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Fine-scale variation in lip and cheek colour according to the timing of ovulation in women

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Abstract

Various animal species have evolved a sexual communication system with females displaying and males discriminating information about the timing of ovulation through sexual signals. More research is now investigating the potential ovulatory signalling function of female red skin colour in human and non-human primates. However, to date it is still challenging to draft satisfying hypotheses about the evolution and function of female red skin colour, due to methodological discrepancies between human and non-human primate studies. The present study used a within-individual design and objective methods to analyse the relationship between fine-scale variation in cheek and lip colour (luminance and redness) and the estimated day of ovulation in 15 cycling women. I found weak evidence that intra-cycle variation in lip luminance only may be less modulated by inter-individual variation, with lips getting slightly darker around ovulation. However, day to day variation in lip luminance is likely imperceptible, meaning that lip luminance may not act as an ovulatory signal in humans. This study adds to the growing research and discussion on the role and evolution of female red skin colour of human and non-human primates in the context of sexual signalling and mate attraction.

Keywords: Red skin colour, Information content, Ovulation, Lips, Human, Primate

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Data availability. The data set generated and analysed during the current study is available in the supplementary material.

Significance statement

In human and non-human primates, red skin and red ornaments are attractive for both sexes and often associated with mating and fertility. While female red skin colour can be informative about the reproductive status and thus influences mating strategies in some primate species, it is not clear if humans share with non-human primates a colourful trait of ovulation. This study shows a weak relationship between lip luminance only and the timing of ovulation. This study adds to the growing research and discussion on the role and evolution of female red skin colour of human and non-human primates in the context of sexual signalling and mate attraction.

Introduction

Do females display visual information about their reproductive status that can modulate mate attraction and mating strategies? This question is central to sexual selection theory. The number of offspring a female can have is constrained by physiological factors such as menstrual cyclicity, gestation and post-partum amenorrhea, as well as reproductive senescence. Reproduction is usually more costly for females due to the production of larger gametes, extended maternal care, and male monopolization (Galef 1983; Gittleman and Thompson 1988). Thus, in several species, females appear to have evolved traits which are attractive to males and can act as probabilistic signals of ovulation to maximize reproduction while balancing its associated costs. Across human and non-human primates (hereafter, primates), there is evidence that female traits influence male behaviours, suggesting common evolutionary pathways and underlying mechanisms for sexual signalling (Campbell 2007; Dixson 2012).

Among the different female traits, there is a growing interest in the potential role of red skin colour in primate sexual communication. Intra-cycle variation in oestrogens induces ovulation and also affects some chromatic (redness) and achromatic (luminance) parameters of skin colour (Rhodes et al. 1997; Thornton 2002). Circulating oestrogens bind to receptors in the skin, causing an increase in blood flow and consequently a decrease in the perceived skin luminance (i.e., darkening of the skin) (Thornton et al. 2003; Pelletier and Ren 2004). Increase in blood flow can modulate the ratio of oxygenated/deoxygenated blood in the skin vessels which may influence perceived redness. Primate studies provide evidence that facial skin colour varies across the cycle in mandrills (Setchell et al. 2006), contains information about the probability of ovulation in rhesus macaques (Dubuc et al. 2009; Higham et al. 2010), and advertises pregnancy but not ovulation in Japanese macaques (Rigail et al. 2015,

2019). Moreover, red skin colour appears to be attractive to males suggesting a role in mate attraction, at least in macaques (Higham and Winters 2015).

