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Tools and Technology

An Alternative Minimally Invasive Technique for Genetic Sampling of Bats: Wing Swabs Yield Species Identification

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ABSTRACT Bat species are traditionally identified morphologically, but in some cases, species can be difficult to differentiate. Wing punches (biopsies) of wing or tail membranes are commonly used to collect tissue for DNA analysis, but less invasive techniques are preferable. As such, DNA acquired using buccal and wing swabs or from fecal pellets are increasingly being employed. We compared a dry swabbing technique with the wing biopsy technique for DNA collection. We compared species identification between tissue biopsies and wing swabs collected from bats in Alberta and British Columbia, Canada, between April and November, 2014, and September and October 2015. Species identification was achieved with varying methods of field collection and lab processing. DNA was extracted, sequenced, and compared with reference sequences and field identifications. We concluded that wing swabs are an effective way to identify bat species genetically and far less invasive than biopsy techniques. These methods should be considered for genetically sampling bats, especially during seasons when wounds from biopsy are slow to heal. © 2017 The Wildlife Society.

KEY WORDS bats, Chiroptera, DNA, genetic, identification, minimally invasive sampling, *Myotis*, swab, swabbing.

Accurate identification of bat species and populations in an area is essential for conservation efforts. However, species identification of captured bats in Canada by morphological features is sometimes difficult, particularly among some *Myotis* species, such as *M. evotis* (long-eared myotis), *M. septentrionalis* (northern myotis), *M. yumanensis* (Yuma myotis), and *M. lucifugus* (little brown myotis; Vonhof 2006). To augment morphological (visual) species identification, tissue biopsies are increasingly being used to provide genetic confirmation of species identification (Worthington-Wilmer and Barratt 1996). Biopsy samples may be taken from the chiropatagium or uropatagium, and preserved for DNA analysis (Lausen 2005, Faure et al. 2009). Wounds resulting from biopsies vary in healing time, which may depend largely on the time of year, with wounds made just prior to hibernation taking significantly longer to heal (Faure et al. 2009, Weaver et al. 2009, Ceballos-Vasquez et al. 2014).

Genetic sampling of bats in autumn and winter has increased in recent years with interest in hibernation ecology, population dynamics, migration, and breeding patterns that might elucidate spread patterns of white-nose syndrome (WNS), a fungal disease (caused by *Pseudogymnoascus destructans*) devastating bat colonies mainly in eastern North America. Species-specific prevalence and impacts of WNS require distinguishing morphologically similar species; swab sampling can be multipurpose genetic identification and WNS surveillance (Walker et al. 2016). Sampling of bats during or just prior to hibernation is increasingly necessary in preparation for the arrival of WNS. Establishing baseline information about species diversity at hibernacula, measuring species-specific prehibernation body mass and other physiological parameters for predictive species-specific disease models, and species-specific sampling for the presence of the fungus that causes WNS is becoming important across the continent (USGS National Wildlife Health Center 2015, Hayman et al. 2016). The effect of genetically sampling bats through biopsy techniques just prior to or during hibernation is unknown, but may be predicted to cause infections or increased water loss in unhealed wounds. There is a growing demand for less-invasive protocols to collect DNA from bats to reduce injury and lengthen the sampling season.

Walker et al. (2016) identified bat species from genetic analysis of bat fecal pellets and from buccal swabs, wing

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swabs and punches, and some internal tissues. As an alternative method, we used a dry swabbing technique of wings for species identification, evaluating this method by comparing with field identifications and replicate samples including wing biopsies and fecal pellets.

STUDY AREA

Samples were taken at 4 sites in British Columbia, Canada, (Victoria, Port McNeil, Creston, and Atlin) in April–November, 2014 and October and November, 2015. Samples were taken at 2 sites in Alberta, Canada (Peace River and Conklin), in July and early August, 2014. All British Columbia sites were roosts, with all but one (Port McNeil) roosts of *M. lucifugus*, *M. yumanensis*, or a mix of the 2 species. Both of the Alberta sites were foraging or commuting areas.

