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Culture medium for flower-breeding drosophilids.

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Despite more than 6,800 species of drosophilids are known (Bächli, 2017), very few species are easily maintained in the laboratory and could be used as research models. Among the reasons, it is relatively difficult to keep some lineages, particularly when they have specific developmental requirements. Flower-breeding drosophilids are one of these species that could not be maintained in the laboratory and extremely depends on living flowers. In this context, the recipe presented below was developed to rear flower-breeding drosophilids that do not grow in already described medium, such as the ones from Bizzo *et al.* (2012), Markow and O'Grady (2006), Marques *et al.* (1966), Schmitz (2016), and Vaz *et al.* (2014).

This recipe was developed and has been used in the *Laboratory of Genetic Diversity and Evolution* of *Universidade Federal de Pelotas* (Rio Grande do Sul, Brazil), prepared with flower extracts from *Brugmansia suaveolens* (Solanaceae) and *Ipomoea alba* (Convolvulaceae). However, it can be performed using extracts of other flower species, or even from mushrooms. It proved to be successful for raising *D. bromelioides*-like species (new species not yet properly described, named '*tipo III*' by Schmitz (2010) belonging to the *Drosophila bromeliae* group, from *Drosophila* genus) and *D. denieri* (belonging to the *Phloridosa* subgenus from *Drosophila* genus). Both species depend on flowers to develop their life cycle (Brncic, 1983; Schmitz, 2010). In our tests the lineages were maintained until F4 generation, in good performance.

The preparation of the medium uses common ingredients and the flowers can be kept in the freezer until their use, overcoming the natural periodicity of the blooms. Also, the equipment used is accessible making the routine preparation of this medium very cheap and easy.

Medium ingredients

0.22 g of agar
0.45 g of sugar
0.02 g of dry yeast biological
0.01 g of methilparaben
10ml of distilled water
1 macerated flower

Procedure

- Mix agar and 5 ml of distilled water and wait 10 min
- Add the remaining distilled water, the sugar, and the dry yeast
- Mix everything and boil on the microwave three times for 1 min

- Add the methylparaben and mix again
- Add the macerated flower and mix
- Transfer the medium to clean vials and let the medium cool protected from contaminants
- The use of a folded tissue in the medium is recommended

Comments

The flowers were collected and frozen at -10ºC, or lower, in identified bags with flower species and collection date. The adults of P generation were collected with entomological aspirator in the mature flowers of *B. suaveolens* (used for *B. suaveolens* medium) and in *I. alba* (used for *I. alba* medium). The adults of P generation were placed in small vials containing the culture medium. These groups of flies are difficult to identify only using stereomicroscope. So, yellow adults (*Drosophila bromeliae* species group) were separated from black adults (*Phloridosa* subgenus species group). Adult females were placed separated in the medium for *B. suaveolens* and for *I. alba* and let oviposit for 5 days. After that, the P generation females were stored in 90% ethanol. Adult males, from all generations, were identified through the analysis of the terminalia using the technique from Wheeler and Kambysellis (1996), modified by Bächli *et al.* (2004). After the first day of transference of the adults to a new medium, it was placed two drops of the flower extract prepared with 5 ml of distilled water, to feed the larvae and adults. All media were kept in a temperature and humidity controlled chamber (±25°C, 60% r.h.) reaching to the F4 generation in both kinds of media (*B. suaveolens* and *I. alba*) in good performance.

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