Evidence indicates that men perceive women dressed in red as more sexually attractive (Elliot & Niesta, 2008; Elliot, Tracy, Pazda, & Beall, 2013; Niesta Kayser, Elliot, & Feltman, 2010; Pazda, Elliot, & Greitemeyer, 2012; Schwarz & Singer, 2013) and that women use red clothing as a courtship tactic, insofar as they are more likely to choose red clothes when they anticipate meeting potential mates (Elliot, Greitemeyer, & Pazda, 2013; Elliot & Pazda, 2012; Prokop & Hromada, 2013). Beall and Tracy (2013) argued further that red clothing may be an observable cue of women’s fecundity; this assertion was based on their finding that women were more likely to wear red during the fertile window than on other cycle days. Furthermore, within-subjects fluctuations in the ratio of estradiol to progesterone statistically mediated the within-subjects shifts in red-clothing choices. Our results appear to represent the first direct demonstration of specific hormone measurements predicting observable changes in women's courtship-related behaviors. We also demonstrate the advantages of hormonal determination of ovulatory timing for tests of cycle-phase shifts in psychology or behavior.
(Roney & Simmons, 2013). Therefore, reasoning that red clothing may reflect enhanced mating motivation, we predicted that the odds of wearing red would be positively associated with within-subjects shifts in estradiol and negatively associated with within-subjects shifts in progesterone. Although our a priori hypotheses pertained to the individual hormones on the basis of previous findings for libido, we also tested the effects of the estradiol-to-progesterone ratio, given our interest in cycle-phase shifts, because this ratio better predicts fertile-window timing (i.e., cycle days when conception is possible) than does either hormone in isolation (Baird, Weinberg, Wilcox, McConnaughey, & Musey, 1991).

Method
Participants and procedure
We took photos of a sample of naturally cycling women who participated in a broader study on the relationship between ovarian hormones and mating behavior (see Grillot, Simmons, Lukaszewski, & Roney, 2014; Roney & Simmons, 2013). A total of 52 women participated in the overall study, but only data from 46 women who consented to use of their photos were analyzed here (mean age = 18.80 years, SD = 1.22). Twenty-five of these women identified themselves as White, 10 as Asian, 7 as Hispanic, and 4 as “other.” Further details regarding this sample can be found in Roney and Simmons (2013). Participants provided written informed consent for their participation, and all procedures were approved by the institutional review board at the University of California, Santa Barbara.

Participants attended four afternoon laboratory sessions during either one or two menstrual cycles. The sessions were spaced approximately 1 week apart, so each woman participated in either four or eight laboratory sessions. During each session, women provided saliva samples via passive drool and were photographed under standard lighting conditions in the clothing that they had worn to the lab. Thirty-six women provided photos across two menstrual cycles (separated by 1–2 months) and 10 women provided photos in one cycle.

As part of the broader study, the participants also collected daily saliva samples each morning. Hormones were assayed from a subset of these to estimate day of ovulation for the fertile-window analyses (see Estimation of Fertile-Window Timing). Only samples collected from the afternoon laboratory sessions were used to test hormonal predictors of red-clothing choices because hormones were assayed from every laboratory-session saliva sample but from only a subset of the daily morning samples.

All data and stimuli from the participants were collected years before the authors considered examining red-clothing choices. Therefore, sample size was determined by the original data collection. The original study was designed to examine hormonal predictors of women’s sexual psychology and behavior, as well as their physical attractiveness. Sample size was determined as the largest number of participants for whom available funding would allow daily hormone assays across two menstrual cycles.

Measures
Hormone measures. Saliva samples were shipped to the Endocrine Core Laboratory of the California Regional Primate Research Center (Davis, CA), where they were assayed for concentrations of estradiol, testosterone, and progesterone. Samples were centrifuged at 3,000 rpm for 20 min to separate the aqueous component from other particles. Estradiol concentrations were estimated in duplicate using the high-sensitivity salivary 17β-estradiol enzyme immunoassay kit (Salimetrics LLC, State College, PA). The estradiol assay has a least detectable dose of 0.1 pg/ml; intra-assay and interassay coefficients of variation (CVs) were 4.52% and 8.15%, respectively. Concentrations of progesterone were estimated in duplicate using commercial radioimmunoassay kits (Siemens Health Diagnostics, Inc., Los Angeles, CA). The procedures were modified as follows to accommodate overall lower levels of progesterone in human saliva relative to plasma: (a) Standards were diluted to concentrations ranging from 0.05 to 4.0 ng/ml, and (b) sample volume was increased to 200 µl. The progesterone assay has a least detectable dose of 0.00914 ng/ml; intra-assay and interassay CVs were 4.57% and 7.36%, respectively. Concentrations of testosterone were estimated in duplicate using commercial double-antibody radioimmunoassay kits (Beckman Coulter Inc., Webster, TX). The testosterone assay has a least detectable dose of 1.3697 pg/ml; intra-assay and interassay CVs were 5.20% and 9.83%, respectively.

