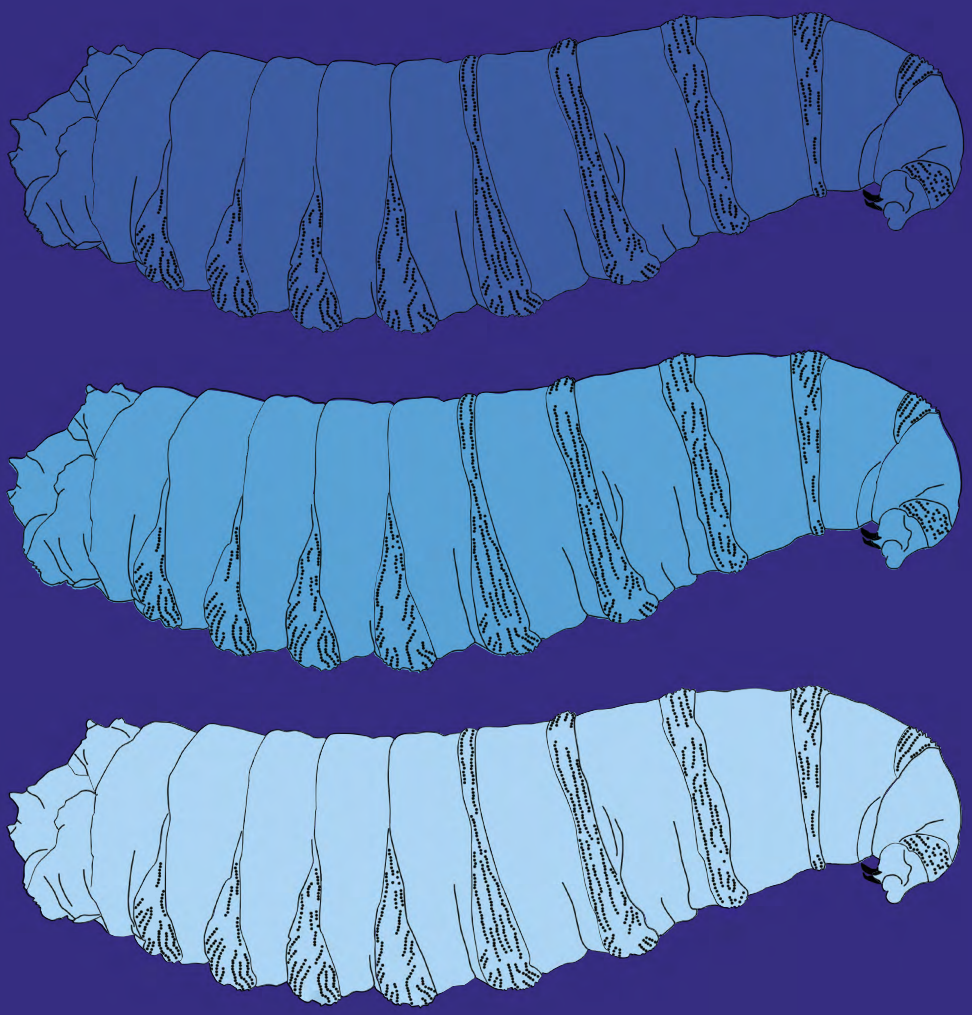
A COMPLETE GUIDE TO MAGGOT THERAPY

Clinical Practice, Therapeutic Principles, Production, Distribution, and Ethics



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14. Medicinal Maggot Production

Frank Stadler and Peter Takáč

This chapter discusses the requirements for adult fly rearing, high-volume egg production, larval rearing and pupariation, and it explains the production of disinfected medicinal maggots for maggot therapy, the quality control procedures that are required to ensure safe and efficacious maggot therapy, and supply chain management considerations arising from the perishability of medicinal maggots. The chapter draws on a broad range of sources including the literature on maggot therapy, forensic entomology, and general entomology. For compromised healthcare settings with limited resources, point-of-care production solutions are discussed that do not rely on sophisticated laboratory and logistics infrastructure.

Introduction

Having covered the laboratory and insectary infrastructure and equipment needs in Chapter 12 [1], as well as the process of medicinal fly colony establishment in Chapter 13 [2], the next step is to provide the information necessary to produce high-quality, efficacious, and safe medicinal maggots. This chapter discusses the requirements for adult fly rearing, high-volume egg production, larval rearing and pupariation. The chapter also explains the production of disinfected medicinal maggots for maggot therapy, the quality control procedures that are required to ensure safe and efficacious maggot therapy, and the

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Summary

There are potentially many fly species that could be utilised for maggot therapy and several species have been successfully applied to treat wounds. Nevertheless, it is recommended that producers and researchers starting medicinal fly colonies only collect species of known efficacy. For the most part this will involve the production of *L. sericata* and *L. cuprina* thanks to their near-global distribution. Producers and therapists interested in developing maggot therapy with other fly species will find Chapter 11 on the identification and testing of new maggot therapy species essential reading [13].

All life stages are suitable for collection, except pupae that are generally hidden from view because maggots bury into the ground for pupariation. Correct identification of the species that are collected and the correct selection of breeding stock is critical. Identification guides provided in this chapter and identification keys published in the literature should be consulted. In addition, identification services offered by natural history museums or molecular barcoding methods should be used where accessible to confirm species identity.

Domestication of the newly established fly colony will proceed via adaptation to the laboratory/insectary environment and the producer's operating procedures. Producers must implement routine quality-monitoring protocols during colony establishment and, beyond that, track life history characteristics like the ones suggested in Table 13.4. There is largely unexplored scope for selective breeding of desirable characteristics of medicinal fly species and molecular tools may also offer opportunities for genetic enhancement of medicinal flies. However, the latter will require considerable research, regulatory approval and patient acceptance before transgenic maggots reach the bedside.

References

1. Firoozfar, F., et al., *Mass Rearing of Lucilia sericata Meigen* (*Diptera: Calliphoridae*). Asian Pacific Journal of Tropical Biomedicine, 2011. 1(1): pp. 54–56, https://doi.org/10.1016/S2221-1691(11)60068-3.

2. Firoozfar, F., et al., *Laboratory Colonization of Lucilia sericata Meigen* (*Diptera: Caliphoridae*) *strain from Hashtgerd, Iran*. Journal of Vector Borne Diseases, 2012. 49(1): pp. 23–26.

Life history characteristic	Measurement
Body length at post-feeding	Length of maggots (mm) at the beginning of the post-feeding period. Immerse maggots in boiling water to rapidly kill them and then preserve in 70–80% ethanol prior to measurement [92].
Weight at post-feeding	Weight of maggots (mg) at beginning of post-feeding period.
Time to pupariation	Time (hours) it takes for post-feeding maggots to pupariate.
Pupariation rate (in %)	Number of pupariated maggots divided by the total number of maggots studied, multiplied by 100.
Pupal development	
Pupal weight	Weight (mg) of pupae just after pupariation (when puparia have hardened and darkened to a glossy brown colour).
Development time	Time (hours) it takes for the fly to emerge after pupariation.
Emergence rate (in %)	Number of healthy flies divided by the number of pupae studied, multiplied by 100.

Strain Improvement

In addition to selecting suitable species with maggots that are benign and highly effective in debriding necrotic tissue, clinical performance could also be improved, and new therapeutic benefits introduced to maggot therapy, if fly strains were to be genetically enhanced. Traditionally, such enhancement is done through animal breeding based on selecting individuals with favourable characteristics. There have been recent attempts to genetically engineer *L. sericata* so that the flies express human growth hormone in larval excretions and secretions. Such genetically modified strains could deliver a variety of growth factors and anti-microbial substances during maggot therapy and thereby enhance wound healing [93]. However, due to the negative perception of genetic modification, regulatory approval for genetically modified flies may be difficult to obtain even if *in vitro* and clinical trials demonstrate efficacy. Therefore, until regulatory barriers have been overcome, the best strategy will be to improve medicinal maggot performance and efficacy through conventional animal breeding approaches.

demand sophisticated instrumentation, software, and skilled laboratory technicians that may not be available to all producers [89–91].

The easiest way for a producer to monitor therapeutic efficacy of medicinal maggots over time is to communicate frequently with maggot therapy practitioners to receive feedback on clinical performance. This must also include an adverse-events reporting system so that any potential quality issues that have led to adverse treatment outcomes can be addressed quickly. The therapeutic performance with respect to debridement can also be tested in the laboratory with a meat-based test that simulates debridement in a wound. A protocol for such a debridement activity assay is given by Thyssen and Masiero [13] in Chapter 11 based on studies published in the literature.

Table 13.4 Quality control activities.

Life history characteristic	**Measurement**
Adult fly performance	
Sex ratio	Ratio of males to females. Determine the sex of 100 randomly sampled adult flies.
Adult weight	Measure the weight of adults directly after emergence and before first meal. There should be a strong correlation between this weight and that of pupae.
First oviposition	Time (hours) it takes from emergence for females to lay their first egg mass (assuming that males are present for mating as soon as females are receptive)
Number of oviposition events	Number of times a female lays eggs (oviposition).
Egg mass size	Number of eggs per oviposition (per egg batch).
Fecundity	Total number of eggs laid in a lifetime.
Adult longevity	Life span (days) of an adult fly from emergence.
Egg development	
Development time	Time (hours) from oviposition to emergence of larvae at a specific temperature.
Survival rate (in %)	Number of emerged larvae divided by the total number of eggs incubated and multiplied by 100.
Larval development	
Development time	Time (hours) from hatching to post-feeding period (when they leave the larval food substrate).

may even increase [85], provided the negative effects of inbreeding can be managed.

