Chapter

Fruit Flies (*Drosophila spp.*) Collection, Handling, and Maintenance: Field to Laboratory

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Abstract

As drosophilids are versatile, low maintenance and non-harming model organisms, they can be easily used in all fields of life sciences like Genetics, Biotechnology, Cancer biology, Genomics, Reproductive biology, Developmental biology, Micro chemical studies, ecology and much more. For using such a model organism, we need to learn capturing, rearing and culturing their progeny along with basic identification and differentiation between males and females. This chapter is being emphasized on techniques of capturing these flies with different and effective techniques. Along with it, most species-specific baits are discussed to catch more yield. Culture food media, a set measurement of different ingredients is used to rear the collected sample. The reasons for using each ingredient are also discussed in this chapter. At last, this chapter highlights the basic clues to identify different species in the field and lab along with learning distinguishing characteristics of males and females easily and effectively.

Keywords: attractive baits, culturing flies, food preparation, identification, sorting out male and female

1. Introduction

In 1911 T.H. Morgan and his students C. Bridges, H. J. Muller and A. H. Sturtevant came across red-eyed insects, fruit-flies. Since then, it has made its way in research labs helping scientists to explore fundamental problems in biological sciences. It has turned out as one of the best metazoan insect model organisms. It is one of the best model organisms for the biological studies ranging from molecular genetics of diseases to the ecosystems and up to the evolutionary scales. Starting from visible mutants and chromosome mapping, today studies of complex genetic networks are possible with the help of multiple genome sequences (the 12 genomes project), systematic gene disruption or knock-down (RNAi stock library), microarray analysis, protein interaction maps and the FlyBase integrated database. High-throughput platform biology and open-source availability are considered as part of modern developments and the *Drosophila* model has integrated them with success. Recent developments allow the analysis of problems and processes previously inaccessible like complex human diseases caused by developmental, neurological or metabolic defects. *Drosophila* has clear-cut advantages in this field of research with its sophisticated genetic techniques. *Drosophila* research also plays an important role in technological transfer to other arthropod models, opening the window to biodiversity

resources and macro-evolutionary scale. It has also been successfully integrated in teaching subjects as diverse as genetics, physiology, ecology and evolution.

Drosophilids are ectothermic insects whose body temperature changes with the ambient temperatures. These insects can easily survive between 12–21 degrees Celsius [1]. Temperature impact on their viability, fertility, developmental period, foraging activity, feeding, and breeding could easily be seen in laboratory stocks and distribution and populations dynamics under field conditions [2, 3]. Even Apart from temperature, humidity and rainfall, sunlight also plays a vital role in distribution of drosophilid species.

The family Drosophilidae encompasses 4,450 species distributed in 75 genera with two subfamilies, drosophilidae and steganinae [4]. The sub family drosophilinae is more diverse, distributed across 47 genera of which genus *Drosophila* is the largest with 1,213 species. The subfamily steganinae is a smaller one and is distributed across 28 genera of which genus *Leucophenga* is the largest with 256 species [4].

In India, more than 347 species are recorded which are spread across 27 genera, of which 58 species belongs to 8 genera of subfamily steganinae and rest 289 species of 19 genera are placed in subfamily drosophilinae (unpublished data).

2. Methods of collection

There are several methods in practice to collect fruit-flies from their natural habitats. Some of the methods are shown below:

2.1 Installing trap-bait

For setting-up traps one could use plastic bottles ranging in the volumes of 250 mL to 1 L. Fruit slices along with a pinch of yeast could be used as a bait. With the use of blade somewhere in the middle of the bottle a section could be carved out for food access. These traps could be hanged on orchard tree at a height 3–4 feet above the ground (**Figure 1**). The collection area must be damp and moist with minimal human interference. After 1–2 days traps must be recovered to collect flies. Same method could be applied to collect all kinds of *Drosophila* species. Some flies prefer to breed in the trash and stay close to ground, and some around the trees.

