MENDELIAN INHERITANCE IN *DROSOPHILA MELANOGASTER*

The following investigation will be used to demonstrate two basic principles of Mendelian inheritance using the fruit fly *Drosophila melanogaster* – the principle of segregation and the principle of independent assortment. You will first learn about the anatomy, development, and the management of *Drosophila* as an experimental organism for genetic analysis. Then, you will conduct crosses to determine the modes of inheritance (autosomal vs. sex-linked) of two different mutant phenotypes and the nature of the mutations (dominant, co-dominant, recessive, *etc.*) that cause these phenotypes. To make these determinations, it will be necessary to generate F_2 progeny flies. This will require a total of six to seven weeks. You will need to work with the flies at times outside of your regularly scheduled lab period. This investigation requires careful observation of the flies, efficient team work to share the work load, and excellent record-keeping to make sense of the data.

Introduction

I. LIFE CYCLE OF *Drosophila melanogaster*

EGG**:** Oviposition takes place on the surface of a larval medium (rotting fruit in nature; prepared "fly food" in the lab). The egg is a small ovoid shaped, shiny white object that can be seen (barely) with the unaided eye. It is structured in such a way that it floats on the surface of the semi-liquid medium. Because fertilization is internal in Drosophila, the "eggs" are actually zygotes, and embryonic development has already begun by the time of oviposition (unfertilized eggs may also be deposited, but these do not develop). There are two distinct phases in development. The embryonic phase is the period from fertilization to hatching of the mobile larvae. The post-embryonic phase consists of larval, pupal and adult stages.

LARVA**:** The larva (maggot) (plural = larvae) is a worm-like segmented creature with black jaws. It is a voracious feeder (indeed, that is about all that it does) which burrows (eats its way) through the medium. At each end of the larva are a pair of spiracles (openings) which lead to the tracheal system. This system enables the larva to exchange gasses by placing one or the other end of itself at the surface of the medium (otherwise it would eventually drown). As the larva feeds, it develops and grows; but, like all insects, its cuticle (skin) can not stretch very much. In order to grow larger, the larva periodically molts (sheds its old cuticle) and rapidly expands in size before the new cuticle hardens. The periods between molts are known as instars. There are three instar stages; first instar = hatching to 1st molt; second instar = first molt to second molt; third instar = second molt to pupal stage.

PUPA**:** After several days, the large third instar larva crawls out of the medium and seeks a dry location in which to metamorphose into an adult (reproductive stage) fly. In the laboratory situation, the larva will crawl to the upper walls of the vial. First the larva contracts and enters a "prepupal" stage in which its cuticle hardens and takes on a darker pigmentation. Another molt occurs (this stage is considered a fourth instar), but the old cuticle is not shed. Instead, it becomes the outer covering of the pupal case. The larva has

become a pupa (plural = pupae). During pupation, specific islands of cells, called imaginal discs (which have remained dormant since the embryonic stage), develop rapidly into the tissues of the adult fly. In many cases, these new tissues completely replace larval tissues, which themselves break down and are absorbed as an energy source.

ADULT**:** After several days in the pupal stage, a soft wrinkled adult fly emerges from the pupal case. This process is called eclosion. At this stage the adult (imago) has a soft cuticle and folded wings, and still looks a bit worm-like. It takes several hours for the fly to unfold its wings, dry out, and harden. No additional development takes place after this stage. Female flies are sexually unreceptive during this post-eclosion period, and for several hours afterwards, but mating may take place as early as 8 to 12 hours after eclosion. Females will begin to deposit eggs within two days.

II. MATING AND THE IMPORTANCE OF VIRGINITY

Mating in *Drosophila* follows an elaborate courtship in which males perform species-specific wing flapping patterns while pursuing (on foot) females. The wing movements create vibrations (courtship songs) which are recognized as correct or incorrect by the female. A receptive females of the correct species will ultimately allow the male to "catch up" to her, and after some chemosensory behavior, copulation will ensue.

Adult female *Drosophila* may store and utilize sperm from a single insemination for weeks. Hence, when making specific crosses of flies it is absolutely crucial that females be virgins. If a female carrying the sperm from one type of male is inadvertently used in a cross with another type of male, it is likely that the offspring will be a mix of individuals with different fathers, or that the offspring will be fathered by the first male. This would almost certainly lead to an erroneous interpretation of the inheritance patterns of the characteristics that are being studied.

III. FLY HUSBANDRY AND HANDLING

Food preparation and general stock handling: Remember, flies ARE animals and require proper conditions to survive. Prepare the food in the following way: using the measure provided, transfer 1 measure of food into each vial; then, using the pump-bottle provided, add a measured volume (depress the plunger ONCE) of antibiotic-supplemented distilled water to the vial. The medium will set in a few minutes. Sprinkle several grains of Baker's yeast onto the surface of the medium to promote fermentation. After the medium has set and the yeast has been added flies are introduced into the vial. If the flies are unconscious, introduce them in such a way that they remain on the side of the vial. Unconscious flies may become stuck to the wet medium and die. As the flies recover, place the vial in an upright position in a tray. The crosses are to be cultivated in the incubators marked BIOS 116. If, during incubation, the medium becomes dry, add a small amount of water. As soon as pupae begin to form, either move the adult flies to a new vial or sacrifice them. Overcrowded vials result in poor growth and fungal contamination.

