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Chapter 6

GENETIC ANALYSES OF NONINVASIVELY COLLECTED FEATHERS CAN PROVIDE NEW INSIGHTS INTO AVIAN DEMOGRAPHY AND BEHAVIOR

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ABSTRACT

The use of noninvasively collected samples for genetic analyses has dramatically impacted studies of species that are threatened, endangered, or sensitive to disturbance. Although the pioneering work employing noninvasively collected samples for genetic analyses used hair or feces to study mammals, naturally shed feathers are increasingly used to study avian demography and behavior. Studies on larger birds such as eagles, vulture, or herons can particularly benefit from the incorporation of research that uses noninvasively collected samples, as these species are often challenging to study by traditional means because they are difficult to capture and mark. Wing-tagging and telemetry studies generally collect information on only a limited number of individuals due to logistical and financial constraints, which means that data on demography and dispersal are usually sparse for these species. A strength of using single, shed feathers for genetic analyses is that DNA markers can provide permanent genetic "tags" for identification; in general, if two feathers are genetically identical they originated from the same individual. Because tens, hundreds, or even thousands of feathers can be collected from nesting and communal roosting or foraging sites, genetic tags can be generated on a much larger number of individuals than has been historically possible. In this chapter we discuss how genetic data generated from feathers can address avian demography (population size, vital rates, and movement) and behavior (habitat use and mating systems), as well as provide guidance on issues that should be considered when incorporating noninvasively collected genetic data into avian research.

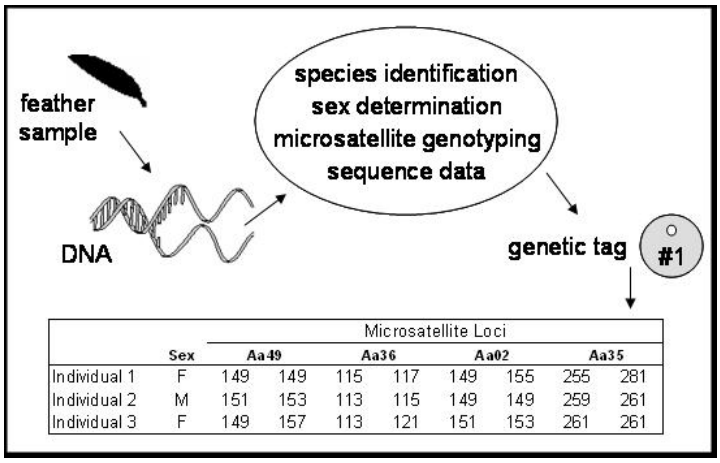
INTRODUCTION

Genetic techniques provide contemporary alternatives to traditional means of studying wildlife. For species that are inherently difficult to study, utilizing noninvasively collected samples makes the application of those techniques particularly powerful. Genetic analyses of noninvasive samples can inform two primary aspects of an organism's biology: population demography (e.g., census size or turnover) and behavioral ecology (mating system, home range size, etc.). Of course, many behaviors like dispersal also influence population processes, so the two are not always decoupled. A wide variety of studies have successfully applied genetic analyses on hair and feces to topics of mammalian demography and behavior (Tables 1). Furthermore, noninvasive genetic samples also have been used to address issues at the species level (e.g., species identification, Rudnick et al. 2007; hybridization, Adams et al. 2003). In this chapter we review (or preview) noninvasive genetic contributions to avian demography and behavior, primarily in relation to large birds that are difficult to catch and mark using traditional field methods.

Table 1. Examples of studies that used noninvasive sampling of hair and/or feces to investigate mammalian demography and behavior

Species	Parameter or Behavior	Citation
African forest elephant	abundance, sex ratio	Eggert et al. 2003
Atlantic spotted dolphin	parentage	Green et al. 2007
badger	abundance	Wilson et al. 2003
Bornean gibbon	parentage	Oka & Takenaka 2001
Bornean orang-utan	migration	Goossens et al. 2005
brush-tailed rock wallaby	population size	Piggot et al. 2006a
brush-tailed rock wallaby	dispersal	Piggot et al. 2006b
Canada lynx	presence/absence in habitat	McKelvey et al. 2006
chimpanzee	parentage, reproductive success	Constable et al. 2001
coyote	population size	Kohn et al. 1999
coyote	population size, survival rates	Prugh et al. 2005
grey wolf	parentage, relatedness	Lucchini et al. 2002
grizzly bear	population size	Romain-Bondi et al. 2004
Louisiana black bear	abundance, gene flow	Triant et al. 2004
red wolves	hybridization with coyotes	Adams et al. 2003
southern hairy-nosed wombat	burrow use, ranging behavior	Walker et al. 2006
wolverine	number of founders, relatedness	Hedmark & Ellegren 2007
wolverine	population size, dispersal	Flagstad et al. 2004