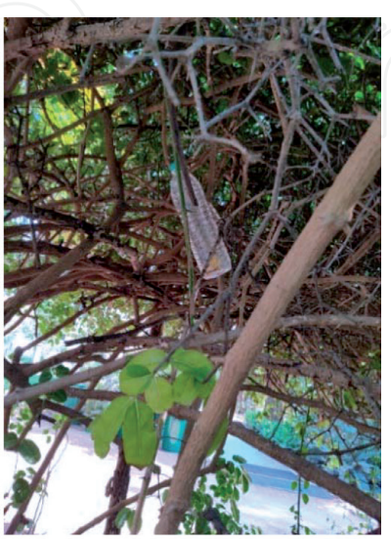
a)  b) 

Figure 1.
Trap bait: a) Transparent plastic bottles can be used as traps, a C-shaped window is made in the center of the bottle which serves as an entry point for the flies. Banana pieces placed inside the bottles attracts the flies. b) The bottle can be placed around small bushes/trees and can be collected next morning.

The methods could be improved based on little understanding of *Drosophila* species ecology (authors observations; unpublished).

2.2 Net swipe

There are many genera that are not attracted to regular traps and need to be captured from their natural food source (wild rotting fruits). Capturing such drosophilid species nets are more effective. The flies hovering over the rotten fruits or piles of organic wastes could be captured using this method (**Figure 2**).

2.3 Use of aspirator

This method is used when fly's numbers are low and also when investigator is familiar with the species identification and targeting particular species individuals. This method is more appropriate when flies are feeding, mating or resting on petals, leaves, fruits etc. Flies feeding on mushrooms and flowers are mostly collected using this method (**Figure 3**). Aspirator is also used to transfer flies from bait bottles or insect net to culture vials.

Some of the common species-specific baits are:

Banana: It is the most commonly used bait. It could be used to collect cosmopolitan *Drosophila melanogaster,* and other commonly found drosophilid species.

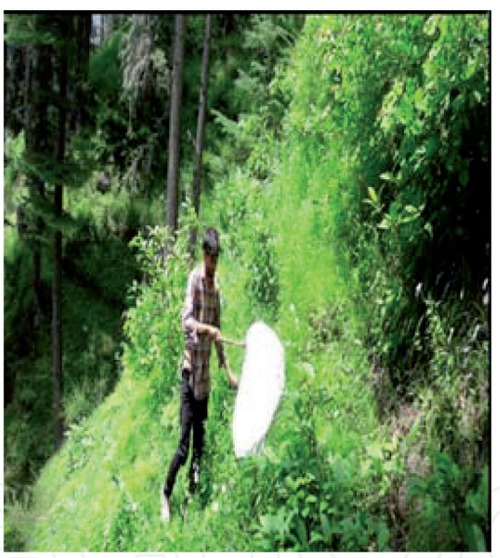


Figure 2.
Net sweeping: The modified insect nets are used for capturing drosophilids which are not attracted toward baits.

Figure 3.
Use of aspirator: The flies performing mating or resting over leaves, mushroom or fruits etc. are captured with help of aspirator.

Tomato: Adding yeast to it forms an attractive bait for *Drosophila suzukii* and *Drosophila busckii*.

Orange: It is also an attractive bait for *Drosophila suzukii* and *Drosophila busckii*.

Grapes: With added yeast grapes act as an attractive bait for species like *Drosophila busckii* and *Drosophila immigrians*.

Usage of vinegar in the baits for *Drosophila suzukii* helps in trapping more flies. Keeping baits in moisture free traps improves the collection yield.

3. Culture handling and maintenance

The culture handling and maintenance is required to be learned for making the collected sample to multiply several times and for making isolines and confirming the species's level identification.

3.1 Rearing and culturing *Drosophila*

For rearing and culturing the drosophilids, the collected samples need to be transferred from bait bottles to the narrow culture vials (with culture media) While shifting flies from the bottles to collection tubes the openings of the bottles must be kept under bright light directing the flies toward the light and thus making their transfer easy. For culturing, the flies are kept at room temperature (25°C). If room temperature is not adequate the culture tubes could be kept in the BOD (Biological Oxygen Demand) incubators. This would help the culture media to last longer until the drosophilid colony begins to establish.

3.2 Preparing *Drosophila* media

1. The commonly used media for various drosophilid stock maintenance includes Agar, yeast, Maize flour, Brown sugar, Nepagin, and Propionic acid.

2. To prepare fly media agar is added to the hot water. Following this yeast, maize flour, and sugar is added. After 20–30 minutes of cooking (with 1–2 boil) heat should be turned off. Once media temperature reaches to close to 50 degrees nepagin and propionic acid could be added. Throughout the preparation food needs to be constantly stirred.

