DNA AVAILABILITY FROM FLY MAGGOTS INFESTING ON VARIED BEEF MEAT CONDITIONS

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UNIVERSITI SAINS MALAYSIA

2022

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by

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Thesis submitted in partial fulfilment of the requirements
for the degree of
Master of Science (Forensic Science)

September 2022

ACKNOWLEDGEMENT

First and foremost, I am thankful to God for his blessings and grace that gave the strength to complete my research project throughout every hurdle and struggles. Secondly, I would like to express my greatest gratitude to both my parents, and my sister for being the best cheerleaders I could have ever asked for throughout this whole journey. They have encouraged me and pushed me to not give up completing this task despite the stressful moments with the problems that I faced during the research project. I would also like to express my appreciation to my module coordinator; Dr Nurasmat, my supervisor; Dr Helmi Bin Mohd Hadi Pritam and my co-supervisor Dr. Nurhaslindawaty for their guidance and advice during this research project. Without them, I would not have been able to complete this research. I would also like to thank all my course mates for the support throughout the project. They have been with me through all the ups and downs during this research project. Special mention to those who were with me in the laboratory almost all the time; Dahir Ali Hersi, Ng Yu-Chen, Durratun Nasuha, Nur Sabrina Shamsha, Sabrina George and Izzah Syahira. A special thanks to my senior Saiful Ammar and my roommate Kasturi Selvam for the guidance and advice given to me throughout this research. Lastly, I would like to give my appreciation towards all the staff in the Forensic Science Laboratory for all the help given.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

%	Percentage
°C	Degree Celsius
∞	Infinity

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ATP	Adenosine triphosphate
bp	Base pairs
Cyt B	Cytochrome B
ddH_2O	Double deionised water
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
EDTA	Ethylene diamine tetraacetic acid
FNAC	Fine needle aspiration cytology
g	Grams
HCl	Hydrochloric acid
Kbp	Kilo base pairs
KCl	Potassium chloride
Kg	kilogram
L	Litres
LAMP	Loop-mediated isothermal amplification
$MgCl_2$	Magnesium chloride
mtDNA	Mitochondrial DNA
mM	Micro molar
mm	millimeter
mg	milligram

mL	milliliters
nm	nanometer
NaCl	Sodium Chloride
NDF	Neutral detergent fibre
PCR	Polymerase Chain Reaction
PMI	Post-Mortem Interval
qRT-PCR	Quantitative real time PCR
rpm	Rotation per minute
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase PCR
SDS	Sodium dodecyl sulphate
STR	Short tandem repeat
Spp	Species
TE	Tris-EDTA
Tm	Melting temperature
UPMS	Science Laboratory Management Unit
UV-Vis	Ultraviolet Visible
V	volts
uL	microliters

KETERSEDIAAN DNA DARIPADA ULAT LALAT YANG MEMBIAK DI ATAS PELBAGAI KEADAAN DAGING LEMBU

ABSTRAK

Kajian ini bertujuan untuk mengenal pasti ketersediaan DNA daging yang boleh diperolehi daripada ulat lalat yang telah makan daging lembu. Penyelidikan ini dijalankan untuk menentukan peringkat belatung lalat yang mana merupakan pilihan yang sesuai untuk pengekstrakan DNA dan analisis DNA daging. Dengan mengekstrak DNA daripada ulat lalat, bidang entomologi forensik dan biologi forensik boleh digunakan bersama untuk mengenal pasti mayat mangsa apabila ulat tidak digunakan untuk anggaran PMI atau apabila mayat mangsa tidak sesuai untuk pengecaman. Dalam kajian ini, tiga keadaan daging lembu diletakkan di tiga lokasi yang berbeza secara serentak dan ulat instar pertama, instar kedua dan instar ketiga telah dikumpulkan. Pada masa yang sama, sampel daging lembu turut dikumpul. Malangnya, sampel daging lembu kering terpaksa dikecualikan kerana ketiadaan lalat dan ulat pada daging sehingga dua minggu dari persediaan awal. Keputusan menunjukkan bahawa kepekatan asid nukleik secara amnya lebih tinggi dalam sampel daging berbanding ulat kecuali ulat instar pertama. Kepekatan asid nukleik yang tinggi dalam ulat instar pertama kemungkinan besar disebabkan oleh kehadiran DNA ulat kerana lebih banyak ulat digunakan untuk pengekstrakan DNA ulat instar pertama berbanding dengan ulat instar kedua dan ketiga. Selepas amplifikasi PCR menggunakan primer bovine cytochrome B, gen sasaran berjaya dikuatkan untuk ulat instar kedua dan ketiga daripada sampel daging lembu segar. Sebaliknya, gen sasaran tidak berjaya diperkuatkan untuk ulat daripada keadaan daging lembu beku. Kesimpulannya, ulat instar

kedua dan ketiga boleh digunakan untuk pengekstrakan DNA apabila daging segar, dan lebih banyak kajian mesti dilakukan untuk daging beku dan daging kering sebelum membuat kesimpulan tentang ketersediaan DNA dengan membuat beberapa pengubahsuaian kepada kajian.

