can be derived from a single injected embryo. Injection of a mixture of transgenic plasmids therefore provides a fast and cheap method of generating multiple transformed fly lines with a relatively small number of microinjections.

The co-injection method requires that transformed progeny be individually genotyped, but this does not slow down the crosses, as red-eyed flies are genotyped after mating. More importantly, the transgenes of all transformed lines should be sequenced, to rule out human error and acquired mutations, regardless of the method of injection. For example, of the 18 flies genotyped here, one showed evidence of mutations in the "A" transgene that was not present in the injected "A" DNA, or in any other fly bearing the same transgene. This may be the result of a PCR amplification error, but alternatively it may reflect a DNA mutation occurring before or after transgene insertion. Mutations aside, without genotyping it is impossible to rule out the possibility that the DNA or the flies could have been mislabeled, either in the lab or by the injection company.

The results presented here almost certainly underestimate the frequency of independent transformation events in different germline cells within a multiplex-injected embryo, for two reasons. First, only three progeny were selected for genotyping; sequencing of additional red-eyed progeny could only have increased the count of embryos giving rise to progeny bearing all three transgenes. Second, only three transgenic vectors were co-injected; it is possible that, for example, the three "B" progeny of injected embryo #3 represent three independent transformation events within that embryo. Taking this into account, it is possible that the number of co-injected plasmids could be increased significantly, further reducing the number of embryos to be injected.

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## An efficient and cheap entomological aspirator to collect mycophylic and anthophilic adult Drosophilidae flies.

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### Introduction

Traditionally, the methodology used to collect drosophilids in Brazil relies on flies' attraction to traps baited with resources, principally fermented fruits like banana (Tidon and Sene, 1988; Medeiros and Klaczko, 1999). However, this collection method attracts mainly frugivorous species of the genus *Drosophila* (Gottschalk *et al.*, 2008), providing a biased sample of subjacent biodiversity, once species with other feeding preferences are rarely recorded. In fact, *Drosophila* encompasses almost 60% of the 304 reported Brazilian drosophilid species, being followed by far by the mycophylic *Zygothrica* (with 54 species) and *Hirtodrosophila* genera (with only 16 species) (Gottschalk *et al.*, 2008).

Species of *Hirtodrosophila*, *Mycodrosophila*, *Paraliodrosophila*, and *Zygothrica* encompass the putatively monophyletic *Zygothrica* genus group (Grimaldi, 1990), which presents different degrees of association with macroscopic fungi. As only part of these species use fungi as resources for feeding or

oviposition (Courtney *et al*., 1990), we generically named them "mycophylic" species instead of "mycophagous", which is more frequently used. In this case, there seems to be a negative correlation between collection frequency and specialization level in the use of macroscopic fungi as feeding, breeding, or oviposition sites. *Zygothrica* is just the more generalist genus (Courtney *et al.*, 1990; Grimaldi, 1987) and the best recorded mycophylic taxon (Gottschalk *et al.*, 2008), although resource specialization may not be the only factor responsible for this scenario. In fact, reduced or skewed sampling and high levels of unregistered diversity seems to be the case for both, mycophilic (Bolzan, 2011) and anthophilic (Schmitz, 2010) species, which seem to be much more diverse in the Neotropics than previously reported.

The traditional methods used to collect mycophylic drosophilid species are entomological nets, mouth aspiration, or through the collection and storage of resources until adult eclosion (Markow and O'Grady, 2006; Gottschalk *et al.*, 2009; Robe *et al.*, 2014). This last method also seems to be widely used in the collection of antophylic drosophilid species (Vilela, 1984; dos Santos and Vilela 2005; Robe *et al.*, 2013). However, these sampling methodologies have important limitations: entomological nets are frequently hampered by fungi or flower disposition (that sometimes block net passage), and tend to be inefficient when the number of available specimens is low; mouth aspiration adds a health risk, once the collector could aspirate potentially harmful fungus spores or flower pollen, and it is also very inefficient. Once only few flies could be collected before breath breaking. Collection and storage of resources is also a biased sampling strategy, since some species can use fungi/flowers for purposes other than oviposition and these will not be collected at all. So, we developed a cheap entomological aspirator in order to make the collection of mycophagous and/or antophylous drosophilids more safe and efficient.

