Wayne D. Lord,[1] *Ph.D. and John F. Burger,*[2] *Ph.D.*

Collection and Preservation of Forensically Important Entomological Materials

REFERENCE: Lord, W. D. and Burger, J. F., **"Collection and Preservation of Forensically Important Entomological Materials,"** *Journal of Forensic Sciences*, JFSCA, Vol. 28, No. 4, Oct. 1983, pp. 936-944.

ABSTRACT: The insects and other invertebrates colonizing corpses as decomposition progresses can provide valuable information concerning the time and manner of death. Accurate determinations are possible, however, only when representative specimens are properly collected and preserved. The protocol developed by the authors describes equipment and techniques for sampling, preserving, packaging, shipping, and rearing forensically important insects. This information should aid medicolegal professionals in data collection, allowing accurate determinations by entomological means.

KEYWORDS: pathology and biology, entomology, sampling, preservation, rearing, Insecta, time of death, Diptera, Coleoptera

Forensic entomology is based on the analysis of insects and other invertebrates sequentially colonizing a corpse as decomposition progresses, and on the developmental stages of their offspring. The use of entomological information in determining manner of death, movement of a cadaver from one site to another, and length of the postmortem interval is well documented [*1-12*]. A comprehensive review of the subject and bibliographies are presented by Nuorteva [*11*] and Smith [*12*], respectively.

Although accurate forensic science determinations depend upon the proper collection, preservation, and rearing of entomological specimens, detailed descriptions of these techniques are not available in the forensic science literature. Brief descriptions of sampling and rearing procedures are presented by Easton and Smith [*9*] and Nuorteva et al [*4*].

The following paper is a protocol for the collection, preservation, and rearing of forensically important entomological materials. Attention is given to the recognition of major carrion-frequenting insect groups, representative sampling, methods of preserving adults and immature stages, rearing techniques, packaging and shipping, and important supplemental data. This information should aid medicolegal professionals in data collection, allowing more accurate forensic science determinations by entomological means.

Collection Procedures

While many insects may provide information of forensic science importance, two groups, Diptera (true flies) and Coleoptera (beetles) are most important during the first two months of

Scientific Contribution No. 1195 from the New Hampshire Agricultural Experiment Station. Received for publication 28 Oct. 1982; revised manuscript received 4 Feb. 1983; accepted for publication 7 Feb. 1983.

[1]Graduate research assistant, Department of Zoology, University of New Hampshire, Durham, NH and Shoals Marine Laboratory, Appledore Island, Isles of Shoals, ME.

[2]Associate professor, Department of Entomology, University of New Hampshire, Durham, NH.

decomposition. One must recognize adult and immature stages of locally abundant carrion-frequenting insects to make meaningful specimen collections. The major families of forensically important insects are given in Table 1. Several authors have described the biology and identification of these and other groups [*12-19*].

Collect representative insects (both adults and immature) on, in, and beneath the corpse. Adult forms may be killed and preserved immediately. Divide representative samples of immature stages into two subsamples. Preserve one immediately, and retain the other alive for later rearing to adult stages. Collect sufficient numbers of individuals to ensure complete representation of the insect populations present. When available, collect a minimum of 100 immatures for rearing. Basic materials necessary for collection of entomological specimens are given in Table 2. These materials can be carried in a small box or kit and stored with other specialized equipment until needed.

Flying Insects

Flying insects can be collected with a standard insect or a short-handled hand net. It is important to make collections *as soon as possible*. Once collected, specimens can be retained indefinitely for analysis. Preserve all forensically important adult insects in 70% ethanol, or isopropyl alcohol diluted 1:1 with water. Higher concentrations of isopropyl alcohol may cause specimens to become brittle. Do not use formalin to preserve insects, unless no other preserving fluid is available. Transfer formalin preserved material to alcohol as soon as possible. A small hand net and preserving fluid can easily be carried as part of standard equipment.