DNA AVAILABILITY FROM FLY MAGGOTS INFESTING ON VARIED BEEF MEAT CONDITIONS

ABSTRACT

The aim of this research was to identify the availability of meat DNA that is available from fly maggots that has been feeding on beef meat. This research was carried out to determine which stage of the fly maggots are a suitable option for the DNA extraction and analysis of meat DNA. By extracting DNA from the fly maggots, the field of forensic entomology and forensic biology can be used together to identify victim cadavers when the maggots are not being used for PMI estimation or when the victims' body is not suitable for identification. In this study, three conditions of beef meat were placed at three different field location concurrently and the first instar, second instar and third instar maggots were collected. At the same time, the beef meat samples were also collected. Unfortunately, the dried beef meat sample had to be excluded due to the absence of flies and maggots on the meat up to two weeks from the initial set-up. The results showed that the concentration of nucleic acid is generally higher in the meat samples compared to the maggots except for the first instar maggots. The high nucleic acid concentration in the first instar maggot is most probably due to the presence of maggot DNA as more maggots were used for the first instar maggot DNA extraction compared to the second and third instar maggots. After PCR amplification using the bovine cytochrome B primer, the target gene was successfully amplified for the second and third instar maggots from the fresh beef meat sample. In contrast, the target gene was not successfully amplified for the maggots from the frozen beef meat condition. In conclusion, the second and third instar maggots can be used for the DNA extraction

when the meat is fresh, and more research must be done for the frozen meat and dried meat on the DNA availability.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Humans and nearly all the other species in the world carry their genetic information in their deoxyribonucleic acid, which is most commonly known as DNA. DNA is a double helix structure that is made up of four chemical base pairs that are attached to a sugar-phosphate backbone. The DNA of an individual can be found in almost all the cells of the individual. The majority of the DNA can be found in the cell nucleus but there is also a small percentage of DNA that can be found in the mitochondria. These types of DNA are known as nuclear DNA and mitochondrial DNA (mtDNA) respectively. A crucial characteristic of the DNA is the ability of the DNA to replicate or generate duplicates of itself. The blueprint for replicating the base sequence can be found in each of the double helix's DNA strands. When cells divide, this characteristic is crucial since each new cell is required to be an exact replica of the DNA found in the old cell. (Medline Plus, 2021). Since the DNA is always replicated and never changing in an individual, this can be considered solid evidence when trying to identify an individual.

DNA evidence is important at a crime scene. It has revolutionized the field of forensic science since it has been made available to aid the justice system in bringing justice to victims and perpetrators. (Foreman et al., 2003). DNA evidence has been playing a vital role in the forensic justice system, especially in homicide cases. (Briody, 2004). With the

exception of identical twins, no two people have the same DNA, making DNA a highly effective investigative tool. As a result, DNA evidence gathered from a crime scene can be used to identify a victim, or a suspect or exclude suspects from further investigation (National Institute of Justice, 2001). Unfortunately, DNA evidence needs to be handled in a proper manner for its validity. Improper collection and storage may cause the evidence to be degraded or unfit as evidence in court. On the other hand, it may be hard to identify DNA evidence at an outdoor crime scene or when the criminal has done through clean-up of a crime scene.

In criminal cases where a victim's body is involved, entomological specimens are often used. This field of forensics is known as forensic entomology where the science of insects and arthropods are used to aid legal investigations. Insect evidence from a crime scene is often used for the identification of the post-mortem interval (PMI) using the age of the maggots and stages of the insect. These larvae and insects can often be found around the area where a body is located. The forensic entomologists often use the oldest aged maggots that are found on a body to give the most accurate estimation of the post-mortem interval. When forensic entomology is used to estimate the post-mortem interval, experts are needed to analyze and interpret the findings in light of the environmental factors that may affect the development of these entomological specimens (Sharma et al., 2015).

For the use of forensic entomological specimens, the collection and preservation of the samples must be done correctly. In many situations, police officers or law enforcement officers are the ones to collect the samples. If the collection of samples is not diverse and not part of the cadaver's entomofauna, it would be inaccurate to estimate the PMI of the victim (Matuszewski, 2021). In cases where the entomological specimens cannot be used

to estimate the postmortem interval, the specimen may be used for DNA analysis to identify the victim.

DNA has been extracted from third instar maggots to help with mtDNA identification of victims in the near past (Chamoun et al., 2020; Chavez-Briones et al., 2013). This field of study is yet to be researched further as the available research only focuses on the third instar maggots of flies that were collected from fresh meat. This paper aims to look into the first instar maggots, second instar maggots and third instar maggots that are collected from three conditions of beef meat: fresh, frozen and dried.

1.2 Problem Statement

Since the advancement of DNA analysis, DNA has been considered very important evidence that can be found at the crime scene. Unfortunately, DNA from evidence such as blood and hair are not often found as criminals are knowledgeable and often clean a crime scene as much as possible.

There are incidences where the body of a victim is moved after several days either from a primary crime scene or a secondary crime scene to remove the evidence before investigation. In cases like this, forensic examiners may find maggots, insects or beetles surrounding an area. Some of these may have been infesting the victim's body before it was moved. Entomological specimens that are not found on the victim's body are not relevant for the PMI estimation. Previous studies have shown that STR analysis has been successful when using maggots for DNA analysis although the STR profiles obtained

were incomplete. According to Chavez-Briones et al. (2013), if it is not possible to retrieve DNA from other sources, investigators may use maggots if they are found nearer to the human remains (Chavez-Briones et al., 2013). From the research by Luise et al. (2008), the autosomal and Y-STR profiles were able to be retrieved using the gut of third instar fly larvae.

The availability of the DNA when the cadaver has been subjected to cold temperatures to a freezing point and hot temperatures that dry up the cadaver has not been looked into therefore it is a gap in the field of research relating to DNA that can be obtained from maggots. Therefore, this research aims to address the research gap.

