Parasitic Hymenoptera as Forensic Indicator Species

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Additional information is available at the end of the chapter

Abstract

Necrophagous insects are the most important ecological evidence associated with a decomposing corpse. Insects provide insight into estimating the post-mortem interval (PMI), assessing whether a corpse has been moved, use in toxicological analyses, and provide utility in surveillance and as sniffer systems. Necrophagous Diptera are regarded as the most important forensic indicator species, largely because they colonize a corpse within minutes of death. Other types of carrion-inhabiting insects also offer value, although more limited than flies, to forensic investigations. Perhaps, the most neglected of these groups is the parasitic Hymenoptera, a group comprising several species that utilize necrophagous flies as natural hosts. Parasitic wasps extend the PMI window to include the period of time after necrophagous flies have emerged to when a corpse is discovered. Wasp host preferences and seasonal occurrences can reveal if a body was moved from another location prior to discovery. Foraging behavior of adults can be used to locate concealed bodies and potentially aid in combating entomological terrorism agents. Presently, the full potential of parasitic Hymenoptera as alternate forensic indicator species has not been explored. This chapter relates the life history characteristics of parasitic wasps to their potential usefulness in forensic applications.

Keywords: forensic entomology, host-parasitoid association, parasitism, synanthropic flies, Calliphoridae

1. Introduction

Forensic entomology is the subfield of forensic science that relies on insects and related arthropods for use in the judicial system. More specifically, the discipline uses information about terrestrial arthropods, namely insects (e.g., their occurrence, activity, seasonality), to draw conclusions about legal matters. Several species of insects are important physical evidence in issues relevant to civil, criminal, and administrative law [1]. By understanding the biology of

necrophagous and synanthropic insects, predictions can be made as to why and when certain insects will become associated with human habitation, stored food, or colonizing a corpse. For example, several fly species are attracted to animal remains, including humans. Deciphering which species arrive in specific biogeographical and artificial (e.g., man-made) locations to a corpse, when during the decomposition process, and how long they take to complete development under varying environmental conditions, these insects become useful pieces of physical evidence in the investigations of homicides, suicides, and unexplained deaths [2]. The use of insect biology in this deductive manner is the basis for forensic entomology. However, forensic entomology is much broader than just death investigations. The legal issues addressed range from insects invading food and related stored products, to infestation of human habitation, to insect attraction to and use of animal remains. The latter also encompasses cases of neglect and abuse of humans and domesticated animals, as well as wildlife poaching. These examples broadly define the three subdisciplines of forensic entomology: stored product entomology, urban entomology, and medicocriminal entomology [1]. Medicocriminal or medicolegal entomology has received a great deal of attention in the past three decades because of the direct impact on the human condition [3] and is the focus of this chapter.

What is the basis for insect usefulness in medicocriminal entomology? The keys are foraging behavior of necrophagous species and subsequent development of immatures on a corpse. Necrophagous insects recognize and are drawn to the odors emitted from the dead, especially from the remains of warm-blooded vertebrate animals [4]. No matter the size of carrion or location of a carcass (inside or outdoors), chemical signals associated with decomposition activate foraging in a wide range of saprophagous, predatory, and parasitic insects associated with carrion [5, 6]. This is particularly true of several families of flies. The linkage between animal death and necrophagous flies can be summed up with just one word: protein. For a female fly belonging to the families Calliphoridae and Sarcophagidae, animal tissues are rich sources of protein that are essential for producing or provisioning eggs [1]. Protein is so important to females that some males have evolved a keen chemical acuity to locate a freshly dead carcass with the goal of finding mates. The "need" for protein as well as other nutrients leads to intense competition for any type of animal remains. The fact that carrion is a finite source of nutrients, and that the occurrence (ephemeral) and location (patchy) of animal death are unpredictable, contributes to the frenzy in securing at least a portion of the nutrient prize [2]. So when a death event occurs in natural environments, many animals take notice and quickly mobilize for action. The same can be said for the occurrence of a corpse in a concealed location, with the realization that detection of emitted odors is slower and reaching a corpse is hindered by physical barriers such as seclusion in a dwelling, burial, or placement in an artificial container [1, 7]. Such barriers favor colonization by small-sized insects, but that is not necessarily what always occurs.

