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BRIEF REPORT

Individuals' Behaviors Following Dye-Marking in Wild Black-and-White Colobus (*Colobus vellerosus*)

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The ability to recognize individuals is a prerequisite for analyzing social relationships. We marked five adult and subadult *Colobus vellerosus* (three in 2002, and two in 2003) at the Boabeng Fiema Monkey Sanctuary, Ghana, to assess the feasibility of dye-marking black-and-white colobus, describe their reactions, and compare some of their behaviors with those of unmarked individuals. We used Nyanzol-D, a nontoxic black dye sprayed on the white tail (or white thigh) of the animal with a spray gun or a tree sprayer. Reactions to the marking procedure ranged from moving away and staring at the observer, without interruption in feeding (in one subject), to fleeing about 5 m away (in four subjects). In 234 hr of ad libitum observations (in 2002 and 2003), marks were scratched or otherwise were the object of attention from the bearer or other individuals on only one occasion. In 2002 we collected 22 hr of observations on the three marked monkeys and some unmarked monkeys in 10-min focal samples. Neither the marked nor the unmarked animals attended to the marks during focal samples. Marked and unmarked individuals displayed similar rates of displacement activities (autogrooming, scratching, and yawning). The proportion of scans with at least one near neighbor varied between marked and unmarked subjects, but the direction of the difference was not the same between males and females. The only aggression observed was displacements, and only in one comparison (out of four) did a difference emerge: the marked subadult male received more displacements than the unmarked males. Overall, marked and unmarked individuals did not differ consistently in our measures. Examination of the potential effects of marking should continue, since changes in pelage coloration may have longer-term social effects in species that rely largely on vision. Am. J. Primatol. 65:197–203, 2005.

Key words: *Colobus vellerosus*; individual recognition; dye-marking; extragroup mating

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INTRODUCTION

The ability to recognize individuals is a prerequisite for conducting detailed analyses of social relationships. By documenting social relationships, one can construct the social structure of a population or species [Hinde, 1976], and in turn determine the extent to which variables such as dominance rank, reproductive status, and kinship influence social interactions. Researchers who study species in which individuals have few natural identifying characteristics have difficulty attaining the same level of understanding of the social structure and variables that influence social interactions.

Dye-marking can be a useful method of identification [Clover, 1954; Gullion et al., 1962; Hansen, 1964; Honess &Macdonald, 2003; Isbell et al., 1998; Savage et al., 1993]. Under some conditions, it can be done without the need to capture, restrain, or anesthetize the subjects (e.g., when dealing with habituated animals that use the lower canopy at least some of the time, and have some light pelage). However, little is known about the effects of the marking procedure and the marks themselves on subsequent behavior. In previous studies, tamarins marked with color hair dye did not differ in intergroup aggression, predation, or integration into a neighboring group [Savage et al., 1993], and captive callitrichids dyed with picric acid did not vary in body weight at 12 months, in survivorship or age at emigration [Halloren et al., 1989]. It is useful to investigate other primates and other possible effects, since in species that rely largely on vision for cues from their environment, markings on the pelage are unlikely to go unnoticed. Indeed, changes in body coloration sometimes give social cues. In colobines, natal coats often contrast sharply with adult pelage, gradually changing to adult coloration as the infants become independent [Hrdy, 1976; Treves, 1997], and in vervets, scrotal darkness is associated with high male dominance [Gerald, 2001].

The black-and-white colobus form a cluster of species in which it is particularly difficult to recognize individuals. Researchers only attain individual recognition of animals in small groups [Fashing, 2001a; Korstjens, 2001]. This may bias research efforts because researchers may tend to select small groups in which to study social behavior. This may influence our understanding of the social dynamics in this genus. In this pilot study we evaluated the feasibility of noninvasively dye-marking black-and-white colobus in the field. We gauged the immediate reactions of individuals when they were marked, and evaluated whether their behaviors differed from those of unmarked individuals in the same group. We investigated whether marked and unmarked individuals varied in frequency of ''displacement activities'' (i.e., behaviors that increase under stress [Aureli et al., 1989; Tinbergen, 1952; Mastripieri, 1993; Mastripieri et al., 1992], such as autogrooming, scratching, and yawning), the proportion of time spent with at least one near neighbor, and the frequency of agonism received or given. We were not in a position to assess the effect of the mark on the behavior of an individual before and after the marking, since we could not individually recognize subjects in the absence of a mark.

