Tools and Technology Article

Using DNA Barcodes to Identify Bird Species Involved in Birdstrikes

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ABSTRACT We determined effectiveness of using mitochondrial DNA barcodes (*cytochrome c oxidase subunit 1* [CO1]) to identify bird–aircraft collision (birdstrike) cases that lacked sufficient feather evidence for morphological diagnosis. From September through December 2006, 821 samples from birdstrike events occurring in the United States were submitted for DNA analysis. We successfully amplified a CO1 DNA barcode product from 554 (67.5%) of the samples; 267 (32.5%) did not contain viable DNA and depended on morphological methods (microscopy) for Order or Family level identification. We deemed 19 cases inconclusive either because the DNA barcode recovered from the sample did not meet our 98% match criteria when compared to the Barcode of Life Database (BoLD) or because the DNA barcode matched to a set of $\geq$2 closely related species with overlapping barcodes, preventing complete species identification. Age of the sample ($\leq$6 months) did not affect DNA viability, but initial condition of the sample and the collection method was critical to DNA identification success. The DNA barcoding approach has great potential in aiding in identification of birds (and wildlife) for airfield management practices, particularly in regions of the world that lack the vast research collections and individual expertise for morphologic identifications. (JOURNAL OF WILDLIFE MANAGEMENT 72(5):1231–1236; 2008)

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Bird–aircraft collisions (hereafter birdstrikes) are a serious safety hazard and a major expense for the aviation industry (Linnell et al. 1996, Sodhi 2002, Cleary and Dolbeer 2005, Blackwell and Wright 2006, Dolbeer 2006). Knowing the exact species involved in wildlife strikes (usually birds) is fundamental to any management plan that aims to adequately control this hazard (Doran et al. 1990, Brom 1991, Dove 2000). Sometimes bird species can be identified by airfield biologists, but most often birdstrike remains are fragmented or minute and require a more thorough examination. Traditional morphological identifications of fragmented feathers are best done in a museum research collection where feather fragments can be properly prepared (Laybourne and Dove 1994), examined microscopically, and compared with museum reference collections. Birds are identified to various taxonomic levels using the morphological feather characters of size, shape, color, and texture of whole feathers or feather fragments and the microscopic characters found in the plumulaceous (downy) feather barbs. The Smithsonian Institution, National Museum of Natural History has been providing species identifications to military and civil aviation industries since the 1960s, and the number of cases submitted for identification now totals >3,500/year. Field sampling of birdstrike remains and recognition of trace birdstrike evidence on the aircraft has improved substantially in recent years. In many cases, only microscopic feather evidence or blood and tissue are present in the unidentified sample. Because these types of forensic identifications require expertise in feather microstructure and access to a large reference collection of downy feather microslides for accurate comparisons, there are few experts in the world who are able to conduct such specific identifications. Attempts have been made to quantify the downy feather microscopic characters that are unique to some groups of birds but these microscopic techniques usually do not lead to identifications at the species level, and simple keys are not practical due to the amount of variation in microscopic feather characters within the plumage of a single bird (Gilroy 1987, Brom 1991, Dove 1997, Heacker-Skeans 2002, Dove and Agreda 2007). The problem with previous attempts at DNA identification of birdstrike remains was not in the molecular methods but rather in the lack of a complete DNA library of bird sequences linked to named voucher specimens (Doran et al. 1990, Ouellet 1994, Hermans et al. 1996, Christidis et al. 2006). Hence, a comprehensive DNA identification system that can be used for blood and tissue samples as well as an independent method of bird species identification is highly desirable and utilitarian.

In 2003 the Federal Aviation Administration (FAA) and the United States Air Force (USAF) joined the Smithsonian in a 5-year project to build a more complete library of mitochondrial DNA sequences of bird species. An additional project goal was to develop a standard protocol for DNA identifications of birdstrike remains that lacked sufficient feather evidence. We chose a 648–base-pair

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portion of the mitochondrial gene *cytochrome c oxidase subunit 1* (CO1), otherwise known as the barcoding gene, as one of our molecular markers because this gene has been shown to work well for bird species identification (Hebert et al. 2003, Yoo et al. 2006, Kerr et al. 2007).

