A Roadmap for Bridging Basic and Applied Research in Forensic Entomology

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conceptual framework, succession, community assembly, quantitative genetics, functional genomics, *Daubert* standard

Abstract

The National Research Council issued a report in 2009 that heavily criticized the forensic sciences. The report made several recommendations that if addressed would allow the forensic sciences to develop a stronger scientific foundation. We suggest a roadmap for decomposition ecology and forensic entomology hinging on a framework built on basic research concepts in ecology, evolution, and genetics. Unifying both basic and applied research fields under a common umbrella of terminology and structure would facilitate communication in the field and the production of scientific results. It would also help to identify novel research areas leading to a better understanding of principal underpinnings governing ecosystem structure, function, and evolution while increasing the accuracy of and ability to interpret entomological evidence collected from crime scenes. By following the proposed roadmap, a bridge can be built between basic and applied decomposition ecology research, culminating in science that could withstand the rigors of emerging legal and cultural expectations.

INTRODUCTION

Daubert **standard:** the court standard used to evaluate scientific information used in court either as evidence or the interpretation of evidence

Population genetics: the study of the flow of genetic material within and among populations of a species

Postmortem interval (PMI): the time from death of an individual to discovery of their remains

The criteria established by *Daubert v. Merrell Dow Pharmaceuticals, Inc.* are used to evaluate scientific evidence prior to its admission in court (124). The *Daubert* decision mandated scientific evidence (*a*) be testable, (*b*) have a known error rate, (*c*) be peer-reviewed, and (*d*) be accepted by the specific scientific community employing the technique (37). This ruling profoundly altered the landscape of the forensic sciences and continues to affect them today.

A 2009 National Research Council (NRC) report (93) indicated a need for major improvements in many forensic science disciplines in order to increase accuracy and meet the *Daubert* standard. In hindsight, this report was inevitable. Calls have been made for at least ten years to restructure the forensic sciences to fit the model of self-criticism and review used by pure sciences (114). Increased media exposure of unconscious and/or fraudulent data analysis, interpretation, and presentation by forensic experts has added to highly visible exonerations (79), raising the awareness of the general public. Now that this issue of objective reliability has been brought to the attention of the forensic science community, it can no longer be ignored (78, 116). Questions such as "What are the limitations and error rates of evidence interpretation?" and "What can be done to reduce these limitations?" need to be answered by all the forensic sciences through basic research in each discipline. Answering such questions gives forensics a rigorous scientific foundation, which provides more objective and reliable evidence interpretation in a legal setting.

The NRC report (93) outlined specific areas where forensic disciplines could improve by addressing basic research questions using methods and standard practices common to basic science research. In this paper, we discuss the field of forensic entomology and propose a roadmap to guide research and practice to address the concerns of the NRC report. We propose a framework for forensic entomology (**Figure 1**) applications with a common language to streamline research questions, techniques, and data output that focuses on basic science. We believe that resulting research will eventually produce guidelines that meet the *Daubert* criteria and provide greater insight into the function of natural ecological systems, thereby developing a stronger link between basic and applied sciences. To demonstrate our concept, we provide examples of research systems that conduct basic scientific studies with direct, practical applications to the field of forensic entomology. These systems can be bridged with the wider forensic sciences, just as the principles of population genetics have been applied to forensic DNA analysis (92).

The Present State of Forensic Entomology

Forensic entomologists are frequently asked to examine arthropod evidence recovered from human remains and determine how long the arthropods were present. This time period has often been interpreted as the postmortem interval (PMI), or time since death (24). Assessment of the PMI has been grounded in arthropod development rates and community succession of arthropods. Following seminal casework by Beregeret (13) and Mégnin (87), the assumption that forensic entomologists provide the actual PMI was widely accepted. Case studies demonstrating entomologists' ability to accurately estimate the PMI have been published in books (49), research articles (10, 69), and national forensic science conference proceedings (64), and have been reinforced by popular media (120). However, arthropod-based PMI predictions are acknowledged to be associated with a number of assumptions, which can lead to severe deviations from the true PMI if violated (25). The European Forensic Entomology Association recognizes that the onset of arthropod colonization does not always coincide with the actual time of death, and in some instances can occur without death (e.g., myiasis) (4). Consequently, Amendt et al. (4) proposed that forensic entomologists reconsider their conclusions

