"Dover" wild-type strain. Single Dover males were crossed with a balanced lethal marker strain (below), to generate isogenic lines for chromosomes I, II and III. BN and BN' designate pairs of balanced marker chromosomes for chromosome number one, two, or three, and Y represents the Y-chromosome:

Cross 1:   B1/B1'; B2/B2'; B3/B3'   ×   +/Y;   +/+;   +/+ (Dover male)

Cross 2:   B1/+;   B2/+;   B3/+   ×   B1/Y; B2/B2'; B3/B3'

Cross 3:   B1/+;   B2/+;   B3/+   ×   +/Y;   B2/+;   B3/+

Isogenic: +/+;   +/+;   +/+   ×   +/Y;   +/+;   +/+

B1 = FM7 *B g v sn w sc y*
B1'= *y pn cv m f*
B2 = SM1 *sp cn Cy al*
B2'= *Pm*
B3 = TM3 *Sb Ser e bx sep p ri*
B3'= TM2 *e Ubx*

        The original Dover wild-type strain and four surviving isogenic lines (53, 60, 63, and 67) were maintained at the University of Iowa from 1978 to 1983 when they were moved to Skidmore College, Saratoga Springs, NY, USA, and maintained by mass culturing in two to five shell vials per strain. Cultures have been maintained on open shelves at an average temperature of approximately 21°C in a room with a 16:8LD photoperiod until 1995 and a 12:12LD photoperiod thereafter. Occasionally, if the population size falls below 10 flies in a vial, flies are transferred from another vial of the same strain to maintain viability of the culture. The Dover isogenic lines have been used to characterize genetic background effects modifying expression of *Antennapedia* (Possidente *et al*., 1990), genetic variance influencing oviposition site preference with respect to the presence of phenylthiocarbamide in the food (Possidente *et al*., 1999), genetic variation and covariation influencing developmental rate (Norton, 1985), and genetic variance affecting latency to copulation in response to developmental lead exposure (Wilson, 2004). The Dover wild-type strain and the four derived isogenic lines are available upon request from B. Possidente.
        References: Norton, C.G., 1985, Ph.D. Thesis. The Timing of Developmental Events in *Drosophila*: Genetic Variation and Covariation, University of Iowa; Possidente, D.R,, J. Greaux, and B. Possidente 1990, Heredity 65: 321-327; Possidente, B., M. Mustafa, and L. Collins 1999, Behavior Genetics 29: 193-198; Wilson, D., 2004, Ph.D. Thesis. The Development of *Drosophila* as an Animal Model for Studying the Behavioral Genetics of Lead Toxicology. State University of New York at Albany.

Assaying basal and ethanol-induced locomotion in flies using a custom built apparatus and freely available software.

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        Wolf *et al*. (2002) developed an apparatus that can be used to monitor the locomotor stimulant and depressant responses to ethanol in fruit flies. In this assay, approximately 20 flies are loaded into each of 8 tubes and placed into a custom-built, horizontal tube holder though which air or vaporized ethanol is perfused. An overhead video camera connected to a computer is used to record the behavior of the flies before and

during the exposure, and locomotor activity is quantified offline using commercially available DIAS software (Solltech, IA). In implementing this apparatus and protocol in my own lab, I wished to retain its essential feature – the ability to track multiple samples of flies before and during an ethanol exposure – while improving on several aspects, including subject throughput, temperature control, and the ability to present air and ethanol simultaneously to separate groups of flies. The design of this apparatus and the analysis of the data by freely available software are described in this article.

Figure 1. The ethanol delivery system and locomotor activity apparatus. A, air pump; B, gas washing bottles; C, dose selector; D, valve bank; E, incubator; F, computer. Details of each component are provided in the text and other Figures.
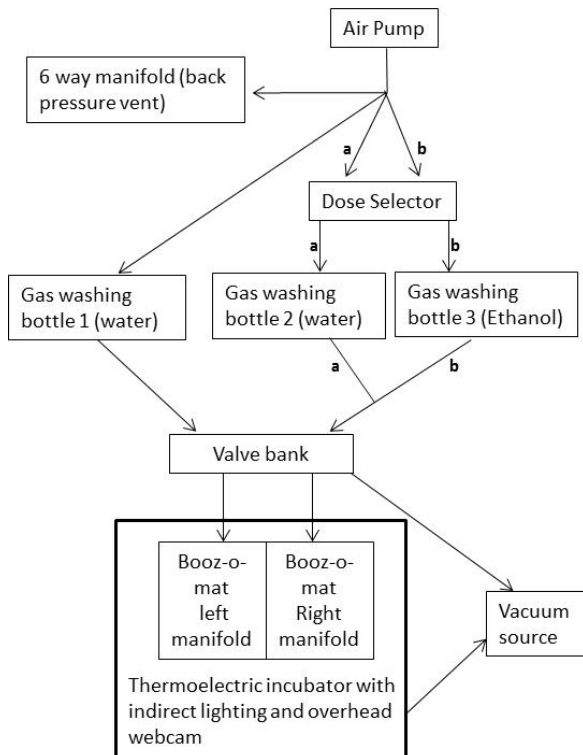
Ethanol Vaporization

