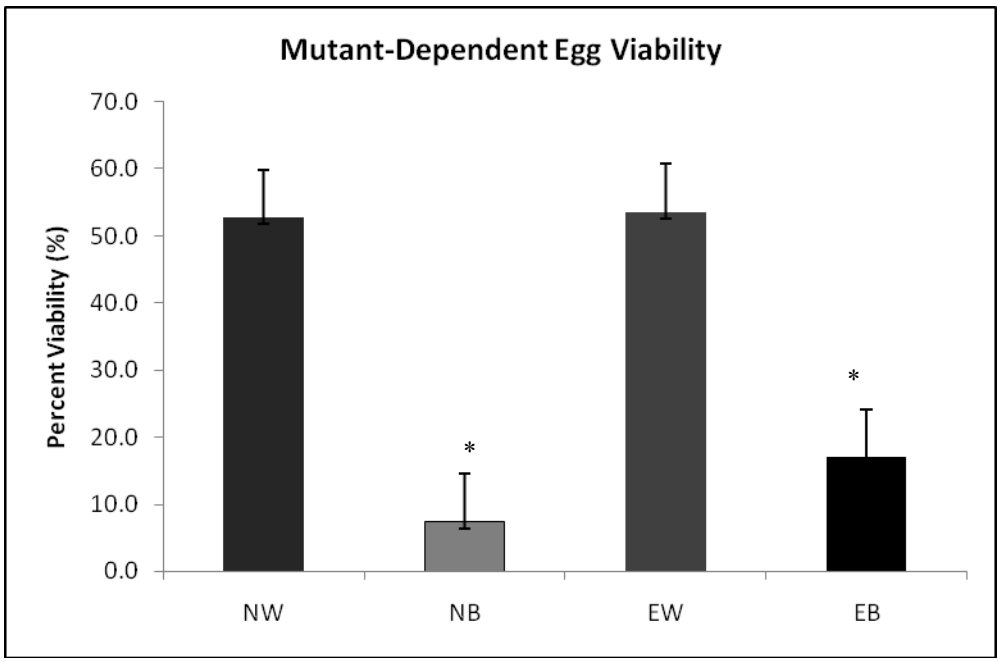

Figure 2. Viability of offspring from wild-type and *Basc* mutants treated with either Fly Nap® or ether anesthetic.

While the level of detrimental effects of Fly Nap® on post-treatment viability of *Basc* offspring remains unclear, mutants from both treatment groups displayed significantly lower ($p < 0.05$) viability than wild-type from either group. These results suggest that treatment with Fly Nap® results in a significant ($p < 0.05$) reduction in egg-laying in both wild-type and *Basc* mutant groups, and that the *Basc* mutants are more highly sensitive to such pertubations from which they fail to recover.

References: Barron, A.B., 2000, J. Insect Physiol. 46: 439-442; Champion De Crespigny, F.E., and N. Wedell 2008, Physiological Entomology 33: 310-315; Greenspan, R.J., 1997, *Fly Pushing: The Theory and Practice of* Drosophila *Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor; Kaiser, M., 1995, Dros. Inf. Serv. 76: 92-93; Nilson, T.L., B.J. Sinclair, and S.P. Roberts 2006, J. Insect Physiol. 52(10): 1027-1033; Tinklenberg, J.A., I.S. Segal, T.Z. Guo, and M. Maze 1991, Ann. NY Acad. Sci. 625: 532-539; Van Dijken, F.R., M.J.P.W. Van Sambeck, and W. Scharloo 1977, Experientia 33: 1360-1361; Volkova, N.E., and L.I. Vorobjova 2005, Russian Journal of Genetics 41(5): 490-494; Walcourt, A., and H.A. Nash 2000, J. Neurobiol. 42: 69-78; Weber, B., C. Schaper, D. Bushey, M. Rohlfs, M. Steinfath, G. Tononi, C. Cirelli, J. Scholz, and B. Bein 2009, Anesthesiology 110: 313-316.

Notes regarding the collection of African *Drosophila melanogaster.*

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Below I describe some techniques and observations concerning the collection of *Drosophila melanogaster* from sub-Saharan Africa. My most recent trip brought me to Ethiopia, Rwanda, and Kenya. Prior to that, I had also collected flies from Cameroon. I find that I'm still improving my collection techniques, but I would like to share some methods that have worked for me (reflecting both my own ideas and many suggestions by colleagues).