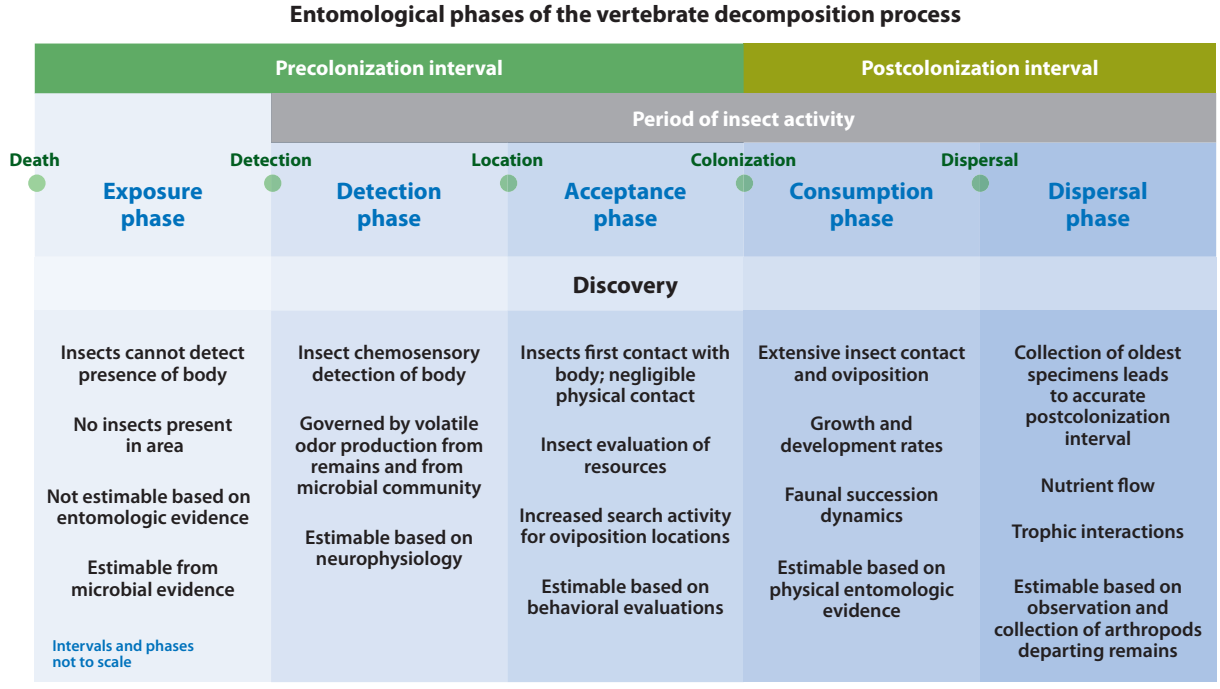

Figure 1

Framework proposed for entomological phases of the decomposition process for vertebrate remains (phases not to scale).

in terms of the period of insect activity (PIA), defined as the time from arthropod colonization until discovery of the remains.

A more explicit recognition that PMI and PIA are usually, but not always, strongly related will promote better interpretation of results and systematic evaluation of potential sources of error, a key feature of *Daubert* standards and the NRC recommendations. Consequently, those implementing current methods need to be mindful of data limitations. The proposed framework will guide research that builds understanding of the sources of error in forensic entomology by applying basic science.

Conceptual Framework for Forensic Entomology

This proposed framework is a generality, meant primarily to guide future research. Forensic entomology should be framed in terms of multidisciplinary ecological concepts to advance understanding of the carrion decomposition process and to explain observed error and variation. The framework is based on these concepts to make it objective and to provide a roadmap for the application of principles of ecology and molecular biology to forensic entomology. Furthermore, we advocate the use of terms that reflect the basic dynamics of decomposition that transcend forensics and reflect concepts that can be used to guide basic research. Undoubtedly, the temporal scale of the given intervals and phases will change depending on the arthropod species and specific environmental factors examined.

Period of insect activity (PIA): the time interval encompassing arthropod association with decomposing remains

Background and rationale. There are important elements composing the behavioral ecology of arthropods that use carrion (e.g., human remains). These include, but are not limited to, (*a*) evolutionary underpinnings of effective foraging, (*b*) carrion signaling

Precolonization interval (pre-CI): the time interval from death of an individual to their colonization by arthropod(s)

Postcolonization interval (post-CI): the time interval from arthropod colonization of decomposing remains to dispersal

characteristics, (*c*) control modes of arthropod behavioral cascades, and (*d*) mechanisms of host location and selection.

According to optimal foraging theory (OFT) as defined by MacArthur & Pianka (85), an organism maximizes its fitness by capitalizing on necessary resources while minimizing energy expenditure; however, its ability to use a resource is restricted by its sensory perception, memory, and locomotion (61). One of the major assumptions of OFT is that, over evolutionary time, the fitness advantage of more efficient foraging drives individual behaviors to converge into a species' characteristic foraging patterns (105).

Although questions about the mechanisms of carrion arthropod location, acceptance, and colonization of new resource patches are relatively new in forensic entomology, these mechanisms are well described for other systems. Host-finding behavior in parasitoids and herbivorous insects has been described as a series of decision steps with important neural events (145). It is likely that arthropods make decisions when locating carrion. We hypothesize that adult carrion arthropods such as blow flies (Diptera: Calliphoridae) emerge under one of three scenarios. In the first scenario, arthropods emerge into a habitat where both appropriate carrion resources and mates are present, requiring limited searching behavior. Alternatively, they may find a habitat that contains appropriate carrion but no mates, or vice versa. In all three cases, arthropods must detect and exploit resources to maximize their reproductive fitness. For the last two cases, they must also disperse from their natal habitat. In the proposed framework, different neurobiological events and the ensuing arthropod choices divide the PMI continuum into five phases: exposure, detection, acceptance, consumption, and dispersal (**Figure 1**). These discrete ecological phases can in turn be used to accurately and more precisely describe the phases of the PMI. It is important to note that each of the phases, particularly the consumption phase, may be prematurely ended by the recovery or destruction of the remains. In these instances, the time of discovery/recovery would function as the endpoint.

Precolonization interval. At the broadest level, the PMI is divided into the precolonization interval (pre-CI) and the postcolonization interval (post-CI). The pre-CI extends immediately after death until colonization by arthropods. Although in **Figure 1** the pre-CI visually accounts for half of the decomposition time, the actual percentage of the PMI for which it accounts will vary depending on specific conditions.

The mechanisms/motivations of neural stimulations and ensuing behavioral cascades have significant implications for the timing of discrete necrophilous arthropod interactions with carrion. Due to inherent variability in arthropod foraging behaviors and the negligible physical evidence of the interaction between arthropod and carrion prior to colonization, estimation of the pre-CI is currently problematic. The pre-CI phase is generally overlooked in the literature (87, 90, 97, 99), indicating a large void in understanding decomposition ecology. Research on this topic will help determine how PMI and PIA relate to each other, leading to a better understanding of error associated with entomologically based predictions.