We assessed the utility of using DNA barcoding and the Barcode of Life Database (BoLD) for identification of birdstrike remains and evaluated current field collecting techniques with respect to DNA analysis. Our objective was to determine if DNA barcodes are useful for species identification of degraded samples such as those obtained from birdstrikes.

METHODS

We conducted our study at the Smithsonian Institution's National Museum of Natural History, Division of Birds, Washington, D.C., and at the Smithsonian's Laboratories of Analytical Biology (LAB), Suitland, Maryland, USA. We received unidentified birdstrike samples from USAF bases and civil airfields representing 46 states including Alaska, Hawaii, and the District of Columbia; no samples were received from Kentucky, New Hampshire, Vermont, Virginia, or Wyoming. Our samples were evenly distributed throughout the United States with 44% coming from the eastern United States (east of the Mississippi River). Only 8% of our samples were from civil airfields. The discrepancy in reporting rates is due to the USAF regulation that requires mandatory birdstrike reporting and submission of bird remains for identification, whereas the FAA only requires civil aviation to voluntarily report birdstrikes.

We received 1,715 birdstrike samples for identification from 1 September through 31 December 2006 and initially sorted them according to whether they could be identified using traditional methods of morphological feather characters. We then submitted to LAB for DNA analysis those samples that lacked sufficient feather material for species-level identifications (821 cases) but had tissue or blood evidence. We also recorded date of strike, the condition of the sample upon receipt, date submitted to LAB, and date of completed identification. This allowed us to evaluate the amount time to complete DNA identifications and helped us evaluate the types of bird remains currently received for DNA identification and associated success rate of DNA identifications.

Using sterile techniques, we processed the recovered samples of muscle, tissue, blood, or debris by placing them in either 96-well DNA plates or individual 1.5-ml tubes. We sampled all tissues prior to any attempts at morphological identification. We extracted DNA from the tissue using the Qiagen DNeasy® Blood and Tissue Kit (Qiagen, Inc., Valencia, CA) or the Qiagen BioSprint® 96 DNA Blood Kit. We conducted polymerase chain reaction (PCR) on extracted DNA with negative controls and $\geq$1 sample positively identified using morphological feather characters with each batch that we processed. The positively identified sample in each 96-well plate was correctly matched by the BoLD database. Additionally, we checked 48 of the 554 samples that were identified using DNA barcodes by examining the microscopic characters that are unique to some species (e.g., swifts [*Chaetura, Aeronautes* spp.]) or the morphological comparisons of feather fragments to verify the DNA identifications. When we encountered false positives (4 cases), we repeated all matches to the species in that batch from the extraction or PCR. If the repeated PCR matched the feather morphology, we considered the identification to be correct. If the repeated PCR was inconsistent with the feather morphology, we repeated the extraction.