1.3 Objective of Study

1.3.1 General Objective

To investigate the DNA availability of fly maggots infested with beef meat on varying study conditions.

1.3.2 Specific Objectives

1. To collect the samples of 1st instar, 2nd instar and 3rd instar fly maggots infesting the beef meat from the varied beef conditions.
2. To extract DNA of the beef meat from the 1st instar, 2nd instar and 3rd instar fly maggots infesting the meat and the fresh, frozen and dry beef meat.
3. To verify the quality and quantity of extracted DNA using spectrophotometer and agarose gel electrophoresis.

4. To assess the DNA availability through PCR amplification by using cytochrome B gene.

1.4 Research Questions

1) Does the DNA concentration vary based on the condition of the decomposing beef meat?
2) Does the DNA integrity of the extracted DNA from all condition satisfactory for the next application?
3) How can the DNA availability from the 3 beef conditions be assessed?

1.5 Hypothesis

It is hypothesised that the DNA concentration obtained from the decomposing meat would be reduced through decomposition with the highest concentration from fresh meat, frozen meat and dried meat. It is assumed that the concentration of DNA is in decreasing trends.

1.6 Significance of Study

The availability of DNA in terms of the DNA concentration that can be extracted from the maggots that are infesting the meat when the meats are in different conditions namely, fresh beef meat, frozen beef meat and dried beef meat is the main aim of this study. The findings of this research would determine whether DNA extracted from entomological specimens can be used for the identification of a victim when the cadaver is found in

different conditions. The findings from this study can be used to resolve the question of whether the condition of the cadaver and the different stages of a fly's life cycle would affect DNA availability. In addition, the usefulness of the different instar stages of the fly maggots can be determined so that the collection of maggot samples from a crime scene would more meaningful when all the stages are collected for DNA analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Current roles of insects in the forensic field

The current field of forensic science that includes the presence of insects is forensic entomology. The study of insects and other arthropods for forensic and criminal investigation purposes is known as forensic entomology. Insects are commonly found in and around a body that has already started the decomposing process. Forensic scientists often use these insects to estimate the PMI of corpses that are found. Forensic scientists can investigate the cause of death, the postmortem index, and any changes in the position of the body by observing the insect population and its growing larval stages. The presence of insect larvae can help in the estimation of the postmortem interval up to one month from the death of an individual if the species and the developmental stage of the insects surrounding the body can be identified (Joseph et al., 2011).

2.2 Flies

Diptera, or true flies, are the insects that are most frequently used in forensic investigations (Syamsa et al., 2017). Within minutes of death, *Calliphoridae* (blow flies) and *Sacrophagidae* (flesh flies) may come near the corpse. The *Muscidae* (house flies) usually postpone colonization until the corpse has decomposed to the point of bloating. The

colonization of the flies is dependent on the surrounding temperature and factors such as the environment surrounding the corpse.

In Malaysia, the common types of flies that are found are the house flies (*Musca domestica*), Bluebottle fly (*Calliphora vomitoria*), Sand fly (*Spiriverpa Lunulata*), Fruit fly (*Drosophila species*), drain fly (*Psychodidae*), flesh fly (*Sarcophagidae* family) and horse fly (*tabanidae* family) (Rentokil, 2022). Based on Syamsa et al. (2017), the common fly species that are found and utilized in outdoor and indoor forensic cases in Malaysia are *Chrysomya megacephala (Calliphoridae), Ch. rufifacies (Calliphoridae), sarcophagid fly (Sarcophagidae), Synthesiomya nudiseta (Muscidae), Megaselia scalaris (Phoridae), Lucilia cuprina (Calliphoridae), Ch. nigripes (Calliphoridae), Eristalis spp. (Syrphidae*) and *Hydrotaea spinigera (Muscidae)* with the highest to lowest frequency. The research was studying the occurrence of the maggot species on corpses. In comparison to outdoor remains, the fly variety was highest indoors with eight species while outdoors had three species. The species that were found indoors were *Ch. Megacephala, Ch. Rufifacies, Sarchophagid, S. nudiseta, M. scalaris, L. cuprina, Ch. Nigripes* and *Hy. Spinigera*. As for the outdoors, the species that were found were *Ch. Megacephala*, *Ch. Rufifacies* and *Eristalis spp*. While single as well as double infestation were frequent in both indoors and outdoors cases, one of the indoor cases also showed multiple infestation of up to six species. Even though many other fly species were discovered on human remains, *Chrysomya* flies continued to predominate, and *S. nudiseta* was only discovered on human remains that had been recovered from indoor locations (Sanchez-Arroyo & Capinera, 2020).

2.2.1 Fly life cycle

The four stages of a fly's life cycle are the eggs, larva, pupa, and adult stages. As shown in Figure 2.1, the larva stage is further separated into the first instar, second instar and third instar. Fly larvae effectively have two separate lives since they occupy various habitats and are always morphologically distinct from adult forms, which allows them to successfully adjust to environmental changes (Oldroyd, 2018).

