TITLE:
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CITATION:
Ono, Eiri ...[et al]. Spectrocolourimetry visualized differences in sexual skin colouration in macaques.. Folia primatologica 2015, 86(3): 178-186

ISSUE DATE:
2015-05

URL:
http://hdl.handle.net/2433/201386

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Spectrocolorimetry visualized differences in sexual skin coloration in macaques

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The number of words
4,106 words

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Running Head
Spectrocolorimetry of macaque sexual skin

Keywords
Macaca fuscata, Macaca mulatta, sexual skin, spectorocolorimetry, CIELAB space
Abstract

Females of some catarrhines develop conspicuous sexual skin transformation in their hindlimbs. Among the macaques, one of the radiated and adapted catarrhine groups with diversified sexual skin transformation, differences in sexual skin coloration between the Japanese macaques *Macaca fuscata* and rhesus macaques *Macaca mulatta* have not been quantitatively analyzed. In this study, sexual skin coloration of these macaques was spectrocolorimetrically measured in the non-mating season (NMS) and mating season (MS) and represented in the CIELAB space with the variables of $L^*$, $a^*$ and $b^*$. In the Japanese macaques, the average±SD of $L^*$, $a^*$, and $b^*$ was 53.61±3.31, 11.51±4.57 and 6.66±2.25 in the NMS, and 46.60±2.78, 19.97±2.99 and 8.80±1.34 in the MS, respectively; while in the rhesus macaques, the average of $L^*$, $a^*$, $b^*$ was 60.09±3.96, 5.99±4.59 and 5.83±2.37 in the NMS, and 52.70±6.54, 13.62±6.86 and 8.07±1.43 in the MS, respectively. Sexual skin of Japanese macaques was consistently much redder (larger $a^*$) and darker (smaller $L^*$) than that of rhesus macaques. The smaller $L^*$ suggested the more dermal melanin content in the Japanese macaques. These closely related macaque species have similar but distinct sexual skin coloration. Spectrocolorimetry is thus useful to suggest histo-physiological background of coloration.
Introduction

Females of some catarrhines develop conspicuous transformation around the perineal skin in their hindlimbs [Collings, 1926; Dixson, 1983; Anderson and Bielert, 1994; Nunn, 1999; Higham et al., 2010]; this “sexual skin” is thought to be tightly involved in socio-sexual signaling [Wickler, 1967; Gerald et al., 2007; Bradley and Mundy, 2008]. Changes in the sexual skin are simply referred as swelling and coloration. Exaggerated swellings are typical for females in the species with a multimale-multifemale social structure [Nunn, 1999]. Because the swelling reaches its maximum size at around the time of ovulation in the baboons [Higham et al., 2008], mandrills [Setchell and Wickings, 2004], chimpanzees [Deschner et al., 2004] and macaques [Higham et al., 2012], sexual skin swelling is thought to indicate female fertility. On the other hand, sexual skin coloration comprises several facets in the context of fertility and mating behavior [Czaja et al., 1975; Baulu, 1976; Waitt et al., 2006; Higham et al., 2008; Dubuc et al., 2009; Wallner et al., 2011; Watson et al., 2012].

The macaques comprising more than 20 species have radiated and adapted to various environments [Thierry, 2010]. Changes in the sexual skin are also diversified among them. Japanese macaques (Macaca fuscata) and rhesus macaques (M. mulatta) belong to the fascicularis lineage and the former live under various environments from warm to cool temperate zones in Japan, whereas the latter inhabit various environments in the mainland Asia such as India, China and southeast Asia. These two macaques share similar reproductive features [reviewed in Thierry, 2010]; for example, ovarian cycle length (Japanese macaques: 26.5 days; rhesus macaques: 28.3 days), a multimale-multifemale social structure, multi-mounting behavior and seasonal breeding but age at first birth delays in the Japanese macaques (Japanese macaques: five years; rhesus macaques: four years). Sexual swellings were recognized in adolescent females of both species [Anderson and Bielert, 1994], and females' red sexual skin draws male attention [Waitt, et al., 2006; Wallner et al., 2011]. However, sexual skin of the Japanese macaques swells less obviously and is much redder than
that of the rhesus macaques [observed by JS], differences in sexual skin coloration between
the two species have not been quantitatively analyzed; these backgrounds prompted us to
introduce spectrocolorimetry into the sexual skin coloration study.