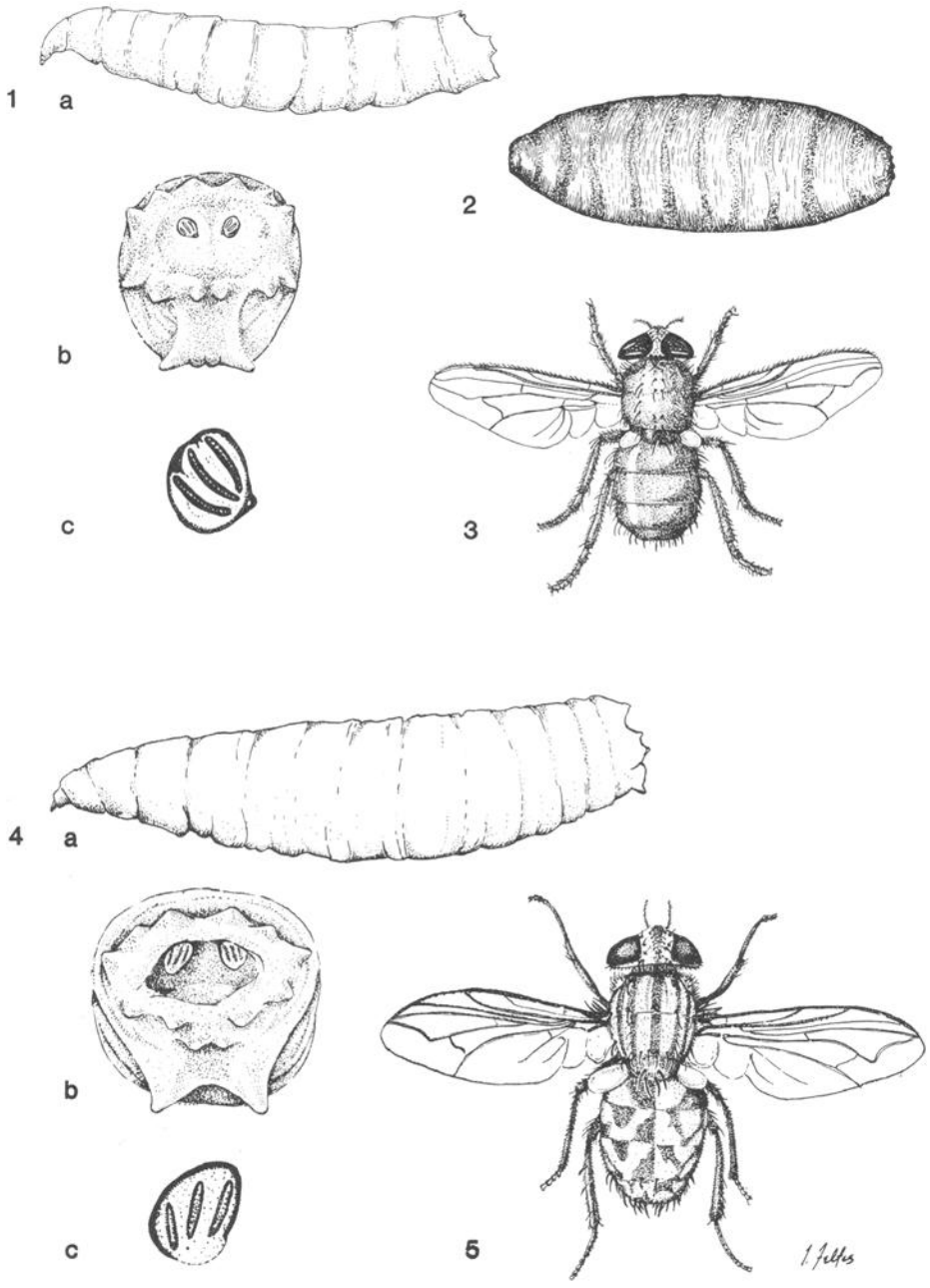
Crawling Insects

Collect crawling insects from the surface of, and within the corpse using forceps or with fingers. During sampling, hands should be protected with surgical gloves at all times. Collect smaller specimens (under 5 mm) with a small artist's paint brush dipped in the preserving fluid.