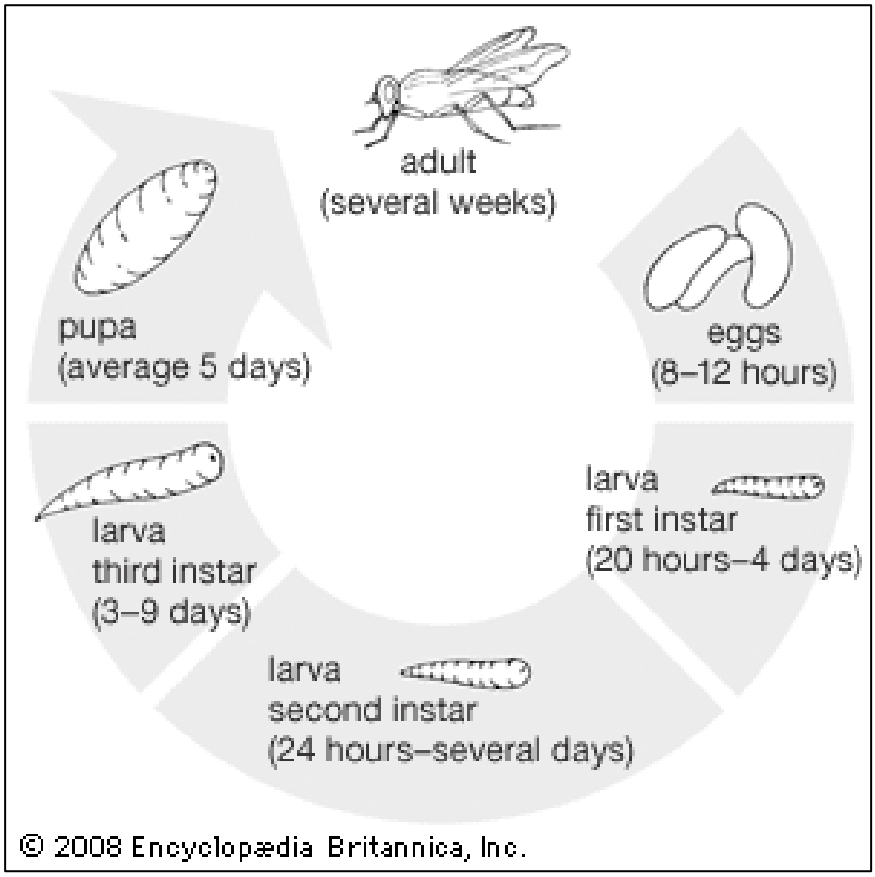

Figure 2.1. Life cycle of housefly (Oldroyd, 2018).

The flies predominantly lay their eggs on decomposing meat as soon as a few minutes to a few hours (Joseph et al., 2011). After a few hours or days, most of the flies' eggs will start to develop into small larvae which is the first instar larvae. The female fly can lay roughly between one to two hundred fifty eggs and many additional batches of eggs may be laid following the initial batch of eggs depending on the surrounding environment, species of fly and the time taken by the fly to find the suitable location to deposit the eggs (Oldroyd, 2018).

The first stage of the fly life cycle is the egg stage. The white egg deposited by the adult fly is usually piled in small groups where each egg is about one point two millimeter (1.2 mm) in length. The amount of eggs laid depends on the size of the female fly, which is mostly a product of larval diet. The ideal egg-laying temperature range is between 25 °C and 30 °C. Several flies will frequently place their eggs next to one another, creating huge mounds of larvae and pupae. Eggs must stay wet in order to hatch (Sanchez-Arroyo & Capinera, 2020).

The second stage of the fly life cycle is the larva or fly maggot stage. This stage is split into three stages which are the first instar, second instar and third instar. For the development of the larva in this stage, A great substrate for development is one that is nutrient-rich, like animal manure. The amount of degraded manure required for larval development is quite minimal, and sand or soil containing a modest amount of it promotes successful belowground development. Once fully grown, the maggot can scurry up to 50 feet towards a dry, cool location close to breeding material where it will change into the pupal stage (Sanchez-Arroyo & Capinera, 2020).

The third stage of the fly life cycle is the pupal stage. On average, the length of a pupa is about eight-millimeter (8mm) long. The last larval skin, which is converted into the pupal case, changes colour as the pupa ages from yellow to red to brown to black. The pupa's form, which is bluntly rounded at both ends, is very different from that of the larva. Pupae mature fully in two to six days at 32°C to 37°C, whereas it takes them seventeen to twenty-seven days at around 14°C. The emerging fly uses a sac located on the front of its head called the ptilinum, which alternately swells and contracts, like a pneumatic hammer, to break through the pupal cover and escape.

The final stage of the fly life cycle is the adult fly. The female house fly is often larger than the male, measuring six to seven millimeter in length. The rather broad distance between the eyes helps to distinguish the female from the male. For the male flies, their eyes almost touch each other. The adult fly's head contains sponging mouthparts and crimson eyes. Four little black stripes may be seen on the thorax, and the fourth longitudinal wing vein has a sharp upward bend. With a black midline and sporadic dark patterns on the sides, the abdomen is grey or yellowish. The male's underside has a yellowish hue. These characteristics may differ according to the various species of flies.

Adult flies typically live between fifteen and twenty-five days, but they can live for up to two months. They only last between two and three days without food. The accessibility of appropriate food, especially sugar, promotes their longevity. They do not live longer as adults if they have access to animal excrement, and they do live longer in cooler climates. To be able to reproduce, the flies have to eat. Copulation might last as little as two minutes or as prolonged to fifteen minutes. About four to twenty days after copulation, ovulation begins. Manure alone is insufficient for female flies to lay eggs; they

also need access to appropriate food such as protein. Fly reproduction has enormous potential, but fortunately it never comes to fruition.