Competition for animal remains has shaped several aspects of the life history characteristics of insects that depend on the dead for survival. Nowhere is this more on display than with the reproductive strategies demonstrated by necrophagous flies. One group, the blow flies and bottle flies (Family Calliphoridae), is recognized as first responders to death [8]. In other words, many species are the earliest colonizers of carrion; often arriving within minutes of death or

just after a corpse becomes detected. This generally means that adults are especially efficient at detecting and finding human or other animal remains [6]. Even in this shared pattern of early colonization by multiple species, there are several adaptations that permit various species to decrease competition with each other. For example, some blow fly species prefer large carcasses over smaller for oviposition; others demonstrate preferences for carrion placed in full sun, shade, or partial shade; and some are influenced by location on the body [7]. The latter is manifested in terms of depositing eggs in concealed locales such as natural body openings versus exposed on skin, hair, fur, or plumage. Many oviposit in exposed wounds, while others only deposit eggs on very moist surfaces such as in saturated soil or carpet under a body, or on clothing or hair/fur/plumage soaked in exuded body fluids. Even eggs and larvae are adapted for competition, as eggs hatch quickly so that neonates can begin a period of rapid and continuous feeding [9]. Larvae feed cooperatively in large aggregations (maggot masses) that maximize the rate at which larval development can proceed [10]. Maggot masses are unique microhabitats that permit larvae to thrive in carrion communities [1]. Importantly, the life history characteristics of these flies and the subtle nuisances that allow each to use a corpse differently, even if just slightly, contribute to resource partitioning. The latter is a necessity for necrophagous insects utilizing an ephemeral, patchy resource as a primary food source.

Multiple groups of insects, not just calliphorids, utilize a decomposing body, arriving in waves to colonize a carcass when it is appropriate for progeny development and/or maximizes fitness of the mothers, or food/hosts is (are) abundant for predatory and parasitic species. For example, flesh flies (Sarcophagidae) can arrive in the first wave of colonizers or later during decomposition, and reduce the competition with blow flies by preferring small carrion instead of larger, depositing larvae (larviposition) instead of eggs, and the young of some species are predatory on other species [11]. Some fly species (families Phoridae and Piophilidae) do not lay eggs until the body is very dry, long after calliphorids and sarcophagids no longer have an interest in the body. Many species of beetles (Order Coleoptera) arrive early but generally after the initial wave of fly colonizers so that they can feed both on the corpse and fly eggs [12]. A few species of beetles (families Dermestidae and Cleridae) prefer dry remains, when the tissues are leathery in consistency and nearly devoid of moisture. Once the body has been reduced to bone, cartilage, and hair, insect interest wains but is not totally absent. A few moths (Lepidoptera) will feed on hair and remnants of clothing, parasitic wasps (Hymenoptera) that specialize on wandering fly larvae or puparia scattered about the remains are present, and a small number of beetle species can be found searching for scraps on the bone [13]. By specializing on different stages of decay, many organisms can successfully utilize the same resource, thereby forming thriving communities in a rapidly vanishing microhabitat.

Few of these "other" carrion inhabitants receive the attention of necrophagous flies. The net effect is that the potential of non-dipteran insects to serve as alternative forensic indicator species has not been examined in any detail. This chapter examines the parasitic Hymenoptera associated with carrion communities that offer potential utility to forensic investigations. The biology of the most common families of parasitic wasps is discussed in terms of how the life history characteristics relate to specific forensic applications.

