

BAT GUANO IS USEFUL FOR MORE THAN DIET STUDIES

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ABSTRACT. Researchers collect bat guano using a variety of techniques, and most samples are used for diet analysis. We provide recommendations for an easily-constructed guano collector that also samples a standard (1 m²) area. Recently, many conservationists have begun using fecal DNA in an effort to identify the species of an unknown donor, or collect demographic data on rare or cryptic species. Most studies have targeted larger mammals such as carnivores that produce large scats; but more recently, researchers have begun to use bat guano to obtain DNA. This DNA can be analyzed using extraction techniques and a suite of highly polymorphic microsatellite loci to provide information about the identity of the species and of individual bats that are present. Other advances in analytical techniques suggest that future samples of bat guano can provide information about stress levels within a colony and even to obtain information about where prey insects were produced.

Keywords: DNA, guano, Indiana bat, microsatellites, *Myotis sodalis*, roosts

Guano is increasingly collected under the roosts of bats for use in a variety of studies. The most frequent purpose of collecting these samples is to examine the diet of bats within the roost (Whitaker 1988). Usually such samples are collected from free-ranging bats that are held until they defecate, or collected at standard intervals beneath roosts. In addition to providing information about diet, advances in modern molecular techniques now make it possible to identify the species (ERDC 2005; Kanuch et al. 2007; Puechmaile et al. 2007) or individual bat (Vege & McCracken 2001) that produced a sample using DNA and to monitor stress levels within a colony by monitoring by isolating glucocorticoid secretions in fresh guano (von der Ohe & Servheen 2002; Millsbaugh & Washburn 2004). Our purpose is to provide an overview of the overlooked potential of guano and to encourage chiropteran biologists to make use of this resource.

METHODS

Techniques for handling guano.—Previous authors have used plywood (Whitaker & Clem 1992), plastic sheeting (Whitaker 1995), and window screening (Kurta & Whitaker 1998; Murray & Kurta 2002). In our experience bridal veil material (often sold as tulle) has several advantages over other materials because it is readily available (from any fabric or larger department store), inexpensive (usually less than \$2.00 per m²), machine washable, easily collapsed for storage or movement, and allows rain and urine to pass through. We recommend that, when possible, guano be collected using quadrats of a standard size. This quadrat-based approach has the benefits of allowing researchers to accurately measure the density of fecal pellets and to design sub-sampling protocols when needed.

We have experimented with several varieties of quadrats including some made from PVC, a variety of lumber and even direct mounting of

the bridal veil to the substrate. The most versatile of these quadrats was made by converting $2.5 \times 2.5 \times 122$ cm guard stakes available from Forestry Suppliers (Jackson, Mississippi). We constructed a frame of 1 m per side by removing the pointed end of the stakes. These pointed ends are then mounted on the side and serve as legs. The frame is joined together and legs attached using wing nuts which allows the entire unit to be disarticulated and reassembled. At such locations we attached the fabric with pushpins or a staple gun which allows the fabric to be easily changed to prevent cross-contamination between sampling bouts.

One source of cross-contamination that is not cured by changing the fabric is the possibility that the sample includes older guano that had been trapped in the roost but has since become dislodged due to more recent movements within the roost. To limit this problem, we recommend presorting fecal pellets and removing older material, which has a faded appearance. If molecular techniques are going to be used, fresh fecal pellets should be placed in microcentrifuge tubes (Lab Depot, Dawsonville, Georgia) using toothpicks to minimize the potential for contamination. Each toothpick is broken off in the vial with the fecal pellet to prevent the loss of epithelial cells that might cling to the toothpick. In the field, the microcentrifuge tubes are stored on ice until they can be placed in an ultra-cool freezer to await molecular analysis.

Advances in molecular techniques allow the identification of individual bats through DNA without handling or disturbing the bats. Fecal samples are obtained under a roost, and DNA is extracted from individual fecal pellets using the Extractmaster Fecal DNA extraction kit (Epicenter) following the manufacturer's protocol with modifications described in Oyler-McCance & St. John (2006). Using these DNA samples, individuals can be identified using a suite of highly polymorphic microsatellite loci (Oyler-McCance & St. John 2006).