For birds, feathers are the primary genetic sample that can be collected noninvasively (although it is possible to extract DNA from egg shells and feces, Pearce et al. 1997; Regnaut et al. 2006; Schamltz et al. 2006). One of the first studies to demonstrate that naturally shed feathers were suitable for genetic analyses was Morin et al. (1994), who utilized feathers collected from the nesting sites of four hornbill species to sequence a portion of a mitochondrial gene for phylogenetic analyses. A number of additional papers further confirmed that feathers yield DNA of appropriate quantities and qualities for genetic analyses (Pearce et al. 1997; Segelbacher et al. 2002; Horvath et al. 2005; Hogan et al. 2007),



		Microsatellite Loci							
	Sex	Aa49		Aa36		Aa02		Aa35	
Individual 1	F	149	149	115	117	149	155	255	281
Individual 2	M	151	153	113	115	149	149	259	261
Individual 3	F	149	157	113	121	151	153	261	261

Figure 1. A diagram that demonstrates how nuclear, mitochondrial, and molecular sexing markers can be combined to create genetic tags for individual identification.

and naturally shed feathers subsequently have been used to study the genetic relationships between Canada goose populations (*Branta canadensis*, Talbot et al. 2003) and assess the genetic variation and differentiation between populations of capercaillie (*Tetrao urogallus*, Rodriguez-Munoz et al. 2007). However, despite this pioneering work on feathers, ornithologists have lagged behind mammalogists in applying noninvasive sampling to their research.

While naturally shed feathers are clearly an appropriate source of DNA for genetic analyses, few avian studies have used large-scale collections of feathers to study the demography or behavior of birds. One of the most powerful, but overlooked, advantages of using feathers for genetic analyses is the possibility of individual identification. Molecular markers can provide genetic "tags" for single, naturally shed feathers that, by extension, identify the donors. Genetic tags have several advantages over physical tags. A primary advantage is permanency, as genetic tags are intrinsic to an individual and cannot fall off or change appearance over time. Additionally, if generated from noninvasively collected samples, genetic tags do not require that an individual be captured for initial marking. This is especially important for species of conservation concern that are rare, inherently hard to catch, or sensitive to disturbance. Data from nuclear, mitochondrial, and molecular sexing markers can be combined to create genetic tags that both identify and reveal informative information about individuals (Figure 1).

Genetic tags can be used to address a variety of topics related to avian demography and behavior. Here, we break those topics into five general categories: population size, vital rates, movement, habitat use, and mating systems. Because few studies have used naturally shed feathers to address these questions, the majority of examples are drawn from research conducted on an eastern imperial eagle (*Aquila helica*) population from north-central Kazakhstan (Rudnick et al. 2005; Rudnick et al. 2008). Research on the demography and behavior of birds of prey is often limited by small sample sizes, due to the difficulties inherent in initially finding, capturing, marking, and re-locating individuals. While much of this chapter is structured with those species in mind, noninvasive sampling also can be applied to most other avian taxa.

DEMOGRAPHY

Population Size

Size is a fundamental aspect of population demography. Furthermore, abundance estimation across a geographic range is vital for assessing the status of threatened and endangered species. Traditional estimates of size and abundance can be difficult to obtain for many species of birds, particularly birds of prey, because most species are highly mobile and individuals can be difficult to locate. Consequently, many abundance estimates are based on the breeding portions of populations because the presence of territorial adults is easier to monitor than the number of "floating" individuals (i.e., non-breeders). Genetic data can improve upon these estimates by incorporating information on non-territorial individuals that feed or roost at communal areas, where large numbers of feathers usually can be collected over relatively short periods of time (e.g., shed feathers collected at clay licks have been used to genetically estimate the effective size of parrot populations; Gebhardt 2007). Genetic tags collected from these feathers can provide a minimum estimate of size and, if samples are repeatedly collected from a given site, can be used to monitor the turnover of individuals. If collected correctly, genetic tags can be analyzed in either a traditional mark-recapture framework (Triant et al. 2004; Prugh et al. 2005; Dreher et al. 2007) or by mark-recapture models specifically designed for genetic data generated from noninvasively collected samples (Lukacs and Burnham 2005).

