Experimental setup for network based analysis of Drosophila melanogaster groups

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ABSTRACT

This protocol describes the breeding procedure of Dtosophila melanogaster for the purpose of studying group behavior. The steps of fly cultivation on a cornmeal medium and preparation of food medium are described. Then the procedure of anesthetization with CO2 and the division of the population into a baseline and an experimental population are explained. It is explained how to add psychostimulants to the food in order to study changes in social behavior. The last part describes how to record fly behavior in the arena, how to set up the arena, and how to extract the position of individuals over time from the video recordings and build the network to be analyzed.

1 Cultivation of flies on cornmeal media without separation male from female. 5d

1.1 Cornmeal food

Cornmeal food is used for breeding large amount of flies repeatedly collected and used during several days in road.

TABLE 1. Ingrediens for different final volumes of cornmeal food.

	Volume			
Ingredient:	1 L	750 mL	500 mL	200 mL
Mixture A - pot				
Tap water	1000 mL	750 mL	500 mL	200 mL
Table sugar*	79,3 g	59,48 g	39,65 g	15,86
Agar Type I**	8,5 g	6,4 g	4,25 g	1,7 g
Mixture B - beaker				
Tap water	400 mL	300 mL	200 mL	80 mL
Yellow cornmeal*	72 g	54 g	36 g	14,40 g
Dry yeast*	50 g	37,5 g	25 g	10 g
Before finishing				
Evaporation-tap water	200 mL	150 mL	100 mL	40 mL
Propionic acid**	8 mL	6 mL	4 mL	1,6 mL
Ethanol solution of nipagine***	15 mL	11,25 mL	7,5 mL	3 mL

*

Tablesugar we used is a product available in Croatia https://www.ppkbjelovar.com/secer-1kg-premijer.aspx.

Yellow cornmeal we used is a product available in Croatia https://okusizavicaja.hr/proizvodi/kukuruzno-brasno/.

Dry yeast we used is a product available in Croatia https://encian.hr/suhi-pivski-kvasac-250g.

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Agar Type I –https://geneseesci.com/shop-online/product-details/66-103/flystuff-66-103-nutri-fly-drosophila-agar-gelidium-100-mesh-5kg-11.02lbs-unit

Propionic acid – https://www.sigmaaldrich.com/HR/en/product/sigald/p1386

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Ethanol solution of Nipagin is 15% solution made by dissolving 15 g of p-hydroxybenzoic acid methyl ester in 100 mL of 96% ethanol.

Nipagine or p-hydroxybenzoic acid methyl ester = https://www.carlroth.com/com/en/aromatic-building-blocks/4-hydroxybenzoic-acid-methyl-ester/p/3646.1

Ethanol 96% = https://www.grammol.hr/ETANOL/Etanol-denaturirani-96--11_1

Steps in cooking procedure for cornmeal fly food:

1. Measure 1 L of tap water and pour it in stainless steel pot. Add sugar and agar (**Mixture A**).
2. Put pot with ingredients on induction plate, set on 6-7 and cook it until point of boiling.
3. In clean beaker mix together cornmeal and dry yeast and after that add 400 mL (in case of 1L) of tap water (**Mixture B**).
4. Add **Mixture B** in the pot. Cook on point of boiling for 10 minutes. Because of water evaporation, add additional 200 mL (in case of 1L) of tap water. Stire food every 1 minute.
5. Take fly food from induction plate. Add propionic acid and nipagin according to prepared final volume of cornmeal food.
6. Take pipettor and sterile plastic pipette of 50 mL. Pore 35-40 mL of fly food in each plastic bottle***. Cover with cotton gauze and leave to cool until morning (Figure 1.).
7. Wash all materials used for preparing of fly food and area in which food was cooked. If necessary, pass over all surfaces with 70% ethanol.
8. Next day plug plastic bottles with plug****.
9. Transfer food from room temperature to cooler @ 4 °C to preserve it before further usage.

