LABORATORY MANUAL of *DROSOPHILA*

Evolutionary Biology Research

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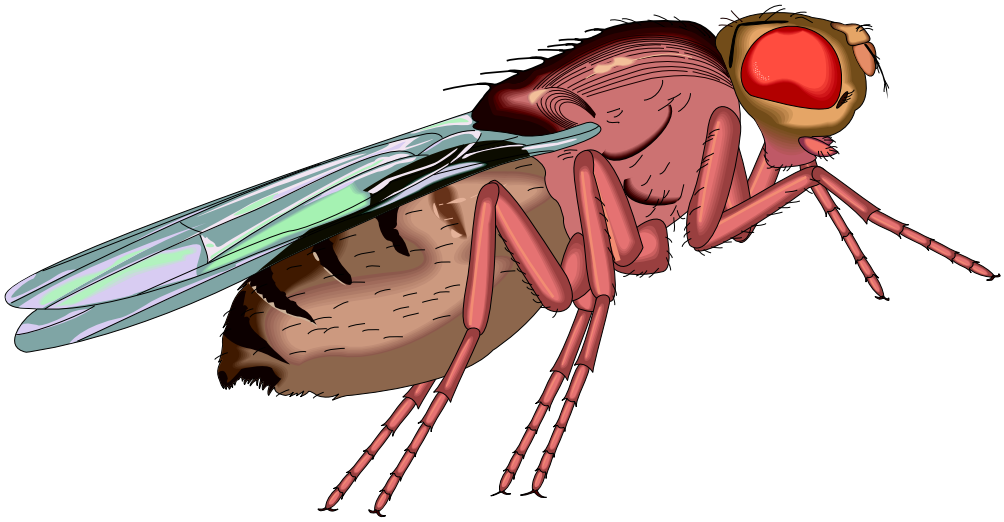

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Authors' Note

This lab manual has taken us many long hours to write. We would not have bothered to do it if we did not see a definite use. This is a guide to the methods employed which should help to standardize protocols and simplify experiments in a number of ways. We also try to give additional background for the student experience. We hope that we have provided enough discussion of the general importance of this work as well as the funky things that flies do to make life in the labs more interesting and rewarding to the students that make it all happen, even when daunting tasks and repetitive jobs seem to have the better of you.

We hope the manual will be helpful to you. The manual has been upgraded periodically. Any contributions, suggestions for improvement, or opinions about it would be greatly appreciated and carefully considered for the next upgrade. Please contact one of us.

Sincerely,

Adam Chippindale, Margaret Archer, Erin Gass, Michael Rose & Laurence Mueller

§1. INTRODUCTION

WELCOME TO THE LAB THAT NEVER ENDS

Our research explores many interesting areas of evolutionary biology; the genetic and physiological basis of aging and stress resistance, the role of crowding on population growth and evolution, the nature of quantitative genetic variation. This research is unified by the use of *Drosophila* as model organism and the by the use of populations that have evolved under well defined environments and demographic schedules.

Our ideal would be for each and every student to be an important and fulfilled contributor to our collective work. We will make every reasonable effort to foster this goal. But the most important person affecting your success is yourself. If you read the material that we give you, attend the lab meetings and work hard in the laboratory, then it should be easy for you to get involved in interesting research projects. But if you do not make that effort, we can't make it for you, and your work in the lab will be pretty boring.

For hard-working students, we hope that you will prosper in our laboratory, experiencing first-hand the thrill of discovery. We all start as undergraduates, and for many scientists it was the opportunity to work in a lab then that started their careers. I sincerely hope that many of you will be the scientists of the future.

§2. GUIDE TO THE FLIES

Life cycle of *Drosophila melanogaster*

Drosophila is a dipteran (two-winged) insect that undergoes a complete metamorphosis from the larva to the adult. Although there is some variation in the lifecycles and development of other *Drosophila* species, here we deal principly with *D. melanogaster*. Other species are discussed in the last section of this chapter.

Eggs typically hatch in our *D. melanogaster* at about 21 hours from laying. However, if females are deprived of a suitable egg-laying environment, eggs may hatch earlier due to advanced development while being held by the female. Fertilized eggs are generally a creamy white. As the embryo within matures, the segmentation of the larva is apparent. Infertile eggs are clear and lack segmentation. Infertile eggs also frequently lack the "antennae" (chorionic appendages) characteristic of the *Drosophila* egg.

The hatchling larva is said to be in its first instar, or stage, of growth. There are three instars in the larval growth of *D. melanogaster*: L1, L2, and L3. Between instars one and two, and two and three, the larvae shed their exoskeletons and secrete a new cuticle to grow into, a process called "moulting". In holometabolous insects, the timing of pupation determines the final size of the fly, because there are no further moults. Because the larval growth period and level of food determines adult size, and size affects a wide array of life-history characters, such as fecundity, controlling the conditions of larval development in our experiments is important.

The larvae munch away at the food with pulsating movements of the mouthparts until they pupate at about four days from hatching. They then search for a good spot to pupate (especially the sides of vials, as anyone who has ever washed a vial will acknowledge). The "puparium" is the fly's equivalent of the caterpillar's cocoon. Like the caterpillar-to-butterfly transition, a major conversion of larval-to-adult tissue occurs in puparium before the larva emerges as a beautiful fly. Since the pupal phase lasts for more than four days (about 100 hours) it represents a major period of starvation stress, in which the larval tissue is converted into adult tissue. Some pupal stages can be easily discerned with transmitted light. There is a complete darkening of the pupa about 24 hours before adult emergence.

Under normal lab conditions (25^{o} C, 24 hour light, and moderate densities) the first adults will emerge about eight days from egg-collection. Emergence continues for several days with an approximately normal distribution. Females always emerge ahead of males by about five hours on average. Average egg to adult development times vary substantially with stock, density, and light and temperature conditions. For example, in the carefully controlled development time experiments the time from egg to adult for a typical B fly is 220/225 hours (F/M), but for a D fly these times are more like 250 hours. It will be especially important in experiments that require collection of very young adults (e.g. virgin flies) that these factors be taken into consideration and controlled as much as possible.

Sexes of the adult are easily determined: Males are usually smaller and have a slender

abdomen, darkened on top by the fusion of the tergites. Females are larger and have a striped and larger whitish abdomen. However, if the flies are malnourished, immature, or from crowded cultures, sexing can be more difficult. When the flies are malnourished or have come from crowded cultures, the sexes may be the same size and the female's abdomen may be as slender as the male's is. Size is not a good character to use when sexing. Another problem is that the adult fly emerges a ghostly white apparition with folded wings, regardless of sex. It is difficult to distinguish the sex of a newly-emerged fly because males and females look similarly pale, bloated and amorphous. Males have darkened genitalia from the earliest stages, so that (at the very tip of the abdomen) a brown spot will be apparent. This genital spot is the only easily visible character that is always dimorphic between the sexes. Another characteristic that can be used, if a microscope is available, is the hairy, brush-like "sex combs", found on the forelegs of males and not females.

The darkening of the cuticle in maturation depends upon the "tanning" of the proteins within the cuticle (a chemically similar process to the tanning of leather). Flies that are newly emerged do not mate for at least eight hours, so the easiest way to ensure that a fly is virgin is to collect it within six hours of emergence. (Some people even refer to the ghost-white flies as "virgins" in common parlance.).

Adult longevity has been extensively explored in our stocks. The average longevity of the adult B fly evolved on a two-week schedule is about 30-50 days when nourished on standard Rose Lab medium. Selection for postponed-senescence has easily doubled the life-expectancy of the selected fly. All other populations in the Mueller and Rose labs have longevities somewher between the B and O populations. Longevity depends strongly upon the food background the flies are maintained on: if fresh yeast is available, flies will lay more eggs and live a much shorter life. Individual females will lay into the hundreds or thousands of eggs in their lifetimes, depending upon the food background and stress factors imposed upon them. More detail on adult life-history is available from the sample publications and recommended readings.

General Stock Maintenance

Most of the procedures we employ are straightforward even though we address sophisticated scientific problems. This procedural simplicity has been one of the keys to the success of our system because it allows us to accomplish large-scale experiments using many students to achieve a high level of replication of individuals, populations and experiments. There are, however, a number of problems that arise from such an operation — most of them relating to the diligence and dedication of students working on the projects. In this section, we outline some basic procedures in stock maintenance and highlight some of the areas in which alertness is especially important.

Emergencies

Things that might seem like minor problems to some can be major ones to others. As an example, many of our experiments are done in the open lab due to limitations on incubator space. If the lab temperature goes up to 30^{o} C some evening, due to a failure in the air-

conditioning system, ... and it has done this ..., experiments that have taken people hundreds of work-hours to set up and maintain, and stocks that have been evolving for years, could be threatened. Even though it may seem like a slight discomfort to you, it could be a huge set-back to someone else.

The most common lab emergency is an incubator overheat. If each population is divided between two incubators this will not threaten a stock. Experiments, however, are often purposely kept in a single chamber. Most of our modern incubators have alarm systems. If you hear an alarm, you should investigate its source. Everyone should be aware of the incubator temperatures and scan the digital readouts occasionally when working in the lab (see figure 1). Often the digital readouts differ slightly from the true temperature, usually reading a degree below true temperature (for reasons known only to refrigeration experts and other divinities). If the temperature reading is much greater (5^{o}) than 25^{o} inform someone immediately. Do not try to adjust the temperature with the control panel dials in any circumstances. The most accuarte method of determining the incubator temperature is to place a termometer in a beaker with water and let it equilibrate for several hours. If this temperature differs by more than 1^{o} from 25^{o}, someone knowlegable should adjust the set temperatures.

Emergency phone numbers are generally posted on the doors of labs for the security patrol or other people likely to notice an after-hours problem, but each lab should also have a more specialized list. This list will likely include phone numbers for the professor, postdocs, grad students, and responsible senior undergrads. Again, if there is any uncertainty, at any hour, you should call someone. You might just save somebody a lot of work, and even if you wake them up unnecessarily they will still appreciate your concern.

It is everybody's responsibility to watch for trouble in the lab. If you notice an incubator with a high temperature reading, someone doing something procedurally wrong, a cage with flies coming out, and so on, you must deal with it. If you don't know exactly what to do you should never be hesitant to call up somebody in charge to tell them — be it noon or 4AM.

Figure 1. The control panel for the large double-door incubators in the Mueller and Rose labs.

Carbon Dioxide

Many experiments require counting, sexing or otherwise manipulating adult flies. This can only be accomplished if the flies are immobile. In the old days flies used to be anesthetized with ether. This was dangerous to both flies (they are easily killed with an excess of ether) and humans. We use CO_2 exclusively to anesthetize flies. While this is a much safer way to knock flies out it is still potentiall

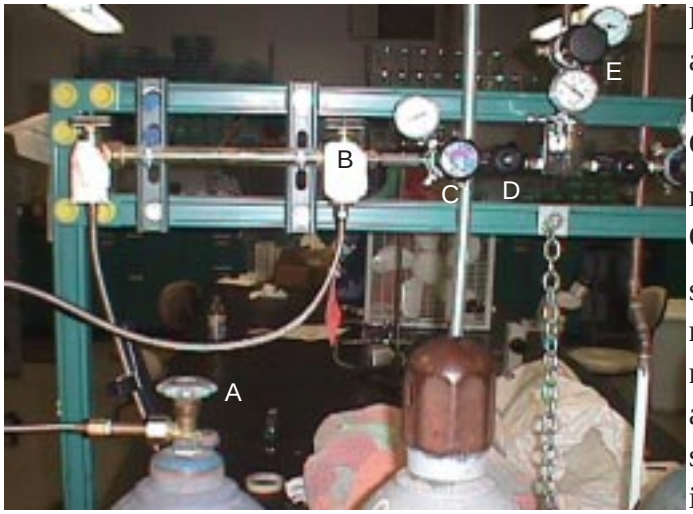

Figure 2. The CO_2 tanks and their associated valves. See text for a description of the regulation of gas flow.

lethal. If you want your flies to be alive after they have been anesthetized they should not be on a CO_2 plate for more than about 10 minutes. In addition the volume of CO_2 flowing through the plate should be kept at an absolute minimum. To determine what that minimum is put some flies on the plate and reduce the flow until you see some flies begin to wake up. Then increase the CO_2 flow slightly above this level. As a general rule of thumb if you can hear the CO_2 flowing out of your CO_2 plate the flow is too great. If you are using CO_2 for a prolonged period you may have to readjust the flow several times since the pressure will change as the CO_2 tank empties. Very young females (several hours old or less) can be sterilized by even modest levels of CO_2. If you are collecting virgins or newly emerged flies you must be very carful and keep exposures down to 1-2 minutes or less.