2. Necrophagous Diptera: Primary forensic indicator species

Before turning our full attention to parasitic wasps for the remainder of this chapter, it is worth briefly examining the features of necrophagous flies that make them especially well suited for use in death investigations. This in turn will serve as a foundation for making comparisons to the traits of parasitic Hymenoptera associated with carrion communities. Certain species of flies are invaluable for estimating a portion of the post-mortem interval and also in assessing whether a body has been moved. The first key characteristic was already addressed, and it lies in the need of adult females to find protein for provisioning eggs. In reality, this is true of only some species, those that are anautogenous. Such flies tend to be first wave colonizers, often arriving shortly after death. Thus, the timing of oviposition often closely approximates the perimortem window after the agonal period. Several species of calliphorids are anautogenous, early colonizers. Many autogenous calliphorids species are also early colonizers, motivated to forage for oviposition sites that maximize female fitness. This essentially means locating carrion while protein resources for progeny development are still abundant. The latter comment leads to a second feature of necrophagous flies: faunal succession is relatively predictable for specific stages of physical decomposition [1]. Really the same is true for non-dipteran insects with the exception that the period of insect activity is often more broad than for flies serving as primary forensic indicator species. What this also means is that the absence of particular species can convey relevant information as well. For example, lack of first wave or early colonizers suggests that the body was not accessible for a set period of time, or that environmental conditions were not favorable for insect activity until a later stage of decomposition. The third key characteristic is that necrophagous fly larvae feed exclusively on the corpse, thereby linking immature development to the post-mortem existence of the deceased, which includes the habitat and environment where the remains are located [14]. A linkage to the environment is also a key feature of flies in that they are poikilothermic and derive thermal energy from the local microenvironment for development. By understanding the duration of development under a range of temperatures and environmental conditions, calculations can be made of how long a fly larva has been present on a corpse [15]. This, in turn, permits an estimation of the minimum length of time that the remains must have been available for colonization for the fly of interest to reach the stage of development found at the time of body discovery. What this means is that larval development of flies can be used to calculate the minimum post-mortem interval [16]. Such predictions based on environmental conditions also necessitate that the stage of fly development can be determined, a process that is aided by the fact that flies exhibit determinant development, meaning that there are a fixed number of larval instars regardless of changes in abiotic and biotic factors in the environment [17]. Other insects that inhabit carrion generally are not exclusively saprophagous/necrophagous such as certain species of flies and/or experience indeterminant development, and thus offer more limited use in criminal investigations.

Despite the value of necrophagous flies to medicocriminal entomology, there are shortcomings or limitations to their utility in death investigations. The most obvious is that after third instar larvae complete feeding, they disperse from the corpse [18]. This does not end the usefulness of flies, but it does reduce their value as physical evidence. Diminished value is due in part to

the fact that the larvae are no longer feeding and thus a direct linkage to the corpse has ceased. The length of the wandering stage also can be highly variable between species, decreasing the precision in estimating a minimum PMI based on any developmental stage post-feeding [19, 20]. As well, recovery of dispersed flies from the crime scene can be challenging dependent on body location, soil type, and fly species, the latter of which show a great deal of variability in terms of distance dispersed from the remains [18]. The most important factors limiting the value of flies as physical evidence are those that impact the rate of immature development [1, 15]. The key to this statement is that use of fly development in predicting the PMI is based on the underlying assumption that a linear relationship exists between the rate of larval development and ambient temperatures. Obviously any factor that influences growth independent of temperature violates this assumption and thus diminishes the value of flies, and most importantly, leads to less precise estimations of the PMI. Such factors as maggot mass temperatures, overcrowding, competition, cannibalism/predation among and between fly species, and nutritional quality of corpse tissues are just some of the influences known to alter the rate of fly development. Each of these factors is highly variable, reflecting the unique conditions of independent death events, which in turn makes them extremely challenging to incorporate into fly growth models. Thus, there is a need to compliment the use of necrophagous Diptera with other ecological evidence collected from crime scenes, such as in the form of alternative forensic indicator species. One such group is the parasitic Hymenoptera, which possess life history traits that overcome some of the limitations encountered with fly larvae.

3. Parasitic Hymenoptera as alternate forensic indicator species

All parasitic wasps that frequent animal remains rely on a parasitoid lifestyle, regardless of the species or developmental stage of host used for progeny development. What this means is that the host will die as a result of the parasitic association, because of the action of venom injected by females at the time of oviposition, envenomation via larval salivary secretions, or through the feeding activity of developing larvae on or in the host [21, 22]. Parasitic wasps are distinct from most carrion-inhabiting flies in that they are necrophilous, or attracted to carrion, but are not necrophagous, and thus do not feed on animal remains. In most instances, the small wasps utilize larvae, prepupae, or puparial stages of flies belonging to the families Calliphoridae, Sarcophagidae, and Muscidae that are feeding on, under, or near a decomposing carcass. The wasps are best known to forensic practitioners through unfortunate encounters during death investigations, when their parasitic efforts compromise attempts to raise flies in the laboratory for species identification or during developmental experiments [1]. While these frustrating occurrences are common, parasitic wasps possess untapped potential to serve as forensic indicator species; filling in key information gaps remaining after fly evidence has been considered.

