MAKING USE OF CAPUCHINS' BEHAVIORAL PROPENSITIES TO OBTAIN HAIR SAMPLES FOR DNA ANALYSES

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Abstract

Genotyping wild and captive capuchins has become a priority and hair bulbs have high quality DNA. Here, we describe a method to non-invasively collect fresh-plucked strands of hair that exploits capuchins' manual dexterity and propensity to grasp and extract food. The apparatus consists of a transparent tube baited with food. Its extraction requires the monkey to place its forearm in contact with double-sided tape applied on the inner surface of the tube entrance. The "tube" method, successfully implemented with captive (N=23) and wild (N=21) capuchins, allowed us to obtain hair bulbs from most individuals and usable genomic DNA was extracted even from a single bulb.

Keywords: Hair collection; DNA; non-invasive method; *Sapajus*

Resumen

Establecer el genotipo de capuchinos silvestres y en cautiverio se ha convertido en una prioridad y los bulbos capilares tienen AND de alta calidad. Describimos un método no invasivo para colectar hebras de cabello recién arrancadas que aprovecha la destreza manual de los capuchinos y su propensión a agarrar y extraer alimento. El aparato esta compuesto por un tubo transparente cebado con alimento. Su extracción require que el mono ponga su antebrazo en contacto con cinta de doble faz que se ha acondicionado en la superficie interior de la entrada del tubo. El método del ¨tubo¨, exitosamente implementado con capuchinos cautivos (N=23) y silvestres (N=21), nos permitió obtener bulbos capilares de la mayoría de individuos y AND genómico utilizable fue extraído aún de un solo bulbo.

Palabras clave: Colección de pelo; ADN; método no invasivo; *Sapajus*

Introduction

Genetic material is a fundamental tool for population genetics and conservation biology, since it allows determining species identification, reproductive success, inbreeding, population size, dispersal and migration (Sunnucks, 2000). Recent phylogenetic and biogeographic evidence indicate distinct evolutionary histories of the two radiations of capuchin monkeys, *Cebus* (untufted or gracile capuchins) and *Sapajus* (tufted or robust capuchins) and a higher number of species than previously recognized (Lynch Alfaro *et al.*, 2012 a, b). As a consequence, captive capuchins of uncertain geographic origin cannot be ascribed to a given species without being genotyped to identify the species and the hybrids. Therefore, genotyping wild and captive capuchins has become a priority for both wild and captive capuchins (Lynch Alfaro *et al.*, 2014).

Samples of fresh-plucked strands of hair have relatively high concentrations of high-quality DNA that preserves well at room temperature if kept dry and produces fewer artifacts during amplification than DNA isolated from other tissues (e.g. Morin *et al.*, 1994; Taberlet and Bouvet, 1994; Valderrama *et al.*, 1999; Améndola-Pimenta *et al.*, 2009). Hair is also suitable to study heavy metal bioaccumulation (e.g., Malvandi *et al.*, 2010), feeding and dietary variations (e.g., Oelze, 2015), and hormone levels (e.g., Fourie and Bernstein, 2011). Consequently, a number of non-invasive methods to collect fresh hair from wild nonhuman primates exploiting species' spontaneous behaviors have been developed. For example, researchers took advantage of the fact that apes build nests and collected shed hair for DNA analyses (e.g., Morin *et al.*, 1994; Jeffery *et al.*, 2007), or of the proclivity of some species to manipulate food wrapped in duct tape to get their hair (Valderrama *et al.*, 1999).

In this study, we describe and test the effectiveness of a new non-invasive method to collect fresh-plucked strands of hair from wild and captive capuchin monkeys that exploits capuchins' manual dexterity, proclivity to manipulate foods/objects and strong natural propensity to exploit resources that need to be extracted from a substrate (Fragaszy *et al.*, 2004). We also evaluate the productivity of the method in terms of bulbs obtained for DNA extraction.

