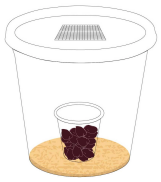
Jun 20, 2024

Protocols for entomotoxicological developmental study of Phormia regina (Megnin)



DOI
dx.doi.org/10.17504/protocols.io.n2bvjnrpngk5/v1

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DOI: dx.doi.org/10.17504/protocols.io.n2bvjnrpngk5/v1

Protocol Citation: Hayden S. McKee-Zech, Charity Owings 2024. Protocols for entomotoxicological developmental study of Phormia regina (Megnin). **protocols.io https://dx.doi.org/10.17504/protocols.io.n2bvjnrpngk5/v1**

Protocol status: Working
We use this protocol and it's working

Created: April 01, 2024

Last Modified: June 20, 2024

Protocol Integer ID: 97615

Keywords: Phormia regina, entomotoxicology, development, blow fly, development, growth rate, morphine, lorazepam, drugs, pharmaceuticals

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Abstract

This protocol was developed specifically for examining the impact of drug exposure on forensically important phenotypes (i.e., developmental duration, size, survivorship) of larval blow flies. This protocol was implemented using a laboratory colony of the black blow fly, *Phormia regina* (Meigen), a common and abundant blow fly in the eastern and southeastern United States. The end goal of this protocol is the generation of drug-specific developmental datasets that can be used for estimating larval age, which can be used in death investigations to then infer a minimum postmortem interval.

Attachments

XLSX

Development Protocol...

10KB

Image Attribution

Image depicting experimental housing created by Graycen C. McKee
Experimental image included in this publication were taken by Hayden S. McKee-Zech

Materials

Phormia regina fly colony
32oz plastic deli cups
3oz plastic cups
1oz plastic cups with lids
rubber bands
6"x6" paper napkins
aluminum trays with min of 3"sides
autoclaved sand
sharpie marker
colored tape to differentiate treatments
drugs of choice/decomposition fluid
lean pork
kemwipes
chicken liver blood castoff
paintbrushes
tally counter
flour sieve
plastic tub
labels
pencil
electric tea kettle
distilled water
plastic bin 12"x9"
EtOH 70%
cryovials
liquid nitrogen
20 ml scintillation vial
freezer (0C)
freezer (-80)
bullet blender - cup and base 1 per treatment
micropipette and tips
stereomicroscope + camera
microscope scale calibration

Safety warnings

Refer to your university guidelines for working with potentially hazardous material and Blood Borne Pathogen (BBP) protocol. This work was performed in a Biohazard Safety Level (BSL)-2 laboratory.

Ethics statement

The use of invertebrates in research activities does not require IACUC oversight.

Before start

1) Growth chamber or laboratory space with a fixed recorded temperature and light:dark cycle is needed.
25C temperature
70% RH
12:12 L:D cycle

2) Printing labels for each step of the experiment that can be filled in prior to starting each step with speed the process along immensely.

Entomotoxicology laboratory experiments have limitations:
1) Inject a single drug into excised tissue after death, neglecting metabolization and thus resulting in a scenario where drug interactions are not comparable to those that would be observed in real-life.
We suggest utilizing treatments that examine a single drug, a single metabolite of that drug, mixture of the parent drug and metabolite, as well as, a mixture of different drugs.
2) These experiments are closed systems and do not allow new energy or matter to enter the system, thereby presenting a single predictable, inevitable solution. An open system, such as field experiments, consistently gain and lose energy and matter.
We suggest performing toxicological screening on decomposition fluid of humans and using this fluid the same as you would a drug mixture to preform experiments.
3) Postmortem redistribution - diffusion, neoformation and metabolism
This is an important factor to consider but a true limitation of a development study of this nature.
Metabolomic analysis may help elucidate these interactions in experiments.

Colony Establishment

1 Fly colonies should be established using local populations of blow flies. Adult flies can be baited with aged organ or muscle tissue, or they can be collected from a vertebrate carcass, refuse, or feces. Flies should be collected with an aerial sweep net, sorted to the desired species, and placed in a BugDorm or other enclosure specifically for insects. Flies can be maintained at ambient conditions in a laboratory and given sugar, water, and cultured buttermilk *ad libitum*. To ensure sufficient genetic diversity and to minimize the risk for genetic drift within the colony, wild fly collections should span multiple timepoints and the total founding generation (G0) should be ~200 individuals.

Egg collection

2 Fly colonies should be presented with a fresh protein source daily for ~1 week prior to the start of experiments. For this experiment, we exposed colonies to a kimwipe soaked in chicken blood in a 3 oz bath cup each day.

3 24 - 48 hours prior to the start of experiments, provide the colony with ~5g chicken liver and a kimwipe soaked with chicken blood in a 3 oz cup.