We amplified the mitochondrial CO1 gene on an MJ Research Tetrad Thermal Cycler® (Bio-Rad Laboratories, Hercules, CA). The 10.0-µl amplification reaction contained 1.0 µl of genomic DNA from a 200.0-µl extraction volume (containing anywhere from 10 ng/µl to 100 ng/µl DNA), 1.0 µl Bioline® dNTP mix at 10 µM, 1.0 µl Bioline 10× NH_4 Reaction buffer, 0.3 µl Bioline® $MgCl_2$ solution at 50 µM, and 0.2 µl BIOLASE® Taq Polymerase (BioLine USA, Randolf, MA), 5.5 µl dH_2O, 0.5 µl CO1 F primer (5′-TTCTCGAACCAGAAAGACATTGG CAC-3′) at 10 µM, and 0.5 µl CO1 R primer (5′-ACTTCTGGGTGGCCAAAGAATCAGAA-3′) at 10 µM. We set the thermal cycler for an initial denaturation at 94° C for 2 minutes followed by 25 cycles of 94° C for 20 seconds, 48° C for 45 seconds, and 72° C for 30 seconds, a final extension at 72° C for 3 minutes, and an indefinite hold at 10° C. We then cleaned PCR products with a diluted solution of ExoSAP-IT® (USB Corporation, Cleveland, OH). We diluted ExoSAP-IT 10-fold in dH_2O with 1.0 µl added to each 10.0 µl PCR sample. We then heated samples to 37° C for 30 minutes and at 80° C for 15 minutes and cycle-sequenced the samples using BigDye® Terminator v.3.1 (Applied Biosystems, Foster City, CA) in both forward and reverse directions. Our 10.0-µl reactions contained a 1.0-µl cleaned PCR product, 0.75 µl BigDye, 1.65 µl 5× cycle-sequencing buffer, 0.5 µl CO1 F or CO1 R primer, and 6.1 µl dH_2O. We conducted cycle-sequencing on the MJ tetrad thermal cycler in 25 cycles of 94° C for 30 seconds, 50° C for 30 seconds, and 60° C for 4 minutes with an indefinite hold at 10° C. We cleaned reactions using Sephadex-G50® (Sigma-Aldrich, St. Louis, MO) and loaded them on an Applied Biosystems 3130 DNA Analyzer® (Applied Biosystems) with a 36-cm array. We analyzed and trimmed trace files on a Finch server v2.20.4 software (Geospiza, Inc., Seattle, WA) and entered sequences into BoLD for quality checks and identification analysis (Ratnasingham and Hebert 2007). We preferred to use the reference library option on BoLD to ensure that the available library sequence was vouchered by specimens housed in museum collections. The full database option on BoLD includes data from blood, feathers, and other samples that are not connected to museum specimens. BoLD contains $\geq$2 individual vouchered samples for >93% of the breeding and pelagic avifauna of the United States and Canada.

Because of the large caseload and time constraints, we

conducted a one-pass attempt at DNA extractions. If we did not successfully amplify DNA off the first DNA extraction attempt, we considered the case unidentified and analyzed it later using traditional morphological identification methods.

Apart from problematic taxa listed in Kerr et al. (2007; Table 1), we considered query sequences that did not match sequences in the database at ≥98% to be unidentified to species level. We selected the conservative value of 98% similarity match of the unknown sequences to BoLD based on nearest-neighbor distance results reported by Kerr et al. (2007) with average congeneric distances of 4.3%. We later identified unknown samples with reported low congeneric distances of 95–98% to various taxonomic levels using a combination of supporting evidence such as microscopic examination, geographic distribution, population status, and by comparing any feather fragments present in the sample using traditional morphologic methods. When we encountered a DNA identification of a species with overlapping barcode clusters listed in Kerr et al. (2007) and could rule out one of the species based on geographic location, date of strike, and other morphologic feather characters, we accepted the DNA identification (see footnote in Table 1 for these taxa).

RESULTS

From 1 September through 31 December 2006, we submitted 821 of the 1,715 birdstrike samples that contained only blood or tissue for DNA testing; 267 (32.5%) had no viable DNA on the single pass amplification–extraction attempt and we subsequently identified them mainly to Order or Family level using traditional morphological microscopic techniques. The remaining 554 (67.5%) samples had viable DNA and 535 (96.6%) of those cases led to species-level identifications. We did not have problems extracting the 648–base-pair segment of CO1 in birdstrike samples.

From the 554 samples that had viable DNA, barcoding identified 128 species representing 14 Orders of birds from birdstrike cases (Table 1). Passeriformes were the most common Order of birds identified, with 79 species represented in 412 identifications. Savannah sparrow (*Passerculus sandwichensis*), horned lark (*Eremophila alpestris*), and barn swallow (*Hirundo rustica*) were the most common passerines identified in 31, 28, and 21 cases, respectively. Of the species identified, 49% were non-passeriform. In addition to birds, we identified 12 bats using DNA barcoding, including 2 red bats (*Lasiurus borealis*), 7 Brazilian free-tailed bats (*Tadarida brasiliensis*), and 2 unidentified bats.