Visual observation, digital photography, and spectrocolorimetry have been used for
skin color measurement [Stevens et al., 2009]. Among them, spectrocolorimetry obtaining
direct color data is applicable to reared animals and the obtained color data are objective
without interferences such as stray light. For the evaluation of the obtained color data, there
are two major protocols; a model of common color space, such as the CIELAB space that was
developed for human color vision and a Receptor-Noise Limited (RNL) model [Vorobyev and
Osorio, 1998] that stands species-specific data on the peripheral visual system. Data for
species-specific visual system were not available in the Japanese macaques yet but were
already obtained for the rhesus macaques [Bowmaker et al., 1978]. The CIELAB space gave
great insights into the relationship between skin color and pigmentation, such as by melanin
and hemoglobin, in humans [Weatherall and Coombs, 1992; Zonios et al., 2001; Alaluf et al.,
2002a; Stephen et al., 2009]; in addition, it was proved in the mandrills (Mandrillus sphinx), a
member of the papionines, that the CIELAB model gave qualitatively similar results to those
in the RNL model [Renoult et al., 2011]. The CIELAB model is thus considered a useful tool
to compare skin color in the catarrhines who have a uniform color vision system similar to
that of humans [Jacobs and Deegan, 1999; Changizi et al., 2006].

In this study, we aimed to evaluate and distinguish sexual skin coloration
objectively in closely related macaques categorized in the fascicularis group whose sexual
skin appears similar [Nunn, 1999]. We thus obtained color profiles of sexual skin of the
Japanese macaques and rhesus macaques with a spectrocolorimeter and analyzed them in the
CIELAB space.

Materials & Methods
Subjects
Twelve female Japanese macaques (*Macaca fuscata*) aged from four to six years and 13 female rhesus macaques (*Macaca mulatta*) aged from three to five years reared at Primate Research Institute, Kyoto University, Inuyama, Aichi, Japan were recruited for this study. When we started the data collection in the non-mating season, reddening and swelling of sexual skin of the subjects were not recognized; they were likely to be menarche. We measured their color profiles in July (non-mating season: NMS) and October (mating season: MS) 2010, or in August (NMS) and November (MS) 2011. Two of 12 Japanese macaques were studied in 2010 and the other 10 were in 2011, while six of 13 rhesus macaques were in 2010 and the other seven were in 2011. The detailed information of the subjects is in the online supplementary table. This study followed the Guidelines for Care and Use of non-Human Primates, Primate Research Institute, Kyoto University, and was approved by the Ethics committee of animal experiments of the institute.

Color measurements
To measure sexual skin coloration, one spot was selected at 2-3 cm under the ischial callosity of each side (two spots for each subject in one season, figure 1). Prior to the measurement, the subjects were anesthetized with ketamine hydrochloride (50 mg/mL Ketalar®, Sankyo-Parke-Davis & Co., Inc., Japan, 2.5 mg/kg) and medetomidine hydrochloride (Domitor® Meiji Seika Kaisha, Ltd. Tokyo, Japan, 0.1 mg/kg), and then the measurement spots were cleaned and shaved. The selected spots were measured in July (NMS) and in October (MS) 2010, also in August (NMS) and in November (MS) 2011. A single color measurement for each spot was conducted with a MINOLTA Spectrocolorimeter CG-411C (for the CIE D65/10° illuminant/observer condition). Three variables in the CIELAB color system, $L^*$, $a^*$, and $b^*$ were extracted for each measurement. The average values of $L^*$, $a^*$ and $b^*$ for each subject were calculated. The variable $L^*$, $a^*$ and $b^*$ represents
position on the light–dark, red/magenta-green, and yellow-blue axis, respectively. The $L^*$ ranges from 100 to 0, whereas the $a^*$ and $b^*$, can take any figure theoretically, ranging from about 100 to -100 in general.