The modified apparatus consists of two main parts: a vapor delivery system and a behavioral observation area. Figure 1 shows the components of the apparatus, and Figure 2 shows a schematic diagram of the connections. The parts of the vapor delivery system include a commercial grade aquarium pump used as a source of compressed air (Alita Model AL-40), a custom built diverter valve for controlling ethanol dose, three gas washing bottles (Bel-Art Scienceware #110300000), and a bank of valves used to direct the air and ethanol streams as needed.

As indicated in Figure 2, an air stream from the pump is divided into 3 streams: one stream is directed to an adjustable, 6-way gang manifold and is used to vent back pressure; a second stream is directed to gas washing bottle that contains water (bottle 1 in Figure 2) and then to the valve bank (Air stream). The third stream (ethanol stream) is split again, and one half directed towards a water-containing gas washing bottle (bottle 2 in Figure 2) and the other stream to an ethanol-containing gas washing bottle (bottle 3 in Figure 2).

Figure 2. Schematic diagram of the ethanol delivery system and locomotor activity apparatus. Each line represents 3/8" ID tubing, and the direction of the air flow is indicated by the arrows. The dose is selected at the Dose Selector by changing the proportion of air flowing through stream 'a' relative to stream 'b.' Air or ethanol can be directed independently to the left or right manifolds, or concurrently to both manifolds.

Figure 3. The dose selector was built from construction grade lumber and plywood. Two 3/8" ball valves are mounted orthogonally to each other to form a constant output diverter valve. The red handles of these valves are visible in this image. The wooden dowel was secured to both handles with two bolts and a zip tie.

In the apparatus described by Wolf *et al*. (2002), ethanol dose is changed by way of calibrated flow meters, which are used to control the amounts of air directed to an ethanol-containing flask and a water-containing gas washing bottle. The greater the amount of air sent to the ethanol-containing flask, the greater the ethanol concentration downstream. Because of the cost of these meters, a custom built diverter valve (the dose selector) that functions similarly to the flow meters was constructed. The dose selector (Figure 3) consists of two orthogonally mounted 3/8" brass ball valves connected by the handles and attached to a rigid support. A single wooden dowel attached to both of the handles serves as a lever for operating the valves and as the dose indicator. Different ethanol doses are produced by varying the proportions of the air stream sent to two gas washing bottles, one of which contains water and the other contains absolute ethanol (bottles 2 and 3, respectively, in Figure 2). Because the valve handles are connected orthogonally to each other, changing the amount of air flow to bottle 2 inversely and proportionally changes the air flow to bottle 3, allowing for a continuous range of ethanol doses to be produced. The dose is read on an attached scale that ranges from 0 to 90 degrees, which can then be converted to a percentage that reflects the amount of the air stream downstream from the gas washing bottles that carries vaporized ethanol. Downstream from washing bottles 2 and 3, the split streams are unified and sent to the valve bank.
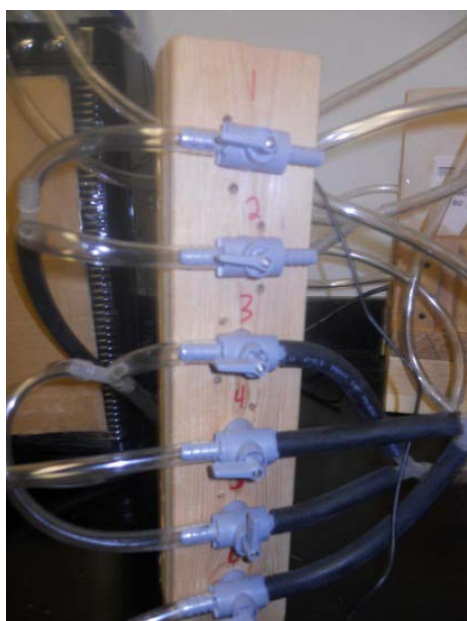

Figure 4. The valve bank consists of a length of lumber into which 6 three-way diverter valves are mounted, and is used to direct the flows of air and ethanol to the left or right manifolds in the testing apparatus. The two barbs visible on each of the six valves were used to direct the output to another valve or to the locomotor apparatus, as appropriate. The third barb on each valve (not visible in the image above) received the input.