All saliva samples from the laboratory sessions were assayed. Mean concentrations were 2.96 pg/ml (SD = 1.23) for estradiol, 0.05 ng/ml (SD = 0.04) for progesterone, and 26.15 pg/ml (SD = 14.98) for testosterone. For the daily samples, all samples in a 9-day window surrounding the initial estimated day of ovulation (computed as 15 days before the end of each cycle) were sent for assay, as were samples from alternating days outside of this window. The estradiol-to-progesterone ratio was computed by first converting the unit of measure for each hormone to picomoles per liter and then dividing the value for estradiol by the value for progesterone.

Red-clothing measures. Photos from the participants’ lab sessions were coded independently by two female research assistants who were blind to the participants’
hormonal status, using criteria from Beall and Tracy (2013). Research assistants were instructed to code the color (black, blue, gray, green, pink, red, white, yellow, or other) of each visible item in seven categories of clothing (dress, shirt/blouse, jacket or sweatshirt, pants, shorts, skirt, and accessories). If an item was multicolored, they were instructed to pick the color that was most prevalent. Because the lower body was not fully visible in all photographs, and because past research on red clothing has focused on the color of shirts (Beall & Tracy, 2013), the dependent variable for our data analyses was whether the subject was coded as wearing a top garment (i.e., shirt, sweatshirt, dress, or jacket) that was red or pink (hereafter referred to as red). Coders discussed each case in which they disagreed on the presence of a red top (n = 2) and then reached a joint decision regarding the final coding.

**Data analysis**

**Estimation of fertile-window timing.** To classify individual photo sessions as being inside or outside of the fertile window, we used the daily hormone values to estimate the day of ovulation in each cycle. We first judged whether cycles were ovulatory by following the procedures in Ellison, Lager, and Calfee (1987), in which cycles that did not achieve a maximum progesterone value of at least 300 pmol/L were judged anovulatory; 53 of 82 total cycles were judged ovulatory. (Note that in the previous articles in which cycle-phase effects on red-clothing choices were assessed, ovulatory status was not confirmed, which was an additional source of error.) Next, for each cycle identified as ovulatory, we determined the day of peak estradiol. Following the method of Lipson and Ellison (1996), we then found the two consecutive days (including or after the estradiol peak) between which there was the greatest decrease in estradiol. The second of those two days was considered the day of ovulation (for further details, see Grillot et al., 2014). The fertile window was then defined as the day of ovulation and the 5 days preceding it (see Wilcox, Weinberg, & Baird, 1995).

To compare findings across different methods of estimating ovulatory timing, we also computed high-fertility days using the method of Beall and Tracy (2013). They specified Days 6 to 14 after the start of menses as high-fertility days and Days 0 to 5 and 15 to 28 after menses onset as low-fertility days. We counted forward from the participants’ self-reported last onset of menses to compute these two time windows.

**Statistical models.** We constructed binary mixed logistic regression models in IBM SPSS Statistics (Version 22) to model the probability of wearing a red top as a dichotomous outcome. Although our data were technically on three levels (laboratory sessions nested within cycles nested within women), we took the advice of statistical consultants and constructed two-level models (sessions nested within women), given the known convergence problems in logistic mixed regression models with few higher level units (in this case, two cycles). The two-level structure meant that the probability of wearing red was modeled across four to eight sessions within each woman, depending on whether she contributed one or two cycles of data. All models included a random subject-level intercept and used robust estimation of standard errors.

To place all variables on the same metric, we first standardized values for hormone predictor variables relative to their respective grand means, but we then group-mean centered the standardized values within cycles. Thus, for women with two cycles of data, each hormone value was centered relative to the mean of the cycle from which it was drawn rather than relative to the woman’s overall mean. This centering decision means that regression models assessed the effects of within-cycle shifts in hormones. Centered hormone values that were more than 3 standard deviations higher or lower than their respective means were excluded to avoid undue influence of outliers (there was one such outlier each for testosterone, progesterone, and the estradiol-to-progesterone ratio), though results were very similar when outliers were included. Fertile-window timing was entered as a dummy variable. Models including the fertile-window variable were computed only for ovulatory cycles, whereas models containing only hormonal predictors were estimated for all cycles. Tests for mediation of fertile-window effects by hormonal predictors were conducted using the Monte Carlo method for assessing mediation as described in Selig and Preacher (2008), with adjustment of coefficients to account for the dichotomous outcome variable (see Herr, 2014).