The objectives regarding colony performance may vary depending on the purpose of the colony. For example, if flies are to be reared in the laboratory for the purpose of controlled-release field experiments or pest control, it will be important to maintain their natural fitness and resilience in the field [86]. In contrast, the objectives for medicinal fly production are:

1. Optimisation of the therapeutic performance of medicinal fly species or strains.
2. Reliability of colony performance via homogenous life history traits, meaning that the individuals in the colony have very similar development times and growth rates, for example.
3. Maximisation of the number of eggs produced by females.

Unfortunately, there has not been much published research on optimal domestication and management of medicinal fly populations in the laboratory and insectary [87]. Much of this information and practical knowledge, as far as it exists, is of proprietary nature and held by medicinal maggot producers. Chapter 14 provides comprehensive guidance on how medicinal flies are best maintained in the insectary and how medicinal maggots are prepared for treatment [88].

Quality Control

Newly collected flies and established colonies should be carefully monitored, and their life history parameters measured on a regular basis. Species-specific characteristics such as morphology, life-history, physiology, and genetic characteristics can all be used as performance indicators. Which sampling regimen and performance indicators are chosen will depend on the producer's resources. For example, it is fairly easy to measure how many eggs females produce, how many of these hatch, how fast maggots grow and how many survive to adulthood. Table 13.4 lists some easily monitored quality control activities that may be included in the standard operating procedures. However, wing morphology or genetic investigations are more complex and

Colony Replenishment

While some laboratory colonies of fly species that are of interest to forensic, agricultural, or medical entomologists have been kept successfully for many years without adding wild flies to the colonies [4], it may be necessary to periodically restock in order to replenish colony genetics and vigour. For consistent performance of production colonies, it will be best to always collect replenishment stock from the same location as that of the founder colony [79–81]. This will avoid introduction of vastly different genetics and resulting life history traits. The aim is to maintain uniform fly performance characteristics in the laboratory.

When collecting from the wild, there is always a danger that flies have been exposed to toxins, and carry disease or parasites, making preliminary quarantine necessary. Careless introduction of these newly caught flies into the laboratory environment can harm established colonies. Many flies including *L. sericata* are also prone to pupal parasites [82] and various fungi and nematodes that kill insects. These diseases must not be introduced into the laboratory and insectary [83, 84]. In practical terms this means that newly collected flies should be kept apart from the established laboratory colonies for at least one life-cycle until their health has been confirmed.

Domestication of Fly Stock

Domestication is the process of cultivating or rearing plant and animal species for human use, pleasure or companionship. During domestication, the animal or plant population adjusts to the new environmental conditions under human cultivation. This is also the case when wild flies are brought into the laboratory. They will need to adjust to the laboratory environment, constant temperature and light regimens, diets, cages, and higher population densities. Initially, individuals of newly collected fly stock will vary in their life history characteristics such as development times, number of eggs produced and timing of maturation. Over a number of generations and as laboratory processes impose selection pressure on the fly population, this variation will diminish, performance will become more and more uniform, and fitness

vein (Figure 13.10e). *Chrysomya* can be distinguished from *Cochliomya* by the absence of any longitudinal black stripes on the thorax (Figure 13.10c).

Chrysomya megacephala. This species can be distinguished from other *Chrysomya* by the combination of entirely clear wings (Figure 13.10e), a blackish-brown anterior spiracle (Figure 13.10b), lower calypter brownish (not white or black) (Figure 13.10b), and many black vibrissal setulae on the face and parafacialia (Figure 13.10f). Males of this species possess touching eyes (holoptic) with sharply distinguished upper and lower facets (Figure 13.10c). In the female, the eyes are separated (dichoptic) (Figure 13.10d), and the fronto-orbital plate is dark rather than red (Figure 13.10d).

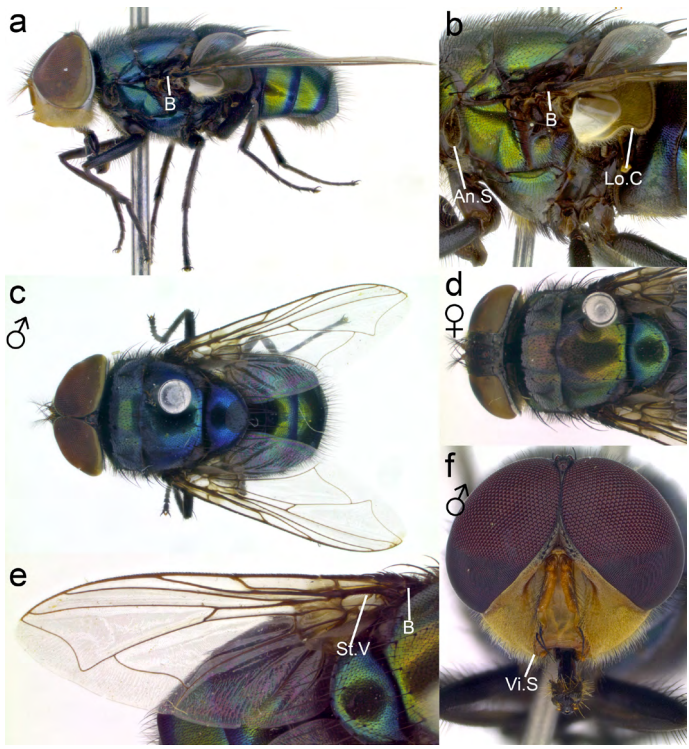

Figure 13.10 *Chrysomya megacephala*. B: basicosta, An.S: anterior spiracle, Lo.C: lower calypter, St.V: stem vein, Vi.S: vibrissal setulae. CC BY-NC.

Cochliomya macellaria. This species can be distinguished from all other species of *Cochliomya* by the combination of genae with only yellow hairs (Figure 13.9a, f), fronto-orbital area with both a row of black bristles (Figure 13.9f) and fine pale hairs, particularly in the lower half (Figure 13.9a, d, f) (if fine black hairs are present, proceed with caution—the specimen could be *Co. hominivorax* and should NOT be used for maggot debridement therapy). The entire abdomen is metallic green or blue (with each segment being identical in colour) (Figure 13.9c).

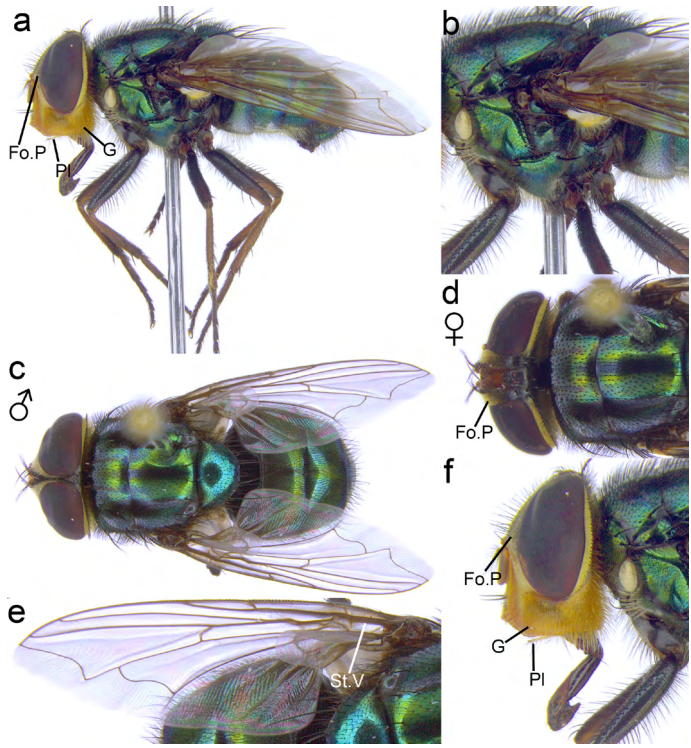

Figure 13.9 *Cochliomyia macellaria*. Fo.P: fronto-orbital plate, Pl: palpi, G: genae, St.V: stem vein. CC BY-NC.

Genus *Chrysomya*

Genus Diagnosis. The genus *Chrysomya* is most closely related to *Cochliomya* and both genera can be distinguished from *Calliphora* and *Lucilia* by the presence of a row of hairs on the dorsal surface of the stem

13.8c), basicosta yellow not fuscous or black (Figure 13.8a-c). This species closely resembles *L. cuprina* but can be distinguished from it by the combination of central occipital area with many hairs (2–8) (Figure 13.8d) and humeral callus (anterior lateral region of the thorax) with three strong bristles and many fine hairs (>5) (Figure 13.8c).

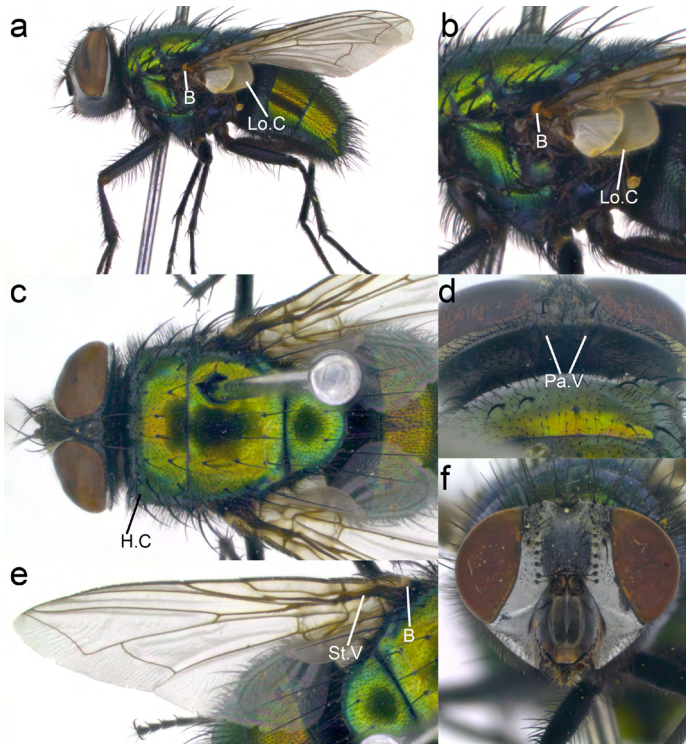

Figure 13.8 *Lucilia sericata*. B: basicosta, Lo.C: lower calypter, H.C: humeral calli, Pa.V: paravertical, St.V: stem vein. CC BY-NC.