Genetic tags generated from hair or fecal samples are now commonly used to estimate population size in mammals (e.g., Wilson et al. 2003; Triant et al. 2004). Furthermore, noninvasive abundance estimation has been greatly beneficial for mammal species that are rare (e.g., Flagstad et al. 2004; Piggott et al. 2006a) or elusive (e.g., Eggert et al. 2003; Walker et al. 2006). Although the size of many bird populations is often hard to estimate by traditional means, few studies have used large-scale collections of naturally shed feathers to estimate abundance from noninvasively generated genetic tags.

Rudnick et al. (2008) demonstrated the power of this technique by estimating eagle abundance from naturally shed feathers. In that study, greater than 1500 feathers were collected over four two-hour collection periods at a nature reserve in north-central Kazakhstan. After a genetic assay was used to verify species of origin (Rudnick et al. 2007), those feathers provided genetic tags for 287 eastern imperial eagles. Beyond the simple estimate of abundance, this genetic tagging study provided several additional key insights into the demography of the population. First, the number of floaters documented at the nature reserve was over an order of magnitude greater than suspected from a decade of visual observations. Second, nearly all of the floaters genetically tagged were non-breeding individuals (see Rudnick et al. 2008 for additional information). Third, the total number of floaters was estimated to be approximately three times the size of the breeding population. As a whole, this study demonstrated that abundance can be noninvasively estimated from naturally shed feathers with minimal field work, particularly when communal use areas yield large numbers of feathers. Furthermore, Rudnick et al. (2008) demonstrated that estimates of abundance should be based not only on the breeding portion of a population, but rather on all individuals present at a study site.

Vital Rates

Vital rates are demographic measures that describe how a population changes over time. They include estimates of survival, recruitment, turnover, and sex ratios. Accurate estimates of vital rates are necessary for understanding the current status of populations, as well as for predicting future changes in population stability. Vital rates for birds are generally estimated through banding, ringing, wing tagging, telemetry, and visual observation. However, these methods are both time-intensive and expensive. These limitations often reduce sample sizes, particularly for large species that are difficult to catch (e.g., birds of prey, some wetland and waterbird species like herons and swans, etc.). Furthermore, sex ratios usually are presented only for nestlings or fledglings rather than for the non-breeding portions of populations because, although the gender of nestlings can be determined by physical examination, juvenile and/or adult plumage often is ambiguous.

Genetic tags generated from naturally shed feathers have the potential to dramatically improve the estimation of avian vital rates, particularly for birds of prey. For example, turnover in a breeding population can only be accurately assessed if the presence of specific individuals can be monitored over time. Rudnick et al. (2005) used shed feathers collected at eastern imperial eagle nests to genetically tag breeding pairs that were present during a four year period (Figure 2). A total of 82 adults were identified over that time interval, and annual turnover in the breeding population was estimated to be 16% (a mean of seven individuals were lost/replaced per year). Because eastern imperial eagles exhibit high nesting site fidelity, this estimate of turnover also was used as a proxy for annual adult mortality in a simple model designed to characterize the stability of the population. The assessment of such a model would not have been possible without the turnover estimate, as no other estimates of mortality were available for the species.

There are numerous avian populations that currently benefit from traditional, long-term monitoring programs. Many of these pre-existing programs are ideal for incorporating genetic tagging via noninvasive sampling because nests are already frequently visited and substantial information is available on both past and present nesting locations. Thus, collecting shed feathers for estimating turnover, as described by Rudnick et al. (2005), would require little additional field work. If nestlings are routinely handled prior to fledging, recruitment also could be estimated with relative ease. Nestlings could be genetically tagged by direct sampling, then compared to breeding adults tagged from shed feathers collected in subsequent breeding seasons. The timing of recruitment for birds hatched in the population could be monitored, as well as the parentage of recruited individuals.

