

Appendix A

Annual Report 2004: FlyWorld

A.1 Introduction of 'FlyWorld'

Observation rather than experimentation dominates the study of animal behavior, limiting our understanding. We require the ability to study behavior in which aspects of an animals environment can be controlled. To meet this goal, we built a multi-chambered, biosphere in which we can control parameters to replicate and examine pertinent aspects of an animals natural environment, while precisely quantifying its behavior. 'FlyWorld' allows both controlled input manipulation and precise behavioral quantification, while capable of parallel, high-throughput analysis essential for neuro-genetic studies in the fruit fly *Drosophila melanogaster*. Whereas we built this tool for studies of the elementary decision processes in *Drosophila*, its design being both general and modular anticipates a variety of future behavioral studies.

A.2 Modular, experimental chambers

'FlyWorld' is designed to give a researcher the control and quantitative means to compare the behavior of *Drosophila* both within parallel experimental runs on a single day

as well between trials run over separate days. This tool incorporates commercially available regulated environmental incubators (capable of creating various ambient temperatures, photoperiods, and monitoring humidity) to house isolated chambers of our own design connected by tubing that may be combined in various conformations (see Fig. A.1). Automated, solenoid-driven gates and infrared sensors together control and quantify locomotor behavior between the chambers described above (see Fig. A.2). The general, modular design allows the experimental flexibility for use in a variety of behavioral studies that include parallel configurations necessary for high-throughput experimentation for anticipated neuro-genetics studies.

A.3 Dedicated circuit boards

Dedicated, programmable microprocessors incorporated in circuit boards of our own design keep computation local and thus allow a single computer the capacity to run over 900 modules (see Fig. A.3 and Fig. A.4). The Atmel ATmega8 microprocessors we use are easily programmed with any compatible Atmel programmer. Activation of the gates between chambers is controlled through H-bridges (L293DNE). And finally, additional connections are incorporated into our circuit design to anticipate future experiments. Some examples of possible inputs are surface temperature readings through a thermocouple and humidity, gas, or vibration sensors. Examples of outputs are solenoid-driven doors to release animals, uncover food, or power to drive countless other devices.

A.4 User-friendly software

The final component of FlyWorld is a home-born computer software package to query and analyze the bi-directional counts from the microprocessors as well as drive gates between chambers to a variety of control networks. For example, we can specify that either all gates or a subset may be opened or closed by a specified time, or a directional count in a specified counter block. A user-friendly GUI (graphical user interface) aids in the setup of experiments (see Fig. A.5).

A.5 High-throughput, quantitative behavioral studies

Abstract: Ethological studies on resource-emigration in the fruit fly, *Drosophila melanogaster*

A new direction for the Dickinson laboratory is to understand how the simple nervous system of the fruit fly *Drosophila melanogaster* supports sophisticated behaviors such as decision making. Specifically, we aim to study decisions involved in resource assessment, an element of habitat selection. We propose a quantitative analysis of specific external, internal, and intrinsic factors that appear to influence emigration behavior from an established food resource. The objective is to establish a research program to assist in the defining of genes and neural hierarchies involved in elementary decision processes (see Fig. A.6 for an example of preliminary results).

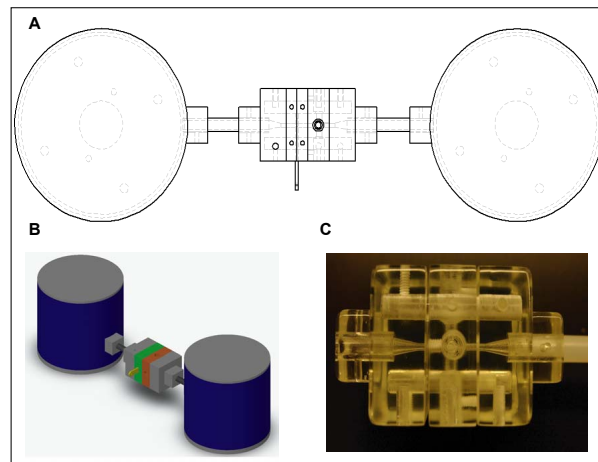


Figure A.1: Prototype components of the ‘FlyWorld’ apparatus. (A) Top schematic view of simple configuration consisting of two canisters, connector tubing, detector, and gate. (B) CAD-rendered view of configuration drawn in A. (C) Photograph of prototype detector block fabrication from stereo lithography.

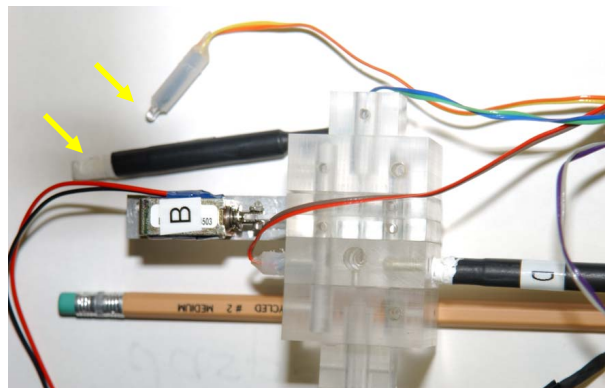


Figure A.2: Photograph of detector block and solenoid-driven gate. One of two infrared LED emitter and infrared photodiode detector pairs (arrows) that make up the bi-directional counter has been removed and displayed along the detector and gate unit.

