International Journal of Primatology, Vol. 28, No. 1, February 2007 (© 2007)
DOI: 10.1007/s10764-006-9110-8

Females Pay Attention to Female Secondary Sexual Color: An Experimental Study in *Macaca mulatta*

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Received October 6, 2005; accepted December 10, 2005;
Published Online January 25, 2007

Researchers have long considered the color of female sexual skin to play a role in attracting or inciting competition among males, or both; however, females may also use color in intrasexual communication. To assess this possibility, we examined whether variation in same-sex sexual skin color is salient to female rhesus macaques (Macaca mulatta)*. We exposed adult females to computerized images of conspecific female faces and hindquarters manipulated for color (red vs. non-red), within the natural range of color variation. Females visually attended more to both reddened faces and hindquarters over the non-red counterparts. We conclude that female color might be biologically meaningful to other females.*

KEY WORDS: *Macaca mulatta*; primate; secondary sexual color; sex skin.

INTRODUCTION

Secondary sexual coloration of the face and/or anogenital region occurs in many diverse species of primates (Dixson, 1998; Gerald, 2003).

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There has been considerable discussion regarding the function of secondary sexual coloration and swellings in female primates, and how color might enhance attractiveness to, or incite competition among males (Bielert *et al.*, 1989; Dixson, 1998; Domb and Pagel, 2001; Hrdy and Whitten, 1987; Nunn, 1999; Stallmann and Froehlich, 2000; Zinner *et al.*, 2004). Although evidence from avian species, e.g., pinyon jays (*Gymnorhinus cyanocephalusi*: Johnson, 1988); Gulanan cock-of-the-rock birds (*Rupicola rupicola*: Trail, 1990) suggests that color is involved in female intrasexual competition there has been no report re whether female color is important in intrasexual interactions in primates.

Rhesus macaque (*Macaca mulatta*) males and females experience reddening of the face and hindquarters coinciding with times of heightened mating activity (Baulu, 1976). As a necessary step to address whether the colored areas play a role in intrasexual communication, we tested whether differences in female face and hindquarter colors are salient to female rhesus macaques. Experiments consisted of exposing adult females to color-manipulated digital images of other females. The method allowed us to isolate female attention to coloration, uncoupled from other potentially color-related cues, including behavior.

METHODS

Subjects

The subjects were 9 adult female rhesus macaques, singly housed at the Caribbean Primate Research Center, Sabana Seca Field Station (SSFS), Puerto Rico, age 3.75–14.25 yr (mean = 6.32, SE = 1.23). A separate research protocol unrelated to the present study required individuals to live in single housing, and at the end of which, all were to be placed in social housing. Subjects were naïve to experimental procedures involving pictorial stimuli. Testing took place at the SSFS between October and early November 2004, during the annual peak in mating activity. No female was pregnant, and we presumed they were cycling normally, but we did not know the stage of the menstrual cycle.

Stimuli

We used a digital video camera (Sony DCR-PC100E) to capture images of 24 individual female rhesus macaques at SSFS, unfamiliar to the experimental subjects. We took facial images while individuals exhibited neutral expressions, with mouths closed and faces and eyes pointed

directly at the camera. As the images showed direct gaze, subjects may not have perceived them as neutral because prolonged eye contact is associated with aggressive intent (Hinde and Rowell, 1962). However, macaques also use different facial attributes to interpret intent, e.g., mouth configuration and brow position (Perrett and Mistlin, 1990). Eye contact also occurs in other communicative contexts, e.g., appeasement gestures (van Hooff, 1967). Controlling for eye gaze direction was essential because it can influence looking behavior (Sato and Nakamura 2001). We took hindquarter images directly from behind while the individuals stood with tails erect, exposing the entire anogenital and surrounding regions of sexual skin. We frame grabbed images from digital video and downloaded them onto a computer (Sony Vaio V505 series) as 640 × 480 pixel images in jpeg format. We calibrated all images in Adobe Photoshop Elements 2.0 via RGB (Gerald *et al*., 2001).

To manipulate coloration, we adapted computer graphics techniques used to alter human facial color (Rowland and Perrett, 1995). Waitt *et al*. (2003, 2006) used the techniques successfully in 2 previous studies examining conspecific response to altered secondary sexual coloration among rhesus macaques. We constructed 2 composite faces—red and non-red—via computerized amalgamations of 8 of the reddest and 8 of the least red images. We used multiple images to control for individual differences in coloration and differences in ambient lighting. To make composites, we calculated the mean RGB color values at each pixel for the color sample and converted the measures into hue and saturation values. We then applied hue and saturation values and combined them with the individual brightness component of each pixel of 8 individual female stimulus faces, from the 8 individuals of intermediate color range, not used in the composites, producing a red and a non-red version of each face. We followed the same procedure to transform hindquarter coloration. All color manipulations were within the natural range of variation, but as the techniques combine the applied hue and saturation with the existing values, there was a small degree of variation between stimuli of the same color category. We standardized backgrounds across images by cropping and placing faces and hindquarters against the same color-calibrated background image taken on Cayo Santiago. We thus created a natural contrast to the stimuli, as is recommended when using artificial visual stimuli to investigate the significance of color in animal communication (Fleishman and Endler, 2000).