Cheek and lip colour also correlate with female attractiveness in human (Stephen and McKeegan 2010; Re et al. 2011; Young 2015; Pazda et al. 2016). Skin colour may thus be involved into human mate attraction, although the colour of the stimuli was artificially manipulated in previous studies, which may impair the ecological validity of the results. Studies of the potential signalling function of red skin colour in women yielded mixed results. Oberzaucher et al. (2012) described that cheeks were redder around ovulation compared to the end of the cycle while Samson et al. (2011) could not replicate these findings. Recently, Burriss et al. (2015) found no intra-cycle variation in cheek colour. Interpretation of the informative role of skin colour in ovulatory signalling is however constrained by some methodological limitations. While most studies used photography to analyse skin colour, which provides accurate measurements, they did not always correct lighting conditions across photography sessions using colour standards, which may have altered measurements (White et al. 2015; Rowland and Burriss 2017). Human studies usually rely on a restricted data set to assess intra-cycle difference: e.g. 2 samples from mid and late cycle, which can underestimate or overestimate cycle effects (but see Burriss et al. 2015). Finally, human studies often failed to confirm ovulation with sex hormone profiles (with the exception of Burriss et al. 2015, ovulation tests), especially when studying perceptions of intra-cycle variation. Taken together, these methodological limitations constrain our understanding of the potential role of female red skin colour in sexual signalling in humans.

To determine if women display a colourful trait that may play a role in ovulatory signalling, there is a fundamental need for more studies of the relationship between fine-scale variation in trait expression and the probability of ovulation. Such studies would benefit from objective and quantitative methods, inspired by primate studies, using standardized photography, regular biological sampling, and hormonal estimation of the ovulation date (Bergman and Beehner 2008; Stevens et al. 2009; Hodges and Heistermann 2011; White et al. 2015). Following this premise, this study aims at investigating whether cheek and lip colour contain information about intra-cycle variation in the probability of ovulation in women. If cheek or lip colour is related to the timing of ovulation within a cycle, I predict that cheeks or lips will be darker/redder around this timing.

Methods

Participants

In total, 18 women participated in the study (mean age = $28.3 \pm \text{SD } 4.3$ years, “Asian” = 11, “Caucasian” = 7, additional information on the supplementary material). All participants were naturally cycling and not taking hormonal contraceptives for at least 3 months prior to and during the study. I collected digital photographs and saliva samples every two days, excluding weekends and national holidays, for the duration of one complete menstrual cycle (i.e., from the beginning of their menstruations until the next ones) between September 2018 and June 2019, to limit the effect of tanning. Sampling occurred at a fixed time of the day for each participant to control for possible diurnal variation in sex hormone concentrations (Bao et al. 2003). I collected a total of 204 photographs and saliva samples (mean per participant = $11.3 \pm \text{SD } 1.5$, range = 8-13). It was not possible to record and analyse data blind, because a single experimenter performed data collection and analyses.

Assessment of cheek and lip colour

Participants removed face and lip make-up a minimum of 30 min before sampling. A single female experimenter photographed the participants (LR), as the sex of the photographer can influence participant facial temperature and potentially skin colour (Hahn et al. 2012). Sampling was conducted in a unique location, i.e., an office room with closed curtains, ceiling lights turned on, and a constant 24°C temperature. Participants sat in front of a beige background and adopted a neutral expression. The camera was placed 2m from the participant’s chair and held at the same height as the participant’s face. The experimenter used a Nikon 5000D camera with a 12.3 megapixel CMOS sensor and a Nikon AF-S NIKKOR 18-55mm f/3.5-5.6G lens. Photographs were collected in NEF format, i.e., Nikon’s RAW format, with the flash disabled, the shutter speed and aperture size determined automatically by the camera, and a 55mm focal to limit distortion (Třebický et al. 2016). The experimenter standardised photographs by manual setting the white balance using a X-Rite White Balance Card (GretagMacbeth ColorChecker) and by photographing the participant’s face along with a Gretag X-Rite Color Checker (colour standards). This technique standardizes photographs such that colour measurements are comparable across all photographs (Bergman and Beehner 2008; Stevens et al. 2009; White et al. 2015). Photographs were converted to uncompressed 16-bit TIFF files. Cheek colour was measured from a pair of points located on the area described in (Burriss et al. 2015). Lip colour was measured from the whole lip skin (supplementary material, Fig. S1). CIELAB values were extracted using Colourworker software (Chrometics Ltd. available at: <http://www.colourworker.com/>) which provided luminance (L^*) and red-green ratio (a^* , hereafter redness) values calculated from the estimated reflectance spectrum according to the standard CIE (Commission Internationale de l’Éclairage) equations (Vorobyev et al. 1997).