RESULTS

Species identification from guano.—Frequently researchers simply want to identify the species of bat that is present. For example, researchers working with artificial roosts often quantify the effectiveness of the artificial roost based on the presence of guano or other sign

(Kanuch et al. 2007; Puechmaille et al. 2007). Similarly, guano is often found in sites when no bats are present and thus species identification is difficult. In both cases, the ability to successfully identify the species that deposited the guano would be valuable. Modern molecular tools make this process relatively simple through the amplification and sequencing of regions of the genome with species-specific signatures.

Fecal DNA for identification.—Behavior of the bats and the low quantity and quality of DNA present in the samples are two major factors complicating the use of fecal DNA to examine the ecology of bats. Tree-roosting bats frequently switch roosts and may use many different trees during a summer season. Thus, obtaining accurate population-level data requires sampling at multiple roosts during a season.

A variety of standard techniques are frequently used to obtain DNA samples from free-ranging wildlife, such as the use of tissue biopsies by bat biologists (Wilmer & Barratt 1996; Stadelmann et al. 2004; Weyandt et al. 2005). These provide high quality samples but require capturing and handling the bat. Hair snags made of barbed wire are commonly used to collect DNA samples from larger mammals such as bear (Woods et al. 1999) and bobcats (Haynes et al. 2005). However, this technique is not applicable to bats because of their tendency to become entangled or impaled on the barbs (Johnson 1933; Iwen 1958; Hibbard 1963; Denys 1972; Wisely 1978).

While DNA extracted from fecal material has been successful on a number of mammals (Kohn et al. 1999; Woods et al. 1999; Ernest et al. 2000), fecal pellets from bats are extremely small compared to feces from larger species. Because of this, studies have investigated the viability of various extraction protocols (Kanuch et al. 2007; Puechmaille et al. 2007). Guano yields small amounts of DNA because there are fewer epithelial cells present (Vege & McCracken 2001). Further, the available DNA rapidly degrades reducing the potential for successful PCR amplification. Additional issues, such as allelic drop out and cross contamination of samples (both during collection and during extraction), can be a problem and must be addressed using strict collection and extraction protocols (Waits & Leberg 2000; Taberlet et al. 1999).

DISCUSSION

Molecular advancements make it possible to use DNA-based identifications in the same manner that wildlife biologists have traditionally used other marking techniques such as banding. To date, this approach has been restricted to much larger species such as brown bears (*Ursus arctos*) (Mowat & Strobeck 2000), coyotes (*Canis latrans*) (Kohn et al. 1999), and mountain lions (*Puma concolor*) (Ernest et al. 2000). We contend that wide-spread application of this approach will provide new insights into the behavior and demographics of colonial species of bats (Vege & McCracken 2001) including the federally-endangered Indiana bat (*Myotis sodalis*).

In addition, because some roosts are frequently re-used both within and between seasons (Kurta et al. 1996; Whitaker 1998), it is possible to have old guano still in the roost. Two other sources of contamination are the result of bats visiting roosts that they are not using. We suspect the most frequent of these occurs during a behavior known as rallying or checking (Gardner et al. 1991; Murray & Kurta 2004), in which bats fly to and often briefly touch a roost. Finally, we have obtained guano from beneath an unoccupied roost where we observed no rallying behavior, indicating that the guano was deposited by a bat that flew past the roost. While we suspect this is a minor source of contamination, it should not be discounted.

Future uses of guano.—Future researchers will likely monitor stress by isolating glucocorticoid secretions in fresh guano (von der Ohe & Servheen 2002; Millspaugh & Washburn 2004). This is important because adrenocortical activity can ultimately alter animal behavior, increase disease susceptibility, and affect overall population performance (Millspaugh & Washburn 2004). Advances in stable isotope analysis may also allow researchers to identify the origin of prey species such as cucumber beetles and whether or not they are coming from corn, beans, or non-crop sources (McKechnie 2004).

Traditionally, bat ecologists have viewed guano sampling as a technique to understand the diet of bats. Recent advances in molecular biology now allow guano to be used for many other purposes including demographic patterns. While we have discussed several limita-

tions to the use of guano, strict collection and extraction protocols can allow a wealth of new information to be obtained from guano. These recent changes, however, also suggest that the time has come to develop a more standardized approach to collecting and processing guano samples.

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