3. Maize powder, brown sugar, and dried yeast are used as food. Yeast holds special nutritional value for drosophilids. While Nepagin is anti-fungal in nature and Propionic acid is a bactericidal and functions as preservative and increases the shelf life of food.

The food could then immediately be transferred to the sterilized vials or bottles. As soon as the media starts hardening the vials or bottles needs to be covered properly with the help of a cheese cloth. The food could be used after a day. The media tubes or bottles could also be stored in a cool place for 1–2 weeks for future use. *Other food recipes:*

There are almost 7–8 different food recipes for drosophilid species. The following link could be further explored for other recepies [5].

However, the standard procedure can be modified for the normal lab conditions and general use.

1. Cornmeal, sucrose, dextrose, yeast and 2-acid medium

This food recipe was described by Lewis in 1960. This uses the phosphoric acid which allows less propanoic acid usage without affecting the fungicidal effects. This recipe has also been used at Caltech since 1955.

2. Cornmeal, molasses and yeast medium

This mixture has to be used fresh. This medium was used at Bloomington for several years. It went through certain modifications in order to prevent bacterial contamination.

3. Cornmeal, dextrose and yeast medium

This recipe was first used by Brent and Oster in 1974. In order to reduce the chances of Leucon Stoc infection of cultures, sucrose was substituted by dextrose [5].

4. Identification (taxonomy)

In a biodiversity rich place, one could come across many small insect species. Identification becomes crucial in sorting a particular species from a pool of collection. Over the years, taxonomists came across various methods to identify species. Therefore, some common morphological diagnostic characters were listed out and based on these keys one could identify drosophilid species. However, closely related or sibling species are hard to distinguish with general morphological characteristics.

Drosophilids are usually small flies, ranging from 1.5–7.0 mm in length; yellowish, golden, brownish or blackish in color. They possess a number of the characteristics, such as red eyes and plumose arista. Body is shiny, often with stripes or spots on the thorax. Wings are hyaline or with black patches or marginal areas with dark lines. Abdominal tergite strip patterns (i.e. pigmentation) vary from species to species (dark or light bands or spots in 2–6 tergites). In some species, sexual dimorphism is clear by wing patch or presence of sex-comb on legs.

However, species are identified by their male genital organs (periphallic & phallic) possessing structural compatibility features [6]. These genital organelle structures are species specific. Example, the two subfamilies viz., drosophilinae and steganinae are differentiated on the basis of the distance between proclinate orbital setae and inner vertical setae from posterior reclinate setae. In subfamily drosophilinae the distance of proclinate orbital seate from posterior reclinate setae is less compared to its the distance of inner vertical setae. While in subfamily Steganinae the distance of proclinate orbital setae from posterior reclinate setae is more than compared to its distance from inner vertical setae.

Flies are too small in size to be observed with naked eyes. Hence magnification is required and this could be achieved with a good hand lens, or a wide-field binocular microscope, or a stereo zoom microscope, or a compound microscope. On a white background pictures emerge better (**Figure 4**).

For identification, anesthesia could be given to flies. This makes flies unconscious. Ether or Carbon dioxide gas could be used as anesthesia. Flies sensitive to ether or CO2 cold treatment is an option.

Generally, females possess larger body size and have swollen abdomen than males. In some cases of males' 5th and 6th tergites are pigmented whereas others have wing patches. In a few genera of drosophilids males also possess sex comb on their fore legs. The posterior end of males body is pointed whereas in females it is pointed (due to the presence of their ovipositor and female genital organ). The male genital organs are species-specific and differ from species to species. Dissection of genital organs is used for conformation of species. Dissection of male genitals is usually done with the help

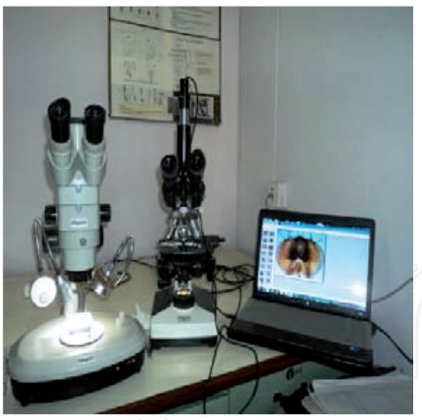
a)  b) 

Figure 4.
Identification: a) after collection of the flies, they are given anesthesia (CO_2) which gives 30 minute time to sort the male and female. b) Identification can also be done by dissecting the male genitalia.

of a needle. It requires washing the tissues in 10% KOH approximately at 100 °C for several minutes. This opens the intact reproductive plates and helps investigators to collect additional details. Few drops of glycerol can be added for better resolution.

