Getting started: An overview on raising and handling *Drosophila*

Overview chapter: non-standard format

Hugo Stocker[1] & Peter Gallant[2]

[1] Institute for Molecular Systems Biology, ETH Zurich, Wolfgang-Pauli-Strasse 16, CH-8093 Zurich, Switzerland, phone: +41-44-6333679, fax: +41-44-6331051, email: stocker@imsb.biol.ethz.ch;

[2] Zoological Institute, University Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland, phone: +41-44-6354812, fax: +41-44-6356820, email: gallant@zool.unizh.ch

Abstract

Drosophila melanogaster has long been a prime model organism for developmental biologists. During their work, they have established a large collection of techniques and reagents. This in turn has made fruit flies an attractive system for many other biomedical researchers who have otherwise no background in fly biology. This review intends to help *Drosophila* neophytes in setting up a fly lab. It briefly introduces the biological properties of fruit flies, describes the minimal equipment required for working with flies, and offers some basic advice for maintaining fly lines and setting up and analyzing experiments.

Keywords: *Drosophila melanogaster*, stock keeping, nomenclature, model organism

1 Introduction

Drosophila melanogaster has served as a genetic model system for a century. It has populated research laboratories all over the planet because of its many advantages: It is modest regarding dietary and spatial requirements, allows easy observation and manipulation at most developmental stages, produces large numbers of offspring and is

robust against plagues and pathogens. Above all, the plethora of sophisticated genetic tools developed by an ever increasing number of “Drosophilists” over many years makes *Drosophila* the model system of choice to study biological phenomena as diverse as pattern formation, behavior, aging, and evolution.

A big advantage of *Drosophila melanogaster* is its rapid development. Under standard laboratory conditions (25°C, see “2.2 Vials and hardware for raising flies”) the whole life cycle does not take longer than some ten days. Embryogenesis occurs within the egg that is deposited into the food, and after slightly less than 24 hours, the first instar larva hatches. Immediately after hatching, the larva takes up its main task: feeding! The growth period lasts four days and includes two molts. During this time, the larva increases approximately 200 fold in weight. This astonishing mass accumulation is aided by the endoreplication of larval tissues, i.e., those tissues that will be destroyed during metamorphosis and will not contribute to the adult fly. In contrast, the so-called imaginal discs consist of diploid cells and during metamorphosis will be transformed into the adult body structures. Towards the end of the third larval instar (about 5 days after egg deposition), the larva stops feeding and leaves the food (“wandering stage”) in search of a dry place suited for pupariation. Metamorphosis takes place in the pupal case during the following four days, and the imagos eclose 9 to 10 days after egg deposition. The emerging adult flies are some 3 mm in length with females being slightly larger than males. The distinctive features of the two genders are illustrated in Figure 1. Females weigh about 1.4 mg, whereas males are only about 0.8 mg (much of this weight difference is accounted for by the ovaries in the female abdomen). The dry weight is about one third of the wet weight. Evidently, both environmental conditions (food quality, temperature) and genetic makeup impact on body size and weight.

The females are already receptive less than twelve hours after eclosion, and they start to lay eggs soon after mating. Therefore, two weeks usually suffice for each generation in a crossing scheme. Egg production reaches up to 100 eggs per day and female (with a fecundity peak between day 4 and day 15 after eclosion). Thus, a single pair of flies can give

rise to a substantial number of offspring. This is, however, an inadmissible simplification, as each stock keeper knows how poorly some fly stocks (usually the most important ones) perform.

2 Handling flies

2.1 Fly pushing

Although fruit flies are not very demanding, each laboratory intending to do fly work should be equipped with certain basic tools. It is possible to start out with minimal equipment, and many of the tools can be self-made with a bit of imagination. Furthermore, personal preferences result in fly laboratories that hardly resemble each other. Nevertheless, some tools are quite essential and will be described in the following sections. A typical collection of such tools is shown in Figure 2. Please contact a local fly laboratory (can be found at the FlyBase web site) or the Bloomington stock center web page for the addresses of local suppliers.

Even though some “fly pushers” recognize the sex of flying flies with bare eyes, the use of dissecting microscopes is essential. Since you will spend many hours observing flies under the stereomicroscope, you should refrain from buying the cheapest one. Good optical quality and a magnification range from 6x (for handling live adult flies and larvae) to 40x (for dissections) are desirable. Transmitted light is not required. Use heat filters or – preferably – either fiber-optic transmission from a distant light source or LEDs to avoid overheating of the flies. A ringlight is appropriate for inspection of flies as it reduces unwanted optical reflections. For dissections, flexible optical fibers – ideally mounted directly on the microscope – are recommended. Since Green Fluorescent Protein (GFP) is widely used as a marker, a stereomicroscope suitable for fluorescence analysis is often required. In order to examine dissected animals or individual tissues, you will also need a

(fluorescence) compound microscope with higher magnification objectives and phase contrast optics.

Obviously, you need to anesthetize the flies prior to inspection. Although the use of ether has a long-standing tradition, modern fly labs are relying on carbon dioxide as anesthetic. Industrial grade CO_2 in tanks of 40-50 liters can be purchased from gas suppliers. The tanks should be secured by solid racks. An automated switch between tanks makes your life easier, as CO_2 tanks tend to run out of gas in the very moment you are chasing the long-sought-after fly. If your laboratory intends to do a large volume of fly work, permanent piping of CO_2 at the individual benches in combination with a large remote CO_2 source (e.g., two batteries of twelve CO_2 tanks each placed in the basement) is an attractive (but expensive) option. Pre-existing air lines can also be adapted to provide the workspaces with CO_2 (contact a professional plumber and check for safety regulations!). The CO_2 source needs to be fitted with a pressure reduction valve. Also keep in mind that the expanding CO_2 cools the environment – without heating, the valves and pipes may freeze.