The flies are found to be fairly inactive at nighttime and rarely lay their eggs. Trees, shrubs, various sorts of electrical wires, and grasses have all been reported as nocturnal resting places for flies, as well as the ceilings, beams, and overhead wires of structures (Sanchez-Arroyo & Capinera, 2020).

2.2.2 Fly maggots

About half of the fly species contain larvae known as maggots. The intricate head capsule of prehistoric flies has been lost by the maggot. The maggots now have one or two mouth hooks on its pointed anterior end. A pair of posterior spiracles which are external airholes on the blunt posterior end can be seen with the unaided eye as black dots. When viewed under a microscope, the spiracles appear as a dynamic system pattern of pores or slits that can be used to identify the different species of fly maggots. With the bare eyes, the instar of the maggots can be determined using the size of the spiracles.

Maggots exhibit morphological consistency, but they have a physiological diversity. The majority of maggots consume decomposing organic debris, although forensic research has revealed significant variations in the feeding preferences of various flies according to their species, environment and a variety of external factors. (Oldroyd, 2018).

Fly maggots appear on a cadaver in eight distinct stages or waves that have been identified. Each wave of maggots attacks deceased animals in a specific order as degradation develops from the freshly dead carcass through rigor and putrefaction to mummification. The enormous, voracious larvae of many blow flies feed on about any type of animal debris, including living tissues, despite the fact that some maggots only develop during a certain stage of animal decomposition (Oldroyd, 2018).

Numerous carnivorous fly larvae undoubtedly reside in the soil and consume any accessible plant or animal debris. During the third instar, when the majority of the growth occurs, some larvae, particularly maggots, that feed on vegetables during the first instar and second instar, turn carnivorous (Washmuth & Bergstresser, 2022).

Early instar larvae of flies are cylindrical but taper toward the head and range in length from 3 to 9 mm. They are typically creamy yellowish in colour. One set of dark hooks can be found on the head. The spiracular apertures are sinuous slits that are entirely enclosed by an oval black border, and the posterior spiracles are somewhat elevated. Within eight to twenty hours in warm temperatures, the legless maggot hatches from the egg. Maggots start to eat and grow in the material where the egg was laid right away. A fully formed maggot, measuring seven to twelve millimeter in length, has a greasy, cream-coloured look. The larva of house flies thrives in high-moisture manure. Although larval survival is highest around 17°C to 32°C, the ideal temperature range for larval stage development is 35°C to 38°C. At ideal temperatures, larvae mature in four to thirteen days, but at temperatures between 12°C and 17°C, they take fourteen to thirty days. (Washmuth & Bergstresser, 2022).

Fly maggots favour moist and wet settings since their body can easily dry out and get dehydrated in dry conditions. Maggots are frequently seen on damp, rotting material. The fly maggots also favour warm or hot conditions since they grow much more slowly in colder climates. The development of the maggots is accelerated by warmer temperatures, which allows them to develop from eggs to fully developed flies considerably more quickly in warm conditions. Since very bright conditions might be fatal to the maggots, they prefer darker environmental settings. On their bodies, maggots have specialized light-sensing cells that enable them to determine locations that may be too bright for their survival. The warmer months are optimal for the development and maturation of these larvae because maggots enjoy warm, moist conditions. Maggots are frequently discovered on rotting fruits, vegetables, or animal corpses because these areas offer the damp climate they require (Washmuth & Bergstresser, 2022).

2.2.3 Structure of Fly Larvae (Maggots)

Figure 2.2 shows the structure of a typical blowfly larvae, *Chrysomya bezziana* (Calliphoridae). The larvae at the top of the figure labelled as A show the complete larvae. The anterior spiracle of the larvae is labelled as B. C shows the cephalopharyngeal skeleton of the larvae and the hooks-like structure at the end is the mouth hooks of the larvae. The part labelled as D are the spines that are used as a movement aid and the part labelled as E is the caudal end of the fly larvae which has a pair of spiracular plates. These are the spiracles that are often used for species identification of the larvae (Hall & Gerhardt, 2002).

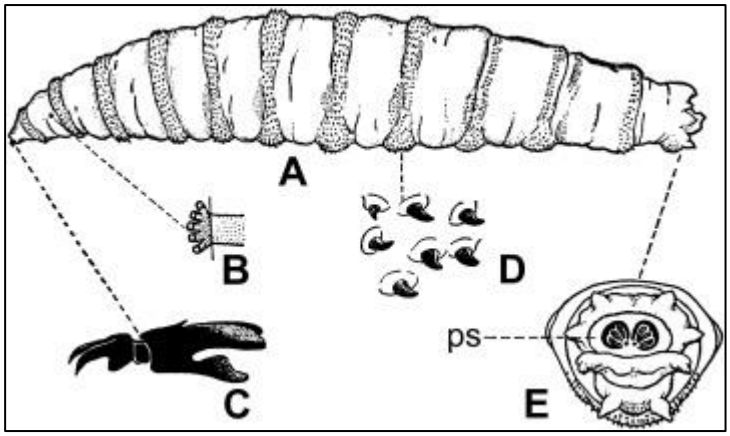

Figure 2.2. Blowfly larvae (Hall & Gerhardt, 2002).