METHODS

Bats were captured using mist nets at all sites, with the exception of one sample (Atlin-02BC) in which a bat was found on a wall and hand-captured. We used standard keys to morphologically identify bats to species (e.g., Nagorsen and Brigham 1993). In most cases, *M. lucifugus* and *M. yumanensis* are difficult to distinguish in hand, requiring acoustic and genetic means (Weller et al. 2007, Luszcz et al. 2016). We processed bats within 1 hr of capture and immediately released individuals following processing. We used a bat detector (EM3; Wildlife Acoustics, MA, USA) to distinguish the latter 2 species by echolocation calls. Guano produced by captured bats collected in the holding bag was transferred to a coin envelope for room temperature dry storage. We washed all holding bags in hot water and 10% bleach between uses. All bat handlers wore disposable latex gloves. Gloves were always changed between bats at foraging and commuting sites, but gloves were not always changed between each bat when processing roost-mates to minimize holding times despite large capture numbers. Capture techniques and animal handling followed Canadian Council on Animal Care protocols (CCAC 2003, 2007), and protocols developed for bat surveys in Alberta (Vonhof 2006). We implemented protocols to prevent the spread of WNS while handling bats (ESRD 2012).

In most cases, we took a wing biopsy and a wing swab from each bat, although in 2 cases, we collected guano instead of a tissue biopsy, and 6 samples were of swabs only. Wing biopsies followed methods outlined by the Alberta Wildlife Animal Care Committee Class Protocol #004 (ESRD 2012), Lausen (2005), and the CCAC (2003). We took a biopsy from one wing near the tibia, using a 2-mm sterile biopsy punch, and preserved it in 95% ethanol. To collect DNA using a swab, we opened the bat's wing and rubbed a swab along the surface of the wing, using a motion that applies slight pressure to drag the swab while rolling it, resulting in most or all of the head of the swab making contact with the skin of the bat. Only enough pressure is applied to make good contact of the skin with the head of the swab, without abrading the tissue. We rubbed the inside (ventral) surfaces of the wings in all sampling sessions except one, when we swabbed both sides of the wings. The Alberta protocol called for sterile rayon–polyester-tipped collection swabs (Puritan HydraFlock®, Guilford, ME, USA), preserved in RNA-Later® (ThermoFisher Scientific, Waltham, MA, USA). The British Columbia protocol called for sterile cotton-tipped (Dynarex, Orangeburg, NY, USA) and polyester (Pur-Wraps, Guilford, ME, USA) swabs stored in paper envelopes, and then refrigerated within 5 days or transferred to a -20° C freezer after 1 month. Samples were processed within 1 year of sample date. For logistical reasons, 2 laboratories were involved in processing the samples: The Matrix Research Testing Service (Matrix RTS; Calgary, AB, Canada) generally was used for samples collected in Alberta; and Wildlife Genetics International (WGI; Nelson, BC, Canada) generally was used for samples collected in British Columbia.

Genetic Analysis

DNA from swabs, wing biopsies, and guano were isolated using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Toronto, ON, Canada). There were 4 DNA purification protocols available using this kit; the protocol titled "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)" was followed (Qiagen 2006). There were minor modifications to the protocols between the 2 laboratories (Table 1). After isolation, DNA was frozen at -20° C ($\pm 2^{\circ}$ C) until used for polymerase chain reaction (PCR) amplification.

Both labs sequenced portions of the 16S rRNA gene, and performed the initial PCR reactions in-house (e.g., Johnson et al. 1998). The Matrix RTS lab used a slight variation of published primers (16Sr; 16SrF/16SrR; Palumbi et al. 2002, Zinck et al. 2004); the WGI lab used a primer developed in-house (Table 1). Matrix sent PCR products to Eurofins MWG Operon (Louisville, KY, USA) to be sequenced, whereas WGI performed their own sequence reactions and electrophoresis. Both labs compared sequence results to GenBank NCBI nucleotide database (NCBI 2015) for species identification, but WGI also referenced its own library of sequence profiles from past projects that included specimens of known species identity. At the WGI lab, results were scored as high confidence or low confidence based on quantitative and qualitative guidelines, first by the person managing the project and then reviewed by a second lab worker. Anything that missed the threshold for high confidence was reanalyzed, and those that failed again were generally analyzed a third time as per the protocol outlined in Paetkau (2003).