At each workstation, an additional valve should allow to regulate the supply pressure of CO_2. From this valve, a pipeline consisting of plastic tubing of about 5 mm inner diameter and bifurcating by means of a Y-junction supplies two devices: One of the two branches leads to a special plate ("fly pad"), the other one ends in a robust syringe needle connected to a spring valve. The needle can be inserted into vials and bottles (see "2.2 Vials and hardware for raising flies") between the stopper and the rim of the culture vessel, and CO_2 is infused by bending and thereby opening the valve. The fly pad consists of a porous plate (made e.g. of polyethylene) surrounded by a metal or plastic rim. The CO_2 passes through the porous plate and forms a sea of gas in the shallow vessel. Thus, flies lying on the pad will be anesthetized by the lack of oxygen and can be readily inspected and handled. Flies can survive several minutes in this unconscious state, which leaves plenty of time for extensive analysis. However, exposure to CO_2 for more than 20 minutes will result in lethality, and even before that, the flies' fertility begins to suffer. A further unwanted

consequence of prolonged exposure to such a CO_2 stream can be dehydration. This problem can be minimized if the CO_2 is passed through a flask of water before arriving at the fly pad.

The use of ether may still be required in certain situations. If you intend to take pictures of the animals or to measure their weights, you need to immobilize the flies for several minutes. This can be achieved either by freezing or by treatment with ether. Leaving flies in an ether atmosphere for about 30 seconds renders them unconscious, whereas a minute suffices to kill them. Be aware of the rapid dehydration that will change the wet weight significantly within minutes. Therefore, the flies should always be treated identically if you want to compare their weights (e.g., one minute in ether atmosphere). If you want to avoid ether, you can also measure the flies' dry weight by placing them into an Eppendorf tube in a 95°C heat block. Once the flies have stopped moving, open the lid and continue the incubation for 10-15 minutes, then put the Eppendorf tube at room temperature to equilibrate with ambient humidity. After such a treatment, flies can be stored for several days without a change in weight.

Although tiny and seemingly delicate, flies are not particularly fragile. They can be moved around with fine paintbrushes or bird feathers. Another convenient tool to transport individual flies and add them to culture vials already containing other flies is the spit-tube. It consists of a piece of plastic tubing (approx. 70 cm long and 5-7 mm in diameter) with a mouthpiece at one end and a small glass (Pasteur) pipet with a wide opening at the other end. The spit-tube allows you to pipet up and down individual flies – just make sure that you place a filter (e.g., a little ball of cotton) between the glass pipet and the plastic tubing, lest you swallow your favorite fly.

In the course of your genetic experiments, a large number of flies will be produced that are of no use (any more). Dump these flies into the "morgue" - a medium-sized glass vessel filled with 70% ethanol, fitted with a funnel. Once the morgue is full, the dead flies should be discarded according to your local biosafety regulations (e.g., autoclaved).

A few other items will support your daily work: forceps (typically watchmaker's forceps, size 5, essential for dissection), a hand-held counter (either mechanical or digital, as also used by tissue culture experimentalists), and a little piece of carpet or a computer mouse pad (to dampen the hits when you bang vials or bottles against the bench). Furthermore, you need some fly traps to catch escapees. Either hang up sticky flypapers or place a reasonable number of unused fly food bottles with a funnel on top all over the fly room (or, even better, do both).

2.2 Vials and hardware for raising flies

Flies need a cozy home and good food. Space is usually not limiting – although maintaining thousands of different lines does require large cultivation rooms. For small cultures (up to about 200 progeny flies), fly pushers make use of different kinds of vials. Standard volumes are 30 - 45 ml (25 mm in diameter, 70 – 100 mm in height), and the vials can be made of plastic or glass. Whereas plastic vials are typically for single use only, glass vials can be reused a number of times (after autoclaving, washing and intense rinsing). The use of disposable plastic vials may be more expensive, and some fly pushers do not like their electrostatic features (flies tend to stick to the walls when you want to push them into the vial). Furthermore, there are anecdotal reports that the fly food detaches more quickly from plastic walls upon drying (although the reason for this phenomenon is unknown). Nevertheless, plastic vials may be preferable if there is no efficient cleaning facility available. Larger cultures (up to 1000 progeny flies) are set up in bottles (volumes of about 200 – 250 ml) that are also made of glass or plastic. Special conditions apply for very large cultures (see chapter by Kunert and Brehm in this Volume).

The vials and bottles can be closed by various kinds of stoppers, the most common ones being paper or foam plugs and cotton. Using nonabsorbent cotton is the only reliable way to keep mites out of the vial (see ”2.5 Plagues”). However, many fly pushers are irritated by cotton fibers in the air. Plugging the vials in the fume hood may offer some relief. Paper and

foam plugs can be washed and reused several times. It is crucial, however, that the stoppers are autoclaved after every use, and this harsh procedure certainly does not contribute to an extension of their half-lives.

The vials can be placed into cardboard boxes, and the bottles are usually transported and stored on trays. Make sure that both the boxes and the trays are regularly cleaned to prevent the accumulation of microorganisms or mites (it is recommended to incubate the trays between uses at 60°C for several hours).