Determination of the estimated ovulation

Participants followed the sampling guideline recommended by Salimetrics (Salimetrics, LLC, USA). Samples were collected during the photo session, labelled and stored within 15 min at -80°C until analyses at KUPRI. Saliva samples were analysed for oestradiol (17 β -estradiol) and progesterone (4-pregenene-3,20-dione) using Salimetrics enzyme immunoassays kits. The oestradiol assay had a sensitivity of 0.1 pg/ml with an intra-assay coefficient of variation (CV) of 6.2 % and inter-assay CV of 10.8 %. The progesterone assay had a sensitivity of 5 pg/ml with an intra-assay CV of 6.6 % and inter-assay CV of 9.7 %.

The onset of the luteal phase was defined as the sample with a progesterone concentration at least 2 standard deviations greater than the mean of the 2-3 preceding baseline values (Hodges and Heistermann 2011). Ovulation was considered to have occurred when a mid-cycle peak of oestradiol was detected around the onset of the luteal phase. Because sampling occurred every 2-3 days, I considered the day of peak oestradiol as the most likely day of ovulation and labelled it as *day 0*. The day directly preceding the estimated ovulation day was labelled as *day -1*, the day directly following it as *day +1*, and so on. Ovulation could not be determined (i.e., abnormal hormone variations) for 3 out of the 18 participants.

Statistical analyses

I used photographs of 15 participants showing ovulatory cycles to analyse intra-cycle variation in cheek and lip luminance and redness (N = 165 photographs, mean per participant = 11.0 \pm SD 1.4, range = 8-13). Although this sample appears small, it is within the range of previous studies of fine-scale intra-cycle variation in skin colour in both human (Samson et al. 2011; Burriss et al. 2015) and non-human primates (Dubuc et al. 2009; Higham et al. 2010; Rigai et al. 2019). That said, the sample size warrants cautious reading of the results.

This study tests for a possible quadratic effect of the days relative to ovulation on cheek and lip colour: higher effect toward the estimated ovulation and lower effect toward the beginning and end of the cycle. I thus constructed general linear mixed-effects models (LMMs) for cheek and lip colour (luminance and redness respectively) which included the linear (DAY) and quadratic (DAY²) effects of the days relative to the estimated ovulation as fixed effects, and fitted by maximum likelihood using lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017) packages in R version 3.6.0 (R Core Team 2019). Inspection of the cumulative distribution functions revealed good fits to the normal distribution for luminance variables and to the lognormal distribution for redness variables (Harrison et al. 2018). Prior to modelling, the days relative to the estimated

ovulation were standardized to mean = 0 and SD = 1 to improve model performance and interpretability (Harrison et al. 2018). On one hand, we can expect that the relationship between the timing of ovulation and colour would be similar across participants; I thus constructed random intercept models (RIM) with random intercepts for participant identity. On the other hand, this relationship may vary between participants; I thus constructed uncorrelated random slope models (RSM) with random slopes for the linear and quadratic terms. I also constructed the respective RIM and RSM null models in which the predictor variables were removed but the random effect structure was maintained. This resulted in 4 candidate models for each colour/skin combination: RIM-full, RIM-null, RSM-full, and RSM-null. I ensured that all relevant model assumptions (homogeneity of the residuals and stability of estimates) were met by visually inspecting histograms of the residuals and plots of residuals against fitted values, and performing Kolmogorov-Smirnov, deviation, and outliers tests (all $P > 0.05$, except the KS test for the RIM-full for cheek luminance: $P = 0.042$) using the functions `simulateResiduals` and `testResiduals` of the DHARMA package (Hartig 2020). I used an information-theory approach to objectively compare and rank the 4 candidate models in terms of how well they fitted the existing data thus assessing the likelihood that one or more models among the candidates is/are best supported by the data (Burnham et al. 2011; Symonds and Moussalli 2011). I used the function `model.sel` of the MuMIn package (Bartoń 2017) to rank models based on the Akaike's information criterion corrected for small sample size (AICc values). I reported the weight of the models which indicates to what extent one candidate model is more likely than another to provide a reasonable explanation of the variance in the data, along with the evidence ratio (ER) which indicates the extent to which the higher-ranked model is better than another candidate model. I also reported the marginal (fixed effects alone) and condition (fixed effects and random structures) coefficients of determination (R^2_m and R^2_c) of all models using the function `r.squaredGLMM` (Nakagawa and Schielzeth 2013). I extracted weighted parameter estimates (β), standard errors (SE), and 95% confidence intervals (95% CIs) of model intercepts and predictors from conditional averaging of the 4 candidate models (function `model.avg`).