Genus *Cochliomyia*

Genus Diagnosis. The genus *Cochliomyia* can be distinguished from *Calliphora*, *Chrysomya* and *Lucilia* by the combination of three black longitudinal stripes on the thorax (Figure 13.9c), short thread-like palpi (Figure 13.9a, f), and at least some yellow hairs on the genae (Figure 13.9a, f). It is most closely related to *Chrysomya* and both genera can be distinguished from *Calliphora* and *Lucilia* through the presence of a row of hairs on the dorsal surface of the stem vein (Figure 13.9e).

dorsal surface (Figure 13.7e, 13.8e) and lower calypter without hairs on dorsal surface (Figure 13.7b, 13.8b).

Lucilia cuprina. This fly has an abdomen and thorax ranging in colour from metallic green to copper (Figure 13.7a, b) with a white head (fronto-orbital and parafacial regions) (Figure 13.7f). Wings are clear (Figure 13.7e), basicosta yellow not fuscous or black (Figure 13.7a–c). This species closely resembles *L. sericata* but can be distinguished from this species through the combination of occipital area with one (rarely 0 or 2) paravertical hairs (Figure 13.7d) and humeral callus (anterior lateral region of the thorax) with three strong bristles and very few hairs (0–4) (Figure 13.7c).

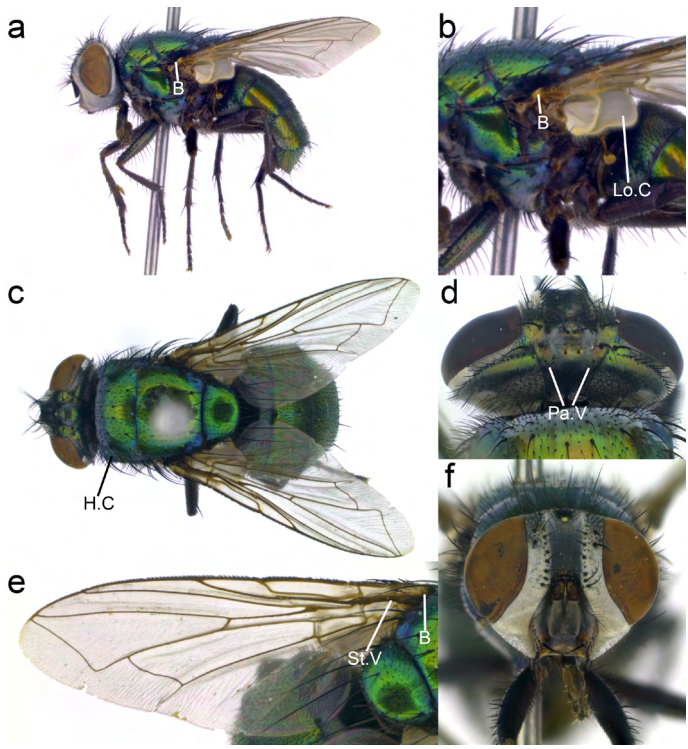

Figure 13.7 *Lucilia cuprina.* B: basicosta, Lo.C: lower calypter, H.C: humeral calli, Pa.V: paravertical, St.V: stem vein. CC BY-NC.

Lucilia sericata. This fly has an abdomen and thorax ranging from metallic green to copper in colour with a white head (fronto-orbital and parafacial regions) (Figure 13.8a, c, e). Wings are clear (Figure

Calliphora vicina. *C. vicina* can be distinguished from other carrion-breeding members of the genus *Calliphora* through the combination of following characteristics: abdomen metallic blue with a silver powdery coating that changes with viewing angle (Figure 13.6c), eyes without any hairs (Figure 13.6a, c, d), parafacials and genae orange, with black hairs on genae (Figure 13.6a, d), basicosta yellow/orange-brown (can appear darker but not black) (Figure 13.6c, e).

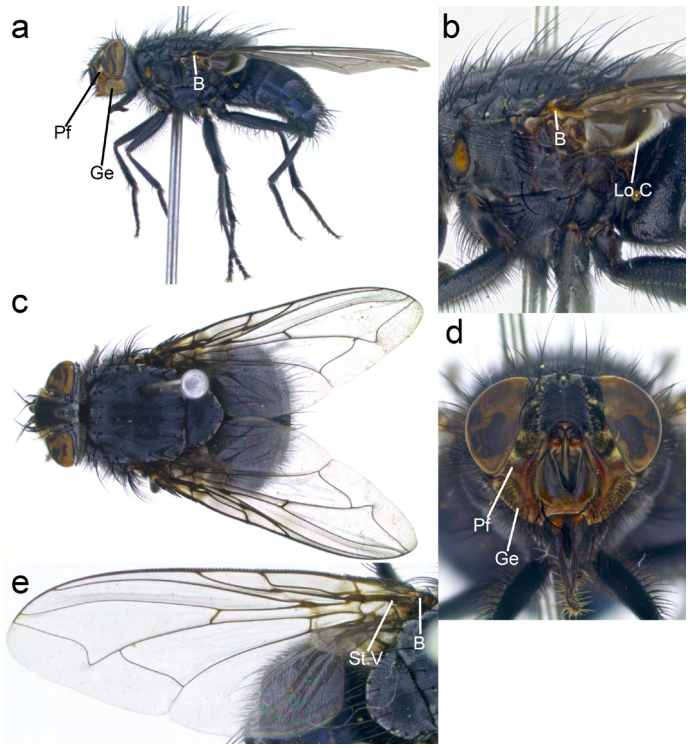

Figure 13.6 *Calliphora vicina.* Pf: parafacials, Ge: genae, B: basicosta, Lo.C: lower calypter. CC BY-NC.

Genus *Lucilia*

Genus Diagnosis. Flies of the genus *Lucilia* can be distinguished from *Calliphora* by the bright metallic green, blue or copper thorax and abdomen without any significant powdery coating (Figure 13.7c, 13.8c). In addition, this genus can be distinguished from *Chrysomya* and *Cochliomya* by the combination of wing stem vein without hairs on

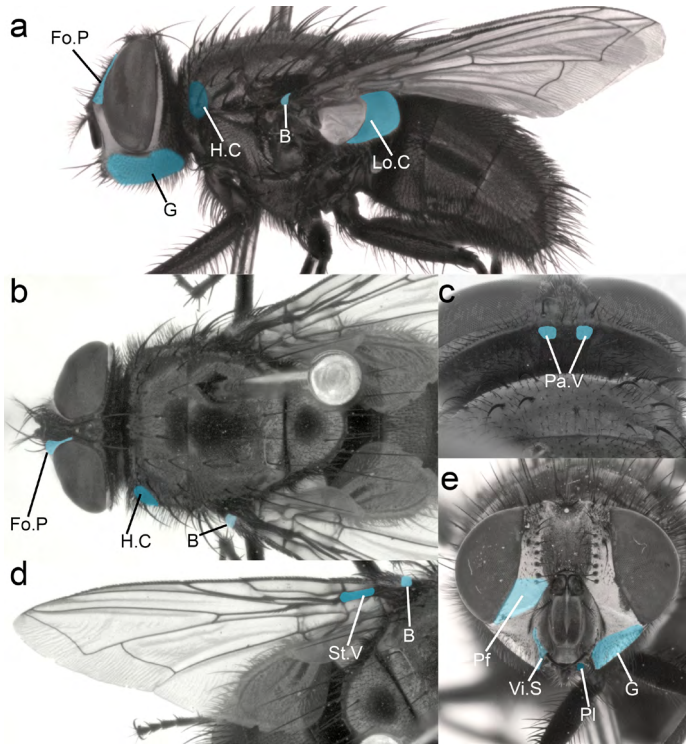

Figure 13.5 Morphological characters required for the identification of the five medicinal fly species. Head: Fo.P fronto-orbital plate, G genae, Pa.V paravertical, Pf parafacials, Vi.S vibrissal setulae, Pl palpi. Thorax: H.C. humeral calli, Lo.C lower calypter. Wing: St.V stem vein, B basicosta. CC BY-NC.

Genus *Calliphora*

Genus Diagnosis. Flies of the genus *Calliphora* (Figure 13.6) are generally black or brown in colour with a blue-metallic abdomen (rarely green), and a powdery coating on their thorax and abdomen. The genus *Calliphora* can be distinguished from *Lucilia*, *Chysomya* and *Cochliomya* by the combination of the following characters: lower parafacial area without strong bristles (Figure 13.6d), stem vein without bristles on dorsal surface (Figure 13.6e), dorsal surface of lower calypter hairy (Figure 13.6b). Note that some of these characters may be present in the other mentioned genera, but the combination of all three is unique to the genus *Calliphora*.

Zoogeographic Region	**Species**	**ID Facility**	**Key**
Neotropical	*Co. macellaria* *L. cuprina* *Ch. megacephala*	Laboratory of Integrative Entomology, Department of Animal Biology, University of Campinas, Brazil	[74, 78]

Calliphorid Fly Morphology Relevant to the Identification of Species

In order to facilitate the identification of flies using the information and figures provided in this section, it will be helpful to first locate the relevant morphological characters on a fly (Table 13.3 and Figure 13.5). The blue highlighting is omitted in subsequent species plates in order not to obstruct the features.

Table 13.3 Key to morphological characters used for species identification.