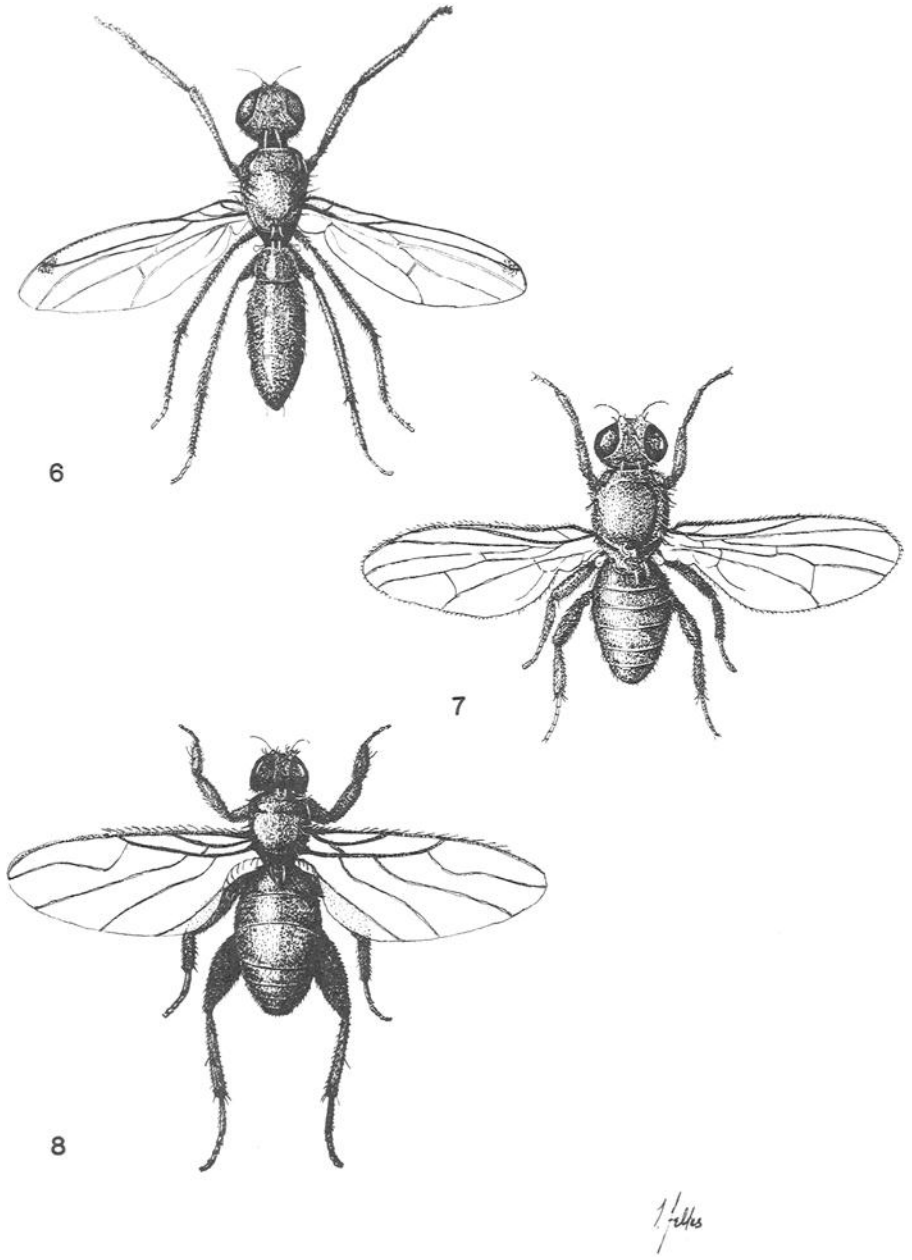
Collect crawling insects on the ground beneath the corpse by scooping the top few centimetres of soil into a plastic bag. Chill the bag of soil, if possible, until the insects are extracted and preserved to prevent further growth or possible predation and asphyxiation. This is particularly important when wandering fly larvae are preparing to pupate and are subject to predation by beetles and other soil-inhabiting insects.

TABLE 1—*Families of forensically important insects.*

DIPTERA (TRUE FLIES)
1. Muscidae (muscid flies)
2. Calliphoridae (blow flies) (Figs. 1*a-c*, 2, and 3)
3. Sarcophagidae (flesh flies) (Figs. 4*a*-c and 5)
4. Sepsidae (scavenger flies) (Fig. 6)
5. Piophilidae (skipper flies) (Fig. 7)
6. Phoridae (scuttle flies or coffin flies) (Fig. 8)
7. Sphaeroceridae (small dung flies)
COLEOPTERA (BEETLES)
1. Dermestidae (skin beetles) (Figs. 9 and 10)
2. Silphidae (carrion beetles) (Fig. 11)
3. Histeridae (hister beetles) (Fig. 12)
4. Trogidae (trogid beetles) (Fig. 13)
5. Staphylinidae (rove beetles) (Fig. 14)
6. Nitidulidae (sap beetles)