We considered 19 cases unidentifiable using our DNA barcoding methods, including 7 cases with overlapping barcode clusters (3 cases blue-wing teal [*Anas discors*] and cinnamon teal [*A. cyanoptera*]; one case mallard [*A. platyrhynchos*] and American black duck [*A. rubripes*]; 3 cases *Empidonax* vs. *Contopus* flycatchers), and 12 cases with <98% sequence similarity match to BoLD. We considered 4 cases false positives due to cross-contaminations.

We sorted sample condition (dry, moldy, tissue, blood, etc.) into 6 categories for 499 of the 821 cases (Table 2). The United States Postal Service subjected 38 samples to irradiation procedures; we extracted DNA and identified species in 39% of those cases. Dried tissue, or feathers with dried tissue attached, resulted in 69% species identifications, and dried blood on cotton swabs worked 72% of the time. Moldy samples, or those that were collected using water and paper towels, resulted in the poorest success rate for DNA extractions (Table 2).

Age of the sample was not a critical factor in DNA extraction success. In 807 cases, we recorded number of days from the birdstrike event to laboratory sampling (age of sample) and we determined average DNA identification success rate for each time period. We categorized samples into 4 groups from 1 to >3 weeks old. Sample size and percent DNA identification success rates for each group were as follows: 1–7 days ($n = 152$; 69%), 8–14 days ($n = 422$; 72%), 15–21 days ($n = 147$; 75%), and >22 days ($n = 86$; 69%) post-strike. Although all of the categories consisted of similar types of samples, some of the oldest samples (116 days) yielded viable DNA, whereas many fresh samples (<7 days) were unsuccessful.

DISCUSSION

Our study of bird identifications mainly during autumn migration 2006 using DNA barcoding as a method to identify birdstrike samples that lacked sufficient feather material resulted in species identifications in 65.2% (535) of the cases. This value is a substantial achievement for our birdstrike identification program, which has relied completely on morphological identifications for >50 years. In our project, which only provides a glimpse of annual activity, 12.5% of the species identified using only DNA barcodes had a mean body mass >1.82 kg and exceeded the maximum bird mass standards established by the FAA that must be tested for airframes, windshields, and engines (Dolbeer and Eschenfelder 2003). A more complete DNA library encompassing larger birds (>1.82 kg) is needed on global scale for accurate DNA identifications of those hazardous birds.

Because of our experience with birdstrike identifications, we were not surprised by the diversity and quantity of the species of birds involved in birdstrikes, but we were impressed with the high number of some individual species such as savannah sparrow, barn swallow, and ruby-crowned kinglet (*Regulus calendula*). Although horned larks are typically the most commonly struck species by military aircraft in overall calculations of birdstrikes (E Leboeuf, USAF, personal communication), the savannah sparrow was equally numerous in our study and indicates that DNA barcoding will increase our knowledge of passerine species involved in birdstrikes. Most species we identified are associated with airport habitats where 90% of total and 66% of damaging birdstrikes occur (Sodhi 2002, Dolbeer 2006). Species that were numerous, such as the ruby-crowned kinglet and the 19 species of wood warblers

Table 1. Total species of birds identified in United States birdstrikes from 1 September to 31 December 2006 using DNA barcodes.