RESULTS

A total of 56 bats were swab-sampled: 38 using the Alberta protocol and analyzed by the Matrix RTS and 18 using the BC protocol and analyzed by Wildlife Genetics International. A total of 44 bats were sampled in duplicate, using swabbing as the test technique and either wing biopsy (42 samples) or fecal sample (2 samples) as the standard technique. Overall, 53 (95%) swab samples yielded sequenceable DNA, of which 48 (91%) produced a single

Table 1. Modifications to Qiagen (2006) Protocol "Purification of total DNA from animal tissues," amplification conditions, and primers for the 16S region of the mitochondrial genome for bats sampled during 2014–2015 in Alberta and British Columbia, Canada. Matrix Research Testing Service (Matrix RTS; Calgary, AB, Canada) generally was used for samples collected in Alberta and Wildlife Genetics International (WGI; Nelson, BC, Canada) generally was used for samples collected in British Columbia.

Protocol used by Matrix RTS facility				
Modifications to Qiagen 2006 Protocol "Purification of total DNA from animal tissues"	**Reaction mixture (25 μL total vol)**	**Primer sequences**	**Reaction conditions**	**Target size (base pairs)**
Step 2: Proteinase K incubation performed at 56° C for 20–24 hr Step 4: Solution added to the spin column, then the swab and microcentrifuge tube were centrifuged at 13,000*g* for 2 min. Additional solution pulled from the swab was added to the spin filter, the spin filter was centrifuged according to kit protocol Step 8: Final elution performed with 2 sequential elutions of 50 μL, for a total final vol of 100 μL	5 μL DNA 10 nmol each primer 5 nmol dNTP mix 1× Phusion Green HF Buffer 0.02U Phusion Polymerase	5′-GTGCAAAGGTAGCATAATCA-3′ 5′-TGTCCTGATCCAACATCGAG-3′	98° C for 30 sec **98° C for 10 sec** **58° C for 30 sec** **72° C for 30 sec** Repeat steps in bold ×39 72° C for 10 min 4° C for 10 min	450
		5′-CGCCTGTTTATCAAAAACAT-3′ 5′-CCGGTCTGAACTCAGATCACGT-3′	98° C for 30 sec **98° C for 10 sec** **60° C for 30 sec** **72° C for 30 sec** Repeat steps in bold ×39 72° C for 10 min 4° C for 10 min	600
Protocol used by WGI facility				
Step 1a: 10 μg of linear acrylamide was added to each lysis mix to enhance DNA precipitation, along with 176 μL Buffer ATL and 20 μL Proteinase K Step 6: Samples were centrifuged for 2 min at 12,000*g*, and then incubated at 70° C for 3 min to dry the membrane	5 μL total vol 3 μL DNA 100 nM each primer 50 mM KCL 10 mM Tris-HCL 0.1% Triton X-100 1.5 mM $MgCl_2$ 160 μg/mL BSA 100 μM each dNTP *Taq* DNA polymerase	5′-AGACGAGAAGACCCTATGGAGCTT-3′ 5′-TTCTCCGAGGTCACCCCAAC-3′	94° C for 80 sec **94° C for 20 sec** **60° C for 25 sec** **72° C for 1 sec** Repeat steps in bold ×40 72° C for 65 sec	120

(*Continued*)

species sequence; the remaining 5 samples produced multiple DNA sequences, suggesting contamination. Success varied slightly with protocol or lab and field techniques. Thirty-five (92%) swab samples processed with the Alberta protocol produced sequenceable amplification products using at least one of the amplification methods (Table 2). All 35 (100%) of these sequenceable swab samples matched both the field identifications and corresponding wing-biopsy DNA identifications.

Wildlife Genetics International had 100% amplification success (18 swab samples). Five (28%) of these samples produced mixed-species sequences, suggesting that field techniques resulted in swabbing of epithelial cells of ≥1 bat species. Four samples (151016-13BC, 151016-08BC, 151010-01BC, 151005-12BC) from a maternity roost known to house both *M. lucifugus* and *M. yumanensis* yielded sequences of both *M. lucifugus* and *M. yumanensis* (Table 2).

All single-species sequences ($n = 48$; 100%) matched field identifications, and where duplicate sampling occurred, also matched corresponding genetic identifications from guano or wing biopsies. One swab sample (141010-01BC) was additionally used in an extraneous population genetics study and successfully genotyped at 14 microsatellite loci by WGI (C. Lausen, unpublished data).