FIGS. 1-5—*Adult and immature stages of Calliphoridae* (1-3) *and Sarcophagidae* (4-5) *where 1*a *is larva of* Calliphora coloradensis; *1*b *is posterior view of* C. coloradensis *larva; and 1*c *is posterior spiracle of* C. coloradensis *larva (third instar). Fig. 2 is puparium of* Eucalliphora lilaea. *Fig. 3 is adult of* Phormia regina. *Fig. 4*a *is larva of* Blaesoxipha plinthopyga; *4*b *is posterior view of* B. plinthopyga *larva; and 4*c *is posterior spiracle of* B. plinthopyga. *Fig. 5 is adult of* Sarcophaga bullata.

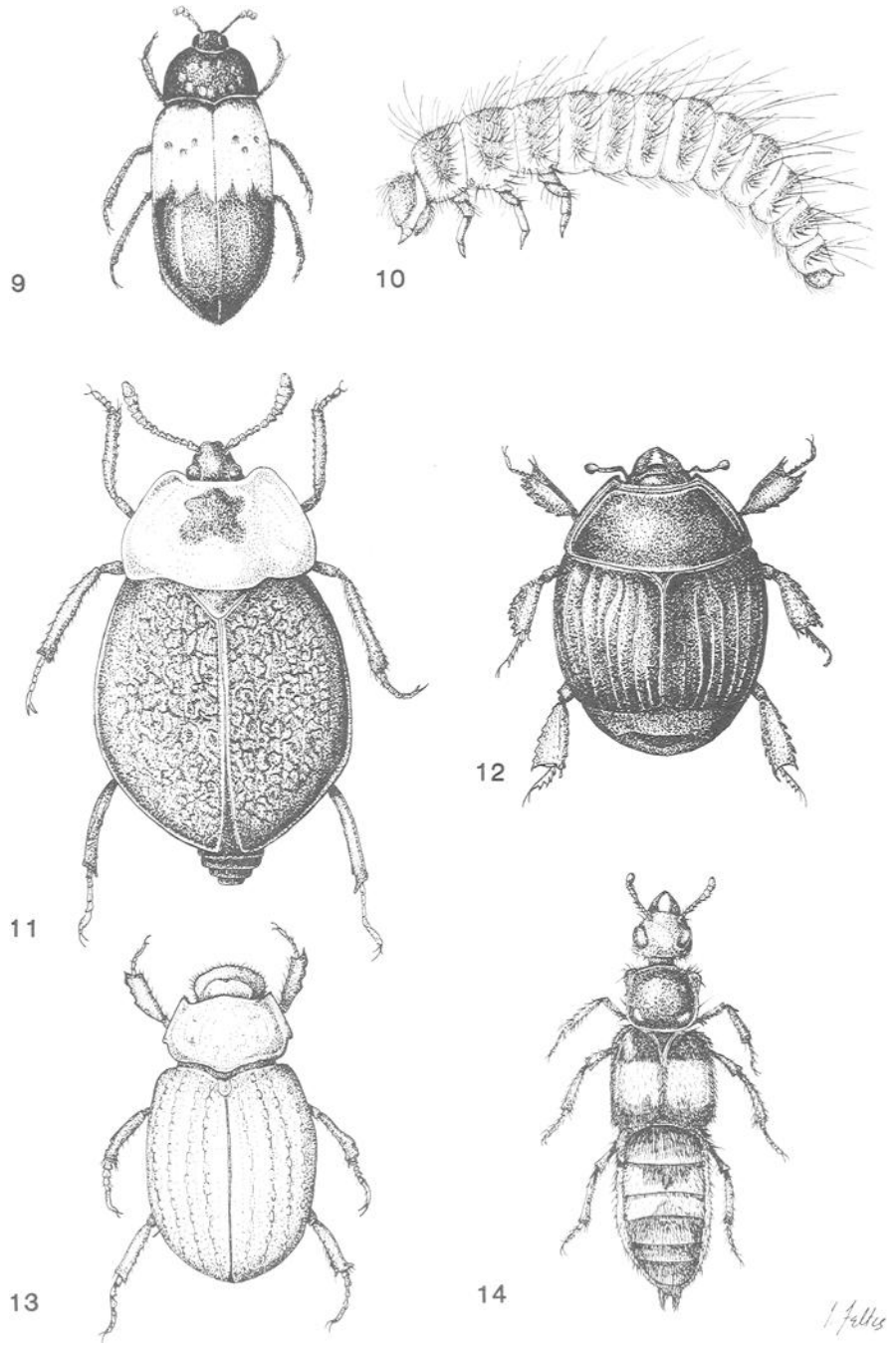


FIGS. 6-8—*Adults of Sepsidae, Piophilidae, and Phoridae where Fig. 6 is* Sepsis vicaria, *Fig. 7 is* Piophila casei *and Fig. 8 is* Borophaga fuscipalpis.