To ensure the reproducibility of the experiments, the fly cultures have to be maintained at standard conditions. A frequently used temperature is 25°C, and the relative humidity should be around 70%. There are two ways to meet these criteria: Either you use stand-alone incubators, or, preferentially, you have access to a climate-controlled room. Incubators have several disadvantages: There is a tremendous exchange of air (and a rapid drop in temperature, unless it is very hot in the laboratory) every time you open the door. Furthermore, incubators capable of controlling temperature and humidity are expensive and noisy. However, incubators are very useful if an experiment requires switching to an unusual temperature or, for example, a repeated incubation at 37°C (e.g., to induce expression from a transgene under heatshock promoter control). Especially the latter is a painful experience without a programmable incubator. For single heatshock treatments, the vials can be placed in a water bath.

Climatized fly culturing rooms are very convenient for both controlled experiments and stock keeping. The temperature should be kept within a narrow range (+/- 0.5°C), and the circulating air needs to be humidified (70% relative humidity is ideal). It is crucial that both overheating and freezing of the climate room cannot occur under any circumstances. Both temperature and humidity should be constantly monitored, and an alarm needs to be triggered whenever the temperature falls outside an acceptable range (e.g., 22 - 27°C for the 25°C room). The inside of the chamber (including the shelves) should be designed such that

it provides maximal accessibility for cleaning and minimal opportunities for hiding (of unwanted guests, see ”2.5 Plagues”). Automated doors are desirable as fly pushers often approach the climate room with both hands filled with fly boxes. Finally, the lighting in the room should be controlled to achieve a 12h light/ 12h dark cycle. Obviously, the transition times between dark and light need not coincide with the outside day/night cycle. Instead, they can be adjusted to the experimenter’s needs, as adult flies tend to eclose around dawn.

2.3 Feeding flies

The well-being of your flies depends on the food even more than on the environment. Our limited survey among fly labs on most continents revealed that there are probably not two laboratories that produce exactly the same fly food. This may cause problems when growth-related aspects are under investigation. Therefore, instead of relying on published findings, you should always carry out the controls under the same nutritional and environmental conditions.

Most fly food recipes are based on similar ingredients: water, agar, sugar, corn meal, yeast, and fungicides. The main difference is the source of carbohydrates. Whereas laboratories in the United States tend to use molasses (a by-product of the processing of sugarcane or sugar beet), fly pushers in Europe and Asia seem to prefer glucose or dextrose. In principle, fly food can be prepared in a simple cooking pot. However, to prepare large quantities, you will need a stirrer kettle (volume up to 100 liters) and a peristaltic pump.

We prepare our fly food as follows (the volume depends on the demand; the following indications are for 1 liter of water): While the water is warming up, 100g of live yeast is added and dissolved. Glucose (75 g), agar (8 g), and corn meal (55 g) are mixed and added to the boiling water under constant stirring. Wheat flour (10 g) is dissolved in 100 ml cold water and added to the boiling mixture. After at least 30 minutes of boiling, the heating is reduced and the mixture is allowed to cool down slowly. The fungicide (either 15 ml of a 1:1 mixture of Nipagin (methyl paraben, 33 g/l ethanol) and Nipasol (propyl paraben, 66 g/l

ethanol) or 5 ml of 8.5% phosphoric acid plus 5 ml of 85% propionic acid) is added at a temperature of approximately 60°C, and the mixture is stirred for another 15 minutes before dispensing into vials (roughly 12 ml per vial) and bottles (roughly 40 ml per bottle). The vials and bottles are placed in open plastic boxes on a table and allowed to cool and dry. Constant subtle ventilation accelerates this process (and keeps hungry flies away). As soon as the fly medium is dry enough (after about 5 hours), a drop of autoclaved yeast paste is added on top. When kept in closed plastic boxes, the fly food can be stored for several days (always check for invaders upon use!).

2.4 Culturing flies

The vials are now ready for use. For most crosses, 5 virgins and 2 to 5 males per vial will give you a reasonable number of progeny. At least 20 virgins and 5 to 15 males are needed to populate a bottle. Carefully check whether the unconscious flies stick to the food (especially when the yeast drop is still wet). Laying the vials on the side until the flies have recovered helps to avoid early losses.

As soon as a culture is set up, the vial must be labeled. Use a waterproof marker to write date and the genotypes of the females and males directly onto the vial. For stocks, the use of labels (e.g., sticky tapes) is convenient.

After two to three days, the flies should be transferred to a new vial. There is no need to anesthetize the flies again – simply shake the flies down, open the old and the new vials, press them together, and shake the flies into the new vial. With a bit of exercise, you will manage to transfer your flies quantitatively. Repeat as needed – and then dump the flies into the morgue.

Rule number one of stock keeping is diligence – to avoid contamination or mixing up of fly stocks. Stocks are usually maintained in vials at 18°C (which slows down development to a generation time of about 20 days). A dedicated constant temperature room is strongly recommended. Again, there are several schedules for stock collections. Many labs prefer to

simply flip stocks into new vials in order to save time. However, we recommend inspecting your flies at least twice a year for their phenotype (to recognize contamination of a stock and allow the rescue of the correct genotype) and for mite infestations. The inspection under the dissecting microscope also has the advantage of an accurate population control. Either way, the cultures should be changed over to a new vial after one week, and a second time after another week. Thus, you will have three copies of each stock. Keep an old vial until larvae are visible in the new ones. Under optimal conditions (vials not overcrowded), you can wait up to 5 weeks before starting the same procedure again.

There will always be some stocks that are difficult to maintain. Keep a special tray for the sick stocks, usually at 25°C because many stocks perform better at this temperature. However, some stocks do prefer lower temperature, especially those that carry genetic elements to achieve Gal4-mediated overexpression.

Finally, good practice of stock keeping involves a database harboring all information about the stocks, including any special requirements for stock keeping.

