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Molted feathers from clay licks in Peru provide DNA for three large macaws (*Ara ararauna*, *A. chloropterus*, and *A. macao*)

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ABSTRACT. Conservation genetic analyses of wildlife have increased greatly in the past 10 yr, yet genetic studies of parrots are rare because of difficulties associated with capturing them and obtaining samples. Recent studies have demonstrated that molted feathers can provide a useful source of DNA, but success rates have varied considerably among studies. Our objective was to determine if molted macaw feathers from Blue-and-yellow Macaws (*Ara ararauna*), Scarlet Macaws (*A. macao*), and Red-and-green Macaws (*A. chloropterus*) collected from rainforest geophagy sites called clay licks could provide a good source of DNA for population genetic studies. Specific objectives were to determine (1) how nuclear DNA microsatellite amplification success and genotyping error rates for plucked macaw feathers compared to those for molted feathers collected from clay licks in the Amazon rainforest, and (2) if feather size, feather condition, species, or extraction method affected microsatellite amplification success or genotyping error rates from molted feathers. Amplification success and error rates were calculated using duplicate analyses of four microsatellite loci. We found that plucked feathers were an excellent source of DNA, with significantly higher success rates ($P < 0.0001$) and lower error rates ($P = 0.0002$) than for molted feathers. However, relatively high success rates (75.6%) were obtained for molted feathers, with a genotyping error rate of 11.7%. For molted feathers, we had higher success rates and lower error rates for large feathers than small feathers and for feathers in good condition than feathers that were moldy and broken when collected. We also found that longer incubation times and lower elution volumes yielded the highest quality DNA when extracting with the Qiagen DNeasy tissue kit. Our study demonstrates that molted feathers can be a valuable source of genetic material even in the challenging conditions of tropical rainforests, and our results provide valuable information for maximizing DNA amplification success rates when working with shed feathers of parrots.

SINOPSIS. **Plumas mudadas de tres grandes guacamayos (*Ara ararauna*, *A. chloropterus*, y *A. macao*) en una colpa en Perú proveen ADN**

Los análisis genéticos para la conservación de la vida silvestre han crecido en gran escala durante los últimos 10 años, pero el análisis genético de los loros son raros por las dificultades asociados con su captura y obtención de muestras. Estudios recientes han demostrado que plumas mudadas podrían proveer una fuente útil de ADN, pero las tasas de éxito varían considerablemente entre estudios. Nuestro objetivo fue determinar si las plumas mudadas de *Ara ararauna*, *A. macao* y *A. chloropterus* colectadas en sitios de bosque húmedo donde estas aves consumen el suelo, llamados colpas, podrían proveer una fuente útil de ADN para estudios de la genética de las poblaciones. Los objetivos específicos fueron determinar (1) como comparan las tasas de éxito de la amplificación de los microsatélites del ADN nuclear y las tasas de error en el análisis del genotipo de plumas, entre plumas colectadas directamente de los guacamayos y plumas colectadas en colpas en el bosque Amazónico, y (2) si el tamaño de la pluma, su condición, la especie o el método de extracción afecta el éxito de la amplificación de los microsatélites o las tasas de error en el análisis del genotipo de las plumas mudadas. Las tasas de éxito de amplificación y error fueron calculados usando análisis duplicados de cuatro loci de microsatélites. Encontramos que plumas colectadas directamente de las aves son una fuente excelente de ADN, con tasas de éxito significativamente más altas ($P < 0.0001$), y con menores tasas de error ($P = 0.0002$) que las plumas mudadas. Sin embargo, tasas de éxito relativamente altas (75.6%) fueron obtenidos de plumas mudadas, con una tasa de error en el análisis del genotipo de 11.7%. Para plumas

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mudadas, tuvimos tasas de éxito más altas y tasas de error menores para plumas grandes que para plumas pequeñas y para plumas en buena condición que para plumas que estaban cubiertos con hongos y quebradas cuando fueron colectadas. También encontramos que mayores periodos de incubación y menores volúmenes de elución proveían el ADN de mayor calidad cuando se extraía el ADN usando el kit de tejido Quiagen DNeasy. Nuestro estudio demuestra que las plumas mudadas pueden ser una fuente valiosa de materia genética, hasta en las condiciones de los bosques húmedos tropicales. Nuestros resultados proveen información valiosa para maximizar las tasas de éxito de la amplificación del ADN cuando se analizan las plumas mudadas de los loros.