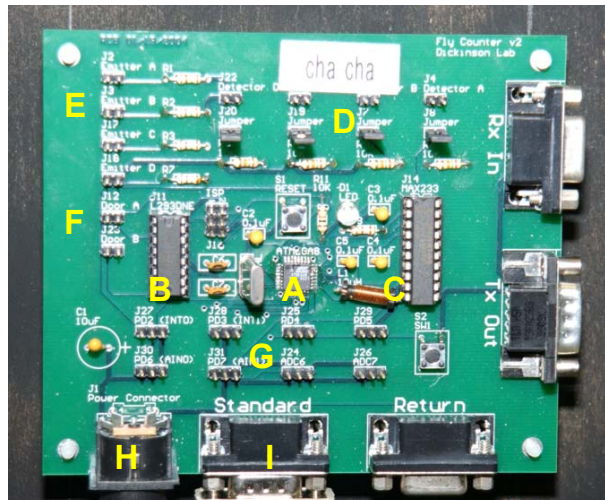


Figure A.3: Photograph of the circuit board with key components identified. (A) Atmel microchip, (B) H-bridge to drive gates, (C) inductor/capacitor act to filter oscillations in power required for the analog to digital converter needed to read signals from infrared detectors, (D) detector jumpers, (E) emitter lines, (F) gate activation lines, (G) extra connections for future experiments, (H) 12V and 5V power input, and (I) serial ports to connect circuit board to computer.



Figure A.4: Photograph of the FlyWorlds in a room with temperature and photoperiod-control. The parallel, two chamber configuration is used for experiments described within this report.

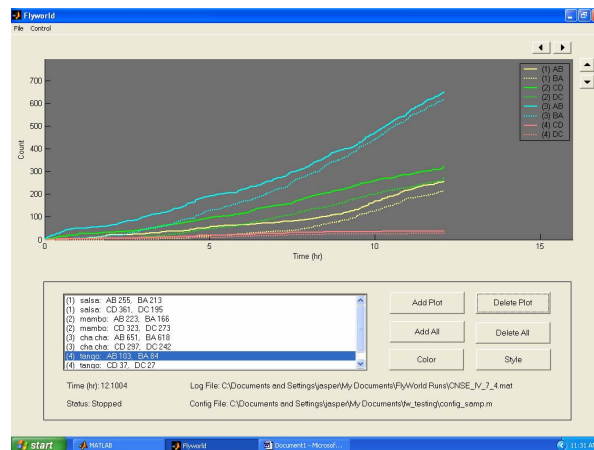


Figure A.5: A computer “screen shot” of our software’s application window. A reader can see pull-down menus and toggle buttons that enable the experimenter to modify experimental parameters. Here we test the influence of food deprivation and genetic variation on the baseline locomotor behavior of 50 flies per experiment between two chambers (“blue” and “red” traces are a natural fly isolate collected from Chicago, IL deprived of food for 12 or 36 hours respectively; the “green” and “yellow” traces are two distinct isolates collected by others from Toronto, Canada and deprived of food for 12 hours).

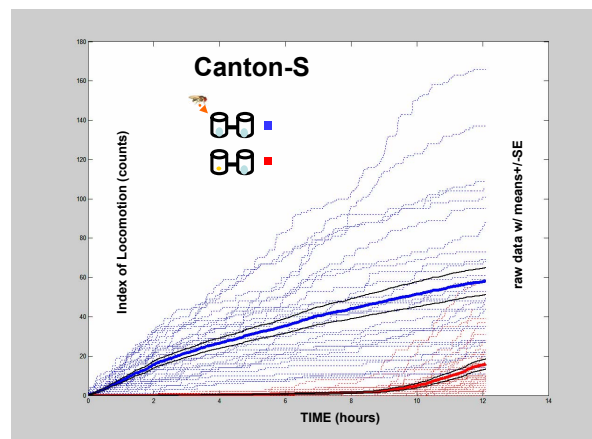


Figure A.6: An example of the results where we establish emigration baselines by manipulating various experimental parameters. Shown above is the baseline locomotor behavior from a first to a second chamber ≈ 50 12-hour food-deprived flies (blue) as compared to the locomotor behavior of ≈ 50 12-hour food-deprived flies introduced to a first chamber that contains a small food resource (red). Here we examine Canton-S the widely-used fly stock from which many molecular-genetic tools have been derived. We manipulate the amount of food resource to be consumed over the time course of the experiment. While initially the flies stay in the first chamber, eventually they become food-deprived and emigrate to the second chamber presumably in search of food.