DECOMPOSITION AND INSECT SUCCESSION PATTERN ON MONKEY CARCASSES PLACED INDOOR AND OUTDOOR WITH NOTES ON THE LIFE TABLE OF *Chrysomya rufifacies* (DIPTERA: CALLIPHORIDAE)

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by

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ABSTRAK

Kajian ini dijalankan di Lembah Durian, USM, Pulau Pinang dari 15 Februari hingga 4 April, 11 Jun hingga 30 Julai dan 10 Oktober hingga 28 November, 2012, dengan menggunakan 18 bangkai monyet; 6 bangkai monyet bagi setiap tempoh kajian. Lima peringkat pereputan dikenalpasti; segar, kembung, penguraian aktif, penguraian melampau di dalam kedua-dua keadaan luar dan dalam rumah, dan yang terakhir, baki kering bertulang; di luar rumah manakala baki bermumia; di dalam rumah pada tiga tempoh kajian. Permulaan peringkat pereputan antara tiga tempoh kajian berbeza dengan signifikan ($p<0.05$) di keadaan luar rumah sahaja; dalam peringkat penguraian melampau dan baki kering bertulang sahaja. Manakala permulaan peringkat pereputan antara keadaan luar dan dalam rumah berbeza dengan signifikan ($p<0.05$), dalam penguraian aktif, penguraian melampau dan baki kering bertulang; di luar rumah manakala baki bermumia; di dalam rumah di semua tempoh kajian. Diptera merupakan Order artropod yang paling dominan yang diperolehi dalam kajian ini. Sejumlah 21 spesies diptera diperolehi sepanjang tempoh kajian. Sesetengah spesies lalat yang diperolehi di dalam rumah tidak diperolehi di luar rumah. Keadaan luar rumah yang terdedah memudahkan lalat untuk bertelur pada bangkai yang berlaku dalam masa 15-30 minit pendedahan. Walaubagaimanapun terdapat kelewatan dalam oviposisi lalat di keadaan dalam rumah selama dua hari. Pengkolonian larva oleh sesetengah spesies adalah lewat pada keadaan dalam rumah. Proses pereputan bangkai dalam rumah adalah lebih lewat berbanding dengan di luar rumah. Faktor utama yang mempengaruhi perbezaan dalam proses pereputan dan pola sesaran serangga antara semua tempoh kajian, adalah curahan hujan. Manakala perbezaan antara bangkai dalam dan luar rumah dipengaruhi oleh suhu persekitaran, kelembapan relatif, intensiti cahaya dan kelimpahan serangga; akses serangga ke atas bangkai, secara signifikan ($p<0.05$). *C. rufifacies* adalah spesies yang paling berlimpah yang dikutip di lapangan sewaktu awal proses pereputan, maka jadual hayat lalat ini dikaji di dalam makmal pada suhu 28 ± 2.5 ºC dan keadaan kelembapan75 ± 10% R.H untuk menentukan dinamiks populasinya. Data kasar yang diperolehi dianalisis berdasarkan teori jadual hayat “age-stage, two-sex’, untuk mempertimbangkan kedua-dua jantina dan perbezaan kadar perkembangan dalam kalangan individu dan antara jantina. Parameter populasi; kadar intrinsik peningkatan semulajadi (r) adalah 0.2361 d^{-1}, kadar peningkatan finit (λ) adalah 1.2663 d^{-1}, kadar reproduksi bersih (R_0) adalah 97.18, dan purata masa generasi (T) adalah 19.36. Parameter populasi mencadangkan bahawa populasi *C. rufifacies* mempamerkan strategi r.