MATERIALS AND METHODS

Study Site and Study Subjects

We have studied *Colobus vellerosus* at the Boabeng Fiema Monkey Sanctuary (BFMS) in central Ghana (7° 43′ N and 1° 42′ W) since 2000. The BFMS is a dry semideciduous forest approximately 1.9 km^2 in size. The villages of Boabeng and

Fiema are located within the forest, which connects to smaller forest fragments via a riparian forest. Details on the site and its population of black-and-white colobus can be found in Saj et al. [in press].

For this study, we used one of our two study groups (WW), which consisted of 33–34 individuals (two adult males, five subadult males, 12 adult females, four subadult females, and 10–11 juveniles and infants) in 2002. In 2003, it had increased to 38 individuals. WW was well habituated, but it was still impossible to recognize group members, particularly females and subadult males.

Marking Procedure

Nyanzol-D (Albinal Dyestuff Inc., Jersey City, NJ), a nontoxic black dye, was sprayed on the tail of the subjects by means of a ''spray gun'' (Hasbro Super Soaker Max-D 4000 Pawtuckat, RI) [Ellis & Ellis, 1975; Isbell et al., 1998] with a range of 2–3 m in 2002. A Hudson Trombone tree and shrub sprayer (model 61224, Hastings, MN; available through www.rittenhouse.ca) with a range of 2–5 m was used in 2003. Nyanzol-D comes in crystal form and was mixed according to a procedure adapted from Melchior and Iwen [1965]. This mixture was taken into the field and put into the tanks of the sprayers, with space left for the addition of hydrogen peroxide immediately before the animals were marked. J.A.T. and S.M. were present during marking (one to spray, and the other to collect data after an individual was marked). The lower portion of the long white tail was targeted, but in the case of one female, the white portion of the thigh was dyed.

The subjects were marked when they came to the low canopy and the ground to feed. They were easiest to mark when they were in an area close to a human settlement where they were accustomed to having people near them. Dominant males sometimes threatened or charged toward observers when the latter approached infants and juveniles, so no attempt was made to mark immature individuals.

Data Collection

We conducted marking in June 2002 and 2003. To minimize any potential disturbance caused by the marking procedure, and to prevent a situation in which we might collect data on an individual whose behavior was affected by marking attempts on other subjects, we decided to mark the animals on two days only (one in each year).

After the subjects were marked, individual dye patterns were recorded and behavioral data were collected. We recorded the immediate reaction of the marked individuals (i.e., whether they looked at the mark, moved away or fled, interrupted the activity performed at the time of marking, and their reaction to the observer). Ad libitum observations [Altmann, 1974] were then conducted between June and August in each year (183 hr in 2002, and 51 hr in 2003), during which time the marked individuals were observed several times a day.

In 2002, we also conducted 10-min focal-animal samples [Altmann, 1974] on the marked animals ($n = 3$) and different unmarked adult individuals. Since we did not recognize individuals, we do not know how many unmarked animals are represented in the unmarked sample. There were 20 adult and subadult individuals from which unmarked individuals could be selected. Although there was no pre-order in the sampling of individuals (because we did not know who they were), it is unlikely that we continually resampled the same individuals, because we made an effort to move around the group, alternating between males

and females, and central and peripheral individuals. Here we analyze only the data pertaining to marked individuals (7.7 hr of observation, 46 focal samples) and to unmarked adult females and subadult males (i.e., the same age/sex classes as the marked individuals; 14.3 hr, 86 focal samples).