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Plastic bottles and plugs = Flystuff 32-130BF 6oz Square Bottom, Polypropylene, w/ Flugs for Stock Bottles, 2000 Bottles & Closures/Unit. https://geneseesci.com/shop-online/product-details/32-130BF/flystuff-32-130bf-6oz-square-bottom-polypropylene-w--flugs-for-stock-bottles-2000-bottles--and--closures-unit

Freshly prepared cornmeal fly food.

1.2 Fly breeding

Flies are kept in incubator with cooling @ 18-25 °C, humidity 60-75% with light-dark cycles, 12 hours of dark from 8pm to 8am, and 12 hours of light from 8am to 8pm (Figure 2.). In case that you want to breed flies in the optimum condition of 25°C and 60% humidity generation time is 9-10 days from egg to adult, while in lower temperature such as 18 °C generation time is longer. Higher temperatures increase aging, useful when in experiment one wants to have old flies and compare it to young. In the case that you do not have automatic setting of humidity in the incubator, a container with distilled water can be placed, which will ensure the necessary humidity, which is then controlled from the inside. Same it is with light and dark control. It is possible to buy timer and LED strip, and place it in the incubator in the way that LED strip is attached to the inside of the incubator, while the lighting is controlled via timer connected to socket inside the incubator.

Incubator with cooling = https://www.pol-eko.com.pl/model/cooled-incubators-st/cooled-incubators-st-4/

Image of inner space of incubator with bottles and cornmeal food (left image) or vials and molasses fly food (right image).

1.3 Molassesfood

Molasses food is used for preserving a small amount of stock flies especial mutants, flies with balancers, USA-Gal4 lines and wild type not commonly used in the lab (Berlin, W1118 or OregonR). This food is suitable for different type of supplements which flies than consume trough food.

Table 2. Ingredients for different final volumes of molasses food.

	Volume			
Ingredients:	1 L	750 mL	500 mL	200 mL
Tap water	970 mL	727 mL	485 mL	194 mL
Molasses *	60 mL	45 mL	30 mL	12 mL
Table sugar*	15 g	11,25 g	7,5 g	3 g
Dry yeast*	35 g	26,25 g	17,5 g	7 g
Agar Type I **	12 g	9 g	6 g	2,4 g
Propionic acid**	7,5 mL	5,63 mL	3,7 mL	1,8 mL
Ethanol solution of nipagin***	7,5 mL	5,63 mL	3,7 mL	1,8 mL

*

Molasses we used is a product available in Croatiahttps://www.tvornicazdravehrane.com/melasa-secerne-trske-organska-450g-nutrigold-proizvod-54431/

NOTE: Molasses is very viscose so in the lab is used as 50% solution of molasses. This means that in 250 mL of pure molasses was added 250 mL of water.

Tablesugar we used is a product available in Croatia https://www.ppkbjelovar.com/secer-1kg-premijer.aspx.

Dry yeast we used is a product available in Croatia https://encian.hr/suhi-pivski-kvasac-250g.

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Agar Type I –https://geneseesci.com/shop-online/product-details/66-103/flystuff-66-103-nutri-fly-drosophila-agar-gelidium-100-mesh-5kg-11.02lbs-unit

Propionic acid – https://www.sigmaaldrich.com/HR/en/product/sigald/p1386

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Ethanol solution of Nipagin is 15% solution made by dissolving 15 g of p-hydroxybenzoic acid methyl ester in 100 mL of 96% ethanol.

Nipagine or p-hydroxybenzoic acid methyl ester = https://www.carlroth.com/com/en/aromatic-building-blocks/4-hydroxybenzoic-acid-methyl-ester/p/3646.1

Etnalo 96% = https://www.grammol.hr/ETANOL/Etanol-denaturirani-96--11_1

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Plastic vials with plug = Flystuff 32-117BC Wide Vials, Bulk Pack (PS), w/ Cotton Balls, Extra Large, 2000 Vials & Closures/Unit https://geneseesci.com/shop-online/product-details/32-117BC/flystuff-32-117bc-wide-vials-bulk-pack-ps-w--cotton-balls-extra-large-2000-vials--and--closures-unit

Steps in molasses fly food preparation:

1. Measure 1 L of tap water and pour it into a stainless steel pot. Add agar and mix in the way to prevent forming of the blobs.
2. Put pot on induction plate, set it on 6-7 and cook it until point of boiling. Add molasses, sugar, dry yeast, mix (Figure 4. Left image) and set induction plate to 3 and cook for 15-20 min. Mix food every 1 minute.
3. Take fly food off induction plate. Wait 30 minutes to cool down and add propionic acid and nipagin.
4. Take pipettor and 50 mL sterile plastic pipette. Pore 10 mL of molasses food in each plastic tube (Figure 4. Right image). Cover with cotton gauze and leave to cool and solidify until next day.
5. Wash all appurtenance and accessorize used for preparing of molasses food and clean area in which food was weighed, cooked and pour. If necessary, pass over all surfaces with 70% ethanol.
6. Next day plug plastic tubes**** with cotton balls. Place plastic tube in additional plastic bag to prevent food dehydration.
7. Transfer food from room temperature to cooler @ 4 °C to preserve it before further usage.

Preparation of mol(right image) Freshly prepared cornmeal fly food.

1.4 Molasses food supplemented with 0.50 mg/mL cocaine hydrochloride

First, a stock solution of cocaine hydrochloride is prepared by dissolving 100 mg of cocaine in 1 ml of distilled water. The stock solution must be stored in the refrigerator at +4 °C. To prepare 0,50 mg/mLcociane contain food take 50 µL aliquot of the cocaine stock solution and transfer it to a plastic vial. Add 10 ml of liquid freshly prepared molasses food to it and vortexed for 30 seconds. Leave food to cool down and solidify. Transfer flies that have recovered from anesthesia to the vial containing 0,50 mg/mL cocaine in molasses fly food.

Cocaine hydrochloride = https://www.sigmaaldrich.com/HR/en/product/sigma/c5776

1.5 CO2 anesthesia

Carbon dioxide (CO2) has traditionally been the anaesthetic of choice for invertebrate species. CO2 provides rapid inductions and recoveries. Flies should not be longer than 5 minutes at CO2 pad, since it was reported that long-lasting episodes of CO2 anaesthesia could have effect on growth, maturation and reproductive parameters.

Anaesthesia protocol. First knockdown flies with CO2 using blowgun (left image). Transfer flies to CO2 pad and separate (right image).

Steps in CO2 anaesthesia:

1. Take bottle with stock line of flies from incubator and flip flies form bottle to empty vial. Clog the vial with cotton ball.
2. Open CO2 tank and press on at Flowbuddy Flow Regulator* to enable CO2 flow from tank to pad placed under microscope and blowgun (after pressing the lever).
3. Take blowgun* and place tip of it inside of vial containing flies.
4. Press the lever on blowgun to start CO2 flow and wait around 5 seconds. Remove blowgun (Figure 3. Left image).
5. Transfer flies from vial to pad* which provide constant flow of CO2 so flies will not escape during separation (Figure 3. Right image).
6. Start to separate flies.
7. Transfer separated flies in new cultivation vial in the way that the flies lie on the walls of the plastic vials so that they do not stick to the food until recovery from anesthesia.

*
Flystuff 59-122WCU Wall-Mount Flowbuddy Complet, With Ultimate Flypad, 1 System/Unit
Flystuff 59-119 FlyStuff Flypad, Large, CO2 Anesthetizing Apparatus, 1 Flypad/Unit
Flystuff 54-104 Flystuff Blowgun, CO2 Anesthesia Accessory, 1 Pistol/Unit

2 Arena for video recording

Arena is 3 millimeters high to restrict the movement in two dimensions, and 120 millimeters in diameter to allow
movement of the flies . The bottom is white translucent Plexiglas back-lit illuminated with white LED strip, and top transparent Plexiglas cover with height of 2 mm. In the
arena_blueprint.pdf the dimensions needed to construct the arena can be found.