2.5 Plagues

Cleanliness is key to healthy fly cultures. Always keep an eye on the places that could convert into sites of infection: the working spaces in the fly room, the cultivation rooms, and the fly food kitchen. Make sure that all lab members keep their working areas clean. Especially the fly pads should be cleaned with ethanol after work. It is crucial that old vials are not given the chance to get spoilt, as rotten cultures are the main source of nasties. Not only should you appeal to the discipline of your colleagues, but you should also appoint a person to regularly inspect the fly room and the cultivation rooms.

Contaminations can also be favored by insufficient precautions taken in the fly food kitchen. Double doors to avoid flies being attracted by the smell of the food are helpful. Also pay attention to the quality of the ingredients of the fly food (especially live yeast is a potential carrier of infectious agents).

Incoming stocks should be treated with special caution. Keep them under quarantine in an isolated place for two generations (e.g., a dedicated incubator far away from your fly room, or even your office may do), and only transfer them to your fly room upon careful inspection.

The main causes for sleepless nights of fly pushers are molds and mites. Molds appear rapidly in the absence of fungicide. Whereas healthy fly stocks can usually cope with mold infections, weak stocks are heavily endangered by the fungi. Make sure that fungicide is always added to the fly food in proper quantities. It is also suggested that two fungicides (e.g., Nipagin/Nipasol and propionic/phosphoric acid) are used in an alternating manner to prevent resistance formation. For example, add propionic/phosphoric acid on a particular weekday and Nipagin/Nipasol on all the others. Furthermore, the relative humidity in the climate room should not exceed 70% and, importantly, all the reused items (vials, stoppers) must be autoclaved after every use. These few and simple rules usually suffice to fight the molds successfully.

Mites can be more renitent. There are two types of mites, those that feed on fly food and those that feed on flies. Food mites are much more common but, fortunately, far less dangerous. They tend to appear out of nowhere and spread rapidly. Probably, they are imported into the laboratory by the raw ingredients of the fly medium (corn meal, flour). If you notice mites, the affected cultures should be quarantined or, if possible, autoclaved. Quarantined cultures should be transferred daily – a procedure that is, however, no option for weak stocks. If the mites persist, manual removal of adult mites and their eggs from fly eggs or pupae may help. Finally, placing dechorionated eggs (by means of “bleaching”, i.e. treatment with sodium hypochlorite) into fresh vials is a promising but tedious strategy to get rid of mites. You may want to choose chemical warfare instead: Filter papers soaked in Tedion (Tetradifon) are effective weapons against some mite species.

3 Experimental use of flies

3.1 Genetic makeup of flies

Drosophila is, above all, a genetic model organism, and working with flies requires a minimal knowledge of their genetic makeup. The fly's genome is distributed onto 8 chromosomes: 2 sex chromosomes (two X chromosomes in females, also called 1st chromosomes; one X and one Y chromosome in males) and 2 sets of autosomes in both sexes (simply called 2nd, 3rd and 4th chromosomes). These chromosomes differ substantially in their sizes: 21.9, 42.5, 51.3, and 1.2 Mb of euchromatin are located on the X, 2nd, 3rd, and 4th chromosome, respectively. The Y chromosome consists entirely of heterochromatin and carries just a few genes that are only required for male fertility, but not for viability. To indicate a specific position within a chromosome, different coordinate systems are used: molecular nucleotide sequence, genetic map, and cytological location. The first is based on the completed 120 Mbp sequence of the *Drosophila* euchromatin. The genetic map is derived from experimentally determined recombination frequencies between genes; the left tip of each chromosome is arbitrarily set to map position 0, and a map distance of 1 corresponds to a 1% recombination rate – notice, however, that the one-to-one relationship between map distance and recombination frequency holds only for closely spaced loci (and, of course, that the maximum frequency of meiotic recombination between any two loci is 50%). The cytological map is based on the appearance of the massively polyploid (and polytene) chromosomes found in larval salivary glands; the alternating darker bands and lighter interbands that can be discerned under a light microscope each have been assigned an identifier of the type "xay", where "x" is the band number, "a" the lettered subdivision (ranging from "A" through "F"), and "y" another number subdividing the lettered subdivision. Each major chromosome arm is divided into 20 such bands (X: 1-20; left arm of the 2nd chromosome: 21-40; right arm of the 2nd chromosome: 41-60; left arm of the 3rd chromosome: 61-80; right arm of the 3rd chromosome: 81-100; 4th chromosome: 101-102).

As an example, the *white* gene is localized close to the tip of the X chromosome at cytological region 3B6, map position 1.5, and it starts at nucleotide position 2'646'755.

3.2 Nomenclature

Genes are often named for the first mutant phenotype observed (frequently the phenotype of a weak, or hypomorphic, mutant allele). If this phenotype is dominant to wildtype, the gene name begins with an uppercase letter, else with a lowercase letter. For example, mutation of the *white* gene has no phenotypical consequences as long as a wild-type copy of the gene is present, but when both copies of the *white* gene are mutant the fly has white eyes. Each gene also carries a unique symbol (or abbreviation), and superscripts or brackets are used to distinguish between different alleles; e.g. w^{1118} or *w[1118]* refer to the allele "1118" of the *white* gene. A "+" designates the wildtype allele (e.g., w^{+}) and an asterisk a mutant allele whose identity is not known (e.g., w^{*}).