Key words: Amazon rainforest, DNA amplification success, DNA extraction, genotyping errors, molted feathers, parrot, plucked feathers

Large macaws can be difficult to study because their habitats are often inaccessible and remote. In addition, macaws inhabit canopy trees (Juniper and Parr 1998), can move long distances (Adamek et al. 2005), and are difficult to capture (Meyers 1994). Despite these difficulties, understanding the life history and population dynamics of parrots is important because populations of many species are declining (Collar and Juniper 1992, BirdLife International 2008). One way to better understand the population structure and dynamics of parrots is by studying their genetic diversity (Moritz 1995, Petit et al. 1998, Sunnucks 2000, Ransom et al. 2001, Martínez-Cruz et al. 2004). Genetic data can also provide baseline information for measuring the response of populations to anthropogenic changes, such as habitat fragmentation, habitat loss, and climate change (Pulido et al. 2001, Réale et al. 2003, Martínez-Cruz et al. 2004).

Studying the genetic diversity of parrots requires DNA samples, and blood is the preferred source. Less invasive techniques, including mouth swabs, eggshell swabs, and plucked feathers, also provide useful DNA samples (Bush et al. 2005, Harvey et al. 2006, Schmaltz et al. 2006). However, all of these methods require capture of birds. To obtain genetic samples from parrots, researchers generally resort to capturing adults and chicks at nests (Brock and White 1992, Wright and Wilkinson 2001). This method is challenging, time consuming, labor intensive, and often results in sample sizes inadequate for population genetics studies. Several studies have revealed the usefulness of molted feathers collected noninvasively for analyses of both mitochondrial and nuclear DNA, but amplification success rates vary considerably (Pearce et al. 1997, Segelbacher 2002, Peterson et al. 2003, Bush et al. 2005, Horváth et al. 2005, Rudnick et al. 2005, Seki 2006, Hogan et al. 2008). As a result, few investigators have used molted feathers as a primary source of genetic material (Segelbacher et al. 2003, Lõhmus and Väli 2004, Rudnick et al. 2005, Asai et al. 2006, Seki 2006) and, in most studies, molted feathers are used to supplement higher quality blood or plucked-feather samples (Duan and Fuerst 2001, Martínez-Cruz et al. 2004, Nittinger et al. 2005, Hailer et al. 2006, Lopes et al. 2007, Banhos et al. 2008).

Using molted feathers for DNA analysis requires the collection of a large number of feathers with good quality DNA. Throughout the southwestern Amazon basin, parrots and other birds congregate along exposed riverbanks to consume soils high in sodium and toxin-absorbing clays (Gilardi et al. 1999, Burger and Gochfeld 2003, Brightsmith et al. 2008). These "clay licks" are often easily observable, readily accessible, and visited by hundreds of individuals of several parrot species daily (Brightsmith 2004). Molted feathers can be found at the base of clay licks throughout the breeding season (D.B., pers. obs.), providing an ideal place to noninvasively obtain genetic material.

However, molted feathers in a rainforest are exposed to high humidity, warm temperatures, frequent rain, and intense sunlight that can rapidly degrade DNA (Lindahl 1993, Piggott 2004, Murphy et al. 2007). Most studies employing molted feathers as the primary DNA source have been conducted in temperate regions (Segelbacher et al. 2003, Lõhmus and Väli 2004, Rudnick et al. 2005, Asai et al. 2006, Seki 2006, Hogan et al. 2008). To date, no one has evaluated the feasibility of using molted feathers collected in the tropics as the primary DNA source for population genetic studies.

