Chapter

Diptera Development: A Forensic Science Perspective

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Abstract

Insects, particularly Diptera, can reveal a great deal of information to investigators. By using known developmental data along with the common pattern of development displayed by flies, the time of colonization (and by logical extension, the postmortem interval) may be determined. This method requires investigators to know the exact development data of the insects at a scene and be able to do some simple calculations based on the concept of degree days (DD) or degree hours (DH). This chapter will give an overview of the methods currently used by forensic entomologists to translate the developmental cycle of flies into usable data for a crime scene.

Keywords: forensic entomology, Diptera, development, time of colonization, PMI

1. Introduction

Forensic entomology is the intersection between the study of arthropods and the justice system [1–4]. The study of arthropods, particularly insects and their close relatives, is entomology. The word comes from the Greek words "entomo," meaning insect, and "logus," meaning research. The area includes a variety of biological disciplines and areas of study, including forensics [5]. Such disciplines' common denominator is that they all include insects as the study subject.

A scene's analysis of entomological evidence has the potential to provide invaluable information about the scene and circumstances surrounding the situation. Interpretation of entomological evidence as the ability to inform an investigator about many different aspects of the case, including: colonization period and, by extension, death time; colonization season; colonization location; potential movement or storage of the remains after death; evidence of neglect; sites of trauma on the remains; and the presence of chemicals in the remains [3, 4, 6, 7]. Such data can then notify the investigation in several stages.

Insects reveal a lot to a knowledgeable researcher about a scene. First, the information most sought after is the time of colonization (TOC) estimate, which is often associated with the postmortem interval or time of death [1–4]. Most essential insects in forensics belong to the ecological class of decomposers—the ones that use the nutrients bound in dead matter [8]. Decomposers locate and exploit dead matter efficiently, and those who respond to ephemeral resources such as animal carcasses excel in the location of resources [8, 9]. Insects may arrive at a newly dead animal within minutes after death [7, 10] and either feed upon the carcass directly or colonize the carcass through egg oviposition [4, 8]. According to the resource

position output, it can be concluded that an insect with unfettered access to a carcass arrived and colonized it within minutes of animal death. The calculation of the age of the insects colonizing the animal body (TOC estimate) can therefore be used as a measure of how long this carcass was available for insect colonization and, by logical extension, how long it was dead (postmortem interval or PMI) [4, 11].

2. The dipteran life cycle

Insect age estimation relies on their basic physiology. Insect are poikilothermic, meaning their growth and development is affected by ambient temperature [12]. Lower ambient temperatures lead to slower insect growth, while higher ambient temperatures lead to faster insect growth. Each insect species has an upper and lower threshold, above or below which the insect no longer grows [8, 13]. We can describe and predict this growth based on ambient temperature by using a mathematical formula [4, 14, 15].

Investigators are able to use the ability of insects to quickly locate and colonize carrion and the ability to mathematically estimate insect age given ambient temperature to estimate a postmortem interval, given some assumptions. If we assume that insects on an animal arrived right after death and that their growth was dictated by ambient temperature, then we can calculate how long it would take an observed insect to reach an observed developmental stage at a given ambient temperature. This calculated time is the postmortem interval [3, 4, 7]. If the exact arrival time of insects at carrion is unknown, however (i.e., before or after death, animal was blocked from insect activity, etc.) this calculated time is the time of colonization interval [4, 11].

There are two genders involved in reproduction in most species. It is the female reproductive system that produces and stores eggs, provides food for eggs, collects and stores sperm, fertilizes the eggs, and positions the eggs in the environment. The male reproductive system is responsible for sperm production and for egg fertilization presentation to the female [16].