Some frequently encountered names of mutations (and, consequently, also of genes) are lethals, steriles, Minutes, enhancers, suppressors, transposon insertions. Lethal mutations in unknown genes are designated *l(x)n*, for a recessive lethal mutation located on chromosome "x" (1, 2, 3, or 4), where "n" either corresponds to a code for the gene or to the cytological location of the mutation; e.g. *l(1)1Aa* corresponds to a lethal mutation mapping to cytological band 1A on the X chromosome. Mutations resulting in male or female sterility are abbreviated ms(x)n or fs(x)n if they act recessively, Ms(x)n and Fs(x)n if they act dominantly; e.g. fs(1)3 would be a recessive female-sterile mutation located on the X chromosome and having the name "3". The *Minute* mutations are characterized by a dominant growth defect manifested (amongst others) as a delay in development and a reduction in bristle size. Most *Minute* mutations disrupt a gene coding for ribosomal proteins – example: *M(3)66D* is a mutation of the *RpL14* gene, which is located on chromosome 3 at cytological position 66D. Enhancer or suppressor mutations were initially isolated based on their ability to modify the mutant phenotype of a different mutation "m" and named

accordingly as e(m)n or su(m)n - E(m)n and Su(m)n if their effect on the mutation "m" is dominant. For example, the mutation *Su(Pc)35CD* is located at the cytological bands 35C/35D and dominantly suppresses mutations in the *Pc* (*Polycomb*) gene (which itself has dominant mutant phenotypes). A special class of modifier mutations has an influence on "position effect variegation", a phenomenon linked to the control of transcription and chromatin structure. Such mutations are called E(var) or Su(var), e.g., Su(var)3-9. Finally, tens of thousands of mutant fly lines have been created using transposable elements, mainly P-elements (see chapter by Hummel and Klämbt in this Volume). Insertions of such transposons are labeled as P{c}n, where "c" describes the "payload" of the P-element (i.e. the transgene carried by the P-element) and "n" a code or (if applicable) the gene into which this P-element has inserted; an example would be P{GawB}h^{1J3} which expresses both *white* and the yeast transcription factor Gal4 as indicated by the term "GawB" and has inserted into the *h* (*hairy*) gene and now constitutes allele "1J3" of *h*. At this point, we should also mention the very large class of genes named "CGz". This name is not derived from any observed mutant phenotype but based on a gene prediction – CG is an acronym for "computed gene", and "z" stands for a 4- to 5-digit identifier.

In addition to mutations affecting a single locus, several types of large-scale chromosomal abnormalities are commonly encountered. *Deficiencies* are denoted as Df(x)n (where x specifies the chromosome arm, i.e.: 1, 2L, 2R, 3L, 3R, 4), and they are characterized by the deletion of large regions of the chromosome, often containing dozens of genes. *Duplications* are denoted as Dp(x1;x2)n, whereby "x1" denotes the chromosome from which a segment is duplicated onto chromosome "x2", and "n" denotes a code or "designator". A combination of duplications and deletions is encountered in *Transpositions* and *Translocations*, denoted Tp(x1;x2)n and T(x1;x2)n, respectively. *Inversion* chromosomes, In(x1)n, contain segments that are inverted in their arrangement as compared to a wild-type chromosome. Importantly, such a configuration suppresses meiotic recombination.

3.3 Balancers

This attribute is exploited in so-called *balancer chromosomes*. Balancers are amongst the most important genetic tools in *Drosophila* (and the envy of non-Drosophilists). They contain multiple inversions to suppress meiotic recombination with an un-rearranged chromosome. In addition, balancers carry dominant mutations with an easily visible phenotype and recessive lethal or recessive sterile mutations. Thus, suppose you are crossing a fly of the genotype "$hippo^{42\text{-}47}$ $yorkie^{B5}$/ SM5, *Cy*" to a wildtype fly. Since "SM5, *Cy*" is a balancer (of the 2nd chromosome) marked with the dominant wing mutation *Curly* (*Cy*), you know that half of the offspring of this cross will be "$hippo^{42\text{-}47}$ $yorkie^{B5}$/ +" and the other half will be "SM5, *Cy* / +". These latter flies will be easily recognized since they have bent-up ("curly") wings, so all the flies with normal wings are heterozygous both for *hippo* and *yorkie* – even though you cannot recognize the presence of these mutations themselves by visual inspection. Importantly, you also know that you will never encounter *hippo* or *yorkie* alone. Now suppose you are crossing this $hippo^{42\text{-}47}$ $yorkie^{B5}$/ SM5, *Cy* fly with a partner of the same genotype. A priori, you might expect to obtain three types of offspring: $hippo^{42\text{-}47}$ $yorkie^{B5}$/ $hippo^{42\text{-}47}$ $yorkie^{B5}$ (homozygous mutant), SM5, *Cy* / SM5, *Cy* (homozygous for the balancer), $hippo^{42\text{-}47}$ $yorkie^{B5}$/ SM5, *Cy*. However, life without *hippo* (or without *yorkie*) is impossible for flies, and the SM5 balancer is also not homozygous viable, hence you only get the third genotype, which is identical to the genotype of the parents – you have just established a *balanced stock*. This means that you can transfer the offspring from the above cross into a new vial, let them have offspring of their own, and repeat this procedure for many generations more – you will always only have one type of flies in your vials, so you can maintain your fly line without having to molecularly genotype them.