Data availability

All data analysed during this study are provided in the electronic supplementary material.

Results

RIMs generally performed better and had between 5.3-37.5 times more empirical support than the competing RSM models (Table 1).

Concerning cheek colour, the RIM-null provided better explanation of the variance in the luminance data than its respective RIM-full (weight = 0.75, ER = 5.4, Table 1). RIM-full provided slightly better explanation of the variance in the redness data than its respective RIM-null (weight = 0.51, ER = 1.4, Table 1). Participant identity explained most of the observed variance in cheek luminance and redness data (R^2_{cs} around 80 %).

Concerning lip colour, the RIM-full provided slightly better explanation of the variance in the luminance data than its respective RIM-null (weight = 0.53, ER = 1.8). The RIM-null provided slightly better explanation of the variance in the redness data than its respective RIM-full (weight = 0.60, ER = 2.1). Participant identity explained most of the variation in lip redness data (R^2_{cs} = 82 %) and around half of the variation in lip luminance data (R^2_{cs} around 50 %).

The timing of ovulation explained none of the variance in cheek luminance or redness (R^2_{ms} = 0%, Table 1, Fig. 1). The timing of ovulation explained very little of the variance in lip luminance only (lip redness: R^2_m = 0%; lip luminance: R^2_m = 2% but with only 1.8 times more empirical support than the null hypothesis, Table 1). Lips became darker around ovulation, with a slight decrease / increase in luminance of 0.21 units on average each day² to / from the day of ovulation (β = 0.21 \pm 0.09 SE, 95% CI = 0.03; 0.39, Table 2, Fig. 2).

Discussion

Using objective methods to analyse fine-scale and intra-individual changes in cheek and lip colour according to the timing of ovulation, I found a weak evidence for a relationship between lip luminance, but not redness, and the timing of ovulation in women. Cheek colour may not be related to the timing of ovulation in agreement with a previous study (Burriss et al. 2015).

Overall, variation in colour data was mostly explained by inter-individual differences (up to 80% of the variance explained). However, when considering lip luminance only, intra-cycle variation appeared to be less modulated by participant identity, which effect dropped around 50%, and slightly more susceptible to the timing of ovulation (around 2%, which is undeniably small but yet twice as much as its effect on any other colour variable analysed). This may suggest that lip luminance respond differently to intra- and inter-individual effects than other colour variables. The luminance of lip skin may be more susceptible to variation in blood flow as a result of its higher vascularization and finer cellular layers (Crouzet et al. 1998) or due to tissue-specific differences in oestrogen receptor expression or sensitivity. The latter has already been suggested to explain the differential effect of cycle phase on facial and hindquarter colour in Japanese macaques (Rigaill et al. 2019). Further studies on tissue-specific expression and sensitivity of oestrogen receptors would help to clarify this question. Alternatively,

skin colour changes, and especially redness, may be more condition-dependent and related to individual effects such as health or heritability (Re et al. 2011; Dubuc et al. 2014; Pazda et al. 2016; Thorstenson et al. 2017).