Head		
Fo.P	fronto-orbital plate	Figure 13.5a, b
G	genae	Figure 13.5a, e
Pa.V	paravertical	Figure 13.5c
Pf	parafacials	Figure 13.5e
Vi.S	vibrissal setulae	Figure 13.5e
Pl	palpi	Figure 13.5e

Thorax		
H.C.	humeral calli	Figure 13.5a, b
Lo.C	lower calypter	Figure 13.5a

Wing		
St.V	stem vein	Figure 13.5d
B	basicosta	Figure 13.5a, b

At least one of these species can be found in every major zoogeographic region (Table 13.2). Importantly, the diagnoses provided here are only intended to be used as a general guide for identifying flies that are associated with and breed in carrion. There will be uncommon and non-carrion-breeding blowfly species that cannot be discriminated by the simplified diagnoses we provide here. It is therefore crucial that specimen identity be confirmed by reference to one of the many published resources such as those cited here (Table 13.2), or by sending your specimen to an expert entomologist at your closest research institution. For a list of recommended institutions refer to Table 13.2. For later reference throughout this section, the term 'bristles' refers to larger hairs (setae) located in a sunken pit in the exoskeleton. The term 'hairs' refers to smaller hairs (setulae) not located in such a pit.

Table 13.2 The six major zoogeographic regions of the world, and which of the five fly species (*C. vicina, L. sericata, L. cuprina, Co. macellaria, Ch. megacephala*) are found there. Also provided are suggested facilities that can provide specimen identification services. The last column lists the references to the relevant taxonomic keys for species identification.

Zoogeographic Region	**Species**	**ID Facility**	**Key**
Afrotropical	*L. sericata* *L. cuprina* *Ch. megacephala*	KwaZulu-Natal Museum, South Africa	[69]
Indomalayan	*Ch. megacephala* *L. cuprina*	Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand	[70–72]
Palearctic	*C. vicina* *L. sericata*	Natural History Museum, UK	[58, 73]
Nearctic	*L. sericata* *L. cuprina* *Co. macellaria*	Smithsonian National Museum of Natural History, USA	[74, 75]
Australasian-Oceanian	*L. sericata* *L. cuprina* *Ch. megacephala* *C. vicina*	Australian National Insect Collection, Australia	[76, 77]

Taxonomic Identification and Confirmation of Species

Great care must be taken in accurate identification and selection of the desired fly species to ensure that the flies added to the existing laboratory stock are of the same species and that no harmful species is inadvertently selected. Morphological characteristics are used for the identification of adult flies [52–58], larvae [59–62], and pupae [63]. Even non-specialists can use reliable morphological character differences between *L. sericata* and *L. cuprina* [64]. In recent times a variety of molecular genetic techniques have been employed for taxonomic studies and identification of species, including *L. sericata*, and have been found to be as reliable as examination of morphological characters [55, 65–68]. Because many maggot therapy entrepreneurs will not have access to molecular laboratory facilities and may find it difficult to access the taxonomic literature, it follows an identification guide that highlights the most relevant medicinal fly species for each zoogeographic region (Figure 13.4).

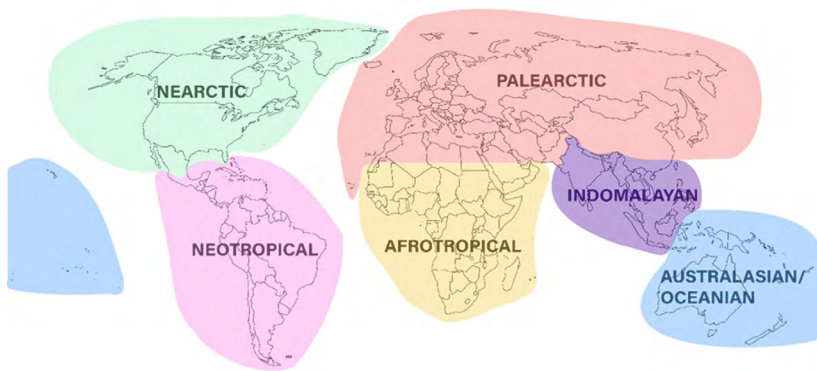

Figure 13.4 The six major zoogeographic regions of the world.

Although a range of species have been used in maggot debridement therapy (Table 13.1), we have chosen to provide identification guides to the following five species because they are well described biologically and broadly distributed throughout the world.

- *C. vicina*
- *L. cuprina*
- *Ch. megacephala*
- *L. sericata*
- *Co. macellaria*

invertebrates can be prevented from accessing the bait by placing the meat container into a larger tray/container partially filled with water. This creates a moat surrounding the meat that cannot be crossed by ants. Rain protection and a dark cover to mimic a cadaver can be achieved by placing a dark sheet of plastic material or similar cover onto the exclusion cage. The bait station may be set up in the morning and by late afternoon the eggs must be collected from the bait or else they are prone to dry out if it is hot, or hatch overnight. Unfortunately, it is not easy to discern what fly species has laid the eggs. One way to narrow down the species is by collecting egg masses from the bait used in a trap as described earlier and in Figure 13.1. It will be highly likely that the eggs will have been deposited by flies that were subsequently trapped. Irrespective of the method, it is best to place individual egg masses laid by a single female into separate rearing containers with larval diet. Once they have been raised to adult stage, they can be properly identified and placed into communal cages along with flies of the same species.

Hand Collecting of Adults, Eggs and Maggots

When hand collecting fly specimens, a bait such as an animal carcass, a quantity of putrid meat, or even faeces can be placed in a convenient spot to attract flies over a period of a few days. The collector returns regularly to the bait to collect adult flies with an entomological sweep net. The cadavers may also be examined periodically for egg masses and maggots. Various utensils such as spoons, forceps and applicator sticks may be used to pick eggs and maggots off the bait. Pathology specimen containers, various test tubes, or even empty food jars may be used to store and transport collected eggs and maggots to the insectary for identification and culture. Containers will not need to be ventilated if this transfer happens quickly. It is, however, best to use containers that are fitted with lids that permit air exchange. For this purpose, larger holes can be drilled into the lid and a sheet of fine-weave fabric can be sandwiched between lid and container-mouth prior to closing. This is especially necessary if very active, minute maggots are to be collected.

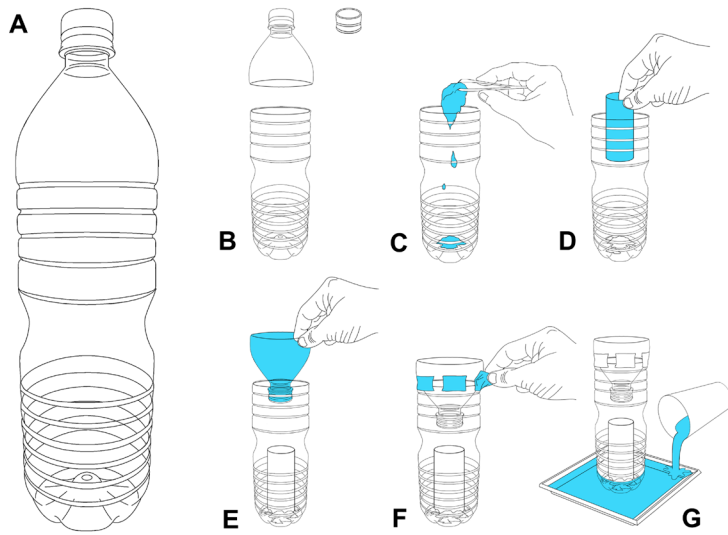

Figure 13.3 Instruction for a DIY plastic container cone trap. A standard soft drink plastic bottle (A). The tapered neck is cut off and the lid is removed (B). The bottle is baited with putrid meat to attract flies (C). A piece of cardboard such as a toilet paper roll is placed on top of the bait to provide a perch for flies as they may not be able to easily grip the plastic bottle walls after contact with the moist bait (D). The tapered neck is inverted and fitted back onto the bottle (without the lid) (E) and secured with a few strips of sticky tape (F). The trap is then placed in a dish of water to keep ants and other crawling insects out (G). The trap should be set up in a spot that is accessible to flies but protected from rain and direct sun. Illustration by P. Busana, MedMagLabs and Creating Hope in Conflict: A Humanitarian Grand Challenge, CC BY-ND.

Collecting Eggs

Female flies are attracted to cadavers to feed on protein-rich food and lay their eggs. It is therefore possible to collect eggs by placing meat bait in select locations during the day. Flies are not nocturnal and will not seek out the bait during the night. However, flies prefer to lay their eggs in dark recesses and orifices of cadavers such as the mouth or nostrils. Therefore, offering the bait in a dark container mimicking a cadaver and covering it while still allowing flies easy access via entry holes will make the bait more attractive. The bait must also be protected from larger animals and ants that will raid this free food. Birds and rats may be excluded with a wire mesh cage of sorts. Ants and other crawling

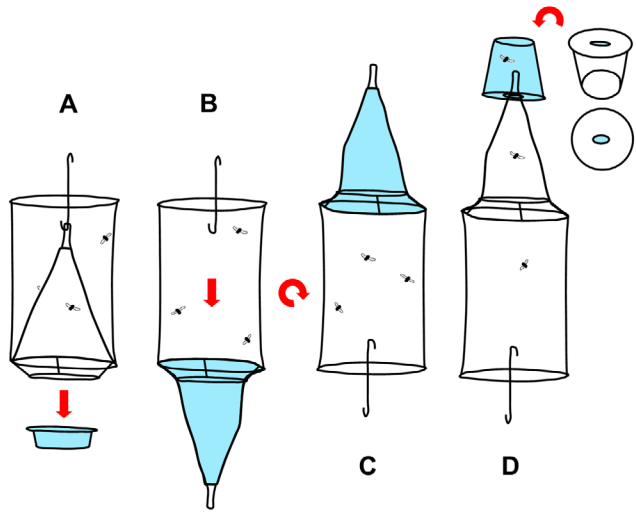

Figure 13.2 Retrieval of flies. Flies may be removed from the trap in Figure 13.1 by exploiting their natural inclination to move upward. Remove the bait tray from the trap (A). Unhook the net funnel and pull it out through the wire basket that held the bait tray (B). Turn the trap upside down (C). Use a transparent plastic container and cut an oval hole into the lid or bottom of it. It has to be wide enough to receive the narrow end of the trap's net funnel but thinner than the diameter of the funnel opening (D). When inserted, the metal-enforced funnel opening will sit on the inside edge of the hole without slipping out. Let the trap hang from the plastic container and make sure the funnel is untwisted to avoid obstruction. Flies will climb upward and through the funnel into the collection container (D). Illustration by F. Stadler, CC BY-NC.