The exposure phase is the time between death and exposure of remains to initial arthropod detection. In most instances, it is of negligible duration, as the body is instantly exposed to the environment. In some cases, however, remains are artificially contained, preserved, or protected from the arthropod activity, including such treatments as cold storage in a morgue, embalming, or deep burial. In criminal cases, wrapping (48), burning (8), and placement of remains in contained facilities (e.g., the trunk of a car) can substantially delay, if not outright prevent, natural arthropod succession. Because arthropods have no interaction with the remains, the duration of the exposure phase cannot currently be estimated using entomological evidence. In the future, it may become measurable, perhaps through microbial and/or biochemical assays. Nevertheless, this

phase should not be ignored as part of the total PMI.

The detection phase is made up of two stages: activation and searching. The activation stage begins when arthropods first detect decomposition cues. Arthropod resource-finding behavior is regulated by two control systems: allothetic, or the processing of external stimuli, and idiothetic, the processing of endogenous stimuli and memory (147). These control systems work in tandem to determine if neuronal stimulation will result in a behavioral cascade (89). As a result, arthropod response to carrion is shaped by external factors such as temperature, precipitation, wind speed, time of day (19), and internal factors such as mating status (39) and ovarian development.

At long distances, cues are likely to take the form of volatile chemicals produced by the carrion itself (141), the endogenous bacterial community (16, 66, 72), or semiochemicals produced by other organisms using the remains (33, 80, 124, 150). Arthropods must differentiate relevant cues from the complex suite of background odors (119), so their sensory systems may be specifically sensitive to particular odors or odor blends (15). This sensitivity to chemical properties of a resource allows individuals to discriminate among available patches and selectively forage (65). Such a mechanism can partially explain characteristic arthropod colonization and succession on carrion. As the chemical profile of the carrion changes over the decomposition process (141), species within later succession waves are activated to seek out the carrion, while the earlier colonizers are no longer activated (148). With identification of chemical cues that activate primary colonizers, and the taphonomic and/or microbiological carrion conditions that produce these cues, the duration of this phase may become measurable.

The searching stage is the time between sensory activation of arthropods and their physical contact with the carrion and is similar to the classic host-finding stage of parasitoid, hematophagous, or herbivorous arthropods (104). Among parasitoids, host microhabitat cues play as important a role in host-finding as cues presented by the hosts themselves (142). Habitat location and searching, therefore, are frequently a preliminary step to host searching. Long-distance cues are often allelochemicals released by host plants, from volatile compounds in frass, and/or by pheromones released by herbivores (146). Other long-distance cues include auditory cues from feeding or mating hosts, visual cues of plant damage, and/or the actual host itself (106). For necrophilous arthropods, long-distance cues take the form of chemical products of decomposition, body fluids, and/or the visual image of the resource. Once the carrion resource is detected, it is likely that shorter-distance cues are used by arthropods, just as is found with parasitoids. This switch is due to higher reliability and informational quality of short-range signals compared to longer-distance cues, the classic reliability-detectability trade-off of parasitoid host-finding (142). Short-distance cues of carrion take the form of low-volatility decomposition products, allelochemicals released by microbial colonists, or kairomones released by conspecific or heterospecific blow flies or other saprophages.

Arthropods may use a variety of related searching strategies and behaviors (147) to identify and track the exact location of carrion. These search strategies and their efficiency may be affected by environmental conditions such as darkness (5), humidity, and/or wind patterns (19). Like the activation stage, the mechanisms of searching behavior have an idiothetic component, which can greatly affect the speed and efficiency of foraging (147). Physiological state and learning can significantly accelerate host-finding (138). Searching behavior of a species is also limited by its sensory performance, memory capability, and structure and locomotion ability (61). Once activated, searching tends to proceed directly regardless of conditions (5), so long as the stimulus remains above the arthropod's activation threshold.

Three components are important for estimating the duration of the searching stage for a species: (*a*) appropriate identification of

the activating cues, (*b*) identification of external and internal factors that modify activation, and (*c*) characterization of spatial distribution and rate of taxis. Once these components have been identified, modeling the system to determine the relationship between cues and attraction of the targeted arthropod to the carrion source can provide an understanding of the nature and variation of insect response to cues, as well as error associated with predictions based on such models.

The first two components could be achieved through neurophysiological analysis of arthropod response to different carrion- and microbe-derived odor blends. The third component could be evaluated through both laboratory behavioral assays, such as locomotion trials and olfactometry, and field trial and observation, including mark-release-recapture studies. Accurately identifying the chemical activators and attractors of carrion is a critical gap in our understanding of necrophilous arthropod behavior and has direct relevance to PIA estimates.

The acceptance phase is the period of time from physical contact of an arthropod with carrion until the arthropod begins to establish residence on that resource. As the searching stage is similar to parasitoid host-finding (145), the acceptance phase of the necrophilous arthropod is similar to the host acceptance phase of parasitoid and phytophagous arthropods. In parasitoids, this takes the form of antennation to identify a host, followed by testing the host for suitability. During this stage, arthropods use close-range cues including color, shape, size, movement, sound, and taste to evaluate the resource (144). Like parasitoids, carrion arthropods must positively identify a resource and then determine its suitability for oviposition, which could include chemotactile contact (26). Blow flies likely use a similar combination of chemosensory taste receptors on their tarsi and labellum.