Lip luminance varied of roughly 0.21 units each day² to / from ovulation. Day to day and overall intra-cycle variation in lip luminance don't achieve a change of more than 2.2 units, i.e., the threshold of colour discrimination in humans (Brainard 2003). Therefore, variation in lip luminance is likely imperceptible (at least in this sample), which is not really surprising since we may have noticed this about our own species. While lip luminance is unlikely an ovulatory signal it could still play a role in mate attraction and mating decision, if darker lips are attractive regardless of being informative about the timing of ovulation. Further studies should assess men and women responses toward inter-individual variation in lip colour to determine if this is the case. However, caution should be used when designing such experiments. Studies should avoid using composite stimuli from different participants and should control for the reproductive stage and sex hormones levels associated with the tested stimuli. Studies should also collect and test multiple samples per individual in order to detect fine-scale variation, and confirm ovulation using hormonal data or at minimum with ovulation detection kit. Such experimental design should reduce the risk of over- or under-estimating a potential cycle effect. However, whether variation in this trait is detectable under "optimal" conditions, i.e., artificial environments and controlled settings, does not necessarily entail consequences on human sexual communication and mate preferences since laboratory studies can have low ecological validity.

It should be noted again that the timing of ovulation explained only 2% of the variance in the data, which is quite a weak effect. This could suggest a correlation effect without causation between lip luminance and the timing of ovulation. However, a biological relationship does exist between skin luminance and variation in circulating oestrogens in humans, although presently weak in this sample, as in non-human primates (Rhodes et al. 1997; Thornton 2002). Thus, humans could have inherited the biological bases and mechanisms of expression for female red skin colour from a primate ancestor. This doesn't necessarily imply that lip luminance plays or does not play an active role in concealing ovulation or in mate choice in our species, i.e., the expression of red skin could have been maintained because it does not impose costs on women. However, investigating the possible different forms (traits) and functions (e.g., signalling/concealing the timing of ovulation) of female red skin colour in human and non-human primate species is necessary to better understand their evolution, i.e., to unveil the different socio-ecological constraints on sexual communication and mate choice.

The differences in red skin traits (face, lips, and sexual skin) and signalling functions probably result from the different socio-environmental constraints on mating across species. Concealing the reproductive status may

have evolved in species facing higher male monopolization or pair-bonding, lower intra-sexual competition, and higher costs on signalling; while higher infanticide risks and intra-sexual competition, limited mating opportunities, and lower costs on signalling may have favoured exaggerated or multimodal signalling (Fitzpatrick and Servedio 2018; Rooker and Gavrilets 2018). However, these proposed effects do not appear to fully explain the inter-species differences observed in female red skin colour functions, as species with similar socio-ecology express traits that may be involved in signalling or not signalling ovulation (Higham et al. 2010; Rigai et al. 2019). More studies using longitudinal and ecologically valid designs are needed to better understand the possible evolutionary pathways and underlying mechanisms leading to the evolution of female red skin colour in primate species including humans. Researches duplicating the present study design with a greater sample size would especially help to clarify the link between lip luminance, ovulation, and potentially mate choice in humans.

Compliance with ethical standard

Conflict of interest. I declare no conflict of interest.

Ethical approval. This study was approved by the Human Research Ethics Committee of the Kyoto University Primate Research Institute with the spirit of the Helsinki Declaration of 1975, as revised in 2000 (KUPRI, project number 2017-13). All participants signed a written agreement concerning their participation in the study, confidentiality, and the use of their photographs for research purposes and illustrations. Participants received financial compensation in return for their participation (5,000 JPY ~ 45 USD).

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367

Figure captions

Fig 1 Relationship between cheek (a) luminance and (b) redness and the days relative to the estimated ovulation.