Procedure and Equipment

Caretakers moved females to the testing area adjacent to their living quarters. We habituated individuals to the testing area for 1–3 h before

testing, during which we provided access to both food and water. Testing took place from 1400 to 1700 h after all feeding and cleaning routines were completed.

We used a Spyder^{TM} colorimeter and OptiCAL software (Pantone ColorVision) to color calibrate the monitor weekly to ensure realistic and consistent color depiction. During testing, the experimenter was situated directly behind the monitor; however, views of the experimenter were blocked with a curtain. We monitored behavior remotely and recorded it for later analysis via a digital camera, placed centrally directly above the monitor, and a laptop computer. A trial began when the individual's eyes were orientated toward the monitor. During a trial, a single image appeared in 24-bit color, enlarged to approximate life size, for 5 s, i.e., individuals viewed 1 image after another. We kept presentation time to a minimum because longer trial durations can lead to boredom and inattention on the part of the subjects (Waitt, 2005). We randomized the order of stimuli and counterbalanced the presentation of red or non-red stimuli between subjects, i.e., individuals viewed each face and hindquarter once, in either a red or non-red version, so that each individual saw 4 red faces, 4 red hindquarters, 4 non-red faces, and 4 non-red hindquarters, as viewing images more than once can lead to a marked reduction in subject interest (Wilson and Goldman-Rakic, 1994). There were 16 trials in total.

We excluded trials if eye gaze was obscured, i.e., individuals were orientated with their backs to the monitors or if their eyes were closed for the entire trial. The mean number of trials included per individual is 14.87 (SE = .48). We tested each subject only once and experiments lasted 2–6.5 min (mean = 3.75, SE = .51).

We programmed Observer software (Noldus, Version 3.0) to record subjects' visual gaze duration, on a continuous basis, as a measure of attention, during frame-by-frame analyses. The order of stimuli was unknown to the experimenter recording these data. To assess intra-observer reliability, we reanalyzed the sessions from 2 randomly selected individuals to compare original and reanalysed scores. We compared scores for gaze duration in trial-by-trial correlations, yielding reliability coefficients of .90 and .87 (Martin and Bateson, 1993). We performed paired *t*-tests to determine whether facial and hindquarter color (red or non-red) influenced gaze duration. All tests are 2-tailed, with a critical statistical significance set at $p < .05$, and performed analyses in SPSS 12.0.

RESULTS

Females had significantly longer gaze durations toward red in comparison to non-red female faces (red: mean/trial = 3.51 s, SE = .44 vs.

non-red: mean/trial = 2.61 s, SE = .39; $t_7 = -3.87$, $p = .006$). Females also gazed significantly longer at images of red vs. non-red hindquarters (mean/trial = 2.68 s, SE = .39 vs. 2.14 s, SE = .33; $t_7 = -2.535$, $p = .039$).

DISCUSSION

Adopting an experimental approach, we examined female attention to color, while eliminating the effects of social interactions, or existing relationships among females, which could be related to color. Results demonstrated that females preferentially attended to both red vs. non-red versions of faces and hindquarters, suggesting that variation in same-sex color is indeed salient to females. In the absence of data linking color directly to social behavior, it is not possible to define how color might be meaningful to female primates.

Females may attend more to red images simply because they appeared to be novel in some way. However, the explanation seems improbable because all stimuli were within the natural range of variation that rhesus macaques exhibit. In addition, including images altered with a novel color, such as blue or green, may act as a way to control for novelty, but it is important to note that having such a condition would introduce the confound of novelty into the equation because they fall outside of the range of natural variation, and could indeed appear as odd, unlike the 2 conditions we presented.

Inferring from data on avian taxa (Johnson, 1988; Trail, 1990), one may speculate as to what types of information color can convey to female primates. First, females could use color as a gauge to monitor other females' reproductive status or cyclic phase, for competitive purposes, as hindquarter color can advertise the relative timing of ovulation (Czaja *et al*., 1977). The possibility might not be plausible for facial color, given that the relationship between face color and cyclic phase is less predictable in rhesus macaques (Baulu, 1976), among other macaques, e.g., Japanese macaques (*Macaca fuscata*; Wallner *et al*., 2002), and toque macaques (*M. sinica*; Dittus, 1975). Alternatively, females could also use face and hindquarter color to convey social intentions toward other females, as suggested by preliminary data collected on free-ranging rhesus macaques (Gerald, unpublished data).

ACKNOWLEDGMENTS

The experiments comply with the current laws of Puerto Rico and the United States. The IACUC of the University of Puerto Rico, Medical

Sciences Campus approved the investigation (Protocol 6810103). A grant awarded to M. S. Gerald by The Leakey Foundation and under NIH, NCRR grant CM-5-P40RR003640-13 awarded to the Caribbean Primate Research Center and awards from the University of Puerto Rico-Medical Sciences Campus supported the investigation. We thank Dr. Janis Gonzalez, Dr. Mario Rodriguez, and the caretaking staff of the Sabana Seca Field Station, particularly Milton Martínez, whose logistical support allowed us to complete our experiments successfully, and Professor David Perrett for providing equipment access.

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