final design alleviated these issues by using copper tubes that do not require a housing for test tube placement. On the first iteration of the PCB, when the motors drew too much current, the voltage across the ATmega328p would dip below the recommended operating level causing the system to reboot back to the beginning of the code. We did not encounter this problem in the breadboarding stage as the inherent capacitance of the breadboard remediated the issue. Adding a 100 μF capacitor to the voltage regulator resolved this issue. Therefore, these decoupling capacitors were added to future iterations of the PCB.

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