Raw data are presented as grey circle. The red line presents the global model prediction; the black lines show prediction for each participant

Fig 2 Relationship between lip (a) luminance and (b) redness and the days relative to the estimated ovulation. Raw

data are presented as grey circle. The red line presents the global model prediction; the black lines show prediction for each participant

Table 1 Characteristics of candidate models for cheek and lip redness and luminance. AICc: corrected Akaike's information criterion, weight: model probabilities, ER: evidence ratios, $R^2_m - R^2_c$: marginal and conditional coefficients of determination. For each colour variable, candidate models are presented from higher-ranked to lower-ranked ones

Table 2 Model averaged parameters estimates (β) \pm standard errors (SE) and confidence intervals (95% CIs) from conditional averaging of all candidate models. Result for which CI does not include zero are presented in bold

Figure 1

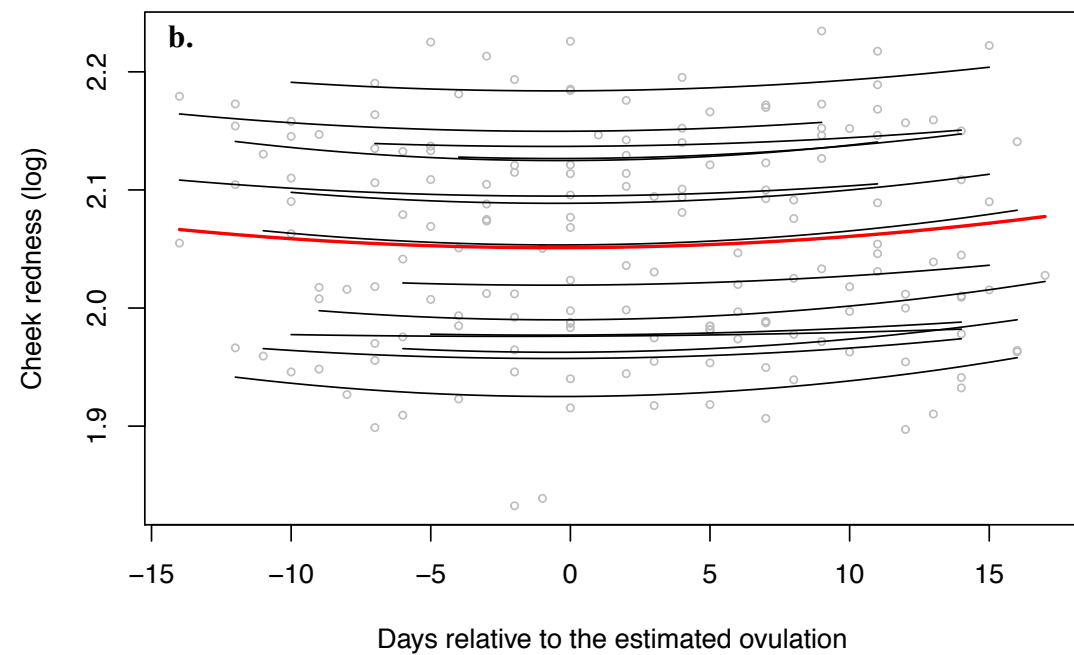
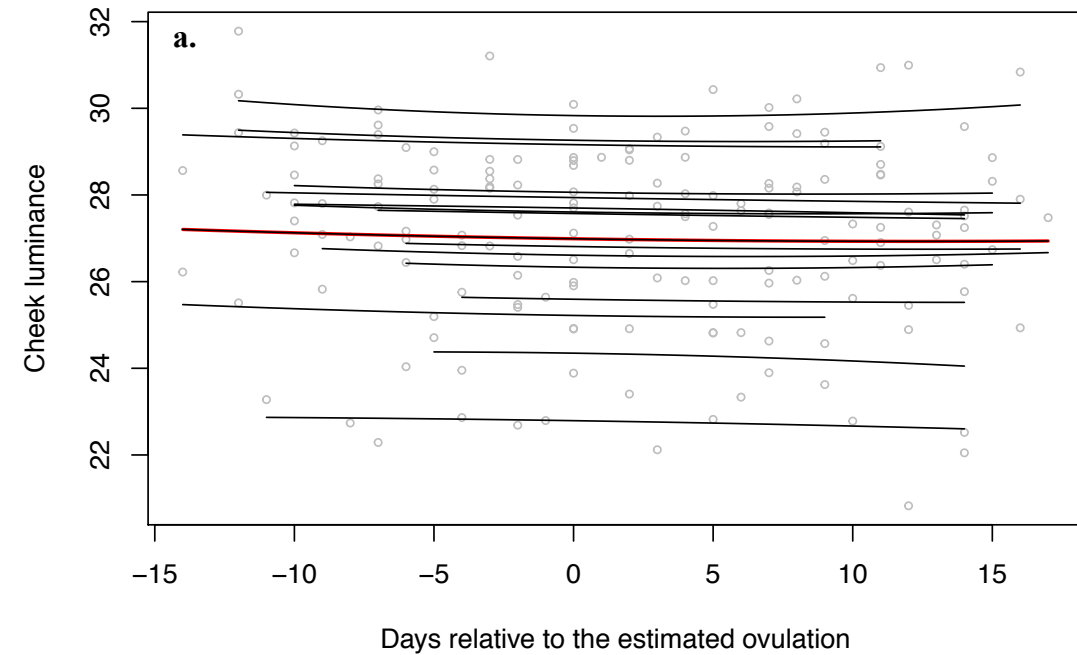