We evaluated the use of feathers from Blue-and-yellow Macaws (*Ara ararauna*), Red-and-green Macaws (*A. chloropterus*), and Scarlet Macaws (*A. macao*) collected at clay licks as

a DNA source. Our objectives were to determine (1) how DNA microsatellite amplification success and genotyping error rates for plucked macaw feathers compared to success rates and error rates for molted feathers collected at clay licks, and (2) how feather size, feather condition, species, or extraction method affected microsatellite amplification success and genotyping error rates.

METHODS

Study area. Our study was conducted at the Tambopata Research Center (TRC, 13°08′S, 69°37′W) in the southwestern Amazon Basin (Fig. 1). The center is located in the Tambopata National Reserve (275,000 ha) near the border of Bahuaja-Sonene National Park (537,000 ha) in the Department of Madre de Dios, Peru. Although human densities are low near the Tambopata National Reserve and parrot populations are healthy and apparently stable, anthropogenic disturbance has led to habitat loss and population declines elsewhere (Karubian et al. 2005). One of the largest clay licks in Peru is located <2 km from TRC (Brightsmith 2004), and 17 species of parrots, including our three focal species, visit this clay lick almost daily (Brightsmith 2004).

Sampling. We collected molted and plucked feathers during the macaw breeding season from November to March in 2004–2005 and 2005–2006. Sampling coincided with the rainy season and peak use of the clay lick by large macaws. Plucked feathers were obtained from macaw chicks at nesting sites and from adults captured using nylon foot snares at the clay lick. We plucked two to four mature feathers from the breast of each bird. We chose breast feathers over larger flight feathers to prevent negative impacts on flight. Trapping involved four people and at least 50 person hours of work per bird captured. Molted feathers were obtained by a single person searching the few hundred meters of clay lick for less than 1 h/d; 10–25 feathers were typically collected daily. Molted feathers were collected during periods when the likelihood of disturbing birds was low, either at 07:30 after the morning activity or after 16:00 (Brightsmith 2004).

On our first visit to the clay lick in November 2004, we collected all feathers. These feathers had been molted over an unknown period of time, but had likely been present for anywhere from less than 1 d to several months. The following week, the clay lick was visited daily to collect feathers that had been molted within the past 24 hr. Later collections occurred about once per month during 2004–2005 season for a

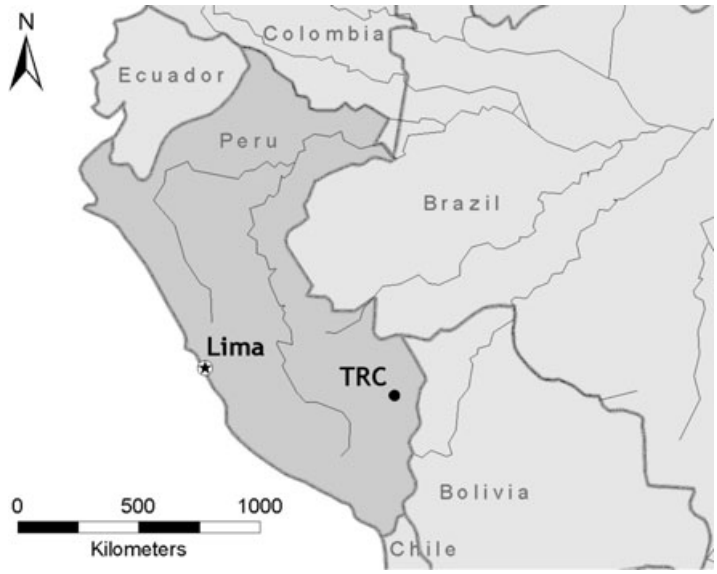

Fig. 1. Location of macaw feather sampling site at Tambopata Research Center (TRC) in Peru.