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ABSTRACT

This study was conducted in the Durian Valley, USM, Penang from 15 February to 4 April, 11 June to 30 July and 10 October to 28 November, 2012, using total of 18 monkey carcasses; 6 carcasses per study period. Five decomposition stages were identified; fresh, bloated, active decay, advance decay in both outdoor and indoor conditions and finally dry skeletal remain; in outdoor and mummified remain; in indoor conditions for all study periods. Onset of decomposition stage between the three study periods were significantly different ($p<0.05$) on outdoor only; for advance decay and dry skeletal remain stages only. While, onset of decomposition stage between the outdoor and indoor were signifantly different ($p<0.05$), for active decay, advance decay and dry skeletal remain; in outdoor and mummified remain; in indoor conditions in all study period. Diptera was the most prominent Order of arthropod obtained in the present study. A total of 21 species of dipterans were collected throughout the study period. Certain species of flies which were obtained indoor, not obtained from outdoor. The exposed outdoor condition made it easier for the flies to oviposit on the carcass, which occurred within 15-30 minutes of exposure. However there was a delay in the oviposition of fly, in the indoor condition by two days. The colonization by the larvae of some of the species was delayed in the indoor condition. The decomposition process of the carcass placed indoor was prolonged compared to the outdoor. The major factor affecting the variation in the decomposition process and insect succession patterns between all the three study periods was solely rainfall. While, variation between indoor and outdoor carcasses were influenced by ambient temperature, relative humidity, light intensity and insect abundance; accessibility of the insects to the carcasess, significantly ($p<0.05$). *Chrysomya rufifacies* was the most abundant species collected in the field during the early stage of decay and the life history of this fly was studied in the laboratory conditions at temperature of 28 ± 2.5 ºC and 75 ± 10% R.H, to establish its population dynamics. The collected raw data were analysed based on the age-stage, two-sex life table theory in order to consider both of the sexes and the variable developmental rate among individuals and between sexes. The population parameters; intrinsic rate of natural increase (r) was 0.2361 d^{-1}, the finite rate of increase (λ) was 1.2663 d^{-1}, the net reproduction rate (R_0) was 97.18 and the mean generation time (T) was 19.36. The population parameters suggest that the *C. rufifacies* population exhibited the *r*-strategy.

CHAPTER 1

INTRODUCTION

1.1 General introduction

The study of entomological fauna which associated with the judicial system is termed as forensic entomology (Hall & Doisy, 1993). This wide field of forensic entomology has been categorised into three divisions. Firstly, urban entomology which encompasses legal events involving insects that is linked to human environment. Secondly, stored products entomology which involves disputes over insects in food and other related kitchen products and lastly the medico legal entomology (Lord & Stevenson, 1986). The term medico legal entomology was soon more familiarly known as medicocriminal entomology for the reasons of its prominent emphasis on the usefulness of entomological evidence in resolving crimes, utmost often crimes of violence (Hall, 1990).

Anderson & VanLaerhoven (1996) defined forensic entomology as a study of insects associated with human corpse which is highly valuable in determining the time elapsed since death or more precisely known as post-mortem interval. Post-mortem interval (PMI) is the time between the death and discovery of a human corpse (Adams & Halls, 2003). By determining the post mortem interval, the time of death that is identified can almost reveal the real scenario of the death or the crime that has occurred.

The pathologists are usually considered to be able to provide a reasonable accurate determination of the time of the death within the first 72 hours after death. This is based on the condition of the body itself and features such as the body temperature (Gennard, 2007). Once the time of death exceeded that time period, forensic entomology comes in to perform its role. As stated by Kashyap & Pillai (1989) forensic entomology became the most precise and recurrently the only available method of determining the time of death when more than one or two days have elapsed from the time of death.

Based on the history of forensic entomology, the training manual on investigating death, *Washing Away of Wrongs*, written by Sung Tz'u in the 13th century and later translated by McKnight (1981) was assumed as the earlier review of forensic entomology. The manual described the investigation of a homicide in which the landing of a number of blowflies on a particular sickle identified the murder weapon and consequently drew a confession from the murderer.

A noteworthy legal case which assisted to recognize and establish the field of forensic entomology as a crucial tool for investigating crime was the mummified body of a murdered newborn baby which was revealed from behind a mantelpiece in a boarding house renovation work undertaken in Paris, France in 1850. The discovery of flesh fly larvae and some moths by Dr. Marcel Bergeret during the autopsy of the body lead to a conclusion that the body of the baby was sealed in 1848 and the moths had gain access to the body in 1849. This estimation of post mortem interval pointed the murder to the occupiers of the house previous to 1848 and exonerated the current occupiers. This case was recorded as the first forensic entomological case in Europe by Smith (1986) which initiated the way for latter studies.

The succeeding significant point in the forensic entomological history was stemmed from the observations and conclusions constructed by J. P.Megnin in his publication, *La fauna des Cadavers* in 1894 (Gupta & Setia, 2004). In the book, he published findings relating eight stages of human decomposition to the succession of insects colonizing the body after death (Greenberg, 1991). The knowledge about insect succession on a dead body became the basis to estimate the post mortem interval by forensic entomologists.

In 1964, William Brittle was accused of the murder of Peter Thomas whose decomposing body was found in a wood near Bracknell, Berkshire, England. Brittle's alibi was totally destroyed when the life cycle of the common blue-bottle whose maggots were determined and consequently established the time of death (Lane & Brian, 1992). A published study on the succession of insect communities found on baby pig carcasses by Payne (1965) began research works which advanced the field of forensic entomology into the science it is today.

