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

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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 cyanocephalus*; 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 manipulated from other stimuli.

regarding the function of secondary sexual coloration among males (Bielert *et al.*, 2001; Hrdy and Whitten, 1987; Nunn, 2001; Pinner *et al.*, 2004). Although evidence from rhesus macaques (*Gymnorhinus cyanocephalus*) and zebra finches (*Rupicola rupicola*; Trail, 2001) suggests that color is important in intrasexual in-

teractions, males and females experience red coloration coinciding with times of heightened social activity. A necessary step to address whether color is important in communication, we tested whether red and blue colors are salient to female rhesus macaques by exposing adult females to color-matched stimuli. The method allowed us to test color perception uncoupled from other potentially

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rhesus macaques, singly housed at Sabana Seca Field Station (SSFS), (mean age = 6.32, SE = 1.23). A separate study required individuals to live in pairs, all were to be placed in social groups. Experimental procedures involving pictorial stimuli began in October and early November. During this time, social activity was high. No female was pregnant during the study, but we did not know

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(Sony DCR-PC100E) to capture images of rhesus macaques at SSFS, unfamiliar to the subjects. Images were taken while individuals exhibited social activity. 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). Waite *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™ 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 (Waite, 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 = 2.6 s, SE = .44). Gaze durations toward red were significantly longer than toward non-red (mean/trial = 2.68 s, SE = .44).

Adopting an experimental design that eliminated relationships among females, we demonstrated that females preferred faces and hindquarters of red color. In behavior, it is not possible to determine the role of male primates.

Females may attend to red color to be novel in some way. Because all stimuli were white, red color may cause all stimuli were white. In addition, macaques exhibit. In addition, such as blue or green, may be important to note that having of novelty into the equation of variation, and could be presented.

Inferring from data of macaques, we speculate as to what type of relationship between face color and reproductive status or eye color. The possibility might not be a relationship between face color and reproductive status (Baulu, 1976), (Macaca fuscata; Wallnerus, 1975). Alternatively, to convey social intention, primary data collected on female macaques (data).

The experiments were conducted in the United States. The data were collected in the United States.

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was obscured, i.e., individuals were nitors or if their eyes were closed of trials included per individual is

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

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REFERENCES

- Baulu, J. (1976). Seasonal sex skin coloration and hormonal fluctuations in free-ranging and captive monkeys. *Horm. Behav.* 7: 481-494.
- Bielert, C., Girolami, L., and Jowell, S. (1989). An experimental examination of the colour component in visually mediated sexual arousal of the male chacma baboon (*Papio ursinus*). *J. Zool.* 219: 569-579.
- Czaja, J. A., Pobinson, J. A., Eisele, S. G., Scheffler, G., and Goy, R. W. (1977). Relationship between sexual skin colour of female rhesus monkeys and midcycle plasma levels of oestradiol and progesterone. *J. Reprod. Fertil.* 49: 147-150.
- Dittus, W. P. J. (1975). Population dynamics of the toque monkeys, *Macaca sinica*. In Tuttle, R. H. (ed.), *Socioecology and Psychology of Primates*. Mount, The Hague, pp. 125-151.
- Dixson, A. F. (1998). *Primate Sexuality: Comparative Studies of Prosimians, Monkeys, Apes, and Human Beings*. Oxford University Press, New York.
- Domb, L. G., and Pagel, M. (2001). Sexual swellings advertise female quality in wild baboons. *Nature* 410: 204-206.
- Fleishman, L. J., and Endler, J. A. (2000). Some comments on visual perception and the use of video playback in animal behavior studies. *Acta Ethol.* 3: 15-27.
- Gerald, M. S. (2003). How color may guide the primate world: Possible relationships between sexual selection and sexual dichromatism. In Jones, C. B. (ed.), *Sexual Selection and Reproductive Competition in Primates: New Perspectives and Directions*. American Society of Primatologists, Norman, OK, pp. 141-172.
- Gerald, M. S., Bernstein, J., Hinkson, R., and Fosbury, R. (2001). A formal method for objective assessment of primate color. *Am. J. Primatol.* 53: 79-85.
- Hinde, R. G., and Rowell, T. E. (1962). Communication by postures and facial expressions in the rhesus monkey (*Macaca mulatta*). *Proc. Zool. Soc. Lond.* 138: 1-21.
- Hrdy, S. B., and Whitten, P. L. (1987). Patterning of sexual activity. In Smuts, B., Cheney, D. L., Seyfarth, R. M., Wrangham, R. W., and Struthsaker, T. T. (eds.), *Primate Societies*. University of Chicago Press, Chicago, pp. 370-384.
- Johnson, K. (1988). Sexual selection in pinyon jays: II. Male choice and female-female competition. *Anim. Behav.* 36: 1048-1053.
- Martin, P., and Bateson, P. (1993). *Measuring Behaviour*. Cambridge University Press, Cambridge, UK.
- Nunn, C. L. (1999). The evolution of exaggerated sexual swellings in primates and the graded signal hypothesis. *Anim. Behav.* 58: 229-246.
- Perrett, D. I., and Mistlin, A. J. (1990). Perception of facial characteristics by monkeys. In Stebbins, W. C., and Berkley, M. A. (eds.), *Comparative Perception, Vol. II: Complex Signals*. New York: John Wiley & Sons, pp. 187-213.
- Rowland, D. A., and Perrett, D. I. (1995). Manipulating facial appearance through shape and colour. *IEEE Comput. Graph. Appl.* 15: 70-76.

- Sato, M., and Nakamura, K. (2002). Facial coloration in female Japanese macaques (*Macaca mulatta*). *J. Comp. Psychol.* 116: 1-16.
- Stallmann, R. R., and Froehlich, R. (1990). Why should females pay attention to facial coloration? *Primates* 41: 1-16.
- Trail, P. W. (1990). Why should females pay attention to facial coloration? *Primates* 41: 1-16.
- van Hooff, J. A. R. A. M. (1967). *Primate Ethology*. Dordrecht: Reidel.
- Waitt, C. (2005). *Facial Attraction and Measuring Preference*. University of Stirling, Stirling, Scotland.
- Waitt, C., Gerald, M. S., Little, A. C., Wolfenson, D., Perrett, D. I. (2003). Evidence for exaggerated sexual swellings in female primate mate choice. *Anim. Behav.* 45: 1-16.
- Wallner, B., Aspernig, D., Dittus, W. P. J., and Kraiselburd, D. (2003). Japanese macaques: A fur color related to memory for conspecifics. *Anim. Behav.* 45: 1-16.
- Wilson, F. A. W., and Goldman, R. L. (1990). Memory for conspecifics related to memory for conspecifics. *Anim. Behav.* 40: 1-16.
- Zinner, D., Nunn, C. L., van Sool, L., and Nunn, C. L. (eds.), *Sexual Selection in*