Alibaba (e.g. Figure 13.1). Traps that have been specifically designed for entomological investigations will allow easy access and collection of trapped flies while the inexpensive traps sold for pest control may be permanently closed and make access to the fly compartment difficult. This requires modification of the fly trap prior to use, or particular retrieval strategies as described in Figure 13.2.

A variant of this trap mechanism utilises the same inability of flies to escape through an inverted, funnel-shaped opening. Inexpensive plastic storage containers may be baited with meat or other attractants and furnished with inward-pointing cones [44]. Commercial traps exploit this approach to capture sheep blowflies that can cause significant stock loss and animal suffering [51]. Figure 13.3 explains how an inexpensive trap based on the same principle can be constructed from a simple plastic bottle.

Figure 13.1 Inexpensive fly trap available from online retailers. The trap is best set up where flies naturally congregate. In an urban setting, this may be domestic or commercial garbage bins or near food businesses (A). In the rural environment, traps may be set up near livestock (B), but flies will detect attractive bait from afar and find the trap even if it is set up away from livestock or food waste. Photos by F. Stadler, CC BY-NC.

best to use meat which has not been treated with preservatives. If fresh store-bought meat is used, give the meat up to 3 days to become putrid (depending on the ambient temperature), at which stage it will begin to attract significant numbers of blowflies. If using meat other than mince, to aid the process of decomposition and the release of volatile organic compounds, it can be beneficial to cut the meat into smaller pieces. The attractiveness of the decomposing meat can be further enhanced when it is mixed with sodium sulphide (Na_2S) [46]. Furthermore, the attractive potential of meat bait is also enhanced once blowfly adults and larvae begin to arrive, as they release attractive semiochemicals when feeding [47]. Occasionally stirring the bait can further aid in the release of volatile organic compounds.

Importantly, the occurrence and distribution of necrophagous calliphorids varies greatly depending on the season and habitat [48, 49]. While some species are cold-tolerant and will remain active in winter in low abundances (e.g. *C. vicina*) [48], the majority of necrophagous calliphorids are only active in the warmer months. As such, fly collection should be undertaken in the warmer months in most geographic regions.

Trapping of Adult Flies

The characteristic behaviour of flies can be used to efficiently trap adults without killing them [50]. Flies in search of food and cadavers on which to deposit eggs have no difficulty searching out small dark holes and crevices as would be found on a cadaver. However, when taking flight, blowflies invariably head straight up and towards light. Cone or funnel traps make use of this behaviour. There are various versions of this trap type, but all work the same. A bait is provided to attract the flies. Suspended above the bait station is a cone/funnel (wide at the bottom and narrowing to a small opening at the top) which ends in a holding compartment made of fabric, a plastic bag or such containment. Flies that visit the bait station fly off vertically into the cone and wander upward through the small opening into the holding compartment. The flies have difficulty finding the small opening again and remain trapped [50]. Although cone/funnel traps are easily constructed, there are now various types of these traps available online via entomological equipment suppliers and popular retail platforms such as eBay and

maggot therapy, including: i) the secondary screwworm *Cochliomyia macellaria* which is found from southern Canada to the South American tropics [21, 22], ii) the tropical African latrine fly *Ch. putoria* that is now also found across the Americas [23], and iii) the South American *Sarconesiopsis magellanica* [24]. There are likely to be many more fly species globally that could be employed to treat wounds and all except the harshest of environments should be home to candidate fly species for maggot therapy.

Table 13.1 Species that have been used in maggot therapy to date and associated references.

Species	**Reference**
Calliphora vicina	[25]
Lucilia caesar	[26]
Lucilia illustris	[27]
L. sericata	[24, 28–30]
L. cuprina	[20, 31–33]
Phormia regina	[26, 29, 34]
Protophormia terraenovae	[35]
Chrysomya megacephala	[36]
Ch. putoria	[23]
Co. macellaria	[21, 22, 37]
S. magellanica	[24]
Wohlfahrtia nuba	[38]
Musca domestica	[39]

Collecting Wild Flies

Several options are available to the collector of live flies for the establishment of laboratory colonies. Female flies can be encouraged to lay eggs on decomposing meat or other suitable bait [40], maggots may be hand-collected from wild carcasses and cadavers [7, 41, 42], and adult flies can be trapped [6, 43–45]. Common choices of bait include minced ovine (sheep), porcine (pork), or bovine (cattle) meat, but a wide range of other mammal and bird meats will attract medicinal fly species. It is

history and morphological traits. This will enable producers to manage the adaptation of flies to the insectary environment and to improve performance through selective breeding, genetic replenishment, and potentially genetic engineering.

Of course, it is not strictly necessary for maggot therapists to use maggots that were reared in the laboratory. Under extremely austere conditions and with no access to medical aid and purpose-produced medicinal maggots, it is possible to collect eggs from the wild or permit free-living females to deposit eggs on a wound [11, 12]. Such controlled myiasis is not without its risks but anecdotal and published case histories of maggot colonisation (myiasis) of necrotic wounds from surgery, violence, neglect, or misfortune demonstrate its potential benefit [14–16].

Medicinal Fly Species

There are many fly species worldwide that could potentially be employed to treat wounds. These belong mostly to the family Calliphoridae of which over 1,500 species have been described to date [17]. There are some prerequisites for species to be suitable for maggot therapy:

- In the first instance, any species used for maggot therapy must not consume or damage healthy tissue, but only debride dead or devitalised tissue.
- Ideally, the species used should not only debride dead tissue but also control infection and stimulate wound healing.
- The species should be easy to maintain in captivity and colonies should retain their vigour over many generations.
- The species should lay eggs and not give birth to live maggots. This is necessary to efficiently harvest and disinfect eggs and rear medicinal maggots prior to treatment.

Although a good number of fly species have been used to treat wounds (Table 13.1) the fly species most widely cited and regularly used for maggot therapy are the greenbottle blowfly *L. sericata* and to a lesser extent the sheep blowfly *Lucilia cuprina* [18–20]. However, other species are also under active experimental and clinical investigation for use in

Moreover, maggot therapy treatment conditions vary between geographic areas and socio-economic conditions. For example, maggot therapy in Sub-Saharan Africa might benefit from the domestication of African fly species or strains such as *Lucilia sericata* or perhaps even the tropical African latrine fly *Chrysomya putoria*, that are already adapted to African environmental conditions. It is known that fly development is temperature-dependent and speeds up as temperature increases, up to a threshold temperature beyond which development is negatively impacted and may even lead to death [7]. While the laboratory and insectary conditions can be regulated, the wound temperature during treatment may be difficult to control. Average chronic wound temperature has been reported to be between 32 and 33ºC under mild climatic conditions [8–10]. Ambient atmospheric conditions in Sub-Saharan Africa may lead to a significant increase in the temperature medicinal maggots experience on the wound. Therefore, rather than importing flies from Europe, it will be beneficial to develop locally collected fly strains that are better adapted to higher developmental temperatures. However, further research to determine the influence of temperature on maggot therapeutic performance in tropical regions is required.

Finally, local quarantine laws and regulations may not permit the introduction of foreign insect material, which means that local flies will need to be collected. If the therapeutic efficacy of the species has not already been shown, its suitability for maggot therapy will need to be established. However, at times of great need when disaster or war is raging, or where there is no access to medical treatment, these restrictions may not apply, particularly regarding the use of local fly species [11, 12].

Chapter 11 is chiefly concerned with the bioprospecting and testing of new fly species for maggot therapy [13], while this chapter provides guidance on the collection and selection of species deemed suitable for maggot therapy. Not all of the species suggested here have been subject to robust testing as proposed in Chapter 11 but have a recorded history of successful medicinal use and at least one of the discussed species can be found in every major zoogeographic region. Because domestication is critical to the reliable supply of high-quality medicinal maggots, some attention must be given to the monitoring of fly colony life

13. Fly Colony Establishment, Quality Control and Improvement

Frank Stadler, Nikolas P. Johnston, Nathan J. Butterworth, and James F. Wallman

This chapter provides guidance on the collection and selection of species suitable for maggot therapy. All life stages are suitable for collection, except pupae that are generally hidden from view. Correct identification of the species that are collected and the correct selection of breeding stock is critical. Domestication of the newly established fly colony proceeds via adaptation to the insectary environment and the producers' operating procedures. Monitoring of fly colony life history and morphological traits enables producers to manage the adaptation of flies to the insectary environment and to improve performance through selective breeding, genetic replenishment, and genetic engineering.

Introduction

The first step in the establishment of a laboratory colony is the collection of flies and their subsequent maintenance in the laboratory or insectary [e.g. 1, 2, 3]. While in some instances blowfly strains have been reared continuously for decades [4], there is concern that relatively small population sizes, limited genetic diversity, rapid selection and adaptation to the laboratory [5] may require genetic replenishment and rejuvenation of lab colonies with newly caught flies on a regular basis to ensure productivity and health of the colonies [6].