In Malaysia the field of forensic entomology was established since the 1950s. Reid (1953) was the first person to use forensic entomology to determine the post mortem interval in a female corpse in Penang in 1950. Ever since many forensic entomology publications regarding entomological fauna obtained from human cadavers and insect succession pattern and decomposition of animal model carrion have been published in the country (Lee *et al*., 1984; Lee, 1989 & 1996; Lee & Marzuki, 1993; Omar *et al*., 1994a, Hamid *et al*., 2003; Azwandi & Abu Hassan, 2009; Heo *et al*., 2007, 2008a & 2008b; Nazni *et al*., 2011).

Other than field study using carrions, the study on the life table of particular species of flies which predominantly arrive on the carrion also contribute a significant amount of knowledge in the field of forensic entomology. A well-established life table of a population of a particular species of interest can be very useful in understanding the growth, survival and fecundity of the species, since they provide the most comprehensive description of those aspects (Abou Zied *et al.*, 2003).

The first detailed example of a life table for the natural populations of insects was presented by Morris and Miller (1954). Fundamentally, a life table describes the development, survival and fecundity of a cohort and provides basic data on the population growth parameters (Gabre *et al.*, 2005). Nevertheless, Fisher (1930) noted that the life table analysis of a population also reveals the contribution of an individual towards the growth of the future population.

1.2 Objectives of the research study

1. To study and compare the effect of weather, and outdoor and indoor conditions on the decomposition process, insect succession and the composition of the dipteran adults of the monkey carcasses in a secondary forest ecosystem, Durian Valley.

2. To study and establish the life table of a forensically important fly, *Chrysomya rufifacies*.

CHAPTER 2

LITERATURE REVIEW

2.1 Insect succession as a valuable post mortem interval indicator

The most primary and vital contribution made by the field of forensic entomology in a death investigation is the estimation of the time elapsed since death or more familiar known as the post mortem interval (PMI). Determination of an accurate PMI is very crucial to narrow the field of suspects and help to identify both the victim and the criminal in a homicide case and also at the same time connecting the deceased with reportedly missing individuals at the same time interval (Catts, 1990; Geberth 1996).

At the moment a death occurs, the dead body will evolve a sequence of decomposition stages and during this process the corpse will undergo physical, biological and chemical changes which act as PMI indicator (Henssge *et al*., 1995). However, as the time since death increased, the physical, biological and chemical changes occur on the cadaver does not provide a precise PMI estimation, where the ecological information provides a more accurate result. Basically insects play an important ecological role in the decomposition of organic material. Often a dead body, disregarding whether it is a human corpse or an animal carcass provide a huge food resource and support many fauna as it decomposed (Byrd & Castner, 2010). This is where forensic entomology plays an important role, since insects are the first witness in a death scene due to their arrival in a predictable sequence (Smith, 1986).

For a long time it was apparent that insects associated with vertebrate carrion exhibit PMI dependent process (Hall, 1990). The basic assumption in applying entomological techniques to estimate the post mortem intervals is that the invasion of the body by insects will begin soon after death (Goff, 1992). As insects are being utilised to indicate the post mortem interval, there are two complementary approaches they can be applied. One of the methods is to recognize the insect's stage of development found on the carrion. The estimated age of the immature insect larvae is able to be backtracked to the time of oviposition, subsequently predicting the estimated time of death. Another method utilizes the knowledge of the insect colonization of the carrion in a predictable manner, which is a good indicator of the time since death. The second method seems to be very useful, when the cadaver is exposed to the insects for a longer period of time (Anderson 2000; Byrd & Castner 2010; Smith, 1986).

An insect succession model usually comprises of information about the time elapsed between death and the arrival of a particular insect species and also the insect's developmental stage. Thus, it can be applied to determine both the minimum and maximum PMI (Schoenly *et al.*, 1992). Such an insect succession data have been used to calculate a post mortem interval (PMI) very accurately as long as 52 days (Schoenly *et al.*, 1996).

2.2 Decomposition process of vertebrate carrion

Decomposition is a natural process in the ecosystem which is essential for the break down and returns of the organic material, such as dead plant, animal carcass or even human cadaver back to the ecosystem itself. The vertebrate carrion either of animal or human, provide a temporary and changing food source for a variety and diverse community of organisms (Putman, 1983). During the process of decomposition, the series of faunal that arrives correlates with the natural changes that occur in the carrion (Smith, 1986).

In general, decomposition is an enduring process where discrete stages do not overlap (Schoenly & Reed, 1987). Kelly (2006) also noted that decomposition as a continuous process in which the transformation of the physical criteria may not always be clearly observed between the stages. In spite of this, many researches segregate this process to different stages for the comfort of discussion and to be better recognized (Anderson & VanLaerhoven, 1996).