The CO_2 in the laboratory is supplied with tanks as shown in figure 2. The valves labelled A, B and D are normal open or closed valves and open in the normal fashion (turn counterclockwise). The valves labelled C and E are pressure values (see associated pressure gauges) and are opened by turning in a clockwise direction. The pressure in the CO_2 tank is very high however the pressure required by the CO_2 outlets is quite low. You should be able to regulate the pressure adequately by opening valves A, B and D all the way and adjusting C and E.

CO_2 tanks should always be strapped to the metal frame. If a tank fell during and earthquake and the top broke off they could behave like a missile. After a tank has been emptied, replace the metal cover and put a peice of masking tape with the words empty on it, so we don't waste our time connecting empty tanks. In addition when the service people come to replace the tanks they need to know which ones are empty.

Feeding

In almost all instances, if we talk about "food", we are referring to standard Rose Lab banana - molasses medium. This food is easy to prepare (see recipe in section D) but also easy to screw up, so the instructions must be followed closely, with care. Controlling the food quality is as important as any other environmental variable, particularly for the developing larva, which bathes in it throughout its entire growth and development. We have attempted to standardize the recipe, but there is still room for variation (bananas vary, evaporation-level varies etc.). However, the most serious problems are failure to kill

the yeast by boiling, and not adding the (anti-fungal) hydrobenzoic acid at the end of the cook. One or both of these mistakes can lead to overgrowth by the yeast and molds that can devastate a stock or an experiment.

Most selection lines share a common two-week pre-selection phase, from the time eggs are collected to the imposition of the selection treatment on the adults. The egg numbers are regulated to try to minimize differences in the level of competition in the larval stages between lines, which can be problematical for the study of other, intentionally selected, characters. Obviously, it is mainly the density (larvae per mL food) which will create differences in the level of competition among larvae, so the amount of food per vial is just as important as the number of eggs. When 60-80 larvae are put in an 8-dram vial, a food level of about 5 mL is enough to ensure low levels of larval competition and leave enough food to humidify the vial after pupation.

All of our selection lines with extended lifecycles are kept as adults in population cages to make feeding easier. Flies are fed three times weekly (M, W, F) with standard banana food plates (see Appendix for recipe). As with the larvae, the feeding of unstressed lines' adults is designed to be ad libidum. Since flies die very quickly of desiccation, it is important that they not be deprived of food and water for very long, except when selection entails the application of this stress. If you change the plates for a selection line, it is incumbent upon you that you check that each and every cage actually gets fed. If you see a cage of starving flies that looks as if it should have food, you should alert whomever is in charge of that stock immediately.

Yeasting and cutting of plates

Yeast paste is applied to most egg-collection plates to boost the fecundity of females. The increase in fecundity may be due to a behavioural cue or it may be due to the protein supplement provided by the yeast, (some data favour the latter). In any event, the addition of dietary yeast is made to all populations, except Rose two-week stocks, about two days before egg-collection.

Yeast Paste

There is no fixed recipe for the paste:

Add dry yeast to distilled water gradually, mixing until a cake-icing texture starts to appear. Add a few squirts of 1% acetic acid to make it smell vinegary — do not add straight glacial acetic acid. Mix until creamy.

The important point about yeasting is that it be excessive for the flies but not excessive for the person collecting eggs. A swirled dollop of about a centimeter across (at the base) is enough for the hungriest of flies, and will allow eggs to be collected without live yeast being put in the vials. If live yeast is put in the vials it may grow and interfere with the larval growth.

caution: populations with extended lifecycles that would normally be yeasted for egg-collection at the end of their stock-cycle are not to be yeasted prematurely for convenience in collecting for an experiment.

Plates are “cut” to expose side area. For some reason the females prefer to oviposit on a vertical surface and in a tight space. Trimming the plates on one side and then tapping the food down to expose the smooth edge is the simplest and most effective way to cut a plate. Another reason to collect eggs from the side of a cut plate, rather than the top, is that the freshly cut side is much less likely to harbor contaminating eggs (e.g. laid by females while food was cooling on the counter).

Egg Collection

The standard density of eggs / vial is supposed to be 60 - 80 eggs per 8 dram vial with 5mL food. Differences in larval density can be severely confounding to the evolution of other characters, such as adult longevity, so it is important that larval rearing densities be consistent among stocks. For most stocks, eggs are collected from pieces of food cut from egg collection plates. There is no “standard size” of cut. The possibility exists for strong and consistent biases between different selection lines due to differences in fecundity. Consider the case of a young unstressed line compared to an old stressed line: the young line is likely to lay more eggs, and therefore more eggs per square unit of egg-collection surface. The size of the piece of food cut must cater to the fecundity of the specific population that is being collected, and it is generally a good idea to use a microscope or magnifier to accurately estimate egg number. It is best to estimate 60 eggs, as you will usually end up with 70 or 80 (due to unseen eggs), which is ideal.

Egg-collection is one of the most likely stages at which cross-contamination can occur. If you are egg collecting, you must be sure that only eggs from the specific population you are collecting go into the bundle of vials you produce. This means cleaning your tools, watching for contamination (esp. by loose flies; don’t leave anything open), checking the correspondence of tags and selection cages, and organizing things so that another person could take over (with no advance notice) if need be ... without any ambiguity.

Population Size

Part of the success of our labs has been the avoidance of inbreeding depression in the study populations. The reasons for avoiding inbreeding are too numerous to list. The study of life-history characteristics is largely the study of interrelations among performance characteristics. If two lines are compared, and one is generally screwed up oweing to inbreeding, the results could have nothing to do with inherent properties of the genetic architecture of those populations in the absence of inbreeding. Inbreeding is a severe problem for the study of life-history evolution.

Inbreeding depression has been minimized in our stocks very simply by keeping population sizes above about 1000 flies per generation. With some stocks, keeping population size up is simply a matter of maintaining enough culture vials each generation. In other stocks, however, it is very easy to have population bottlenecks. Stress-selected lines are particularly vulnerable to bottlenecks in population size because selection normally involves the mass-killing of flies under the stress. If populations are not watched closely, such over-selection will be a problem. Another problem general to all stocks is escape by the flies, which usually happens because a cage was carelessly assembled. Leaving a hole

large enough for a fly to squeeze through can be enough to lose a population. Most commonly the “hole” is actually a gap between the cage and its plexiglass cover at one corner. Evidence of escaped flies may also be the presence of many dead flies on the bottom of the incubator, especially if these have formed over a short period of time.

Make it a rule to check carefully the cage from a fly’s eye vantage point, checking also for gaps around the sides of the front plate. If you ever notice a selection line falling to low population size, you should alert whomever looks after that line. But, most obviously, you should be on the alert for problems with the containment of populations from the first step: building the cage.

Backups

In the Rose Lab, there have been a few incidents in which over-selection has occurred. In case a population level falls down to unacceptable levels, we have to resort to back-up stocks. The backups are usually vials (20) saved from the beginning of the selection procedure after dumping. Even though the adults were dumped into the cage, the vials they came from will always contain eggs and larvae of the next generation. Although these are sub-standard flies by normal criteria, they still possess the genetic make-up of the selection line at the previous selection generation. We save some of these vials as backups until it is clear that the selection procedure has gone smoothly. Backups should be maintained from the time a stock is dumped to the time it is next dumped. When backups are used, a selection generation is skipped to restore population sizes, and all populations (including controls) are restored from the back-up to maintain synchrony of the stocks. It is undesirable to resort to backups, but it is generally better to save a population at any expense.

Other forms of back-up may be employed. In the A-lines, for example, the back-up is maintained by keeping the adults of the previous generation in their cages after egg-collection until development of the new generation is nearly complete. If a problem occurred with the new generation, the adults of the previous generation could be used to restore the stock with only a slight glitch in the selection progress. Again, the maintenance of large population sizes to avoid inbreeding depression is more important than the maintenance of high selection pressures.

In the case that a selection line falters and someone has flies from that line ready for an experiment, the experimental flies can be used to restore the stock. Regardless of whether or not it interupts that person’s experimental schedule, if needs be, the flies will be used to restore the laboratory stock. Stocks are more important than experiments.

As a safety measure, each population of a stock should always be split in two and placed in two different incubators. Should one chamber overheat and kill the flies, it would only kill half of them, and the stock could be recovered.

Contamination

The fly system we are using depends upon the maintenance of pure breeding populations without complications caused by disease and interbreeding of populations. Contamination can occur at several different levels: between species (parasites etc.), between selection

lines, and between replicate numbered population within a selection line.

Disease has never been a problem within our stock system, however this is no reason to relax precautionary measures. *Drosophila* can have a number of diseases, but ectoparasite infestations are the most common source of trouble for a long-established set of laboratory flies. A mite infestation can cripple a fruitfly lab because, not only do the mites kill flies and compete for the food, but they obviously make comparisons of life-history characters (like fecundity or longevity) impossible, and interfere with routine selection. Mites can move quickly from vial to vial; the stoppers do not stop mites. As Ashburner points out in his *Drosophila* Handbook, the simplest way to encourage a disaster is to let old culture containers lie about and decay. Wild flies are likely to harbour parasites, and this is good reason to prevent decaying fruit and garbage from lying around too long. Everybody knows that the Albertson's banana is the only known repeatable source of spontaneously generated life.

Ectoparasites, viruses, and internal parasites can easily be transmitted by personnel from lab to lab, and this is the primary reason for placing restrictions on people's movement between labs. Nobody who has been working in the Ayala or Bradley labs, where there are recently wild-caught populations, is to enter the Rose or Mueller labs unless they have observed the quarantine rules set forth. In general, nobody may enter a laboratory which is potentially contaminated and return to a "clean" lab witout a change of clothes and a shower. Personnel who wish to pursue projects in any other lab may only do so with permission of the professor, postdoc, or lab manager of their lab. Any student who is found to be in violation of these rules will be discharged from their lab with a failing grade.

The independence of replicate population numbers within a selection line (e.g. the separation of the five replicate B's) is crucial to our system: when five evolutionarily independent populations show the same pattern of response to selection, we have much greater confidence in our result than if had we created only one of each type. It is therefore important that the purity of a population be regarded sacred when handling stocks.

Here are some rules that must be followed whenever you work with the stocks. All of these rules are simple common sense, but their importance cannot be over-emphasised. Each population must be a completely independent evolutionary unit, so any source of contamination should be considered a problem of the highest order.