DISCUSSION

There is growing interest in genetic confirmation of bat species in North America, necessitating a minimally invasive method of obtaining genetic material. When bats enter into hibernation, a period of immune compromise (Weaver et al. 2009), and slowed body maintenance and repair occurs, so that wound-healing from tissue biopsies is significantly delayed (Worthington-Wilmer and Barratt 1996, Andrews 2007, Ceballos-Vasquez et al. 2014). Our results indicate

Table 2. Alignment of sample amplification products with 16S sequences in the National Center for Biotechnology Information or Wildlife Genetics International databases. Genetic identification by swab was verified with a duplicate sample: tissue biopsy (T), or guano pellet (G) for bats sampled in British Columbia (BC) and Alberta (AB), Canada, during 2014–2015. All amplified samples that produced a single aligned sequence matched field species identifications. Species identifications: MYLU, *Myotis lucifugus*; MYSE, *M. septentrionalis*; LANO, *Lasionycteris noctivagans*; MYYU, *M. yumanensis* (morphologically cryptic with *M. lucifugus*); MYEV, *M. evotis*. Site of sampling was either at a roost, or mist nets were strung along bat fly-ways to capture foraging or commuting bats. "Other individuals" includes bats handled in the same capture session at a roost or were observed at, or suspected of being in, the same roost.

Swab sample ID	Sampling date	Genetic ID	Field ID	Duplicate sample	Sampling site type	Other individuals	Swabbing location
14-0021-01AB	2014/07/16	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-02AB	2014/07/16	MYSE	MYSE	T	Fly-way	–	Inside wing
14-0021-03AB	2014/07/16	LANO	LANO	T	Fly-way	–	Inside wing
14-0021-04AB	2014/07/16	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-05AB	2014/07/16	MYSE	MYSE	T	Fly-way	–	Inside wing
14-0021-06AB	2014/07/16	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-07AB	2014/07/16	MYSE	MYSE	T	Fly-way	–	Inside wing
14-0021-08AB	2014/07/16	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-09AB	2014/07/16	MYSE	MYSE	T	Fly-way	–	Inside wing
14-0021-10AB	2014/07/16	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-11AB	2014/07/16	MYSE	MYSE	T	Fly-way	–	Inside wing
14-0021-12AB	2014/07/16	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-19AB	2014/07/18	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-20AB	2014/07/18	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-21AB	2014/07/18	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-22AB	2014/07/19	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-23AB	2014/07/19	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0021-24AB	2014/07/19	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0030-01AB	2014/07/30	LANO	LANO	T	Fly-way	–	Inside wing
14-0030-02AB	2014/07/30	Did not amplify	MYLU	T	Fly-way	–	Inside wing
14-0030-03AB	2014/07/30	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0030-04AB	2014/07/30	LANO	LANO	T	Fly-way	–	Inside wing
14-0030-05AB	2014/07/30	Did not amplify	MYLU	T	Fly-way	–	Inside wing
14-0030-06AB	2014/07/30	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0030-07AB	2014/07/30	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0030-08AB	2014/07/30	LANO	LANO	T	Fly-way	–	Inside wing
14-0030-09AB	2014/07/30	LANO	LANO	T	Fly-way	–	Inside wing
14-0030-10AB	2014/07/30	LANO	LANO	T	Fly-way	–	Inside wing
14-0030-11AB	2014/08/01	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0030-12AB	2014/08/01	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0030-13AB	2014/08/01	Did not amplify	MYSE	T	Fly-way	–	Inside wing
14-0030-14AB	2014/08/01	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0030-15AB	2014/08/01	LANO	LANO	T	Fly-way	–	Inside wing
14-0030-16AB	2014/08/02	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0030-17AB	2014/08/02	MYLU	MYLU	T	Fly-way	–	Inside wing
14-0030-18AB	2014/08/02	MYSE	MYSE	T	Fly-way	–	Inside wing
14-0030-19AB	2014/08/02	MYSE	MYSE	T	Fly-way	–	Inside wing
14-0030-20AB	2014/08/02	LANO	LANO	T	Fly-way	–	Inside wing
141014-05BC	2014/10/14	MYYU	MYYU	–	Roost	Roost not well-documented; MYYU known	Inside wing
141026-01BC	2014/10/26	MYLU	MYLU		Roost	MYLU and MYYU known from roost	Inside wing
141026-08BC	2014/10/26	MYYU	MYYU	–	Roost	MYLU and MYYU known from roost	Inside wing
141108-11BC	2014/10/08	MYYU	MYYU	–	Roost	MYLU and MYYU known from roost	Inside wing
141127-02BC	2014/11/27	MYLU	MYLU	–	Roost	MYLU and MYYU known from roost	Inside wing
140411-04BC	2014/04/11	MYYU	MYLU	T	Roost	MYLU and MYYU known from roost	Inside wing
140411-05BC	2014/04/11	MYYU	MYYU	T	Roost	MYLU and MYYU known from roost	Inside wing
140411-07BC	2014/04/11	MYYU	MYYU	T	Roost	MYLU and MYYU known from roost	Inside wing
140411-13BC	2014/04/11	MYYU	MYYU	T	Roost	MYLU and MYYU known from roost	Inside wing
141024-03BC	2014/04/24	MYYU	MYYU	G	Roost	MYLU and MYYU known from roost	Inside wing
141010-01BC	2014/10/10	MYEV	MYEV	–	Fly-way	No other bats captured or seen	Inside wing
Atlin-02BC	2015/09/08	Multiple species detected; ID not conclusive	MYLU	G	Roost	3 other *Myotis* bats seen; species not determined	Inside and outside wing

