On the retention compartment, holes of 5 mm diameter are made arranged in sets of four parallel series around the bottle on the painted part (Figure 1C), close to the bait compartment. The holes can be done with iron to solder. The holes will be the entrance of flies to the trap and from where the bait smell will spread. Moreover, to optimize the attractiveness, it is suggested to isolate the capture compartment using a clothing like voile affixed with hot glue in the opening of bottom bottle (Figure 1C), replacing the holes in the base of the bottle suggested by Roque *et al*. (2013).

Regarding the assembly of the trap, for the engagement between the capture and bait storage compartments, two opposing holes are made on each compartment, and screws are used to hold the parts together after laying the bait (Figure 1D). Finally, the two compartments are attached with scotch or masking tape to prevent the entry of flies in the bait compartment for possible openings in the slot.

Trap test

To test the traps, particularly regarding attractiveness, ten pairs of traps suggested by Roque *et al*. (2013) were set on the field and ten pairs of traps as suggested by us. Each pair of traps was spaced 50 m from each other, while each trap in a pair was spaced 5 m. The bait used in each trap was 250g of smashed banana with yeast (*Saccharomyces cerevisiae*). The test was performed in *Restinga* forest area in southern Brazil (31º48'S; 52º43'W).

A paired Wilcoxon test was performed, and the test reliability was calculated through the Monte Carlo test with 100,000 iterations using the Past 2.17c program (Hammer *et al*., 2001).

Results

In total, 319 individuals were collected in the trap model proposed by this work and 79 individuals with the model suggested by Roque *et al*. (2013). The abundance was significantly higher in the trap proposed here ($w = 47$, $df = 9$, $p = 0.0499$), suggesting that the adjustments made in the trap provides an increase in the attractiveness for Drosophilidae.

Acknowledgments: We thank the Universidade Federal de Pelotas for research grant.

References: Hammer, Ø., D.A.T. Harper, and P.D. Ryan 2001, Palaeontologia Electronica. 4: 9; Roque, F., S.C.F. de Oliveira, and R. Tidon 2011, Dros. Inf. Serv. 94: 140-141; Tidon, R., and F.M. Sene 1988, Dros. Inf. Serv. 67: 90.

Efficient high-throughput cuticle preparations from fly lines yielding both viable and unviable embryos.

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Introduction

Analysis of *Drosophila* cuticular structures is a classic genetic tool to infer the efficiency of developmental processes via careful morphological evaluation. When performing genetic screens in pursuit of mutations with developmental effects or during mutational scanning to identify functionally important protein domains, high throughput analyses of cuticles from numerous fly lines becomes necessary. To increase throughput ingenious structures of fly containers have been devised such as fly "condominiums", that allow separate housing of relatively small numbers of flies of different genotypes and parallel embryos collections. The earliest of such devices were artisanal-made by cylindrical chambers the size of fly vials linked into a single structure (Nüsslein-Volhard *et al*., 1984). Modern commercial versions resemble scaled up microtiter plates with vial-like containers organized in regular arrays lodging onto specialized collection and feeding plates.

To obtain enough embryos for statistically significant analyses, embryos are normally collected on apple or grape plates 1-24 hours, the longer times to accommodate for fewer and/or less fertile flies. Embryos are then incubated 18-36 hours to allow for development and embryos that did not hatch are picked and processed for analyses. While certainly feasible, these procedures can be lengthy and may give rise to accidental sample mixing or confusion when multiple collections are done on the same plate in an attempt to increase throughput. An important additional complication can arise with mutations that produce a distribution of defects that are not all lethal. In such situations, different amounts of viable larvae can develop and can travel around the plate and disturb, eat, or move non-hatched eggs despite a "bait" of yeast paste strategically placed on the collection plate. This may become particularly problematic when long incubation times are required to allow full terminal development of delayed development mutants. Additionally, at collection time such larvae are much bigger than the non-hatched embryos and can hinder collection of the unhatched siblings.

During a recent screen of linker-scanning mutations of a gene of developmental interest I found that the modified protocol described below was particularly easy to follow, practically eliminated the problem of interfering larvae and sample cross-contamination, and did not require any additional equipment to that already used daily in a fly laboratory.

Methodology

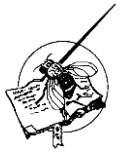
Embryo collections were carried out with regular cornmeal-agar vials, where 10-60 adult flies were placed and collection time was tailored to the number of flies in the vials and embryos laid on its surface. As the viable embryos were hatching into larvae, they could quickly burrow in the cornmeal mixture leaving only the unhatched embryos at the vial's surface. Vials were incubated at the desired temperature and length of time. To harvest the unhatched embryos ~2 ml of diluted bleach (1:4) from a squirt bottle was used to detach embryos from the surface and collect them into small strainers lined with nytex screen. It is important that this step is carried out quickly to prevent larvae buried in the cornmeal agar to float to the surface and pollute the embryo collection. For embryo dechorionation the strainers were then immersed in a shallow container with 50% bleach and incubated for 2 minutes, then washed with PBS 0.04% Triton-X100, PBS, and finally water. Embryos were carefully picked up from the strainer with a paintbrush and placed directly in 1.5 ml conical tubes with 250 µl of lactic acid/70% (ethanol 9:1) and incubated 60° C overnight. If needed, embryos could be prior devitellinized with equal volumes of heptane and methanol and vigorous shaking. Skipping vitelline membrane removal was rapid and most appropriate when studying mutations that can damage the cuticle, for example by creating holes that would cause fragmentation upon removal of the vitelline membrane. Processed cuticles were laid on cleaned slides via a P1000 pipette equipped with a cut tip, and gently placed with tweezers. Excess solution was removed either with a Kimwipe tissue or very light suction, then one drop of Hoyer's mounting medium (below) was added, gently mixed with the remnant of lactic acid/ethanol solution on the slide surface, and the coverslip was placed on top avoiding trapping air bubbles beneath. Excess solution extruded from the coverslip was carefully removed with light suction and slides were incubated overnight at 60°C on a leveled slide warmer with 10 g weights on top of the coverslips to ensure proper sample flattening. Incubation could be prolonged to 1-2 days placing 50 g weights onto the coverslip to flatten the preps. Cooled down slides were sealed with nail polish and could be stored for prolonged periods without any loss of quality.