The idea of using parasitic Hymenoptera as forensic indicator species is not new, but in practice, their biology is rarely applied to criminal investigations [23–25]. Why? Part of the problem stems from the fact that parasitic wasps often go unnoticed at crime scenes. The oversight is attributable to their small size (most that frequent carrion are less than 2 mm in

length) and tendency to arrive during later stages of decay, when the early fly colonizers have already dispersed or are nearing the wandering stage associated with post-feeding. In practice, early colonizers are favored as ecological evidence for all the reasons given for necrophagous Diptera. More significant than size or period of activity on carrion is that the life history characteristics of most parasitoids, other than the pteromalid *Nasonia vitripennis* (Walker), that frequent carrion have not been examined or only limited aspects of the biology and behavior of a few species are known [26, 27]. For example, developmental thresholds and temperature-influenced developmental data have been worked out for only two parasitoids, *N. vitripennis* and *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae) [28–31]. Even less is known about seasonal occurrences of parasitoids, with the most extensive work being conducted on *N. vitripennis* and to a much lesser extent with *T. zealandicus* and *Alysia manducator* (Panzer) (Hymenoptera: Braconidae) [29, 31–34], and the parasitoid fauna of most biogeographical regions has never been examined [35–37]. The data available for most species relates to their potential as biological control agents of filth flies, namely muscids, which generally do not translate to carrion communities, or the parasitoids of such flies are not encountered on animal remains [29, 38]. Despite these limitations, several parasitoids have been collected from forensically important flies in Australia, Europe, South America, and United States, and thereby are purported to be potential forensic indicator species [25, 29, 36, 37, 39–41]. In Section 3.1, an examination of whether such potential truly exists for parasitic Hymenoptera is discussed, as will the areas of parasitoid biology in need of further investigation to put them in line as alternative forensic indicator species.

3.1. The case for forensic relevance

Many species of parasitic wasps do show promise as alternative forensic indicator species, especially pupal parasitoids. The question that must be asked is if parasitic Hymenoptera were not overlooked at crime scenes (and, of course, the needed life history data were available), what information could they reveal about death? There are at least four pieces of information that can be derived from parasitic wasps in forensic investigations: (1) Parasitic wasps can extend the PMI window to include the period of time after necrophagous flies cease feeding to when a corpse is discovered, (2) wasp host preferences and seasonal occurrences can reveal if a body was moved from another location prior to discovery, (3) artifacts of past wasp activity remain at the scene for many years after they have dispersed permitting interpretation of period of activity and seasonality, and (4) foraging behavior of adults can be used to locate concealed bodies [1, 23, 42, 43]. In some instance, parasitic wasps have already been useful in case studies (i.e., PMI estimations; [23, 25, 40, 44]), and in yet others, the full potential of fly parasitoids has not been realized because key aspects of their biology remains poorly understood.

3.1.1. Extending the PMI

In many ways, parasitoids offer similar evidence as necrophagous flies, only with regard to a different period of time. For instance, many species of Hymenoptera parasitize older larvae, prepupae, or puparial stages of fly hosts, and do not emerge from the host until a few to several