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Packing and dispatch. The equipment required to support the dispatch of orders will vary depending on the volume of consignments processed per day and the business logistics systems employed. At the very least, the room will have a good amount of bench space to comfortably handle products and packaging, and process orders. For producers shipping their maggots further afield using couriers, equipment is needed to package and handle cool chain consignments (e.g. tape dispensers). Vials holding medicinal maggots are placed into insulated shippers along with phase-change cool elements which have been pre-cooled in a fridge.

Summary

The take-home-message from this chapter is that there is no one typical production laboratory. Production laboratory infrastructure needs will depend on pre-existing building and laboratory infrastructure, on the current research and/or production activities, and on the production objectives—whether only for research or therapy, or a combination of both. There are clearly opportunities to optimise infrastructure and equipment and thereby also work processes when setting up a lab specifically for commercial production. However, it is important to understand that reliable production of safe and high-quality medicinal maggots does not necessarily require sophisticated and expensive laboratories and equipment. Maggot therapy has been performed for millennia in tribal cultures and innovations such as mobile laboratories and community-based laboratories utilising locally-available resources have the potential to produce safe medical-grade maggots where there is little to no access to reliable wound care.

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use, which is taking place in the general lab space. The small amounts of waste water and disinfection solution are collected and disposed of afterward when the disinfection equipment and utensils are taken to the general lab space for cleaning and autoclaving.

The actual work processes of egg disinfection, quality control, and packaging of medicinal maggots generally require various reagent bottles, measuring cylinders and pipettes, various spatulas or applicators, and a vacuum filtration system (electric or handpump-driven) for the filtration of eggs and perhaps maggots. The exact equipment needs will have to be established through trial as they depend on the volume of work, the disinfection procedure chosen by the lab, and personal preference.

General lab. The general lab area is the central hub of the production laboratory. It provides the support for activities that take place in the insectary and the clean lab. Consequently, the lab should be equipped with cupboards and shelves to hold utensils and consumables and provide ample benchtop workspace for diet preparation and quality control activities.

The general lab has a dedicated wet area with tap, sink and drying racks to clean equipment and utensils. Diets for the incubation of disinfected eggs and for the rearing of fly stock are prepared in the general lab area. A fridge and a freezer should be available to store perishable, meat-based diet ingredients and pre-prepared diets. A kitchen food blender may be used for diet preparation. All sterilisation of equipment, diets and water is conducted with an autoclave, or a medical-grade steam steriliser which is a cost-effective alternative to expensive autoclaves [10].

Quality control work such as monitoring the fly colony performance requires an analytical scale with a sensitivity of at least 0.0001g [25] to be able to determine the weight of individual flies, pupae and older larvae. A stereo dissecting microscope with a magnification range of 10 to 40 times is essential for visual observation of flies and their life stages. Measurement of morphological characters such as wing features may be conducted with digital image analysis software [26] but the length of puparia or maggots can be measured relatively simply with a fine ruler (0.1mm scale) [27], or a geometrical micrometre [28].

with a cellulose sponge or rolled cellulose wicks (ideally organic and unstained to avoid poisoning of flies from residues).

Clean lab. The clean lab is where harvested and pre-cleaned fly eggs are disinfected and incubated, where hatched maggots are packaged into primary packaging containers, and where the microbial safety of medicinal maggots is examined via microbial assays. The clean lab should be as uncluttered as possible and may feature some cupboard and benchtop space as required for convenient operations. In practice, however, most activities undertaken in the clean lab take place within the laminar flow cabinet.

The production of medicinal goods such as medicinal maggots requires clean room conditions to maintain sterility during production processes, handling and packaging. There is no need to maintain the entire lab under clean room conditions. The comparatively small production volumes and the small size of the product handled in medicinal maggot production makes it feasible to use laminar flow cabinets, also known as clean benches, for the work that requires a sterile work environment. The number of laminar flow cabinets installed in a medicinal maggot clean lab depends on the volume of production and the time available to undertake required work. For most producers, one such work bench will suffice. When planning the clean lab, enough space should be allocated to be prepared for higher production volumes and to allow for additional clean benches.

There are a number of microbial testing protocols that may be employed by producers to make sure medicinal maggots are adequately disinfected [21]. These tests require a laboratory incubator that can be set and maintained at a constant temperature conducive to the growth of clinically relevant microbes (32°C to 37°C). Such an incubator is therefore an essential piece of equipment for any sophisticated production facility. It is also convenient to be able to store pre-prepared consumables such as egg incubation media and blood agar plates in a refrigerator within the clean lab to maximise efficient workflow and to maintain hygiene in the clean lab area. A sink and water supply to the clean lab is not essential because clean lab activities do not necessarily require access to running water. Eggs should be already separated and prewashed when they arrive in the clean lab and all water used during the disinfection and packaging processes must be autoclaved prior to

maggots have pupated, they need to be separated from the substrate by sieving. A variety of expensive metal sieves and inexpensive plastic colanders are readily available so long as they achieve separation of the puparia. This can be a dusty business and workers should wear dust masks or perform the sieving where particulates are quickly removed, either in the open air or under a laboratory exhaust system. Fly larvae shy away from light and therefore maggots are best reared in darkness. In addition, depending on the diet used, maggot rearing can be a smelly business. To make insectary work bearable, maggot containers can be stored in converted cupboards, fridge bodies or similar furniture that is fitted with ventilation that draws room air in and vents it to the outside. Alternatively, such units can be fitted with air filtration systems to limit odour in the room. If air is vented to the outside, it is important to ensure that the exhaust piping is screened with fine mesh to prevent wild flies and fly parasites from accessing the rearing cupboard.

Maintenance of fly colonies will require the removal of cages from the shelves and placement on a work bench. Like the shelving, the work bench or table should be sufficiently large to provide ample room for cages and other equipment. When flies have reached their useful life span and begin to die off, it is time to replace them with young flies. At this point the cage is placed in a freezer for a few hours to euthanise the flies and kill any pests such as mites. For more info on the humane treatment of flies please consult Chapter 19 [24]. The insectary should be fitted with a sink unit and drying racks to allow for easy cleaning of cages and food containers. Where feasible, shelving and other insectary furniture should be fitted with castors so it can be easily rolled about and moved for regular cleaning of the room surfaces.

Diets for maggots or bait to encourage egg laying may be prepared in the insectary or the general lab space. For most producers a household food blender will suffice to allow homogeneous mixing and maceration of various dietary ingredients for either plant- or meat-based diets. Adult flies are fed with a variety of protein-based and carbohydrate-rich food depending on the preference of the producer. These diets and egg-harvesting baits are usually offered with inexpensive and easy-to-clean or disposable plastic containers small enough to fit through the sleeve access in the fly cage. In order not to drown flies accidentally, liquid foods and water are offered with lidded plastic containers that are fitted

affords a great deal of flexibility regarding the scale of operations and the production approaches. Much can be achieved with very little by way of facilities and equipment which makes maggot therapy such an attractive proposition for the low-resource healthcare setting.

The following discussion of equipment needs will guide the prospective producer through the production areas and specific process:

1. insectary activities (e.g. adult fly colony maintenance and maggot rearing)
2. general lab activities (e.g. diet preparation and quality control)
3. clean lab activities (e.g. disinfection, medicinal maggot rearing, and packaging)
4. packaging and dispatch activities (packaging of consignments).

The general equipment and furnishing needs for staff support areas (e.g. restrooms, locker rooms, meeting rooms), reception and offices is omitted here because these are not unique to medicinal maggot production facilities. By necessity, the following discussion of equipment and material needs for production facilities foreshadows content discussed in subsequent chapters, particularly those on medicinal maggot production (Chapter 14) [21] and transport packaging (Chapter 16) [22].

Insectary. The insectary is a dedicated space for the maintenance of medicinal flies and the rearing of new fly stock. Disinfected eggs and medicinal maggots should not be incubated in this space. Adult flies can be housed in a variety of cage systems [21]. The main consideration for choosing the right cage model, home-made or commercial, is adequate ventilation, light penetration, volume, and ease of handling and cleaning. These cages are best stored on easy-to-clean shelving that is water resistant and withstands a wide range of cleaning reagents such as bleach and alcohol-based disinfectants.

The maggots are conventionally reared in an inexpensive double-container system. A smaller plastic container holding the diet and maggots is placed inside a larger container holding a pupariation substrate such as sand, sawdust or vermiculite. The larger container is securely covered with fine-mesh muslin or synthetic fabric to provide ventilation but prevent wandering maggots from escaping. Once

the facility reception and office areas. This ensures good communication with sales who will want to oversee order fulfilment. Shipping-ready consignments also need to be picked up by couriers who may access the premises via the main entrance and reception unless a dedicated service entry exists.

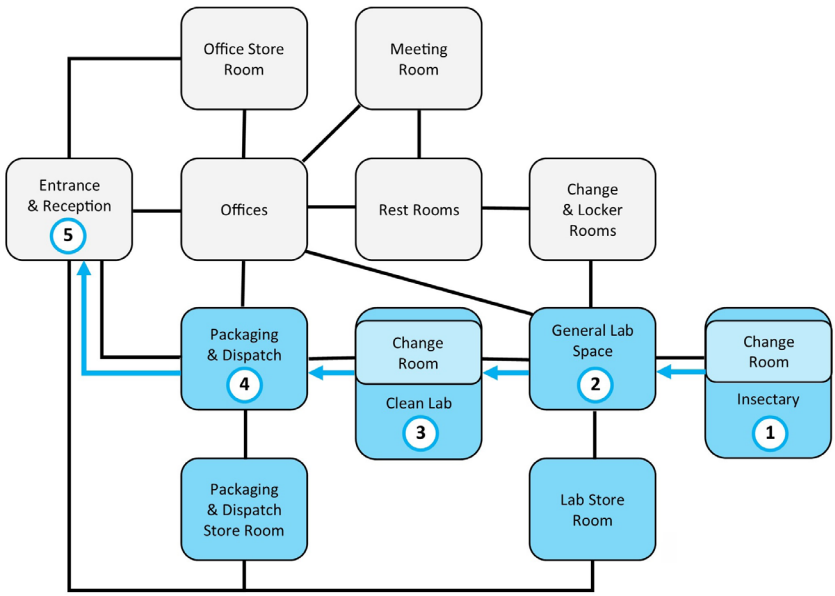

Figure 12.1 Proximity and workflow diagram for production facility. Relative position of bubbles in diagram suggests the spatial layout and proximity of workspaces in the facility. Black lines describe the movement of staff and materials around the facility according to production and administrative workflows. The blue arrows describe the unidirectional production path of medicinal maggots from (1) egg harvest in the insectary to (2) the general lab area for pre-cleaning and perhaps de-agglutination, to (3) the clean lab for disinfection, quality control, and primary packing, to (4) the packing and dispatch area, and (5) the reception for pickup by couriers.