4. Morphological study of drosophilid

Like in other arthropods, the adult drosophilids body is comprised of three major parts i.e., head, thorax and abdomen. Drosophilids have one pair of wings and halters each. Wings help in flight whereas halters help as balancing organs.

4.1 Head

The head of drosophilids has distinguishing parts like ocellar triangle, post-ocellar setae, inner and outer vertical setae, fronto-orbital plates, cibarium and arista. Arista is a distinctive character as it possesses varying dorsal and ventral branches in different genus. The genus *Drosophila* possesses three elongated orbitals with proclinate setae inserted into the anterior most part. The ratio, length and placement of orbitals, ocellar, vertical and post-ocellar setae is used for the identification of different genera of drosophilids (**Figure 5**). In genus *Chymomyza* the anterior reclinate setae is present in front of the proclinate setae whereas in genus *Liodrosophila* anterior reclinate setae present is small in size. Facial carina is also an important diagnostic characteristic in the species. The chaetotaxy and color of the palps is used to identify the sibling species [7]. The setae on either side of the face known as vibrissae and sub-vibrissae are important for the identification of species. The number of anterior & posterior sensilla and sensillacampaniformia of the cibarium are important diagnostic characteristics for the identification of the species.

4.2 Thorax

The thorax has three main segments: prothorax, mesothorax and metathorax. Mesothorax is significantly enlarged which aids the wings, while prothorax and metathorax are generally reduced. Most of the dorsal surface of the mesothorax is covered with mesonotum. There are numerous regular and irregular rows of

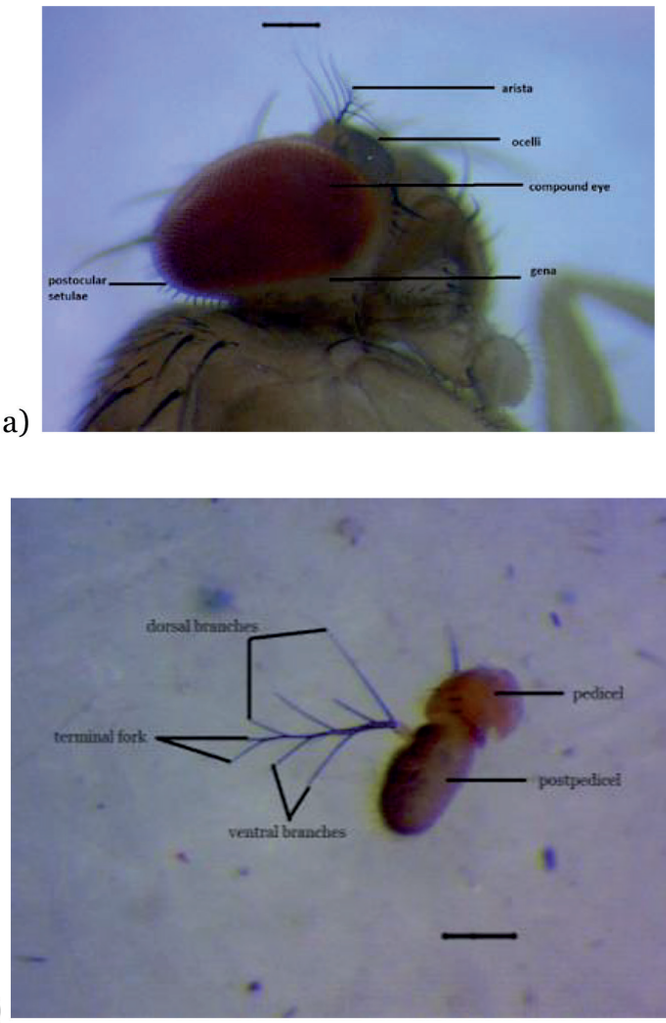
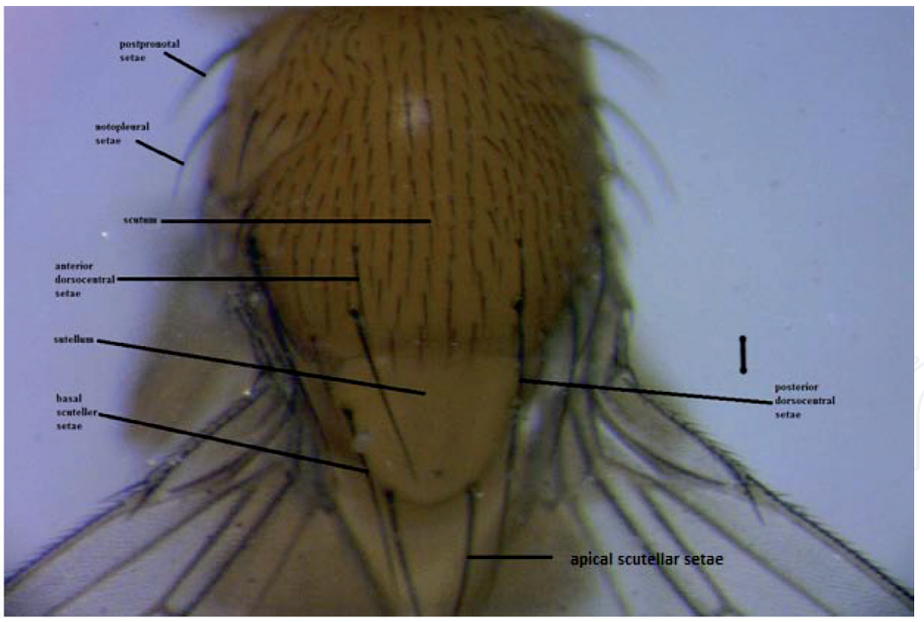