weeks after unparasitized necrophagous flies have eclosed. Thus, if the developmental parameters of a given parasitic wasp are fully understood, such species can extend the PMI window from the time flies initiate dispersal behavior until the corpse is discovered. This window of time can potentially represent 2–4 weeks or longer depending on the environmental conditions and season. The best example is with *N. vitripennis*, a wasp that parasitizes the puparial stages of flies predominantly in the families Calliphoridae and Sarcophagidae, although muscids are readily utilized if discovered by foraging females. Fly hosts cannot be parasitized prior to hardening of the puparium; meaning after pupation is complete, but prior to the onset of eclosion behavior. Thus, a precise window exists into the minimum length of host development on a corpse prior to parasitism by *N. vitripennis*. A similar relationship exists for other pupal parasitoids in the genera *Trichomalopsis*, *Muscidifurax*, *Pachycrepoideus*, and *Spalangia*, but they rarely are associated with carrion, and even then, parasitism is typically restricted to muscids [35, 45]. Larval parasitoids from the families Braconidae, Diapriidae, Encyrtidae, and Ichneumonidae show less host specificity than pupal parasitoids, preferring post-feeding larvae as hosts, but also ovipositing in younger larvae and pre-pupae [26, 46, 47] (**Table 1**). Their use in PMI estimations would yield broader time ranges than those based on pupal parasitoids development on fly hosts. A similar trend is true for members of the family Figitidae that deposit eggs in young fly larvae [48], although their host age preferences are narrower than more common larval parasitoids found on carrion. The reality is that PMI estimations based on parasitoids is markedly more complex than those derived from flesh-eating flies. It is also not as simple as the suggestion by Frederickx et al. [37] that the developmental time of a given parasitoid is just added to the duration of host development. Why not? Parasitoid progeny development is influenced by multiple factors beyond just ambient temperatures, including host age, size, physiological state, species, whether the fly has been previously parasitized or not, and the size of larval feeding aggregations experienced by the host [31, 49–52]. Developmental data for a particular species of parasitic wasp must also be derived from each relevant host species for use in PMI or period of insect activity calculations [29]. This thought must be extended to also include a wide range of developmental parameters that influence parasitoid growth for *each host utilized by each parasitic wasp* discovered at a crime scene. Such data are available for very few parasitic wasp species.

Family	Fly hosts	Host stage parasitized
Braconidae	Calliphoridae	Larvae
Diapriidae	Calliphoridae	Larvae
Encyrtidae	Calliphoridae, Muscidae, Sarcophagidae	Larvae, prepupae
Figitidae	Calliphoridae	Young larvae
Ichneumonidae	Calliphoridae, Sarcophagidae	Larvae
Pteromalidae	Calliphoridae, Muscidae, Sarcophagidae	Pupae, pharate adults

Table 1. Common families of parasitic Hymenoptera collected from human remains

Further complicating the host-parasite relationship in terms of predicting wasp development times is the physiological state of the parasitoids and conditions of parasitism. Female age directly influences efficiency of foraging behavior for hosts, the length of time needed to parasitize a host, which can be especially long for parasitoids using concealed hosts, and the quality of eggs deposited on a host. Eggs from older females may fail to hatch or larvae may spend more time feeding than is typical of progeny produced by younger adults [53, 54]. A similar effect is associated with larval development on flies that have been previously parasitized by conspecifics or allospecifics [55–57]. Failure to take into account each of these influences can lead to inaccurate calculations of developmental thresholds and estimations of wasp development times, as in most instances unfavorable host conditions increases the duration of parasitoid development [51, 58, 59]. The complex interactions between parasitoids and their hosts underscore the need for standard protocols in collecting wasp development data for use in forensic entomology [31].

3.1.2. Host preferences

The limitations discussed in terms of information required to improve the precision of PMI estimations using parasitoids can be overcome with more focused research. It is also important to note that the forensic potential of parasitic wasps is not diminished by gaps in basic knowledge of life history characteristics. In fact, parasitoids have far more potential as alternative indicator species for estimating the PMI than predatory species because of their specificity as parasites. What this means is that though some species may show variability in host preferences, progeny development is entirely tied to feeding on the host. As the fly hosts are linked directly to a corpse, the parasitoid's immature development is a second-level linkage to the conditions associated with the deceased. The same features are not true with predators that visit carrion. Another key difference is that the window of time in which a host is suitable for parasitism is more predictable and generally represents a narrower time period than that in which suitable prey are available for predation. The later is reflective of necrophilous beetles that may feed on animal remains as well as prey upon eggs and fly larvae for several days to weeks, depending on the season and ambient conditions. In comparison, the window of opportunity is quite short when pupal parasitoids (e.g., pteromalids) use puparia of calliphorids. Hosts are only of useable age for 3–5 days during warmer months, and somewhat longer as temperatures decline. Even this assessment is complicated by the observation that puparial development of *Lucilia sericata* is shorter in the presence of *N. vitripennis*, thereby reducing the window for parasitism by almost 1 day at temperatures near 25°C [60]. By contrast, the puparial stages suitable to serve as hosts for some sarcophagids extend over a much longer duration (1–2 weeks) [51], decreasing the precision of a PMI calculated from wasp developmental data as the age of the host at the time of parasitism is not known (**Table 2**).