The Diptera, or the true flies, are the main forensically significant insects. Because Diptera is typically the first insect to colonize animal remains, the most widely used in forensic cases is the dipteran life cycle. Therefore, the dipteran life cycle is considered the most important to understand [3, 7, 17]. All flies start as eggs normally laid in the environment, although some eggs remain in the female's body and are placed as larvae in the environment [18]. The adult flies enter a dead animal and lay their eggs or larvae on that animal's natural body openings and wounds. The resulting larvae feed on the body until it is ready for pupation. Flies have what is known as a complete life cycle or metamorphosis: beginning as embryos, hatching into larvae, pupating, and eclosing from the pupae into an adult. The larvae are in three stages or instars, before they finish feeding on the body and move into the dispersal or wandering stage. This dispersal stage takes the larvae 15–20 ft away from the carrion and to a protected place in which to pupate [8]. The dispersing larvae will crawl under things in the environment or dig a few centimeters down into the soil, where the outer cuticle of the larva hardens into a protective shell or pupal case. Inside this pupal casing, enzyme transforms the larvae into an adult [6].

3. Calculating time of colonization

Dipteran larvae are poikilothermic, so they do not generate their own body heat [5, 16, 19]. Rather, the ambient temperature affects them, which determines how

rapidly or slowly they develop [12, 16, 19]. The warmer the temperature in the area, the faster they go through their instars and pupal stage. The higher the ambient temperature, the faster their instars and pupal stage are going through. This estate allows forensic entomologists to use information on insect growth to assess the time of colonization estimate of animal remains. This also makes temperature the most important density-independent factor for insects [4, 19].

Temperature has a profound effect on the metabolic of dipteran and the speed of development [16]. Warmer temperatures generally result in faster development, within certain temperature ranges. But, this is not accurate at extreme temperatures. Insects have both upper and lower thermal limits, below and above which the insect either dies or no longer develops. These are known as threshold temperatures [16, 19, 20]. In different species, these thermal levels are naturally different. Organisms that have evolved in tropical and warmer areas will have higher limits than those that have developed in temperate or colder areas [21]. Therefore, when it comes to temperature scales, there is a huge variation between species. The lower limits are better known than the upper limits and are significant when measuring a colonization estimate period mathematically [17, 20]. Developmental thresholds for forensically important flies are usually between 6 and 10°C and are experimentally determined. If a specific fly species developmental threshold is not available, a general rule of thumb is to use 6°C for winter species or flies in cold areas and 10°C for warm weather species or flies in warmer areas [7, 22–24].

Each stage of insect growth requires a certain amount of heat above the minimum temperature and below the maximum temperature, from egg to an adult [12, 25]. This growth rate can be represented with a linear model that represents the amount of heat necessary for a given fly to go through each of its developmental stages. These linear models are called degree day or degree hour models, because development is recorded as the temperature above the minimum developmental threshold multiplied by time (days or hours) [13, 14, 26, 27].

This model allows us to calculate the time a fly takes to develop using ambient temperature, and this formula is

$$(\text{average ambient temperature} - \text{minimum threshold}) \times \text{unit of time}$$

This formula takes the average ambient temperature over a given time period, subtracts the minimum threshold for the insect species, and multiplies the result by unit of time. The result is called a "degree day" (if the unit of time used was a day) or a "degree hour" (if the unit of time used was an hour). These are the two most common units of time seen in this type of model, simply because weather stations often record ambient temperature at daily or hourly intervals.

The formula may be used to calculate how many degree days or degree hours are necessary for an insect to get through various stages in its life cycle, as well as how many degree days have been accumulated over time. The DD/DH necessary for an insect to get through its lifecycle is determined by experiment. The data are recorded as hours needed for a species to develop at a given temperature, and these data may be converted into DD or DH simply by using the formula.

Table 1 shows several forensically important insects and the time it takes each insect to develop at various temperatures. Take, for example, the hours necessary for the black blow fly, *Phormia regina* (Meigen), to develop at 27°C. According to research, it takes *P. regina* eggs 16 h to go from freshly laid to hatching, 18 h to go through the first larval instar, 11 h to go through the second larval instar, and 36 h to go through the third larval instar. To turn this information into degree hours, simply plug the data into the equation

Egg DH:

$$(27°C - 10°C \times 16\ h$$
$$(17°C) \times 16\ h$$
$$272\ DH$$

In this case, it takes *P. regina* 272 DH to go through its entire egg stage. This same calculation may be used to determine DD:

Egg DD:

$$(27°C - 10°C \times 0.67\ days$$
$$(17°C) \times 0.67\ days$$
$$11.39\ DD$$

The number of DD or DH necessary for each stage of *P. regina* development may be calculated in this same way:

First instar DH/DD:

$$(27°C - 10°C \times 18\ h$$
$$(17°C) \times 18\ h$$
$$306\ DH \quad or \quad 12.75\ DD$$

Second instar DH/DD:

$$(27°C - 10°C \times 11\ h$$
$$(17°C) \times 11\ h$$
$$180\ DH \quad or \quad 7.5\ DD$$

Third instar DH/DD:

$$(27°C - 10°C \times 36\ h$$
$$(17°C) \times 36\ h$$
$$612\ DH \quad or \quad 25.5\ DD$$

The results may be summed up to determine the accumulated degree days (ADD) or accumulated degree hours (ADH) necessary for *P. regina* to develop from freshly oviposited egg through the end of the third instar:

$$11.39\ DD + 12.75\ DD + 7.5\ DD + 25.5\ DD = 57.14\ DD$$

This may be calculated for any range of developmental stages in an insect's life cycle.

Once the ADD or ADH necessary for insect development is known, it is possible to calculate the time necessary for the DD to accumulate at recorded ambient temperatures. The species threshold temperature is used in the same DD formula, but the average temperature is a weather station recording over a given period of time.

If, for example, a weather station recorded an average temperature of 15^0C over 1 day, and then the DD accumulated over that 1 day may be calculated as

$$(15°C - 10°C) \times 1\ day$$
$$(5°C) \times 1\ day$$
$$5\ DD$$

This calculation shows that 5 degree days accumulated over that 1 day. Each passing day may be calculated in the same way, and the results are added up to produce the accumulated degree days over any given period of time:

Average temperature (°C) on 4 separate days: 15°C, 20°C, 21°C, and 18°C
DD for each day: 5 DD, 10 DD, 11 DD, 8 DD
ADD over the 4 days: 5 DD + 10 DD + 11 DD + 8 DD = 34 DD

Once all this data is determined, the DD required for the growth of an insect species is added to the total DD accumulated over a period of time to decide how long it would take for an insect to develop into an observed stage of life given at the reported ambient temperature. It is best to display this data in table form:

Date	Average temp (°C)	DD	ADD
May 15	21	11	11
May 14	25	15	26
May 13	22	12	38
May 12	18	8	46
May 11	22	12	58
May 10	20	10	68
May 9	15	5	73

Table 1.
Example of average ambient temperatures and calculated DD/ADD from a weather station.

Note that the dates are described in descending order; this method allows an entomologist to begin with the date on which evidence of insects was collected and to work back in time to determine the minimum period of insect colonization. In **Table 1**, 11 DD were accumulated on May 15, 15 DD on May 14, 12 DD on May 13, etc. In total, it takes 57.14 DD to develop from egg through the end of the third instar.

If maggots at the very end of the third instar were found on an animal at the end of the day on May 15, the ADD necessary for those maggots to develop through the third instar may be used to determine when the eggs were laid. In this case, *P. regina* needs 57.14 DD to develop through the third instar. There were not enough DD accumulated on May 15 to reach that number, so the eggs could not have been laid on May 15 and have had time to reach the observed life stage. Since only 25 DD accumulated between May 14 and May 15, there was still not enough time for the fly to reach its third instar. Enough DD is accumulated between May 11 and May 15, however, for the insect to reach its observed life stage. In this instance, we can say the eggs had to have been laid sometime on May 11 or earlier to reach the third instar by May 15. This gives a minimum time of colonization estimation, based on the life history of the flies colonizing an animal.

The feasibility of this approach of the colonization estimation time degree day model depends on the availability of growth rate information for different necrophagous species. Such data are collected experimentally and appear to be reported as a life table or the rate of development of different insect species.

Arthropods associated with carrion are associated with ecological groups: necrophagous species, which feed upon the animal remains; omnivorous species, which feed upon both the animal remains and other organisms colonizing those remains; predators and parasites of the necrophagous or omnivorous species;

adventive species, which use the remains as an extension of the environment; and incidental species which are associated with animal remains due to happenstance [3, 28].