**Results**

**Fertile-window timing**

Across all ovulatory cycles, the participants wore red 17% (8 of 48 days) of the time during the fertile window but only 8% (13 of 160 days) of the time on other cycle days. A mixed regression model confirmed that, within subjects, the odds of wearing red were higher during the estimated fertile window than on other cycle days, \( b = 0.93, p = .040 \), odds ratio (OR) = 2.53, 95% confidence interval (CI) = [1.04, 6.14]. The 2.53 odds ratio indicates that the odds of wearing a red top were about 2.5 times higher inside the fertile window, but there was a wide confidence interval.

Using Beall and Tracy’s (2013) counting method of estimating high fertility (see Method), we found that red was worn on 11% (8 of 76) of high-fertility days and on a virtually identical 10% (13 of 129) of low-fertility days.
A mixed regression model confirmed the absence of a within-subjects fertility effect when using this method, \( b = 0.09, p > .250, \text{OR} = 1.09, 95\% \text{CI} = [0.47, 2.55] \). These results pertain only to ovulatory cycles, but application of this method to all sessions still produced a null result (i.e., a 10\% rate of wearing red on both high- and low-fertility days).

### Hormonal predictors

Table 1 presents results of separate mixed logistic regression models to test the zero-order effects of each measured hormone and the estradiol-to-progesterone ratio on the odds of wearing red (we had no predictions for testosterone, but we included it given the common belief that testosterone affects women’s libido; however, see Roney & Simmons, 2013). Only the estradiol-to-progesterone ratio had a confidence interval for the odds ratio that excluded 1. For each 10\% increase in the estradiol-to-progesterone ratio above a cycle mean, the odds of wearing red nearly doubled. Null results for the three individual hormones persisted when all were entered simultaneously into the same regression model (without the estradiol-to-progesterone ratio), whereas the effect size for the estradiol-to-progesterone ratio was even larger when added to a model that included the three individual hormones, \( b = 0.84, p = .009, \text{OR} = 2.33, 95\% \text{CI} = [1.24, 4.36] \).

Next, for the subset of ovulatory cycles, we simultaneously entered fertile-window timing and the estradiol-to-progesterone ratio into a logistic mixed regression model. In this model, the confidence interval for the odds ratio associated with fertile window timing no longer excluded 1, \( b = 0.47, p > .250, \text{OR} = 1.60, 95\% \text{CI} = [0.63, 4.03] \), whereas the interval for the estradiol-to-progesterone ratio did exclude 1, \( b = 0.65, p = .041, \text{OR} = 1.92, 95\% \text{CI} = [1.03, 3.58] \). In a Monte Carlo simulation with 20,000 random draws, the estradiol-to-progesterone ratio did in fact mediate the effect of fertile-window timing on the probability of wearing red, indirect effect = 0.042, 95\% CI = [0.002, 0.09].

Figure 1 presents the mean estradiol-to-progesterone ratio and mean probability of wearing red aggregated across all ovulatory cycles and plotted against cycle days. The fertile-window effect is visible as the increased probability of red-clothing choices in the late follicular phase relative to most other regions of the cycle. The correspondence between the estradiol-to-progesterone ratio and frequency of red clothing is also clearly visible; both variables show sharp declines from their follicular-phase peaks after the onset of the nonfecund luteal phase.

### Discussion

The present findings support the idea that women more frequently choose to wear red clothing during the fertile window than on other days of their menstrual cycles. Previous studies had provided mixed evidence for this effect (Beall & Tracy, 2013; Prokop & Hromada, 2013; Tracy & Beall, 2014) but were limited by between-subject analyses, error-prone methods of estimating ovulatory timing, and an inability to exclude anovulatory cycles. Here, we used hormonal confirmation of ovulatory timing to demonstrate this effect within subjects. These results suggest that women are more likely to exploit men’s preference for women wearing red (see the introduction) on days when conception is possible than on other days in the cycle. This complements other findings of women’s increased motivation to enhance their attractiveness during the fertile window (e.g., Durante, Li, & Haselton, 2008).

It is not entirely clear why red in particular is used to enhance attractiveness. Women’s facial redness is positively related to shifts in estradiol (Jones et al., 2015), and red flushing has been associated with sexual arousal (Katchadourian, 1984). Men may therefore prefer red as a marker of hormone production or sexual interest, which women may exploit when their sexual motivation is higher. Further research on ultimate causes is necessary.