3.1.3. Seasonality

Seasonality of parasitoids is especially useful for the determination of whether a body has been moved. As with other aspects of parasitoid biology, seasonal occurrence of parasitic wasps is directly linked to fly hosts. For pupal parasitoids, those that enter winter dormancy in the form

of diapause generally do so within the hardened puparium of a fly host. Depending on the timing of diapause onset, which is influenced by latitude, several species of calliphorids are eliminated as potential hosts as they diapause as either larvae or in an adult reproductive diapause [1]. As a consequence, sarcophagids often serve as the overwintering host for pupal parasitoids that frequent carrion. Collection of parasitized hosts with diapausing wasp larvae or parasitism of hosts by a wasp that should ordinarily be in diapause can be an indicator that the body has been moved from another region in which the seasonal conditions are substantially different from the site of discovery. Widespread use of seasonal information for pupal parasitoids is quite limited at present because diapause details are only firmly established for *N. vitripennis*, and even then, only for specific biogeographical locales within North America and parts of Europe [32, 61]. The situation for larval parasitoids is similarly bleak in that though the seasonal occurrence of a few species has been reported [29], diapause has only been examined in the braconid *A. manducator*, which synchronizes dormancy with that of its calliphorid hosts [33]. Presumably other species synchronize diapause with that of their hosts and/or enter dormancy during a time that certain fly species are not available for parasitism. It is also quite likely that in certain biogeographical regions, a true diapause does not occur and that senescence similar to that of calliphorid adults that rely on reproductive diapause occurs. Much more research needs to be devoted to examining the seasonality of parasitic Hymenoptera, as the resulting data would open new opportunities for applying parasitoid biology to medicocriminal investigations.

Idiobiont	Koinobiont
Utilize puparial stages as hosts	Utilize young or old larvae, or prepupae as hosts
Manipulate host through maternal and/or larval venom injection	Host manipulation has not been examined but presumed to rely on endosymbiotic viruses of maternal origin
Host typically dies shortly after parasitism	Host continues to fed and develop following parasitism
Parasitoid development not synchronized with host and generally lasts from 2–4 weeks	Parasitoid development is synchronized with host in some cases, not in others, and development is generally longer than idiobiont species (4–6 weeks)
Adult parasitoids emerge from puparia	Adult parasitoid emerge from puparia

Table 2. Characteristics of idiobiont and koinobiont parasitoids associated with carrion breeding flies

3.1.4. Wasp artifacts

The vast majority of parasitoids utilizing carrion-inhabiting flies as hosts emerge as adults from host puparia, regardless of whether they are larval or pupal parasitoids. Exit holes

chewed in puparia reflect species preferences for emergence location and the size of the hole often typifies species [1]. Pupal exuvia also remain within the puparium, along with any unemerged larvae or pupae, providing clues regarding species identity and possibly developmental duration, which are useful in establishing a PMI based on a particular wasp species and host. Such information has also proven useful to the specialized discipline of forensic archaeoentomology, in which parasitized puparia provided insight into the burial practices of pre-Columbian civilizations in Peru [42].

