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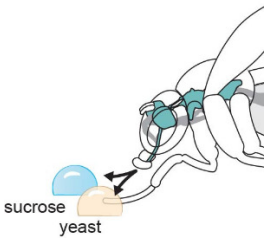
Rearing of gnotobiotic Drosophila on Holidic Media (HM) for feeding behavior assays V.1



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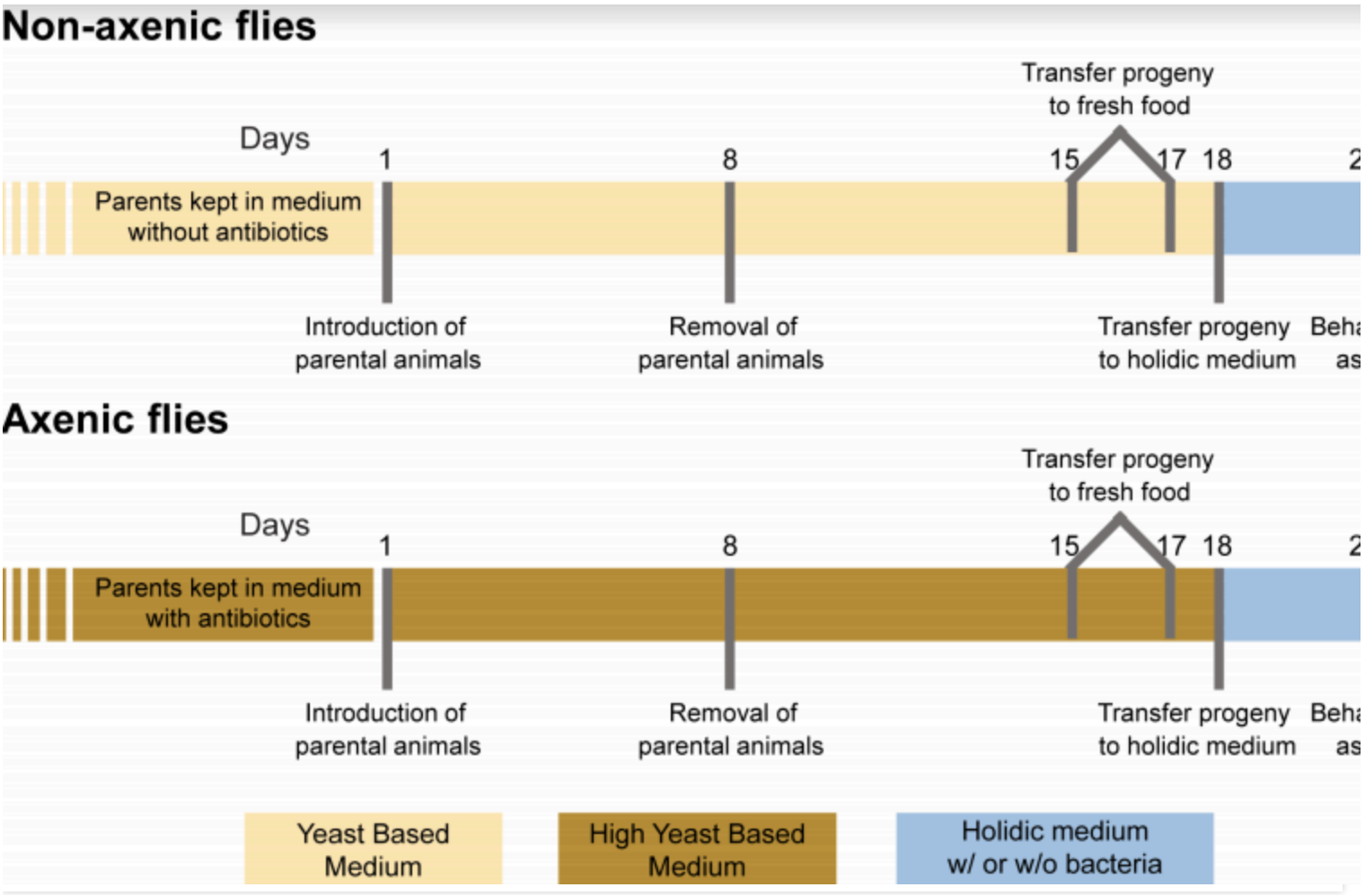
Abstract

This protocol is part of the manuscript: **Gonçalves et al. Commensal bacteria and essential amino acids control food choice behavior and reproduction. Plos Biology. 2017 Apr 18.**

Guidelines



Prepare high yeast-based medium (HYBM) **WITHOUT** antibiotics and **NO** yeast granules for rearing the experimental flies as follows:

- mix 8 g agar, 80 g barley malt syrup, 22 g sugar beet syrup, 80 g corn flour, 10 g soya flour, **41.67 g instant yeast**, 8 ml propionic acid, and 12 ml nipagin (15% in 96% ethanol) and fill up to 1000 ml using milliQ filtered water
- autoclave before pouring into polypropylene fly culture vials (VWR, #734-2261)
- **DO NOT supplement the food with live instant yeast granules on the surface**

Perform all fly rearing, maintenance, and behavioral testing at 25°C in climate-controlled chambers at 70% relative humidity in a 12-hr-light-dark cycle (Aralab, FitoClima 60000EH).

Before start

Prepare the required fly and bacterial media.

1 Prepare High Yeast-based Medium (HYBM) without antibiotics according to the **Guidelines**

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> Note
>
> This step can be prepared in advance as HYBM can be stored at 18ºC up to 3 days before use.

2 Set up fly cultures using 6 females and 4 males per vial to ensure a homogenous density of offspring among experiments. Experimental flies are generated by crossing parental flies on sterile HYBM **WITHOUT** antibiotics and **NO** live yeast granules. Parental flies come from HYBM containing antibiotics (check protocol for **Generating and Rearing Axenic Drosophila**).

Fly culture

3 Keep the crosses in a dedicated incubator at 25º C, 70% relative humidity, and a 12-hr-light-dark cycle. Remove parental flies after 3 to 7 days and wait 14 days (since the day the crosses were set up) to obtain adult flies.

If you are using temperature sensitive alleles adjust rearing temperature accordingly and plan your experiments to account for the delay in the development.

Preparing adult flies to be tested for feeding behavior

4 Sort the progeny according to the desired genotype, and collect 16 females into fresh HYBM **WITHOUT** antibiotics and **NO** live yeast granules. Add 5 wild-type males to ensure that the females are mated. When testing males collect 20 males into fresh HYBM **WITHOUT** antibiotics and **NO** live yeast granules.

> Note
>
> Check the **Guideline** section for details on the preparation of High Yeast-based Medium (hYBM).

5 To generate gnotobiotic flies start the liquid bacterial cultures following the protocol **Growing Drosophila gut bacteria.**

6

7 After 24 hours on fresh HYBM tansfer the flies to the different HM. For this prepare all the different HM needed according to the **Holidic media (HM) preparation** protocol. If required innoculate HM with the commensal bacteria following the **Inoculation of Holidic Media (HM) with bacteria** protocol.

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