Methods

Subjects

The method was tested with wild and captive tufted capuchin monkeys. The former, 21 (out of 23; Table 1) wild bearded capuchins (*Sapajus libidinosus*) living in one social group at Fazenda Boa Vista (hereafter FBV) in the State of Piauí (Brazil; Visalberghi and Fragaszy, 2013). The latter were 23 captive-born tufted capuchins (*Sapajus* spp.; Table 2) derived from individuals of different geographical origin. These individuals were housed at the Primate Center of the ISTC-CNR in Italy. They belonged to four groups, each housed in an indoor–outdoor enclosure (indoor: 5 m^2 × 2.5 m high; outdoor: 40-130 m^2 × 3 m high). Water was freely available at all times. Fresh fruit, vegetables and monkey chow were provided in the afternoon after testing. This study complied with protocols approved by the Italian Health Ministry (DM133/2014 C to E. Visalberghi) and by SISBIO (Number: 51258-1) and adhered to the ASP Principles for the Ethical Treatment of Non Human Primates.

Apparatus and Procedure

FBV, Piauí, Brazil. We presented four transparent acrylic tubes of different lengths and diameters. The four tubes were 18, 18, 27, and 34 cm long, with inner diameters of 3.3, 2.6, 2.6, and 2.0 cm, respectively. The inner diameter of tubes 3 and 4 were obtained by using PVC-adapters in tubes with larger inner diameters (Fig. 1). One end of the tube was open. Small food items were placed inside the tube at a variable distance from the opening. Food items were sufficiently small to allow the monkey to easily move the hand inside the tube when holding the food during retrieval. Finally, if necessary, the depth of the tube was adjusted by inserting in the tube blocks (circular blocks made of the rachis of the local *burití* palm) and/or pebbles so that the food was within the subject's reach but far enough to require it to insert the arm fully.

Double-sided tape (19 mm width) (Pattex extra strong Henkel Italia SPA ©) was attached to the inner circumference of the tube at the opening (Fig. 1). To allow removal of the tape after the hair was collected, one cm of the tube circumference was left bare. Immediately prior to use, the protective covering of the exposed surface of the tape was removed. Finally, the tube was fastened with two straps to a tree trunk or thick branch; each strap was 3 cm from the end of the tube (Fig. 2). A firm anchorage prevented the monkeys from dislodging the tube. Wooden wedges were

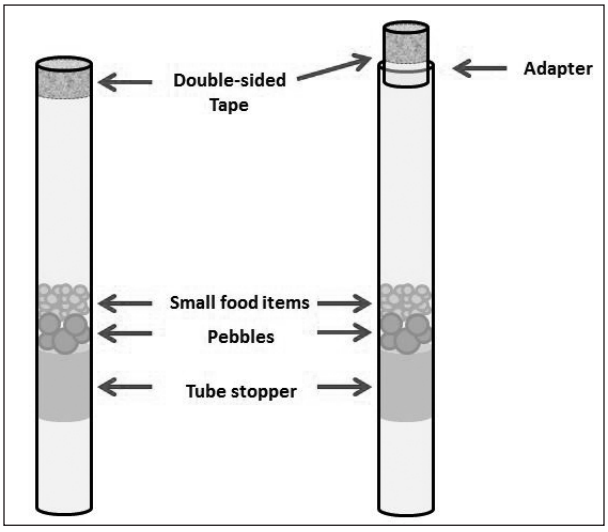

Figure 1. Schematic representation of the tube apparatus baited with small food items. Double-sided tape was applied on the inner surface of the tube entrance. The inner diameter of the tube could be reduced by tightly inserting a PVC-adapter inside the tube. One ending of the tube is blocked to change its depth as necessary to fit the individual's arm length. Pebbles were also used for the same purpose.

sometimes useful for this purpose. Tubes were placed so that a monkey could see the food inside the tube from its opening and could easily introduce its arm into the tube (see video at https://vimeo.com/162360240). The tube was oriented to increase the probability that the dorsal surface of the monkey's arm contacted the tape because the dorsal surface has longer hairs than the volar surface, and has a greater density of hair.