Molecular artifacts may also be associated with parasitic wasps, potentially revealing information regarding developmental conditions for the wasps and/or host species. The artifacts are in the form of heat shock proteins (hsps), produced in response to various stresses experienced during development, most frequently while progeny are feeding on fly hosts. For example, larvae of *N. vitripennis* demonstrate up-regulation of hsp 23, 60, and 70 when developing on hosts that have experienced overcrowded conditions in larval aggregations [52]. Hsp expression levels correspond with species and size of maggot masses experienced by the host. Similarly, fly hosts synthesize specific hsps in response to maggot mass dynamics, and the expression continues during pupal and early pharate adult development [62], a window of time in which pupal parasitoids oviposit on discovered flies. These observations suggest the possibility that molecular markers associated with hsp expression or associated with altered gene expression of other proteins may be useful in deciphering the developmental conditions experienced by parasitic wasps prior to discovery, and may also reveal limited but useful information concerning ambient temperatures realized by their fly hosts [52]. Much more research is needed to determine if such possibilities are feasible.

3.1.5. Biosensors

Parasitic wasps do offer some advantages to forensic entomologists not yet exploited with necrophagous Diptera, namely in the form of chemical detection of decomposing bodies [43]. Under natural conditions, most species of parasitic wasps rely on chemical cues during foraging to locate potential hosts and their food resources [63]. At least with one species, *Microplitis croceipes* (Braconidae), a wasp that utilizes the larval stages of agricultural pests (Lepidoptera: Noctuidae) as hosts, can be trained through Pavlovian conditioning to associate a wide range of factitious chemicals with food [64, 65]. Odorants common to decomposition of animal tissues, that is, cadaverine and putrescine, have been used to successfully condition adult females, which, in turn, demonstrate foraging behavior in the presence of the odors [66]. Thus far the research is still in its infancy and has not yet been tested in a field setting to determine if *M. croceipes* can successfully locate a decomposing corpse emitting odors recognized by the wasps. For practical use, the parasitoids need to be tractable like has been done with honeybees, *Apis mellifera* (Hymenoptera: Apidae) equipped with GPS or radio signal detection when used as a sniffer system [67]. Other species have not been tested yet to determine how widespread is the ability of parasitic Hymenoptera to be used for the location of decomposing bodies. The technique would be especially valuable for finding concealed remains, such as buried, trapped in secluded locations, or those hidden in artificial containers.

4. Future directions

Though parasitic wasps show tremendous potential to serve as alternative forensic indicator species, their full utility cannot be realized until key gaps in information are understood. Developmental thresholds and durations of development for most parasitoids are unknown. Any experiments performed to fill this void must take into account factors that influence the host-parasite relationship, as outlined earlier, and also consider optimal host-parasite ratios during parasitism [31]. The parasitoid fauna and seasonality based on biogeographical distribution are poorly understood throughout most regions of the world. What is known represents just a few parasitic wasps from a limited number of locations (Brazil, the United States, and parts of Australia and Europe), and even then, species from just a few locales within a region have been examined. This represents a considerable deficit in background information for application of parasitoid biology to medicocriminal entomology. Parasitoids are also known to alter the development of their fly hosts dependent on the parasitic strategy adopted yet few details are understood in terms of how this impacts the use of parasitic wasps in PMI estimations. For example, larval parasitoids such as *A. manducator* and *T. zealandicus* rely on a koinobiont strategy, whereby the host remains alive and in some cases, continues to feed following oviposition [26, 47]. Parasitism is not evident usually until pharate adult development of the fly host, which means that parasitized hosts may be used for estimations of the PMI. What is not known is whether the development of such hosts has been significantly altered by comparison to unparasitized flies. There is, thus, a need to examine the impact of koinobiont parasitoids on fly development and to uncover developmental markers relevant to staging both the flies and wasps. The latter is especially critical for species that utilize multiple stages of the same host, which likely are not equally suited for parasitoid development, and consequently would be expected to yield different developmental rates for a given parasitic wasp [26]. Host manipulation by idiobiont parasitoids is much different than occurs with a koinobiont strategy in that the fly typically does not continue to develop or only in a limited capacity following parasitism, and host death usually ensues quickly after oviposition [56]. That said the only detailed work with idiobiont parasitoids of carrion flies is with *N. vitripennis*, which is a pupal ectoparasitoid. Venom injected by females during oviposition alters several keys aspects of host physiology and development [68, 69]. In less desirable hosts for progeny development, such host alterations do not occur and larval development requires a significantly longer period of time to complete [70]. This again emphasizes the need for collecting data on developmental thresholds and durations of development for each host species of interest for each parasitoid encountered at a crime scene.

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