Solid acceptance criteria are critical to fitness, particularly for females evaluating oviposition sites (96). Reproductive strategies may be dictated by exposure to resources. The number of eggs deposited may vary depending on the female physiological state and the size, nutritional quality, or age of the resource (96). Experienced parasitoid females evaluate a potential site more quickly than naïve females (138); however, older females and females with a high egg load accept lower-quality oviposition sites more readily and are less likely to leave a suboptimal patch (43). Aggregative semiochemicals may play a role in influencing host acceptance, particularly for species that oviposit gregariously, such as *Cochliomyia macellaria* (Fabricius) (32) (Diptera: Calliphoridae); alternatively, semiochemicals from competitors, predators, or prey items may also shape acceptance phase behaviors (45). For species that merely feed on carrion, rather than using it to rear offspring, the acceptance behavior may be under much less selection and is a balance of phagostimulatory and deterrent inputs either as volatile or contact chemicals (26).

Accurate estimation of the activation phase requires understanding inter- and intraspecies variability in innate behavior of carrion arthropods. The behavior of primary colonizers is particularly important, as acceptance marks the onset of direct arthropod contact and extensive physical colonization of carrion.

Postcolonization interval. The second of the broad divisions of the PMI, the post-CI, is initiated at colonization (i.e., oviposition) and lasts until the departure of arthropods, either following complete decomposition or upon discovery and removal of remains in the case of forensics. It commences when arthropods begin to leave discrete evidence of their presence on remains, either as feeding damage or through oviposition. The post-CI currently represents a minimum postmortem interval (4, 128, 134).

The consumption phase is the time between the onset of colonization and arthropod departure from the remains when they no longer provide appropriate resources for sustenance or development. The consumption stage is characterized by successive waves of arthropods extensively using the carrion as a food source for themselves and/or their offspring. It is currently the best understood of the proposed

phases of decomposition relevant for PIA estimates, including studies on principles of larval development rates (24), seasonality and carcass size (31), taxon structure of successional waves (118), presence of antemortem toxins (70), and effects of intraguild predation (112). This phase is most often estimated using species-specific known larval and pupal development rates of Diptera (24) using mass (154), length (152), or stage of development (24). However, increasing evidence of biogeographic variation in forensically important species makes use of locally derived development data critical for accurate assessment of this phase (101, 128).

The dispersal phase includes the movement of arthropods previously feeding on the remains to their departure. Dispersal can occur due to the need to pupate (58, 131); to disturbance of the remains; to lack of resources (51, 53); or to interactions with abiotic factors, such as temperature, rain, or sunlight (52). Although not initially thought to affect the development cycle of dispersing individuals, dispersal prior to completion of the consumption phase could result in extended development times (11).

This proposed framework provides a flexible list of terms to describe ecologically relevant phases of decomposition, allowing researchers to describe and communicate the temporal and physical aspects of studies. It can apply to the use of carrion by an individual arthropod, by a single population, or by the entire necrophilous arthropod community.

Universal application of this framework in future research would allow for a more concrete understanding of ecology and evolution within the practice of forensic entomology. We suggest that an overall model for characterizing each phase be taken from Tinbergen's (132) "four questions" of animal behavior: causation, ontogeny, phylogeny, and adaptation. The proximate causes of "How does the behavior occur?" and "How does it change over the organism's life?" are significant primarily from a forensic perspective, and the ultimate causes of "How did it develop?" and "How does it affect reproductive fitness?" are important to understanding the natural variation associated with decomposition. Within this framework most of the underlying ecological and genetic mechanisms remain unknown or understudied. Not until the mechanisms driving carrion location, acceptance, and colonization in arthropods are better understood will we have the foundational science required to make predictions that better meet the *Daubert* criteria.

ECOLOGY OF DECOMPOSITION

As an example of how this framework can be used to facilitate basic and applied research, we consider an important issue for forensic entomology: variability in carrion succession under natural conditions. **Figure 2** illustrates the visual differences in decomposition among six swine carcasses that were euthanized at the same time and photographed on the same day during decomposition.

A fundamental question of ecology asks how and why communities of organisms assemble under various environmental conditions. Living organisms act as discrete patches of space, nutrients, and energy that are made available soon after death. In many ecosystems, these remains are food-falls that act as resource subsidies to the local habitat, often with a functional impact on the surrounding ecosystem (16). The process by which these resources become available to the ecosystem is limited by the natural succession of organisms that occupy and modify the patch over time. This succession is defined by the species that assemble from the wider regional species pool (139). As species modify resources in ways limiting or facilitating additional use by other species, they affect the rate and permanence of species assembly. The biological interactions that occur during colonization are complex and often habitat dependent (16).

Abiotic variables affect networks of local community patches (i.e., metacommunities) by habitat modification and production of conditions that limit species distribution, competitive ability, and persistence in the landscape. Understanding how abiotic conditions interact with biotic communities can be important for