1- to 3-d collection period and between 4 and 7 times per month during the 2005–2006 season. Feathers were not collected daily during these periods and, therefore, the age of feathers was not recorded. To prevent cross-contamination, collected feathers were handled by the vane and stored in individual coin envelopes with desiccant at ambient temperature. Feathers were identified to species by comparison with specimens at the Museo de Historia Natural in Lima, Peru. Feathers were then sorted by size, with breast, head, and back feathers (1–7 cm) classified as small, primary, and secondary wing coverts and some tail coverts (7–15 cm) as medium, and remiges and rectrices (15–80 cm) as large. The condition of feathers was classified as good (clear, clean, undamaged calamus), dirty (caked with clay that was wiped off prior to DNA extraction), moldy (opaque, brittle calamus), or broken (in good condition, but with a split or hole in the calamus).

DNA extraction and microsatellite amplification. DNA extraction and PCR set up were performed in a laboratory free of concentrated DNA and post-PCR products. Negative controls were extracted alongside feathers to monitor for contamination. DNA was extracted from 1 to 1.5 cm of the calamus tip of large feathers, including pulp cells inside the feather shaft if available, and from the entire shaft for small feathers using the Qiagen DNeasy tissue kit (QIAGEN, Valencia, California). Modifications to the manufacturer's protocol included incubating overnight at 56°C in Buffer ATL, incubating at 70°C in Buffer AL for 45 min, incubating in 100 μl Buffer AE at 70°C for 15 min, and recycling Buffer AE through the membrane with an additional 5-min incubation at 70°C. Samples extracted before the final optimization of the DNA extraction protocol were incubated in AL Buffer for only 10 min at 70°C, and were resuspended in 200 μl of Buffer AE for 5 min at room temperature followed by recycling the AE through the membrane without additional incubation. These two extraction methods were accounted for in the statistical analysis (see below) by including the extraction method as a variable.

Because DNA quantity and quality from molted feathers are low (yields likely 0–40 ng/μl), typical photometric means of quantification are not accurate and do not provide a reliable method for predicting possible use of DNA in further analysis. Thus, we used PCR amplification success to quantify DNA quality. Four primer sets from microsatellite loci UnaCT21, UnaCT32, UnaCT43, and UnaCT74 (Caparroz et al. 2003) were redesigned to amplify smaller fragment sizes and optimized to work in a single multiplex. Our redesigned primer sequences and PCR conditions for this study are described by Gebhardt and Waits (2008). All PCR runs included a negative control to monitor for contamination. PCR products were visualized on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California) and analyzed with the associated Genemapper software. Multiplex PCR was run in duplicate for a total of eight trials per sample to evaluate microsatellite locus amplification success and genotyping error rates.

Scoring successes and errors. Success was evaluated for each amplification attempt at each locus and scored either as a 1 (amplification of a fragment greater than 100 fluorescent units in the expected size range) or 0 (failure to amplify). A success proportion was calculated for each sample based on the number of successful microsatellite loci amplifications out of the total number attempted (8) per sample. Mean amplification success rates were calculated by averaging over the individual sample success proportions. Because we did not know the true genotype of each sample, a genotyping error was defined as an inconsistency in the genotype for an individual at a given locus between successful duplicate PCRs and scored as either 1 (inconsistency between genotypes) or 0 (genotypes matched). Genotyping error proportions were calculated for each sample by totaling the number of inconsistencies divided by the total number of successful loci. Mean genotyping error rates were calculated by averaging over the individual sample genotyping error proportions.

Statistical analyses. Sample success and error proportions were transformed to stabilize variance using the arcsine square root method with a correction for proportions at 0 or 1 (Ott and Longnecker 2001). Differences in the transformed success and error proportions between the plucked and molted-feather groups were tested using two-sample *t*-tests assuming unequal variances (SAS 1989–2005). To determine if either amplification success or genotyping error proportions were associated with feather

Table 1. DNA amplification success rates for plucked and molted feathers from Blue-and-yellow Macaws, Red-and-green Macaws, and Scarlet Macaws.