In a study of the arthropod succession in a pig carcass, Bornemissza (1957) recognized five stages of decomposition as follow; initial decay stage, putrefaction stage, black putrefaction, butyric fermentation and dry decay. In the United States, Payne (1965) recognised six stages of decay; fresh, bloated, active decay, advance decay, dry and remain stages on pig carcass decomposition. In the decomposition study of large animal carcasses conducted in Southern Queensland, O'Flynn (1983) divided the decomposition stages into five categories: those that are slightly decomposed, bloated, active decay, advance decay and stage after advance decay.

Tantawi *et al*. (1996) divided the decomposition of rabbit carrion into four stages which resemble the decomposition stages divided by Early and Goff (1986) from a study conducted using pig carcass on the island of Oahu, Hawaiian Island, United States. The stages are fresh, bloated, decay and dry. In Malaysia, a study conducted by Omar *et al*. (1994a) on monkey carrion in a rubber plantation categorised the decomposition process into five stages; fresh, bloated, decay, post decay and remain stage. In a comparative study on monkey carrion conducted in a semi-forested area by Nazni *et al*. (2011), five stages of decomposition were also documented; fresh, bloating, decay, advanced decay and remains.

However, for the convenience of discussion the decomposition process is commonly apportioned into five stages and these are: fresh, bloat, active decay, advanced decay and dry remain (Tullis & Goff, 1987; Anderson & VanLaerhoven, 1996; Wolff *et al*., 2001; Grassberger & Frank, 2004; Gennard, 2007; Heo *et al*., 2007; Nazni *et al*. (2011),). All the way through the decomposition process, a pattern of insect succession resulted since various insects attracted to the carrion due to the varying chemical, biological and physical changes that the carrion endures (Anderson & VanLaerhoven, 1996).

In the decomposition process the phase from the second death occurs is described as the fresh stage. This stage will last until the first indication of bloating becomes visible. During this stage, no odour is associated with carcass (Anderson & VanLaerhoven, 1996; Grassberger & Frank, 2004). Once the heart stop beating, death occurs (Carter *et al*., 2007). During this period the body will undergo a bluish-purple discoloration termed as livor mortis as the blood is no longer being pumped.

The body temperature falls immediately after death as heat is loss to the surrounding environment. This changes lead to an overall cooling and this state is called algor mortis (Janaway *et al*., 2009).

In spite of this, the primary major recognizable changes that occur during the fresh stage, is the stiffening of the muscle fibres due to the breakdown of the glycogen and the accumulation of lactic acid. The duration of this biochemical process which is known as rigor mortis seems to be highly dependable on the metabolic state at death such as body temperature and size of the body (Smith, 1986).

Following this, a series of biochemical fermentation processes known as autolysis occur. In this process of natural breakdown, the body cells begin to be digested by the enzymes which results in the discharge of gases such as ammonia (NH_3), hydrogen sulphide (H_2S), carbon dioxide (CO_2) and nitrogen (N_2) (Smith, 1986). This process can be most rapid in the organs such as brain and liver (Vass *et al*., 2001). Although the internal bacteria have been inaugurated to digest the organ tissues which may lead to the appearance of blisters on the skin surface, there are limited external symptoms of physical changes that can be observed during the decomposition at this stage (Kreitlow, 2010; Knight, 1991). The first insect to arrive during this stage is the blowfly (Turner & Howard 1992; Bourel 2003). Throughout this stage the flanks become greenish and the abdominal region become bloated (Smith, 1986).

The bloating stage is the second prevailing stage in the decomposition process; a slight inflation of the abdomen and some blood bubbles from the nose are the primary noticeable sign (Payne, 1965). The occurrence of the abdominal bloating in this stage is due to the activity of the anaerobic bacteria in the abdomen which produce gases that will accumulate later (Anderson & Vanlaerhoven, 1996). This anaerobic bacteria activity is known as a putrefaction process and is the most distinctive part of the decomposition process in vertebrate carrions (Early & Goff, 1986). Adult calliphorids and muscids become the most abundant flies during this period. It can be observed that the larvae of those adult flies concentrate on the natural orifices such as mouth, nose, anus and the wound (Richard & Goff, 1997).

The deflation of the carcass from the perforations in the abdominal wall occurs when the preceding decomposition phase, the active decay stage begins (Tullis & Goff, 1987). The carcass is associated with a strong odour of putrefaction (Anderson & VanLaerhoven, 1996) and also has a wet appearance due to the liquefaction of tissues. During this stage, the number of the adult calliphorids and muscids decreased (Payne, 1965). A rapid biomass loss of carrion is perceived and the major significant factor which is responsible for this was the larvae which feed on the carrion vigorously (Putman, 1978). Right through this stage, a massive temperature variation might occur in between the internal carcass temperature and the ambient temperature. However the internal temperature of the carcass begins to complement the ambient air temperature more closely later (Tullis & Goff, 1987). The completion of this stage of decay is indicated by a massive migration of maggots away from the carrion to begin the pupation phase (Carter & Tibbett, 2007).