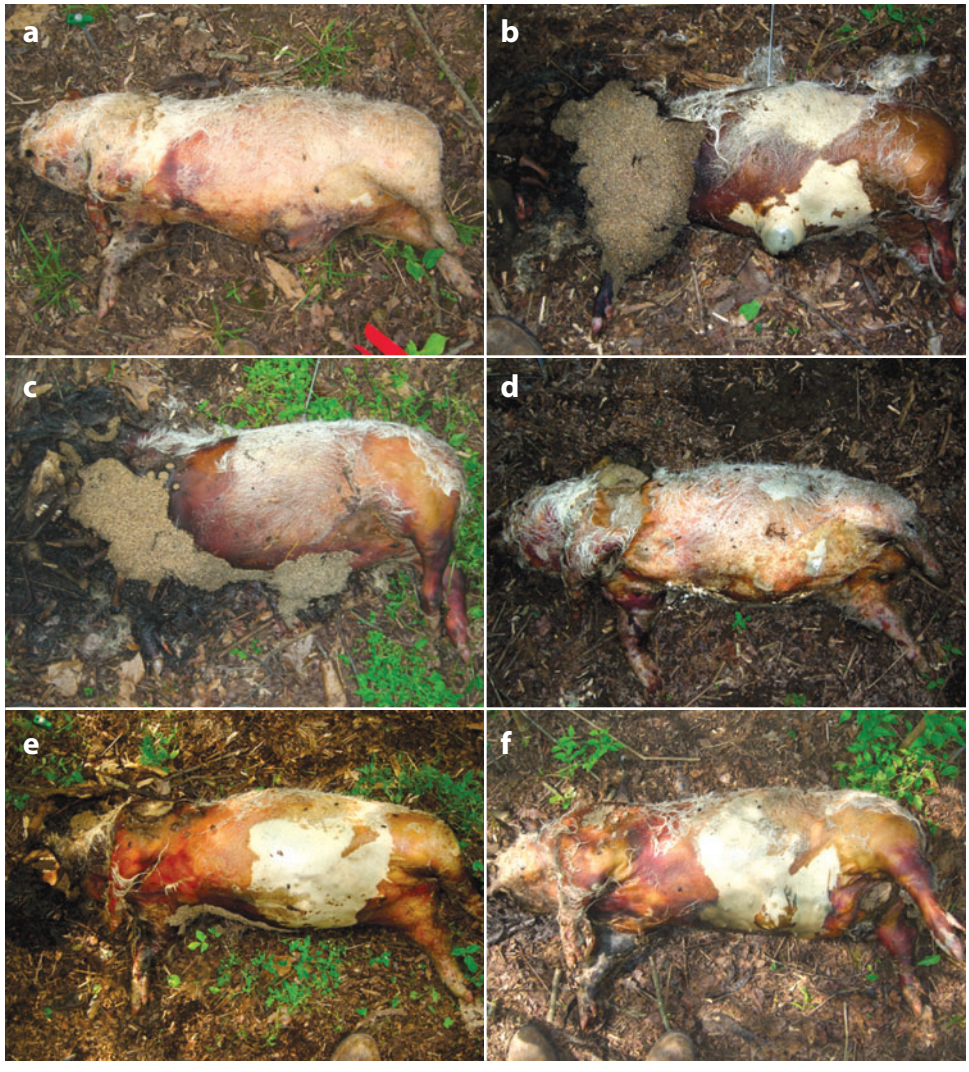

Figure 2

Six replicate swine carcasses all euthanized and placed in the forest on the same day and then photographed here on the same day during decomposition. Note that two of the six (panels *b* and *c*) have large larval blow fly masses, indicating differences occurring during decomposition.

Metacommunity: several ecological communities that are linked together through the dispersal and gene flow of multiple, often interacting, species in a local geographic area

predicting metacommunity assembly (16, 103), with implications for identifying and describing variation in arthropod assembly on human remains under different conditions (139). By further expanding our understanding of how these mechanisms operate over space and time, we will be able to predict more accurately how and in what way a carrion resource will be colonized.

ECOLOGICAL APPLICATIONS TO FORENSIC ENTOMOLOGY

In the criminal justice system, forensic entomology uses data derived from arthropods that have evolved to colonize and consume decomposing animal remains (19, 59). Understanding the timing of arthropod colonization of a body is useful in estimating a post-CI. As

previously mentioned, many abiotic factors can affect entomologically based post-CI estimates (23). Substantial variation in the arrival and succession of arthropods on remains can reduce the effectiveness of using entomological succession data in criminal cases (**Figure 2**).

Because arthropods have predictable life histories, habitats, known distributions, behaviors, and/or developmental rates, the presence/absence and developmental stage of certain species at a crime scene can provide important information about when, where, and how a particular death occurred (19). Arthropods play a natural role in the decomposition of carrion by consuming organic material and recycling energy and nutrients. They are thought to follow predictable rules of community assembly, but this idea has not been robustly tested in replicated field and/or laboratory studies. When an organism dies, bacteria that were once held in equilibrium by the immune system immediately begin to digest proteins, lipids and carbohydrates as energy sources, creating both gaseous and liquid by-products that act as olfactory cues for colonization by arthropods (16, 72). In most instances initial colonizers are adult blow flies that feed and lay eggs or live larvae on the remains (6, 19, 88).

Decomposing remains act as a food resource patch for newly hatched larvae that develop at temperature-dependent rates (**Figure 2**). The presence of blow fly larvae attracts predators and parasites such as beetles (113), mites (99), ants (50), wasps, and spiders (99) that parasitize or feed on the eggs, larvae, and/or pupae of the flies. This suite of species is followed by other species that come to feed on previously eaten or conditioned (e.g., dry skin) remains in a succession of arthropod species that colonize and ultimately decompose the carrion to dry bones and hair (19).

In most studies of forensic entomology, swine carcasses have been used as models for human decomposition (99). Many studies have evaluated arthropod colonization of swine remains during the post-CI (**Figure 1**). Few have examined the time interval from death to initial insect contact (140) or colonization (56, 87, 90, 97). Based on the few studies examining initial contact, the temporal variation can span from 30 s (140) to several hours (149) or days (121, 133) after death. Lacking information on this phase limits our understanding of the ecological variation of the entire decomposition process.

Quantitative genetics: the study of the inheritance of complex traits

QUANTITATIVE GENETICS AS A MEANS OF DECREASING ERROR IN FORENSIC ENTOMOLOGY

The analysis of DNA has set the standard by which other forensic sciences are measured (115). Molecular research is well established in forensic entomology species identification (153), but an understanding of the role of genetics in development and behavior of necrophilous arthropods will help decrease error in forensic entomology. Although this section focuses on blow fly biology, the principles discussed in this section can apply to other forensically informative arthropods.