Antidote to medetomidine, atipamezole hydrochloride (Domitor® Meiji Seika Kaisha, Ltd. Tokyo, Japan, 0.5 mg/kg), was administered for fully recovering after the measurement.

**Data analysis**

The data in July 2010 and August 2011 were combined for the NMS, while the data in October 2010 and November 2011 were combined for the MS. Changes in sex skin coloration between NMS and MS were tested with the paired t-test. The differences between the two species in each season were tested with the paired t-test, two-sample t-test and Welch's two-sample t-test if necessary. The relationship between the seasonal differences (MS - NMS) in $L^*$ and $a^*$ was analyzed with a linear regression. P value less than 0.05 ($p < 0.05$) was considered significant.

**Results**

Sexual skin coloration of Japanese macaques and rhesus macaques in the NMS and MS is visualized in figure 2 with the $L^*$, $a^*$, and $b^*$ variables. In the Japanese macaques, the average±SD of $L^*$, $a^*$, and $b^*$ was 53.61±3.31, 11.51±4.57 and 6.66±2.25 in the NMS, and 46.60±2.78, 19.97±2.99 and 8.80±1.34 in the MS, respectively; while in the rhesus macaques, the average of $L^*$, $a^*$, $b^*$ was 60.09±3.96, 5.99±4.59 and 5.83±2.37 in the NMS, and 52.70±6.54, 13.62±6.86 and 8.07±1.43 in the MS, respectively. In the MS, decreases in the $L^*$ and increases in the $a^*$ and $b^*$ were consistently observed in each subject of both the Japanese and rhesus macaques (table 1).
Differences in sexual skin coloration by the species and season are also represented in figure 2. In both seasons, the significant species differences were observed for the variable $L^*$ (NMS: $t = -4.22$, $p < 0.001$ by two-sample t-test; MS: $t = -3.08$, $p < 0.01$ by Welch’s two-sample t-test) and the variable $a^*$ (NMS: $t = -3.01$, $p < 0.01$ by two-sample t-test; MS: $t = -3.04$, $p < 0.01$ by Welch’s two sample t-test) but not for the variable $b^*$ (NMS: $t = 0.89$, $p > 0.05$ by two-sample t-test; MS: $t = 1.31$, $p > 0.05$ by two-sample t-test). In both species, the significant seasonal differences were observed for the variable $L^*$ (Japanese macaques: $t = 6.55$, $p < 0.001$ by paired t-test; rhesus macaques: $t = 6.48$, $p < 0.001$ by paired t-test), the variable $a^*$ (Japanese macaques: $t = -6.20$, $p < 0.001$ by paired t-test; rhesus macaques: $t = -8.41$, $p < 0.001$ by paired t-test) and the variable $b^*$ (Japanese macaques: $t = -3.58$, $p < 0.01$ by paired t-test; rhesus macaques: $t = -3.76$, $p < 0.01$ by paired t-test).

The correlation coefficient between the seasonal differences in $L^*$ and $a^*$ was -0.94 ($r^2 = 0.89$, $p < 0.001$) in the Japanese macaques, and -0.82 ($r^2 = 0.67$, $p < 0.001$) in the rhesus macaques.