The tubes were baited with pieces of palm nuts (common in the diet of the capuchins living in FBV). To retrieve them the monkey had to place the full length of its arm inside the tube one or more times. The food items were located inside the tube at different distances. When the distance was short, the reward could be obtained by large individuals with some effort and high probability of hairs sticking to the tape. To collect the hairs of the smaller individuals the food had to be positioned further away from the opening. The tubes were located at 8-12 m from one another and each was closely monitored by at least one observer to detect the presence of hair(s) on the tape after an individual reached inside the tube (one or more times). If this were the case, the tube was removed, and the tape was carefully detached from the tube inner surface, attached to a clean paper sheet, covered with protective film and stored in a plastic bag marked with the date and subject's identity. Hair samples were collected in an area frequently visited by the group and used for field experiments (Visalberghi and Fragaszy, 2013), or opportunistically in other locations. The latter option was adopted for peripheral individuals. The hairs left in the tube by a single monkey were collected; when certain identification of the source of hairs on the tape could not be made, the samples were discarded. Before starting hair collection capuchins were familiarized with

Figure 2. The tubes were fastened to tree trunks or branches by means of two straps located close to the extremities of the tube. A firm anchorage was necessary to prevent monkeys from dislodging the tube (from a video taken by Luca Antonio Marino).

Figure 3. Before starting hair collection, young capuchins were allowed to explore the tubes together (A) and, only after they became more confident, each individual was opportunistically attracted to the tube for sample collection when no other individuals were nearby (B) (Photos V. Truppa).

the tubes by exploring them (Fig. 3a, b). Testing occurred between 7.00 and 16.00. We presented the tubes on days without rain because wet hair does not adhere to the tape.

The number of hair bulbs of each sample collected in FBV was counted at the Department of Experimental Psychology, University of São Paulo using a stereo-microscope (Nikon AxioCam MRm Zeiss, SMZ 745T).

Primate Center of the CNR. Tubes 1, 2, and 3 were used (see previous section). The tubes were baited with small pieces of peanuts that required the monkey to reach into the tube one or more times to retrieve. Hair samples were collected from a single individual while the individual was separated briefly from the group for this purpose. Captive capuchins housed at the Primate Center of the CNR are familiar with tubes, Testing occurred between 9.30 and 15.15.

To evaluate the quality of genomic DNA from the hair bulb samples of the 23 captive capuchins was extracted using NucleoSpin® Tissue (Macherey Nagel, MN) and Chelex® 100 (Sigma) at two amounts of resin (5% and 20%) and their yield of genetic material assessed (see Table 2). Amount and purity of DNA extracted were evaluated using the optical reader Multi-Mode Microplate Readers (Biotek).

Results

FBV, Piauí. Data collection was carried out during 12 days for about 40 min per day. Seventy-four hair samples were collected from 21 individuals tested, including infants (Table 1). The individuals from whom we did not obtain hair were an adult female and a 6 month-old infant. An average of 3.4 samples (range 1-7 samples) was obtained per individual, yielding an average number of 8.8 usable bulbs per individual (range 0->35).

Primate Center of the CNR. Data collection was carried out during 5 days during a total of 3-4 hours. Fifty-one samples were collected from all the 23 individuals tested (Table 2). An average of 2.1 samples (range 1-4 samples) was obtained per individual, yielding an average number of 6.9 usable bulbs (range 0-20). The number of bulbs collected and the mean yield from each individual in relation to the DNA extraction methods are reported in Table 2. Usable genomic DNA was extracted even from a single bulb hair.

Table 1. Number of hair samples and hair bulbs collected from wild capuchins at Fazenda Boa Vista.