Given their usefulness, balancers have been developed for each major chromosome: the FM6/7 series for the X chromosome (where F stands for the **f**irst chromosome and M for the **m**ultiple inversions), CyO and SM5/6 for the 2nd chromosome (S for **s**econd), TM2/3/6 for

the 3rd (T for **t**hird). There is no need for a balancer chromosome for the 4th chromosome as it does not undergo meiotic recombination – and there is also no meiotic recombination in males (so theoretically balancers are only needed in female flies). Amongst the dominant markers found on these balancers –as well as on other marked chromosomes - are mutations affecting adult eye shape (*Bar*/*B*, on the X; *Glazed*/*Gla*, on the 2nd), wing shape (*Curly*/*Cy*, 2nd; *Serrate*/*Ser*, 3rd), bristle shape (*Stubble*/*Sb*, 3rd), bristle number (*Sternopleural*/*Sp* and *Scutoid*/*Sco*, both on the 2nd; *Humeral*/*Hu*, 3rd). To mark earlier stages of development one uses *Tubby*/*Tb* (carried on the TM6B chromosome; *Tb* makes larvae short and fat, but it is only suitable for older larvae) or transgenes expressing *Drosophila yellow*/*y* (this requires the use of a y^- background), a fluorescent protein (typically GFP), or bacterial lacZ. Such transgene insertions exist for several different balancers.

Despite all the enthusiasm about balancers, we should add some words of caution. Depending on the chromosomal location and on the particular balancer, considerable meiotic recombination on the "balanced" chromosome may still be possible. Moreover, the recombination rates on the other chromosomes are increased by the presence of a balancer (e.g., a fly carrying the 2nd chromosome balancer CyO will have increased recombination between the two homologous 3rd chromosomes). Also, flies carrying balancers are not as fit and do not produce as many offspring as wild type flies. This is particularly obvious when balancers for two different chromosomes are used at the same time – and it is virtually impossible to work with flies that are simultaneously balanced on the 1st, 2nd, and 3rd chromosomes. Furthermore, some visible markers cannot be combined, either because they interact genetically or because they affect the same trait. For example, *singed*/*sn* and *Sb* both destroy bristle architecture and the mutant phenotypes cannot be scored simultaneously. Along the same line, a balancer chromosome (i.e., one of the mutations carried on this balancer) can also modify the phenotype one is trying to study (e.g., the rough eyes that are caused by overexpression of your favorite gene), and hence it is advisable to analyze such phenotypes in flies lacking any balancer chromosomes.

After all this talk of mutations and mutant chromosomes, we also need to mention wildtype lines that are commonly used for comparison purposes, typically Oregon R (OreR) and Canton S (CS). In addition, many researchers use "w^{1118}" and "y^{*} w^{*}" lines as reference lines, since many transgenes (marked by the expression of *white* or *yellow*) have been generated in these backgrounds.

Finally, if we want to put together all the genetic elements mentioned above into a coherent genotype, we need to observe a few rules of syntax. These can be illustrated with the genotype "*y w*; *Kr*[If-1]/CyO, *Cy*; *D*/TM3, *Ser"*. First, only genes with mutant alleles are mentioned (and none of the 14'000 other genes). Second, the mutant alleles are listed according to their cytological position without intervening comma, whereby different chromosomes are separated by semi-colons. Third, two homologous chromosomes are only listed if they differ, and then they are separated by a forward slash "/". Fourth, a "named" chromosome (e.g., a balancer such as "TM3") is followed by a comma and a list of specific mutations on this chromosome. You will also notice in the example shown above that the different chromosomes are not explicitly numbered, but if you know that "*y*" and "*w*" are located on the first chromosome, that CyO is a 2nd chromosome balancer, and that TM3 is a 3rd chromosome balancer, you will figure out which chromosomes are described here. Occasionally however, the situation is less clear; e.g., without any further information you cannot know whether the P-element in "w; P{w+}xxx" flies is inserted on the 2nd, 3rd or even the 4th chromosome.

3.4 Crossing flies

Only rarely will you obtain flies of exactly the right genotype from an outside source. Instead, you will usually need to cross different mutant flies together in order to generate the desired flies. This will confront you with one of the most common tasks in fly husbandry: virgin collection. Since you want to force the females to mate with the partners you have chosen for them (rather than with their brothers or fathers from the stock), they have to be

virgins before you introduce them to their selected mates. Female flies start mating only a few hours after eclosion, therefore you can safely identify virgins by collecting freshly eclosed flies. Such flies can be recognized by the light color of their cuticle (as it tans only later) and by a greenish spot that can be easily seen through the white abdomen – the meconium (waste products the fly will get rid off with the first defecation). Alternatively, you can empty a vial or bottle of all adult flies and then wait for 8-10 hours (at 18°); all females that have eclosed in the meantime will be virgins. It is a good idea to keep virgin females in a separate vial for a few days. Virgins will lay a small number of eggs, but if any of these hatches into a larva you know that (at least) one of the flies had already lost its virginity. Whenever possible, you should also include markers in your crosses such that illegitimate offspring (e.g., originating from non-virgin mothers) can be recognized.

Many crossing schemes involve more than one generation. In such cases it is important to start with enough flies (e.g., by setting up the first cross with many flies in bottles rather than in small vials). Otherwise you risk collecting fewer and fewer flies with each passing generation (and end up with none in the end) because the "correct" flies typically make up only a small fraction of all the offspring. Also, you should make sure that all the used mutations are mutually compatible. It could be that one marker cannot be recognized in the presence of another one (see above), or that flies carrying a combination of two particular mutations are not viable. Often it is not possible to predict such problems beforehand and test-crosses might be required.

3.5 Basic phenotypic analysis

Several chapters in this volume describe the generation of mutations, starting either with a mutant phenotype (*forward genetics*) or with a gene of interest (*reverse genetics*). Below we provide some suggestions for a general and basic characterization of such mutants. Since there is always a risk of unrelated background mutations, in particular if the mutation of interest was generated using chemical mutagens, it is essential to carry out such an analysis

in a heteroallelic situation, i.e., in an animal carrying mutant allele 1 over mutant allele 2 (or over a deficiency uncovering the mutant gene). If only one allele is available, one should try to rescue the mutant phenotype with a transgene carrying the wildtype version of the gene or a cDNA.