Order	Species	Total
Anseriformes	Canada goose (*Branta canadensis*)	3
	Wood duck (*Aix sponsa*)	1
	Gadwall (*Anas strepera*)	2
	American wigeon (*Anas americana*)	2
	Mallard (*Anas platyrhynchos*)[a]	1
	Northern shoveler (*Anas clypeata*)	1
	Ring-necked duck (*Aythya collaris*)	1
	Lesser scaup (*Aythya affinis*)	1
	Ruddy duck (*Oxyura jamaicensis*)	1
Podicipediformes	Pied-billed grebe (*Podilymbus podiceps*)	1
Pelecaniformes	American white pelican (*Pelecanus erythrorhynchos*)	1
	Double-crested cormorant (*Phalacrocorax auritus*)	1
	Anhinga (*Anhinga anhinga*)	1
Ciconiiformes	Great blue heron (*Ardea herodias*)	3
	Black vulture (*Coragyps atratus)*	5
	Turkey vulture (*Cathartes aura*)	3
Falconiformes	White-tailed kite (*Elanus leucurus*)	1
	Mississippi kite (*Ictinia mississippiensis*)	1
	Cooper's hawk (*Accipiter cooperii*)	1
	Broad-winged hawk (*Buteo platypterus*)	2
	Swainson's hawk (*Buteo swainsoni*)	1
	Red-tailed hawk (*Buteo jamaicensis*)	4
	Ferruginous hawk (*Buteo regalis*)	1
	American kestrel (*Falco sparverius*)	1
	Merlin (*Falco columbarius*)	1
Gruiformes	Clapper rail (*Rallus longirostris*)	1
	American coot (*Fulica americana*)	1
Charadriiformes	Pacific golden-plover (*Pluvialis fulva*)	1
	Killdeer (*Charadrius vociferus*)	17
	Western sandpiper (*Calidris mauri*)	2
	Least sandpiper (*Calidris minutilla*)	4
	Baird's sandpiper (*Calidris bairdii*)	2
	Pectoral sandpiper (*Calidris melanotos*)	1
	American woodcock(*Scolopax minor*)	1
	Wilson's phalarope (*Phalaropus tricolor*)	1
	Franklin's gull (*Larus pipixcan*)[a]	1
Columbiformes	Rock pigeon (*Columba livia*)	4
	Mourning dove (*Zenaida macroura*)	13
Cuculiformes	Yellow-billed cuckoo (*Coccyzus americanus*)	1
	Black-billed cuckoo (*Coccyzus erythropthalmus*)	1
Strigiformes	Barn owl (*Tyto alba*)	1
Caprimulgiformes	Common nighthawk (*Chordeiles minor*)	5
Apodiformes	Chimney swift (*Chaetura pelagica*)	15
	Vaux's swift (*Chaetura vauxi*)	1
	White-throated swift (*Aeronautes saxatalis*)	1
	Ruby-throated hummingbird (*Archilochus colubris*)	3
Piciformes	Red-headed woodpecker (*Melanerpes erythrocephalus*)	1
	Yellow-bellied sapsucker (*Sphyrapicus varius*)	3
	Northern flicker (*Colaptes auratus*)	1
Passeriformes	Western wood-pewee (*Contopus sordidulus*)	2
	Hammond's flycatcher (*Empidonax hammondii*)	1
	Great crested flycatcher (*Myiarchus crinitus*)	1
	White-eyed vireo (*Vireo griseus*)	2
	Warbling vireo (*Vireo gilvus*)	3
	Philadelphia vireo (*Vireo philadelphicus*)	1
	Red-eyed vireo (*Vireo olivaceus*)	9
	Horned lark (*Eremophila alpestris*)	28
	Tree swallow (*Tachycineta bicolor*)	6
	Cliff swallow (*Petrochelidon pyrrhonota*)	8
	Cave swallow (*Petrochelidon fulva*)	2
	Barn swallow (*Hirundo rustica*)	21
	House wren (*Troglodytes aedon*)	3
	Winter wren (*Troglodytes troglodytes*)	2
	Sedge wren (*Cistothorus platensis*)	1
	Golden-crowned kinglet (*Regulus satrapa*)	6
	Ruby-crowned kinglet (*Regulus calendula*)	19
	Blue-gray gnatcatcher (*Polioptila caerulea*)	1
	Mountain bluebird (*Sialia currucoides*)	1
	Veery (*Catharus fuscescens*)	4
	Gray-cheeked thrush (*Catharus minimus*)	2
	Bicknell's thrush (*Catharus bicknelli*)	1
	Swainson's thrush (*Catharus ustulatus*)	10
	Hermit thrush (*Catharus guttatus*)	5
	Wood thrush (*Hylocichla mustelina*)	6
	American robin (*Turdus migratorius*)	13
	Varied thrush (*Ixoreus naevius*)	1
	Gray catbird (*Dumetella carolinensis*)	8
	Brown thrasher (*Toxostoma rufum*)	2
	European starling (*Sturnus vulgaris*)	1
	American pipit (*Anthus rubescens*)	2
	Cedar waxwing (*Bombycilla cedrorum*)	11
	Tennessee warbler (*Vermivora peregrina*)	6
	Orange-crowned warbler (*Vermivora celata*)	7
	Nashville warbler (*Vermivora ruficapilla*)	1
	Yellow warbler (*Dendroica petechia*)	1
	Chestnut-sided warbler (*Dendroica pensylvanica*)	3
	Magnolia warbler (*Dendroica magnolia*)	3
	Cape May warbler (*Dendroica tigrina*)	1
	Black-throated blue warbler (*Dendroica caerulescens*)	1
	Yellow-rumped warbler (*Dendroica coronata*)	17
	Black-throated green warbler (*Dendroica virens*)	4
	Pine warbler (*Dendroica pinus*)	3
	Palm warbler (*Dendroica palmarum*)	4
	Bay-breasted warbler (*Dendroica castanea*)	2
Passeriformes	Blackpoll warbler (*Dendroica striata*)	5
	American redstart (*Setophaga ruticilla*)	2
	Ovenbird (*Seiurus aurocapilla*)	11
	Northern waterthrush (*Seiurus noveboracensis*)	3
	Mourning warbler (*Oporornis philadelphia*)	2
	Common yellowthroat (*Geothlypis trichas*)	9
	Yellow-breasted chat (*Icteria virens*)	4
	Scarlet tanager (*Piranga olivacea*)	1
	Spotted towhee (*Pipilo maculatus*)	3
	Chipping sparrow (*Spizella passerina*)	6
	Clay-colored sparrow (*Spizella pallida*)	1
	Brewer's sparrow (*Spizella breweri*)	1
	Field sparrow (*Spizella pusilla*)	3
	Vesper sparrow (*Pooecetes gramineus*)	3
	Savannah sparrow (*Passerculus sandwichensis*)	31
	Grasshopper sparrow (*Ammodramus savannarum*)	4