The valve bank (Figure 4; see Figure 2 for air flow diagram) consists of six 3-way valves that are used to route the air and ethanol streams to the behavioral observation apparatus. The valve bank functions by splitting the air and vaporized ethanol-containing streams into half, then sending one half of each stream to either the left or right manifold of the testing apparatus. To avoid the difference in flow rate that will result when delivering air or ethanol to a single manifold compared to both manifolds, the valve bank was connected to a vacuum source (a faucet aspirator), which is used to vent a portion of each stream when air and

ethanol are presented concurrently. Three-way valves were used in order to allow the air and ethanol streams to be sent to either the locomotor activity apparatus or to a second apparatus not described here. Two-way valves could be used instead with a slightly different connection configuration; quick connectors could also be used if a fume hood is used to vent the ethanol.

Behavioral Observation Apparatus

        The components of the behavioral apparatus consist of two 10-way aquarium manifolds (Sunlight Supply) that can receive an air or ethanol-saturated air stream from the valve bank, a custom built tube holder that accommodates 20 horizontally arrayed tubes, and a temperature controlled reptile egg incubator (Exoterra, PT2499), into which a webcam (Agama V-1325R) and LED panel light (Bescor LED-70) were mounted (see Figure 5). The tube holder measures 6" × 7" overall, and was made from low-glare, translucent polypropylene divided into 10 slots, each 6" × ½", to accommodate the barb spacing on the 10-way manifolds. The barbs were wrapped with PTFE tape, and ¼" ID polypropylene ID acorn nuts, predrilled with three small holes to allow air flow, were threaded onto the barbs. During testing, flies are tapped into the tubes, which are then slid onto the rounded end of the acorn nut.

        Two one-half inch holes were drilled through the walls of the incubator to connect the valve bank to the manifolds. A vacuum line connected to a faucet aspirator was inserted into the incubator by way of a third hole drilled into the upper left wall of the incubator. The webcam and light panel were attached to predrilled angle iron connected to one wall of the incubator with bolts, creating an adjustable overhead rigging. The cables for the camera and light were run through a separate hole drilled through the incubator wall.

Figure 5.   The behavioral apparatus is housed in a thermoelectric incubator containing two manifolds: A, fitted with nylon acorn nuts that fit into the mouths of disposable test tubes; B, used to contain the flies. The tubes are placed horizontally into a custom-built holder in the bottom of the incubator. A LED light panel, C, and webcam, D, are attached to an overhead angle iron rigging, E.   The cables for these components exit the incubator through a hole in the upper right corner of the incubator.
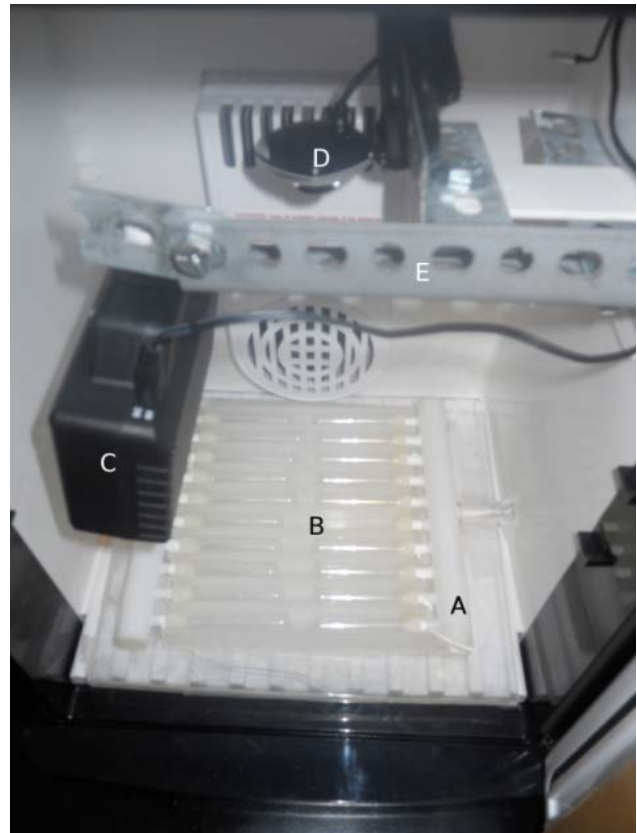
Video Recording and Analysis