Arguably, the most distinctive aspect of a mutation is its effect on viability. If mutant adult flies are viable, they can be compared to control flies with respect to their external morphology (e.g., size and shape of their wings, eyes, legs, bristles), weight, and fertility. Also, the duration of development from egg to adult should be determined, since a number of mutations significantly delay larval development (by up to several days). A method for weighing flies has been described above. To determine fertility, set up several parallel single fly crosses between a mutant fly and a wildtype tester mate and count the number of offspring. A reduction in fertility can be caused by different defects which can be investigated specifically, e.g., behavioral or morphological abnormalities that prevent the adults from efficiently mating, developmental abnormalities that disrupt gametogenesis within the parent, maternal effects that interfere with the development of the offspring (zygote). Note that for any of the analyses mentioned here it is important to raise the flies under controlled conditions (temperature, humidity, day/night cycle). Furthermore, variations in the number of flies developing in a culture vial can strongly influence several parameters – overcrowding delays the duration of development and results in small flies.

If a mutation causes partial or complete lethality, it will be important to establish the lethal phase - or phases, as lethality is often not confined to a single moment during development. To detect a possible embryonic lethality, a large number of flies with the appropriate genotype (e.g., 20 ⬃ ⇩ ⬇ ⇧ ➘ ⇩ ▷ *allele 1* / + and 20 ⬇ ⇧ ➘ ⇩ ▷ *allele 2* / +) are placed in an empty culture vial (or a plastic yoghurt beaker) and placed on a Petri dish with apple (or grape) agar, topped with yeast paste. Let the flies lay eggs onto the agar for a few hours, then remove the adults and place the covered Petri dish at 25°C for at least 24 hours. During this time, wild type and heterozygous zygotes will complete embryogenesis

and hatch as larvae, leaving empty egg shells behind. If the examined mutation results in embryonic lethality, at least 25% of eggs containing only partly developed, unhatched embryos will remain behind; in a wildtype control cross, a few eggs also suffer this fate, but unless the “wildtype” stock is in extremely bad shape this fraction is below 10%. In case of embryonic lethality, it will be interesting to examine the cuticles of such dead mutant embryos (see chapter by C. Alexandre in this Volume). Cuticular structures are secreted by the developing embryo and they reflect its segmentation pattern; thus, mutations in numerous patterning genes (e.g. *wingless* (*wg*), *decapentaplegic* (*dpp*), *hedgehog* (*hh*)) result in characteristic cuticle defects, and any mutation with similar phenotype is likely to function in the corresponding pathway.

Many lethal mutations allow survival to larval or pupal stages, though. Death during metamorphosis can be easily determined by scoring the fraction of empty pupal cases (normally >>95% for a control cross) at a sufficiently late time point when all normal flies have eclosed (e.g., at 20 days after egg deposition at 25°C). To characterize larval lethality in more detail, a similar cross as described above for embryonic lethality determination can be set up. In this case, however, the non-mutant chromosomes should carry a fluorescent marker. At >24 hours after egg deposition the non-fluorescent first instar larvae are collected - these must be of the genotype *mutant 1* / *mutant 2*; they are then transferred at controlled densities to normal food vials. At regular intervals, the food (including the larvae) is extracted from these vials and submerged in glycerol; this floats the living larvae to the surface so they can be counted. Larval stages can be determined by examining the mouthhooks or the anterior spiracles (for a detailed description the reader is referred to Ashburner et al. 2005). In many instances, however, such a detailed analysis is not required and researchers are happy to state that their mutation causes death during larval development.

3.6 Stock centers

Drosophila biologists have a long-standing tradition of sharing their animals freely. Many of these lines have been deposited at one of the official stock centers (and that is where you always should look first before contacting individual researchers): Bloomington, Indiana (http://flystocks.bio.indiana.edu/), Szeged, Hungary (http://expbio.bio.u-szeged.hu/), Kyoto, Japan (http://www.dgrc.kit.ac.jp/), Ehime, Japan (http://kyotofly.kit.jp). Additional large collections of P-element insertions and deficiencies are accessible at Baylor College of Medicine, Texas (http://flypush.imgen.bcm.tmc.edu/pscreen/), at Harvard Medical School, Massachussetts (http://drosophila.med.harvard.edu/), University of Cambridge, UK (http://www.drosdel.org.uk/). A commercial collection of P-element insertions is found at http://genexel.com/eng/htm/genisys.htm. Of interest is also the Drosophila Genomics Resource Center (DGRC; http://dgrc.cgb.indiana.edu/), which distributes cDNA clones, cell lines, and microarrays. The conditions of use of these facilities are described under the different home pages.

3.7 Sending flies

If you want to ship flies yourself, you can do so quite easily – flies are sturdy and usually survive the hardships of international travel quite well. However, especially during the year-end's holiday season such a travel can take quite a long time, and any shipment in the midst of Winter (or Summer) risks exposing the freight to extreme temperatures. To maximize the chances of survival under these conditions, for each line send two vials that contain flies at different stages of development. Importantly, make sure that one vial contains embryonic and larval stages and do not only send adult flies, as extreme temperature can deprive them of their fertility quite easily. The lids on the vials should be secured with adhesive tape, without blocking air access. The vials can be sent with regular mail (in our experience in 95% of the cases this works well for the trip from the US to Europe) or with an express carrier (if this carrier accepts the transport of life animals – check beforehand). If your parcel

crosses borders you should include a customs declaration stating that it contains *Drosophila melanogaster*, which are to be used for research purposes only, are non-hazardous and of no commercial value. Import into the US additionally requires an "import permit" from the USDA - detailed information about which is provided at the Bloomington home page (see above).