Our results highlight limitations of counting methods of cycle-phase estimation (see Gildersleeve, Haselton, & Fales, 2014). Our classification of fertile-window timing via frequent hormone sampling (see Baird et al., 1991; Lipson & Ellison, 1996) agreed with the counting method used by Beall and Tracy (2013) in only 64\% of total cases, and 46\% of hormone-identified high-fertility days were classified as low fertility by the counting method. Beall and Tracy’s classification of Days 6 to 14 as high-fertility days may be especially problematic given evidence that later days are often fertile (Wilcox, Dunson, Weinberg, Trussell, & Baird, 2001). However, other counting methods could be more accurate. Our data nonetheless demonstrate errors associated with common counting methods and thus lead us to recommend hormonal confirmation of ovulatory timing.

### Table 1. Zero-Order Effects of the Hormone Variables on the Odds of Wearing Red

<table>
<thead>
<tr>
<th>Hormone variable</th>
<th>( b )</th>
<th>( p )</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>-0.03</td>
<td>&gt; .250</td>
<td>0.97 [0.59, 1.60]</td>
</tr>
<tr>
<td>Progesterone</td>
<td>-0.27</td>
<td>&gt; .250</td>
<td>0.76 [0.36, 1.63]</td>
</tr>
<tr>
<td>Testosterone</td>
<td>-0.24</td>
<td>&gt; .250</td>
<td>0.79 [0.31, 1.98]</td>
</tr>
<tr>
<td>E:P ratio</td>
<td>0.63</td>
<td>.010</td>
<td>1.88 [1.17, 3.03]</td>
</tr>
</tbody>
</table>

Note: The values in square brackets are 95\% confidence intervals. Each row represents a separate binary mixed logistic regression model predicting red clothing as a dichotomous outcome. E:P ratio = estradiol-to-progesterone ratio.
We also found that the estradiol-to-progesterone ratio positively predicted the odds of wearing red clothing, and that this ratio mediated the fertile-window effect. This is the first direct demonstration of statistical mediation of a fertile-window effect by hormone variables, but it should be interpreted with some caution. Contrary to our hypotheses, we did not observe main effects of estradiol and progesterone but rather a nonadditive effect produced by their ratio. Previous work examining daily fluctuations in women's libido demonstrated only additive effects of these hormones (Roney & Simmons, 2013), whereas studies on the motivational salience of sexually dimorphic faces also found effects of the estradiol-to-progesterone ratio (Wang, Hahn, Fisher, DeBruine, & Jones, 2014). Variables that are tightly coupled to the fertile window may be especially sensitive to this ratio, given how closely it tracks fluctuations in fecundity (Baird et al., 1991), but future research will be necessary to test this possibility and to systematically map the additive and nonadditive psychological effects of ovarian hormones.

In conclusion, we found positive effects of the fertile window and the estradiol-to-progesterone ratio on women's odds of wearing red. The finding of a fertile-window effect replicates a contested effect (Beall & Tracy, 2013; Gelman, 2013) and complements other studies in suggesting that the motivational salience of mate attraction increases on fecund cycle days (e.g., Durante et al., 2008). The findings regarding hormones complement recent studies that have provided evidence for hormonal predictors of cycle-phase shifts in mate preferences (e.g., Pisanski et al., 2014; Roney & Simmons, 2008; Roney, Simmons, & Gray, 2011) and extends this literature to include hormonal prediction of women's observable courtship behaviors.

**Author Contributions**

A. B. Eisenbruch and J. R. Roney designed the study. Z. L. Simmons and J. R. Roney collected the original data set. A. B. Eisenbruch and J. R. Roney conducted the data analysis and drafted the manuscript, and Z. L. Simmons provided critical revisions. All authors approved the final version of the manuscript for submission.

**Acknowledgments**

We thank Shelly Gable for statistical advice, Ellise Fallon and Samantha Wilcoxon for data assistance, and Rachel Grillot for comments on the manuscript. Other analyses from this data set have been published in Roney and Simmons (2013) and Grillot, Simmons, Łukaszewski, and Roney (2014).
Funding
This research was funded by a Hellman Family Faculty Fellowship; by a University of California, Santa Barbara (UCSB), Academic Senate grant (both to J. R. Roney); and by a grant from the Global Centers of Excellence Program of Hokkaido University to the Center for Evolutionary Psychology at UCSB.

Open Practices
All data have been made publicly available via Merritt and can be accessed at http://n2t.net/ark:/13030/m5bc629d. The complete Open Practices Disclosure for this article can be found at http://pss.sagepub.com/content/by/supplemental-data. This article has received the badge for Open Data. More information about the Open Practices badges can be found at https://osf.io/tvyxz/wiki/1.%20View%20the%20Badges/ and http://pss.sagepub.com/content/25/1/3.full.

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