In the subsequent advance decay stage, the greatest amount of the flesh and the cadaveric material is removed though some flesh might remain in the abdominal cavity and the strong odour of the decay begins to disappear (Payne, 1965; Anderson & VanLaerhoven, 1996). The first mass migration of the third instar calliphorid larvae from the carrion to undergo pupation is also occur in this stage (Anderson & VanLaerhoven, 1996; Gressberger & Frank, 2004). Activity of the insects is reduced during this stage (Janaway *et al*., 2009). The remaining cadaver is drying out although there are some soft tissue and adipocere presence (Bornemissza, 1957).

The final stage of decomposition is the dry remains. The carrion is almost dry in this stage with very little bones with dried cartilage, small bits of dried skin and hair or fur remain. The habitat of the carrion will dry up rapidly though the skeletal remain will not become clean completely and will be sun bleached for months (Bornemissza, 1957). There is no or very little odour associated with the remains (Anderson & VanLaerhoven, 1996; Gressberger & Frank, 2004) and if there is any odour present, then it might be from the dry skin or wet fur (Payne, 1965).

The rates and pattern of the arthropods mediated decomposition can be impacted by multiple factors which include elements such as temperature, humidity, rainfall and precipitation and the degree of insulation (Smith, 1986; Galloway *et al*., 1989; Ayila & Goff, 1998). The access by the insects itself and the depth of burial are also important environmental factors which influence the rate of decomposition. In fact the constitution of the carrion associated insects being the second most important parameter which influences the rate of the decomposition of a human cadaver as temperature being the first variable (Mann *et al*., 1990).

2.3 Insect succession in carrion

Arthropods which are associated with carrion can be classified into four ecological categories. First are the necrophagous species which feed on the carrion itself and being the most essential group in establishing the time of death. The blowflies are the most prominent necrophagous species and this is followed by beetles such as skin beetles and some carrion beetles. Secondly, the predators and parasites on the necrophagous species comprised the vital entomological fauna. These are rove beetles, some carrion beetles and also some of the dipteran larvae such as calliphorid and muscid which become predacious in later instars. Next are the omnivorous species which consists of wasp, ants and again some coleopteran beetles which feed on the corpse and inhabitants and finally the adventive or incidental species which use the corpse as the extensions of their environment such as springtails (Payne, 1965; Smith, 1986; Catts & Goff, 1992).

Insect are the first organisms that arrive on a cadaver after death, and these insects which are associated with carrion display PMI-dependent process (Catts & Haskell, 1990). From the early phase of decomposition the insects colonized the carrion in particular successive waves. Each of the waves modifies its own substratum, which has the impact to make it attractive to the next wave (Bornemissza, 1957). Though the line of division might not be clear, but the presence of a recognisable succession waves is undeniable (Smith, 1986). Therefore the insect species do not appear at the same time on a dead body but they do possess specific succession patterns which do exhibits some variations in diverse habitations (Tullis & Goff, 1987).

It is noteworthy that, the insect colonization of vertebrate carrions varies with many factors, such as the geographical region, weather, vegetation and the environmental conditions (Anderson, 2001). In spite of this, the parameters that give the foremost impact on the insect succession are temperature, humidity, rainfall and the assessment of the insect itself (Shean *et al*., 1993) Therefore when the pattern of insect succession on a dead body in a particular region and condition is able to be established, this data can be utilised in an actual death investigation or circumstances which closely resemble the insect succession study (Goff, 2000).

2.4 Flies (Diptera) of forensic importance in Malaysia

Flies has predominate the world till it become very common for human to get in contact with them. They become an important carrion feeder as they eat dead vertebrate bodies, including that of humans which is one of their foremost features (Aggarwal, 2005). Flies being the first insects to appear at a crime scene and therefore they are well known for their utility in the detection of crimes for a long time and numbers of articles have been written about the history of forensic entomology (Benecke, 2001; Greenberg & Kunich, 2002).