Based on Figure 2.3, (a) shows the Cephalopharyngeal skeletons of the first instar *Hemipyrellia ligurriens* larvae, (b) shows the Cephalopharyngeal skeletons of the second instar *Hemipyrellia ligurriens* larvae, (c) shows the Cephalopharyngeal skeletons of the early third instar *Hemipyrellia ligurriens* larvae, (d) shows the Cephalopharyngeal skeletons of the late third instar *Hemipyrellia ligurriens* larvae, (e) shows the posterior spiracles of the first instar *Hemipyrellia ligurriens* larvae, (f) shows the posterior spiracles of the second instar *Hemipyrellia ligurriens* larvae, (g) shows the posterior spiracles of the early third instar *Hemipyrellia ligurriens* larvae, and (h) shows the posterior spiracles of the late third instar *Hemipyrellia ligurriens* larvae (Bunchu et al., 2012).

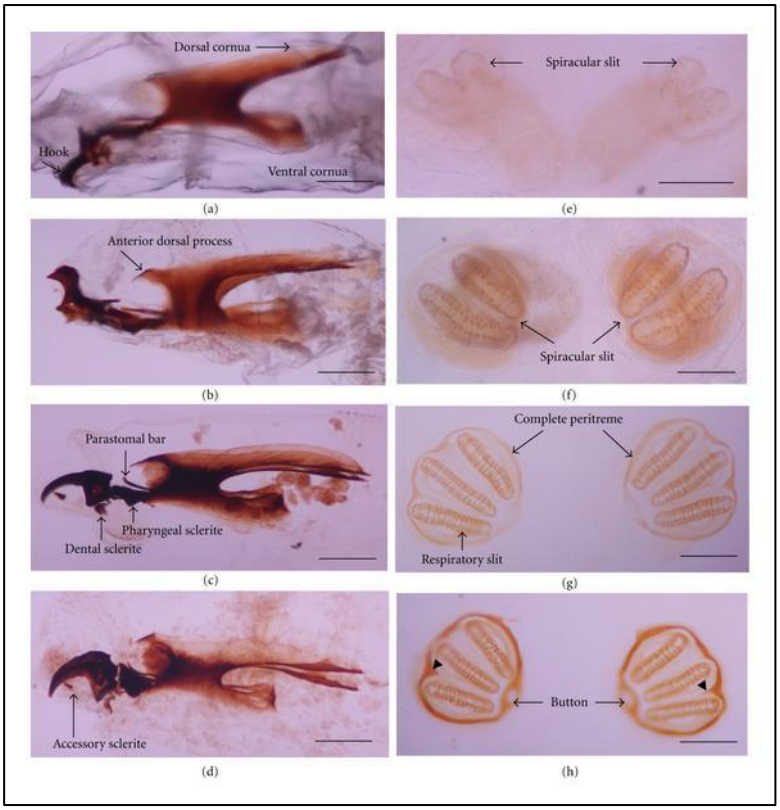

Figure 2.3. Cephalopharyngeal skeletons and posterior spiracles of *Hemipyrellia ligurriens* larvae (Bunchu et al., 2012).

Fly larvae lack wings, externally display no signs of wingbuds (endopterygote insects), they also lack segmented thoracic legs. Most Nematocera and Brachycera larvae have a fully developed head with eating mouthparts. Evolution has favoured the decrease of the head capsule and the substitution of a pair of vertically moving mouth hooks for the chewing mouthparts. Nematocera or Brachycera are typically the home of externally adaptable larvae, such as prolegs. Except for their black mouth hooks and posterior spiracles, the Cyclorrhapha maggots have very little visible anatomy. Most of these larvae cannot be identified above the family level, while a few of them do exhibit secondary complexity. (Oldroyd, 2018).

One characteristic that all fly larvae have in common is the absence of real, jointed thoracic legs. Many fly larvae produce "fake legs" (pseudopods or prolegs) that resemble the caterpillar's support structure for its fleshy abdomen. Compared to caterpillars, flies are significantly more adaptable and can have prolegs on any part of their bodies. Prolegs enable the larvae to push through dirt or soil or crawl through tight places. The larvae of more highly evolved flies, which exhibit higher physiological adaptability, are often less organised than those of more primitive flies since the evolutionary trend among fly larvae has been toward structural reduction. The majority of the Nematocera suborder's larvae have well-developed heads with antennae, palpi, and sophisticated mouthparts resembling those of several adult insects. They frequently have their own way of life so structurally ingrained in them that they are impossible to adjust to any other. This is particularly true of aquatic larvae, such as mosquito larvae, and may be taken to its logical conclusion by mountain midge larvae, which dwell in raging streams and crawl on submerged rocks. Each of their bodily parts has suckers and clinging mechanisms (Oldroyd, 2018).

2.2.4 Composition of fly maggots

Currently, maggots are consumed by many around the world as a source of protein in their diet. The maggots are also used by farmers as a cheaper source of proteins for their breeding animals such as chickens. Maggots are now recognised as a sustainable option that is easily available, cheap and a non-conventional source of protein feed on farms. These maggots contain a range between 39% to 65% of crude protein, between 12.5% to 21% of lipids and between 5.8% to 8.2% of crude fiber. The maggots also have a high content of vitamin B complex, trace elements and phosphorus (Dillak et al., 2019). According to

Kiendrebeogo et al. (2019), concentrated maggot that were produced by their team as animal feed contained 37.69% of crude protein, 5.12% of fat, 11.04% of crude cellular, 50.4% of neutral detergent fiber (NDF), 15.08% of acid detergent fiber (ADF), 4.37% of lignin, 12.72% of mineral matter, 2.14% of sodium chloride (NaCl), 0.08% of calcium and 0.32 of phosphorus. The composition of fly maggots differs according to the species of the flies. According to Gadzama et al. (2019), housefly larvae meals are mainly composed of crude protein, dry matter and crude fiber. They also have a well balanced composition of essential amino acids and non-essential amino acids.