Discussion

Sexual skin coloration of female Japanese macaques and rhesus macaques was spectrocolorimetrically measured and represented in the CIELAB space with which we can biologically interpret sexual skin coloration (table 1 and fig. 2). We analyzed the obtained color values from two aspects: seasonal change and species difference. Seasonal changes in the sexual skin coloration in the Japanese macaques and rhesus macaques were more or less similar; that is, sexual skin became darker, redder and yellower in the MS compared to NMS in both species. In detail, we also found that sexual skin of Japanese macaques was much redder (larger $a^*$) and darker (smaller $L^*$) than that of rhesus macaques through the NMS and MS (fig. 2); this is consistent with the general observations that sexual skin of Japanese
macaques appears to be redder than that of rhesus macaques. In terms of CIELAB values, similar dynamic changes in $L^*$, $a^*$ and $b^*$ were observed and it is thus conceivable that all the color values in the NMS as well as in the MS in both macaques are under the control of the same factor(s). The sexual skin color in the NMS is considered to represent the basic conditions without the surge of reproductive hormones, such as estrogen and progesterone [Nozaki et al., 1995]. Since in the NMS the variable $L^*$ and $a^*$ but not $b^*$ were significantly different between the two species (fig. 2), it is suggested that the presence of different basic conditions that give lighter sexual skin to the rhesus macaques and redder sexual skin to the Japanese macaques. We thus demonstrate that the closely related these macaque species have similar but distinct sexual skin coloration.

Among various factors modifying skin coloration, two major pigments, melanin and hemoglobin, are of our interest. Melanin content modifies all the three color values, in particular, $L^*$ [Takiwaki, 1998; Alaluf et al., 2002b]. In human skin coloration, changes in $L^*$ is mainly due to epidermal melanin content [Alaluf et al., 2002b] that is negatively correlated with the $L^*$ among human population [Alaluf et al., 2002a]. The seasonal decrease in the $L^*$ in the Japanese and rhesus macaques may be attributed to the increase in dermal melanin content. The lower $L^*$ values in the Japanese macaques both in the NMS and MS indicate more dermal melanin contents in the Japanese macaques. Melanin production is induced by ultra-violet (UV) light [Pathak et al., 1962; Jablonski and Chaplin, 2000; Jablonski and Chaplin, 2010] and the epidermal melanin content is subject to adaptive responses to UV light intensity; however, 17-ß estradiol for example stimulates melanocyte tyrosinase activity in the human skin [Ranson et al., 1988; McLeod et al., 1994]. We thus cannot rule out possible contributions of other factor(s) to the melanization, and effects of estradiol on the dermal melanin content in macaques should be also elucidated to explain the consistently lower $L^*$ in the Japanese macaques.

The $a^*$ represents the red-green color axis and the higher $a^*$ values were observed in the Japanese macaques in the NMS and MS. In humans, red color of the skin is mainly due to
the superficial vascular plexus in the upper dermis [Takiwaki, 1998; Alaluf et al., 2002b]

where reproductive hormones also play certain roles such as improved vascularization by
estrogens [Thornton, 2002]. As the reproductive hormones can modulate blood vessels in the
sexual skin in the MS [Czaja et al., 1977], the increases in $a^*$ in the MS may be due to
vascularization and/or vasodilatation in the Japanese and rhesus macaques. Consistently lower
melanin contents in the rhesus macaques were indicated by their higher $L^*$ values both in the
NMS and MS. This may correspond to the observation by Montagna et al. (1964) that rhesus
macaque has little melanin in the epidermis as well as to the prediction by Collings (1926)
that the sexual skin reddening is not due to the pigmentation but to the dynamics of
sub-epidermal blood vessels. We conclude that melanin contents support the baseline of the $a^*$,
whereas the increased $a^*$ in the MS is due to blood hemoglobin in both species. The seasonal
differences in $L^*$ and $a^*$ were highly correlated in both species (fig. 3). The high correlation
indicates $L^*$ and $a^*$ are mostly under the same factors to change, namely, melanin and blood
hemoglobin. The $r^2$ of regression line in the rhesus macaques was lower than that in the
Japanese macaques. It indicates other factor(s) of the coloration in the rhesus macaques, for
example, sexual swelling. The structural change of sexual skin may also modify the

coloration.