Quantitative genetics attempts to identify and understand variation in continuously variable phenotypes (28, 38, 84, 86). The basic premise underlying quantitative genetic research is that phenotypes can be affected by genetic differences among individuals, environment, and/or by interactions between the two. This basic concept is demonstrated with a reaction norm (**Figure 3**), which can be used in part to identify the contributions of each component and interaction to a trait. This concept is shown by the equation

$$P = G + E + G \times E$$

where P is the phenotype, G is the genotype, E is the environment, and G × E is the interaction between genotype and environment.

Forensic entomologists use two quantitative traits, body size and development time, to estimate the post-CI using blow fly evidence (127). Accordingly, it stands to reason that forensic entomologists should approach the study of these phenotypes and their use in the forensic setting within a quantitative genetic framework. Doing so will enable the discipline

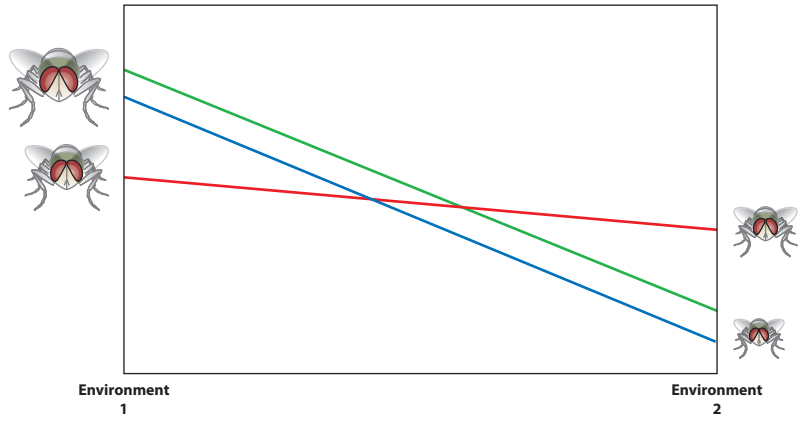

Figure 3

A theoretical reaction norm for blow fly body size. E1 and E2 represent different environments. The colored lines connect average phenotype scores for different genotypes. A significant difference in the phenotypes (the larger flies in E1) between environments means that the trait is plastic with respect to the environments tested. A significant difference between genotypes (*green and blue lines*) means there is a genetic component to body size variation between these two groups. A significant difference in the slopes of the lines for each genotype (*red line versus the green and blue lines*) is a genotype by environment interaction. Genotype by environment interactions observed among populations are indicative of local adaptation, when the phenotype affects the fitness of an organism.

to reduce error in estimates with blow fly evidence by helping practitioners understand how deviations from published developmental data may occur and subsequently allow them to account for these factors in analyses. To date, the discipline has addressed some aspects of the basic quantitative genetic equation but overall has not considered blow fly development in a quantitative genetic context.

The concept of plasticity, an environmental response, in blow fly developmental phenotypes is widely appreciated, and such studies will likely continue. The effect of temperature on development rates has resulted in numerous studies of species-specific developmental times under laboratory-controlled treatments (17, 18, 91). Such studies aid investigators in predicting development rates under field conditions experienced in real-world casework (57). However, other environmental and biological factors, including larval density and food moisture (54, 127), can influence fly development (27, 75).

The genetic side of the quantitative genetic equation has been less fully appreciated in forensic entomology. Because each species has its own unique developmental profile, it is important to correctly identify the species collected as evidence. Accordingly, there is a body of research designed to enable the use of gene sequence as a means of species identification (153). However, it may also be important to determine population-specific development. Grassberger & Reiter (57) made reference to zoogeographic differences among *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) populations to explain differences between development rates of a local Viennese population and that reported by other studies. Likewise, Greenberg (58) found intraspecific differences in development rates of *L. sericata* between Russian and midwestern United States populations. A more recent comparison of *L. sericata* populations from California, Michigan, and West Virginia found significant differences in pupal length, pupal weight, and minimum development times (126) and further found that body size differences among populations could be a result of intraspecific variation in the point at which larvae physiologically commit to pupation (128). These

observations are also supported by evidence that *L. sericata* populations from Sacramento and San Diego, California, and Easton, Massachusetts, develop at different rates (44). Although the field of forensic entomology is beginning to conduct studies of intraspecific variation in development, it is clear that, even within relatively small geographic areas, there are differences between populations of forensically informative flies. This variation means that, just as it is important to know which species of blow fly was collected, it may also be important to know the originating source populations.

These observations of blow fly population differentiation in developmental rates are supported by a wealth of quantitative genetic observations in other Diptera. There are repeated occurrences among *Drosophila* species of population-based differences in development times and body size (71, 73, 95, 98, 136). Some of the most striking observations are chromosomal inversions exhibiting population frequencies that strongly correlate with latitudinal clines of body size. These inversion clines occur in both the ancestral and newly inhabited continents occupied by *Drosophila melanogaster* (Meigen) (22, 77, 108, 109) and *Drosophila subobscura* (Collin) (21, 47), indicating the presence of selective pressure maintaining latitudinal variation in these traits. Among nondrosophilids, different populations of *Scathophaga stercoraria* L. (Diptera: Scathophagidae) exhibit heritably variable body sizes and development times (14, 34, 110), and populations of *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) exhibit a developmental duration cline from Mexico to Michigan (40).