(*Continued*)

Table 2. (*Continued*)

Swab sample ID	Sampling date	Genetic ID	Field ID	Duplicate sample	Sampling site type	Other individuals	Swabbing location
151016-13BC	2015/10/16	Both MYLU and MYYU detected	MYYU	–	Roost	MYLU and MYYU known from roost	Inside and outside wing
151016-12BC	2015/10/16	MYYU	MYLU/MYYU	–	Roost	MYLU and MYYU known from roost	Inside and outside wing
151016-08BC	2015/10/16	Both MYLU and MYYU detected	MYLU	–	Roost	MYLU and MYYU known from roost	Inside and outside wing
151010-01BC	2015/10/10	Both MYLU and MYYU detected	MYLU	–	Roost	MYLU and MYYU known from roost	Inside and outside wing
151005-12BC	2015/10/05	Both MYLU and MYYU detected	MYLU/MYYU	–	Roost	MYLU and MYYU known from roost	Inside and outside wing
151020-11BC	2015/10/20	MYLU	MYLU	–	Roost	MYLU and MYYU known from roost	Inside and outside wing

that simple swabbing of bat wings to collect epithelial cells for DNA analysis can be an effective minimally invasive method for genetic sampling of bats.

Not all samples successfully yielded a species identification, and this may stem from field techniques; 5% of swabs did not produce an amplifiable product. All of these swabs were preserved in RNAlater. Submersion of the swab in liquid following contact with the wing could have resulted in the loss of epithelial cells from the swab to the surrounding fluid; however, it cannot be determined whether this was the cause of nonamplification in some samples. Based on the 100% success of amplification from swabs stored dry, we recommend that swabs be placed in dry envelopes in the field for transport to the laboratory. We also recommend that swabbing be done with enough pressure to lift cells from the skin surface. Walker et al. (2016) reports 100% amplification success of DNA from swabs from collaborators; although the method used in swab collection was not stated, it is likely that many were collected using moistened polyester swabs rolled on bat skin and then stored in sterile water, the United States national protocol for *P. destructans* surveillance (USGS National Wildlife Health Center 2015).