The modified cuticle prep protocol described here is a convenient and expedited way to increase throughput of cuticle analyses without recurring to specialized items such as commercial fly condos and specialty collection plates. Combined with the retaining of the vitelline membrane, this protocol allows the rapid evaluation of all cuticles formed by the embryos, including those remaining incomplete, for example because of mutations causing cuticle holes that could cause disintegration during processing and whose disappearance from the pool could potentially mislead subsequent analyses.

Hoyer's mounting medium

Dissolve 30 g of gum arabic in 50 ml distilled water by stirring overnight. Very gradually add 200 g chloral hydrate. Add 20 g glycerol. Clear by centrifugation for at least 3 hours at 12000g. Can be stored for very long times at room temperature without any loss of quality.

References: Nüsslein-Volhard, C., E. Wieschaus, and H. Kluding 1984, Roux's Arch. Dev. Biol. 193: 267-82.



Safe, fast, cheap, and efficient procedures to collect and deposit in vials large and little numbers of flies in a short time.

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In Faculty of Medicine, Universidad de Chile, Santiago, Chile, eight Genetic and Biology Courses each employ *Drosophila* species for several practicals. A similar situation occurs in the Faculty of Pure Sciences in Universidad de Playa Ancha. The mean number of students per course fluctuates between 110 and 55. This means that our laboratories must have ready in an exact day and time 110 – 55 vials. That is, one vial per alumni × course × per eight weeks. Each vial must contain about 10 flies; N = 880 vials. The work is done 5 days a week per two months. To perform this task we have developed efficient procedures to distribute the flies into vials. Our modus operandi saves time taking a few minutes to deposit the flies into vials.

Handling a large number of flies

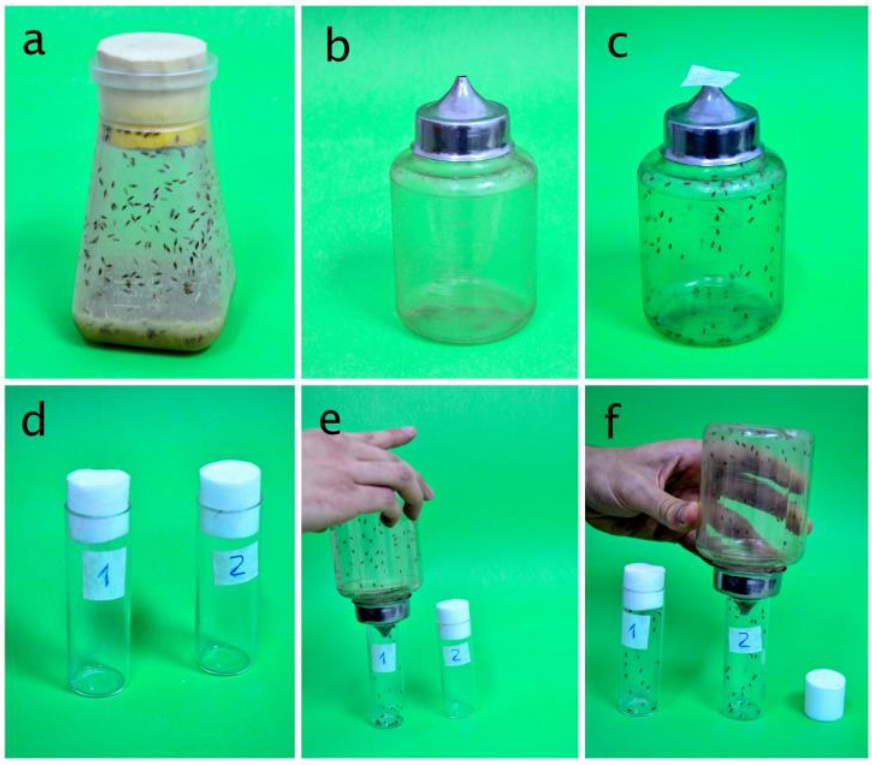

Figure 1. Photographs showing the procedure to handle a large number of flies. 1 a, a rearing bottle; 1 b, a feeding bottle, see text for a description; 1 c, a feeding bottle with adult flies; 1 d, empty vials; 1 e –f, transferring flies.

Figure 1 a- f shows a sequence of photographs describing the procedure to distribute large numbers of flies (Figure 1 a) into a set of 110 vials; each vial must contain 10 flies. For this task, we use plastic feeding bottles of 6 × 12 cm (diameter × height) similar to those employed to give water to mice, hereafter called feeding bottle. Each feeding bottle has a