1) There are always loose flies in the lab. Plates or vials left uncovered will attract the rogue fly and give it a place to lay its eggs. If there is ever even the suspicion that plates or vials involved in an egg collection for stocks have been contaminated, these must be thrown out. There is no room for uncertainty — you cannot always see the eggs and contamination is often very patchily distributed (so "spot checking" a few vials is no good). If a vial comes from the incubator without a sponge, it is no good. If flies are observed in a rack of vials designated for egg collection purposes, they are no good, none of them.

2) When the source of a vial / plate / fly is uncertain, that vial / plate / fly must be thrown out . For example, if you are dumping the SB4 population, and someone beside you is dumping the SB5 population into cages, and a vial is ambiguously placed in "no man's

land", you cannot say "I sort of remember putting it there" and put it into a cage unless you know which population it belongs to. There simply cannot be doubt about the source of a fly. Double check labels. Treat yourself as if you are really dense, because it is very easy to get lulled into a catatonic state when doing repetitive jobs — it goes with the territory. Lay things out so that they are clearly separated from the start, and so that someone else would understand your organisation and could pick up your job if you were called away.

3) Always clean off your tools between populations. Tools like spatulas, used for egg-cutting and transfer to fresh vials, can carry eggs that are difficult to see. You should always rinse and wipe off the spatula before switching to a new population. This also goes for any other thing used to collect eggs.

Fly traps

Escape of flies is inevitable, and fly labs tend to harbour a standing crop of loose flies. These flies are a threat to the maintenance of independent replicate lines because they quickly home in on sources of fresh food. Fly traps should be laid out in any lab culturing *Drosophila*, because the traps not only capture loose flies but serve as diversions to flies that might otherwise seek fresh (stock) food. The menace of loose flies can be overcome if traps are frequently changed.

Fly traps can consist of anything that effectively attracts the rogue fly, but most frequently we have employed two designs:

1) The classic food-plus-yeast and acetic acid vial

and

2) The Megadeth jar: this is a jar with some mulched banana, sweetener (karo or barley malt, acetic acid, alcohol, and whatever else is kicking around; live yeast must be used very sparingly (a "dash" at most), else it will grow and overflow the container. The Megadeth Trap usually features a paper funnel which makes escape difficult once a fly has found its way into the trap.

Either type of trap is effective because it tricks the flies into believing they have found a good egg-laying site. And of course they have found a good place to lay eggs and meet other single flies if the traps are not frequently changed and frozen. If they are neglected, they quickly become breeding chambers, thus working exactly against their purpose.

Cleaning and organization

It is of the utmost importance for all fly laboratories that old cultures not be allowed to quietly decay in some forgotten corner of a culture room . — M. Ashburner

All labs covered by this manual are places shared by many hands. It is obviously important to the functioning of big labs that all who participate are responsible enough to clear up after themselves and return tools to their right places. Unfortunately, the more people that share a space, the more the responsibility for its upkeep tends to be diluted. Keeping

the lab clean and organised is important to its functioning at all levels — from the prevention of contamination to psychological factors. Students who do not show responsibility towards cleaning and the maintenance of order in the shared workspace will be downgraded.

New students should make it their first priority to familiarize themselves with the organisation of the lab. All workers must contribute to the maintenance of order or bad vibes arise quickly. The frustration of not being able to find a $#!@**! stir-bar or having to start a cook from an encrusted dirty pot can be intense. If you prepare medium, you clean the pots, the blender, the counter, the scale, the thermometer, and so on. If you collect eggs, make sure your space has been cleaned up. The order that tends to take hold in a large lab is that a few careful people take up the slack for the slob majority. Some people have to live in the laboratory through long and stressful experiments, and others simply care more about organization and cleaning, and it is important for the sanity of everyone to keep the workspaces clean. Be generous in cleaning up after yourself by cleaning up a little for others, and don't hesitate to complain when other students leave a mess — they are making the work environment less pleasant for everyone.

Description of Stocks Originating in the Rose IV Population

All Rose and Mueller Lab stocks are descended from a single population of Massachusetts *Drosophila melanogaster* studied by P.T. Ives and maintained in the lab at large population sizes with discrete generations at 25^{o} C and 24hr light for close to 20 years. All stocks listed below have ultimately been derived from this population, designated IVES (IV).

After approximately 110 generations of maintenance in the lab on a two-week schedule, the IV population was used to found our two oldest selection treatments: B and O, each line having a subscript 1 - 5 to indicate replicate numbers. The "B", or "baseline", populations were maintained on the same two-week schedule as the ancestral IV's. The "O", or "old", populations were maintained with an increasingly long adult phase: two weeks of standard larval culture vial plus two, then three, then four, then six, and finally eight weeks of extended adult existence; since December 1981, the O's have remained at a total of ten weeks generation time (from egg to egg). O selection was aimed at increasing late-life net-fertility, as natural selection would favour females that could lay fertile eggs late in life.

When the B's and O's were founded, all ten populations were derived by random sampling of the IV population, so there is no special relationship between populations of the same replicate number; B1 and O1 are no more closely related than B5 and O2 . All subsequently derived selection lines have been taken from existing populations (usually B & O) and designated with the same number; for example, BC1 and SB1 are specially related by their common origin in the B1 population. In general, the selection lines created after the B's & O's are given a letter indicating the type of selection and a letter indicating the source treatment (e.g. SB = "S" for starvation & "B" for B-derived). This nomenclature is lost in a number of lines, for example the D & C lines. All lines described here have 5 independent replicate populations under identical treatment, each with census pop-

ulation sizes in the thousands of flies.

All selection has involved the application of a stressor. Demographic selection is simply selection on the duration of the lifecycle with no intentional environmental stress applied; selection is therefore operating on biologically internal stress-resistance. The best known demographically selected lines are the Rose B's and O's. Environmental stress selection involves the application of an external stress, such as a desiccation conditions (0% humidity; no food or water). Generation times in the stress-selected lines depend upon the time to attainment of an approximate selection-coefficient of 80% mortality. When this estimated ratio of 80% (dead : alive) is obtained, the survivors (the "fittest") are recovered with fresh food and yeast and allowed to lay eggs for the next generation.

Because most selection has been performed on the adults, the only common feature of most selected lines is the developmental & young adult (pre-selection) environment: Eggs are collected at regulated moderate densities (60-80 eggs / 6 mL food) and larvae provided with excess food (banana-molasses) in an 8 dram vial before the adult treatment is applied. This allows the completion of development by all flies without any threat of overlapping generations.

Table of Rose selection lines originating from the IV population. Selection treatment applied at two weeks, unless otherwise indicated.

Designation	Selection treatment	Generation Time
Rose Lab		
IV	Ives. Immediate egg-collection.	2 weeks
B	Baseline: Immediate egg-collection.	2 weeks
O	Old: 8 weeks in population cages.	10 weeks
RU**(O-derived)	Reverse-selected from O's.	2 weeks
SB** "	Starvation B". Starvation period of 12 - 16 days; recovery time ~2 days.	4 - 5 weeks
CB**	Fed control to SB	4 - 5 weeks
SO "	Starvation O" (As SB)	4 - 5 weeks
CO	Fed control to SO (As BC)	4 - 5 weeks
D (O - derived)	Desiccation period of 72 - 120 hrs; recovery time ~48 hours.	3 weeks
C (O - derived)	Control to D; watered but not fed	~3 weeks
DS** (D- derived)	"Starvation D" period of 7 - 10 days; recovery time ~ two days	~4 weeks
CS** (C - derived)	"Starvation C". (As DS)	~4 weeks
ACB**	"Accelerated CB": first 20% of emerging adults harvested; allowed to feed & mate ~ 36 hrs before egg collection.	9-10 days
ACO	"Accelerated CO". (As ACB)	9-10 days
BH (B-derived)	"B High": Control. Reproduction after caged adults fed on yeasted plates.	3 weeks

****** ***Lines marked with an asterisk no longer exist.***

Designation	Selection treatment	Generation Time
Mueller Lab		
CU	Derived from B's, crowded larvae. uncrowded adults first in vials then cages	~3 weeks
UU	Like CU's except larvae uncrowded	~3 weeks
UC	Like UU's, except adults crowded in vials	~3 weeks
AUC	Controls for the ammonia and urea selected stocks. derived from UU's. Adults emerge in cages with fresh food.	~3 weeks
UX	Larvae raised in food with added urea	~3 weeks
AX	Like UX except larval food contains ammonia	~3 weeks

Specific Stock Maintenance Descriptions

Two-Week Stocks (IV, B,)

Stocks maintained on a two-week schedule are kept in the larval culture vials for two-weeks (minus a few hours). There is no adult cage phase. Flies of a single replicate population are gassed with CO_2 and mixed together on a gas-plate, then distributed into fresh food vials to lay eggs. Never put all the flies of a population on the plate at once because the survival of the population is then threatened by a sneeze or a CO_2 tank running out.

Put one-third of the vials on the gas plate at one time. Distribute the mixed adults into the egg-laying vials in approximately equal numbers to the same number of vials you dumped out (e.g. 25 for B's). Lay the vials on their sides so that flies don't stick to the food when they're sleeping. After a time, the flies will wake up and lay enough eggs for the next generation — this takes about 40 minutes in a typical B-population. The flies should be allowed to slightly over-lay. The adults should then be discarded either by gassing them or dumping them out. Egg density is regulated by scooping out the excess eggs to the standard level of about 80 eggs. The stocks are then incubated for two weeks at 25

Extended adult phase (O)

At two weeks, the O vials are dumped into their selection cages. The caged adults are given fresh banana food plates three times weekly (M,W,F). The over-weekend plates must be filled high or doubled in number. This basic routine is carried out for eight weeks. The O's are given a yeast supplement three days before egg collection to boost fecundity. Normally, this is the Friday plate change. On the last Sunday of the life-cycle, new plates are cut (and yeasted) for egg laying. On day 70 of the lifecycle, the O's are collected at standard densities of about 80 per vial. This involves cutting off pieces of egg-laden food from the plates to make up 60 vials for each population. As always, the vials are split into two even-sized bundles and incubated in two different incubators.

Starvation-Selected Lines (SO, CO,)

After the standard two week vial phase, the S-Lines and their controls are dumped into cages. The starvation lines are provided only with pure agar plates, while their controls are maintained with banana food plates (exactly as O's are; see above) in parallel to the selected lines. Agar plates should be changed three times per week in the selected lines, and food plates should be changed at the same time in the controls. The recipe for agar plates is simple.

Pure agar plates:

Add 15g agar for each litre of distilled water.

Boil vigourously. Cool enough to pour.

Starvation selection is stopped at about 80% mortality, or when 500 flies are left, whichever comes first, by the addition of a plain (unyeasted) food plate to the selection cage(s). If the selection line has two cages per population, the combined total number of flies in the two cages is what counts. When the last starvation population is stopped in the selection

phase, the plates of all selected populations and their controls are yeasted (all at once). Eggs for the next generation are collected synchronously for vial-rearing and given the standard 14 days before the next generation.

Note that the males die first in both starvation and desiccation selected lines; females left at the end are, however, fertile for some time. The number of genotypes left is therefore about double the number of flies remaining in the cages. A good rule of thumb when dealing with stress selected lines is about 300 females per population, minimum. A corollary, however, is that the females cannot be left too long before egg-collection, otherwise they exhaust their sperm supplies and lay infertile eggs.