Figure 5.
Head: a) D. suzukii, showing major portions of head region. b) Arista and its parts.

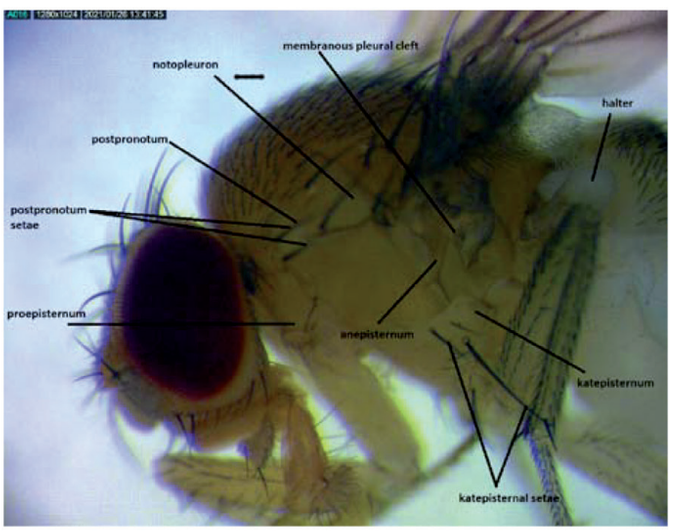
acrostichalsetulae and numeral pairs of dorsocentral setae present on the mesonotum. The number and position of the Acrostichalsetulae is taxonomically important for differentiation of the species (**Figure 6**). Majority of the *Drosophila* species have six or eight regular acrostichal rows. While most of the *Scaptomyza* species possess two or four rows. However, other genres are characterized by ten or more irregular rows. In a few species of mycophagous *tripunctata and testacea* group a set of enlarged acrostichals are located more anteriorly near to the transverse suture known as presutural setae and are also used for identification [7]. The length and orientation of basal and apical scutellar setae (present on the scutellum) are also taxonomically important for the species identification. Number and length of the katepisternal setae present on katepisternum are also significant for differentiation of the species and species group.

4.3 Legs

Legs in drosophilids are divided into coxa, trochanter, femur, tibia and tarsus. Tarsus have 5 tarsal segments. Color and arrangements of bristles (chaetotaxy) of male foreleg and relative length of first tarsus is important for distinguishing traits among different species. Their presence and the number of spines on hind leg, tibia and tarsomere (genus *Impatiophila)* both are considered for the identification of closely related species [8]. In some species such as *Liodrosophila angulata* a number of taxonomically important spines are present on the femur of the foreleg.



a)



b)

Figure 6.
Thorax: D. suzukii a) dorsal b) lateral.

4.4 Sex Combs

Some species of *melanogaster* and *obscura* group are characterized on the basis of sex combs on the male foreleg. The numbers of sex combs are taxonomically important for the distinction of the different species. *Drosophila* males use their sex combs to grasp the females' abdomen and genitalia. They also use them to spread their wings prior to copulation.