Genetic variation in plasticity, resulting in a genotype by environment interaction, has also been demonstrated for fly populations. The leaf miner *Liriomyza sativae* (Diptera: Agromyzidae) exhibits genotype-dependent shifts in development time on different plant hosts (143). Similarly, the goldenrod gall midge, *Dasineura folliculi* (Felt) (Diptera: Cecidomyiidae), shows different host preferences between genotypes (35). These host races also have differences in wing size, abdominal segment allometry, and ovipositor length. Clearly, populations of nonforensically informative flies can have variable development times, body sizes, and/or morphology, warranting more detailed investigation of the quantitative genetics of life-history traits in forensically informative flies.

POPULATION GENETICS IN FORENSIC ENTOMOLOGY

As evidence mounts for intraspecific development rate differences among forensically informative populations, it becomes necessary to account for such variation, just as interspecific developmental differences are presently considered. Doing so will lead to greater acceptance of entomological evidence under the *Daubert* criteria, allowing for more confident and accurate estimations of the PIA.

Fortunately, there is a wealth of research outlining how distinct populations within a species are identified by population genetic analysis. It is routinely done in conservation genetics (reviewed in Reference 12), in many gene-mapping experiments when population structure correlates with a phenotype (156), and within the forensic sciences, as human populations have different frequencies of the same alleles, requiring the use of population-specific databases to more accurately identify individuals that belong to those groups (92). Tests of deviation from Hardy-Weinberg (HW) equilibrium (76), inbreeding coefficients/fixation indices such as Wright's F_{st} (68, 155), and isolation by distance (3, 42, 111) can all be used to identify differentiated populations. F_{st} can also be compared to quantitative trait divergence among populations (Q_{st}) to identify the effects of selection on a trait (83).

Often inbreeding coefficients are assessed with a small panel (tens of loci) of neutral markers such as microsatellites, describing the general level of differentiation among groups. It is possible for populations to diverge genetically without diverging in phenotype (i.e., F_{st} indicates different populations, but Q_{st} does not). Similarly, populations may phenotypically

Functional genomics: the genome-wide study of gene function

diverge (i.e., F_{st} indicates no population structure, but Q_{st} differs) if gene flow is high and the trait of interest experiences differential selection among the environments encountered by specific populations (as in the *Drosophila* examples). In these cases, more detailed evaluations such as functional genomic studies may be necessary to find regions of fly genomes associated with causal variation in phenotypic differences. When such variations are observed, it is critical that the forensic science community makes genetic observations in forensically informative fly species, as this will identify error in PIA estimates due to population-level differentiation.

The presence of rare or unique combinations of genetic material will thus aid in identifying distinct populations of a species and potentially reveal portions of the genome that correspond to phenotypic divergence. Genomic tools have already been used to identify regions of the genome that have differentiated between specific canine breeds (151), human populations (94), and reproductively isolated forms of *Anopheles gambiae* (Giles) (Diptera: Culicidae) (137). Such data could be used as markers of phenotypic differences between populations and will enable functional genomics studies (9) that are useful for predicting blow fly age (129, 130). Currently, genomic data are publically available for only one potentially forensically informative species, *Sarcophaga crassipalpis* (Macquart) (Diptera: Sarcophagidae) (62); unfortunately, this species is rarely used as a forensic indicator (2).

Clearly, at least some of the population genetic analyses described here must be conducted on fly species of forensic importance if unique/divergent populations are to be identified. Unfortunately, these types of analyses have not been routine practice in forensic entomology. To date, there have been a number of analyses of conserved mitochondrial and nuclear DNA sequence variation (153), but these particular analyses have not reliably identified specific populations of forensically informative flies. Cuticular hydrocarbon profiles have been evaluated in *Phormia regina* (Meigen) (Diptera: Calliphoridae). These traits vary extensively across *Drosophila* populations (30, 36, 135) and could be used to differentiate between sexes and populations (20), but initial work has not been pursued. There have been several amplified fragment length polymorphism (AFLP) and microsatellite studies of *P. regina*, *L. sericata*, *Lucilia illustris* (Meigen), and *Cochliomyia hominivorax* (Coquerel) (41, 60, 101, 102). Results in the United States indicated that nonselected markers are unlikely to change significantly across populations and that developmental data collected from flies caught at the same time may result in genotype-biased results (101, 102). In Sweden (41) and Brazil (60), a large degree of inbreeding has been found, but genetic variation was maintained across the populations, likely due to large effective population sizes. Additional studies such as these will be necessary to gain an understanding of how the flow of genes within and among populations of blow flies might affect variation in traits of interest.

A NEED FOR MOLECULAR TOOLS IN FORENSIC ENTOMOLOGY

One limitation to population and functional genetic analyses of forensically informative species is the lack of sufficient genetic sequence data. Frequently, genetic analyses of blow flies are restricted to mitochondrial DNA or ribosomal sequences, which are typically similar enough in sequence among species to enable comparison but different enough to distinguish close relatives. However, genetic tests that are useful for identifying populations rely either on genotyping a panel of neutral loci, such as microsatellites (41) and AFLPs (101), or on a dense array of genomic data to evaluate genome-wide patterns of relatedness (94, 151). As these genomic resources are generally lacking in the forensic entomology community, there should be a focus on the identification and evaluation of genetic markers (41, 101), which can be used to identify population structure and regions of the genome that have undergone selection.