Desiccation selected lines (D, C)

Desiccation selected lines are put into population cages at two weeks of age with no food and no water. The selection involves dumping the flies into cages and adding a bag of Drierite™ desiccant (150 - 175g), then sealing the cage with plastic wrap. Controls are given pure agar plates but no food. When about 80% of the flies have died in a desiccation selected population, a plain banana food plate is added. At the same time, a food plate is added to the control. In the D lines, the paired controls of the same number are handled in exact parallel to the selection line: starvation selection is ceased on the control at the same time that desiccation selection is stopped on the experimental population. If D1 is stopped, so too is C1. When all populations are stopped, the plates are yeasted for two days preceding egg collection.

As with the starvation lines, desiccation selection kills the males first. Therefore it is important to collect eggs soon after the selection is complete — while females are still fertile. On the other hand, the fertile females at the end represent about twice as many genotypes as the number of flies remaining; about three hundred females should be regarded as a lower limit to population size, with 400 being about ideal.

caution: Of all selection stresses currently used in our lab, desiccation selection is the fastest, and so most dangerous to the continuation of a line. Elevations in temperature can accelerate d-selection dramatically. Mortality checks on populations undergoing d-selection must be made frequently, and are particularly important on weekends, when things like lab over-heating tend to happen (and can occur unnoticed). Populations under desiccation selection should be checked at least once every twelve hours.

Accelerated Lines (ACO)

Selection for an accelerated lifecycle involves selection on two basic components of the life cycle: development time and early fertility. The first type of selection is accomplished by careful monitoring of populations for adult emergence; when the first ≤ 20% of emerging adults has come out, they are dumped. As a rule, when 15 or more A-flies are present in a vial, they are dumped into their selection cage. Each vial is individually checked for emerging adults — the overall average of the population is not important. The used vials are frozen after dumping, and so a population bundle shrinks with each dump. Checks / dumps are done at least every 8 hours, and preferably more frequently.

When the last vials have been dumped into cages, the plates are yeasted. Adults are then given 24 - 36 hours before egg-collection plates (cut & yeasted) are inserted for about 8 hours. Density of eggs in rearing vials is especially important in these lines as variation in density will lead to asynchrony of adult emergence (crowded vials take longer).

**Extended adult phase on varied nutrition (BH)

The BH treatment serves as a control for both the timing of reproduction and for adaptation to the cage (rather than vial) environment. (Studies of the B and O treatments by Leroi, Chippindale, and Rose (in press) have shown that adaptation to the two different environments can obscure fundamental trade-offs.).

Note: Because the “H” populations lay so many eggs, it was necessary to implement a procedure of once daily plate changes. The plates can be 1/2 depth poured in the bottom, or full depth poured in the lid, because humidity loss is not substantial in one day. The plates must be changed every day.

Crowded Larval Selection

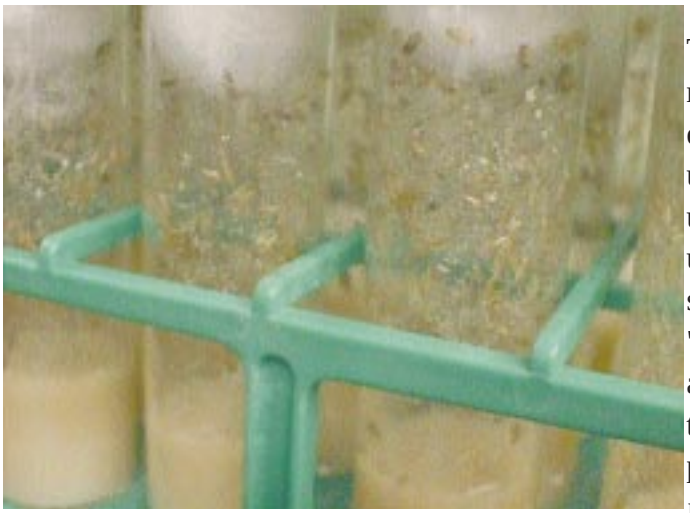

Figure 3 CU cultures. One CU population consists of 40 vials each with initially 1000 eggs.

The CU population consists of five replicate populations that experience crowded larval conditinos but uncrowded adult conditions (see figure 3). We label these replicates under CU1 to CU5 populations. "C" stands for "crowded as larvae" and "U" symbolizes "uncrowded as adults". The control population for this selection treatment is the UU population that is described in the next section.

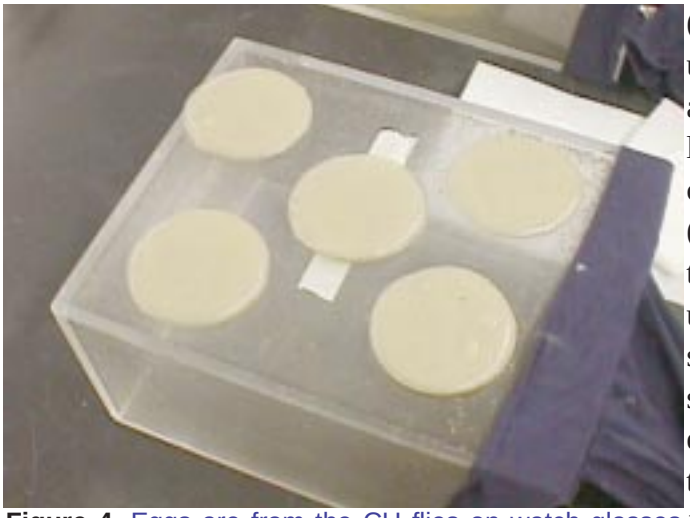

Figure 4. Eggs are from the CU flies on watch glasses with standard food. Each cage receives five watch glasses for 24 hours.

Egg collection: Collect one rack (40 vials) for each population. We use are 6-dram vials to raise the ggs and larvae in since this enhances the levels of larval crowding. In each cage, there will be 5 watchglasses (see figure 4). If the egg density on the watchglasses is evenly distributed, divide each watchglass into 8 slices (5x8=40 slices) and insert the slices into the vials faced up. If the egg density is not evenly distributed, then try your best to estimate about 1000 eggs (I suggest you use the microscope for the first try just to

see how much is 1000 eggs). If there is excess amount of eggs, you do not need to use all of them. Just collect enough for 40 vials and discard the extra eggs. If there is not enough eggs, then collect as many as you can and come back the next day to finish up the rest. Be sure to wash you spatula before starting a new population to eliminate contamination! After you are done with collecting eggs from the watchglasses put two half-cut plates with yeast into each cage. These plates will be used the next day for back-up egg collection. The racks will be kept in the first shelf on Incubator #4. The incubator should have 24-hr light and temperature of 25^{o} C. If you detect anything wrong with the incubator, please notify Dr. Mueller, or the current stock leader immediately so we can remove the racks into a new incubator.

Back-up egg collection: We collect back-ups as insurance in case of emergency. For this egg collection, we only need to collect 20 vials per population at regular 60-80 eggs/vial. Backups can usestandard, 8-dram vials. The back-ups will be kept in Incubator #5. After collection, you can discard the flies (kill them in any way you like). If anyone needs the CU's for an assay, then we need to continue to maintain these flies. That means changing food plates in the cages every two days.

Racks of vials are designatied as either IN or OUT. IN and OUT means that we rotate the racks that we use for egg collection in and out every other day in the incubator. The Black dash on the rack's label means OUT. If there are no dash marks on the CU label then it means IN. The reason for rotating the racks is to give the vials the same amount of light. We don't want any other variables such as uneven light to have any affect on the flies.

Adult collection: Eight days after egg collection, we will start collecting the adults. We want to transfer about 60 adults from the 6-dram vials into 8-dram low-pour vials. We collect the adults in two sets: the BLACKS and the REDS. These are just black and red CU labels that we put on the racks. The reason for having two sets is that if something wrong happens to one set, we still have flies from the other set. We start off the first day of adult fly collection by collecting approximately 60 flies into either the BLACK or RED set only (see figures 5-6). The next day we would collect the same amount of flies in each vial in the other set. We alternate between the red set and Black set for eight days. When collecting the adults into the 8-dram vials, if a larva or pupa gets dumped into the vial by accident, you need to transfer the flies into a new vial. We don't want any larvae or pupae living in the same vial as the adults. The adult collection is carried out for eight consecutive days. Our goal is to collect about one rack of adult flies for each population (the vials from both the BLACKS and the

Figure 5. Flies from the CU cultures are transferred to fresh cultures by inverting the old culture and rapidly tapping the old and fresh vials together. It is not neccessary to get every single fly out of the old culture.

Figure 6. Emerging CU adults are transferred from the crowded larval cultures to fresh vials at densities of about 50-80 per vial.

REDS should add up to more than a rack). After the eighth day, if we don't have one rack yet, then we need to continue collecting the adults until we have enough. The BLACK and RED racks will be kept in Incubator #4. (Note: Having 60 adults in 8-dram vials is the uncrowded condition).

Dumping: Make five cages with labels CU1 to CU5 . Dump all the flies from the racks, both BLACKS and REDS, into the cage with the appropriate population name label. Do not dump the flies into the wrong cage because we do not want them to interbreed. Put one plate with yeast into each cage.

Watchglasses: Two days after dumping, put into each cage five watchglasses (without any yeast). Exactly 24 hours later, collect eggs from these watchglasses. Here the cycle starts all over again!

Transfer back-ups: When you are busy with the stock, don't forget about the back-ups. Thirteen days after back-up collection, the flies from the back-ups vials will emerge. So we need to transfer them into new food. Do a 1:1 transfer. The back-ups will be transferred into new food every two days. And these flies will be discarded after we collect new back-ups for the next generation.

Cooking: Cooking is an important part of stock maintenance because we don't want to starve the flies. For every generation, there will be two cooks.

1) This cook must be done the day before egg collection in order to keep the watchglasses fresh. This cook includes: 5 racks of 6-d high-pour for egg collection (approximately 5mL of food for each 6-d vial), 2.5 racks of 8-d high pour for back-up egg collection (approximately 6mL of food for each 8-d vial), and 30 watchglasses with approximately 8mL of food per watchglass. Put the racks in the refrigerator. For the watchglasses, prepare a tray with wet cotton in it, put the watchglasses on top. Cover the tray with plastic wrap to prevent contamination. The wet cotton will keep the watchglasses moist.

2) The second cook must be done before the day of first adult collection. This cook includes 20- 25 racks of 8-d low pour which will be used for adult collection and transferring back-ups, and 10 food plates for maintaining the cages later.

Crowded Adult Selection & Controls

There are five control populations called UU, that are maintained under **u**ncrowded larval and **u**ncrowded adult conditions. The UU populations serve as control for the CU populations described previously and the five UC populations that are maintained under

uncrowded larval but **c**rowded adult conditions. The UU/UC populations were derived from the five B (baseline) populations, which were originally derived form the IV (ancestral) populations in the Rose Lab.

Figure 7. After the UU and UC flies have laid eggs on food plates, the food is cut into small sections with 60-80 eggs per section.

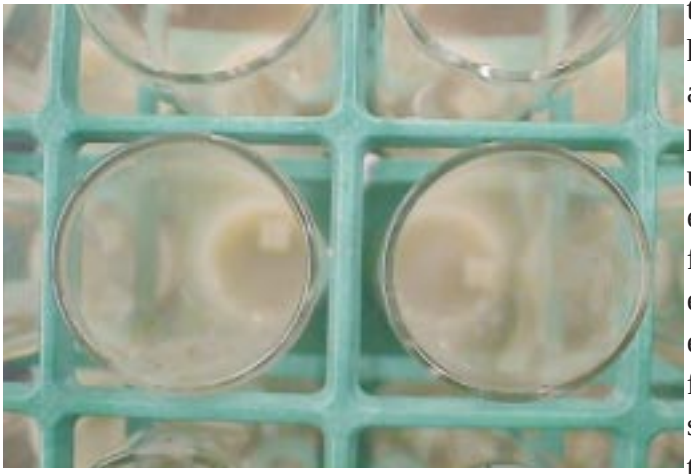

Figure 8. Each small piece of food is then put into a fresh vial. These eggs will hatch and develop in the vials over the next two weeks.