In the focal samples, we recorded scratching, autogrooming, touching, sniffing, and mouthing of marked and other areas. We also recorded yawning. The measure of affiliation that we collected was proximity (since allogrooming is infrequent in *C. vellerosus*). Instantaneous samples [Altmann, 1974] to record the proximity of individuals within 1 m of the focal individual (defined here as ''near neighbors'') were taken at the end of focal samples. We also recorded aggression, which could include displacements, threats, chases, lunges, and contact aggression.

Scratching, autogrooming, and yawning are known to increase in stressful situations [Aureli et al., 1989; Mastripieri, 1993; Mastripieri et al., 1992]; hence, in the analysis we grouped these behaviors to assess whether these ''displacement activities'' varied between marked and unmarked individuals.

RESULTS

Reactions to the Marking Procedure

Two adult females (*An* and *La*) and one subadult male (*Ma*) were marked on 27 June 2002. A third adult female (*Hn*) and a second subadult male (*Sc*) were marked on 14 June 2003. The female marked in 2003 was probably not one of those marked in 2002, and the male surely was not one of the previously-marked monkeys (see below). All of the individuals were sprayed on the tail, except for female *La*, who was marked on the white part of her thigh. Female *An* was sprayed three times to produce a unique pattern on her tail.

Female *An* did not react when sprayed with the dye, except to move a few meters and stare at the researchers. The other two females (*La* and *Hn*) and subadult males (*Ma* and *Sc*) were feeding when they were marked. They interrupted feeding and fled. They moved to a taller tree nearby and climbed away from the researchers. None of the subjects vocalized or fled more than 5 m. Subadult male *Ma* and female *Hn* resumed feeding within 5 min. Subadult male *Sc* and female *La* then moved into bushier trees and were not seen until the next day, when they were observed feeding and resting with the rest of the group.

Behaviors Directed Toward the Mark

The subadult male *Ma* was observed to scratch the mark on his tail once on day 19 after marking. Otherwise, the marked animals were not seen to mouth, scratch, touch, groom, or sniff their marks immediately after marking, during subsequent focal animal samples, or during ad libitum observations. No mouthing, scratching, touching, grooming, or sniffing of the marks by other individuals toward the marked animals was observed immediately after marking, during focals, or during ad libitum observations.

Displacement Activities

The marked adult females displayed a mean rate of 0.24 displacement activities (scratching, autogrooming, and yawning) per minute (mode: 0.2; range of rates/min/focal: 0–0.9). The unmarked adult females showed a rate of 0.24 bouts/min (mode: 0.1; range of rates/min/focal: 0–0.8). The marked subadult male

showed displacement activities at a rate of 0.18 bouts per minute (mode: 0.0; range of rates/min/focal: 0–0.5), while the unmarked subadult males exhibited a rate of 0.21 bouts per minute (mode: 0.1; range of rates/min/focal: 0–0.8).

Proximity

The proportion of scans in which adult females had at least one near neighbor was 0.29 for the marked adult females (8/28 scans, not including one scan with the focal animal out of sight) and 0.35 for the unmarked adult females (25/71 scans, not including one scan with the focal animal out of sight). The proportion of scans in which the subadult males had at least one near neighbor was 0.35 for the marked individual (6/17 scans) and 0.21 for the unmarked subadult males (3/14 scans).

Agonism

The only agonistic acts that occurred during focal samples were displacements. The marked females did not displace others. Displacements caused by unmarked adult females occurred at 0.001 per minute (0 displacement in 29 focals vs. 1 in 72 focals). The marked subadult male caused displacements at a rate of 0.006 per minute (1/17 focals), and the unmarked subadult males did not displace others (0/14 focals).

The marked adult females were not displaced during focal samples (0 displacements received in 29 focals), while the unmarked adult females were displaced at a rate of 0.004 per minute (3/72 focals). The marked subadult male was displaced at a rate of 0.018 per minute (3/17 focals), and the unmarked subadult males were displaced at a rate of 0.007 per minute (1/14 focals).