Equipment Requirements

The equipment needs for medicinal maggot production are best discussed in relation to the specific work processes and functions they relate to. This chapter will provide an overview of the equipment needs without being prescriptive regarding specific brands, makes or suppliers. As has been explained earlier, medicinal maggot production

Offices. For management and administration of operations including marketing, sales, customer support and customer relations, finance/accounts, quality control, and regulatory compliance. An open-plan office accommodating most office staff may not be advisable due to the different functions performed by staff. For example, it is likely that the sales and marketing team members spend a lot of time on the phone which can be disruptive to colleagues sharing the same space. Therefore, it is necessary to consider ahead of facility establishment which arrangement is most productive.

Actual floor area is not being considered in this discussion because it depends, among other things, on the available overall space, the size of operation/production volume, the number of workers using each workspace at the same time, local health and safety regulations, and building codes. It is, however, a good idea to be generous with floor area allocation when planning a production facility so that future growth in staff and production volume can be accommodated without disruption to operations.

An important consideration that does demand some discussion is the relative position of each workspace in relation to the overall facility layout. The physical infrastructure must facilitate the efficient flow of workers, information, products, and materials. This also includes resources entering and leaving the organisation. Figure 12.1 illustrates this relationship for a medicinal maggot production facility and shows the major flows between the spaces. There should be a clear spatial separation between the production areas and the office and support areas. Access to the labs should be via a single point and the clean lab as well as the insectary should be accessible via the general lab area rather than directly from the office area. There are consequently two main hubs, the office area and the general lab area, for administrative and production facilities, respectively. The medicinal maggot production workflow should be one-directional: 1) eggs are passed from insectary to the general lab for separation and prewashing; 2) from there, they are passed to the clean lab where disinfection, incubation and primary packaging takes place; 3) finally, the packaged maggots are moved on to the dispatch room for order fulfilment and shipping. Other activities such as quality control processes involve workflows that are less directional. It is also desirable that the dispatch room is close to

General lab. A general lab space for food and media preparation, cleaning of laboratory utensils after use, autoclaving of equipment used for clean-bench work, and quality control work other than microbial assays of eggs and maggots. The basic space requirements are identical to those of the clean lab. Again, basic services such as air conditioning, adequate lighting, plumbing and potable water supply should be installed.

Packaging and dispatch room. Product packaging and dispatch room for order processing. Medicinal maggots that have been packed into vials (primary packaging) in various unit sizes and treatment modalities (free-range or bagged) are collated according to customer orders and packed into insulated cool-chain shippers (packaging) and processed for dispatch. Requirements for this space are not as stringent as for the lab spaces but worker comfort should be considered regarding lighting levels, ventilation and air conditioning.

Store room. To hold supplies until they are required. It is best to have separate store rooms for laboratory supplies and office/general operations supplies. This is mainly to locate them in close proximity to the respective work area and facilitate easy access by the main user groups. It may also be necessary for facilities with high production volumes and many courier shipments to store a significant volume of insulated shippers and cool elements. If the packaging and dispatch room is not large enough then an additional store for this inventory may be required. Although hazardous chemical use is minimal in medicinal maggot production laboratories, larger volumes of corrosive or flammable chemicals need to be stored in dedicated safety cabinets according to local regulations.

Restrooms. These may be unisex or separate male/female amenities.

Change and locker rooms. These may also be provided for staff to change into lab uniforms and protective clothing and to store valuables that staff cannot or will not want to take into the labs.

Meeting rooms. Meeting rooms provide staff the opportunity to gather and socialise during breaks and prepare and consume food. A separate board or business meeting room should also be considered. If space is at a premium, the staff room can also serve as a formal meeting room.

need to be installed. If possible, circulated air should be filtered to remove fly-generated dust from the insectary atmosphere. If this is not possible, a stand-alone air purifier can be installed, or lack of air filtration can be compensated for with more frequent cleaning protocols and/or increased personal protection with gloves, dust masks, hair nets, and lab gowns for prolonged insectary activities. Ventilation ports for fresh air intake and air exhaust should be screened with fine insect screens and doors should be fitted with door sweeps to prevent vermin entering the room. All surfaces should be washable and all cracks, gaps, joints, etc. sealed to avoid refugia for vermin and build-up of dirt. The insectary should also have potable water taps and greywater plumbing for a lab sink to be fitted. The insectary is entered via a small room that serves as a change room for protective clothing. The same protective clothing must not be worn in the general and clean lab to maintain hygiene and avoid contamination of disinfected medicinal maggots.

Clean lab. Here, a clean lab is defined as the part of the production laboratory that is dedicated to disinfection of eggs, quality control, and packaging of medicinal maggots into primary packaging. It is 'clean' in the sense that no activities such as washing up of dirty equipment, fly diet preparation and regular lab maintenance tasks are performed in this space. The clean lab should not be confused with a clean room, which in medical and pharmaceutical production laboratories provides a sterile work environment. Although possible, clean rooms are not necessary in medicinal maggot production. The sterile environment for work activities is usually provided by laminar flow cabinets (clean benches) that fulfil the same role but cost only a fraction to establish and operate (see equipment requirements).

Overall, the specifications for the clean lab are similar to those for the insectary but lighting is not automatically controlled and there is less stringent requirement to keep temperature and humidity in the optimal range. However, if disinfected eggs and medicinal maggots are to be incubated in the clean lab without an incubator, the temperature must also be kept at around 25°C, unless accelerated growth and development of medicinal maggots at higher temperatures is required. Like the insectary, the clean lab is also entered via a small room used for workers to change into protective clothing.

Building and Space Requirements

Key activities conducted in the operation of a medicinal maggot production laboratory include the rearing and maintenance of fly colonies, the disinfection of fly eggs and rearing of larvae, the packaging of maggots into primary packaging containers, packaging of consignments for customers, quality control activities, storage of inventory, as well as office-based activities. Each of these has its own space, equipment and consumables requirements (Table 12.2 available at https://hdl.handle.net/20.500.12434/2f7d12xl). This chapter pre-empts by necessity some of the discussion that follows in subsequent chapters explaining fly colony establishment (Chapter 13) [20], medicinal maggot production (Chapter 14) [21], and packaging technology (Chapter 16) [22]. However, the focus is on space and equipment requirements for the establishment and operation of a medicinal maggot production laboratory.

Production spaces are less defined in shared research laboratories that are not exclusively set up for the purpose of medicinal maggot production. Research laboratories must often meet the needs of many users, and maggot therapy-related research and production activities sharing this space need to adapt accordingly. However, when dedicated production facilities are to be established, there is the opportunity to account and plan for the distinct work area requirements, and the workflows between them. It follows that a production facility should have:

Table 12.2 Production facility resources (e.g. equipment, space requirements and consumables) required to undertake production processes and sub-processes. https://hdl.handle.net/20.500.12434/2f7d12xl.

Insectary. An insectary for the rearing and maintenance of medicinal fly colonies. Its specifications arise from the environmental preferences of calliphorid flies, and the need to keep the space clean and pest-free. A constant temperature of 25°C and 12 hours of light per day will ensure that the flies continue to produce eggs over their useful life span and that their offspring do not enter a diapause or resting phase during pupariation [23]. Humidity should range between approximately 40 and 60% RH. If the air conditioning system is not able to maintain this range, additional humidification or de-humidification systems may

today when wounds get accidentally colonised by fly maggots. Many cases of such fly colonisation (myiasis) are benign and even beneficial [12, 13], although maggot colonisation can be distressing for the patient, family, and carers because flies and maggots are usually associated with death, decay and filth. Controlled therapy rather than wild infestation is also desirable because of the potential risk of harmful microbes carried in by wild flies above and beyond the microbial burden that is already in the wound [14]. Moreover, a few species of fly such as screw worms can colonise wounds and cause damage to live tissue [15]. Chapter 7 [16] explains in detail the natural history of medicinal flies and related species and Chapters 4 to 6 [17–19] are concerned with the treatment of wounds with maggot therapy.

It follows that if isolated communities are to be encouraged and enabled to produce their own maggots and practice maggot therapy, then any instructions and guidelines need to be based on best practice. Community-based producers will need to be trained in the construction of basic production facilities and equipment, in the identification and culture of safe fly species, in the disinfection of eggs, and the safe treatment of wounds. Such training needs to overcome the material and social constraints of the low-resource setting. Instructions need to be provided in highly visual format to overcome language barriers. Any written material should ideally be provided in multi-lingual format. Minimum requirement would be instructional material in the official national language of the country and easy-to-understand English. The suggested solutions need to be supported by the resources that are locally available, which may vary from place to place. This means the instructions will need to be sufficiently flexible. This will give the end user the freedom to adopt and adapt local resources to achieve production and medicinal maggot quality objectives. The ingenuity and resourcefulness of communities in compromised healthcare settings must not be underestimated. For further information on our own efforts to give isolated communities the wherewithal to treat chronic wounds with safe maggot therapy, please go to www.medmaglabs.com.