Stock Maintenance

The UU/UC populations have a three-week generation time (Exactly 21 days).

1) Day 1: Stock egg collection. 60-80 eggs/8-dram vial are collected from the previous generation of flies (see figure 7-8). For the UU populations (UU_1-UU_5), 1 rack (40 vials) of eggs are collected for each population. For the UC population (UC_1-UC_5), 2 racks (80 vials) of eggs are collected for each population. The food for egg collection is high pour. After the egg collection, a plate of plain banana food is placed in each population for back-up egg collection. (Note: Back-ups egg collections are always done the following day after the main stock egg collections. Never use the same eggs collected from the main stock for back up egg collection. The reason is that, if the food plate used for the main stock egg collection got contaminated then this will kill main stock as well as back-ups.)

2) Day 2: Back-ups Stock egg collection. For both the UU and UC, 20 vials (1/2 rack) of 60-80 eggs are collected in case something goes wrong with the main stock egg collection. (Note: Back-ups populations are maintained in a different incubator than the main stock population.) After the back-up egg collection is a waiting period for 11 days until the adult flies emerges. During this waiting period a food cook of 4.4L is normally done on day 11th. (Note: Do not use food that was kept in the refrigerator for more than three weeks to transfer the flies.)

3) Day 11th: Food Cook 4.4L. Exactly 20 low pour racks are needed for transfer next week. (Note: 20-22 racks are needed because there will be accidental breakage during transferring.)

4) Day 14th: Food transfer (main stock + Back-ups). When the adult flies emerges, they

will be transferred into new food vials. The transfer for the UU and the UC are at different ratio on day 14th. The following transfer on day 16th is at the same ratio for the UU and the UC. For the first transfer on day 14th, the UU flies are transfers from old to new food at a 1:1 ratio (1 vial of adult flies per new food vial). However, the UC flies are transfers from old to new at a 4:1 ratio to crowd the adults (4 vials of adult flies per new food vial). There should be approximately 200-240 flies/vial for the UC first transfer on day 14th. All back-ups transfers are done at a 1:1 ratio on both days 14th and 16th.

5) Day 16th: Food transfer (main stock + Back-ups). For the following transfer on day 16th, the ratio is 1:1 for both UU and UC. All back-up transfers at a 1:1 ratio on both days 14th and 16th. (Note that adult crowding density selection stress decreases as the number of times you transfer because some flies die as a result of crowding.)

6) Day 18th: Food Cook 4.4L. Exactly 15 racks of high pour food are needed for egg collection of the new stock cycle. (Note: 15-17 racks are needed because there will be accidental breakage.) Exactly 30 plates of banana food are needed for dumping and egg collection. Also make 10 cages for dumping on day 19th.

7) Day 19th: Dumping. All the flies from UU and UC are dumps into cages with yeasted banana plates for two days without changing the plates. Dumping usually occurs every third Saturday. There will be 10 cages total. Five cages are for UU_1-UU_5 populations and the other five are for UC_1-UC_5 population.

8) Day 21st: Cut-plate. The old yeasted plates are removed and new non-yeasted plates are replaced. Before replaced with the new plates, the plates are cut in half and place into two petri dishes. This will increase the surface area and allow the flies more room to lay eggs. The plates are left in the cages for 24 hours to allow the flies to lay eggs. After 24 hours, the plates are removed and stock egg collection begins once more. The cycle will start again just like day 1.

Food Requirements

1) Stock egg collection: 10 hi pour racks (UC)
5 hi pour racks (UU)
20 plates

2) Back-up egg collection: 5 hi pour racks

3) Serial transfer: 10 low pour racks (UU) [5 racks on day 14th and 5 on day 16th]
5 low pour racks (UC) [2.5 racks on day 14th and 2.5 on 16th]
5 low pour racks (back-ups) [2.5 racks on day 14th and 2.5 on 16th]

4) Dumping: 10 plates
10 cages

Ammonia & Urea Selection

The Wasted Stocks are composed of two selection regimes and one control regime. All three groups are kept as five-fold replicates, with discrete generations. The UX1-5 lines are exposed to high levels of urea added to their larval food environment. The AX1-5 lines are exposed to high levels of ammonium chloride added to their larval food environment. The AUC1-5 lines serve as controls, and experience no nitrogenous waste products added to their larval food medium.

All populations were initially derived from the UU1-5 lines. All replicate populations bearing the same subscripted indices were derived from the UU replicate population bearing the same number. Initially, the UX lines were kept at a urea supplemented level of 12g/L, which has gradually been increased depending on observed adult survivorship at the end of each generation. The AX lines were begun at 0.25mol NH4Cl, which has also been gradually increased depending on observed adult survivorship at the end of each generation. Currently, the levels of the UX lines are 23g/L added urea, while the AX lines are at 0.82mol added NH4Cl.

Methods and Materials. Every generation requires that this below followed protocol be followed explicitly. Deviations may cause the force and magnitude of selection to be altered dramatically.

Food Preparation. Food is prepared by the same methods used to prepare normal 6.6L banana food cook. The only difference is that after the hydroxy-benzoic acid has been added, 2L of food is set aside, and either a set amount of urea or ammonia is added to the food.

Two liters of food is poured into the two liter plastic beaker, which is to be placed on a hot plate that has a built in magnetic spinner. A thick stir bar is centered in the middle of the beaker, and the known amount of ammonia or urea is added to the banana food. The food is allowed to stir rapidly in the beaker, in order to insure that the nitrogenous waste product is evenly dispersed in the banana food.

The food is poured into vials using the Hydrostatic Pump, delivering an exact pour into each vial. After pouring all the vials for one waste compound, thoroughly wash the beaker and pump tubing, and, then, reassemble to pour the next compound. Again, two liters of banana food are poured into the 2L beaker, and the next nitrogenous waste product is added to the banana food. The stirring mixture is then poured into the vials.

Afterwards, extra food may be used to pour the AUC vials, plates, or back-up vials. Sometimes a second cook (usually 2.2-3.3L) may be necessary to get enough plates and back up vials.

Supplies: Every generation you will need

AUC: 40 vials/pop. @ 5 pops = 200 vials (5 racks)

AX: 60 vials/pop @ 5 pops = 300 vials (7.5 racks)

UX: 60 vials/pop @ 5 pops = 300 vials (7.5 racks)

-15 cages & approximately 30 banana food plates.

-8 agar plates (Do a 1.1L cook, but only boil the water and agar, the pour into plates).

-800 plastic sleeves.

Back-ups: 20 vials/pop @ 15 pops = 300 vials (7.5 Racks).

After the cook, when the food has dried, the vials are to be flipped over, and put into the refrigerator. When we are approximately 2-3 days from collecting the next generation, we may begin to insert plastics into the vials, as to make our lives easier.

Egg Collection: Eggs are laid on the edges or top of a cut agar plate. This non-nutritive agar plate is used to insure that no banana food is transported into the vials which may contain either urea or ammonia. Eggs are collected at a density of 60-80 eggs per vial. It is IMPORTANT that this density is maintained. Vials are sealed with a sponge, not cotton, and placed in Incubator #5.

For every AX and UX line, 60 vials of 60-80 eggs are collected. Many racks are split between two populations, so BE AWARE of which population you are collecting eggs. The For every AUC population, 40 vials of 60-80 eggs are collected. It is critical that when you are finished collecting eggs from one population, you wash your spatula and petri-dish, before moving on to collect eggs from another population.

A half-cut banana food plate is inserted into each cage, after the agar plate is removed; so we can collect eggs for the back-up vials. Generally, you can collect back-ups within 2-3 hours after placing the banana food plate in the cage. Twenty vials of 60-80 eggs per vial serve as back-ups in case something goes wrong with the selection lines. These back up populations are obviously kept in a different incubator than the stock populations, in case of an incubator mishap.

During Selection: One day after egg collection, plastic sleeves will be inserted into every vial from all populations, to allow the larvae to pupate on the sleeves (figure 9). Forty-eight hours after egg collection, the racks are to be rotated in the incubator (front to back), in order to better homogenize the exposure to light of all the vials. After approximately 5 days, the vials are to be checked for pupation.

Figure 9. Plastic sleeves are inserted into each vial after eggs have been added. The fit must be tight and the sleve should be pushed all the way down to the food.

Plastic Pulling. When it appears that a majority of the larvae have pupated, seen by few larvae left feeding in the food, we may begin the process of removing the plastics and placing them in cages. Generally, the AUC lines will be ready first, as they will not experience a prolonged developmental time period brought about by expo-

sure to nitrogenous waste. Plastics are removed and stacked in the back of a cage. Then a food plate is placed into the cage.

Generally 7-8 days after egg collection, the UX and AX lines are ready to be have their plastics removed, but this may vary depending on the effect of the nitrogenous waste. After stacking the plastics in the cage, a food plate is inserted into the cage.

After approximately 2-4 days, most of the flies will have eclosed. This may be determined by observing whether the pupae that have blackened are now clear, indicating that the fly has emerged from its puparia. At this time, a yeasted food plate is put into each cage for two days, in order to boost fecundity. About 12 hours before egg collection, a cut agar plate is put into each cage, for the flies to lay the next generation's eggs upon. A longer egg laying period may be required, if the number of adults are low.

§3. ASSAY PROCEDURES (and recipes)

General Stuff about Experimental Assays

Assay versus stock maintenance

Assays are conducted to closely measure the evolution of characteristics of different selection lines. Stock maintenance and experimental assay are often confused in the working environment, even though there are many differences. In routine selection, control over conditions is limited. The primary concern in selection is the perpetuation of a line. In an assay of a character, flies are separated from the parent stock and used to measure that character of the line. Usually assay flies are cultured more carefully with respect to density of eggs and duration of the egg collection. Flies in assays are usually measured to the point of death (as in longevity, starvation &c) and discarded after the data have been collected whereas stock flies are not generally measured for anything with any precision, but are never discarded. Because assay flies are separated from the selection line, they are less critical to the lab and always give way to the maintenance of a stock if resources conflict; assays can be repeated.

Absolute vs. relative measures

Life-history characteristics are noisy things to measure, depending strongly on exact experimental assay conditions. For this reason, a fecundity or development time (for example) of a population is never an absolute measure. The characters we measure are always taken as a difference or a proportion, relative to a control. Absolute fecundity, for example, can vary by a factor of 50%, depending upon the assay conditions. The absolute number is therefore nearly meaningless. We always assay an evolved character relative to a control.

Pre-Assay maintenance

Experimental assays are usually designed to detect the genetic effects of selection, and so an attempt is made to minimize “noise” generated by the environment and the direct effects of selection (such as parental effects). For example, in comparing a starvation line to its control, an assay performed on flies from eggs taken directly from the selection lines might be biased by a maternal effect generated by the stress imposed during selection. Another example would be the comparison of two lines kept asynchronously, such that the larvae were reared at different densities or on different food. Any non-genetic effects that might bias an assay in a particular direction or add noise should be controlled as much as possible.

Flies that are to be experimentally assayed are always put through at least one full generation of synchronizing and standardizing maintenance away from the parent stock. The standardizing generation is employed to ensure common pre-assay conditions of the flies

to be measured. They are given the same food, the same age of adults, the same density of larvae, and so on. This procedure also helps synchronize the emergence of adults for the experimental set-up. Two generations of two to three week propagation is standard procedure, but after more than three generations of common propagation, selection may start acting on the assay flies in a counter-productive way, so assay flies cannot be too many generations away from the parental stock regimen.