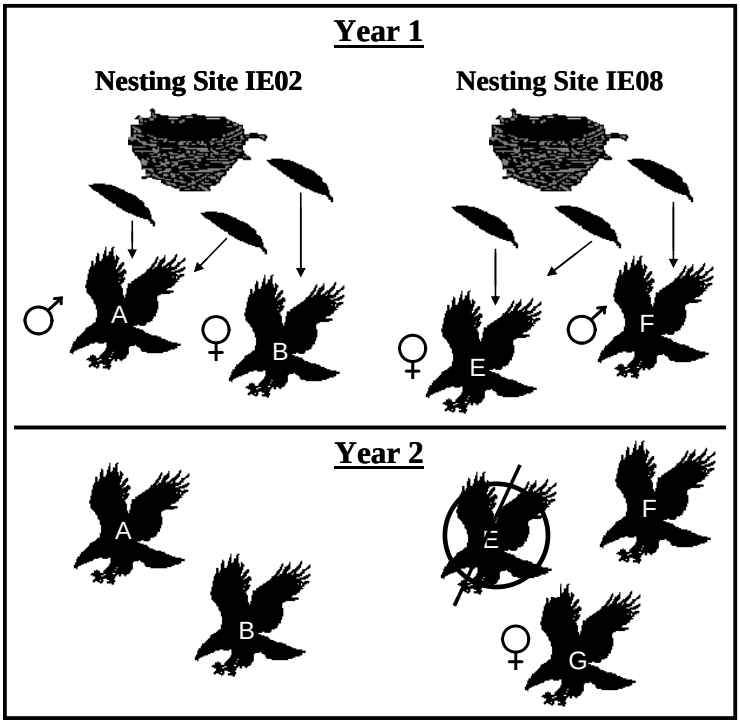


Figure 2. A method for measuring turnover in a population by using noninvasively collected feathers to genetically tag breeding adults. Adult feathers were collected at nesting sites during two breeding seasons, microsatellite genotyping and genetic sexing were combined to generate a genetic profile for each feather, and feathers were assigned to individuals based on their genetic profiles. Male A and Female B were present at nesting site IE02 in both Year 1 and Year 2; no turnover occurred at that site between those years. At nesting site IE08, Female E and Male F were present in Year 1 but Female E was replaced by Female G in Year 2. This scenario represents one turnover event. (Reprinted from Rudnick et al. 2005.)

This type of study would be particularly informative and beneficial for species with delayed sexual maturity (i.e., most long-lived avian species), because large numbers of nestlings would not need to be physically tagged and there would be no concern over physical tags falling off or being unreadable years after they were applied. Finally, if juveniles congregate in communal areas that facilitate the collection of large numbers of feathers, genetic tagging could be used to direct genetic sex determination of the identified individuals and sex ratios for the non-breeding portions of populations could be assessed.

Computer modeling has demonstrated that adult survival and the juvenile:adult ratio may be important variables to incorporate into conservation programs designed to monitor and predict the future stability of many bird populations (Katzner et al. 2006, 2007). Furthermore, if adult turnover is estimated from genetic analyses, then models predicting demographic changes in avian populations can be developed in a timely manner (Kenward et al. 2007). Using naturally shed feathers to genetically tag individuals has the potential to significantly improve the estimation of these and other avian vital rates, by increasing the number of individuals sampled per species and, potentially, by increasing the number of species monitored.

Movement (Dispersal, Migration, and Roaming)

The movement of individuals can reveal many aspects of a species' demography, particularly when movement relates to how population size and composition change due to dispersal, migration, or roaming. The movements of many birds, however, are inherently difficult or expensive to monitor because species often are vagile and capable of traveling long distances over relatively short periods of time. Traditional means of monitoring movement generally depend on visual observations or expensive telemetry applications. Although visual observations seem inexpensive, they require significant time and labor, which is costly, and observations can only be collected if a bird can be physically located. Telemetry, on the other hand, can be used to collect movement data remotely without any *a priori* information about an individual's location. However, the expense associated with both radio and satellite telemetry usually severely limits the sample size. Telemetry also requires that individuals be invasively captured and fitted with a transponder and most visual observations require that individuals be marked initially for subsequent identification.