Visibility of the Marks

The marks were visible and recognizable (with the use of binoculars) up to 100 m away, even when the monkeys were high in the canopy. The marks were still visible 5 months after the animals were marked, but had disappeared by the time J.A.T. returned to the site 12 months after marking (assuming the marked individuals remained in the group). The marks allowed reliable recognition of individuals, even when they left their focal group. For instance, adult female *Hn* was seen on two occasions in different groups participating in extragroup copulations.

DISCUSSION AND CONCLUSIONS

In this pilot study, we marked five individual *Colobus vellerosus* using nontoxic dye to determine whether this could be an option to improve individual recognition in our study groups. We used two types of sprayers (the Hasbro Super Soaker Max-D 4000 in 2002, and the Hudson Trombone tree and shrub sprayer in 2003). The device used in 2003 sprayed farther but was more cumbersome to carry.

Dye-marking did not affect the degree of habituation. Upon being marked, the individuals showed low-intensity reactions. They did not flee or otherwise change their behaviors toward the observers in the days following the marking procedure. The marks were visible up to 100 m away, and lasted for at least 5 months (they have been reported to last up to 1 year in some contexts [Rasmussen, 1991]). Re-marking may sometimes be necessary; however, the

subadult male (*Ma*) marked in year 1 was still recognizable by the same observer in year 2 by the shape of his eyebrows. The marking in the first year allowed the observer to determine that he was the only individual with that eyebrow shape in his age/sex class in this group. The fact that he was still recognized the next year was linked to his presence in a particular group, however. The case of female *Hn* showed the importance of individual recognition, regardless of the context of the group, since her mark allowed us to confirm the presence of extragroup copulations in *C. vellerosus* [Sicotte & MacIntosh, 2004], which were also reported in *C. guereza* [Fashing, 2001b].

We marked only a small number of individuals for this pilot study because we did not want to proceed with more marking without knowing whether the procedure affected behavior. Marked individuals did not display higher rates of displacement activities, which suggests they do not differ from unmarked individuals in terms of stress levels [Mastripieri et al., 1992]. The marks may have had an effect on proximity, since marked and unmarked individuals showed some difference, but the direction was different in males vs. females. The only agonistic events observed during focal samples were displacements, and they were rare. Marked females did not cause or undergo displacements, and the rates for unmarked females were very low as well, suggesting the lack of a difference. There appeared to be no difference between the rates of displacements caused by the marked and unmarked males. However, the marked subadult male seemed to be displaced at a higher rate than his unmarked counterparts. It is difficult to assess the biological significance of this difference. It is tempting to link the fact that the marked subadult male was more often in the proximity of at least one adult or subadult neighbor with the fact that he was displaced more often than the unmarked subadult males. Displacements are rare in *C. vellerosus*, so a difference of a few instances in a small period of time is likely to have a disproportionate effect on the reported rates (particularly because the number of focal samples with subadult males was small). It is also possible that some of the individuals we marked had characteristics that made them easier targets and also more or less likely to be displaced, or to be in the proximity of others, such as being in the process of dispersing from their natal group, or having different confidence levels around humans or in group progressions [c.f., Rhine & Tilson, 1987]. More data are necessary to evaluate which of these scenarios is occurring.

Because this marking system is opportunistic and relies on spraying white areas from a distance, there is a limit to the number of different sizes, shapes, and locations of the marks that can be attained. Other factors in association with the mark (mainly sex and size) can be used to identify an individual. However, in large groups and in species whose pelage lacks large light-colored areas, it may be necessary to use different colors to quickly and reliably recognize individuals. As we reported previously, color hair dye did not seem to affect intergroup behavior and susceptibility to predation in tamarins [Savage et al., 1993], a species in which some females are trichromatic [Smith et al., 2003]. The possible effects of these color marks on within-group behavior of individuals in a fully trichromatic species should be assessed if color marks are to be used for individual recognition.

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