Geographical sampling strategy – which towns to collect from:

It seems likely that *D. melanogaster* originated in eastern Africa, so I visited the first three countries named above in the hope of finding genetically diverse populations that would inform us about the history of the species. Within any given country, it is advisable to collect flies from town areas, unless you harbor hopes of being the first person to discover a true wilderness population of *D. melanogaster*. I try to collect from towns of moderate size, avoiding tiny outposts where fly populations might go through frequent local bottlenecks, but also bypassing large cities and ports that may allow introgression from distant populations. In each country I've visited, I've collected at least two samples. This is partly in case one sample has poor population genetic properties (reduced diversity from a recent local bottleneck, or cosmopolitan admixture). An additional way to make the second sample worthwhile is to collect from sites with some environmental contrast, potentially enabling studies of local adaptation. In terms of climate, one might tend to head for a warm, humid area to find lots of flies, potentially in a fruit-growing area. But I haven't found this to be the best strategy. In warmer sites with moderate to high rainfall, *D. melanogaster* sees more competition from other Drosophilids, especially *D. ananassae* (an invader from southeast Asia), and often comprises a smaller portion of the total flies caught (but usually still fairly common). To enrich for *D. melanogaster*, I've found it very effective to either (1) go higher, or (2) go drier. Compared to most tropical Drosophilids, *D. melanogaster* has good resistance to both cold and desiccation. I've found repeatedly that when I collect from either cool mountain towns above 2000m, or dry and dusty lowland towns where you wouldn't expect to find any flies at all, I get a much higher proportion of *D. melanogaster*. I've read some reports of *D. melanogaster* not being found above a given altitude, but in general this seems to reflect the upward limit of human cultivation in those areas – if there's a town of any decent size up there, you're likely to find *D. melanogaster*. As for when to collect: most parts of sub-Saharan Africa have a dry season, which makes travel more practical. If the fly populations are somewhat reduced in this season, at least they're more likely to visit your traps if it's not raining, and if other food is scarce your traps may do all the better.

Collecting the flies – how to construct traps:

The traps I use are of a simple design. I obtain some empty half-liter plastic bottles (this is easy because I go through bottled water at a prodigious rate in Africa). I slice a window into the side of each bottle: about halfway between top and bottom, cutting about 3 cm along the sides and bottom of a square window. Then I add bait – banana is always available and works well for me (best if they're fairly ripe). I slice each banana into two or three sections (depending on length), then cut each section down the middle. I add a pinch of baker's yeast to each banana section, then cover the exposed fruit with a layer of damp cheesecloth (Figure 1A), and drop two banana sections into a trap. I also tie a half-meter string around the neck of the bottle to hang it from (hanging is advisable to avoid a trap full of roaches or ants; Figure 1B).

Local sampling strategy – where to place traps:

I always put traps indoors: partly to protect them from the elements, but also to enrich for *D. melanogaster* at the expense of other species that are less likely to go indoors, such as *D. simulans*. Having said that, I did obtain some *D. simulans* from most towns. Proportions of *D. simulans* (in

terms of the mel+sim total) ranged from zero to about one third. In any town, my strategy is to walk around and identify businesses that seem like promising trap sites. This includes fruit stands (if covered/enclosed), restaurant and café kitchens (mainly if they have fruit or tomatoes around), and most of all, bars. In one Kenyan town, the weather had turned rainy and the singular trap that saved the collection was placed in a beverage distributorship: right next to crates containing thousands of empty beer bottles. Once I've identified a business, I talk to the person in charge, explaining why I'm there and asking if I can hang this bottle-with-banana somewhere inside. I usually offer a small amount of money as compensation (up to a few dollars). As for how long to leave the traps out: even one day can be productive, though a second day might yield more flies per trap. Traps can be left for at least four or five days, depending on how dry the weather is (leaving the trap window mostly closed can help slow the bait drying out). One travel strategy is to leave traps in one town, move on to another town, and after finishing a collection there, come back for the traps in the first town. When picking up the traps, closing them without letting flies escape can be a bit tricky – I just try to close the window quickly with some wide tape (Figure 1C). Then I bring traps back to the hotel for processing. I don't recommend traveling very far with traps full of flies – this usually leads to significant mortality and/or escapes during transport.