4.5 Wings

Wings are attached to the mesothorax of the abdomen. In many drosophilids wings possess spotted patterns. Also,hylanine or fuscous are taxonomically important. Although there is a consistent pattern on wing's venation but in some taxa additional cross veins could be present eg *planitibia* species group. Different types of cross-veins (bm-cu, dm-cu, r-m and cuA2), subcostal break, humeral break, wing cells (bm, dm, cup), and wing veins (CuA1, A1, R1, R2 + 3, R4 + 5, M1) occur in the wings (**Figure** 7). In genus *Impatiophila* the setae of the middle row on the

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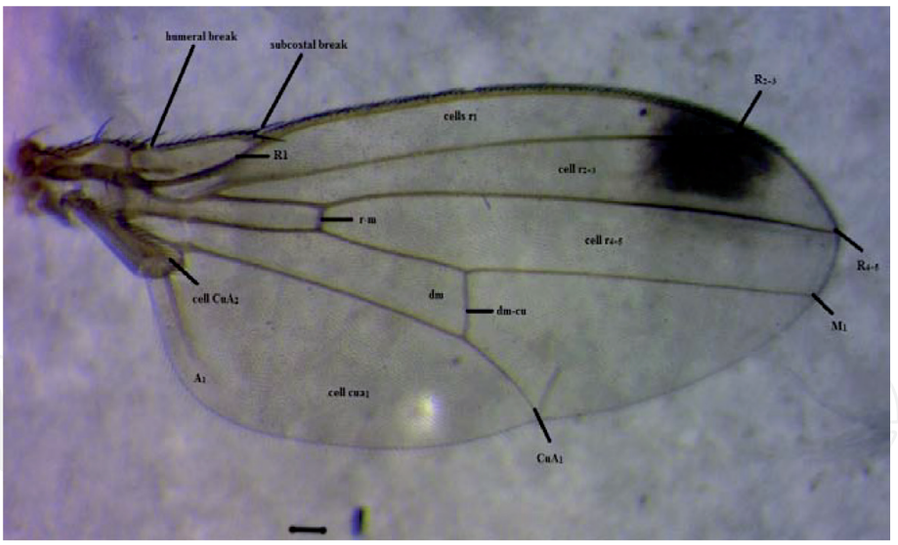

Figure 7.
Wing: Abbreviations; h = humeral, hum brk = humeral break, R1 = anterior branch of radium, C1 s = apical seta(e) on 1st costal section, sc brk = subcostal break, r2 + 3 = second + third radial, r4 + 5 = fourth + fifth radial, M1 = 1st posterior (sectorial) branch of media, dm-cu = discal medial-cubital, CuA2 = 2nd anterior branch of cubitus, CuA1 = 1st anterior branch of cubitus, A1 = 1st branch of anal vein, r-m = radial-medial. (source; terminology by professor M.J Toda).