Figure 2

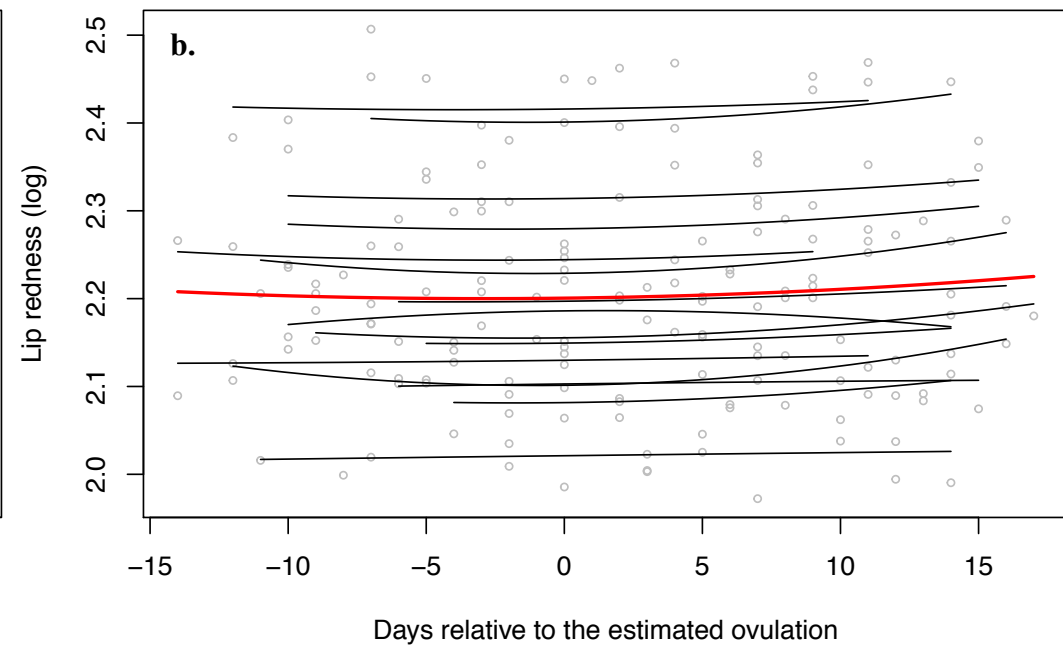
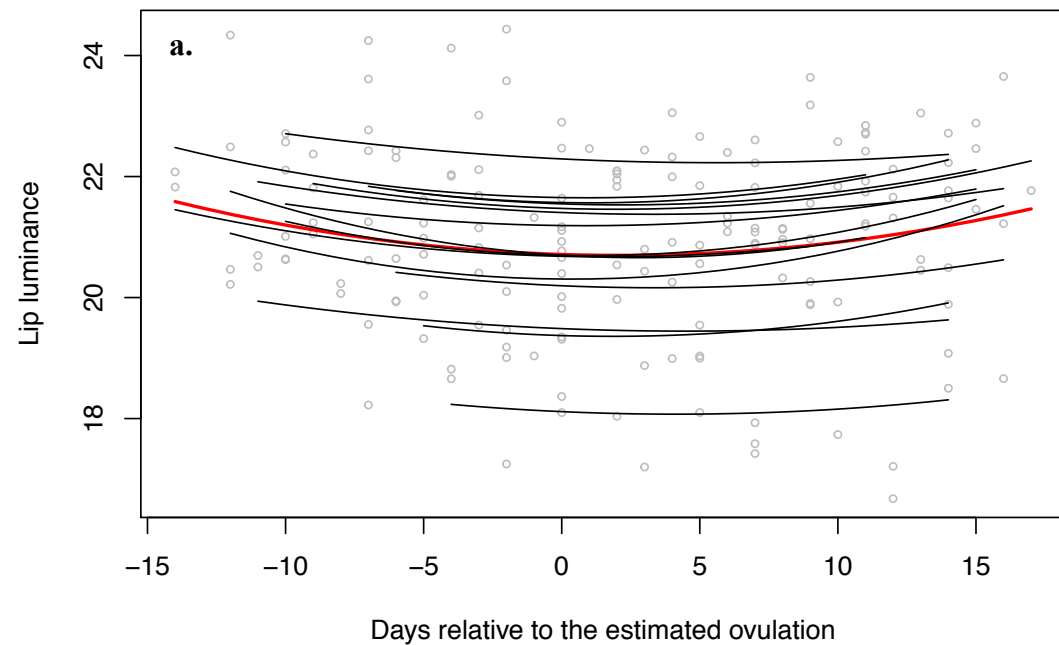