Processing the flies – from traps to isofemale lines:

I've tried a couple techniques for getting flies out of the trap bottles. I've often used FlyNap (an anesthetic from Carolina Biological) to knock out a bottle of flies by setting a trap on its side and applying some of the liquid just inside the top. FlyNap puts the flies to sleep for a relatively long time (up to an hour or more, depending on exposure), which facilitates close examination, but direct contact with liquid Flynap is fatal to the flies. To expose flies to the vapors only, I apply Flynap to a large cotton ball that is in the middle of a 50mL Falcon tube (leaving a gap between the cotton and the top of the tube). Next I put a trap bottle on its side and cover the top half (encouraging the phototaxic flies to move away from the cap). Then I remove the cap and quickly replace it with a square of cheesecloth (Figure 1D) and then the Falcon tube with Flynap (Figure 1E). Vapors disperse through the cheesecloth into the trap bottle and safely anesthetize the flies. Then I slice the trap in half (with scissors, starting from the window; Figure 1F), remove flies with a paintbrush (Figure 1G), and examine them under a simple plastic photographer's loupe (15× lens; though clearly more sophisticated field scopes are available). Near the end of my last trip I ran out of FlyNap. Instead, I replaced a bottle's cap with a cotton ball, then used an aspirator/pooter to take out the flies one small batch at a time. Since these flies were awake, it was harder to look at them closely, but in general I was able to sort them by eye and I could still immobilize a fly by pinning it with a cotton ball against the side of a tube. Each putative *D. melanogaster* female then goes into a separate tube. The plastic tubes I used were 16×75mm test tubes (Lake Charles Manufacturing) with medium size cotton balls. These tubes fit into 3" tall freezer boxes (5×5" wide) with 7×7 dividers for space-efficient transport (Figure 1H). Before the trip, I wrapped about 3 ml of dry instant fly food (Carolina Biological) in a square of cheesecloth, then pushed this into the bottom of each tube. In the future, I may mix some dry antibiotic into the food before putting it in tube (choosing an antibiotic that doesn't kill yeast; bacterial growth was a problem during my last trip). In Africa, I added 3 ml of bottled water to each tube (sterility is important here), let it soak in for a couple days, then removed excess water and let it dry a bit before adding a few tiny pellets of yeast and then the flies. I'm mainly interested in starting isofemale lines, but I also bring back one or more vials containing multiple wild-caught males (they can occasionally be useful for "rescuing" an isofemale line that doesn't yield any male progeny, or for other experiments).