When an assay is planned, flies often have to be derived from asynchronous stocks; for example, if B and O are going to be compared for fecundity, one must plan to have saved one set of populations until the other is available for collection. Collection for an assay cannot interfere with the maintenance of the stock. As an example, if you want B's and O's ASAP, it is likely that the O's will be somewhere in the middle of their cage phase of the life cycle — lets say at five weeks. The B's come up for egg-collection every other week, so its easy to ask for them to be saved for you and get them in fairly short order. But if the O's are in week five in their cages, they will be laying very few eggs. To collect from the O's there is no other way than to cross your fingers and hope that the routinely changed feeder plates will provide you enough eggs. You can cut the plates to make collection easier, but you cannot yeast the stock in the middle of their lifecycle. By the same token, you must wait for the completion of stress selection to acquire these lines for an assay.

A crucial factor that is often overlooked in our system is the importance of larval density in the flies being prepared for an assay. Larval density differences can have pervasive effects on adult performance. It is well known, for example, that larval density strongly influences adult size, and thus also fecundity, starvation resistance, desiccation resistance, and so on and so on. If one wants to compare fecundities of two lines, and larvae are not reared at carefully controlled densities, considerable noise could enter into the data. This problem is particularly insidious when lines have different fecundities, such that a less than careful egg-collection could lead to a systematic bias between lines compared: if O's lay fewer eggs than B's, and egg-collection is not really careful, the assay flies for O will come from lower density vials, be larger, probably have higher stress tolerances, and so on. An artifact has created a result. It is recommended that assay flies be taken from close to or exact density vials (60 per vial). Exact densities will increase confidence in the results of the assay and also lessen error and make sampling easier due to more synchronous emergence of adults.

The importance of checks

Large amounts of time are devoted to the derivation of assay flies, and the assay set up and maintenance. Time checks and time-dependent maintenance are crucial to the success of the project and cannot be treated with a casual attitude.

If you agree to do a check at hour X on somebody's experiment, it is your responsibility to see that the check gets done. What this means practically is that if a crisis arises, or anything or anyone comes between you and your check, you must find a substitute (one who knows exactly what to do on that exact experiment) or inform the person supervising the assay. It is therefore advised that you know the telephone numbers of the person in charge

and several other people closely involved in the experiment. Failure to do a time - check will reflect very badly upon you. Hours and hours are invested in experiments, particularly by the people supervising the project, and if you miss a check you promised to make, you could very easily destroy the experiment and get a lot of people pissed off with you.

If you are managing a project involving odd-hours checks, it is strongly advised that you check up on people doing the checks. If this means waking up and calling in to the lab at 6AM, its worth the trouble. People screw up and miss checks, and unless you are willing to wake up and be ready to dash in if they do so, you will have experiments fail. At the same time, if the people that help you are aware that you are watching over the experiment closely and will be greately inconvenienced by their screw-ups, you will find they are more motivated to be there on time. One way to encourage fidelity is to tell helpers that you will call precisely at the check time, and, if there is no answer, go to the lab to do the check (and not be happy about it).

Conditioning

A conditioning period is often employed before the assay of adult characters is set up. The conditioning period simply refers to a period of fixed duration in which flies are handled with carefully controlled conditions. For stress-resistance characters, this generally means four days with a controlled number of flies on a well defined food background. The conditioning environment and measurement environment may differ, as, for example, when flies are measured for early fecundity on charcoal food but conditioned on banana medium beforehand. In the last example, it may seem inappropriate to measure the character (fecundity) under different environmental circumstances than the conditioning environment of interest (counted on c-food instead of b-food), and indeed, if the character changes rapidly in the new assay environment, it would be inappropriate. It is therefore necessary to know something of the response-rate of the character to the change of environment.

Some Protocols

FECUNDITY

Fecundity is simply the measurement of the number of eggs laid per female per unit time. Fecundity has, however, become an extremely complicated character with the work of Leroi and Chippindale over the past few years. What was considered the “standard” fecundity assay in the early evolution of Rose Lab stocks gives results that are strongly dependent upon the stocks and conditioning environments of the flies assayed (see below).

Fecundity is strongly dependent upon the conditioning environment of the females. As would be expected, against a rich food background (e.g. high yeast), females lay lots of eggs, while on plain food, or old culture medium, fecundity is relatively low. This should not be a problem, because we seldom concern ourselves with absolute measures. We are interested in the performance of an evolved line compared with its control line. In other words, if in one assay of early fecundity B’s layed 82 eggs / day and O’s 69 eggs / day, and in another, B’s = 105 and O’s = 87, the results are the same: B’s layed more eggs than O’s early in life. Yet, to continue this example, this rank-ordering of selection lines has not

proven durable with time in the "standard assay": in 1984, B's did lay more eggs than O's early in life, but, to the shock & surprise of Chippindale and Leroi, in 1990 the O's layed significantly more eggs than the B's in early life. The reversal called into question the theory of antagonistic pleiotropy — more specifically Rose's (1984) classic demonstration of trade-offs: how could the O's live longer and lay more eggs than the B's?

It turns out that fecundity is a character that has evolved in both the experimental line (O) and its control (B) to be sensitive to exact conditioning environment. Each line enjoys a "home-court advantage", and the "standard" fecundity assay more closely approximates the home-court of the O's.

Standard fecundity assay

Flies are collected as newly emergent adults at less than 12 hours from emergence. Pairs are put in charcoal-food vials with yeast prepared as follows:

Charcoal food vials (see recipe in appendix Z) are dotted with a pasteur-pipette's drop of yeast solution (20 mL distilled water, 3 mg yeast, and 1 mL 1% acetic acid), which is then dried overnight in front of a fan.

The assay flies are transferred daily at the same time of day to new food vials and eggs are counted for days 3, 4, and 5. Usually "early fecundity" is taken as the mean over these three days. However, if a different conditioning environment from the medium the eggs are actually counted on is used, then the 24 hour fecundity of day four might be used.

Egg-counting is difficult and cannot be entrusted to the inexperienced worker without careful checks on accuracy. Eggs look like terds look like larvae look like yeast globules; the importance of skill and experience in egg counting cannot be over-emphasised, and it is better to start slowly and carefully and improving on speed later.

cautions: Flies easily stick to the medium, especially when they are anaesthetized, and so hand transfers should be gentle and flies that are gassed should be allowed to wake up for about 15 minutes with the vial on its side before being put in racks so flies are not stuck to the bottom.

If vials are poured too low, they may be difficult to count under the microscope; a depth of 1-2 cm is recommended.

If vials are yeasted and dried in front of a fan, they may start to dry to the point that the substrate medium will shrink away from the side of the vial. The narrow gap produced is very dangerous as it will trap flies and ruin an experiment.

LONGEVITY

Longevity of stocks has been assayed in almost as many different ways as fecundity, but the standard assay remains as follows:

Newly emergent flies are placed in groups of eight (4 males, 4 females) on plain banana food. The flies are transferred three times weekly (M,W,F), but are scored for mortality every day, always at the same time.

In some assays widows and widowers have been shuffled, but in the “standard assay” this complication is not considered.

cautions: Sticky, wet, and cold food can create problems in the transfers. But so too can differences in the violence level employed in the hand transfer. Tapping the flies into the new vials is best done with reserve. Attempts should be made to standardize all factors that could cause the sticking of flies, which is problematic to the assay of longevity.

STARVATION RESISTANCE

The standard assay of resistance to food-deprivation consists of sealing four single-sex flies in a humidified vial. Specifically, the flies are trapped by a thin-cut sponge (half-sponge), two absorbent cotton balls are stuffed in, and 3 mL of water are squirted into the vial before it is sealed with parafilm (see figure 10). The vials are then inverted to make checks easier and to prevent the water from running down into the fly-end of the vial. Mortality is easily scored on the data sheets as the number of flies dead (out of four). Checks on starvation time are generally performed at six or eight hour intervals.

Variations on the standard starvation assay usually involve the numbers and sexes of flies put into a vial. In some assays, a male-female pair has been used, and in others a single fly has been used. The datasheets used will reflect the design of the project.

cautions: When setting up, care must be taken to ensure that all vials are properly watered and sealed. Failure to add the water will lead to very rapid death by desiccation, and failure to seal the vial (or a hole in the parafilm at any time in the assay) can also cause premature death because the water will evaporate. When a vial is observed with anomalously quick death rate, it is a good idea to check that the seal is good and that the cotton is moist; if the cotton is dry, the vial must be thrown out of any sort of analysis.

Chipped vials should be avoided when setting up, as they tend to tear the parafilm immediately or at some point in the assay.

If there is not enough cotton, or (for some other reason) water runs down into the space occupied by the flies, they may get stuck in the droplets. Flies that die in this manner should be excluded from the analysis, and therefore noted on the data sheet.

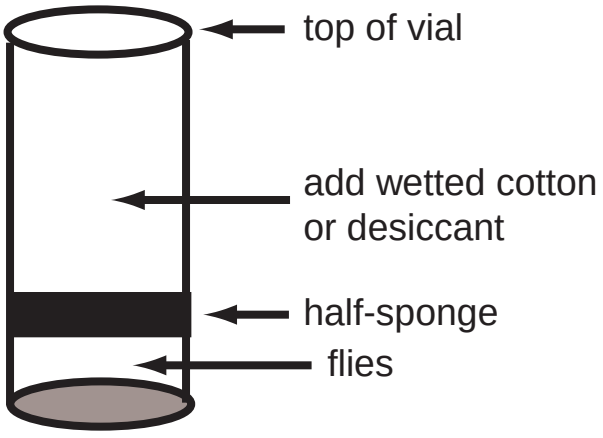

Figure 10. Flies are placed in vials and then civered with a half-sponge. After desiccant or wet cotton has been added the vial is sealed with parafilm and inverted.

DESICCATION RESISTANCE

Desiccation resistance is measured almost exactly as starvation resistance is, except that, instead of wetted cotton, five mL of drierite desiccant is dumped into the vial atop the half sponge before sealing it up. Proper sealing of the vials is even more critical than in the starvation assay, and indicator (blue) desiccant is strongly recommended over the non-indicator type (white). Desiccation death procedes very

rapidly in all stocks other than the D's, and checks have always been done every hour in the Rose lab; this frequency may be excessive, but unless one is comparing an unselected stock to a D (or to desert species), there is little convenience to be gained from a less frequent check schedule.

cautions: Mostly the same as for starvation resistance (above), except that the indicator desiccant can easily give away bum vials. If desiccant turns pink, indicating saturation, the vial should not be incorporated in analyses.

In a frequent check schedule, the sequence of populations checked should be strictly uniform from check to check, and assay racks should be returned to the incubator ASAP, because temperature has a strong effect upon desiccation time.

DEVELOPMENTAL TIME & VIABILITY

Development time is a very fussy assay. The eggs collected must come from a tight collection window, because stock differences are small and larvae hatch fairly quickly. It is recommended that the eggs come from a one to four hour interval of laying by at least 1500 flies in cages, and that, if additional eggs are required, they are taken from another such round of collection. In other words, if you don't get enough eggs from the first collection, you change the plates for another round several hours later. Careful notes should be kept for each population sample.

Eggs should be placed in vials at exact densities. The standard number is 60 eggs in a vial with carefully poured banana food. The eggs are brush-transferred to small cubes of food, in six piles of ten, which are then put into the development vial. The cubes of food must go into the vials right-side-up or they tend to smother. Similarly, the eggs should not be heaped in piles of larger size than ten. Dr. Mueller counts larvae hatched on pure agar to avoid problems with egg hatchability. When the larvae have pupated, checks on adult emergence are done every six hours.