Variable		*N*	Success rate[a] (%)	SE	Least square mean[b]	Differences[c] ($\alpha = 0.05$)	*P*
Sample type[d]	Plucked	23	100.0	0.00	–	A	<0.0001
	Molted	118	75.6	0.03	–	B	
Species	Blue-and-yellow Macaw	33	75.8	0.08	51.9	A	0.85
	Red-and-green Macaw	15	53.3	0.10	58.4	A	
	Scarlet Macaw	70	80.4	0.06	55.0	A	
Feather size	Small	40	66.9	0.06	39.3	A	0.0039
	Medium	23	78.3	0.09	58.4	AB	
	Large	55	80.9	0.08	67.4	B	
Feather condition	Good	74	82.4	0.05	84.4	A	<0.0001
	Dirty	26	81.7	0.08	62.3	B	
	Moldy	7	50.0	0.15	49.6	ABC	
	Broken	11	31.8	0.11	22.3	C	
Extraction method	Preoptimization	39	51.9	0.07	35.8	A	<0.0001
	Long incubation	79	87.3	0.07	73.6	B	

[a]Success rate was calculated as percent successful PCR runs divided by total PCR runs averaged over each individual and for four molted-feather variables.
[b]Least square means represent back-transformed adjusted success rates output by the model after accounting for effects of the variables.
[c]Different letters denote significant differences determined by a Tukey comparison of least square means, alpha = 0.05.
[d]Sample type was not included in the model of effects of variables on molted feathers so there are no least square means.

characteristics, such as species, feather size, sample age, feather condition, or extraction method, multiple regression analyses were conducted on the transformed success and error proportions (SAS 1989–2005).

RESULTS

We collected 23 plucked feathers, including feathers from seven Blue-and-yellow Macaws, four Red-and-green Macaws, and 12 Scarlet Macaws (Table 1). We also collected 118 molted feathers, including 33 from Blue-and-yellow Macaws, 15 from Red-and-green Macaws, and 70 from Scarlet Macaws (Table 1). Of the molted feathers, 40 were small, 23 medium, and 55 large, with an equal number newly molted feathers (within 24 hr of collection) and older feathers (present at the clay lick for anywhere from less than 1 d to several months; Table 1). Because of uncertainty about how long older feathers had been present at the clay lick, this variable (time between molt and collection) was not included in the final model. Thus, four variables (parrot species, feather size, feather condition, and extraction method) were included in the multiple regression analyses.

Success rates. Mean microsatellite amplification success rates were lower for molted feathers than plucked feathers (75.6% vs. 100%; $t_{117} = 7.2$, $P < 0.0001$; Table 1). The overall model testing the effects of species, feather size, feather condition, and extraction method on amplification success rates was significant ($F_{8,117} = 9.5$, $P < 0.0001$; Table 1).

We found that amplification success rates were higher for large feathers than small feathers ($F_{2,117} = 5.8$, $P = 0.0039$; Table 1), higher for feathers in good condition than dirty or broken feathers, and higher for dirty feathers than broken feathers ($F_{3,117} = 11.3$, $P < 0.0001$; Table 1). Amplification success rates did not differ for moldy feathers and feathers in good, dirty, or broken condition, but overall success rates were low. Amplification success rates were also higher using the extraction method with the longer incubation times than when using the preoptimized extraction method ($F_{1,117} = 21.2$,

Table 2. Microsatellite amplification error rates for plucked and molted parrot feathers from Blue-and-yellow Macaws, Red-and-green Macaws, and Scarlet Macaws.

Variable		*N*	Error rate[a] (%)	SE	Least square mean[b]	Differences[c] ($\alpha = 0.05$)	*P*
Sample type[d]	Plucked	22	1.1	0.01	–	A	0.0002
	Molted	97	11.7	0.02	–	B	
Species	Blue-and-yellow Macaw	28	21.4	0.05	29.2	A	0.0196
	Red-and-green Macaw	10	9.2	0.07	17.7	AB	
	Scarlet Macaw	59	7.5	0.04	18.6	B	
Feather size	Small	31	22.0	0.05	33.4	A	0.0002
	Medium	16	1.6	0.06	15.8	B	
	Large	50	8.5	0.03	17.1	B	
Feather condition	Good	65	10.9	0.03	13.5	A	0.0024
	Dirty	24	8.3	0.05	17.3	A	
	Moldy and broken	8	28.1	0.07	36.3	B	
Extraction method	Preoptimization	25	19.0	0.05	25.7	A	0.072
	Long incubation	72	9.1	0.04	17.8	A	