Burrowing Insects

Some insects (that is, some beetles and mature fly larvae) burrow into the soil beneath the corpse. When the postmortem period is greater than three weeks, remove several samples of soil (0.25 m^3 each) from the area beneath the corpse. Place such samples carefully in plastic bags and analyze for beetles, mature fly larvae, and other soil arthropods.

To ensure complete sampling, it may be desirable for a person familiar with entomological



FIGS. 9-14—*Beetles commonly associated with animal carrion where Fig. 9 is* Dermestes lardarius—*adult (Dermestidae), Fig. 10 is* Dermestes lardarius—*Larva (Dermestidae), Fig. 11 is* Silpha americana *(Silphidae), Fig. 12 is* Margarinotus cadaverinus *(Histeridae), Fig. 13 is* Trox suberosus *(Trogidae), and Fig. 14 is* Creophilus maxillosus *(Staphylinidae).*

TABLE 2—*Materials for collection of forensically important insects.*

Forceps
Glass vials
Ethyl alcohol (70%) or isopropyl alcohol (cut 1 : 1 with water)
Ice cream cartons or equivalent
Vermiculite or other inert material
Insect net (standard or hand net)
KAA[a] or similar larval perservative
Surgical gloves
Small artist brush
Plastic bags
Trowel

[a]KAA: kerosene (refined), 1 part glacial acetic acid, 1 part 95% ethyl alcohol, 30 parts immerse larvae 10 min; rinse in 70% ethyl alcohol; and store in 95% ethyl alcohol.

techniques to collect soil samples subsequent to removal of remains. Be certain that the position of the corpse is clearly indicated, so that complete samples can be taken, since fly larvae may wander some distance from the corpse to pupate.

Immature and Soft-Bodied Insects

Collect immature and soft-bodied insects by the methods described for crawling and burrowing insects. Special care is needed, however, to ensure proper preservation and rearing.

Place specimens to be immediately killed/preserved in vials of KAA solution (Table 2) for 10 min, then transfer to vials of 70% ethanol or isopropyl alcohol cut 1 : 1 with water. If larval fixing fluid is not available, place larvae in hot water (76.7°C [170°F]) for 2 to 3 min and then transfer to 70% ethanol. Various alternative larval preservatives can be used, depending on availability of chemicals [*20*].

Rearing specimens of immature flies or beetles to the adult stage requires proper food and laboratory conditions, but is essential for confirming identification of species and ensuring accurate estimates of postmortem duration. Rearing is most easily conducted by entomologists with appropriate materials and facilities.

Place living specimens in a 0.24-, 0.47-, and 0.95-L (½-pt, 1-pt, or 1-qt) ice cream carton or similar container ¼ to ½ filled with a coarse, inert material such as vermiculite. Moist soil can be used, if other materials are not available. Do *not* put living specimens to be reared in sealed plastic bags or sealed vials for longer than 12 h, since they do poorly in such environments, especially in warm weather. Transport living material by the fastest possible means to the rearing facility. Use of regular mail service usually is not suitable for transporting living material.

Immature flies can be successfully reared on diets of beef liver, or on small pieces of musculature obtained from the corpse. Transfer larvae gently via forceps onto the dietary material, which has been previously placed atop a 4 to 8-cm deep container filled with damp, coarse soil or vermiculite. Small glass dishes 8 to 10 cm in diameter, or beakers (250 mL), are suitable. Small cultures each containing 15 to 25 larvae afford maximum rearing success. Place these larval cultures inside standard insect rearing cages until adult flies emerge. Check larvae daily and record larval size and instar. Add additional liver as needed. Take care to allow only minimal culture disturbance. Mature larvae will migrate downward into the substrate and pupate. Adult flies will eventually emerge, crawl to the surface, and fly in the cages.