A "check" is accomplished by the hand-transfer of emergent adults to a "holding vial" before sexing and counting. If enough sets of holding vials are made up, the counting of emergent adults can be done during normal waking hours.

After the completion of adult emergence, the vials should be left for a few days (3-7). The pupae on the sides, dead flies on the bottom, and very late emergent adult should then be scored. This gives informtion about egg to pupa survivorship; the difference gives the number of deaths in puparium.

Fly Maintenance For Physiological Measurements

HANDLING FLIES

For the comparisons, all flies are raised in a common environment, described below, for two generations prior to the experimental generation.

Generation 0

1- Eggs or adults are obtained from the appropriate stocks from the Rose lab.

2- Egg density is controlled by placing approximately 60-80 eggs into each of 25 vials per treatment.

3- Adult flies are placed in cages 14 days after egg collection, where fresh standard Rose banana food is available.

4- Adults are kept in a cage and receive standard Rose banana food in Petri dishes with no additional yeast every other day.

5- On day 18 after egg-laying, flies are given Rose banana food in Petri dishes plus a layer of yeast every day for three days. On day 21 flies are allowed to lay eggs for 4 hours after which eggs are collected for the next generation as described below.

Generation 1

In this generation flies are cultured the same way as in generation 0 with the exception that:

1- Egg density is strictly controlled by counting 60 eggs into each of 100 vials per treatment.

2- On day 18, flies are given Rose banana food plus a layer of yeast every day for three days. On day 21, flies eggs are collected over a 4 hours period.

Generation 2

Generation 2, the generation on which physiological measurements, are made, is handled as follows:

1- For generation 2, egg density is again strictly controlled at 60 eggs each per 150 vials per treatment.

2- Vials are checked for pupae formation every day.

3- Upon pupae formation, vials are checked every 12 hours for darkened pupae and emerging flies.

4- Within 12 hours of emergence some flies are removed for physiological measurements (day 0).

5- At the same time other flies are placed in clean food vials at approximately 60 flies per vial and placed in the incubator until the flies were 24 hours of age for physiological measurements (day 1).

6- All other flies are transferred to cages within 12 hours of hatching (day 4 and other)

7- New food plates with no additional yeast are added on Monday, Wednesday and Fridays.

LARVAL FEEDING RATE

Preparation of larvae

Collect eggs from the experimental and conrol populations and raise at standard low den-

sities. Collect eggs from these adults on petri dishes with agar and a small dab of yeast paste. After the eggs have been laid remove the yeast paste and let the eggs hatch 24 hours later. Remove 50 first instar larvae per population and place these larvae on a petri dish that has an agar base and a generous amount of yeast paste (37.6 g yeast in 100 mL of water). The petri dishes should be placed in an enclosure that prevent the yeast from drying out. This can be a glass caserole dish covered with saran wrap, for instance. These larvae should be allowed to develop in an incubator for 48 hrs.

Setting up the Video Equipment

Microscope

· Put black light splitter on microscope with the double lens on top.

· Set openings to: 100% PHOT and #5.

· Put the metal (silver) lens in camera holder.

· Screw camera into silver metal lens. Align camera position to coincide with microscope.

Camera

· Plug in power supply, green light should go on.

· Connect the TDCT to the camera throught the video connection on the camera.

· Connect the TDCT to the VCR at the video input plug.

VCR

· To display microscope on the TV screen hit input select until the VCR shows A1 (external recording source.

· Record on SP

Recording Feeding Rates

Prepare a petri dish with agar only. Cover the surface of the petri dish with a yeast suspension (10 g live yeast in 100mL of water). This suspension may have to be added several times during the course of the measurements if it is absorbed into the agar. Place a single larva on the dish. Place a written note in view of the camera that describes the population and replicate of this individual larva. Record the larva for at least 60 seconds. Make sure the larva stays in the field of view of the camera by moving the petri dish when necessary. The video tapes are later replayed to count the feeding motions per minute. Two different people should independently count each larva over the same time interval. If the two counts differ by more than 5 feeding motions. Both measurements should be repeated.

ADULT FEEDING RATE

Preparation of the flies

1- For these measurements, an equal sex ratio of flies is used. Counts are made of flies

exposed to food for 1 minute (time 0) or for 2 hours. For each of these measurements, 60 males and 60 females are used.

2- On the day of the measurement, anesthetize the flies using CO2.

3- Count 60 males and 60 females for background and the same number for actual measurements.

5- Move 60 flies at a time to clean food vials and let recover for 1 hour.(Do not separate males and females.)

4- After the recovery, transfer 60 males and 60 females to a cage for time 0 measurement and the same number to another cage for feeding rate measurement.

Preparation of the food

1- You can prepare the food while the flies are recovering from anesthesia

2- For each population that is being measured, 2 nonradioactive dishes of food and 2 radio-active dishes are needed.

3- Use small weigh boats as dishes to reduce the amount of radioactive waste.

4- Fill 8 scintillation vials with counting cocktail.

5- Label the first vial as background.

6- The next two vials can be labeled cold food 1 and 2

7- The last 5 vials should be labeled hot food 1-5

8- In a microwave, melt half a Petri dish of food (the food in four vials can also be used). Watch the food, it tends to boil over.

9- The melted food needs to be clump free and completely melted

10- Stir food and to each of the cold-food vials add 5 microliter of food

11- Pour food into two weigh boats labeled cold food

12- To 250 microcuries of inulin, add 500 l of water and mix well

13- Add 50 l of inulin to food and stir

14- To each of the vials labeled hot food, add 5 l of the food

15- Pour food into the weigh boats labeled hot food and let solidify

Feeding rate measurements

1- Once flies have been transferred to cages, add cold food and let stand for 2 hours.

2- After 2 hours, remove cold food and add hot food to feeding rate cages, both the time-zero cage and the 2-hour cage.

3- To time-zero cages, add hot food for 1 minute

4- After 1 minute, remove food and anesthetize flies

5- Place a filter paper inside a funnel and wet the paper.

6- Heat distilled water in a squirt bottle

7- Remove flies with aspirator onto the filter paper

8- Wash flies with hot water

9- While flies are feeding, prepare 10 vials for counting cocktail and 10 vials for hyamine hydroxide

10- To each Scintillation vial add 5 ml of cocktail

11- To the other 10 vials add 0.5 ml of hyamine hydroxide. Be careful; both of these fluids are corrosive.

12- Once flies are washed, separate the sexes. Place 10 females in each of 5 hyamine hydroxide vials.

13- Repeat for the males.

14- Grind flies with a glass rod.

15- Wash rod with counting cocktail from a vial using a Pasteur pipette.

16- Transfer the rest of the counting cocktail and discard that vial into cold trash.

17- Cap vial with flies and set aside.

18- Repeat this procedure until all vials have been ground.

19- You should have 5 vials of 10 females and 5 vials of 10 males each for time zero.

20- Once flies are done feeding (2 hours) repeat steps 5-19 for flies which have fed on radioactive food.

21- Vials are then taken to a counter and counted on a C14 channel for 5 minutes each.

LIPID MEASUREMENTS

Required Equipment

1- Soxhlet apparatus,

2- Whatman thimbles 10 x 50 mm size

3- Petroleum Ether

4- Oven that maintains 60 Celsius

5- Cahn balance that measures to 0.001 mg

Fly Preparation

1- All flies used for this measurement are removed from the colony and frozen

2- Separate flies by sex and place 10 of each sex on a small weigh boat

3- For each sex prepare 6 such weigh boats.

4- Place boats in oven at 60oC for 45 minutes

5- Calibrate balance using 200 mg weight following instructions closely.

6- Weigh each boat and record as weight before extraction

7- Label the required number of thimbles with a pencil (not a pen or sharpie)

8- Place the contents of each boat (10 flies) in a thimble

9- Fold and staple the end of the thimble

Extraction

1- Make sure the Soxhlet is placed in a hood with no flames or flammable substances

2- Attach condencer to water. Turn water on and make sure it is cold

3- Carefully separate the flask containing petroleum ether from the condenser and extractor and set aside

4- Then separate the condenser from the extractor.

5- Place the thimbles in the extractor, making sure that they are below the small glass side arm.

6- Thimbles have to be placed in the extractor such that they do not float once the extractor is full.

7-fill flask with petroleum ether until it is 2/3 full

8- Reattach extractor to condenser and then flask to extractor

9- Make sure all seals are tight

10- Turn on heating pad

11- Let stand for at least 20 hours

Lipid Measurements

1- After 20 hours turn off heating pad

2- First carefully separate flask from extractor and then extractor from condenser

3- Remove thimbles in a hood and let them dry

4- Remove staples carefully so that thimbles can be re-used

5- Place flies from each thimble into a separately labeled weigh boat

6- Place in oven at 60oC for 30 minutes

7- Weigh flies after calibrating balance and record as weight after extraction

8- The difference between the weight taken before and after extraction is the amount of lipid lost by the fly

9- Keep in mind that this method measures total lipid, which include triglycerides and

cuticular hydrocarbons.

RESPIROMETRY MEASUREMENTS

Required Equipment

1- Sable System SA3 combination oxygen and carbon dioxide analyzer attached to a computer

2- Respirometry chambers

Preparation of Flies

1- On the day of the measurement anesthetize flies and count out the number of flies needed.

2- Transfer flies to a respirometry cage and let recover for an hour.

3- If needed, prepare food by melting half a Petri dish of standard banana food and pouring into small weigh boats.

4- Once solidified, place food in cage

5- Make sure cages are well sealed

6- For each measurement, an absolute zero, an empty cage, and a cage with food only are measured along with fly cage(s).

Using the Sable System SA3

1- Attach cages to the multi-sampling unit.

2- Turn on vacuum

3- Turn on flow meter and make sure it reads 100 ml/minute

4- Turn on pump that flushes chambers

5- Make sure driright is fresh

6- Record the order of cage attachments and any other pertinent information in log book

7-Call up appropriate setup file

8- Record your measurements.

STANDARD ROSE LAB BANANA FOOD

The laboratory is a controlled environment with respect to temperature and so on, and we must take care that the food given the flies is equally uniform in quality. Most of the adult phenotype is determined by the larval stages, and the larvae feed, bathe, and live in the banana food. It is therefore absolutely critical that the food be consistent, particularly when the flies are to be assayed in an experiment. Limited variation in banana ripeness & quality, slight burning (&c) can be tolerated for routine stock maintenance, but should be avoided as much as possible for experimental-generation flies. The ideal banana is ripe but not rotting, with full yellow colour and the first hints of browning spots.

INGREDIENTS

Cook size:	"4.4L"	"6.6L"
step 1		
Distilled water	4.4L	6.6L
Agar	66.7g	100g
step 2		
Bananas (unpeeled/peeled)	900/600g	1350/900g
Distilled water	267mL	400mL
Light karo syrup	1&1/3 scoops*	2 scoops*
Dark karo syrup	1&1/3 scoops*	2 scoops*
Barley Malt	2 scoops*	3 scoops*
step 3		
Distilled water	307mL	460mL
95% Ethanol	107mL	160mL
Yeast	160g	240g
step 4		
95% Ethanol	104mL	156mL
p-Hydrobenzoic acid	10.4g	15.6g

* A "scoop" refers to the ice-cream scooper used in the Rose Lab, which is 55 mL, volumetrically.