[a]Error rates were calculated as percent inconsistencies per successful duplicate PCR run averaged over all individuals and for four molted-feather variables.
[b]Least square means represent back-transformed adjusted success rates output by the model after accounting for effects of the variables.
[c]Different letters denote significant differences determined by a Tukey comparison of least square means, alpha = 0.05.
[d]Sample type was not included in the model of effects of variables on molted feathers so there are no least square means.

$P < 0.0001$; Table 1). Mean amplification success rates varied among parrot species, but, after accounting for the effects of the other variables, differences were not significant ($F_{2,117} = 0.2$, $P = 0.85$; Table 1).

Error rates. Mean genotyping error rates were lower for plucked feathers than molted feathers (1.1% vs. 11.7%; $t_{117} = 5.8$, $P = 0.0002$; Table 2), and the overall model testing the effects of four molted-feather variables on error rates was significant ($F_{7,95} = 6.0$, $P = 0.0004$; Table 2). Genotyping error rates were lower for large and medium feathers than small feathers ($F_{2,95} = 9.4$, $P = 0.0002$; Table 2), and were also lower for feathers in good condition than moldy or broken feathers ($F_{2,95} = 6.5$, $P = 0.0024$, Table 2). Among species, the error rate for Blue-and-yellow Macaws was substantially higher than the other two macaws ($F_{2,95} = 4.1$, $P = 0.02$; Table 2); however, it was not statistically different when compared to Red-and-green Macaws, likely due to small sample size. The extraction method did not affect genotyping error rates ($F_{1,95} = 3.3$, $P = 0.072$; Table 2).

DISCUSSION

Our study demonstrates that molted feathers can serve as a good source of DNA for large macaws in the Amazon. By evaluating multiple extraction methods and feathers of different size and condition, we were able to identify the factors that increase the probability of successful and accurate amplification of nuclear DNA from feathers. In sum, we found that success rates were highest and genotyping error rates were lowest when using large feathers in good condition and a modified Qiagen extraction protocol. Our results from molted feathers are consistent with findings from some previous studies, yet differ from others. In a study of Capercaillie (*Tetrao urogallus*), Segelbacher (2002) found that amplification success was greater for plucked feathers than molted feathers and for large molted feathers than small ones. In contrast, Hogan et al. (2008) found no differences in amplification success rates for small and large feathers, but, as in our study, noted that success rates were higher for feathers in good condition. Bush et al. (2005) found amplification success rates of 60% for

small molted feathers from Greater Sage-Grouse (*Centrocercus urophasianus*), but had no success using large molted feathers.

Our microsatellite genotyping error rates for molted feathers varied considerably (1.6–28%) by species and with feather size and condition, were generally higher than those previously reported (1–4%) for molted feathers from other species (Segelbacher 2002, Horváth et al. 2005, Rudnick et al. 2005), and were comparable to error rates observed in fecal DNA analysis of birds (8.3–21%; Nota and Takenaka 1999, Regnaut et al. 2006). Differences among studies in success and error rates using molted feathers are likely due to variables known to affect the quality of DNA from noninvasive sources, such as age and condition of the sample, size of the feather, environmental conditions, and DNA preservation and extraction method (Waits and Paetkau 2005).

Investigators often do not know the age of the samples collected, but feather condition is likely a good indicator of age. Feathers are more likely to become moldy or broken with increasing exposure time, and less likely to yield usable DNA. We excluded feather age as a variable in our study because all broken or moldy feathers belonged to the "unknown" age group, indicating that feather condition and feather age were not independent variables in our dataset. Interestingly, excluding moldy or broken feathers, amplification success rates for molted feathers of unknown age were still high (79.4%). These results indicate that molted feathers of unknown age, but in good condition, can provide a good source of DNA and increase the diversity of potential sampling protocols.