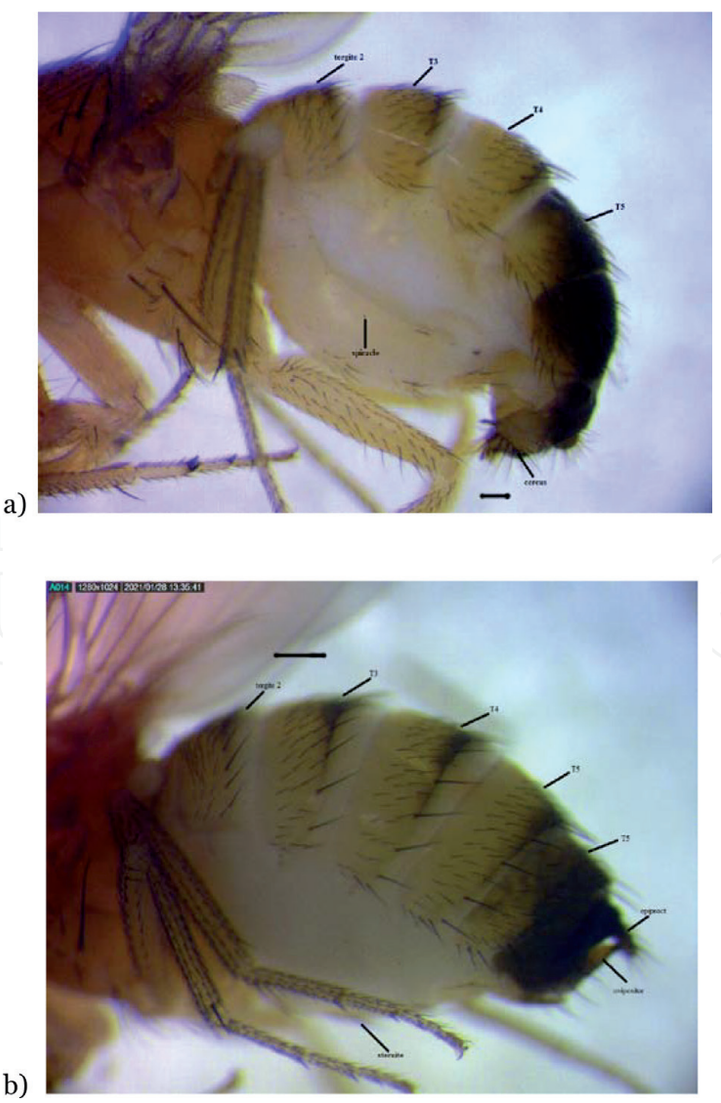

Figure 8.
Abdomen: D. suzukii (a) male abdomen T1 to T6 = Tergite 1 to 6; (b) female abdomen S1 to S6 = Sternite 1 to 6.

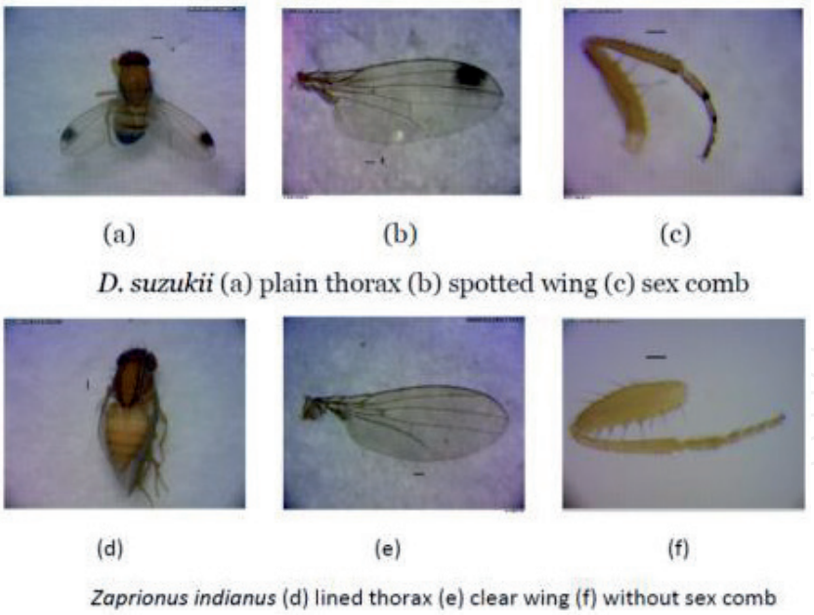

Figure 9.
Species level distinguishing characters.

second costal section is considered important for the differentiation of the species into different species group [8].

4.6 Abdomen

The dorsolateral portion of abdomen is known as tergite and it is segmented and chitinized. The ventral portion is called sternite. It is generally hairy, chitinized and quadrilateral in shape. The length and width of the sternite is important for species identification (**Figure 8**).

4.7 Terminalia

The male terminalia possess many internal and external characters useful for species characterization. In drosophilids the male genitalia exhibit speedy and divergent evolution while female genitalia are thought to evolve slowly among closely-related species. In drosophilids female copulatory structures have been claimed to be mostly invariant compared to male structures. In most animal species with internal fertilization, male external genitalia are the most rapidly evolving organs and they usually are the first organs to diverge morphologically following by speciation. Because of their rapid evolution and species-specificity, their illustration is a common feature of taxonomic literature to discriminate between closely-related species. The morphology of male genitalia can differ dramatically even within very closely related animal species. The male terminalia is further divided into epandrium and hypandrium.

4.8 Epandrium

In males, 9th tergite is known as epandrium and it possesses a number of characteristics such as pair of cerci and surstyli which is present on the posterior and posteroventral of the epandrium respectively. The size, color, shape, morphology and number of setae on the cerci are significant for the distinction of species. Surstyli bears a number of distinct prensistae. The numbers of prensistae vary from species to species and are important for the species identification.