DIRECTIONS

STEP 1

Start by measureing the water & agar and combining them in the cook pot with a stir-bar (a large one) and put the hot-plate on "high". Don't turn the stirring speed too high or the stir-bar will come off-centre. Allow the pot to boil; this may take better than an hour, so you should start the cook while still attending to other things. The bunsen burner may be used at this stage to speed up the boiling, but even at this stage over-boiling and burning are possible. Do not leave the lab with the flame on.

STEP 2

When the agar-water has boiled vigourously for at least 5 minutes you may proceed with step two; do not start this step too early, or else the mixture will congeal into nasty lumps and the bananas will oxidize. For the small (4.4L) cook one blender is sufficient, but two are necessary for the 6.6L cook. Add the water to the blender(s), which should be well cleaned, and then the weighed bananas one by one while blending on low. Make sure the bananas are smoothly blended in by switching to a high setting. Then add the other ingre-

dients (karo & barley malt). Mix everything well and then add to the boiling agar-water. Do not wash the blenders. Using a wooden spoon to scrub the areas of the pot outside of the stir-bar's reach (don't knock stir-bar off centre) and rotate the whole pot slightly help prevent burned spots which slow down re-boiling.

STEP 3

While it was critical in step one to allow the mixture to boil, the third step may be started before the food re-boils. Add most of the water to one of the blenders (at least 3/4), saving a bit in the graduated cylinder, and rinse it out before pouring it into the other blender (it only takes one) to get most of the residual banana mix out. Add ethanol, and, gradually while blending, the yeast. Blend until all particles of yeast are dissolved (5-10 minutes); too short and the yeast will precipitate out, too long and it will start growing in the blender. Use the bit of water you saved to rinse out the blender and add it to the cook. Allow the final mixture to boil for at least five minutes. As the food boils at the final stage it will foam up and overflow if the lid is on; you must watch the food as it nears a boil. Generally it is best to take the lid off when the food comes to a boil and let it boil for at least five minutes. It is absolutely critical that the food boil after the yeast has been added.

STEP 4 anti-fungal solution

Remove the food and allow it to cool -- a cold water bath speeds this up. Mix up the ethanol and hydrobenzoic acid and cover it to prevent evaporation. The food should be stirred occassionally while cooling. When the temperature hits 48^{o}C the anti-bacterial solution should be added and mixed in thoroughly before pouring.

STEP 5 - pouring

Vials: For most purposes we require vials for egg-collection/larval rearing. The standard depth in an 8 dram vial is about 2cm (or 6mL), which is well in excess of the amount eaten by the larvae; within reason, any level of food above about 1cm is adequate. A 6.6L cook will make about 41/2 racks of e.c. food if carefully poured; a 4.4L cook will make about 3 racks. Vials which are only for temporary feeding of adults (transfer vials) can be poured low.

Plates: Plates are usually poured up to the upper lip of a 15x100mm petri dish. A cook will produce about 12 - 14 plates / litre. Plates are almost always needed in the lab, so if you have extra food pour plates.

STEP 6 - cooling & storage

Putting the food in front of the fan will speed its cooling and help prevent stray flies from getting into it. Egg-collection vials should be covered with the screening and plates should not be left out for flies to lay on. When vials are room temperature (1/2 - 1 hour), they should be inverted with paper towel on top into a new & clearly labelled & dated rack. Plates should be closed, bagged, labelled & dated. All food to be kept for more than two days should be refrigerated. Don't expect a fan or a screen to keep out loose flies: check.

CHARCOAL FOOD

Cook roughly 1 litre of food per rack of vials (or 8-10 petri plates) required. The cook size (3 1/2, 4, & 6 L) refers to the total volume of water added to the food; how this amount is allocated is somewhat flexible.

For obvious reasons the quality of the food is critical to our studies of nutritive metabolism... the medium is the message. For this reason, although the recipe is very staight forward, one must be careful to avoid certain easily made mistakes: measure carefully; make sure that the pot and blender are free of soap by rinsing with distilled water; don't allow the food to burn by either adding ingredients too quickly or by letting them stick because the stir-bar got fouled or went off centre.

INGREDIENTS

	3 1/2 L	4 L	6 L
FOOD			
Distilled water	3 1/2 L	4 L	6L
Sucrose	189g	216g	324g
Agar	66.5g	76g	114g
Yeast	112g	128g	192g
Charcoal	17.5g	20g	30g
ANTI-FUNGAL SOLUTION			
p-Hydroxy-benzoic acid	10.5g	12g	18g
Ethanol (95% ETOH)	105mL	120mL	180mL

INSTRUCTIONS

1) Start by heating all but about 1 1/2 litres (less for a 2L cook) of the distilled H_2O in the cook pot with a stir bar (large); the remaining water will be used for blending the yeast. The small pot should be used for any cook of 4 L or less, and the larger one only if a large hotplate is available. Set the hotplate on "high" unless making 2 L or less, in which case heat more gradually. While the water is heating, slowly add the sucrose, making sure that it dissolves and does not burn on the bottom of the pot; a wooden spoon may be helpful.

2) As with the sucrose, add the agar slowly by sprinkling it into the pot. The agar will clump and/or aggregate on the bottom and burn if added too abruptly. The yeast can be blended (step 3) while the agar is being added.

3) Fill one of the blenders up about half way, using roughly half of the water left over from step (1), and add the yeast. Blend for 30 seconds or a minute on a low setting ("grate") while measuring the appropriate quantity of charcoal in a watch glass or petri dish. Add the charcoal and blend again.

4) Add the yeast/charcoal blend to the cookpot and use a wooden spoon to make sure that

nothing is burning onto the bottom of the pot. Use some more of the extra water to rinse out the blender by blending on high (e.g. frappé) and pour into the cookpot. Add the remaining water (if there is any) to the cookpot; note that you have added no more or less water than the total called for in the ingredients section.

5) Allow the food to come to a boil; the time this takes depends upon the cook size, but should be between 20 minutes and an hour.

6) Place the pot in a cold water bath (i.e. fill sink part way) and cool down, stirring occasionally. While the food is cooling, prepare the antibacterial solution. Cool the food to 60^{o}C (the temperature is important) and add the antibacterial solution. It is easy to forget this step or to mix the wrong quantity of the solution. Get it right -- an error in this step can be disasterous.

7) Remove the food from the cooling bath and pour. If pouring a large quantity it is desirable to keep the food warm on the hotplate during pouring.

§4. WARNINGS

MOVEMENT OF PEOPLE AND EQUIPMENT BETWEEN LABS

It is very important to keep flies parasite-free because these parasites may alter the life history characteristics of the fly. Flies that are brought into the lab from the field have to be carefully checked for both ectoparasites living on the back and abdomen of the flies, as well as endoparasites such as protozoans and viruses. Some parasites are easily transported on flies, people, or in food. It is extremely important that the following guidelines be followed.

In order to prevent the contamination of the flies in the Rose and Mueller labs, no equipment should be moved between those two labs and the Ayala, Bradley or Gibbs laboratories. Furthermore no person that has been in the Bradley, Gibbs, or Ayala lab should walk into the Rose or Mueller lab. The reason for this precaution is that flies and their larvae plus parasites will travel on people's clothes, hair etc.. There are NO EXCEPTIONS: no person who has so much as walked through the labs can return to the Rose & Mueller Labs within two weeks. Furthermore, any person who desires to go into the excluded labs may only do so with permission of Dr. Rose or Dr. Mueller, or one of their postdocs or lab managers. Transport of flies is absolutely out of the question.

Nothing that leaves the Rose/Mueller premises is ever returned there, unless it can be disinfected. Any equipment, flies etc. that have gone to any other lab should never return to any of the other labs. So be careful what you loan to people in other labs.

ROCK AND ETIQUETTE

Few things make lab work go faster than music by your favorite group or artist. Few things make lab work go slower than music by a detested group. Most labs have stereos for the first reason. Many lab fights arise from the second factor.

This illustrates one of the most basic features of life in a lab. You have to get along to

achieve a pleasant working environment. That involves making some compromises that allow others their "space". In other words, you must behave with good etiquette.

Here are some points of good lab etiquette.

1- Clean up your mess after you finish work. Be generous in your estimate as to how much of the mess you made.

2- Do not hog the stereo. Let other people hear their music, too. Do not turn up the stereo too loud, particularly when there are meeting rooms or classes next door. Don't use people in the lab as a "captive audience" to torture with your own tastes (unless they are country and western fans) -- settle on something palatable to all even when its not exactly what you'd like to hear.

3- Finish you assigned task, even if it takes a little longer than you thought. Do not abandon your work for the next person.

4- Share supplies when you can. A little generosity can go a long way.

5- If you can't make a scheduled time for your work, be sure your group leader knows as soon as possible.

6- If you are senior, explain as much as you can to new 199s.

7- Do not smoke in the lab.

8- Do not bump the arms, shoulders, etc. of others as they work at the bench, especially when that person is using a microscope or other equipment.

9- Do not yell needlessly.

SEX AND DRUGS

Sexual harassment is one of the most difficult issues that face labs that mix undergraduate students, graduate students, post-docs, and faculty. In order to help us cope, the following are some explicit guidelines as to what is, or is not, appropriate behavior.

Faculty may not have sexual relations with project students or technicians, whether or not they consent. Nor should they make sexual advances to these individuals. Similarly, such students or technicians should not place faculty etc. in jeopardy by initiating or suggesting sexual interactions.

A number of practical rules for everyday conduct follow

a- faculty should not invite out individual undergraduate students or technicians of their sexually preferred gender(s), whether to their homes or some other social setting.

b- Students should not be required to work alone after normal lab hours.

c- Faculty should not initiate or accept physical contact with undergraduate students or technicians.

d- No one should threaten any student or technician because of a denial of sexual favors on the part of the student or technician, even when the sexual relationship was initially

consensual.

In the event of any type of sexual harassment, please contact the University Ombudsman. We will try to help all individuals, irrespective of gender or orientation, with any difficulties arising from sexual harassment in the work environment.

In compliance with federal regulations and the regulations of the University of California, we must actively work to eliminate substance abuse and its effects from the laboratory. The following rules are intended to reflect this objective.

a- No controlled substances ("drugs") or alcohol are to be consumed or stored within the laboratories involved in the Program project, except for alcohol stored for use in scientific experiments.

b- No laboratory worker is to come to the lab in a state of chemical intoxication, of whatever kind, except as arises from prescription medication.

c- Drugs are not to be sold by laboratory personnel or to laboratory personnel. Similarly, drug "deals" are not to be discussed on laboratory premises.

d- Drug use shall not be encouraged by any laboratory worker.

LATE HOURS

Experiments often demand working strange hours. We do not want students to perform lab duties that would put them at risk of attack or rape in the lab or on campus.

Sign up sheets are posted in labs frequently, and you may feel pressure to sign up for hours at which you are not comfortable to go in. Nobody in the lab will look down upon you for being safe. We can't stop you from coming in late, but we can urge you not to put yourself at risk. The campus escort service is very good and will happily offer escort.

Don't be naive about the danger you can put yourself in by walking to and staying at the lab after dark. If anyone in a supervisory capacity pressures you to work hours that might put you at risk, please report to the professor in charge of the lab. \

§5. READINGS

Two general references that will provide more background into the scientific issues addressed in our labs are:

Rose, M. R. 1991. *Evolutionary Biology of Aging*, Oxford University Press.

Mueller, L. D. 1997. Theoretical and empirical examination of density-dependent selection. *Annual Review of Ecology and Systematics* **28**: 269-288.

A general reference about the laboratory ecology of *Drosophila* is:

Mueller, L. D. 1985. The evolutionary ecology of *Drosophila*. *Evolutionary Biology* **19**: 37-98.