Table 1. Continued.

Order	Species	Total
	Le Conte's sparrow (*Ammodramus leconteii*)	2
	Fox sparrow (*Passerella iliaca*)	1
	Song sparrow (*Melospiza melodia*)	9
	Lincoln's sparrow (*Melospiza lincolnii*)	9
	Swamp sparrow (*Melospiza georgiana*)	12
	White-throated sparrow (*Zonotrichia albicollis*)	11
	White-crowned sparrow (*Zonotrichia leucophrys*)[a]	10
	Dark-eyed junco (*Junco hyemalis*)[a]	13
	Smith's longspur (*Calcarius pictus*)	1
	Rose-breasted grosbeak (*Pheucticus ludovicianus*)	1
	Indigo bunting (*Passerina cyanea*)	6
	Painted bunting (*Passerina ciris*)	2
	Red-winged blackbird (*Agelaius phoeniceus*)	1
	Eastern meadowlark (*Sturnella magna*)[a]	5
	Western meadowlark (*Sturnella neglecta*)[a]	1
	Rusty blackbird (*Euphagus carolinus*)	1
	Hoary redpoll (*Carduelis hornemanni*)[a]	1
	American goldfinch (*Carduelis tristis*)	1
Unidentified		19
Total		554

[a] Species with overlapping barcode clusters that we identified based on additional information such as geographic location, date of strike, and any morphological feather evidence in the sample.

(Parulinae), are probably a result of migration patterns rather than habitat preferences.