Another important variable that may explain differences in success and error rates among studies is the DNA extraction method. We found that the extraction method influenced amplification success rates, but did not affect genotyping error rates. Other studies have demonstrated that the extraction method can affect DNA yield and quality from feces (Flagstad et al. 1999, Piggott and Taylor 2003, Wehausen et al. 2004), but the impact of the extraction method on DNA yield from feathers has been directly compared in only one other study and only modifications to a single protocol were evaluated (De Volo et al. 2008). A number of extraction methods have been used for feathers, including phenol-chloroform (Bello et al. 2001), extraction buffers containing NaOH (Duan and Fuerst 2001), dithiothreitol (Rudnick et al. 2005, De Volo et al. 2008), $CaCl_2$/SDS (Hogan et al. 2008), Chelex (Pearce et al. 1997, Nittinger et al. 2005), and IsoQuick (Orca, Bothell, Washington; Seki 2006). Recently, the most common method has been the manufacturer's protocol in the Qiagen DNeasy tissue kit (QIAGEN, Valencia, California; Segelbacher 2002, Martínez-Cruz et al. 2004, Bush et al. 2005, Horváth et al. 2005, Asai et al. 2006). We examined the performance of this standard Qiagen protocol compared to changes we implemented to increase DNA yield. Decreasing final elution volumes and increasing incubation times and temperatures substantially improved our success rates, and our modified protocol should be evaluated for other species. Since the completion of our study, QIAGEN has developed a new feather extraction protocol that adds dithiothreitol (DTT), and an evaluation of the performance of this method is needed.

Surprisingly, we found that genotyping error rates were substantially higher for Blue-and-yellow Macaws than for the other two species of parrots, even after controlling for differences in age, feather size, and condition. Such differences in error rates may have resulted from differences among species in the performance in our PCR primers or differences among species in DNA quality or stability. Another potentially confounding factor was that feathers were classified to species by comparison to museum specimens and some misclassification was possible. However, misclassification of feathers of Red-and-green Macaws and Scarlet Macaws is more likely because the color of Blue-and-yellow Macaw feathers is distinct compared to those of the other two species.

Implications for genetic studies in the tropics. Our study demonstrates that molted feathers collected at clay licks provide a promising alternative for obtaining DNA samples from parrots and other tropical birds. We found that amplification success rates were higher and genotyping error rates lower for plucked feathers than molted feathers. Thus, when plucked feathers can be easily obtained and disturbing birds is not a concern, they should be favored over molted feathers. However, capturing species like large parrots or macaws that are often found in the canopy of tropical forests is difficult (Adamek, unpubl. data), and alternative sources

of samples will likely be necessary to obtain the large samples sizes needed for population genetic, landscape genetic, and mating system studies.

Collecting molted feathers will be particularly effective in areas where macaws visit clay licks. However, species composition at clay licks varies. All three of the large macaw species visit the clay lick at TRC, but most clay licks are used by only one or two large macaw species. Other alternatives include sampling molted feathers from roosting sites. For example, Blue-and-yellow Macaws roost communally at night, and feathers can be found below these roosts. One potential disadvantage of using molted feathers is the possible collection of multiple feathers from the same individuals, reducing sample sizes and increasing cost per bird. However, analysis of our samples revealed that only 5 of 108 feathers were duplicates (Gebhardt 2007).

When molted feathers are available, large feathers in good condition should be targeted due to their increased success rate over small feathers and feathers in poor condition. Our results indicate that microsatellite genotyping errors are relatively common in molted feathers. Thus, researchers will need to design laboratory protocols that detect and remove these errors by repeating PCR amplification multiple times and identifying and removing error prone samples as discussed extensively in previous noninvasive genetic sampling studies and reviews (Taberlet et al. 1996, Ewen et al. 2000, Miller et al. 2002, Frantz et al. 2003, Waits and Paetkau 2005, DeWoody et al. 2006).

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