Genetic tags collected from naturally shed feathers offer an alternative for indirectly monitoring avian movements. Both short and long distance movements can be assessed, permitting the investigation of a variety of topics. In principle, if suitable numbers of feathers are collected from breeding and wintering grounds, seasonal movements can be studied by identifying the changing locations of specific individuals. Noninvasive sampling also can be combined with direct sampling to study natal philopatry and dispersal. If a breeding population is closely monitored and chicks are routinely handled prior to fledging (e.g., for measuring and weighing), samples can be directly collected and genetic tags can be generated for nearly all individuals hatched at a study area. Genetic tags generated from juvenile or adult feathers collected in subsequent years, either from the same study area or elsewhere, can be compared to the chicks' genetic tags to determine natal origins (Rudnick et al. 2008). Although dispersal and population connectivity is commonly studied in a broad sense by using blood samples to characterize the genetic differentiation between bird populations (Kretzmann et al. 2003; Martinez-Cruz et al. 2004; Bollmer et al. 2005; Koopman et al. 2007), the type of study just described would be unique in providing behavioral data on individual dispersal in addition to more conventional demographic data.

BEHAVIOR

Habitat Use

Genetic tags generated from feathers collected throughout a study site can provide infomation on the habitat use and short-distance movements of specific individuals. Habitat use can include both the temporal and spatial use of nests, roosts, and foraging areas, as well as the characterization of territory size. For example, Meyburg et al. (2007) used naturally shed feathers collected from lesser spotted eagle (*Aquila pomarina*) eyeries to disprove the prevailing hypothesis that females remain within a few kilometers of their nest while raising young. Genetic tags were generated for 36 birds from 24 eyeries, through a combination of direct and noninvasive sampling. Shed feathers collected from nests were subsequently

compared to the genetic tags to determine which birds were visiting the eyeries, revealing that non-resident females were visiting nesting sites.

In another study, Rudnick et al. (2008) used genetic tags generated from noninvasively collected feathers to study roost usage in eastern imperial eagles. More than 1500 feathers, representing 287 individuals, were collected from four communal roosting sites over a two week period. All roosting sites were sampled four times during the two week period to determine how individuals were using the study area. Individuals were found to utilize multiple roosts, rather than preferentially frequenting a given roost. Furthermore, although an effort was made to collect only eastern imperial eagle feathers due to permitting considerations, a genetic species identification assay (Rudnick et al. 2007) indicated that a small percentage of the feathers collected at each roosting site were misidentified and were actually from white-tailed eagles. This indicated that the eastern imperial eagles were communally roosting with at least one additional eagle species. If all feathers had been collected from roost sites (rather than targeting a single species), a more complete inventory of the species using the sites would have been possible.

Mating Systems

Genetic evaluations of mating systems have become increasingly common, and can be informative with regards to reproductive success, sex-ratio adjustment, cannibalism/predation, and dispersal (DeWoody 2005). For birds, one of the most prominent areas of research has been the elucidation of the difference between "social" and "genetic" mating systems. Conventional theory regarding avian mating systems was primarily driven by field observations that suggested a pair of individuals, one male and one female, was generally responsible for producing and raising a nest of offspring. Thus, historically, most bird species were believed to be monogamous (Lack 1968). This belief was refuted when molecular markers revealed extra-pair parentage in broods produced by supposedly monogamous species, indicating that the individuals were socially monogamous but genetically promiscuous (Avise 1996). In fact, when data were summarized across 99 socially monogamous or polygynous bird species (Griffith et al. 2002), less than 25% of species were found to be genetically monogamous.

Rates of extra-pair fertilization vary substantially across bird species. Because over 50% of interspecific variation in rates of extra-pair fertilization occurs between families or orders, a continuing characterization of rates in diverse taxa is necessary to understand the evolution of avian mating systems. Many eagles, hawks, owls, and vultures were historically believed to be monogamous, but few studies have assessed their degree of monogamy with genetic data. Unlike passerines, many birds of prey exhibit characteristics consistent with hypothesized correlates of genetic monogamy (Neudorf 2004). For example, numerous species are long-lived, display social monogamy both within and between breeding seasons, and require significant paternal investment in offspring. While this suggests that many birds of prey may indeed be both genetically and socially monogamous, genetic analyses are necessary to corroborate behavioral observations.