4 Check for eggs every 3 hours

5 Once eggs are observed, remove them from the cage and record the time.

6 For each observation period, place eggs on ~10g lean pork muscle and incubate under ambient conditions.

7 Check for egg hatch after 8 hours and then hourly thereafter.

Rearing substrate set-up

8 Weigh out 200g of lean pork.

9 Blend pork in treatment specific blenders for 45 seconds.

10 Add treatment specific drug to lean pork.

11 Blend pork in treatment specific blenders for 1 minute.

12 Divide blended pork into 4 individual pre-weighed 3oz bath cups with 50g of drugged pork in in each.

Note

Record the exact weight of the 3oz bath cup and the drugged pork using a balance.

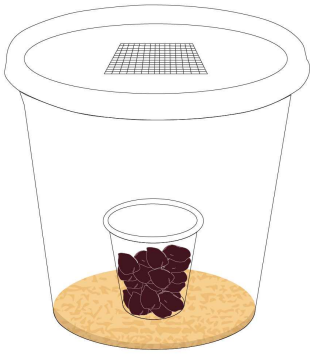
Replicate set-up

13 Use a camel-hair paintbrush and a hand counter to add 100 individual larvae onto each replicate. Record the time when complete.

14 Place the 3oz bath cup in 3" of sterile sand within a 32oz deli cup and cover with a paper napkin and 2 rubber bands.

Note

See image of experimental housing under description



Experimental housing example

Data collection - Larvae

15 Observe the development of larvae every 8 hours and record results until wandering larvae are observed

We sampled at:
12:00 AM
8:00 AM
4:00 PM

16 Record behavior of larvae in each replicate at every observation. Do not disturb the pork, only record what you can visibly see. Record any visible larval masses, dead or presumed dead larvae observed in the cup and movement/behavior of larvae.

17 Once larvae are observed dispersing from the resource, remove feeding cup and sieve sand through a flour sieve (40mm mesh) into a cleaned plastic bin.

17.1 Record the number of wandering larvae present.

17.2 Collect 50% of the wandering larvae present in a labeled 20ml scintillation vial, add boiling water to kill, then strain off water and add 70-85% ethanol.

Note

Two labels should be included with preserved samples: one written on the vial cap and one written on paper within the vials. The following information should be present on the labels:

Treatment:
Replicate:
Date:
Collection time:

17.3 Label the cryovial with the same information from previous vial.Take a 10% sub-sample of wandering larvae for metabolomic analysis via UHPLC-HRMS.
Freeze samples at -80 °C using liquid nitrogen in a cryovial.

Note

Label the cryovial with the same information from previous vial.

18 Return sieved sand and feeding cup to the replicate

18.1 Replace paper napkin and rubber bands

19 Wash plastic 12"x9" bin used for sand cast off with distilled water and dry before sampling the next replicate/treatment

Data collection - Diet

20 After all larvae have dispersed away from the pork, take a final mass of the pork using an analytical balance and record the date and time.

20.1 Freeze remaining pork for metabolomic analysis via UHPLC-HRMS.

Data collection - Pupae

21 Once pupae are observed during the sieving process, use soft forceps to place each individual pupa into 1oz plastic cups with lids and appropriate labels.

Note

Label each lid with:

Treatment:
Replicate:
Date:
Sampling collection time:

21.1 After collecting all pupae from all treatments during the sampling period, immediately weigh each individual pupa to the nearest microgram (0 µg).

Note

Pupa must be weighed shortly after forming as the duration of pupation may be shorter than anticipated due to the drug treatment.

21.2 Check for adult eclosion every 8 hours and record results observed

21.3 Record the emergence date and time for each individual.

Data collection - Adults 2h

22 Allow flies to completely sclerotize after eclosion.

Note

This may take a few hours.

22.1 Freeze adult flies at -20C.

22.2 Record mortality of pupae.

22.3 Record sex of the adult flies. Blow flies exhibit sexual dimorphism that can be easily seen with the naked eye or a dissecting microscope. The eyes of males touch, or are spaced very close together. Females typically have a large gap between their eyes.

23 Record any abnormalities in fly morphology (e.g., wings not formed).

23.1 Dry flies at 60 °C 02:00:00 2h

23.2 Weigh each individual adult to the nearest microgram (0 µg)with a microbalance and record.

Data Collection - Larval Length

24 Calibrate stereomicroscope and camera using the appropriate scale

25 Place all larvae from a scintillation vial under the microscope and take a photo

26 Digitally measure each larvae to the nearest mm and record

Protocol references

These protocols are loosely based on the methods and materials outlined in:

Byrd, J. H., & Allen, J. C. (2001). The development of the black blow fly, Phormia regina (Meigen). *Forensic Science International*, *120*(1-2), 79-88.