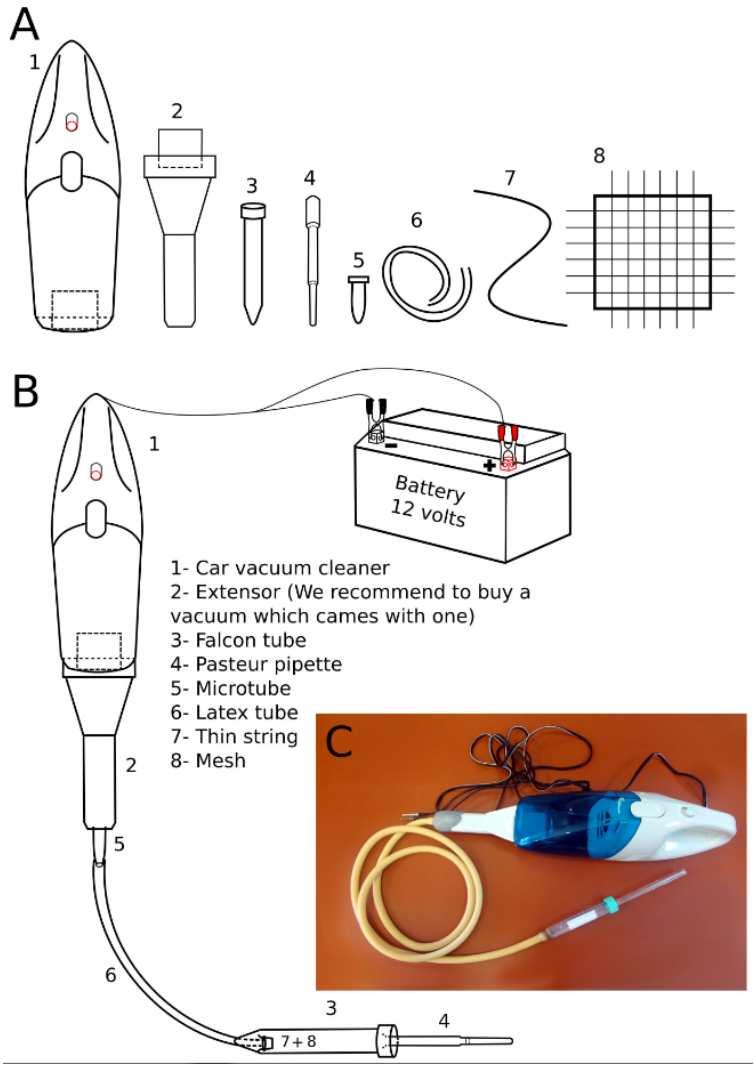


Figure 1. Entomological aspirator scheme showing the required components (A), the assembly design (B), and the final equipment photography (C).

**Aspirator Design**

The aspirator construction is based on the use of a car vacuum cleaner (preferentially with an extensor), which is coupled to a 1.5 ml microtube (or other small rigid tube), approximately 60-120 cm of a latex or plastic tube with a diameter around 0.5 cm (the first is preferred due to its higher flexibility), a thin string and a fine mesh, a 15 ml Falcon tube, a plastic Pasteur pipette (or other rigid tube as a piece of glass pipette or a Bic pen), epoxy adhesive Araldite and Durepox, two alligator battery clips, and a motorcycle battery (Figure 1).

First of all, the Falcon lid needs to be cut and connected with Araldite to a rigid tube provided, for example, by a Pasteur pipette. Both, the Falcon tube and the Pasteur pipette should have their extremities cut. In parallel, one end of the latex or plastic tube needs to be covered by a mesh with the use of a thin string. This region of the latex or plastic tube should then be passed through the cut end of the

Falcon tube in a way that the mesh is placed within the tube. Araldite glue should be used to connect these pieces firmly, without leaving any air passage. The 1.5 ml microtube needs also to be cut at both of its extremities, and its major diameter end needs to be connected to the vacuum cleaner extensor or, in its absence, directly to the car vacuum cleaner with the use of Durepox. The minor diameter extremity of the cut microtube should be firmly connected to the latex tube. The vacuum cleaner plug to a car's cigarette lighter needs finally to be changed to alligator clips or electrical plugs in order to connect the entire equipment to the motorcycle battery (Figure 1). In order to fasten and easily transport this manufactured entomological aspirator, it is important to leave the extensor unconnected to the main piece, so that the equipment is mounted in the field and readily connected to the battery.

**Advantages and Disadvantages**

The manufactured entomological aspirator presented here is easily constructed, transported, and handled. Besides, it is safer than a mouth aspirator, allowing the sampling of adult flies in spaces difficult to access with the use of entomological nets. According to flies' availability, it allows effective capture of a great number of species and specimens (tens to hundreds) in a short period of time. This equipment was used by Robe *et al*. (2014) in the sampling of mycophilic drosophilid species across different Brazilian biomes, where it was shown to be highly efficient, leading to the capture of more than 300 individuals encompassing 22 species (besides approximately 180 individuals belonging to undescribed species) in no more than 45 hours (15 collection sites with only 2-4 hours of active search + aspiration activity). The only disadvantages of the equipment refer to battery weights and the need for battery charging before going to the field, although the time of duration of battery generally compensates.

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