3.8 Further reading

By necessity, this text can only provide a brief introduction to the use of *Drosophila melanogaster* as a laboratory animal. We refer you to the references listed below for extensive (and highly readable) information about fly pushing (5), about the development of flies from eggs to adults and back (2, 3, 6), and about everything else you possibly ever wanted to find out about these critters (1, 4, 7).

4 Acknowledgements

The authors want to thank Christian Dahman, Pierre Leopold, Laura Johnston, Susan Parkhurst, John Roote, Florenci Serras and K. VijayRaghavan for sharing information. Work in the authors' labs is supported by the Swiss National Science Foundation, the University of Zurich, the ETH Zurich and the Swiss Cancer League.

5 References

1. Ashburner, M., Golic, K. G., and Hawley, R. S. (2005) *Drosophila: a laboratory handbook* (Vol 1, 2nd ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

2. Bate, M. and Martinez Arias, A. (1993) *The development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

3. Campos-Ortega, J. A. and Hartenstein, V. (1997) *The Embryonic Development of Drosophila melanogaster*. Springer Verlag, Berlin Heidelberg.

4. Flybase-Consortium: http://flybase.bio.indiana.edu/.

5. Greenspan, R. J. (2004) *Fly pushing - The Theory and Practice of Drosophila Genetics*, (2nd ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

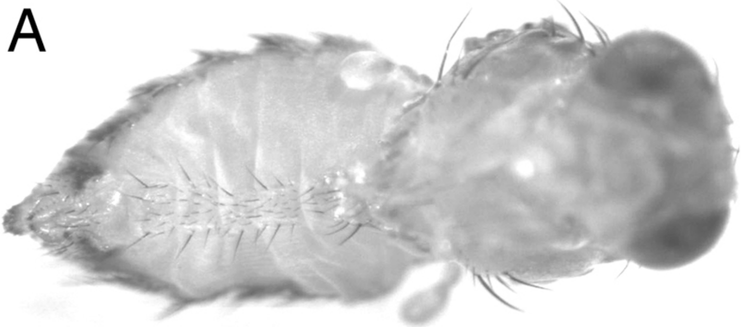
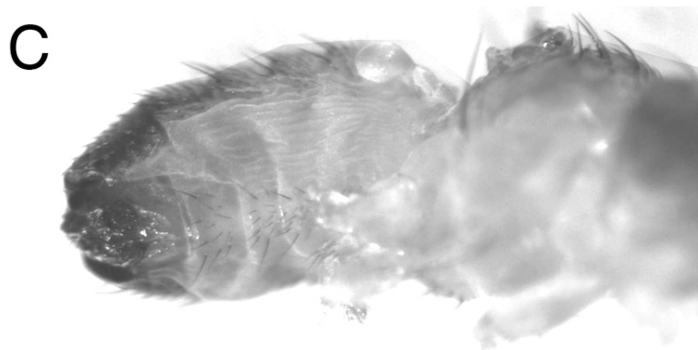
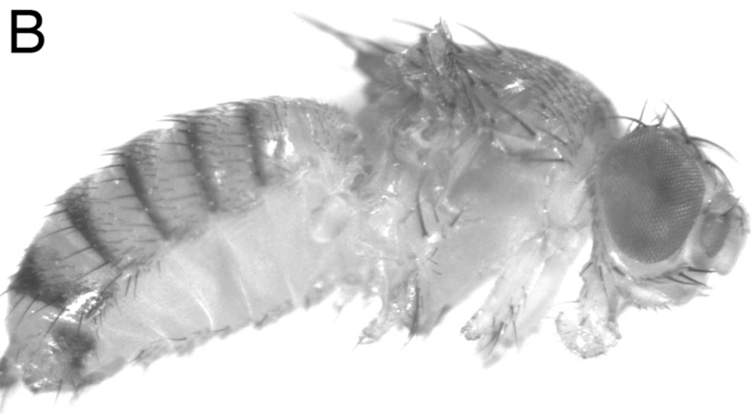
6. Lawrence, P. A. (1992) *The Making of a Fly - The Genetics of Animal Design*. Blackwell Scientific Publishing, Oxford.

7. Lindsley, D. L. and Zimm, G. G. (1992) *The Genome of Drosophila melanogaster*. Academic Press, San Diego, New York, Boston, London, Sydney, Tokyo, Toronto.

Figure legends

Figure 1. Bottom (A, C) and side (B, D) views of a female (A, B) and a male (C, D) abdomen. Males can be recognized by the chitinous structure at the ventral side of their abdomen (the clasper, used during copulation), by their continuous pigmentation at the posterior end, and by the round shape of the abdomen. Wings and legs have been removed for better visibility, and therefore the *sex combs*, found exclusively on the male forelegs, are not shown.

Figure 2. The figure illustrates the essential tools of a fly pusher: brush (1), feather (2), forceps (3), and spit-tube (4) for moving flies, stereomicroscope for looking at flies. Standing on a mouse pad are a culture bottle (5), a vial (6) (plus the tools to label them), and the final destination of most flies – a morgue (7). The fly pad (8) and the "CO_2 needle" (9) (containing a valve that is opened by bending the needle) are located under the microscope.


A

C

B

D

Leica

5

6

8

9

7

www.lifescience-zurich.ch

4

Permanent

3

2

1