Whenever possible, rear larvae in climatic conditions approximating those to which the corpse was exposed. Environmental chambers are useful, if available. Temperature is the most critical factor. Calculations of the average time interval required for each developmental stage (larval instars, prepupae, pupae, and adult emergence) allow accurate determination of corpse colonization, approximating the time of death.

Allow emerged adults to feed for 24 h on a cotton pad soaked with a 10% sucrose solution. This ensures exoskeletal hardening. Species identifications can then be made. Preserve adult flies in 70% ethyl alcohol or pin them dry [*19*] and store in insect boxes.

Immature beetles need not be reared and should be preserved in 70% ethyl alcohol for identification.

Other Biological Materials

Other observations on the kinds of animals and plants found in, on, and around the corpse may provide supplementary information about the time, cause, and location of death. Collect samples of any "unusual" specimens (that is, leaves from branches of trees used to cover a corpse, plants beneath the corpse, fleas, body lice, seaweeds, and so forth). Likewise, collect representative samples of specimens encountered at autopsy for analysis. Process any insect specimens observed during autopsy as described above and carefully record sites where found. Aquatic plants and marine specimens are best preserved in 10% neutral buffered formalin.

Labeling

Label containers, vials, and packages of specimens individually with the following information:

(1) date collected;
(2) time collected;
(3) location of remains (as precise as possible);
(4) area of body infested; and
(5) name, address, and telephone number of collector(s).

Shipment of Specimens

Package containers and vials of preserved specimens in well-cushioned containers to avoid breakage, and ship by the most convenient means. If shipped by regular post, wrap each vial individually in cellucotton and place in a box with *at least* 50.8 mm (2 in.) of styrofoam chips surrounding all sides, top, and bottom. This will minimize possibility of breakage. Clearly mark LIQUID IN GLASS on the container. This usually will receive gentler handling by the post office.

Package soil samples and other living specimens in containers that maintain relatively cool, humid, well-ventilated environments. Time is critical if accurate information is to be obtained from living material. Ship such material by the most rapid means available.

As with other types of physical evidence, take care to ensure a continuous chain of legally acceptable evidence possession.

Description of Locality

An accurate, detailed description of the habitat in which a corpse was found is important to forensic biologists. Whenever possible, written descriptions should be accompanied by a series of photographs. Descriptions and photographs should illustrate the following:

(1) general habitat type—woods, pasture, field, beach, swamp, roadside, parking lot, dump, and so forth;
(2) terrain—hillside (north- or south-facing slope), valley, plateau, and so on including elevation if possible, and location on topographic maps;
(3) vegetation—trees, shrubs, grasses, tall or short grass, and so forth; and
(4) type of soil—sandy, rocky, clay, mud, gravel, asphalt, and so forth.

Description of the Corpse

A detailed photographic and written description of the corpse is necessary, including the following:

(1) sex, age, height, weight;
(2) presence and extent of clothing;
(3) orientation (sitting, prone, and so on);
(4) cause of death;
(5) physical damage (lacerations, abrasions, gunshot wounds, and so forth);
(6) extent of decomposition; and
(7) insect fauna (close-up photographs of adult and immature insects whenever possible).

Microclimate

Because microclimatic conditions have a profound effect on the development of immature insects, the most accurate data available describing these conditions at the location where the corpse is found are of critical importance. Whenever possible, record maximum and minimum temperature values at the scene as soon as possible after discovery. Obtain climatic data from the nearest National Oceanic and Atmospheric Administration (NOAA) weather station for the entire estimated postmortem period and for a two-week interval before estimated time of death. Photographs depicting relative amounts of sunlight and shading of the corpse during the day are useful if available.

Summary

Insects associated with carrion display successional stages closely related to time of death, with each group arriving at specific times following deaths. These intervals can be precisely correlated for each geographic area and used to aid investigations into time and manner of death. It must be recognized, however, that many factors can alter the normal time sequence of carrion insect succession. The most important of these are:

(1) time of day or night that death occurred;
(2) location of corpse following death;
(3) exposure of corpse, including burial, clothing, covering by vegetation, and so on;
(4) presence of external wounds and their position;
(5) temperature;
(6) sunlight and other weather conditions;
(7) length of time corpse in sun or shade;
(8) time of year;
(9) change of location following death;
(10) immersion in water (fresh or salt); and
(11) freezing and thawing.

Any of the above factors will alter the normal course of succession in carrion insects. However, this alteration can often be used to determine if the corpse has been disturbed outdoors following death, if buried and later exhumed, and other details of possible evidentiary importance.