The general life cycle of the flies shows a complete metamorphosis, which means every stage in the life cycle of this fly, are different in morphology and characteristics. The life cycle begins when an the adult female fly lays eggs and then the eggs hath into larvae; 1^{st} instar, 2^{nd} instar and 3^{rd} instar; and later on, enter the pupal stage and finally emerge to become an adult (Gennard, 2007). Payne (1965) stated that 60% of the total fauna collected from carrion comprised of Diptera. A number of studies related to forensically important flies have been conducted in Malaysia (Omar *et al.*, 1994b; Chen *et al.*, 2008; Nazni et al., 2008; Ahmad Firdaus *et al.*, 2010; Kumara *et al.*, 2010)

2.4.1 Family Calliphoridae

The flies from the family of Calliphoridae are commonly known as the blow flies. Their common name is derived from their egg laying behaviour (Crosskey & Lane, 1993). Blowflies are the first insect to arrive on a dead body due to their attraction towards the odour of the fresh blood, particularly the blood from opened wound and body fluid of the carrion (Anderson *et al.*, 2000). A research study showed that blowflies arrived to corpse within a minute of their exposure to the carrion (Shean *et al*, 1993; Byrd & Castner, 2010).

Therefore blow flies typically referred to as the first wave of insects because of their ability to locate the cadaver and begin ovipositing within minutes of death (Kintz *et al*., 1990). Lord (1990) considered blowflies as one of the foremost important forensic indicators during the initial decomposition of a dead body, since they complete each stage of their life cycle on the carrion (Putman, 1983). They were also the most common family of insect evidence that were collected throughout the death investigation (Catts & Goff, 1992; Zehner *et al*., 2004).

The main factors that influences and affect the establishment of this larvae on carrions are competition, carrion size, temperature and humidity (Denno & Cothran, 1976; Introna *et al*., 1991).

Blowflies of the genus *Chrysomya* plays an important role in the field of forensic entomology as they can be used in determining the PMI (Ullyett, 1950; Smith, 1986; Greenberg, 1990; Gomes *et al*., 2006; Ahmad Firdaus *et al*., 2007). It was stated that the flies from this genus usually are the first group of flies found in corpses that undergo early decomposition. This *Chrysomya* species are also the most dominant flies among the large number of various fly species found on human dead bodies in previous studies conducted in Malaysia (Lee, 1984; Omar *et al*., 1994a; Lee *et al*., 2004).

The most common blowfly in Malaysia is the oriental latrine fly, *Chrysomya megacephala* (Fabricius). This fly is bright metallic green in colour and it has a pair of big bright red eyes which makes to stand out in contrast to other flies (Kurahashi *et al*., 1997). The larvae of this species were found to be the primary carrion feeders, and the adult flies show a preference for fresh remains (Byrd & Castner, 2010). In a succession study by Omar *et al*. (1994a), they discovered this fly in a monkey carcass within 30 minutes of placement in a rubber tree plantation in Malaysia. Heo *et al*. (2007), (2008a), (2008b) described that this fly is the dominant species encountered in an insect succession study using pig carcass in an oil palm plantation in Malaysia. Their study is similar that of Tullis & Goff (1987) and Richards & Goff (1997).

In various studies conducted in Malaysia the species *C. megacephala* are usually followed by *Chrysomya rufifacies* (Macquart), another forensically important fly species from this genus (Lee & Marzuki., 1993; Heo *et al*., 2007, 2008a, 2008b, 2010). The common name of this species is hairy maggot blow fly or hairy sheep maggot (Byrd & Castner, 2010). The larvae of this fly species was the most dominant larvae present on the carcass (Heo *et al*., 2007). This might be due to the predacious activity of this larva which predates other fly larva (Byrd & Castner, 2010).

Additionally, the larvae of *C. rufifacies* can tolerate higher temperature in mixed species of maggot mass (Williams & Richardson, 1984). This fly was the primary species found at death scenes in Thailand (Sukontason *et al*., 2003a). In

Hawaii, C. *rufifacies* arrived in a pig carcass on the first day of placement (Early & Goff, 1986; Tullis & Goff, 1987).

Another species of fly from this genus is *Chrysomya villeneuvi* Patton whose larvae morphologically show close resemblance with *C. rufifacies*. However *C. villeneuvi* can be differentiated by their tubercles on each body segment bearing sharp-ended spines that encircle the entire tubercle (Sukantosan *et al*., 2003b). In a review of forensically important entomological fauna collected from the human cadavers in Malaysia in the period from 1972-2002, Lee *et al*. (2004) reported that this fly species comprised of 2.23% of total of 448 specimens that were received. Though this percentage was found to be small, but yet *C. villeneuvi* was the third dominant specimen collected during that period where *C. megacephala* and *C. rufifacies* were the first and second dominant fly species obtained, respectively. Azwandi and Abu Hassan (2009) also stated that the adult of *C. villeneuvi* was never found to colonize carcass as early as the *C. megacephala* in a study conducted in oil palm plantation using monkey carcass.