Table 1

	logLik	AICc	weight	ER	R ² m – R ² c		logLik	AICc	weight	ER	R ² m – R ² c
<i>Cheek luminance</i>						<i>Cheek redness</i>					
RIM - null	-247.751	501.7	0.75	–	0 – 81	RIM - full	279.799	-549.2	0.51	–	0 – 83
RIM - full	-247.292	505.0	0.14	5.4	0 – 81	RIM - null	277.351	-548.6	0.37	1.4	0 – 82
RSM - null	-247.751	505.9	0.09	8.3	0 – 81	RSM - full	279.799	-544.9	0.06	8.5	0 – 82
RSM - full	-247.292	509.3	0.02	37.5	0 – 81	RSM - null	277.621	-544.9	0.06	8.5	0 – 83
<i>Lip luminance</i>						<i>Lip redness</i>					
RIM - full	-272.607	555.6	0.53	–	2 – 50	RIM-null	221.984	-437.8	0.60	–	0 – 82
RIM - null	-275.332	556.8	0.30	1.8	0 – 49	RIM-full	223.354	-436.3	0.29	2.1	0 – 82
RSM - null	-274.316	559.0	0.10	5.3	0 – 54	RSM-null	222.055	-433.7	0.08	7.5	0 – 82
RSM - full	-272.345	559.4	0.08	6.6	2 – 53	RSM-full	223.356	-432.0	0.03	20	0 – 82

Table 2

	Intercept	DAY	DAY ²
<i>Cheek luminance</i>	26.99 ± 0.50 (26.01; 27.97)	-0.06 ± 0.07 (-0.21; 0.08)	0.03 ± 0.07 (-0.11; 0.17)
<i>Cheek redness</i>	2.05 ± 0.02 (2.01; 2.10)	0.00 ± 0.00 (-0.00; 0.01)	0.01 ± 0.00 (-0.00; 0.01)
<i>Lip luminance</i>	20.77 ± 0.32 (20.13; 21.40)	0.01 ± 0.09 (-0.18; 0.19)	0.21 ± 0.09 (0.03; 0.39)

Lip redness

2.20 ± 0.03 (**2.14; 2.27**)

0.01 ± 0.00 (-0.00; 0.01)

0.00 ± 0.00 (-0.00; 0.01)