Forensically important insects can be a powerful tool in investigations of homicide and other deaths, particularly if care is taken to collect specimens and record information. The preceding protocol should allow professionals to collect enough data to ensure the most accurate forensic science determinations possible by entomological means.

Acknowledgment

We wish to thank Tess Feltes for the illustrations of insect adults and larvae associated with animal carrion.

References

[1] Megnin, P., "La Faune des Cadavres, Application l'Entomologie a la Medicine Legale," *Encyclopédie Science Aide-Mémoire*, Paris, 1894.
[2] Motter, M. G., "A Contribution to the Study of the Fauna of the Grave. A Study of One Hundred and Fifty Disinterments, with Some Additional Experimental Observations," *Journal of the New York Entomological Society*, Vol. 6, No. 4, Dec. 1898, pp. 201-231.
[3] Payne, J. A., "A Summer Carrion Study of the Pig *Sus scrofa*," *Ecology*, Vol. 46, No. 5, 1965, pp. 592-602.
[4] Nuorteva, P., Isokoski, M., and Laiho, K., "Studies on the Possibilities of Using Blowflies as Medico-legal Indicators in Finland. I. Report of Four Indoor Cases From the City of Helsinki," *Annales Entomologici Fennici*, Vol. 33, 1967, pp. 217-225.
[5] Nuorteva, P., "Age Determination of a Blood Stain in a Decaying Shirt by Entomological Means," *Forensic Science*, Vol. 3, 1974, pp. 89-94.
[6] Leclerq, M., *Entomological Parasitology—The Relations Between Entomology and the Medical Sciences*, Pergamon Press, London, 1969.
[7] Leclerq, M. and Watrin, P., "Entomologie et medecine legale. Acariens et insectes trouves sur un cadavre humain en decembre 1971," *Bulletin et Annales de la Societe Royale D'Entomologie de Belgique*, Vol. 109, 1971, pp. 195-201.
[8] Leclerq, M., "Entomologie et medecine legale. Etude des insectes et acariens necrophages pour determiner la date de la mort," *Spectrum*, Vol. 17, Nov.-Dec. 1975, pp. 1-6.
[9] Easton, A. M. and Smith, K. G. V., "The Entomology of the Cadaver," *Medicine, Science and the Law*, Vol. 1, 1970, pp. 208-215.
[10] Lane, R. P., "An Investigation into Blowfly Succession on Corpses," *Journal of Natural History*, Vol. 9, No. 5, Sept.-Oct. 1975, pp. 581-588.
[11] Nuorteva, P., "Sarcosaprophagous Insects as Forensic Indicators," in *Forensic Medicine: A Study in Trauma and Environmental Hazards*, C. G. Tedeschi, W. G. Eckert, and L. G. Tedeschi, Eds., W. B. Saunders Co., Philadelphia, 1977.
[12] Smith, K. G. V., *Insects and Other Arthropods of Medical Importance*, British Museum (Natural History), London, 1973.
[13] Aldrich, J. M., *Flies of the Genus Sarcophaga in North America*, Thomas Say Foundation, Lafayette, IN, 1918.
[14] James, M. T., *The Flies That Cause Myiasis In Man*, Miscellaneous Publication 631, U.S. Department of Agriculture, 1947.
[15] Hall, D. G., *The Blowflies of North America*, Thomas Say Foundation, Baltimore, 1948.
[16] Oldroyd, H., *The Natural History of Flies*, William Cloves & Sons, Inc., London, 1964.
[17] Dillon, E. S. and Dillon, L. S., *A Manual of Common Beetles of Eastern North America*, Vols. I & II, Dover Publications, Inc., NY, 1972.
[18] Borror, D. J., Delong, M., and Triplehorn, C. A., *An Introduction to the Study of Insects*, 5th ed., Saunders Publishing Co., Philadelphia, 1981.
[19] McAlpine, J. F., Ed., *Manual of Nearctic Diptera*, Vol. I, Agriculture Canada Monograph 27, Research Branch, Ottawa, 1981.
[20] Peterson, A., *Larvae of Insects*, Edwards Brothers, Inc., Ann Arbor, MI, 1962.

Address requests for reprints or additional information to
Dr. John F. Burger
Department of Entomology
Nesmith Hall
University of New Hampshire
Durham, NH 03824