        A webcam (Agama V-1325R) connected to an attached PC running Mint Linux is used to record the experimental sessions. Videos are saved in avi format using GUVCView (http://guvcview.sourceforge.net), which can be configured to record at various frame rates and resolutions. For the experiments conducted so far, a resolution of 640 × 480 at 10 FPS has been adequate to capture the activity of the 5-10 flies per tube that are typically tested. Jpg frames are extracted from the videos using Avidemux (fixounet.free.fr/ avidemux) or ImageJ (imagej.nih.gov/ij).

        We have used two methods to analyze locomotor activity in this apparatus. The first method uses the procedures and Perl scripts developed by Ramazani *et al*. (2007). Briefly, the "nested_window" script is used to generate a series of images for each user-defined zone, such that movement that occurs between subsequent frames is depicted as white pixels on a black background for each zone. For the apparatus described above, there are 20 zones. A second script, "quantify630," then counts the number of white pixels that are above a user-defined intensity level for each zone, generating a text file oriented with the zones ("cells") as columns

and the locomotor activity that occurs from one frame to the next in rows. In the standard 28 minute videos at 10 FPS collected in my lab, 16,800 jpgs are generated, which results in 16,799 rows × 20 columns outputted by the script. A third perl script is then used to bin the data outputted from the "quantify630" script into more manageable chunks.   Baseline activity, area under the curve scores, and other descriptive data are then calculated from these extracted data. The data outputted by the quantify630 script can also be copied and pasted directly into a spreadsheet for analysis, although this operation can be extremely unwieldy if a large number of frames is analyzed.

The second method uses the "MTrack3" plugin for ImageJ. Given a sequence of frames, this plugin detects all objects (subjects) above a user-defined threshold and assembles tracks for each object across frames as a series of time-stamped x-y coordinates. Similar to the commercially available DIAS, empirically-derived values are used to specify the size of the objects to be tracked, the minimum number of frames a single object must be tracked, and the maximum distance that a single object can move from frame to frame and still be counted as the same object. We run MTrack3 in a macro that imports a user-defined number of frames according to a user-defined array of time points (*e.g.*, frames 1-200, 201-400, 401-600). This macro analyzes all of the experiments contained in a directory and outputs the data into separate directories corresponding to the names of the experiments. These data are analyzed using an Awk script, which groups individual object tracks by tube, calculates the total distance moved by all of the flies in a tube at each time point, and divides the total distance by the number of objects and frames that each object was tracked.

While these two methods of analysis produce comparable results under most conditions, the underlying methods of analysis are different and can produce different results. We have found that the nested_window script can produce unexpected results when comparing subjects that differ in body size, such as can occur when comparing strains or male and female flies. This occurs because larger flies will necessarily produce more white pixels from frame to frame than will smaller flies, resulting in larger flies showing more apparent movement than smaller flies between any two frames. To correct this, observed distances traveled can be corrected for body weights by division or analysis of covariance. In contrast, the Mtrack3 plugin calculates movement as the location of the centroid of an object in one frame compared to the location of the same centroid in the next frame. Consequently, this method seems to be less sensitive to differences in body size of the subjects. The scripts and macros used for either of these analyses are available upon request.

References: Ramazani, R.B., H.R. Krishnan, S.E. Bergeson, and N.S. Atkinson 2007, J. Neurosci. Methods 162: 171–179; Wolf, F.W., A.R. Rodan, L.T.-Y. Tsai, and U. Heberlein 2002, J. Neurosci. 22: 11035–11044.

Co-injected ΦC31 transgenes frequently produce **multiple independent germline transformation events in a single *D. melanogaster* embryo.**

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Introduction

Injecting *Drosophila melanogaster* embryos with P element or ΦC31 plasmid vectors to generate single-copy transgenic fly lines is a common and well-established procedure (*e.g.*, Rubin and Spradling, 1982; Bischof *et al.*, 2007). Today, many labs employ outside injection services, which charge by the construct, to