Walker et al. (2016) does not report any contamination, although the extent of replication of samples from the same individual was not clear. As with our study, verification was to species, not individual level, thus precluding a thorough analysis of potential contamination of samples by roost-mates. Although the exact method of swab sampling is not always described in studies (e.g., Johnson et al. 2014), the 2014/2015 U.S. Geological Survey National Wildlife Health Center WNS submission protocol describes "rolling" the swab (USGS National Wildlife Health Center 2015). Rolling the swab over the skin is likely to obtain epithelial cells in smaller quantities, decreasing the amount of DNA available for extraction and increasing the risk of contamination by amplification of cells from roost-mates. The amount of DNA extracted from swabs has yet to be quantified in any study to date. Based on our findings and those of Walker et al. (2016), swabbing of bats could be multipurpose, providing both fungal and genetic material for both white-nose syndrome surveillance and species identification. We suggest that if species identification is the main goal of the swabbing, that the tip of the swab be lightly rubbed rather than rolled, to pick up sufficient surface epithelial cells, thus ensuring enough DNA from the target individual for a successful species or individual identification. This method of sampling is most appropriate when the individual is being handled for other reasons, and especially when this handling occurs outside of the hibernation season. If the individual is being handled during the hibernation period, minimal disturbance would be the goal and a single swab sample (dual purpose for species identification and WNS surveillance) would be most appropriate.

Eight percent of our samples yielded mixed species sequences and all of these were from 2015 sampling in British Columbia; in this sampling session, bat wings were rubbed on both outside (dorsal) and inside (ventral) surfaces. We recommend that only the ventral surface of wings be swabbed to minimize the chance of sampling epithelial cells of roost-mates. This is of particular concern for species such as *M. lucifugus* and *M. yumanensis*, 2 species that are known to roost together (e.g., Davis et al. 2010). Four of our 5 mixed-species-sequence samples yielded sequences for both of these species; these 4 samples were from a maternity colony consisting of both species, which may indicate that contamination of cells from roost-mates occurs. Our field sampling at maternity roosts did not always involve changing of disposable gloves between handling of each roost-mate, and this may also have contributed to potential cross-contamination of epithelial cells; we thus recommend changing latex gloves between swabbing of roost-mates.

Other minimally invasive methods of obtaining DNA from bats have been developed, including buccal swabs (Corthals et al. 2015). Sufficient amplification success has been reported in population genetics using buccal swabs as source DNA for microsatellite genotyping (e.g., Ramirez 2011); however, some damage to buccal lining, causing bleeding in smaller bats has been reported with this technique (Corthals et al. 2015). In our experience, buccal swabs introduce risks to bats that wing swabbing does not. We observed that bats chew on buccal swabs, sometimes swallowing portions of them; having bats release these swabs from their mouths can be challenging, and it is possible that their small teeth could be damaged. The main advantage of using buccal swab sampling is that the risk of stray cells from nontarget individuals is substantially reduced.

Although methods of swabbing are likely to always obtain less DNA than tissue biopsy (Corthals et al. 2015), field logistics support use of wing swabbing over buccal swabbing. During seasons when metabolic and healing rates of bats slow (just prior to and during hibernation; Andrews 2007, Ceballos-Vasquez et al. 2014), swabbing, rather than tissue biopsy, should be considered because this method may be least likely to negatively affect bats. During seasons of low metabolic rates, fecal production is also likely to be slow, precluding fecal production as a reliable method of obtaining DNA. In contrast, during seasons when bats are actively foraging and metabolic or healing rates high, obtaining DNA from fecal pellets, wing swabs, or wing biopsies are all viable options, in order of increasing invasiveness.

Increasingly, bats are being removed from hibernation surfaces to swab for microflora that may lead to a greater understanding of white-nose syndrome resistance in some bats or to methods for treating infected bats (e.g., CWHC 2015, Hoyt et al. 2015). If and when it is necessary to disturb bats from hibernation, dual-purpose swabbing reduces handling time and uses minimally invasive techniques; this method obtains not only microbial information, but a genetic record of these bats either for active research (e.g., species identification, population genetics) or archiving purposes. Although it is possible that 1 swab could provide genetic material for both *Pseudogymnoascus destructans* screening and genetic identification, 2 swabs of the same bat might be considered for laboratory logistics.

Our study focused on species identification, and only a single swab sample was used as a source of DNA for an extraneous study of population genetics. This sample was successfully typed at 14 microsatellite loci; therefore, this swabbing technique could be used to collect DNA for genetic analyses beyond simple species identification, as has been done with buccal swabs (e.g., Ramirez 2011). However, a more rigorous study would be needed to assess genotyping error rates using this method (e.g., Broquet et al. 2007), including the possibility of extracting DNA from extraneous epithelial cells from clustering individuals. We acknowledge that wing swabbing of bats that roost with other bats could introduce potential contamination.

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