Once genetic and genomic tools are available, there are some steps for the next generation of forensic entomology research. There should be efforts to characterize the degree of phenotypic divergence among fly populations. In cases in which genetic variation for forensically informative traits is demonstrated, studies should be conducted to determine if population identity correlates with variation in phenotype and to characterize the population structure present in the species. If no correlation between trait variation and population membership exists, then finding the genetic variation that correlates with phenotypic divergence must be attempted. If populations develop differently and markers for these divergent phenotypes can be found, evidentiary flies should be considered only locally informative. As appropriate, flies should be assigned to their proper population and/or phenotypic class before their ages are estimated with developmental data. This practice should result in lower error rates for PIA estimates, due to a better fit of predicted development rates to true development rates. In the cases in which genetic variation in forensically informative traits is demonstrated but marker loci are not found, efforts must be made to explore the full range of expected variation, which should then be used to calculate confidence intervals on predictions with arthropod evidence. Although this will likely result in larger confidence intervals for entomologically derived predictions, they will be more realistic and solidly supported by basic science.

FORENSIC ENTOMOLOGY IS AN APPLICATION OF ECOLOGICAL GENETICS

Ecological genetics/genomics is devoted to understanding the inheritance of ecologically relevant phenotypes. Accordingly, the field relies heavily on studying the quantitative and population genetics of adaptations (7, 46, 67, 74). Natural variation in developmental rates, life-history traits, stress tolerance, oviposition preferences, mating behaviors, disease resistance, and predator avoidance behaviors (to name a few) affect the survival and/or reproductive output of an organism in its natural environment. Furthermore, this variation affects the duration of each phase or stage of our proposed framework (**Figure 1**). Understanding the underlying causes of natural variation would lead to a greater appreciation of the variation associated with both pre-CI and post-CI.

Ecological genetics: the study of the inheritance of ecologically important phenotypes

Ecologically relevant phenotypes are also part of the forensic entomology roadmap presented in this paper. Like many organisms, necrophilous flies must find and attract mates. They need to detect, locate, and evaluate a resource to colonize and ensure that their offspring gain access to that resource. Once the flies have colonized a resource, they must compete with conspecifics and heterospecifics for an ephemeral food source. All the while, they must avoid predation, parasitism, disease, and more successful competitors in a variety of environmental settings. These challenges are not unique to forensically informative flies but are encountered in a wide variety of organisms employing a range of adaptations to meet those challenges. Many of those adaptations (as discussed above) have been studied within the context of ecological genetics with great success. A detailed understanding of the sources and consequences of variation in fly development could provide the discipline of forensic entomology with needed valid error rates in blow fly development time. Forensic entomologists should not consider their research just as a problem in applied developmental biology, but rather a problem in applied ecological genetics.

CONCLUSION

In the late 1970s and early 1980s, evolutionary and ecological scientists evaluated the state of their fields and resolved to develop a more hypothesis-driven approach to research (1, 29, 55, 63, 81, 82, 100, 107, 122, 125). This movement provided the foundation for improved science that led both to notable advances in evolutionary ecology and to a maturation of the field. This change paved the way for rapid advances in technological and theoretical

developments, as seen in the successful application of sophisticated ecological genomics research discussed above. We propose that the state of forensic entomology has reached a similar point of development. The field has made great strides in documenting and characterizing many aspects of terrestrial carrion decomposition through observational studies. Although there is always a need for better descriptions, it is equally important to ask quantitative and mechanistic questions that address why carrion decay proceeds as it does and why the participants behave as they do. These are not questions that can be answered under the current observational paradigm and are addressed in this review.

The publication of the NRC report has brought the field to a crossroads. It can either continue down the current path of conducting research that limits the application of data to the traditional assumptions of forensics, or it can embrace a research agenda devoted to identifying and understanding the underlying mechanisms and sources of error associated with arthropod-based predictions. Pursuing the course suggested here will align the field with standards and requirements imposed by the *Daubert* standard, by the 2009 NRC report, and by basic science. We have provided a roadmap for the field to make choices that will continue to strengthen the science at a more efficient and rapid pace with a unified language and scientific philosophy.

> "*Although obstacles exist both inside and outside forensic science, the time is ripe for the traditional forensic sciences to replace antiquated assumptions of uniqueness and perfection with a more defensible empirical and probabilistic foundation.*"
>
> *Saks & Koehler* (*115*)

SUMMARY POINTS

1. The NRC released a report in 2009 calling for forensic-related research built on a stronger scientific foundation.
2. We propose a new roadmap and framework to unify basic and applied decomposition research.
3. The roadmap is intended to advocate the use of terms that reflect the basic dynamics of decomposition that transcend forensics and reflect concepts that can be used to guide basic research.
4. The framework is grounded in understanding the ecological, evolutionary, and genetic mechanisms happening during carrion decomposition.
5. The framework advocates a standard language for describing the ecological activities occurring during decomposition and identifies major intervals and phases that characterize ecologically important transitions of the process.
6. We demonstrate that the pre-CI is largely understudied and could hold the key to the basic parameters regulating community assembly.
7. Forensic entomology can be aligned with basic biology research by studying the phenotypes inherent to the proposed framework in an ecological genetic context.

FUTURE ISSUES

1. The role of microbes associated with decomposing remains as mediators of colonization by arthropods needs to be further explored.

2. The ecological role of microbes on decomposing remains and how they mediate trophic interactions, nutrient processing and ecosystem services should be described and quantified.
3. Common mechanisms of arthropod detection, attraction and use of organic living and nonliving resources should be identified.
4. Error rates for predicting the duration of carrion decomposition by understanding sources of abiotic and biotic variation during the process should be developed.
5. Estimates of the PIA as it relates to the PMI of a corpse should be validated.
6. Genetic and genomic tools for necrophilous arthropod species need to be developed.
7. Ecological genetic studies of phenotypes used to make PMI estimates and that are important for colonization of, and survival on, carrion should be conducted.

DISCLOSURE STATEMENT

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