2.2.5 Fly maggot consumption

According to the study on the food consumption by blow fly maggots carried out by Amin et al. (2019), the food consumption of the fly maggots is not affected by the area where the meat is placed. In their research comparing the meat consumption by blowfly maggots showed that the meat consumption per larvae from the rabbit meat were almost identical. It was also confirmed by this research that the method of killing the rabbit had an effect on the amount of meat consumed by the fly maggots. The highest amount of meat consumed by the fly maggot was collected from the rabbit that was slaughtered and the lowest amount of meat consumed by the fly maggot was collected from the rabbit that was killed by injection.

2.3 Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid, or DNA, is an organic molecule with a complex molecular structure that is present in both prokaryotic and eukaryotic cells, as well as in a variety of viruses. For the transfer of inherited traits, DNA codes genetic information (The Editors of Encyclopaedia Britannica, 2022). The structure of the DNA molecule is formed in a double helix manner as shown in Figure2.4. A DNA molecule's strands are made up of a lengthy chain of monomer nucleotides. DNA nucleotides are made up of a deoxyribose sugar molecule with a phosphate group attached and one of four nitrogenous bases: two purines which are guanine (G) and adenine (A) and two pyrimidines which are thymine (T) and cytosine (C). Covalent bonds develop between the phosphate of one nucleotide and the sugar of the next, generating a phosphate-sugar backbone from which the nitrogenous bases extend. Hydrogen bonding between the bases hold one strand to another; the order of this bonding is important. The adenine only bonds with the thymine and the guanine only with the cytosine.

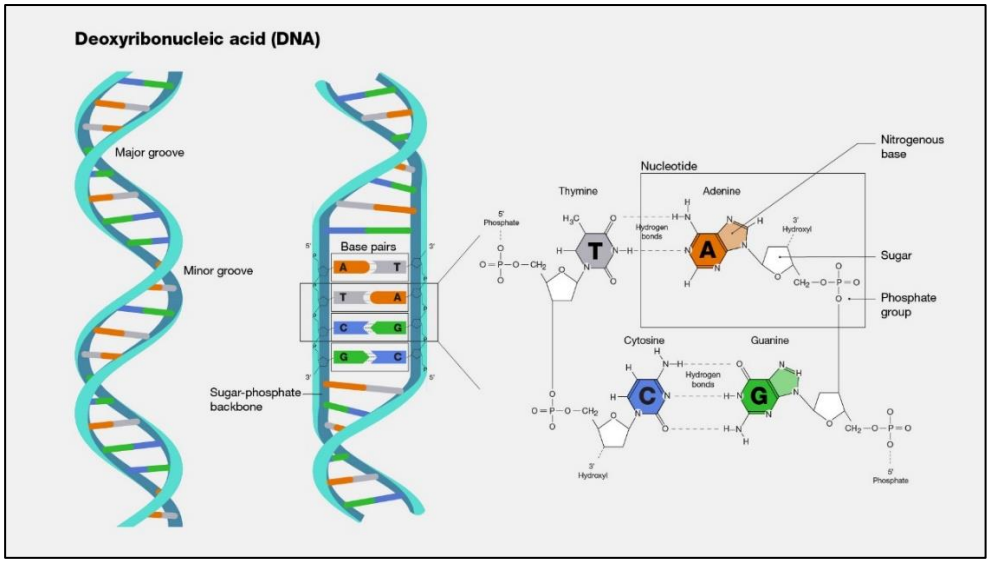

Figure 2.4. Double helix structure of DNA molecule (Bates, 2022).

The DNA molecule's configuration is extremely stable, allowing it to serve as a blueprint for the replication of new additional DNA molecules as well as the creation (transcription) of the corresponding RNA (ribonucleic acid) molecules. A gene is a DNA fragment that codes for the synthesis of a certain protein by the cell.

When DNA replicates, it divides into two single strands, with each of which acts as a model for a DNA strands. The new strands are formed using the same hydrogen-bond pairing process that exists within the double helix. Two new double-stranded DNA molecules are formed, each with one of the original strands and one new strand. The key to the sustained inheritance of genetic features is "semiconservative" replication.

DNA is structured within cells into dense protein-DNA complexes known as chromosomes. Chromosomes are present in the nucleus of eukaryotes, however DNA can also be found in mitochondria and chloroplasts. In prokaryotes, which lack a membrane-bound nucleus, DNA is present in the cytoplasm as a single circular chromosome. Some prokaryotes, including bacteria, and a few eukaryotes include plasmids, which are fully independent, self-replicating genetic makeup. Plasmids have been extensively used in gene expression research using recombinant DNA technology.

In the field of forensic DNA, the most common types of DNA markers that are used for analysis are the three types of short tandem repeats (STRs); autosomal STR, Y-STR and X-STR, amelogenin and mitochondrial DNA (mtDNA) (Kowalczyk et al., 2018). These analyses are carried out through DNA fingerprinting and DNA profiling. DNA. The usage of DNA taken from biological samples is used in DNA fingerprinting. A restriction enzyme is used to digest the genome, and the digested DNA profile is compared between the scene of crime samples and suspected individuals. A specific section of the DNA is

used in DNA profiling. Polymerase chain reaction (PCR) is used to amplify the desired region of the gene. PCR replicates the targeted DNA segment numerous times (Norrgard, 2008). These processes are able to help in forensic science to link a suspect to a crime scene or to place an individual at a crime scene. Even though more than 99.1% of the human genome is the same across the population, the remaining 0.9% of human DNA varies from individual to individual. Polymorphic markers are changeable DNA sequences that can be utilised to distinguish and correlate people (Giardina, 2013).