Research on genetic mating systems is frequently hampered because samples must be collected from both parents and offspring. For birds of prey, samples can generally be collected directly from offspring prior to fledging, but direct sampling of parents is often

impossible or incredibly challenging due to the difficulties inherent in capturing adults. Adult feathers collected from nesting sites provide an alternative to direct sampling, as long as a sufficient number of feathers are collected to "capture" both parents. Table 2 summarizes research on the genetic mating systems of socially monogamous birds of prey. Out of 15 studies, only one utilized noninvasively collected feathers to sample adults. A striking feature of the remaining studies was that, for all but one exception, the species under investigation have average weights of less than 1000 grams. Thus, it is likely that small size facilitated capture and direct sampling in those cases. Using feathers to genetically investigate mating systems in birds of prey, rather than relying solely on direct sampling, would quite likely increase the number and variety of species represented.

TECHNICAL GUIDELINES

Research utilizing naturally shed feathers as a source of DNA has the potential to significantly impact future studies of birds of prey. However, there are several issues that should be considered when noninvasively collected samples are employed. A number of papers thoroughly review the application of noninvasive sampling to wildlife research (Taberlet et al. 1999; Piggott and Taylor 2003; Waits and Paetkau 2005), but a brief description of the primary concerns relevant to naturally shed feathers is provided here.

DNA isolated from noninvasively collected samples is generally of both low quantities and qualities. Consequently, genetic research using that DNA is prone to contamination, the amplification of false alleles, and allelic dropout. Contamination can generally be mitigated by the implementation of rigorous sample handling and data collection protocols, while the amplification of false alleles (i.e., incorrect genotypes) is likely infrequent (Gagneux et al. 1997; Goossens et al. 1998). Consequently, the primary concern for genetic research using noninvasively collected samples is usually allelic dropout, which occurs when only one allele from a heterozygous individual is detected. Allelic dropout is thought to be due to stochastic sampling error when low concentrations of template DNA are pipetted for molecular marker amplification (Taberlet et al. 1999).

A number of methods have been proposed for improving the quality of genetic data generated from noninvasively collected samples. Because most data problems are related to the concentrations of DNA used for analyses, one way to improve genetic data is to pre-screen DNA extracts (Morin et al. 2001). If DNA concentrations are estimated at the beginning of a study, extracts with concentrations below a given threshold can be removed from the study before genetic data collection begins. Furthermore, concentration estimates for all remaining extracts can be used to guide subsequent molecular marker amplification protocols. For example, Ball et al. (2007) used a fluorescence assay to estimate the total DNA concentration and the target DNA concentration present in extracts from fecal samples. Results from the assay guided the amount of template needed from each extract for molecular marker amplification and identified extracts that required a genotyping protocol specifically modified for low target DNA concentrations. The "multiple-tubes approach" (Navidi et al. 1992; Taberlet et al. 1996) is an alternative method for improving the quality of genetic data generated from noninvasively collected samples.

Table 2. Estimates of the percentage of extra-pair offspring (% EPP Offspring) and the percentage of broods that contain at least one extra-pair chick (% EPP Broods) for socially monogamous birds of prey. Numbers in parentheses are sample sizes and all studies but one (marked with an asterisk) used invasive blood sampling. Rudnick et al. (2005) used naturally shed feathers to genetically tag breeding adults for genetic parentage analyses

Family	Species	Common Name	% EPP Offspring	% EPP Broods	Citation
Accipitridae	Aquila heliaca	eastern imperial eagle	0 (166)	0 (86)	Rudnick et al. 2005*
Ciconiidea	Coragyps atratus	black vulture	0 (36)	0 (16)	Decker et al. 1993
Falconidae	Falco columbarius	merlin	0 (47)	0 (18)	Warkentin et al. 1994
Falconidae	Falco eleonorae	Eleonoras falcon	0 (60)	0 (17)	Swatschek et al. 1993
Falconidae	Falco naumanni	lesser kestrel	3.5 (87)	3.9 (26)	Negro et al. 1996
Falconidae	Falco naumanni	lesser kestrel	7.29 (96)	9.67 (31)	Alcaide et al. 2005
Falconidae	Falco sparverius	American kestrel	11.2 (89)	9.5 (21)	Villarroel et al. 1998
Falconidae	Falco tinnunculus	European kestrel	1.9 (319)	2.7 (75)	Korpimaki et al. 1996
Strigidae	Asio noctua	long-eared owl	0 (59)	0 (12)	Marks et al. 1999
Strigidae	Athene noctua	little owl	0 (53)	0 (16)	Muller et al. 2001
Strigidae	Otus asio	Eastern screech-owl	0 (80)	0 (23)	Lawless et al. 1997
Strigidae	Otus elegans botelensis	Lanyu scops owl	1.5 (200)	1.85 (108)	Hsu et al. 2006
Strigidae	Otus flammeolus	flammulated owl	0 (37)	0 (17)	Arsenault et al. 2002
Strigidae	Strix aluco	tawny owl	0.7 (137)	2.7 (37)	Saladin et al. 2007
Tytonidae	Tyto alba	barn owl	0.474 (211)	1.85(54)	Roulin et al. 2004