It has been reported that adolescent females of both Japanese and rhesus macaques
exhibit sexual swellings [Anderson and Bielert, 1994]. However, no Japanese macaques aged
from four to six years exhibited sexual swelling, while 11 of the 13 rhesus macaques aged
from three to five years did in this study. If the sexual skin coloration and swelling play a role
in socio-sexual signaling, possible compensation of their bright red coloration for the lack of
sexual swelling should be taken into consideration; it has been reported that hindlimb bright
coloration might contain meaningful physiological information in Japanese macaques
[Wallner et al., 2011].

For the analyses of spectrocolorimetric data of mandrills, the RNL model gave
similar results as the CIELAB model [Renoult et al., 2011]. We also preliminarily analyzed
data by the RNL model, which were consistent with those in the CIELAB model (data not shown) but the CIELAB model gave higher resolutions. The CIELAB model is suitable for such coloration studies; however, the RNL model that is based on the species-specific visual system may have a potential to approach to understand cognitive consequences in the receiver of color signals. In conclusion, our study has opened objective and quantitative color measurements of sexual skin coloration and demonstrated their similar and dissimilar profiles in the closely related two macaque species. More detailed histological and molecular studies for these macaques’ sexual skin will provide evolutionary insights in the mechanism of the sexual skin development in the primates. Spectrocolorimetry should help a further research on species-specific, sex-specific, and age-related coloration in various body parts especially bare skin among primates.

Acknowledgments

We are grateful to Prof. Kenichi Aoki, Department of Biological Sciences, University of Tokyo for his generous permission to use a spectrocolorimeter. We also thank Prof. Roscoe Stanyon for his comments on this manuscript. This work was supported by the Cooperation Research Program of Primate Research Institute, Kyoto University.

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Table 1. Sexual skin color of the Japanese and rhesus macaques represented with $L^*$, $a^*$ and $b^*$ variables.

<table>
<thead>
<tr>
<th>Subject</th>
<th>NMS</th>
<th>MS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$L^*$</td>
<td>$a^*$</td>
</tr>
<tr>
<td>Mf 1</td>
<td>53.31</td>
<td>9.21</td>
</tr>
<tr>
<td>Mf 2</td>
<td>51.08</td>
<td>10.39</td>
</tr>
<tr>
<td>Mf 3</td>
<td>55.39</td>
<td>15.45</td>
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<td>Mf 6</td>
<td>55.23</td>
<td>5.23</td>
</tr>
<tr>
<td>Mf 7</td>
<td>57.08</td>
<td>10.85</td>
</tr>
<tr>
<td>Mf 8</td>
<td>49.27</td>
<td>19.55</td>
</tr>
<tr>
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<td>13.30</td>
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<td>48.00</td>
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<td>9.92</td>
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<td>Mf 12</td>
<td>58.79</td>
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</tr>
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<td>Mm 2</td>
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<tr>
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<td>58.88</td>
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</tr>
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<td>Mm 11</td>
<td>54.51</td>
<td>11.70</td>
</tr>
<tr>
<td>Mm 12</td>
<td>55.26</td>
<td>10.36</td>
</tr>
</tbody>
</table>

Mf: Japanese macaque; Mm: rhesus macaque; NMS: non-mating season (July, 2010 and Aug, 2011); MS: mating season (October, 2010 and November, 2011)
Legend to figure 1

Measurement spots were selected inside the yellow circles under ischial callosities.

Legend to figure 2

Species and seasonal differences in the sexual skin coloration. The dashed lines represent the interspecific comparisons in each season. The black bold lines indicate the means and the black circles indicate the deviated values.

***P < 0.0001; **P < 0.001; *P < 0.05; NS: not significant.

Legend to figure 3

Correlation between the seasonal differences (MS-NMS) in $L^*$ and $a^*$ for the Japanese macaques (Mf) and the rhesus macaques (Mm).
figure 1
Seasonal difference of $a^*$

Seasonal difference of $L^*$

Species
- Mf
- Mm