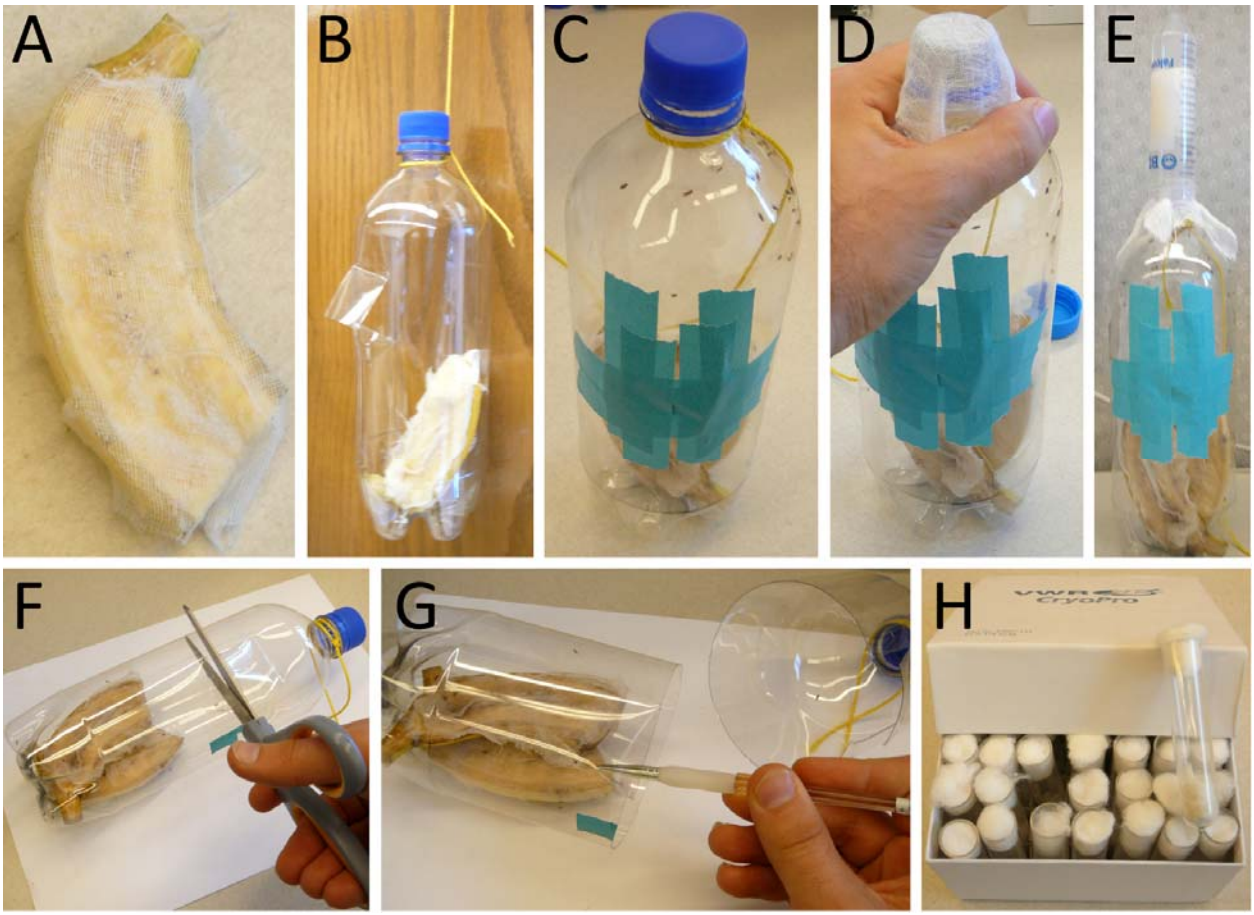

Figure 1. Photographs illustrating collection techniques, as described in the text.

From field to lab – getting the flies home:

Once in the tubes, flies travel quite well. For travel within Africa and for the journey back home, I always wrap the boxes of fly vials in a "space blanket". They're very light-weight, take up almost no space, cheap if ordered online, and highly insulative against heat and cold. I have two ways of getting flies from Africa back to the U.S., and I generally use both by duplicating each line in Africa (transferring each female to a new tube). One duplicate I ship back via DHL (they have a good African network and, last I checked, the best policy toward shipping insects). The other duplicate I travel with in my checked baggage. I haven't had any problem with things freezing in my checked bag, and the flies get less scrutiny there than they would in my carry-on.

Red tape:

Actually collecting *D. melanogaster* is about the easiest field work a biologist could ask for. But navigating layers of bureaucracy to get permission to take the flies home is a far more daunting task. I have succeeded in getting research and export permits a couple of times (Cameroon and Kenya), but this has always involved sacrificing a large portion of my time in Africa (even after initiating the process well before the trip) and barely receiving the export permit before my flight home. Also in both cases, I would not have been able to obtain permits without the help of local scientific contacts. In other countries, the permit process never became clear to me, even after

consulting researchers based there. When leaving a country, flies in my checked baggage have never received any interest at the airport. Once in Ethiopia, a baggage scanner wanted to look at the empty tubes of fly food in my wife's bag because she thought it looked like a carpet (perhaps those are subject to export tax), and was then suspicious about the tubes, but eventually they let us through (after I tasted the instant fly food in front of them and assured them that it was "flakes" and not "powder"). When shipping flies, convincing an African DHL office to accept a shipment of live flies is not guaranteed, but my success rate has been >90%. The primary documents of interest to DHL have been my U.S. import permit and an official-looking letter from my home institution). For bringing or shipping flies to the U.S., an import permit from the USDA PPQ office is needed, and can be applied for online. Note that if you're flying in with flies: you should let the USDA know when you're coming, you have to enter the U.S. at the "port of entry" listed on your permit, you must have one of your permit's "mailing labels" with the flies, and the flies should be sealed inside a second box.

Armchair collecting:

In case you'd like to have some flies collected but no one is available to make the trip, I've also had fairly good luck contacting researchers in Africa and asking if they'd be willing to send me some flies. Basically, I sent these kind people some simple collection equipment, compensated their travel expenses (a few hundred dollars at most), and they shipped flies to me. Results were variable but generally good, especially when I could find an entomologist to help. The instructions I sent to them resemble the methods described above. An alternative to the isofemale line preparation for less experienced collectors (which I have not yet tested) would be to receive a mixed-sex batch of flies, age and transfer the wild-caught females until they run out of sperm, and then pair each with a different wild-caught male.

Correction

Johnson, David A. 2007. Amplification of DNA from 30-year-old aceto-orecein stained salivary gland squash slides. Dros. Inf. Serv. 90: 156-158.

Two corrections are highlighted in bold below in the procedure steps 23 and 26. Step 23 should indicate –70°C, not 70°C; and step 26 omitted 70% before the word ethanol.

23) Incubate at **–70°**C for 5 minutes

26) Add 1000 µl **70%** ethanol, mix gently, and centrifuge for 1 minute at 13,000-16,000 ×g.

Call for Papers

Submissions to Drosophila Information Service are welcome at any time. The annual issue now contains articles submitted during the calendar year of issue. Typically, we would like to have submissions by mid-December to insure their inclusion in the regular annual issue. but articles can be accepted for this volume until 31 December. Details are given in the Guide to Authors or on the DIS web site: www.ou.edu/journals/dis.