When this method is applied to microsatellite genotyping, each locus is amplified multiple times from the same DNA extract and a given genotype is not accepted until a specific number of replicates provide identical results. Because the suggested number of replicate genotypes varies, Miquel et al. (2006) developed a standardized quality index that can be used to compare the reliability of genotypes generated from the multiple-tubes approach across studies.

Regardless of the steps taken to ensure data quality, estimates of genotyping error rates are necessary for evaluating a study's results. Error rates provide information on the reliability of genotypes and can be used to guide the appropriate number of replicates needed for the multiple-tubes approach. Broquet and Petit (2004) discuss numerous methods for estimating error rates. Additionally, McKelvey and Schwartz (2004) describe two tests designed to ascertain the impact of allelic dropout. Both tests are applicable to datasets that contain multiple samples from the same individual; one test examines the bimodality of genetic tags and the other examines capture histories derived from genetic tags.

When noninvasively collected samples are used to generate genetic tags for individual identification, estimates of the probability of identity associated with those genetic tags provide information on their accuracy. The probability of identity is the probability that two individuals are genetically identical due strictly to chance, or the probability of detecting identical genetic tags for two different individuals. Probabilities of identity are dependent on the number and variability of microsatellite loci employed, so they can be lowered if additional loci are added to a study. Waits et al. (2001) describe a number of algorithms for estimating the probability of identity and suggest a method for determining upper and lower bounds for the number of loci required to identify individuals at a specified level of statistical confidence. Reporting the estimated probability of identity is particularly important for any study that uses genetic tags because it provides essential information on the accuracy of individual identification.

In general, the quality and reliability of genetic data generated from noninvasively collected samples can be established if a rigorous data collection protocol is employed and carefully described. Pilot studies also can be used to estimate and demonstrate amplification success, genotyping error rates, and the number of times genotypes should be duplicated (Valiere et al. 2007). One data collection protocol, proposed by Bonin et al. (2004), was designed to limit and quantify errors at each step of the genotyping process by including blind samples, using negative controls, performing pilot work or a pilot study to gather initial information on amplification and error rates, using reference samples to control scoring, and identifying and removing problematic data before final analyses. This is just one example of a rigorous data collection protocol, however, and alternate methods of assuring data quality should be created and modified to fit the needs of specific studies (e.g., Morin et al. 2001; Paetkau et al. 2003; Rudnick et al. 2008). One of the best ways to insure high-quality data when using noninvasively collected samples is "deep" sampling, whereby most individuals are sampled more than once (Figure 3). This is of course not always possible, but in our view deep sampling lends a great deal of support to data quality.

Figure 3. An example of "deep" noninvasive sampling, which uses multiple samples from the same individual to generate genetic tags. A total of 307 individuals are represented and over 65% of the individuals are identified on the basis of two or more feathers. (Reprinted from Rudnick et al. 2008 with kind permission of Springer Science and Business Media.)

CONCLUSION

To date, few studies have used large-scale collections of naturally shed feathers to genetically tag birds. This application of noninvasive sampling has the potential to significantly impact future avian research, particularly for species that are inherently difficult to study by traditional means. Work on eagles (Rudnick et al. 2005; Meyburg et al. 2007; Rudnick et al. 2008) already has demonstrated that genetic tags generated from naturally shed feathers can provide critical data on avian behavior and demography. Currently these topics often are investigated through wing-tagging and telemetry, but we predict that (as in mammals) future avian research will increasingly utilize noninvasively generated genetic tags to provide insights into bird biology. When appropriate measures are taken to ensure high-quality genetic data, the genetic tags generated from noninvasively collected samples can be used for analyses of the topics described here, as well as other interesting aspects of avian biology.

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