4.9 Hypandrium

The genitalia are the structure linked with 9th sternite or Hypandrium and possesses aedeagus, basal processes of the aedeagus, parameres, and gonopods on it. The posterolateral portion of the hypandrium is known as gonopods. Posterolateral to the gonopods are paraphyses which possess a number of setae. The aedeagus is placed centrally with respect to the rest of hypandrium.

The major distinguishing character at species level are the presence and numbers of sex combs and bristles. Different types of spots present on wings and abdomen, and lining present on thorax of different drosophilids etc. are the characters that provide cues to identify flies (**Figure 9**).

5. What makes *Drosophila* a great model organism?

5.1 *Drosophila* as a model organism

The different characteristics of *D. melanogaster* make it an ideal model organism, which are following:

Smaller Size and Short lifespan

Shorter life span facilitates large quantities of flies to be produced in a short time.

Minimal culturing requirements

Due to the smaller size and minimal requirements, *Drosophila* can be cultured and tested in limited resources.

Genetic manipulation

The fly genome has been sequenced and well characterized. It has 100+ years of literature available. Besides this it has four pairs of chromosomes only which makes an ideal system to do genetic crossing and gene editing simpler.

5.2 Basic research

As drosophilids are versatile, low maintenance and non-harming model organisms, they are used in all fields of life sciences like Genetics, Biotechnology, Cancer biology, Genomics, Reproductive biology, Developmental biology, Micro chemical studies, ecology and much more. For more than a century, the low cost, rapid generation time, and excellent genetic tools have made the fly indispensable for basic research. Also, the recent advancements in the field of molecular tools have allowed the organism to be used more efficiently. From human disease modeling to the dissection of cellular morphogenesis and to behavior and aging, the current usage of flies greatly influences fly research. However, this field remains vibrant and exciting, with labs using flies in drug discovery, bioengineering, regenerative biology, and medicine. The future use of fruit flies as a model organism in research is bright.

6. Stock centres

Bloomington stock centre maintains various fly stocks including aberrations, balancers, deficiencies, duplications, clonal, chemically induced mutations, human disease model, mapping, teaching stocks, wild-type lines, transgenes and various other stocks. Further details about the stock centre could be found at site dedicated to stock centre [9].

Here is the list of other stock centres around the globe:

1. Kyoto Stock Centre, Kyoto Institute of Technology, Kyoto, Japan.

2. Harvard Medical School, Boston, MA, USA.

3. FlyORF, University of Zurich, Zurich, Switzerland.

4. NIG-FLY, National Institute of Genetics, Mishima, Japan.

5. THFC, Tsinghua University, Beijing, China.

6. Vienna Drosophila Resource Centre (VDRC), Vienna, Austria

7. Fly maintenance stuff

A detailed information about fly maintenance materials and accessories are valuable at several commercial vendors. The provided links provide access to it [10].

Important websites for Identification of drosophilids

Taxonomic information database for the world Drosophilidae:

DrosWLD Species (Taxonomic information database for world species of Drosophilidae, maintained by Masanori J. Toda) https://bioinfo.museum.hokudai.ac.jp/

Japan *Drosophila* Database: JDD http://www.drosophila.jp/jdd/index_en.html

7. Conclusions

This chapter highlights the basic clues to identify different species in the field and lab along with learning distinguishing characteristics of males and females easily and effectively.

8. Recommendations

These protocols will act as baseline data for handing and maintaining drosophilids for young taxonomist. As these processes are easy ones so these could be used at graduation level to let students get familiar with its taxonomy.

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Conflict of interest

The authors declare no conflict of interest.

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Acronyms and abbreviations

Wing h	humeral
hum brk	humeral break
R1	anterior branch of radium
C1 s	apical seta(e) on 1st costal section
sc brk	subcostal break
r2 + 3	second + third radial
r4 + 5	fourth + fifth radial
M1	1st posterior (sectorial) branch of media
dm-cu	discal medial-cubital
CuA2	2nd anterior branch of cubitus
CuA1	1st anterior branch of cubitus
A1	1st branch of anal vein
r-m	radial-medial. (Source; Terminology by Professor M.J Toda). Abdomen: T1 to T6 = Tergite 1 to 6; S1 to S6 = Sternite 1 to 6;

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