Necrophagous insects arrive on and in a corpse in a somewhat predictable sequence and are arguably the most useful for forensic entomology. This is the ecological succession of insects and is influenced by the environment, season, and the decomposition state of the carrion. The insects arrive in blending waves, called seres, of organisms, each comprised of different species attracted to a particular state of decay [3, 29].

Early research in entomology indicated that the number of seres varies according to the placement of the carrion. Mègnin [30] showed that animals exposed to the environment yielded eight distinct insect seres, while those that were buried attracted only three distinct insect seres. This difference speaks to the availability of the tissue for colonizing insects, along with the ability of those insects to reach tissue that is blocked from easy access. Different insects are attracted to different stages of generalized decomposition. While stages of decomposition are not discrete and can sometimes be difficult to characterize with precision, historically scientists have broken up decomposition of animal remains into five major stages: fresh, putrefaction, active decay, butyric fermentation, and dry decay [31]. Each of these stages attracts one or more seres of insects and other arthropods in a predictable sequence.

From the moment of death, the insect fauna of an animal body begins to change. Any ectoparasites associated with the body leave relatively quickly as the body cools and the blood ceases to circulate [18]. Myiasis-causing flies may or may not die—it is dependent upon if they are obligate parasites of living tissue or facultative parasites that can feed on both living and dead animals. Botflies, for example, are dependent upon a living host, and if the host dies, the botfly dies. On the other hand, *Cochliomyia* sp. can feed on living or dead hosts and may just continue to feed after the animal dies [4, 18].

Necrophagous insects or those attracted to dead tissue are attracted to the body within minutes of death and are associated with the fresh stage of decay. In general, the first adults are observed on a newly dead animal within an hour after death, as long as there is an adequate access to the body [4]. Some investigators report female flies arriving within 15 s of death [7]. Eggs and early instar maggots tend to appear with the onset of autolysis. In general, the animal will be characterized in this early sere by the presence of adult flies and fly eggs. Eggs will be found near natural bodily opening, on wounds, and sometimes in protected folds of skin or coverings. Those eggs will hatch to first instars quickly in warm environments, but there will not be a large maggot mass on the body; the tissue will still look fresh. The most common groups of insects found during this fresh stage include species from the fly families of Calliphoridae, Muscidae, and Sarcophagidae [32, 33].

As the carrion begins to putrefy, the young maggots will move throughout the body, spreading bacteria, secreting digestive enzymes, and feeding on tissue. They move as a maggot mass, benefitting from communal heat and shared digestive secretions. Larvae first feed between muscles then on the muscle fibers themselves as the maggots grow and the digestive juices get to work. The rate of decay increases, and the odors emitted from the body attract more blow flies, flesh flies, beetles, and mites. They are joined by parasitic wasps that lay their eggs inside maggots and later inside pupae. The most common insects associated with this putrefaction stage, however, are species in the fly families of Calliphoridae and Sarcophagidae [3].

Once the carrion enters in active decay, there will be several generations of maggots present on the body. Some of the maggots will be large well and enter into

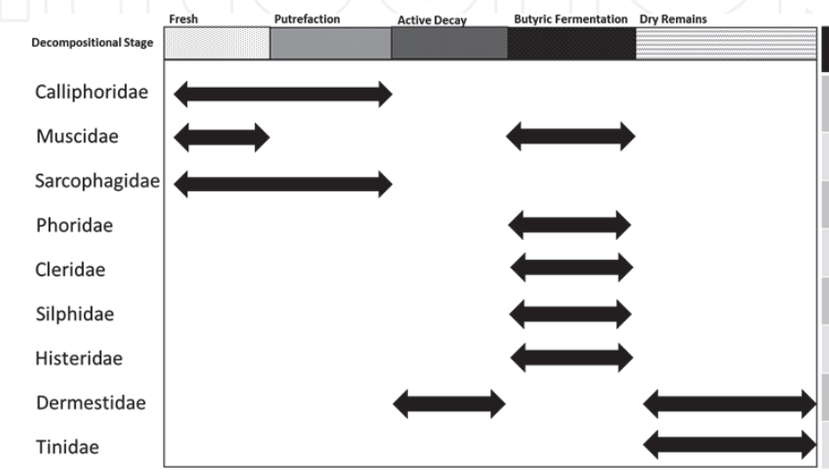
the third instar. The oldest maggots will begin to disperse from the carrion to find pupation sites. These maggots will crawl under objects in the environment or burrow into the soil to become pupae. The early seres or pioneer flies cease to be attracted to the corpse, making way for those insects that prefer later stages of decay. Predatory maggots are much more abundant at this stage and may be feeding upon other species in the maggot mass [34]. Predatory beetles lay their eggs in the corpse and their larvae then hatch to feed on the dead animal and other insects colonizing that animal. Parasitic wasps are even more common, attacking the huge maggot masses and developing pupae. Active decay attracts a new sere characterized by beetles in Dermestidae family and grease moths. As active decay moves into butyric fermentation, two additional seres are attracted: first, a sere consisting of cheese skippers, *Fannia* sp.; Silphid beetles; and beetles in the family Cleridae arrives at the body. The second sere consists of dump flies, flies in the family Phoridae, beetles in the family Silphidae, and clown beetles [3].