Subject	Sex	Age class*	N samples	N bulbs
Jatobá	M	Adult	5	13
Mansinho	M	Adult	5	9
Teimoso	M	Adult	3	2
Piaçava	F	Adult	2	7
Teninha	F	Adult	2	6
Chuchu	F	Adult	1	2
Dita	F	Adult	3	1
Amarelinha	F	Adult	7	8
Tomate	M	Adult	2	17
Catu	M	Adult	2	1
Doree	F	Adult	7	15
Pamonha	F	Adult	5	8
Coco	M	Juvenile	6	35
Taís	F	Juvenile	2	>35
Presente	M	Juvenile	5	15
Chani	F	Juvenile	1	0
Cachaça	M	Juvenile	2	3
Titia	F	Juvenile	1	0
Patrícia	F	Infant	6	5
Divina	F	Infant	1	0
Donzela	F	Infant	6	8
MEAN			3.4	8.8

* Age class was assigned in this way: Adults (individuals older than 5 years); Juveniles (individuals between 18 months and 5 years); Infant (individuals below 18 months).

Discussion

Both wild and captive capuchins were attracted by the tubes and their contents. Our non-invasive method to collect fresh-plucked strands of hair, including the hair bulb, proved to be effective and simple. The number of samples obtained depended primarily on the subject's propensity for inserting its forearm inside the tube to retrieve the food and, in wild individuals, from social constraints. Sampling was easy for high-ranking capuchins, especially males, and more difficult for subordinates (because dominant individuals tended to monopolize access to the tube) and young infants (because of their reluctance to venture to a tube alone). Finally, peripheral individuals were relatively difficult to encounter/detect but since they were often alone or far from dominant individuals, samples were easily collected out of view of the rest of the group.

Our "tube" method uses materials that are easy to find, inexpensive and reusable (with the obvious exception of the tape and the bait); it also guarantees easy retrieval of the sample with low risk of contamination. Moreover, and in contrast with methods requiring shooting (e.g., Valderrama *et al*., 1999, 'shooting tape' method; Améndola-Pimenta *et al*., 2009), or traps (Stone *et al*., 2015), our method does not disturb monkeys and is safe also for very young ones. In addition, food extraction requires the individual contacting the sticky tape with the volar part of the hand (rich in hair) and not with the hand and mouth as it happens when the food is wrapped in tape attached to a tree limb, or in tape-covered food baskets, or wrapped directly with inverted tape, as done by Valderrama *et al*. (1999). In short, our "tube" method seems ideal to obtain the high quality DNA contained in hair samples and thus to contribute to the very much needed genotyping of both wild and captive capuchins (Lynch Alfaro *et al*., 2014). Finally, as described for humans (Higuchi *et al*., 1988), capuchins' usable genomic DNA can be extracted from a single bulb hair.

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Table 2. Number of hair samples and hair bulbs collected from captive capuchins at the CNR. For each subject the method of extraction and the yield of DNA extraction (expressed in nanogram/microliter) are reported.

Subject	Sex	Age class	N samples	N bulbs	Extraction	ng/µl
Peonia	F	Adult	2	10	MN	2
Rucola	F	Adult	1	9	MN	2
Penelope	F	Adult	1	nr	MN	1
Vispo	M	Adult	4	nr	MN	2
Cognac	M	Adult	1	9	MN	2
Robiola	F	Adult	3	7	MN	5
Paprica	F	Adult	3	5	MN	2
Carlotta	F	Adult	2	1	Chelex 5%	12
Robin Hood	M	Adult	1	10	Chelex 5%	18
Ulisse	M	Juvenile	3	1	Chelex 5%	5
Cammello	M	Adult	2	3	Chelex 5%	9
Robinia	F	Adult	2	10	Chelex 5%	9
Brahms	F	Adult	3	5	Chelex 5%	7
Totò	M	Juvenile	1	10	Chelex 5%	32
Rame	F	Adult	1	8	Chelex 5%	10
Pepe	M	Adult	2	15	Chelex 5%	14
Roberta	F	Adult	3	3	Chelex 5%	4
Quincy	F	Adult	1	3	Chelex 5%	10
Pacajà	F	Adult	2	20	Chelex 20%	22
Saroma	F	Adult	2	10	Chelex 20%	8
Robot	M	Adult	3	5	Chelex 20%	7
Virginia	F	Adult	2	1	Chelex 20%	9
Sandokan	M	Adult	3	0	na	na
MEAN			2.1	6.9		8.7

(nr = not recorded; na = not applicable)

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