2.3.1 DNA of human remains from maggots

In cases where a victim's body is found and the DNA from the cadaver is too damaged to be analysed for identification purposes, DNA has successfully been extracted from the digestive system of entomological specimen; maggots, that were on the cadaver and feeding on it. In the study by Li et al. (2011), third instar maggots were used for mitochondrial DNA and STR analyses from the contents of a maggot in a case that occurred in central-southern China. Their research was successful as they were able to prove that there is an association between the maggots that were feeding on the human cadaver and the specific remains of the human. Based on the STR analysis of human DNA extracted from maggots that were feeding on decomposing bodies in the assessment done by Njau et al., in 2016, the maggots were only valuable in identification up to the second and fourth days in the fed and starved cases, respectively.

2.4 DNA quantification

The DNA or RNA quantification of often known as the quantification of nucleic acid. Prior to downstream tests, DNA and RNA quantification, is routinely conducted to quantify the average content of DNA or RNA in a sample. Sample purity is also an important factor to consider when determining the amount of RNA or DNA in a sample. To quantify nucleic acids, two optical technologies are typically utilised: UV-Vis measurement and fluorescence measurement. (ThermoFisher. 2022)

When using UV-Vis photometry, the fundamental absorptivity properties of nucleic acids are used in photometric measurements of nucleic acids; DNA and RNA. Nucleic acids absorb light with a distinctive peak at 260nm when an absorption spectrum is observed. For a fluorescence measurement, the fluorometric evaluation of nucleic acids is based on the use of fluorescence - based dyes that bind to DNA or RNA selectively.

2.4.1 DNA concentration

The Beer-Lambert equation can be utilised to directly determine nucleic acid concentrations from observed absorbance values at 260nm. The Beer-Lambert equation is $A=\varepsilon bC$; where A is the UV absorbance, ε is the wavelength dependent molar absorptivity, b is the length of the light path and c is the concentration of nucleic acid. This formula is only used when a UV-Vis measurement is done. For a fluorescence measurement, the nucleic acid concentrations are evaluated using the sample's fluorescence intensity, and a calibration curve is produced from standard samples with a known concentration and fit to suitable regression models (ThermoFisher, 2022).

According to some reports, the solvent chosen affects how much DNA is absorbed. A basic solution will overrepresent the 260/280 ratio by 0.2–0.3 compared to an acidic solution that will underrepresent it by the same amount. Therefore, it's crucial to make sure that the pH and ionic strength of the elution buffer employed is the same when comparing the 260/280 ratio for various DNA samples. Furthermore, when low-salt buffer rather than water is utilised as the elution buffer, the absorbance at 260 nm and the 260/280 values can be accurately repeated (Lucena-Aguilar et al., 2016)

2.4.2 DNA Purity

Before employing DNA samples in analytical procedures, the quality and usefulness of the samples must be confirmed using DNA quality indicators such as DNA purity and integrity. The ratio of absorption at 260nm and 280 nm is used to determine DNA purity. A ratio of approximately 1.8 is considered "pure" for DNA. If indeed the ratio is significantly smaller; below 1.6, it may suggest the presence of proteins, phenol, or other pollutants that absorb heavily at or near 280nm. On the contrary, because absorbance readings cannot distinguish between DNA and RNA, the presence of RNA can cause the ratio to increase, and this probability must be addressed to prevent DNA over quantification.

The 260/230 ratio is a common secondary measurement of DNA purity. The ideal 260/230 values for "pure" DNA are typically between 2.0 and 2.2. The presence of impurities that absorb at 230nm, such as proteins, guanidine HCL that is used for DNA isolations, EDTA, carbohydrate, lipids, salts, or phenol, may be indicated if the ratio is noticeably lower than predicted. Because of its fragility when DNA is dissolved in a saline

elution buffer, the 260/230 ratio is regarded as a suspect measure of DNA quality. This is because the concentration of salt in the sample increased more rapidly than the concentration of DNA. Since salts absorb light at 230nm, out of two DNA samples that have the same purity, the less saturated sample will have a lower 260/230 ratio (Lucena-Aguilar et al., 2016).

2.5 DNA Extraction

DNA extraction is a technique to separate DNA from cellular membrane, proteins, and other biological components from a sample using chemical and/or physical processes. The employment of a DNA isolation process should result in an effective extraction of DNA that is clean, abundant, and free of impurities like RNA and proteins. Manual techniques as well as kits that are readily available commercially are utilized for DNA extraction. DNA can be extracted from a variety of tissues, such as frozen tissue sections, formalin-fixed paraffin-embedded tissues, blood, bodily fluids, direct fine needle aspiration cytology (FNAC) aspirate, as well as more. Lysing the cells and solubilizing the DNA is the first step in the extraction of DNA. Chemical or enzymatic techniques are then used to remove macromolecules, proteins, RNA, or lipids. Techniques for extracting DNA include salting out and proteinase K treatment, which is the non-organic method, the organic extraction method using phenol-chloroform, and adsorption method using silica–gel membrane.