Senior-White *et al*. (1940) stated that the adult habit of *C. villeneuvi* is unknown, but it is not encountered in an indoor or synanthropic environment. However in a comparative insect succession study on monkey carcass in Malaysia, conducted by Nazni *et al*. (2011); the maggot of this species was found to be the most dominant species obtained during the bloating, decay and advance decay stages. The pupae of this species have been found during the dry stage of decomposition along with other *Chrysomya* sp (Omar *et al*., 1992).

Subsequently, *Chrysomya nigripes* Aubertin and *Chrysomya pinguis* Walker are also blowfly species with substantial forensic importance due to its occurrence in human cadavers in Malaysia as reported previously (Omar, 2002; Lee *et al*., 2004). Furthermore, a lot of insect succession studies conducted in our country by using pig or monkey carcasses have described the presence of *C. nigripes* and their larvae on the carcasses (Azwandi & Abu Hassan, 2009; Heo *et al*., 2010; Nazni *et al*., 2011). Besides explaining the absence of this species in the indoor monkey carcasses Nazni *et al*. (2011) also mentioned about the presence of *C. pinguis* and *Chrysomya chani* on the carcasses.

Other than the genus *Chrysomya*, blowfly from the genera *Hypopygiopsis* and *Hemipyrellia* are also forensically important flies from the Calliphoridae family in Malaysia. *Hypopygiopsis violacea* Macquart is considered as the forensically important calliphorid since this species was reported as the first fly to arrive in monkey carcasses (Omar *et al*., 1994a; Chen *et al*., 2008; Nazni *et al*., 2011). Ahmad Firdaus *et al*. (2010) described the important diagnostic features of the second and third instar larvae of this species which collected from a corpse found lying on the ground in an oil palm estate in Johor Bahru, Malaysia. It was the first study ever in Malaysia which reported the presence of this larva on a human cadaver. *Hemipyrellia ligurriens* Wiedemann and other *Hemipyrellia* sp have been reported to be found on human cadaver by Lee *et al*. (2004).

2.4.2 Family Sarcophagidae

The common name for members of this family is flesh flies. The family comprised of over 2000 species and are found throughout the world, with most of the species occurring either in tropical or warm temperate regions (Byrd & Castner, 2010).

The larvae of the flesh flies were frequently collected from human remains (Benecke, 1998). Therefore they do occupy a high significance in forensic investigations (Zumpt & Patterson, 1952). The size of the larvae are generally larger which makes it easier for the collection from corpse at crime scene (Byrd & Castner, 2010). However the larvae of species of flesh fly are very similar to each other in appearance, making it difficult to identify them up to the species level (Smith, 1986). It requires a taxonomic specialist to make an accurate species identification of either the larvae or adults (Zumpt, 1965).

The life cycle of the flesh flies is found to be generally shorter than that of calliphorids (Denno & Cothran, 1976). Most of the flies of this family are viviparous (Crosskey & Lane, 1993), which means the female fly give birth to live youngs; the first instar larvae hatch from eggs in the reproductive tract of the female fly (Gunn, 2006). The viviparous females of sarcophagids are limited by their fecundity compared to the blow flies and house flies. They also do not deposit all their larvae in the same corpse, but rather prefer to distribute them evenly among several corpses (Galante, 2008).

Smith (1986) stated that flesh flies prefer sunlight rather than shaded conditions. However Tantawi *et al*. (1996) suspected that some of these flesh flies are not as tolerant to high temperatures in the summer which results in the decline of their breeding activity, On the other hand, sarcophagids are considered to be unimpeded by rain and fly around regardless of the weather (Erzinclioglu, 2000). In fact, study showed that they are able to fly more during the wet climate and became the first to colonize a cadaver under this condition rather than blowflies (Byrd & Castner, 2010). In studies conducted in South Carolina and Hawaii, sarcophagid flies were the first species to arrive in carrion (Payne, 1965; Early & Goff, 1986; Tullis & Goff, 1987).

The most common flesh fly found in Malaysia is of the genus *Sarcophaga*. Various species of flesh fly under this genus are characterized by a checkerboard pattern of spots on its abdomen that changes from black to grey and back with the light incidence (Chong *et al*., 1999). Lee *et al*. (2004) reported that the larvae of the *Sarcophaga* sp comprised 6.25% of the total entomological specimens obtained from the human cadaver in Malaysia from the period of 1972-2002. Few studies that were conducted using model carcasses also described the presence of this fly on the carcasses (Heo *et al*., 2007, 2008a; Azwandi & Abu Hassan., 2009; Nazni *et al*., 2011).