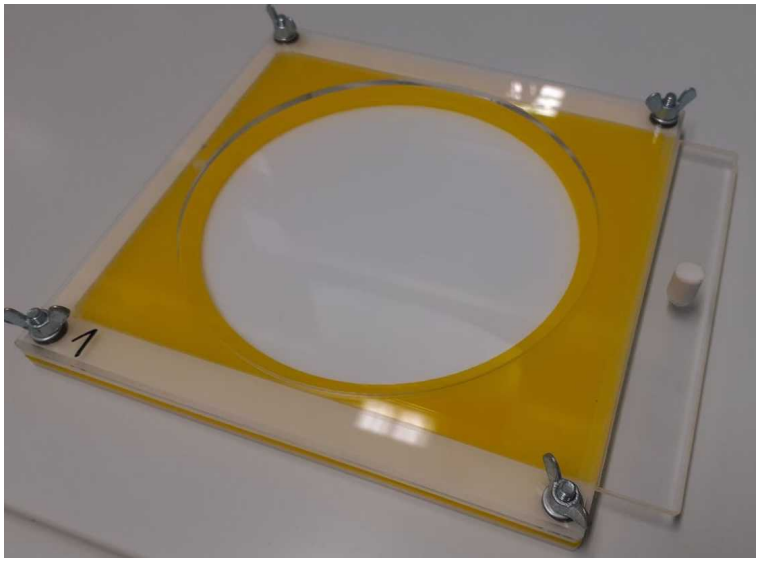


Image of the arena used for experiment recording

Camera for experiment recording:

Equipment	
Logitech C920HD Pro	NAME
camera	TYPE
Logitech	BRAND
1234	SKU
1920 x 1080 pixels video resolution and 24 frames per a second	SPECIFICATIONS

file

3 Transfer flies from cultivation vials to the arena using aspirator

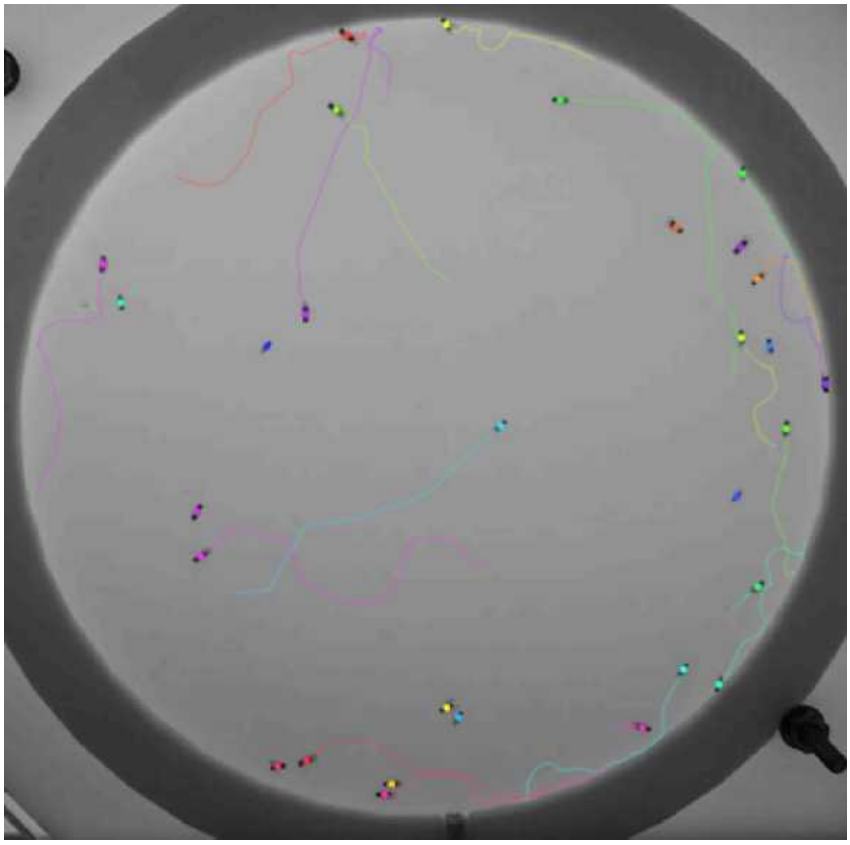
4 15m

Habitation flies in the arena

5 Record flies in the arena

Frame from the video of experiment

6 Track recorded video



Visual display of tracking within the video, where each fly is assigned a unique identity

7 Preprocess .csv data and convert track data to networks

https://github.com/milanXpetrovic/my_module