Unlike results reported by Christidis et al. (2006) for experimental laboratory samples, age of the sample (time from the birdstrike incident to the time of DNA collection) did not seem to affect identification success of our samples. However, condition of the birdstrike sample at time of DNA collection was critical (Table 2).

We found DNA barcoding substantially improved our ability to identify minute samples from birdstrike cases. Because the DNA library for CO1 is now almost complete (>93%) for the birds of North America (north of Mexico) and is increasing on a global scale, this molecular marker is ideal for birdstrike identification. Due to the broad availability of universal CO1 primers, this molecular marker will also benefit other wildlife identifications such as deer, coyote, and bat aircraft strikes.

When problematic species that have overlapping barcode clusters are discovered in unknown samples, or when percent similarity of the unknown sample is not indicating acceptable level (98% sequence similarity in our study), DNA identifications are not reliable and morphological identifications are required. DNA barcoding has great potential on a global scale in regions of the world where research collections and expertise for morphological identifications are unavailable but university or research laboratories are available.

Management programs to reduce wildlife strikes to aircraft depend on accurate species identifications as the fundamental first step in birdstrike prevention and aircraft safety design, yet only about 24% of the civil birdstrikes are identified to the species level (Cleary et al. 2006). Furthermore, improving birdstrike species identification has been cited as a critical action needed to improve the ranking of wildlife hazards to aviation (Dolbeer et al. 2000) and will add to our knowledge of bird migration heights and timing by species (Dolbeer 2006). Until now, many of the birdstrike samples were too minute or lacked sufficient evidence to identify to the species level, making participation in reporting programs frustrating and habitat management or engineering decisions less precise. Knowing that species-level identifications are possible with minute samples will improve birdstrike reporting on a global scale and ultimately aid in the improvement of aviation safety by further defining our knowledge of the exact species of birds that are hazardous to aircraft.

Table 2. DNA identification success of 499 United States birdstrike samples surveyed from 1 September to 31 December 2006. We grouped the samples based on 6 categories of condition at the time of DNA collection. The table lists the condition of the sample, percent success for the category (% success), number of cases that contained DNA (DNA), number of cases that did not contain DNA (No DNA), and total number of samples in the category. Samples collected using current recommendations (category 4–6) of wiping the aircraft with paper towels had the lowest levels of DNA extraction success.

Condition of sample	% success	DNA	No DNA	Total samples
1. Blood on cotton swab	72	52	20	72
2. Dry tissue or feather	69	135	60	195
3. Irradiated	39	15	23	38
4. Dry paper towel	24	17	55	72
5. Moldy wet paper towel	23	20	67	87
6. Wet paper towel	17	6	29	35

MANAGEMENT IMPLICATIONS

Our current field-sample collecting methods involve wiping the aircraft with a damp paper towel when the remains appear to include only tissue and blood. However, we found that those types of samples, regardless of the age of the sample, often had mold growing on the tissue and did not result in viable DNA extractions. For blood and tissue samples, we now recommend wiping the aircraft with alcohol rather than water and allowing the samples to completely dry before shipping as soon as possible. New technologies for collecting samples in the field (e.g., Whatman® FTA cards; Schleicher & Schuell, Whatman International Ltd, United Kingdom) are now being tested to determine if this will improve the quality of the DNA in these forensic samples, whereas others remain to be examined (e.g., Allprotect, Qiagen® Inc.; RNA*later*®, Ambion, Austin, TX).

Because traditional morphologic and microscopic methods continue to be used in approximately half of the >3,500 annual identification cases, and morphological expertise is still needed to identify nearly 33% of the birdstrike cases that currently lack viable DNA, we recommend using a combination of morphological and molecular methods such as DNA barcoding for efficient, cost-effective birdstrike

identifications. Improving species identification is crucial to proper implementation of species management plans on airfields, the design of aircraft parts and engines, and in making computer models to assess the risk of birdstrikes. The addition of a DNA identification tool such as DNA barcoding improves the ability to make species-level identifications for these applications and could also be useful for avian conservation implications in aiding our understanding of flight patterns of migratory bird species of concern.

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