2.4.3 Family Muscidae

The family Muscidae has a world-wide distribution. It includes the housefly, face flies, horn flies and latrine flies. The tendency of these muscid flies to be available in domestic situations, their ubiquitous nature and close association with humans and their wide distribution accord them significantly great amount of forensic values (Byrd & Castner, 2010).

The flies from this family will visit a body soon after death, due to the attraction by any exudates rather than the corpse itself (Smith, 1986). They are found to arrive at carrions after the flesh flies and blow flies and often lay their eggs in natural body openings, at wound sites, or in fluid-soaked clothing. The larvae usually feed directly on carrions; however upon maturity, some of the species do exhibit predacious behaviour (Byrd & Castner, 2010).

This muscid flies are very common in a variety of habitats and some are strongly associated with human environments. Species from this family are found to breed and feed on refuse, excrement and carrion (Smith, 1986). Due to their food preference, they are more commonly associated with carrion during the decay and post-decay stages when the internal organs begin to be exposed. Tullis and Goff (1987) reported their findings of five muscid flies during the decay and post decay stages in an arthropod succession study conducted by using pig carcasses in a tropical rainforest on Oahu Island, Hawaii.

The house fly, *Musca domestica* Linnaeus has a worldwide distribution and is one of the most common flies in Malaysia (Chong *et al*., 1999). In a succession study conducted in an oil palm plantation in Malaysia Heo *et al*. (2008b) reported that this species have been found to oviposit on the dorso-lateral part of the body surface of a pig carcass on the very first day of placement. Apichat *et al*. (2007) noted the occurrence of adults *M. domestica* on a pig carcass in a study conducted in Thailand. However the presence of this species was of minor significance in the estimation of post mortem interval (Goff, 1991). Besides, in a review of Malaysian forensic cases from 1972 – 2002, not a single case involved the presence of this larvae.

Synthesiomyia nudiseta Wulp is another species of fly from the family Muscidae which can be found throughout the tropical and subtropical regions of the world (Byrd & Castner, 2010). It is also being one of the forensically important species in Malaysia ever since it was first recovered as a decomposer of a human remain found indoor by Omar *et al*. (1994b). Following that, Nor Afandy *et al*. (2001) recovered the maggot of this species from the bloated human remains which was found inside a building. The adults of *S. nudiseta* exhibit eusynanthropic characteristic in Malaysia, reason for they being found only near human premises (Nazni *et al*., 2007). While the maggot of this species exhibit a predacious behaviour, it has been known to even feed on *C. rufifacies* larvae (Greenberg, 1971).

Another forensically important muscid fly is from the genus *Ophyra* Robineau-Desvoidy which comprises nearly 20 species of small flies and distributed in warm climates worldwide. The fly species from this genus are recurrently associated with decaying matter, particularly on corpses (Centeno *et al*., 2002; Couri *et al*., 2009). The larvae of flies from this genus were found to predate other dipteran larvae and recovered from human cadaver during the late or active decay stage (Byrd & Castner, 2010). In Malaysia, *Ophyra* species were reported to be found on human cadaver (Lee *et al*., 2004). The species *Ophyra spinigera* were frequently obtained in a number of research studies conducted in Malayia (Omar *et al*., 1994a; Heo *et al*., 2008a, 2008b; Nazni *et al*., 2011).

2.4.4 Family Fannidae

Formerly this family of flies was classified under the subfamily of Muscidae, until they were latterly classified under separate family. Many species from this family have contributed a considerable amount of information in forensic investigations (Smith, 1986). The genus *Fannia* is the most common known fly from this family, where they are often recovered from the decomposing human cadaver when the excrement or gut contents begin to be exposed (Byrd & Castner, 2001). In a recent study in Argentina, these flies were found in decaying pig carcasses (Dominguez & Aballay, 2008; Quiroga & Dominguez, 2010).

The species *Fannia canicularis* (Linnaeus), which is also known the lesser house fly, have been documented in a case of child neglect in central Germany (Benecke & Lessig, 2001). The larvae of this fly species have been noted to migrate to drier habitats to undergo pupation. These flies are more likely available in an indoor condition rather than another forensically important fly species from this genus which has a close resemblance to it, *Fannia scalaris* (Fabricius). The larvae of this latter species can develop in anything from dung, other insects and carrions (Byrd & Castner, 2010).

2.4.5 Family Piophilidae

This family of flies are moderately small but widely distributed throughout the world (Byrd & Castner, 2010). However it is more represented in the cooler and temperate region of the Northern Hemisphere (McAlpine, 1977). They are commonly named as 'skipper flies' due to their leaping behaviour during their larval stage (Bonduriansky, 2002). The phiophilids shows a special attraction to