Anaesthetizing the flies: Several methods can be used to render *Drosophila* unconscious: diethyl ether, FlyNap, or CO_2. The current method of choice is CO_2. CO_2 can be obtained as a compressed gas or generated by dissolving antacid tablets (Alka-Seltzer). **To use compressed CO_2**: materials necessary include an ice pack (freezer, Rm 217), a petri dish, a file card and a manipulating brush. Place the petri dish atop the ice pack with the file card in between and allow the dish to chill. Meanwhile, examine the tank of compressed gas. The gauge on the cylinder indicates the pressure remaining in the tank. The valve on the tank is open, allowing the gas to reach the regulator. Turn the round knob on the left side of the regulator and release a VERY LIGHT FLOW of CO_2 from the tank. Insert the end of the attached hose in the INVERTED vial by sliding it between the plug and the vial wall. The flies will tumble onto the surface of the plug. Quickly, **turn off the CO_2**, withdraw the hose and pour the flies into the chilled petri dish. As long as they are kept cold, they should remain unconscious.

Determining the gender of a fly: Adult *Drosophila* exhibit clear sexual dimorphism, but it takes a little practice to be able to sex flies quickly and without error. The best way to determine sexual identity is to examine the external genitalia. A second method, which is also reliable, is to look for sex combs. Sex combs are small patches of bristles which appear as black knobs on the first tarsal segment of the forelegs (first pair of legs) of male flies.

A less reliable, but quick and easy approach, is to compare body size and abdominal shape and color of the flies. In wild-type strains, males are generally smaller than females, and the posterior end of the abdomen appears more rounded than that of the female. Most important, black bands of pigmentation on the dorsal side of the last abdominal segments appear to be fused (continuous) in males, but not in females. This gives the posterior portion of the male abdomen a solid black appearance. In the female the band on each segment appears separate from those of the adjoining segments. Hence, the female abdomen appears to be striped. Because some of the variant phenotypes you will study in this class involve body color this method of gender determination may not be appropriate in all cases.

Collecting virgin females: Examination of the external appearance will not reveal whether a female has mated. However, depending upon the temperature at which the flies are maintained, the time since eclosion (emergence from the pupal case) is a very reliable indicator of the sexual receptivity and possibility of mating. The fly stocks to be used for virgin collection should be kept at 18? C. Because *Drosophila* are poikliotherms, the cooler temperature slows down the rate of development, allowing more time for virgin collection. At 18? C females will not become sexually receptive until at least 12 hours after eclosion. Therefore, if a vial is completely cleared of all flies at a specific time, one can be certain that any females that eclose within the 12 hours following vial clearance are indeed virgins. This means that a rigorous work schedule must be maintained when virgin females are being collected. Virgins are very valuable and should be isolated from any males (in a separate vial) until used in a mating.

A good collection schedule is as follows:

Day 1: Clear the vials of any adult flies. This should be performed at about 8:00 - 9:00 PM. Place the cleared vials at 18? C.

Day 2: At 8:00 - 9:00 AM, return to the lab. Anesthetize the flies and separate males and females. Place the sorted flies into freshly prepared vials. Record the time and number of each gender collected on a data sheet. Return to the lab at 8:00 - 9:00 PM and repeat the clearing and collection procedure. Keep the females obtained during each collection session in a separate vial. Males may be combined in one or two vials. If such a schedule is followed conscientiously, the isolation of sufficient females to perform a cross should take no more than 4 - 5 days.

If more than twelve hours have passed since the last collection, do not throw the flies away. Anaesthetize the flies and separate males from females. From among the females, select those with little or no pigmentation (except for a gray patch on the ventral side of the abdomen). These females are young enough to be considered virgins. Again, keep these females separate from other collections. All collected males and females may be moved to the 24? C incubator. Keep the females for 3 - 4 days prior to using them in a cross. By that time, if one of the females was not a virgin, small larvae will be seen crawling around. In this case, discard the entire vial. Remember that one non-virgin female will ruin an entire cross.

NOTE: Don't panic if there are eggs in the virgin vials; even virgin females will lay some eggs, but these are unfertilized and will not hatch. If in doubt, watch the eggs for a couple of days. If the eggs in the virgin vials hatch into larvae, these "virgins" are not virgins!

Assignment:

Two fly cultures will be provided to each team of students. Each vial contains a pure breeding population of flies. Possible combinations include wild type, ++, flies and a second vial with flies exhibiting two phenotypic variants (mutants) that are clearly different from the ++ flies; OR, two vials each containing flies with a single visible phenotypic variation that differs from the ++. Examine the flies in order to determine how they differ from the wild type. Each vial containing a mutant stock will be marked with a numerical code. Record this code number.

Remove adult flies to a new stock vial and label. Begin virgin fly collection. When sufficient flies have been collected, prepare reciprocal crosses between the two strains. It is the responsibility of each team to maintain stock cultures. No new stocks will be provided. While collecting virgin females, cultures should be placed in the 18°C incubator. After crosses have been made, vials should be placed in the 24° C incubator.

The experimental objective of this laboratory is to determine the modes of

inheritance (autosomal or sex-linked) and the pattern of gene action (dominant, recessive, partial dominance, *etc.*) of the variants provided. The pattern of inheritance in the present exercise can only be determined after two generations. If crosses are done properly and efficiently, the experiment will take six to seven weeks, that is, two fly generations. Always remember to do the reciprocal crosses; female of type A X male of type B *and* male of type A X female of type B, in separate crosses. (Although it may be unclear how to proceed at this time, the TA will provide basic instruction in beginning the crosses. Within a short time, lecture material and text readings will make everything comprehensible.)

Keep complete records of all procedures and results (number of flies counted, phenotypes, sexes, etc.). Data sheets are available in the laboratory. Record all procedures, phenotypes and numbers in your lab notebook. Be especially careful to record the dates and times of collection, and when the vials are cleared. Fly vials and data will be checked periodically during conferences with the TA. Each team member is responsible for collecting virgins, preparing matings and counting adult flies. Cooperation is essential for the success of the experiment.

NOTE: All vials must be labeled with the date, group initials and code indicating the type of fly and the type of cross.

A lab report will be due in Week 8 of the semester.