As the carrion is stripped of most of its soft tissue, the remaining tissue begins to dry out. The reduction in soft food makes the body less palatable to the mouth hooks of maggots and more suitable for the chewing mouthparts of beetles. Beetle adults and larvae feed on skin and ligaments, while certain late stage flies, such as the cheese fly, arrive to feed on whatever moist flesh remains. Predators and parasites are still prevalent at this stage, feeding on the straggling maggots or other soft-bodied insects in the vicinity [3, 35].

Once the carrion has completely dried out, the body attracts insects that can feed on hair and dried skin. This dry stage of decomposition is highly attractive to a new sere consisting of mites, followed by a sere of beetles in Dermestidae and Tingidae, and finally a sere of primarily Ptinidae and Tenebrionidae. This stage tends to last for a long while, since the business of feeding on dried out tissue takes a great deal of time and digestive enzymes. The beetles will remain on the bones as long as the carrion is undisturbed and has dried tissue [36].

After the dried tissue has been cleaned by the beetles, moths, and mites, only the bones remain along with empty pupal casings. There is no longer any major insect activity, and any further decomposition is accomplished by bacteria and physical factors [31].

By way of comparison, a buried animal has fewer seres with lower species diversity [37]. Fresh buried animals tend to attract flies in Calliphoridae, Muscidae, Sarcophagidae, and Phoridae. Buried animals in active decay may attract root-eating beetles, and those in dry decay are populated by rove beetles.



Sere #	Decomp. stage	Families
1	Fresh	Calliphoridae, Muscidae, Sarcophagidae
2	Putrefaction	Calliphoridae, sarcophagidae
3	Active Decay	Dermestidae, Pyralidae
4	Butyric Fermentation	Muscidae, Phoridae,
5	Butyric Fermentation	Silphidae, Histeridae
6	Dry Remains	Acari (Mites)
7	Dry Remains	Dermestidae, Tineidae
8	Dry Remains	Ptinidae, Tenebrionidae

Figure 1.
General arrival of forensically important insect families by phase of decomposition.

Since Meginin's original experiments, there have been many, many attempts to characterize the number of seres that show up on animals of different sizes. The consensus is that these successional waves range from 8 to 10, and any attempts to discreetly define the arthropod community associated with the seres is confounded by the continual nature of decomposition. There is a broad general agreement of orders and families that show up on a decomposing body [3, 4, 7]. There is also a general agreement of sequence and the idea that the first sere is primarily composed of Dipteran species. Succession is affected by the location of the carrion, any covering, animal type, local insect species, time of year, and a myriad of other factors. At the family level, the succession looks like as shown in **Figure 1**. Knowing the general succession of insects on a corpse in a particular area enables the entomologist to extend the time of colonization estimation even if the primary colonizers of the first sere have come and gone.

4. Conclusions

Diptera are currently the most important insect when it comes to forensic entomology. Detailed knowledge of common flies colonizing a body and the time taken by those flies to develop is the bedrock of the science. While much is known about this work, there is always much more to do.

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