The provision of medical services in the field close to the point of care is not a new idea. Portable healthcare facilities provide life-saving emergency care during war and disasters such as earthquakes, tsunamis, infectious disease outbreaks. They may include independently functioning medical units like airborne, floating, or terrestrial truck-mounted infrastructure. Temporary medical infrastructure is transported in parts and assembled and disassembled as required [6]. Tents have been traditionally used for temporary field hospital shelters to house emergency and surgical care, hospital wards, and support services, but their ease of deployment comes with significant disadvantages. For example, they have no rigid flooring and require level ground, they are prone to the elements because they are not insulated, they may leak during rain. In addition, they are difficult to keep clean [7].

In recent times, military field hospitals have been developed and deployed that are composed of a combination of soft and hard portable infrastructure. The new British Army front-line field hospitals use inflatable dome tents in conjunction with pre-configured, fully equipped, containerised units for services such as sterilisation departments [8]. Converted shipping containers have also proven cost-effective and practical solutions for the provision of medical and scientific laboratory services in low-income country healthcare settings [9, 10]. For example, 40-foot modified shipping containers are used by the President's Malaria Initiative to provide low-cost laboratory infrastructure for mosquito control programmes in Mozambique, Mali, Angola and Liberia [9]. These innovations in mobile healthcare and laboratory services provision suggest strongly that there is a place for mobile medicinal maggot production close to the point of care.

For extremely isolated communities that are cut off from clinical supplies and advanced clinical care by armed conflict, disaster, or simply remoteness, the only way to provide effective limb- and life-saving wound care may be via do-it-yourself medicinal maggot production and maggot therapy—similar to the way maggot therapy had been practiced for millennia in ancient and tribal cultures [11]. The overriding concern, of course, must be patient safety. In today's age, it would not be acceptable to let wild flies lay eggs on wounds for the maggots to treat the wound of their own accord without quality control—although, this is essentially what had been done in the past and what still happens

Table 12.1 Typology of organisations that produce medicinal maggots.

Factor	**Scale**			
	Small-scale mostly not-for-profit			**Large-scale or diversified and for-profit**
	Research laboratory	Mobile laboratories	DIY laboratories	Commercial laboratory
Financial resources	Limited, not core business	Supported by aid agencies or government	Very limited	Investment capital and industry development grants
Pre-existing infrastructure	Biological, forensic, or biomedical laboratory	No building infrastructure	No building infrastructure	Laboratories, convertible buildings, new buildings
Proximity to point of care	Close, in-house or local hospitals	Close, but far from major population centres	Close, but far from major population centres	Irrespective of proximity
Distribution logistics	No need for sophisticated logistics	Container deployment logistics	No distribution logistics	Sophisticated distribution logistics
Wound burden	Low	Up to approx. 250 wounds per day	Low but flexible	High wound burden/ market size
Trained workforce	Present	Present	Untrained laypersons	Present
Institutional support	Within the scope of research	Strong institutional support	Grass-roots community support	Independent
Healthcare-worker endorsement	Limited to collaborating hospitals	Endorsement restricted to agency and care setting	Endorsed by local lay- and trained healthcare workers	Widely endorsed
Patient awareness and acceptance	Low in greater community but high in treating hospitals	High	High	Generally high
Regulatory approval	Not approved	Not necessarily approved	Not necessarily approved	Approved

clinical trial support and infrastructure. However, once the regulatory agencies have approved maggot therapy, it is unlikely that the host research organisations have the agility, entrepreneurial mindset, and expertise required for start-up and commercialisation. At this point, the producer ought to create a largely independent spin-off company that still maintains strong research ties with the parent organisation.

Up to recently, there has been little effort to provide high-quality maggot therapy to hard-to-reach patient populations such as in remote locations, at times of disaster or in armed conflict. However, the author's group has developed a mobile shipping container that is fitted with an insectary and laboratory capable of producing medicinal maggots in remote locations and where logistics infrastructure is failing to guarantee 24- to 48-hour delivery of medicinal maggots. We also developed and tested production and treatment manuals that allow isolated communities to establish and run do-it-yourself (DIY) laboratories with limited resources so they can produce safe medicinal maggots for efficacious maggot therapy (Stadler et al. QVSA). All project material including container lab construction plans, DIY lab instructions, and the treatment manual are available online (www.medmaglabs.com). These resources provide all necessary information for isolated communities to produce safe medicinal maggots.

Mobile and Do-it-yourself Medicinal Maggot Laboratories for Compromised Healthcare Settings

The discussion of production facility establishment has largely focussed on fixed infrastructure such as existing labs, purpose built, or building infrastructure that can be converted into medicinal maggot production facilities. When production is tied to a specific place then this means that the availability of maggot therapy to patients will be dependent on their proximity to the production facility. In advanced economies with highly effective and reliable logistics networks, rapid 24-hour distribution of medicinal maggots over long distances and under cool chain conditions is feasible and standard practice [5]. However, this is not the case where economic disadvantage, poverty, war and conflict, or disasters disrupt logistics infrastructure and supply chains. The answer to this problem may well be mobile and low-resource do-it-yourself medicinal maggot laboratories as mentioned earlier.

Production Laboratory Typology

Thanks to the robust and adaptable nature of calliphorid fly species suitable for maggot therapy, laboratory and equipment infrastructure can be tailored to suit conditions, needs and resources. What a production facility will look like depends on several factors including a) financial resources, b) pre-existing building infrastructure, c) proximity to the point of care, d) transport infrastructure and distribution logistics, e) the wound burden amenable to maggot therapy, f) access to trained medical or scientific workforce, and more social factors such as g) institutional support for maggot therapy, h) healthcare-worker endorsement of maggot therapy, i) patient acceptance, j) regulatory approval of maggot therapy, and k) insurance cover for maggot therapy either via national health insurance or private health cover.

In general, laboratories that produce medicinal maggots either conduct maggot therapy-related research, forensic research, or they are dedicated medicinal maggot production laboratories. Research laboratories may produce medicinal maggots on a small scale to supply lab experiments or small clinical trials. The market size for medicinal maggots is largely determined by the wound burden, the general acceptance of maggot therapy, regulatory approval, and reimbursement of treatment costs via health insurance schemes. It follows that the scale of operations depends on the actual and/or potential demand for maggot therapy, i.e. from small-scale, not-for-profit production by research laboratories to fully independent and for-profit enterprises. The latter may specialise exclusively in the production of medicinal maggots or have a diversified product range that includes medicinal maggots. Table 12.1 presents a typology of medicinal maggot production organisations and their characteristics.

It appears that commercial producers emerge more often than not from university medical research laboratories that investigate clinical and biochemical aspects of maggot therapy. Large institutions such as universities afford a safety net allowing budding producers to slowly test and build the market through awareness-raising and educational activities, and to initiate the regulatory approval process. Since the regulators require clinical evidence of safety and efficacy, such trials are also best conducted from within a biomedical research organisation with

laboratory planning, construction, and management—particularly the following books:

- **Laboratory Design, Construction, and Renovation: Participants, Process, and Production.** Committee on Design, Construction, and Renovation of Laboratory Facilities; Board on Chemical Sciences and Technology; Commission on Physical Sciences, Mathematics, and Applications; National Research Council. National Academy Press, Washington D.C. (2000). [1]
- **The Sustainable Laboratory Handbook: Design, Equipment, Operation.** Dittrich, E. & ProQuest, Ebooks. Wiley-VCH, Weinheim, Germany (2015). [2]
- **Guidelines for Laboratory Design: Health, Safety, and Environmental Considerations.** DiBerardinis, L. J. & ProQuest, Ebooks. Wiley, Hoboken, New Jersey (2013). [3]

The first is an excellent guide on the planning of laboratories from predesign to postconstruction, and the technical aspects that need to be considered. As a free online resource, it should be essential reading for any prospective producer needing to build or refurbish laboratory facilities—ideally, before planning has commenced! The other two books are much more technical and detailed in nature and are intended to provide professionals involved in the planning, design, construction, and operation of laboratories with essential information to facilitate collaboration.

A detailed discussion of all aspects of laboratory planning, construction and operation are beyond the scope of this book. Instead, this chapter will focus on key considerations as they apply specifically to medicinal maggot production laboratories. First it is important to understand the different types of laboratories that are typically concerned with the production of medicinal maggots and related research, and how these differ depending on their primary objectives and history. This discussion also includes an introduction to the production of medicinal maggots in compromised healthcare settings with mobile and community-based do-it-yourself medicinal maggot laboratories [4]. The remainder of the chapter explains the building and space requirements for research- and commercial-production laboratories, and the equipment needed.

12. Laboratory and Insectary Infrastructure and Equipment

Frank Stadler

Medicinal maggot production laboratory infrastructure requirements depend on pre-existing infrastructure, the current research and/or production activities, and on the production objectives—whether medicinal maggots are to be produced for research, therapy, or a combination of both. This chapter provides a typology of production facilities and describes the physical insectary and laboratory infrastructure and equipment necessary to maintain medicinal fly colonies and prepare medicinal maggots for use in human and veterinary medicine. Importantly, reliable production of safe and high-quality medicinal maggots does not necessarily require sophisticated and expensive laboratories and equipment.

Introduction

This chapter is concerned with the physical insectary and laboratory infrastructure and equipment necessary to maintain medicinal fly colonies and prepare medicinal maggots for use in human and veterinary medicine. It focusses specifically on the building, infrastructure, and equipment requirements for medicinal maggot insectaries and laboratories (from now referred to as production laboratories). The establishment of any laboratory can be described as a process that begins with the decision to establish a laboratory, followed by preplanning, planning, design, construction, and post-construction activities. Prospective producers are advised to consult the literature on

 https://doi.org/10.11647/OBP.0300.12

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